



An Infant in Swaddling Clothes. This practice, which dates from antiquity, was probably done with the idea of preventing rachitic deformities. From the plaque by Andrea della Robbia (1435-1525) in the Ospedale degl' Innocenti, Florence. By kind permission of the Director, Prof. Arrigo Galeotti-Flori.

Frontispiece.

THE VITAMINS IN MEDICINE

by

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PREFACE TO THE THIRD EDITION

IN the preparation of the third edition of this book most of it has been rewritten. This has been necessary owing to the changing state of our knowledge and the advances made in certain directions, notably the physiology and biochemistry of the vitamin B complex. Although the number of references has been increased from about 4,500 to 5,500 and the illustrations from 208 to 245, the size of the book remains about the same.

Our work has been greatly helped by suggestions and criticisms and also by the generosity of those who have allowed us to reproduce their original illustrations. We are especially indebted to the many workers in England, the Continent and the Americas who besides sending us reprints of their published articles have kept us informed about their current research and its results.

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CHAPTER I
VITAMIN A
THE ANTIXEROPHTHALMIC VITAMIN
THE ANTI-INFECTIVE VITAMIN
AXEROPHTOL

VITAMIN A is the term used to include vitamin A₁ found in animals and sea fish, and vitamin A₂ found chiefly in fresh-water fish. Biologically and chemically they are so very similar that generally no distinction is made between them. The little that is known about vitamin A₂ is discussed on p. 85. Axerophthol was the name given by Karrer in 1938 to vitamin A₁, but it has not come into common use, except on the Continent.

Provitamin A is the name given to those plant carotenoids which can be converted by animals to vitamin A.

HISTORY

The most clear-cut effect of lack of vitamin A is night blindness, which often occurs suddenly after long exposure to a day's bright sunlight. In rural communities inability to see in the dusk is a very serious condition: fishermen, for instance, may walk off the rocks into the sea after landing in the evening. Night blindness can be cured, often in twelve hours, by eating food rich in vitamin A, such as liver. The dramatic quickness both of the onset and the cure explains why liver has been used for centuries for night blindness.

The Ebers Papyrus [1], written about 1600 B.C., probably referred to night blindness when liver was recommended for the eyes, while the Chinese in 1500 B.C. were giving liver, honey, flying fox dung and tortoiseshell, all of which would have cured night blindness [5]. Hippocrates advised the whole liver of an ox dipped in honey, and liver was known to later Roman writers. Jacob van Maerland, a Dutch poet of the fourteenth century, may be thus translated [2],

He who cannot see at night,
Must eat the liver of the goat
Then he can see all right.

Guillemeau in France in the sixteenth century besides clearly describing night blindness, advised liver for its cure [3], which was also advised by other writers at that time [5].

Drummond and Wilbraham [2] find that the first mention of liver for the eyes in England was in Muffett's "Health's Improvement" (1655), though Bayly, at one time Queen Elizabeth's physician, in his book on eyes recommends "rawe herbes" among which is "eie bright"; but the only evidence of night blindness being common at this time is references to mists and films over the eyes. "Rawe herbes" would of course provide provitamin A.

Aykroyd [1] in his accounts of Newfoundland and Labrador fishermen says they not only recognize how bright sunlight may bring on night blindness, but also use liver, preferably the raw liver of a gull or puffin, for a cure.

The beginning of the present century saw the realization that more

serious eye affections—especially “conjunctivitis” in children—were due to lack of some food factor. Mori [4] in Japan in 1904 treated juvenile conjunctivitis with cod-liver oil, believing the diet was inadequate in fats, while Monrad in 1917 thought that the outbreak of conjunctivitis which occurred in Danish children at that time was due to a deficiency of a fat soluble factor, caused by the export of the country's animal fats to England and Germany.

Animal experiments as early as 1909 had shown that rats on deficient diets developed conjunctivitis [6]; that a fat soluble factor was involved was proved by Osborne and Mendel [7] in 1913, and McCollum and Simmonds [8] in 1917. The latter workers called the factor “fat soluble A” and pointed out the similarity between xerophthalmia in rats and the conjunctivitis found in children on fat-deficient diets.

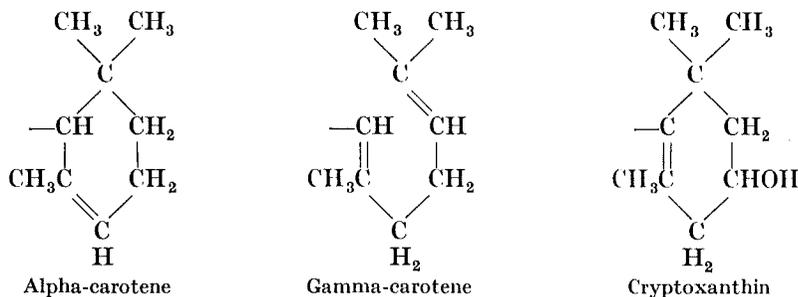
Conjunctivitis and xerophthalmia are, however, only the most noticeable examples of the change in the epithelial surfaces of the body brought about by lack of vitamin A. Wolbach and Howe [9] in 1925 found that “the specific tissue change due to deprivation of fat soluble vitamin A is replacement of various epithelia by stratified squamous keratinizing epithelium.” This replacement of a specialized epithelium by a primitive type leads to a lowered local resistance to infection.

The name “anti-infective” vitamin was first given to vitamin A by Green and Mellanby [10] in 1928 because they found that animals killed by lack of vitamin A showed multiple foci of infection in those areas where the epithelium had altered. At this time the infection was regarded as the direct, and not secondary, effect of lack of vitamin A.

The separation of vitamin D from vitamin A was not complete before 1925. As early as 1909 Stepp [11] had found that there was some unrecognized factor in fats necessary for growth, and in 1913 McCollum and Davies [12] and also Osborne and Mendel [7] confirmed this, the latter workers also stressing that different fats varied in their value for growth. Mellanby in his work on rickets from 1918 onwards originally believed that the antirachitic factor, whose existence he discovered, was the same as the fat soluble A factor of McCollum and Davis. But in 1922 and the following years several very important papers appeared, all showing that there were two separate factors in fats—the growth promoting or anti-xerophthalmic factor and the antirachitic factor. Thus Hume [13] and also Goldblatt and Soames [14] found that ultra-violet irradiation, while it cured rickets, would not prevent xerophthalmia or maintain growth in animals on fat deficient diets. A year later in 1923 Goldblatt and Zilva [15] found that the growth-promoting and antirachitic functions of cod-liver oil were destroyed by heat and oxidation at different rates, and they also observed that spinach was excellent for growth but not for preventing rickets. Mellanby [16] in 1926, comparing the diets of a series of puppies which had died or survived an epidemic of bronchopneumonia, reported that the protective value of the diet against infection was not related to its protective value against rickets.

The carotene content of plants was found by Rosenheim and Drummond [17] in 1920 and by Coward [20] in 1923 to vary with their vitamin A potency, a relationship which was further emphasized by Rosenheim and Drummond's [18] observations in 1925 on the similarity of the colour reactions of the two [19]. Between 1929 and 1930 Moore [21, 22, 23] and Capper [24] were largely responsible for showing that carotene could be used by animals as a source of vitamin A, into which it is converted in the body.

The chemistry and isolation of vitamin A and its relationship to carotene was settled chiefly by the work of Karrer [25, 26, 27] and Heilbron [28, 29] and their co-workers and of Holmes and Corbett [30] between 1930 and 1937. The final synthesis [31] was the result of English, Dutch, Swiss and American research which, stimulated by the threat of a shortage of vitamin A during the Second World War, came to a successful conclusion in 1947.



but in its place the various terminal groups [39] shown above, cannot at best form more than one molecule of vitamin A.

Stereomeric isomers of both vitamin A and its carotenoid precursors occur naturally. In theory vitamin A—since it has but two “stereochemically effective” double bonds [41]—can have only four different spatial configurations: *trans-trans*, *trans-cis*, *cis-trans* and *cis-cis*. Vitamin A is believed to be the *trans-trans* form, and neovitamin A (p. 6), the only other form of vitamin A so far known to occur naturally, the *trans-cis* form.

The carotenoid stereoisomers were first described by Gillam and El Ridi [44] in 1936, when they found that, during adsorption on alumina, beta-carotene was spontaneously transformed into “pseudo alpha-carotene”—the neo-beta-carotene B of later workers. Since 1936 much research has been devoted to the carotene isomers, but they are mostly artificial products, almost all the naturally occurring carotene fortunately being in the all-*trans* form, so that, for instance, the thirty-two possible alpha-carotenes can remain a fascinating academic study without being a nightmare in practical nutrition. The physiology of the carotenoids being discussed on p. 10, it is only necessary here to emphasize that the tendency to spontaneous isomerization may complicate analyses involving carotenoids.

Vitamin A and its carotenoid precursors being fat soluble and unsaponifiable are found concentrated in the unsaponifiable extracts of fats. Separation of vitamin A from the carotenoids can be carried out by dissolving both in petroleum ether and adding alcohol, when the latter will dissolve the vitamin A but not the carotenoids. Since alcohol and petroleum ether are not miscible, the alcohol layer containing the vitamin can be easily separated. Vitamin A esters, however, remain with the carotenoids. Chromatographic adsorption is extensively used for the separation of vitamin A from other substances such as kitol [50], for the separation of the alcohol from the ester [77] or for the separation of the carotenoids from each other [78].

Pure crystalline vitamin A alcohol was first isolated—from fish-liver oil—by Holmes and Corbett [30] in 1937, though it was subsequently shown that the pale yellow crystals were solvated, their true melting point not being 6° C. but 60° C. The blue value (p. 6) was 100,000 and $E_{\frac{1\%}{1\text{ cm.}}}^{328}$ equalled 2,000. The biological activity was reported to be 3,000,000 I.U. per gram, which gave a conversion factor nearer 1,500 than 1,600 (p. 9). Mead, Underhill, and Coward [45] in 1939, using two crystalline esters of vitamin A, reported that the biological activity was 3,181,000 and 3,424,000 I.U. per gram, and since $E_{\frac{1\%}{1\text{ cm.}}}^{328}$ equalled 1,600–1,800, the conversion factor was about 2,000. Many subsequent workers have confirmed these findings [39, 46], apart from very minor differences, and the crystalline vitamin A acetate is now used in the U.S.A. as a Reference Standard (p. 10). Some American reports [46] about abnormally high biological values and conversion factors for the crystalline alcohol and ester were due to one of the older undervalued U.S.A. Reference Oils having been used for the assays (p. 10).

Three other forms of vitamin A besides vitamin A₂, which is discussed on p. 85, may occur in fish-liver and other oils: anhydro or "cyclized"

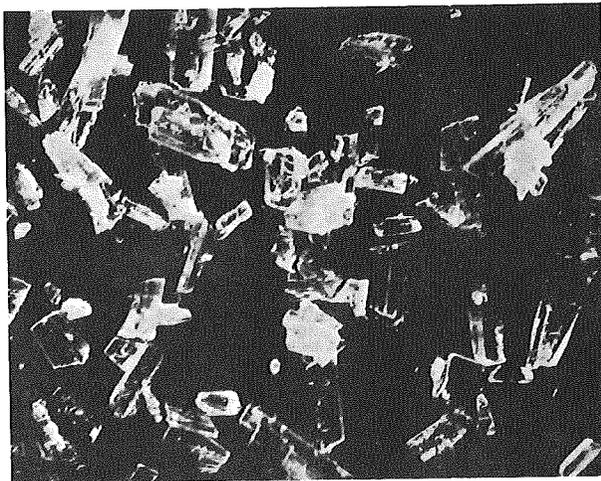


FIG. 1. Vitamin A—Alcohol crystals. Magnification: 10×.

vitamin A, kitol and neovitamin A. The first of these [47] is formed spontaneously in fish-liver oils which have been "maltreated"; it is not a



FIG. 2. Neovitamin A—Alcohol crystals. Magnification: 15×.

cyclized vitamin A alcohol as was at one time thought, but is a hydrocarbon formed by dehydration which has no biological activity. Its only practical

importance is that it robs oils of some of their value and its absorption bands with the Carr-Price reaction may complicate assays by overlapping those due to vitamin A.

Kitol [48, 49, 50], on the other hand, is a di(vitamin A) normally present in large amounts in fresh whale-liver oil and has been reported in that of sharks, dogfish and lambs, while in that of pike is a substance similar to kitol but related to vitamin A₂. Kitol has no biological activity and causes considerable difficulty when estimating vitamin A in whole liver oils owing to its colour reactions, though it can be removed by chromatography [50]. Barua and Morton [50] have suggested that it may be formed to detoxify excessive accumulations of vitamin A. When heated it forms vitamin A and degradation products, a reaction of great value in increasing the yield of the vitamin.

Neovitamin A [51, 54] forms about thirty-five per cent. of the vitamin A in many common fish-liver oils. It is a stereoisomer of vitamin A, probably being in the *trans-cis* form, and it has the same biological potency for rats, being converted into the usual *trans-trans* form and stored as this in the liver after feeding. It has maximum absorption in the ultra-violet region of 328 millimicrons, a conversion factor of 1645 and it reacts more slowly than vitamin A with maleic anhydride, this forming the basis of its assay in fish-liver oils.

The synthesis of vitamin A became of practical importance during the Second World War because of the risk that supplies from fish-liver oils could not be maintained. The consequent wave of research, besides showing that all earlier reported syntheses—such as that of Kuhn and Morris [40] in 1937—could not be confirmed, did also produce, in amounts too small to have any dietetic value, a number of substances with vitamin A activity; but Milas [41], writing in 1947, ended his very detailed review of the highly complicated chemistry of this subject by pointing out that “in no case has a synthetic product been shown to be identical in *every respect* with the natural product.” However, by 1948 Sir Ian Heilbron [51], in a very lucid summary of current research, was able to talk of “the large-scale production of the synthetic vitamin.” For further progress the reader should consult the papers by Isler, Wendler or Milas and their collaborators [42], and that of Sobotka and Chanley [43], who give a preliminary account of research designed for the total synthesis of vitamin A without starting from the natural beta-ionone, mostly obtained from lemon-grass oil, which all other workers have used.

Vitamin A gives a band of maximum absorption in the ultra-violet region at 325 millimicrons [39], but as it was originally thought [52] that the maximum absorption was at 328 millimicrons—which is now known to be due to the neovitamin A of fish-liver oils discussed above—this is still generally used when determining vitamin A in fish-liver oils by spectrophotometry [53].

The colour reactions of vitamin A are of great practical importance. They occur with sulphuric acid [17] and with the chlorides of polyvalent metals [18, 19]; the most satisfactory reagent was found by Carr and Price [19] to be antimony trichloride dissolved in chloroform. This test is often called the Carr-Price colour test. With vitamin A a blue colour develops rapidly and then fades. The blue colour, however, is not only given by vitamin A but also by carotenoids [18] and “oxycholesterol” [54]. Therefore to confirm the presence of vitamin A the absorption spectrum of the Carr-Price reaction must be examined, when, if vitamin A is present, specific absorption bands will be found. Activated glycerol dichlorohydrin has been used instead of antimony trichloride [55], but it would appear to have no advantages [56].

Vitamin A exhibits fluorescence when irradiated by a mercury vapour lamp [57]: this has been used by Popper to demonstrate vitamin A in the tissues (Figs. 3, 4, 5) by the technique which he describes in the *Archives of Pathology* [58]. The differences in the fluorescence of the alcohol and the

ester have been used to determine the amounts of each in preparations of vitamin A [57.]

Biological activity is lost when vitamin A takes part in chemical reactions which affect double bonds, such as oxidation, hydrogenation and bromination [59]. Heat does not destroy vitamin A in butter at temperatures below about 120° C. in the absence of oxygen [60]; when oxygen is present slow destruction occurs in oils even at room temperatures [61] and rancidity accelerates this, though it probably causes no appreciable loss in human food [64]. Many fats when heated develop an "anti-vitamin A" factor which destroys the biological activity of vitamin A; whether this is a chemical or biological effect is unknown [65, 66]. Exposure of cod-liver oil to light reduces its vitamin A content, so the oil should be stored in dark bottles [62, 63]. Vitamin A in "cod-liver oils" prepared from concentrated fish-liver oils diluted with cottonseed, peanut or maize oil is less stable than vitamin A in genuine cod-liver oil, nearly twice as much being destroyed after exposure to winter sunshine for eleven days [67].

ESTIMATION OF VITAMIN A AND CAROTENE

Vitamin A can be estimated biologically; physically, by absorption spectra estimations; and chemically, by colour reactions. Since essentially the same methods are used in the estimation of carotene, these will not be fully described.

The Biological Estimation. In biological estimations—which have been fully discussed by Coward [68] in her classical book on the subject—either the curative or the prophylactic method can be used; that is the potency of the substance being tested is estimated either by its capacity to cure the symptoms of vitamin A deficiency or by its capacity to prevent their onset. Of the two methods, the curative is generally considered more satisfactory, though it has been criticized firstly on the grounds that the ill-health of the vitamin A depleted animals at the beginning of the estimation varies, this variation altering their subsequent response to vitamin A, and secondly on the grounds that the "depletion period" during which a vitamin A deficient diet is given is avoided in prophylactic tests. Coward [68] has pointed out that neither criticism is valid because firstly deficient rats show no greater individual variations than do normal rats in their response to vitamin A, and secondly in the prophylactic test the animals at the beginning have different amounts of vitamin A already stored, thus introducing a variation in response which can only be ignored if the average results from a large number of rats are used. Various symptoms may be taken for indicating the onset of cure of the deficient state. Of these change in weight has been the most widely used, and has the great advantage that it is easy to measure. Against it, has been urged that it is not specific for vitamin A—even though vitamin A has a specific action on growth [69]—since any other deficiency has the same effect. Xerophthalmia (p. 33) and the changes in the desquamated epithelial cells of the vagina (p. 36) are sometimes used, being only caused by lack of vitamin A. Deciding, however, when early xerophthalmia is present or when it is cured depends on what criteria each worker adopts, and so is liable to great variation. The changes in the vaginal epithelium are difficult to interpret [70] unless the animals are previously spayed to prevent the changes due to oestrus [71].

Irving and Richards [72] in 1940 found that after seven weeks on a vitamin A deficient diet young rats had a constant degeneration of nervous tracts in the medulla. There was only a very small difference between the amounts of vitamin A which could and could not protect the rats against this degeneration. This method of assay was very carefully checked by Coetzee [73] in 1949, who reported that it was possibly more accurate than the standard curative method and took two weeks less to perform.

Guggenheim and Koch's method of assay [74] is based on the amount of vitamin A stored in the livers of rats which, after the usual depletion period, are dosed with the test substances for two days and are killed on the fourth day. This again is a much quicker method than the curative method and is claimed to give as accurate results.

Since the most widely used method of biological estimation is the curative based on the growth response this will be discussed fully; the same principles apply to the other methods [68]. But whatever biological method is used it cannot be too strongly emphasized that when comparing the relative potencies of two substances the diet must contain ample of everything necessary for the absorption and metabolism of *both*, and further that both must not only be dissolved in the *same* solvent but also that this solvent must not favour the absorption of one more than the other. Failure to recognize these principles has greatly decreased the value of many assays; the subject is fully discussed on p. 13.

The young rats used for estimations have to be first given a diet deficient in vitamin A until they stop growing or start to lose weight. This is called the depletion period; it can be shortened by having given the suckling mothers a diet lacking in vitamin A. At the beginning of the estimation the rats should weigh about 70 to 90 grams, and should not have severe xerophthalmia. Their diet must of course contain all essentials apart from vitamin A so that their lack of growth and its resumption can only be caused by it. The particular importance of ample vitamin E in the diet is discussed on p. 23. When the rats have been depleted of their stores of vitamin A they are divided into two groups, each containing similar proportions of males and females. The most accurate results are obtained by dividing pairs of littermate rats between the two groups. One group is fed the substance under investigation and one the standard preparation of vitamin A. By comparing the growth of the two the vitamin A content of the first can be determined. The method is accurate to within thirty-three per cent. The period of the test feeding should last four weeks or longer, the vitamin A preparations being given daily or twice weekly. The mouse [611] may prove a satisfactory alternative to the rat for biological assays.

Gain in weight, however, is not directly proportional to the amount of vitamin A given; doubling the vitamin A does not double the growth. Therefore if 1 gram of an oil of unknown potency causes a gain of weight of 8 grams and 1 gram of the standard oil causes a gain of 4 grams, the unknown oil is not twice as potent as the standard oil, but possibly only about one and a half times as potent. To enable a comparison to be made between substances causing different gains in weight Coward has made a curve of reference (by feeding groups of rats on varying amounts of the same oil) which shows how weight increases in proportion to the intake of vitamin A. By referring the gain of weight on two different oils to this curve, the relative values of the two to each other can be decided.

Even in rats from the same colony under the same conditions the rate of growth or other response [73] for the same amount of vitamin A may differ at different times; so the response to the standard vitamin A preparation must be worked out afresh for every fresh group of biological estimations. A laboratory cannot once work out the response of its animals to the standard, and use the results for reference in all subsequent work; Coward, for instance, found that had she done this, one sample of oil would have appeared to contain five times the amount of vitamin A it did nine months earlier.

Physical Estimation. Absorption spectrum estimations of vitamin A have been found to be reliable, giving results in harmony with biological estimations as long as the unsaponifiable fraction is used when the substance being tested contains less than 10,000 I.U. per gram of vitamin A. The reason for using the unsaponifiable fraction is that in oils there are substances other than vitamin A which increase absorption in the region of 328 millimicrons.

This irrelevant absorption has been investigated by Morton and Stubbs [53], who have explained in their outstandingly important papers how it may be measured and discounted.

It is necessary to be able to convert the results of absorption spectrum estimations into International Units. "It has been found that, within certain defined conditions, measurement of the coefficient of absorption (E) at 328 millimicrons affords a reliable method for measuring the vitamin A content of liver oils and concentrates. As a means of converting values obtained for E $\frac{1\%}{1 \text{ cm.}}$ 328 millimicrons into a figure representing the International Units of vitamin A per gram of the material examined, the factor 1,600 is recommended for adoption" and "the intensity of absorption at 328 millimicrons may be determined to within ± 2.5 per cent. by any of the recognized methods of spectrophotometry" (Report of the Second International Conference, 1934, on Vitamin Standardisation of the League of Nations). But the vitamin A sub-committee of the Accessory Food Factors Committee of the Medical Research Council [337] reported in 1943 that the conversion factor should be 1,740, a figure in agreement with that of Irwin [75], who in 1944 analysed statistically the results of vitamin A assays, carried out to determine the conversion factor, by nine or ten different laboratories on halibut-liver oil, the U.S.P. reference oil and vitamin A naphthoate. The average for the halibut-liver oil was 1,570, for the U.S.P. reference oil 1,820, and for the naphthoate 1,770. These values, from their logarithms and their standard errors, were consistent with the same conversion factors for all three substances. Pooling these results gave the conversion factor, mentioned above, of 1,740 with limits of error of ± 120 . The problem has been further clarified by Morton and Stubbs [53], who in 1947 showed that when corrections were made for irrelevant absorptions in oils 1,800 was the correct factor, but that when no such corrections were made 1,600 gave the correct result for the average oil, though of course this introduces an error for those oils which have more or less irrelevant absorption than the average. In spite of early German claims [76] that the natural vitamin A ester was twice as active as the alcohol, it is now certain that both have the same activity [36, 46] when assayed correctly (p. 8), and so have the same conversion factor. The conversion factor of 2,000 which has been used in America in the past was due to the U.S.P. reference cod-liver oil being over-valued (p. 10).

Chemical Estimation. Colour reactions (p. 6) for the estimation of vitamin A are chiefly used for oils and concentrates and, with an instrument such as the photoelectric spectrophotometer described by Thompson [79], can give accurate measurements of the amounts of vitamin A and of carotene when these occur separately or together. With the Carr-Price reaction two bands of maximum absorption due to vitamin A are found at 572 and 606 millimicrons, which in concentrated solutions are displaced to 583 and 620; the latter is that used when estimating vitamin A [68, 79]. For carotene, when dissolved in chloroform, the band at 463 is used.

UNITS OF VITAMIN A

The International Unit of vitamin A—generally abbreviated to I.U. or i.u.—is that amount which has the same vitamin A activity as 0.6 microgram of the international standard beta-carotene, when both are assayed biologically under standard conditions (pp. 8, 12).

The reason for this absurd unit is that in 1931, when the International Unit was defined for the first time, no pure preparation of vitamin A had yet been made though supposedly pure beta-carotene was available. Since carotene is converted into vitamin A in the body the former was chosen as the yardstick for the biological assay of the latter, and the International Unit was defined as the vitamin A activity of 1 microgram of a special sample of

carotene. By 1934 this sample of carotene was known not to be a single substance, and so in its place was put a sample of what was thought to be pure β -carotene, of which 0.6 microgram was found to be equal in biological activity to 1 microgram of the 1931 standard carotene. To avoid confusion the biological value of the International Unit of vitamin A was kept the same, so that it was defined as the biological activity of 0.6 microgram of the 1934 standard carotene, though this also was later found to be only ninety per cent. pure [80].

Now that pure crystalline vitamin A is available the International Unit will be redefined in terms of a given weight of vitamin A or, more probably, sink into oblivion; an International Unit becomes redundant when a vitamin can at last be defined by weight and not only by biological activity.

In the U.S.A. crystalline vitamin A acetate dissolved in cottonseed oil is used as a reference standard, being known as the United States Pharmacopoeia or U.S.P. Vitamin A Reference Standard. Units based on this standard are known as U.S.P. units and are equal to International Units [81]. The solvent used in assays based on this standard is of the greatest importance (p. 12).

In the past U.S.P. units were based on the U.S.P. Reference Cod Liver Oil. The vitamin A in this oil was unstable and so the potency of the oil, and with it the value of U.S.P. units, declined. Therefore all work based on the earlier U.S.P. units tends to be inaccurate, conversion factors, for instance, being too high. In an attempt to keep the U.S.P. units constant in value a succession of Reference Oils were introduced one after the other, but even the third and last by 1949 had lost about twenty-five per cent. of its activity [81].

Other units, such as "blue units Moore," based on the depth of the blue colour of the Carr-Price reaction, were used in the early days of vitamin A, but they could not be accurately converted into International Units and now have only an historical interest; they and their probable values are reviewed by With [82].

PHYSIOLOGY OF CAROTENE OR PROVITAMIN A

Vitamin A is formed in the body from certain of the red or yellow fat-soluble plant pigments known as carotenoids. No animals can apparently make carotenoids for themselves, nor form vitamin A from any other source.

Only a few of the carotenoid pigments, however, have the chemical groupings (p. 3) essential for their conversion into vitamin A. These are generally collectively referred to as carotene.

In calling carotene provitamin A there is the unfortunate implication that, as regards nutrition, it and vitamin A are the same. This is nonsense. The vitamin itself is only found in fatty fish or animal foods; during digestion it, like its accompanying fat, is virtually completely absorbed; once absorbed it is ready for use by the body. Carotene, on the other hand, has none of these advantages: generally locked within the cells of fat-free vegetables, it has first to be liberated during digestion, which often does not occur; next it has to be absorbed into the wall of the intestine, which again may not occur, especially in the absence of fat; once within the intestinal wall it has to be converted *there* into vitamin A, since if carotene once reaches the blood unconverted it remains unconverted and valueless; finally its conversion is largely dependent on age and on the general health of the body. It is therefore not surprising that carotene, with all these reasons why it may fail to be converted into vitamin A, is never the equivalent of the vitamin in practical dietetics.

Morton [39] gives an excellent account of the structure of the common and rare provitamin A carotenoids and also of those which have no provitamin activity. Of the former only alpha-, beta- and gamma-carotene

and cryptoxanthine are important. All these can exist in many stereoisomeric forms, but the all-*trans* forms are virtually the only ones which occur naturally, the others either being formed artificially during, for instance, absorption on alumina [44] or by plants under artificial conditions; thus the buds of *Mimulus*, or monkey plant, opening in diffuse light in jars of water form *cis* compounds [78]. The biological activity of the carotenoids and their isomers is discussed on p. 15.

Function of Carotene. Plants contain many carotenoid pigments which are valueless to mammals and to birds and so are not germane to the subject of this book, but in passing it is interesting to note that, though they are so widely distributed not only in bacteria [83] but also in multicellular organisms, yet their function is still obscure. Goodwin [607] has studied their synthesis in bacteria and fungi. Wald [84] has pointed out that their only universal value in plants seems to be to act as a receptor in photokinetic systems—that is, those concerned with directed movements in response to light. He has enunciated the profoundly significant general theory that: “Within the entire range of living organisms, animal and plant, light-sensitive structures regularly, and perhaps universally, contain carotenoids, the class of substances which include the vitamins A. In this respect the appearance of vitamin A₁ in the mammalian retina continues an association which extends so far as is known throughout the eyes of vertebrates and the photosensitive organs of multicellular plants, algæ and fungi.”

For mammals and birds carotene itself has no value except as a precursor of vitamin A: the control animals in innumerable experiments on vitamin A requirements, etc., have shown that animals develop and remain healthy on diets containing vitamin A but devoid of carotene, while even on normal diets rich in carotene virtually no carotene is absorbed into the blood by many animals, such as the sheep, goat or rabbit [85], while in other animals, such as the cow and man, the carotene in the blood is only due to fortuitous and valueless permeation through the intestinal wall. The presence of “carotene” in bone marrow, the corpus luteum and suprarenal glands has been held to suggest it has some function of its own, but the only recent work on this subject shows that the pigment in these organs—at least in the goat—is not carotene [85]. Pigments which are able to enter into hens’ eggs must contain two or more OH groups [87] and so are not provitamin A carotenoids [86, 87], and the embryonic chick can develop normally with virtually no pigments [87]. Claims that carotene is present in eggs [88] are probably due to the investigators not differentiating between the absorption spectra due to carotene and those due to substances such as lutein and zeaxanthine. With’s contention [89] that some carotenoids are themselves vitamins—at least for the chick—is incorrect; some of its fallacies have been pointed out by Hickman [90], while others, such as the different efficiency of conversion of the various provitamin A carotenoids to vitamin A under different dietary conditions (p. 8), will occur to the reader amused by this biochemical red herring.

Absorption of Carotene. In man the absorption of carotene *from normal diets* is extremely poor; so poor that food tables can only give the vaguest guide as to how much vitamin A is indirectly provided by vegetables. As a rough guide it may be said that the carotene in green leafy vegetables is two to three times as well absorbed as that from red or yellow vegetables like carrots [91], but at very best more than half will probably be wasted in the faeces. The whole matter, however, is very confused: one investigation [92], for instance, showed that twenty per cent. of the carotene in raw carrots is absorbed, and only five per cent. if the carrots are cooked; a second investigation [91] showed the opposite, absorption being one per cent. for raw carrots and nineteen per cent. for cooked carrots; while a third investigation [93] showed that about twenty-five per cent. is absorbed however carrots are cooked, this amount being doubled if carrots are homogenized.

Figures for spinach vary as widely [94, 95], but probably its carotene and that of green peas [95] and cabbage [93] is relatively well absorbed. One vegetarian did not absorb carotene better than men on normal diets [92]. There is no evidence that carotene is one of the vitamins synthesized by the bacteria of the bowel [93, 104], though its excretion in the faeces may continue for a week after it has been eaten [96], and its absorption for two or three days [97, 98].

Children and infants absorb or at least convert carotene into vitamin A very inefficiently. Nicholls and Nimalasuria [99] report that carotene is not satisfactory for the cure of children suffering from lack of vitamin A, while Van Zeben [100] does not even admit that carotene acts as provitamin A for infants, since he found that only five per cent. of the carotene in cooked spinach was absorbed, and eight per cent. of that in canned tomato juice.

The placental barrier [101] is not easily passed by carotene ; in new-born infants the level of carotene in the blood is about one-fifth [102] or one-tenth [103] of that in the maternal blood, though there appears to be some definite relationship between the two [103].

Animals differ widely in their capacity to absorb carotene ; this is very poor in the cat [104], excellent in the rabbit, sheep and goat [85], while in the rat it is known to depend on so many factors, such as the amount of fat in the diet [104], that ninety per cent. of ingested carotene may be absorbed or ninety per cent. excreted [104].

Fat is necessary for the efficient absorption of carotene. Wilson [105] reports that in man on a fat-free diet the absorption of carotene is nearly halved, but increasing the fat in the diet above thirty per cent. does not lead to any further increase in carotene absorption [106]. Kreula [107] finds that ninety per cent. of the carotene in finely grated carrots is excreted in the faeces when no fat is given, but this falls to fifty per cent. if carotene is given dissolved in oil, and falls as low as thirty per cent. if such carotene is given not in one dose but in two. Similar results have been obtained in rats [104], though colloidal carotene in a glucose solution is well absorbed from fat-free diets [108]. Probably the importance of fat is due to its dissolving the carotene and so presenting it for absorption in a fine emulsion.

Bile is necessary for the absorption of carotene, since in animals after ligaturing the common bile duct, or short circuiting it into the colon, carotene is not absorbed by mouth unless it is given with bile salts [109], while Irvin and others [110] have shown by using isolated intestinal loops that lipase is as important as bile.

Liquid paraffin seriously interferes with the absorption of carotene by dissolving it from the food in the intestine, with the result that it is excreted with the paraffin in the faeces. Curtis [111] and his collaborators have shown by careful work on men that on a high carotene diet the carotene level in the blood only rises to half the normal value when 20 ml. of paraffin are taken thrice daily after meals ; taking it twice daily has nearly as bad an effect, and even taking it once at night has some effect. Emulsions of paraffin and agar-agar acted in the same way as paraffin. Alexander and others [113] have confirmed these findings for ordinary commercial salad dressings, which may contain over eighty per cent. of mineral oil, and similar results have been obtained in work on animals [110, 112]. This example of the injurious effects of liquid paraffin will be found to be reduplicated with all the fat-soluble vitamins, but in spite of repeated protests in the medical journals ignorant doctors and rapacious food manufacturers will probably continue to prescribe or sell liquid paraffin overtly or covertly.

The solvent in which carotene is given or in which it is dissolved during digestion appears, from the work reviewed above, to be the chief factor determining its absorbability. But none of the solvents normally available during digestion are ideal for the absorption of carotene ; this is of more

than academic interest because realizing it enabled Koehn [126] in 1948 to perform a completely revolutionary piece of work which showed that given the perfect solvent—*n*-hexane—all-*trans* beta-carotene was as active, weight for weight, as vitamin A itself. This work—which has been confirmed [606]—was revolutionary because until it was published, and even much later, whenever the biological activity of pure vitamin A was assayed by comparing it to that of the beta-carotene of the International Standard (p. 9), both were given dissolved in oil, with the result that roughly all the vitamin A but only about half the carotene were absorbed. Therefore it was erroneously concluded that, weight for weight, vitamin A was twice as active as carotene when in fact they were both equally active. Fortunately methods of assay were so standardized that this fundamental error over the solvents employed occurred in all laboratories, so that they all gave congruous results when using carotene in the assay of vitamin A in oils, etc.

Illness may prevent the absorption of carotene; thus diarrhoea [97], fever [97, 114] and coeliac disease [114] have been found to increase the faecal loss of carotene, probably because in these conditions fat absorption is impaired. Other factors which impair carotene metabolism are considered below when discussing the conversion of carotene to vitamin A.

The transfer of carotene across the gut wall probably depends, according to Drummond and MacWalter [115], on the formation with bile acids of a water-soluble diffusible complex, though it would seem from Frazer's work [116] that it might also be absorbed dissolved in any fat which was so finely emulsified that it did not require lipolysis for its own absorption. Having passed into the lacteals from the intestine [117] the carotene is transported in the blood as a protein compound, the protein probably being albumin [119].

Storage and Excretion of Carotene. Many animals (p. 14) never absorb carotene itself into the blood, while others absorb and store very large amounts—chiefly in the liver, though the body fat may also be impregnated with this pigment; we have been told, for instance, that at operation the fat of prisoners of war in the Far East, who had to eat huge amounts of red palm oil, was a startling scarlet. The paper by Jensen and With [120] should be read for a very interesting account of carotene storage in men, mammals, birds and reptiles; there may be the widest variations even between closely related species living on similar diets.

After injecting carotene into the tissues it may remain unabsorbed [121] or, if directly injected into the circulation, it is treated as a foreign body and is removed from the circulation by the reticulo-endothelial system [58, 118].

The fate of carotene after it has been injected or has been absorbed from the gut, but not converted into vitamin A during this absorption, is obscure. A very small amount may perhaps be re-excreted into the gut [122], some may be excreted by the skin, though not the kidneys, when levels in the blood are abnormally high (p. 78), but when very large amounts are consumed the greater part, at least in the rat, is destroyed by the body [123].

Conversion of Carotene to Vitamin A. One molecule of all-*trans* beta-carotene is converted into two molecules of vitamin A, this conversion taking place in the wall of the intestine.

The above is what happens when carotene is fed to an animal under ideal conditions; it is not what happens under ordinary conditions for either man or laboratory animals. The subject is most simply reviewed by considering firstly what chemical changes occur in the carotene molecule during conversion; secondly what is the site of this conversion in the body; thirdly what factors hinder or facilitate this conversion.

The chemical change which converts beta-carotene to vitamin A is probably oxidative cleavage of the molecule (p. 3) at the middle of the central aliphatic chain, two identical molecules of vitamin A aldehyde being

formed which are then reduced to vitamin A alcohol, that is, to vitamin A itself. In support of this theory is the work of Hunter [37], who succeeded in making small amounts of vitamin A by the oxidation of carotene, and of Glover and his colleagues [124], who have shown that vitamin A aldehyde is converted to the alcohol in the gut wall. Koehn [126], however, holds that the beta-carotene molecule is not oxidized but undergoes hydrolytic fission. The changes which occur in the other provitamin A carotenoids are considered later when discussing the factors which affect conversion.

The site of conversion of carotene to vitamin A is the wall of the gut. That this is so in the rat was first shown by Glover, Goodwin and Morton in 1947, and it has been thoroughly confirmed by their own and their collaborators' subsequent work on the rat [127], and on the sheep, goat and rabbit [85], and by the work of Thompson, Ganguly, Coates and Kon on the rat and pig [128, 132] and chick [132], and of Americans, again on the rat [122, 129]. All this research has proved that when carotene is fed by mouth (*a*) it is not converted to vitamin A within the lumen of the gut; (*b*) it causes storage of vitamin A but not carotene in the liver; (*c*) vitamin A appears within the wall of the gut in large amounts before it appears in the liver; (*d*) vitamin A disappears from within the wall of the gut as it increases in the liver; (*e*) no carotene appears in the lymph from the thoracic duct or in the portal or systemic blood *but* vitamin A appears in the lymph. Further, the injection of carotene into the circulation or tissues causes no hepatic stores of vitamin A, and animals so injected die from lack of vitamin A when there still remains in their livers enough of the injected carotene to keep them alive for many months were it converted to vitamin A [121, 122]. No explanation of all these results is possible except that carotene is converted to vitamin A only within the wall of the gut.

However, there are some observations that at first sight do not appear to be congruous with those just reviewed. Until 1947 it was believed that the liver—by virtue of a hypothetical enzyme called carotinas—was the site of the conversion of carotene, this belief resting partly on experiments in which vitamin A was said to be formed after carotene was perfused through the liver or liver tissue was incubated with carotene, and partly on experiments in which carotene when rubbed or injected into animals showed vitamin A activity. The perfusion or incubation experiments have been reviewed by Glover and others [127]; they were contradictory and the yields of vitamin A so small that they were within the limits of experimental error. But the experiments based on the inunction or injection of carotene cannot be so easily ignored; thus Eddy [130] showed in a very careful experiment that carotene solutions when rubbed for four minutes into the shaven skin of rats—the skin being thereafter carefully washed—promoted growth, even though about three times as much carotene was required as if it were taken by mouth. Lease [121], and later Sexton and others [122], showed quite definitely that injected carotene could cure ophthalmia and restore growth in vitamin A deficient rats for a short period of time. But the animals of both these investigators ultimately died of lack of vitamin A when there was enough carotene in their livers to have kept them alive for many months had this been convertible to vitamin A. The probable explanation of this definite but transitory effect of carotene when introduced directly into the body is that, while most remains at the site of the injection or is captured and destroyed by the Kupffer cells of the liver, yet a little, while still circulating in the blood, is excreted into the gut and is then reabsorbed and converted by the gut wall to vitamin A: in support of this is the recovery from the faeces of 5.5 per cent. of injected carotene [122]. Further, vitamin A depleted animals, whether they are injected with one large or one relatively small dose of carotene, die, after a brief recovery, within the same period [121] though repeated injections enable animals to grow normally [131], which suggests that it is only during the short time when carotene is circulating in the blood,

and so being excreted in the intestine, that it is available to form vitamin A. In other words, the ingestion or injection of carotene is merely a most wasteful method of introducing carotene into the gut.

The factors affecting the conversion of the provitamin A carotenoids to vitamin A are firstly the stability of the carotenoids within the lumen of the gut, secondly their absorbability (p. 11), and thirdly their convertibility after absorption.

The stability of the carotenoids within the lumen of the gut largely depends on vitamin E protecting them against oxidative destruction by rancid fat [133]; but the matter is complicated by vitamin E also protecting vitamin A within the body, so some of the apparent protection of the carotenoids—as judged by their growth-promoting power—may really be due to vitamin A not being destroyed after its formation. The subject is discussed fully in relation to vitamin A on p. 23, but here should be mentioned the work of Quackenbush and his collaborators [134] on linoleic acid, which is one of the essential unsaturated fatty acids (p. 671); they report that carotene dissolved in ethyl linoleate is unstable *in vitro* and is ineffective in curing vitamin A deficient rats unless protected against oxidation by vitamin E or hydroquinone. This is contradicted by Sherman [135], who states that carotene is stable in ethyl linoleate even when both are incubated with the contents of the rat's stomach, and ethyl linoleate does not decrease the amount of carotene in the faeces. Sherman [136] therefore believes that the inhibitory action which the esters of linoleic and linolenic acids have on the metabolism of carotene occurs after absorption even though he reports that this inhibitory action does not occur if the esters are fed six hours after the carotene [135] or vitamin E is given [136]. On the balance of evidence it would appear that the unsaturated fatty acids only disturb the metabolism of carotene by oxidizing it before it can be absorbed.

The convertibility of the carotenoids—apart from that of all-*trans* beta-carotene, which has been discussed on p. 13—is still unknown. From their chemical structure (p. 4) it is obvious that all isomers of beta-carotene could be converted into an equal weight of vitamin A, while the other carotenoids could form half this. But from the reported biological activities of the carotenoids and of their stereoisomeric forms it would appear that, even when allowances are made for differences in absorbability [137], some carotenoids do not form as much vitamin A as their structure suggests is possible. In other words, they are not easily converted, a large proportion being destroyed before or after absorption or, possibly, some part of the carotenoid molecule which does not form vitamin A—such as the beta-ionone nucleus—may destroy vitamin A after its formation and so give a false picture of how much was originally made [137]. Zechmeister [78] in 1949 discussed some of the particular problems raised by the stereoisomers and suggested that their different biological activities are due to whether or no they are the right shape to fit into the enzyme system responsible for their conversion. Deuel and his co-workers [138] found, judging by the gain of weight in rats and ignoring differences in absorption, the following figures of relative activity: β -carotene 100, neo- β -carotene B 53, neo- β -carotene U 38; α -carotene 53, neo- α -carotene B 16, neo- α -carotene U 13; γ -carotene 28, pro- γ -carotene 44; cryptoxanthine 58, neocryptoxanthine 42. Johnson and others [139] have largely confirmed these findings both for the rat and for the chick, studying respectively storage, and growth and storage. That α -carotene has roughly half the activity of β -carotene judged by its effect on growth [138] and only one-quarter judged by hepatic storage of vitamin A [137] is probably explained by these findings being reported from different laboratories at different times, when the dietetic background of the investigations cannot have been the same. The activity of cryptoxanthine in the chick given above, and confirmed by other workers [140], is of interest because With (p. 11) largely based his theory about carotenoids being vitamins in

their own right on his erroneous claim that cryptoxanthine had double the activity of β -carotene.

Many other factors, at least in animals, affect the value of carotene as a source of vitamin A. Thus myxœdema (p. 45), hepatic disease [101], phosphorus poisoning [141], some anæsthetics or drugs [128] and impaired bowel motility [128] may all have an adverse effect, as does lack of vitamin E (p. 23) and possibly of choline (p. 24) and also the consumption of soya beans [142]. Lutein or "xanthophyll," when fed in large amounts—with ample vitamin E—at the same time as carotene [137, 143] or vitamin A [143], reduces the amounts of vitamin A laid down in the liver [137, 143], though it does not hasten the depletion of preformed stores [143]. Since lutein does not increase the excretion of carotene [137], it presumably accelerates the destruction of carotene and vitamin A within the lumen of the gut or, by virtue of its alpha-ionone ring, within the wall of the gut [137]. The contrary evidence that lutein in the absence of vitamin E protects carotene from destruction [144] is probably incorrect [126].

Utilization of Carotene. Moore [145] found that in animals small amounts of carotene added to diets deficient in vitamin A were utilized as well as vitamin A, but that increasing the carotene decreased the percentage stored, till at very high intakes only about one to two per cent. was converted into vitamin A; five to ten per cent. was lost in the fæces, the remainder apparently being destroyed in the body [123]. He also states that it is impossible to cause the symptoms of over-dosage with vitamin A by giving carotene. Gray [146] reported that in rats at levels of intake above the minimum to prevent symptoms of a deficiency, vitamin A was six to ten times better utilized than carotene, while Guilbert and others [147], and English workers [93, 148], have also emphasized that at optimum levels of intake 1 I.U. of vitamin A is equivalent to about 3 I.U. of carotene. Children utilize carotene very badly (p. 12). From all this it appears to be certain that carotene is a far less satisfactory source of vitamin A than is vitamin A itself (see also p. 50).

PHYSIOLOGY OF VITAMIN A

Sources. Herbivorous animals depend entirely on carotene for their vitamin A. Omnivorous animals like man obtain vitamin A partly from carotene, partly from animal foods in which the vitamin itself is present. Some purely carnivorous animals like cats are almost unable under normal conditions to convert carotene to vitamin A [104]. How fish acquire such large stores of vitamin A in their livers, or of what use it is when stored, is obscure, though in some fish it may possibly be necessary for the transference of fat across the gut wall [149], though this does not appear to be so in animals [150, 151]. The seaweeds and plankton, which form the basis of marine food, contain carotene, but the small fish and crustacea which act, as it were, as intermediaries between the plankton and the larger fish do not contain vitamin A or carotene, though they do contain carotenoid pigments. The fish may use these as precursors of vitamin A, carefully storing it up, so that it increases in amount in the liver with increasing age [152], or they may themselves apparently synthesize the vitamin [153], also converting carotene should it be available [154]. The crustacea, however, on which Antarctic whales feed appear to contain vitamin A itself and so may be the source of the large hepatic stores of the vitamin in these mammals [123, 602].

Absorption. Vitamin A only occurs naturally as the ester; therefore fat and vitamin A, from the point of view of absorption, behave in the same way, both being in essence esters of fatty acids and alcohols. Hence any conditions which impair the absorption of fat equally impair the absorption of the vitamin. Further, Fraser [116] has shown that while fat must normally be hydrolysed before absorption, yet it is possible for it to be

absorbed chemically unchanged if it is very finely emulsified ; the same is true of vitamin A.

Vitamin A undergoes no change during intestinal absorption, since it is active when placed in the eyes [189] or when given by inunction through the skin [130] and is as effective when injected (p. 27) as when given orally, though for injection the solvent is most important : propylene glycol, but not cod-liver oil, being satisfactory [190].

The transfer of vitamin A across the gut wall is in the form of the alcohol, the naturally occurring vitamin A esters being first hydrolysed, like the other esters of the fatty acids, by the enzymes of the gut ; the evidence for this is discussed below under *Lipolytic enzymes*. Before passing into the lacteals [132] the alcohol is again combined with fatty acids, since in the chyle from a patient with a chylous fistula Drummond and his collaborators [117] found the vitamin in the form of an ester ; this has been fully confirmed for animals [85, 128, 132, 156], the absorption taking place in the upper or middle part of the small intestine [156, 164]. As all the vitamin A esters found in the rat's liver appear to be very similar as regards their fatty acids it is probable that there is a selective utilization of certain fatty acids for esterifying the vitamin [146]. In the blood vitamin A is present not only as the ester but also as the alcohol (p. 24), probably forming a compound with albumin [119].

Factors Influencing Absorption. Before discussing these it must be emphasized that in all the clinical work quoted below—unless otherwise stated—the efficiency of the absorption of vitamin A has been judged by how great a rise has occurred in the level of the vitamin in the blood (p. 25) after it has been given by mouth. But such a rise merely shows that absorption has occurred and has temporarily outpaced storage ; it does not show how great the total absorption has been. For instance, in normal adults the rise following the consumption of an aqueous dispersion of the vitamin is two to three times as great as that following the consumption of the same amount of the vitamin dissolved in oil, but the total absorption in both cases is the same, all the vitamin being absorbed. Not realizing how fallacious are rises in blood levels as guides to absorption has caused much confusion, claims repeatedly being made, for instance, that dispersing agents improve absorption when all that can be justifiably stated is that such agents hurry absorption—for good or more probably for ill (p. 18).

The ester and the alcohol—and the acetate [192] for all practical purposes—are equally well absorbed by man [160, 163, 176] except in certain diseases (p. 17). Both are also equally well absorbed by the cow [179], though the laying hen [180] is reported to utilize the ester far better at high levels of intake, while certain solvents, on the other hand, hinder the hydrolysis and so the absorption of the natural esters by laboratory animals and chicks [178]. The solvent, indeed, is very important when assaying vitamin A since, for instance, some solvents make the natural ester appear to be only half as biologically active as the acetate or alcohol [178]. To avoid this confusion all substances before being assayed and also the standard of reference should be saponified.

Lipolytic enzymes appear to be necessary for the absorption of vitamin A, since it seems probable from work on rats [128, 155] and bovines and sheep [156] that the naturally occurring vitamin A esters, if unemulsified, have to be hydrolysed to the alcohol—like other esters of fatty acids—before they can be absorbed. This is supported by the poor or slow absorption of the ester compared to the alcohol by children who lack lipolytic enzymes owing to cystic fibrosis of the pancreas [157, 158, 159], and the improved or more rapid absorption of the ester by such children when pancreatic enzymes are given [158, 159].

Bile assists the absorption of vitamin A, since adults [160] and children with jaundice have a poor or slow absorption which is improved, at least in

the latter, when bile salts are given [166, 167], and children dying of congenital atresia of the bile ducts have been reported to show signs of a deficiency of vitamin A [168] which was probably not due to the jaundice, since jaundiced animals utilize vitamin A [109]. In animals, however, ligaturing the common bile duct or anastomosing it with the colon does not interfere with absorption [109].

Decreased intestinal motility probably impairs absorption of vitamin A—as it does of carotene (p. 16)—since neostigmine and cascara increase or hasten absorption in fibrocystic disease of the pancreas [169], while in normal subjects atropine delays or decreases it [170].

Liquid paraffin hinders the absorption of vitamin A in the same way as it hinders the absorption of all the fat-soluble vitamins. Thus Anderson [171] found that when liquid paraffin was given with large doses of vitamin A about a quarter of the latter was excreted with the fæces, being dissolved in the paraffin; one patient, for instance, when given 20,000 I.U. by mouth normally only excreted 800 I.U., but this amount rose to 5,000 I.U. when liquid paraffin was taken. The level of vitamin A in the blood may not be depressed even by large doses of paraffin taken for many weeks [113], but this is no argument in favour of this harmful aperient [172], since the blood levels may remain normal after months on a vitamin A free diet [93] if there are ample hepatic stores on which to draw.

Emulsifying agents apart from bile, which has been discussed above, have been known for some years to hasten and sometimes to improve absorption of vitamin A. Thus Aldersberg and Sobotka [173] in 1943 showed that when vitamin A was given with 10 grams of impure lecithin the subsequent rise in serum vitamin A was roughly double that which occurred when no lecithin was given. Vitamin A as it occurs naturally emulsified in milk is almost perfectly absorbed [163], and the same is probably true of vitamin D (p. 537). It is most improbable that natural, common and physiologically normal emulsifying agents could be harmful, but it is far otherwise with the far more efficient artificial agents such as sorbitan monolaurate, polyoxyethylene sorbitan monolaurate and polyoxyethylene monolaurate, which were first used about 1947. Claimed by the makers to be safe and harmless, it was not until 1950 that Sherman [174] and Schweigert and his co-workers [175] showed that all these compounds when fed to hamsters were highly dangerous, causing especially diarrhoea and marked changes in the intestinal tract. It is true that these compounds formed five or fifteen per cent. of the animals' food, while in children they are never given in any but relatively minute doses, but even so it is difficult to believe that a substance which kills animals in a few weeks is a desirable addition to vitamin preparations which are often taken by infants and children for many years. Further, an emulsifying agent which, when used to make an "aqueous dispersion" of vitamin A, is so efficient that it quintuples the normal rise in serum vitamin A which follows the giving of ordinary halibut-liver oil [160] cannot but open the door to the entry into the body of a multitude of substances—such as liquid paraffin—which in normal circumstances are excluded. Again, the abrupt flooding of the body with vitamin A may overwhelm or pervert storage [181]. All this of course does not mean that emulsifying agents are not of value, when given under careful medical supervision, for the treatment of children and adults who cannot absorb fat-soluble vitamins in a normal manner. But it would seem that vitamin preparations sold to the public should never contain emulsifying agents, and warnings have already been given that such preparations—apart from other dangers—enhance the risk of vitamin A poisoning [163].

Emulsifying agents such as those mentioned above and also possibly dextrimaltose [160], when used to turn oils containing vitamin A into aqueous dispersions, enormously hasten and may increase absorption both by man at all ages and by animals [160, 161, 163, 164] and chicks [165]. Thus in

animals about three times as much vitamin A may be absorbed [164] and stored [161] from aqueous dispersions as from oils, absorption from the former being quicker and taking place higher in the small intestine [164]. In premature infants Lewis and others [160] report that 35,000 I.U. of vitamin A in oil causes an average rise in the serum vitamin A of 62 I.U., while the same dose in an aqueous dispersion causes an average rise of 274 I.U.; the comparable figures for normal infants are 200 and 1,000; for children given 5,000 I.U. per pound of body weight, 600 and 4,000; and for adults given 500,000 I.U., 1,750 and 4,500 (Figs. 6, 7). The normal infants excreted in their faeces an average of thirty-eight per cent. of the vitamin A in the oil, and seven per cent. of that in the aqueous dispersion; the percentage excreted was the same whether 12,500 or 35,000 I.U. was given, and the absorption from the oil was not improved when it was diluted from 60,000 to 10,000 I.U. per ml.

The amount of the emulsifying agent is important; thus in children 6 ml. of an aqueous dispersion containing roughly 50,000 I.U. and eleven per cent. of sorbitan monolaurate polyoxyalkylene raises the serum vitamin A to 400 I.U., while if the emulsifying agent is increased to twenty per cent. the serum level is raised to 13,000 I.U. [176].

Findings congruous with the above as regards the hastening or enhancing of vitamin A absorption by aqueous dispersion have been reported by many workers for adults in health [160, 164, 176] and with most types of illness [177], for children [158, 160, 162, 163, 176] and for infants [160, 176]. Children with fibrocystic disease of the pancreas [158, 160, 162, 163] and coeliac disease [158] and adults with obstructive jaundice [160] absorb vitamin A from an aqueous dispersion—judged by serum levels—better than or as well as normal patients absorb vitamin A from oil (Figs. 6, 7).

Age has an important effect on absorption, which is virtually complete in adults [96] but poor in infants, who, judging by faecal excretion, only absorb about two-thirds of a dose of 35,000 I.U. given in oil [160, 163].

The placental transfer of vitamin A to the foetus appears to be controlled by various factors which have not been fully investigated. Byrn and Eastman [102] found the average level of vitamin A in the blood of fifty newborn infants was 91.3 I.U. and that of the mothers 106.3 I.U., but there was no constant relationship and wide differences occurred; this has been confirmed in 143 mothers and children by Lund and Kimble [103], who also noted that the foetal blood vitamin A might be higher than the mother's when this was low, and further that the levels in a pair of identical twins were 30 and 71 I.U. per 100 ml. Human foetuses and infants tend to have low stores of vitamin A (p. 22).

In animals [188, 193, 194, 195] foetal stores depend on the richness of the maternal diet in vitamin A and, to a slight extent, fat [193]. The foetal rat can only store about 5 to 10 I.U. however much the mother has consumed [195], but the foetal calf does not appear to be so limited in its storage capacity [188], while the hen can transfer to the egg very large amounts [86, 180], especially when given vitamin A as the ester [180]. Vitamin E does not increase placental transfer to the kid or piglet, but possibly does to the lamb [194].

Diseases of many different types are often stated to decrease or delay absorption. But the evidence for this is almost wholly based either on the amounts of vitamin A found *post mortem* stored in the liver or on the levels of vitamin A in the blood during life. Therefore the reader should consult the following sections on storage and on blood levels as well as the sections on the thyroid, kidney, etc., for information as to how disease may affect the metabolism of vitamin A.

Storage. Storage of vitamin A is confined to the liver for all practical purposes (Fig. 3), Moore [181] finding in his early work that the liver of rats on a high carotene diet contained 100,000 times the amount of vitamin A

present in the storage fat of the body, while no other organs contained any. Later work (p. 48), however, showed that with moderate stores in the liver small amounts of vitamin A were always present in the kidneys and sometimes in the lungs.

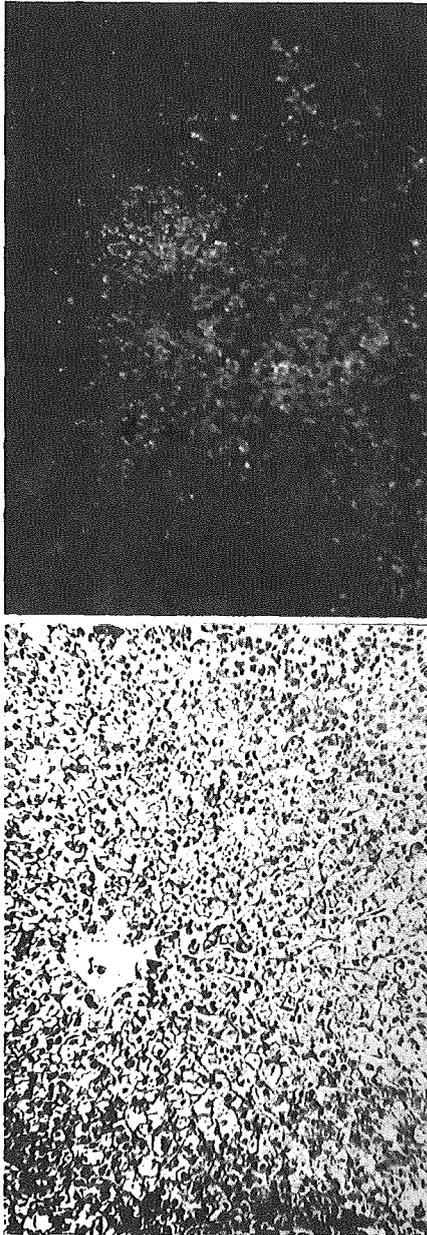


Fig. 3. Photomicrographs of a biopsy specimen from the liver of a patient with peptic ulcer without damage to the liver. A, routine histologic examination: normal structure of the hepatic parenchyma. B, examination with fluorescence microscope: vitamin A fluorescence imparted by fine lipid droplets at the edge of the liver cells and in the Kupffer cells. (See also Figs. 4 and 5.)

When the diet was very rich in vitamin A, so that the liver stores were five per cent. of its dry weight and could in theory have lasted the rat for 200 years, the lungs stored more than the kidneys, the amount being larger in both organs than those found in normal livers. The suprarenal glands also, but inconstantly, stored vitamin A in large amounts, while all the other tissues of the body contained traces. In the pig [128] also the liver is virtually the only storehouse for vitamin A, the walls of the stomach and small intestine, the intestinal lymphatics and lymph nodes, the pancreas and gall containing only traces, and the kidneys very small amounts. In man the adrenals, testes and lactating breast are all said to store vitamin A [58], as well as the ovaries from infancy to the climacteric [182]. Moore and others [183] have shown that it is the healthy human kidney which stores vitamin A: in nephritis and respiratory diseases the kidney generally contains none. Popper [58], however, states that it is only the diseased kidney which contains vitamin A, though this is not congruous with the distribution in healthy animals (see also p. 48). Tumours only store vitamin A if it is present in the parent cells, and the retina if it is light adapted [58].

The level of vitamin A in the blood and the cerebrospinal fluid is discussed on p. 24.

The average storage in human adults from the ages of fifteen to fifty-nine was given by Moore [184] between 1931 and 1935 as being 220 I.U. per gram of wet liver, but between 1941 and 1944 this storage had increased to 324 I.U. [93], though the figures are not strictly comparable since the specimens

in the two surveys came from different areas in England. For children the respective figures were 130 and 550 I.U., while for the elderly they were 158 and 300 I.U. In Scotland [185] in 1945 the average figure between the ages of twelve and eighty was 504 I.U. It is a moot point whether an increase in storage means better nutrition or worse; whether it means more dairy produce or more vegetables eaten to stave off hunger. The differences between individuals is remarkable; figures range from 23 to 2,400 I.U. in adults dying in accidents [93], while in infants and young children dying at birth [186] or later from various causes [187] figures have varied from 14 to 134 I.U. In the five months' embryo the stores of vitamin A have been reported to be high [58]. Braun and Carle [188] in 1943 reviewed the literature on foetal and infantile stores in man and animals.

Both the true hepatic cells and the Kupffer cells store vitamin A [58, 196], so that in phosphorus poisoning of the hepatic cells good stores remain [118, 184], while "blocking" the Kupffer cells reduces storage [197]. Popper [58] states that his fluorescence microscopy shows that the Kupffer cells (Figs. 3 and 4) are the last cells to give up their stores of vitamin A and the first cells to replenish them, but Glover and Morton [191], from a review of the literature, believe that the evidence points to the Kupffer

cells only storing vitamin A after the normal storage space in the true hepatic cells has been filled. They also suggest that, while the true hepatic cells contain a lipolytic enzyme which can hydrolyse the stored vitamin A ester and so release it as it is needed into the blood as the active alcohol (p. 24), the Kupffer cells contain no such enzyme and so can only slowly

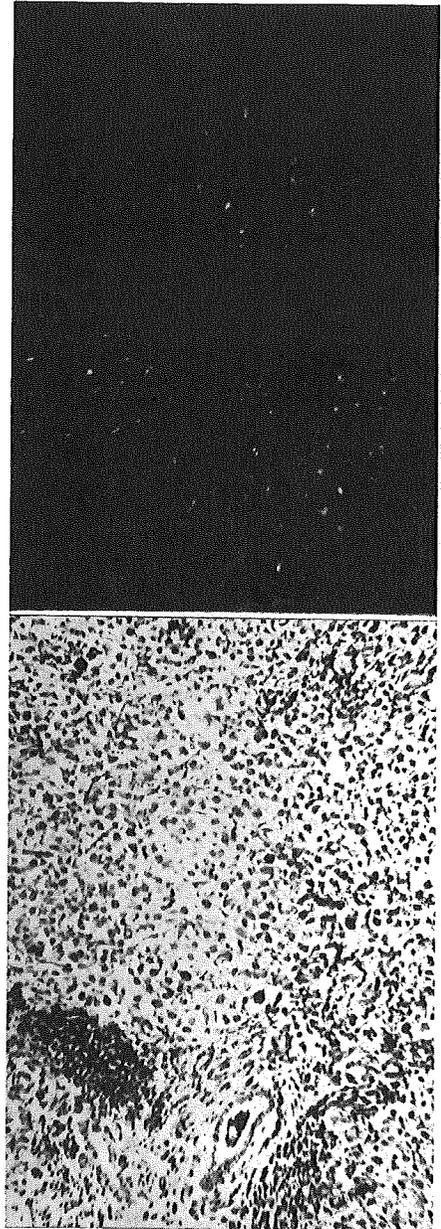


FIG. 4. Photomicrographs of a biopsy specimen from the liver of a patient with disease of the gallbladder with jaundice of short duration and without clinical signs of damage to the liver. A, routine histologic examination: extensive lymphocytic infiltration in the enlarged periportal field. No visible damage to the parenchyma. B, examination with fluorescence microscope: irregular vitamin A fluorescence is imparted almost only by Kupffer cells. The bile casts in the enlarged bile capillaries appear black. (See also Figs. 3 and 5.)

release the inactive ester. In other words, storage in the Kupffer cells is physiologically valueless, playing no part in keeping vitamin A alcohol at a constant level in the blood.

Factors, apart from Intake, affecting Storage. *Disease.* Moore [184] gives

the following figures, in International Units of vitamin A per gram of wet liver, for adults dying of various diseases: thyrotoxicosis 310, diabetes 300, poisoning 170, hypertension 120, conditions of the gall bladder 110, gastric ulcer 110, coronary thrombosis 110, tuberculosis 96, syphilis 95, endocarditis 90, bronchiectasis and bronchitis 80, subacute nephritis 75, peritonitis 75, enteritis and colitis 74, meningitis 73, pneumonia 63, empyema 60, valvular disease of the heart 60, septic diseases 51, prostate 40, chronic nephritis 25, kidney and bladder infections 19. From Scotland [185] low stores have been reported in renal disease, congestive heart failure, chronic infections and, especially, diseases of the alimentary tract. In syphilitic [184] and other forms of cirrhosis [198, 199] storage is low, and generally [58, 184, 185, 198] but not always [59, 200, 201, 202] in "hepatic diseases" (see Figs. 4 and 5). Fox [203] reports that large doses of vitamin A given to patients with pneumonia, who subsequently died, did not increase their hepatic stores.

In children under the age of fifteen [189] the values were: tuberculosis 140, measles 110, pneu-

monia 78, meningitis 68, septic diseases 47, heart disease 15. Some of the children who died from measles had been given large amounts of vitamin A, but by comparison with other children not given vitamin A it appears that they only had stored about seven per cent., a figure comparable to the storage of only ten per cent. reported by Woo and Chu [204].

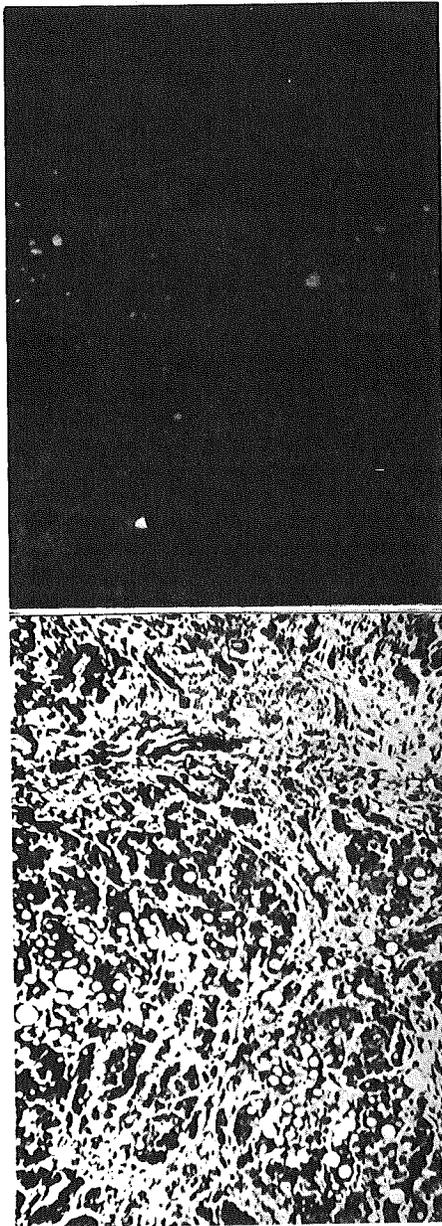


FIG. 5. Photomicrographs of a biopsy specimen from the liver of a patient with cirrhosis with jaundice. A, routine histologic examination: extensive proliferation of connective tissue with lymphocytic infiltration and proliferation in the bile ducts. Many fat droplets of various sizes in the hepatic parenchyma. The cytoplasm of the hepatic cells is dark. B, examination with fluorescence microscope: vitamin A fluorescence imparted by a few irregularly shaped Kupffer cells and by the large fat droplets, the fluorescence of which is very low. (See also Figs. 3 and 4.)

A disease may affect storage by decreasing absorption, increasing utilization, hindering the conversion of carotene to vitamin A, impairing the storage capacity of the liver or increasing excretion by the kidney. All or any of these factors have been the cause of the figures given above, but it may be tentatively suggested: (1) that all the diseases where low storage occurs would tend to decrease absorption by a poor appetite, fever (p. 30), and an unhealthy gut; (2) that the low figures found in heart disease are partly due to anoxæmia interfering with conversion, and partly to the engorgement of the liver with blood which decreases the proportion of vitamin A to liver weight; (3) that the yellow liver found in patients dying from the uræmia of chronic nephritis (p. 47) is due to the presence of carotene, which presumably could not be converted to vitamin A because of the toxic condition of the gut; (4) that though many of the pulmonary infections with a low storage are similar to those from which vitamin A deficient animals die, yet the low storage is not the cause of the infections but the result of poor absorption or poor conversion, and increased renal secretion (p. 47), all of which might then tend to set up a vicious circle by decreasing the power of resistance of the lungs (p. 38).

Vitamin E. The sparing effect which vitamin E has on the metabolism of vitamin A may have more than academic importance *since when human diets contain insufficient vitamin A or carotene an increase in the consumption of vitamin E would decrease this insufficiency* [205].

Further, when vitamin A or carotene are being assayed biologically, equal amounts of vitamin E must be included in the diets of the experimental and control animals, as the response to vitamin A will be affected by the amount of vitamin E in the diets [205].

Davies and Moore [206] first suggested that the reason why vitamin E spares hepatic stores of vitamin A [207, 208], is that it protects them against oxidation within the liver. This idea has been amplified by Hickman and his collaborators [205, 209], who believe that vitamin A in vitamin E deficient animals is drained away from the liver to replenish the blood vitamin A which is lost through oxidation within the blood vessels of the gut. This oxidation is due to oxidants from within the lumen of the gut diffusing into the blood vessels; normally such oxidants are destroyed within the gut by the vitamin E of the food, which thus also preserves vitamin A and carotene from oxidation before absorption. (Compare the destruction of vitamin E by rancid fat, p. 615.)

The original work which drew attention to the relationship between vitamins A and E was done by Moore [206, 207] and by Bacharach [208]. The former found that the storage of vitamin A—given weekly as halibut liver oil—in vitamin E deficient rats, was increased from two to ten times when the deficiency of vitamin E was removed by giving large amounts of wheat germ oil daily or synthetic vitamin E alcohol weekly. Vitamin A storage from carotene was not so greatly affected. Bacharach [208], keeping rats for shorter periods than Moore on a vitamin E deficient diet, found storage could be increased from twenty-five to thirty-three per cent. by giving large amounts of synthetic vitamin E ester daily; physiologically adequate amounts of the ester had no effect.

In both the above experiments the intake of vitamin A was high; Hickman and his collaborators [205, 209] investigated the subject further by feeding vitamins A and E in small and varying amounts to rats. Their extensive work should be read in full, their chief conclusions being: vitamin E increases the growth-promoting power of vitamin A and carotene and the survival and depletion times of vitamin A deficient rats; there is an optimum ratio between vitamins A and E in the diet, which was also noticed by Moore [207]; vitamin E is most effective when fed together with vitamin A; injections of vitamin E are ineffective; the effect of injected vitamin A is enhanced by oral vitamin E; a mixture of the three naturally-occurring

tocopherols is slightly more effective than α -tocopherol alone and much more effective than tocopherol esters; the action of vitamin E is enhanced by reducing agents such as ascorbic acid. In man vitamin E does not affect the vitamin A tolerance curve (p. 25) or excretion in the milk (p. 52), though it may raise the level in cows' milk [608]. Further relationships between vitamins A and E are discussed on pp. 15 and 36.

Choline. Popper [58] reports that in rats on a diet deficient in choline with ample carotene no vitamin A is stored in the hepatic or Kupffer cells, though it is present in abnormally large amounts in the kidney. When vitamin A itself is given in the diet the Kupffer cells but not the hepatic cells contain vitamin A. Other workers [210] have thought that a deficiency of choline may hasten the depletion of hepatic stores of vitamin A. The relationship of vitamin A to the other vitamins is described on p. 49.

Other Dietetic Factors. Dann and Moore [211] observed that in rats extreme wasting from lack of aneurine had no effect on the storage of vitamin A, and beriberi in man does not cause night blindness [214]. Moore [184] found that in man there was no relationship between the stores of vitamin A and the general nutrition. Storage in animals is increased by a high fat diet [212], and not affected by a low protein diet [207]. In rats [210] and chickens [213] a deficiency of vitamin K has no effect on storage. Carcinogens may or may not cause depletion of vitamin A in the liver [215], and depletion is not hastened by 4-hydroxycoumarin, carvone, dimethylaminoazobenzene or flushing out hepatic fat [210]. The factors influencing absorption, and so storage, have been discussed on p. 17. Atebrine given to rats impairs both storage and absorption [612].

Blood Levels. The ester and the alcohol of vitamin A are both present in the blood, the former according to Hoch and Hoch [216] forming ten to seventeen per cent. of the fasting total in both men and women, while Popper [177] gives the figure as twenty per cent. The alcohol appears to be the active form of vitamin A, the ester merely being used for transport from the gut to the liver (p. 17) and for storage in the liver. The arguments in favour of this are firstly that Glover and his co-workers [217] have shown that in the rat the plasma vitamin A is proportional to the amount of vitamin A alcohol in the liver but is not proportional to the total stores, which are chiefly in the form of the ester. Secondly, whenever a large dose of any form of vitamin A is fed to man [159, 177, 192, 216] the amount of the alcohol in the serum remains almost constant, whatever slight rise there may be occurring later than the very large rise in the ester [216]. Thirdly it would seem probable that there would be some protection for the body against the enormous amounts of vitamin A which for a short time after absorption may circulate in the blood; this could be achieved by vitamin A being transported to its storage in an inactive form, such as the ester is thought to be. The factors which influence the levels of the alcohol and the ester are discussed later when considering the factors which influence the total levels of vitamin A.

The average fasting level of the total vitamin A in the plasma of men is about 130 I.U., or 39 micrograms, and of women about 110 I.U., or 33 micrograms, per 100 ml. These are the figures given in 1949 by Moore and Leitner [93], being based on their own work in England and on the reports of other surveys in England and the U.S.A. Later surveys in the U.S.A. give slightly higher figures; thus the average for 126 men between the ages of forty and ninety was 150 I.U., age making no difference (218); for 18 men and 7 women, 162 and 120 I.U. [192]; for 100 men and women, 150 I.U. [219].

Children under sixteen years of age are said to have slightly lower levels than adults in the same family [220], which is supported by the average level of 394 school children in the U.S.A. being 114 I.U. [221]. In Brussels [93] the average for 41 boys and girls aged about fourteen years was 134 I.U.;

for 60 boys in Dundee [93] aged about fifteen it was 88 I.U. ; for 11 youths aged about seventeen in the south of England [93] it was 137 I.U., while in Italy [222] values for infants and children have been reported as being between 52 and 210 I.U., the lower levels occurring most frequently in infancy. Premature infants a few weeks old have been found in the U.S.A. to have values ranging from 52 to 164 I.U. [160]. At birth the average level of vitamin A in one series [103] of 143 infants in the U.S.A. has been reported as 49 I.U., in another series [102] of 50 infants as 91 I.U., and in a third series [186] of 108 infants as 76 U.S.P. units, which fell to 37 at the end of the second day and then rose again to 61 on the fourth day. The factors which affect the levels in adults and infants are discussed later.

The individual's fasting blood level may vary so greatly from the average that though it tends to remain a personal characteristic—as is discussed on p. 28—it has none of the clinical value of, for instance, a fasting blood sugar level. Thus for apparently healthy adults values have been reported of 30 to 264 I.U. [218], 66 to 231 I.U. [192] and under 40 to over 280 I.U. [93], while for children values have been from 52 to 210 I.U. [222], and for infants 24 I.U. [103] to over 100 I.U. [102]. Therefore all that can be deduced from fasting serum levels is that when these are above 300 I.U. there has probably been within the last few days a very large or toxic consumption of vitamin A (p. 81), while readings below 70 I.U. should suggest the possibility, in an apparently healthy man, of a genuine deficiency of vitamin A, since Lindquist [223]—though his figures may be too high [93]—and Cowell [224] and Pett and La Page [225] have all reported that dark adaptation (p. 60) is impaired when levels are as low as 70 I.U., while the Medical Research Council report [93] states that the three subjects whose dark adaptation had “deteriorated significantly” had values below 50 I.U.

Vitamin A absorption or tolerance curves are similar to blood sugar curves, showing how the level of the vitamin in the serum alters after an oral dose. To construct such a curve the patient, who has fasted all night and has had no large dose of the vitamin in the preceding twenty-four hours, has his “fasting” level measured by any of the methods which have been fully described [93]. An oral dose of the vitamin is then given and serum levels are measured hourly for the first six to eight hours, and sometimes over longer periods. There is no standardized oral dose: for adults the doses have been 50,000 I.U. [220] or 75,000 I.U. [177], or 100,000 I.U. [218] or 134,000 I.U. [192], or 250,000 I.U. [220] or 500,000 I.U. [160]. For children the doses have been 25,000 to 50,000 I.U. [221] or 5,000 I.U. [160, 222] to 6,000 I.U. [162, 226] per pound of body weight, and for infants 35,000 I.U. [160] irrespective of weight. The maximum rise, when the vitamin is given dissolved in oil, occurs within three to six hours, after which the level falls rapidly till about the eighth hour and then very gradually, not returning to normal until about the end of twenty-four hours. The larger the dose the higher the level reached, though the shape of the curve is not altered [221]. When the vitamin is given as an aqueous dispersion—a subject very fully discussed on p. 18—the rise is far greater and the maximum level is attained sooner. Figs. 6 and 7 show the kinds of curves which are obtained and how they differ with sex, the size of the dose, etc.

But, while it is possible to draw an “average” curve, this curve may differ very considerably in its shape and in the maximum level reached, etc., from that obtained from a normal healthy individual. The series of curves, for instance, given by Lewis and others [160] for adults, children and infants show this very clearly; or, again, Erling and Sevigne [192] found the maximum levels attained in adults all given the same dose was between 133 and 1,234 I.U.

In disease, however, the value of tolerance curves is not vitiated by their normal lack of uniformity, since they are only used clinically as an aid in confirming—in the unlikely event of such confirmation ever being needed

—a diagnosis of mongolism [227], lambliasis or giardiasis [229], sprue [173], coeliac disease [158, 159, 162], congenital atresia of the bile ducts [168, 228], intestinal tuberculosis [159] and cystic fibrosis of the pancreas [158, 159, 160, 163]: in all these conditions the tolerance curve (Fig. 7) with the vitamin dissolved in oil differs far more from the average than does the curve of any normal individual. The level of vitamin A rises very slowly to a relatively very low maximum in about nine hours and then falls so gradually that at the end of twenty-four hours it may still be four times as high as it was at

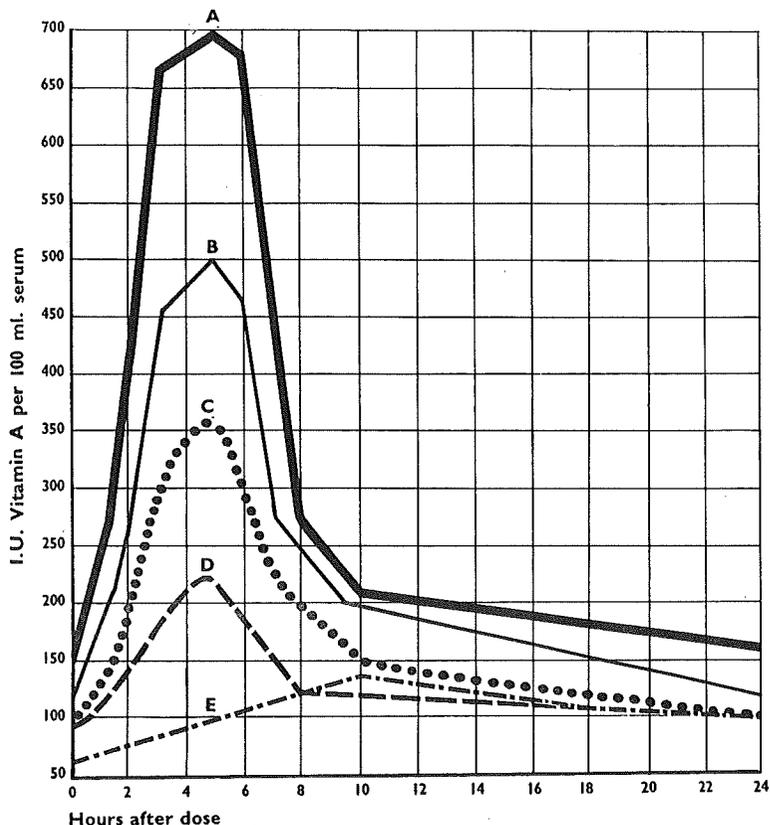


FIG. 6. Vitamin A absorption curves, the vitamin being given orally dissolved in oil.

- A. Male adults after 250,000 I.U.
- B. Female adults after 250,000 I.U.
- C. Children after 50,000 I.U.
- D. Children after 25,000 I.U.
- E. Coeliac disease after 50,000 I.U.

the beginning. The same type of curve has sometimes been found in ulcerative colitis [244], while the types of curve found in diseases which affect mobilization are discussed later.

All these flat curves, with the probable exception of those in intestinal tuberculosis, become virtually normal if the vitamin is given in an aqueous dispersion (p. 18), while in cystic fibrosis of the pancreas absorption is normal from oil if vitamin A alcohol and not the natural ester is given (p. 17). In nephrosis [226] the curve is at a startlingly high level; thus in one very severe case the fasting level was 1,359 I.U., which rose after 6,000 units in oil per pound of body weight to 3,197 units in three hours; to 8,365 units in six hours; to 8,591 units in twenty-four hours and was still 6,094 units in forty-eight hours. It must again be emphasized, as it has been on p. 17, that

absorption curves give no information as to the total amount of vitamin A absorbed.

Intramuscular injections of vitamin A in oil, though physiologically as active as by mouth (p. 17), cause no rise in the level in the blood [162, 222, 250] because absorption from the site of the injection is so slow; thus Masi [222] showed that in guinea-pigs such an injection caused a steady increase in hepatic stores up to the sixtieth day, though during sixty days

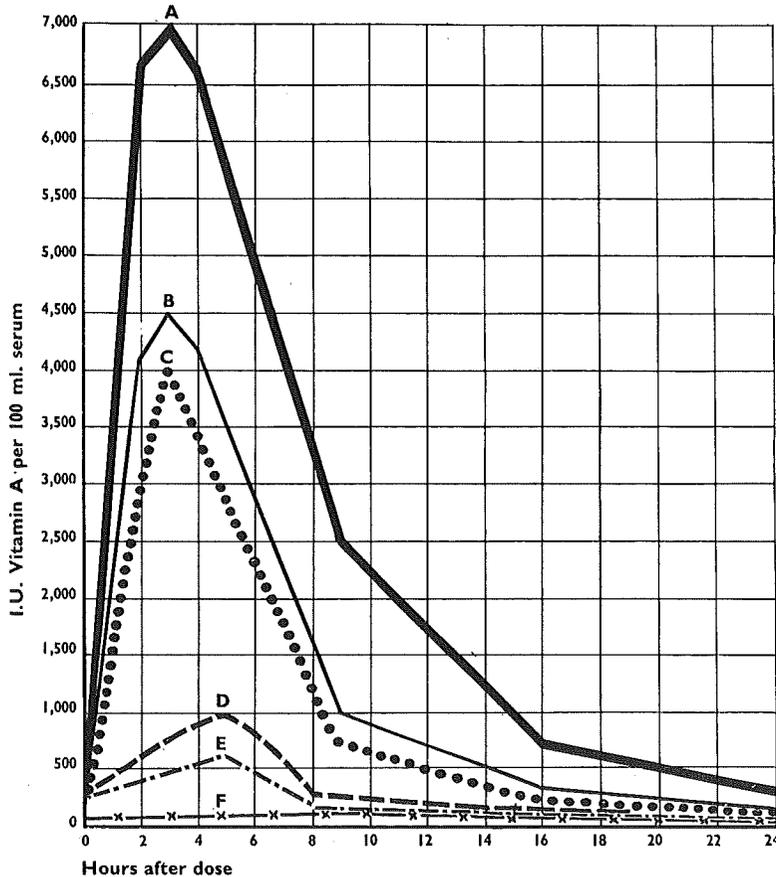


FIG. 7. Vitamin A absorption curves, the vitamin being given orally dissolved in oil or as an aqueous dispersion (p. 18).

- A. Adult after 500,000 I.U. as an aqueous dispersion.
- B. Child after 300,000 I.U. as an aqueous dispersion.
- C. Celiac disease after 250,000 I.U. as an aqueous dispersion.
- D. Adult after 500,000 I.U. dissolved in oil.
- E. Child after 300,000 I.U. dissolved in oil.
- F. Celiac disease after 250,000 I.U. dissolved in oil.

after the same dose had been given by mouth stores steadily fell to a low level. He therefore suggests that it is better to give single large doses of vitamin A by injection rather than by mouth, since the effect is more prolonged. Injections of an aqueous dispersion (p. 18) of vitamin A are painful; they cause a very slight and delayed rise [162].

The Mobilization of Hepatic Stores. The balance between *storage* and *mobilization* must play an essential part in the maintenance of the level of vitamin A in the blood, since the liver is virtually the only storehouse of the body. But how such mobilization is controlled is obscure. Glover (p. 21) has suggested that the true hepatic cells contain an enzyme [610] which

converts the stored inactive esters into the active alcohol, and it is the level of this alcohol in the liver which keeps the alcohol in the blood constant (p. 24). Many factors are known which affect this production of the alcohol or, at least, the maintenance of the level of the total vitamin A of the blood.

Diet, after a period of normal nutrition, causes mobilization or replenishing of the hepatic stores with no alteration in the blood levels. Thus in the rat [151] the level in the blood remains constant until the stores are depleted, and a review of the literature [93] suggests that, when levels in man have fallen very rapidly because of a deficient diet, this has been due to low reserves being rapidly exhausted. This is supported by the work of Callison and Orent-Keiles [238], who showed that while depletion on a deficient diet, as judged by dark adaptation, took from two to six months—an unusually short time [93]—yet depletion of the same subjects could be achieved a second time, after only a short period on a normal diet, in thirteen to sixteen days. Conversely a very high consumption of vitamin A does not increase the level in the blood, a group of nurses, for instance, who took 50,000 I.U. daily for nearly two years having no higher blood levels than nurses on ordinary diets [239].

The *individual* tends to preserve a particular level which is characteristic of himself; thus after single large doses of the vitamin his blood level reverts to his normal after about twenty-four hours [220], and even after taking 3,000,000 I.U. over twelve days the level in the blood is back again to the individual's normal in another ten days [220]. Also, deprivation of vitamin A may leave the individual's level unaltered; for instance, the blood levels in the deprived subjects investigated by the Medical Research Council [93] varied very little from month to month over many months. Reports that a diet low in the vitamin may within a week almost halve the vitamin A alcohol in the blood [177] while the ester may fluctuate in either direction [237] or fall [177] may possibly be congruous with the monthly investigations of the Medical Research Council, because after "prolonged periods" of deprivation the ester and the alcohol may both rise [237].

Pregnancy, according to Bryn and Eastman [102] and to Aron [220], who in 1949 reviewed his own work and that of several others, causes a slight fall in the level of vitamin A, most marked in the last trimester; after delivery the level rises to normal within forty-eight hours even when no vitamin A is consumed. In other words, pregnancy decreases mobilization from the liver, though individual levels, according to Cayer and others [245], may remain above the average and are related to the level of lipoids in the blood. After parturition the alcohol but not the ester rises [216].

Drugs may mobilize stores of vitamin A. Thus according to Hoch and Hoch [216] a dose of 60 ml. of ethyl alcohol leaves the level of the inactive vitamin A ester unchanged, while after two and a half hours the active vitamin A alcohol has risen by ten to fifteen per cent. and is still rising. The smallest dose which causes a significant increase is 20 to 40 ml. Pett [246] states that in dogs ethyl alcohol quadruples the level of vitamin A after forty-eight hours, while Clausen [251] states there is a considerable rise in the ester. Somewhat similar results have been recorded for man by Clausen [501] and several workers whose papers have been summarized in the Medical Research Council's report on vitamin A [93], though the original work described in this report failed to show that vitamin A has any effect whatsoever on the level of vitamin A in the blood. Even so the balance of evidence is heavily in favour of alcohol mobilizing vitamin A.

Adrenaline and stimulation of the greater splanchnic nerve has been claimed to raise the level of vitamin A in the blood of rabbits [247], but Goodwin and Wilson [248] could not confirm that adrenaline had any effect in either rabbits or rats, and work purporting to show that adrenaline mobilizes vitamin A in man is most unconvincing [249]. Clausen [251] found that adrenaline, insulin and mecholyl chloride all had no effect in rabbits.

Illnesses which interfere with the absorption of vitamin A, such as those mentioned above when discussing tolerance curves, all tend to cause low fasting levels, though these are often within the lower normal limits; for instance, Kramer and others [162] found the normal for children was 135 I.U., and the average for five children with cœliac disease was 117 I.U. In nephrosis the fasting level is high or very high; for instance, levels of 1,665 I.U. have been reported [226], the latter being found in the severer cases with the higher lipæmia, though there was no quantitative relationship between the degree of lipæmia and the level of vitamin A. It is the ester which rises in nephrosis [177]; why it does so is obscure, though from the very slow fall in the absorption curve (p. 6) it is obvious that hepatic storage is grossly impaired. In nephritis, on the other hand, the alcohol rises [177]; this may be due to increased mobilization occurring to counteract the excretion in the urine—which is discussed later.

Many other illnesses, often because of the associated fever discussed below, cause a low level of vitamin A in the blood. Spector and his collaborators [167] in 1943 gave an excellent review of their own work and that of others; in acute and chronic infections the serum vitamin A is low and cannot be raised by giving vitamin A by mouth, and this was also found in an infant with infected adenoids until the adenoids were removed, in children with uncompensated mitral stenosis and also in children with eczema or asthma and hay fever. Even feverish colds halve the level of the vitamin [230]. Popper [177] in 1948 stated that vitamin A alcohol falls in cardiac diseases, malnutrition, severe illness, phthisis, hepatitis of all kinds, and especially in cirrhosis and pneumonia, though after the latter a rise occurs. The ester may rise or fall in cirrhosis [177]. The level of vitamin A in skin diseases is discussed on p. 65, and in diseases of the thyroid on p. 45.

Diabetics in the early days of insulin used to be given a very restricted diet, the only foods they were allowed to satiety being green leafy vegetables. These were eaten in huge amounts in an effort to stay their gnawing hunger. The result was that the level of carotene in the blood was often very high [98, 240], which led to the idea that diabetics were unable to convert carotene to vitamin A, this being supported by the poor dark adaptation said to be found in diabetics [240]. But Kimble and her colleagues [241] in 1946 examined the levels of carotene and of vitamin A in the blood of 116 diabetics and, while finding that the levels of vitamin A tended to be low, they did not find high carotene levels nor did they find that the level of vitamin A was low when that of carotene was high, as would have been the case had there been any inability to convert carotene into vitamin A. Further, the large stores of vitamin A found in English diabetics (p. 22) suggest that neither absorption of vitamin A nor its formation from carotene is impaired. Probably the correct explanation of the earlier observations on diabetics is that their carotenemia and yellow skin was normal for the large amounts of carotene consumed (p. 78); that the low levels of the vitamin and the high levels of carotene in children [98] were due to the normal difficulty all children have in converting carotene to vitamin A, combined with a diet probably deficient in fats containing vitamin A; that the impaired dark adaptation, which was said to respond to vitamin A only as long as it was given and never to carotene [240], was due to impaired mobilization, since vitamin A, once given, should have had more than a transitory effect were there a genuine deficiency of vitamin A and not only a blood level "set" too low. In support of this is the low levels found in diabetics [241]. Of course dark adaptation would be impaired both by hypoglycæmia and hyperglycæmia, which means that any studies on dark adaptation in diabetics need the control of many complicated factors, which was not done in the cases mentioned, so that the importance of the findings must not be over emphasized. In other words, diabetics, like everyone else, need a diet containing ample vitamin A, but while they may have impaired mobilization they do not have difficulty in

converting carotene to vitamin A, nor have they the particular need for it which they have for the water-soluble vitamins.

Hepatic disease when it is chronic causes low reserves of vitamin A (p. 22) and low total levels in the blood [199, 201, 202] at the expense of the alcohol [177], but when there is acute hepatitis, though the level falls [200, 201, 202, 252], again at the expense of the alcohol [177], reserves remain normal [58, 201, 202, 252]. After a large oral dose of vitamin A in oil, a large amount may be lost in the faeces or all of it may be absorbed [252]. There generally is a flat absorption curve [160, 166, 167, 200, 220]—though not always [252]—which Aron [220] holds is a very delicate indicator of hepatic function, appearing several days before the onset of jaundice and reverting to normal several days before it fades. But Harris and Moore [252] found there were too many fluctuating factors—temperature, absorption, storage, mobilization—in infective hepatitis for vitamin A absorption curves to give definite evidence about hepatic function, though they did find that the levels of vitamin A tended to improve with improvement in the hippuric acid tolerance test and the prothrombin value. Aqueous dispersions of vitamin A are absorbed normally in obstructive jaundice [160].

Fever depresses the level of vitamin A in the blood whether it is caused by drugs [98, 231] or by infections. This may partly be due to its effect on absorption (p. 17), but it must in essence be due to impaired mobilization, since after the fever abates the level in the blood returns again to normal or above without any vitamin A having been given; this has been confirmed for fever from drugs [231], from typhus [233], from rheumatic fever [234] and from pneumonia [235]. Since fever has this effect on vitamin A levels it may well be that the prevalence of night blindness in consumptives [232, 236] is not due to an inadequate intake of the vitamin but to impaired mobilization.

Levels in the cerebrospinal fluid and in exudates and transudates of the body are of no practical importance. Abderhalden and Elsaesser [242] could find no vitamin A in the cerebrospinal fluid even after dosing by mouth, while Tomaszewski and Dzialoszynski [243] could only find traces when using larger amounts of the fluid than those normally obtained by lumbar puncture. The latter workers also report that they found up to 30 I.U. per 100 ml. in 21 of 25 specimens of pleuritic fluid, in 12 of 13 specimens of ascitic fluid, in 2 specimens of pericardial fluid, and in 2 of 6 specimens of amniotic fluid. The presence of vitamin A in these various fluids was not related to their protein content nor to the presence of vitamin A in the urine.

Excretion and Destruction. Lawrie, Moore, and Rajagopal [183] in 1941 reviewed the work on excretion of vitamin A by the kidney and also gave the results of their own research. The following is chiefly based on their paper. Vitamin A is never excreted by man during good health, except possibly as a breakdown product [212], but it may appear in the urine during illness, being found most frequently and in the highest concentrations in pneumonia. A daily output of 3,200 I.U. has been recorded, which is said to cease abruptly with the crisis. In chronic nephritis vitamin A is common in the urine though in smaller amounts than in pneumonia. Still smaller amounts have occasionally been reported in chronic infections, rheumatic fever, skin diseases, diabetes, pernicious anæmia, asthma, cancer, normal pregnancy and also infective hepatitis [252].

How vitamin A is dissolved in human urine is obscure. It is always associated with protein, but not all urines containing protein contain the vitamin, even though they are capable, unlike normal urines, of taking it up from halibut-liver oil. Apparently the excretion of vitamin A in the urine is dependent on a functional abnormality of the liver causing a diminished capacity for absorbing or retaining the vitamin. This alters the equilibrium between the liver and the blood, vitamin A passing to the latter. But the blood and kidneys will only yield up the vitamin to the urine if their capacity to retain vitamin A is impaired. In pneumonia this is frequently so to a

marked degree. In nephritis the damage to the kidney probably leads to an accumulation in the blood of substances which increase the solubility of vitamin A and so partially hold it back from excretion.

The healthy dog constantly excretes vitamin A. It is interesting to note that the level of vitamin A in the dog's blood may be extremely low without causing any signs of a deficiency [253] and that its metabolism appears to differ from that in other animals (p. 50). Rats never excrete vitamin A, even when taken in toxic amounts [123, 183] or when their kidneys are damaged by lack of vitamin E [207], and rabbits only when diseased [183]. The occasional presence of vitamin A in the faeces is presumably due to incomplete intestinal absorption (p. 17). Secretion in the milk is discussed on p. 52.

The large stores which accumulate during a diet rich in vitamin A are depleted with great rapidity if the diet becomes deficient in the vitamin [212, 254], or in vitamin E [206], the depletion being greater than can be accounted for by the needs of the body. Moore [254] suggests that there are two ways in which vitamin A is destroyed, one depending on the normal and economical physiological use of vitamin A in the processes of the body, and the other depending on some mechanism for destroying vitamin A when it is stored in excessive amounts.

Factors Influencing Requirements of Vitamin A. *Age, Weight, Metabolism.* It often appears to be accepted as axiomatic that children and young animals require more vitamins than adults, but the reverse of this is true as regards vitamin A, since the requirements, being proportional to the weight, are higher in adults. Thus Irving and Richards [255] noted that old rats need more vitamin A than young rats both to prevent deficiency lesions only demonstrable at autopsy, and to keep the development of the continuously growing incisor teeth normal. Guilbert and his co-workers found that in cattle, sheep and swine [256] and also in rats [257] the minimum carotene and vitamin A requirements were dependent on the weight alone, being 25 to 30 micrograms of carotene, or 6 to 8 micrograms of vitamin A per kilogram of body weight, and they suggest this figure holds true—apart from carnivores—for all animals, including man [147] for whom it is in agreement with requirements based on clinical work (p. 58). Other workers [266, 267], using rats, have shown that three to four times these minimum amounts throughout life increase longevity and health and delay the onset of senility. Guilbert and his co-workers also state that the requirements are not affected by the amount of energy expended by the animals, their work being confirmed both as regards the effect of weight and increased metabolism by Guerrant and others [258], who found that when rats on a deficient diet were made to exercise they gained less weight but developed less severe deficiency symptoms than control animals. These results confirm Wolbach's [259] suggestion that vitamin A is necessary not for the metabolic activities of the cells, but for the maintenance of the structure of the cells, so that the requirements of the body would depend on its weight, that is its number of cells, and not on its activity. The changes in metabolism resulting from a low protein diet [207] and a high fat diet [261] do not influence, respectively, storage and consumption while thyrotoxicosis in man (p. 45) appears to increase storage. Birds [290, 291] appear to need five to ten times as much vitamin A as animals.

All these investigations which run counter to the belief that the young need more vitamin A than the old have admittedly been done on animals, but it is generally believed that the effect of vitamin A is broadly the same in all species [260], so it seems most probable that increasing age and so increasing weight raises the requirements of vitamin A, but that youth, exercise and a raised metabolism have no effect.

Sex. Sex does not greatly influence the requirements of vitamin A, since in countries where xerophthalmia is common both sexes are equally affected [262], and in England Harris [263] found poor dark adaptation equally

common in boys and girls between the age of eleven and twelve, though between the age of twelve and thirteen girls were slightly less affected than boys. Coward [264] after many years' work on rats has shown that the male requires more vitamin A than the female, and she suggests this may explain the higher mortality among human male than female infants. The less uniform requirements of the female rat [68] may possibly be related to the fluctuating vitamin A metabolism which occurs in the ovaries, at least of women [182]. Depletion of hepatic stores of vitamin A occurs more rapidly in the male rat than the female [265].

Illness. If the list of the amounts of vitamin A stored in the livers of patients dying from various diseases is referred to on p. 22, it will be seen that in some diseases—notably in chronic genito-urinary conditions, chronic sepsis, infections of the lungs and alimentary tract and hepatitis—there are low reserves of vitamin A which suggests that, whatever is the cause (p. 23), the requirements of vitamin A are increased. Some diseases hinder absorption (p. 17) or cause excretion in the urine (p. 30) or impair mobilization (p. 27), and so in these large amounts of vitamin A, possibly in the form of an aqueous suspension (p. 18), may be continuously required if it is desired to keep the level in the blood at a normal level.

Poor Utilization of Carotene. When discussing the absorption of carotene and its conversion to vitamin A (p. 13) it was seen that many factors impair the value of carotene to the body and so increase the need for vitamin A itself.

Action of Vitamin A in the Body. Nothing definite is known about what is the fundamental part played by vitamin A in the metabolic processes of the body: all that can be said is that a consideration of its chemical structure with five unsaturated bonds (p. 3) suggests its probable function as an oxidation-reduction catalyst; while the needs of the body depending on weight, and not on metabolic activity, seem to imply that it is chiefly necessary for cellular structure rather than function. French work on *chronaxie* does not help in understanding the action of vitamin A; it corrects disturbances in *chronaxie* due to acidosis [268] and also causes disturbances similar to those of acidosis when it is deficient in the diet [269]—but such effects can have many explanations other than those concerned with the fundamental action of vitamin A itself. There is some evidence that vitamin A has a specific effect on the growth of animals [69] and quickens growth and prolongs the life of tissue cultures [270, 271], which is reminiscent of work showing that a very plentiful supply of vitamin A throughout the life of the rat increases longevity and delays the onset of senility [266, 267].

The Effects of Lack of Vitamin A on the Epithelial Surfaces of the Body

“The specific tissue change due to deprivation of fat-soluble vitamin A is replacement of various epithelia by stratified squamous keratinizing epithelium” (Wolbach and Howe [272]). If this statement is broadened to include all tissues of epithelial origin be they from entoderm, or ectoderm like the skin and nervous system and retina, a valuable generalization is made which harmonizes many of the more important functions of vitamin A, always remembering that some tissues are damaged earlier than others by a deficiency. The epithelial changes are the direct effect of lack of vitamin A and are not the secondary result, as was suggested by Mellanby, of the degeneration of the nervous system which also occurs; the subject is discussed fully on p. 42.

The change in the epithelium is the same in all areas where it occurs, the normal epithelium becoming undermined by stratified epithelium, which starts to be formed by the basal layer of cells in many places at the same time. The basal cells themselves, however, are not changed, maintaining their individual properties, so that when vitamin A is given to a deficient animal the basal cells promptly start to replace the stratified epithelium with epithelium of the correct type.

In considering in detail the changes induced in the various surfaces of the

body Wolbach and Howe's [272] description of the rat will be followed, references being given where later work has amplified their observations, or extended it to other species, whilst post-mortem observations in man are described on p. 78. The effects of a deficiency in birds will not be given in detail, as these were reviewed in 1950 by Hogan [290]; they include failure to grow, changes in the nervous system (p. 42), changes in the nasal epithelium with a nasal discharge, degeneration of the nictitating membrane, ophthalmia, pustules in the mouth and œsophagus and deposits of urates.

Young animals develop symptoms first, due probably to their naturally low stores of vitamin A (p. 19), the most striking features at the time of death being a humped posture, rough fur, emaciation, and encrusted eyelids.

Glands. The epithelium lining the ducts of the glands is involved before that lining the acini, and in general the changes are not very pronounced. The shedding, however, of desquamated cells by the new keratinizing epithelium into the ducts blocks these with the result that retention cysts occur in the glands, and then secretion is impaired even when the secreting cells are not yet affected. This accounts for the early observations on the common occurrence of abscesses or cysts at the base of the tongue, which are really salivary retention cysts that may become infected. As well as all the salivary glands the conjunctival and lachrymal glands are involved, soon and constantly, while the glands of the duodenum are only slightly affected and the pancreas escapes apart from some late changes in the duct. In Wilson and Du Bois' [273] child, however, the pancreas was more affected, the ducts being blocked and the acini cystic, though the islets

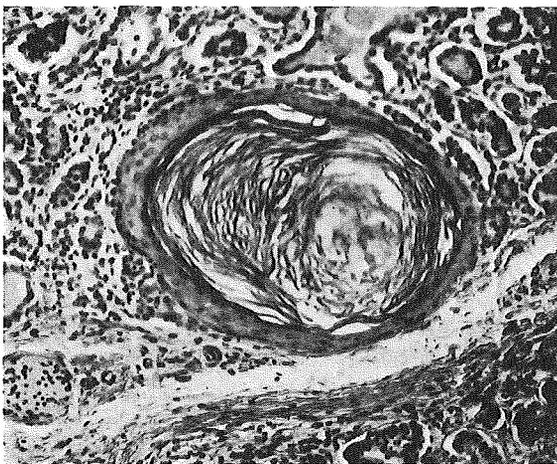


FIG. 8. Pancreas of an American infant showing duct occluded by keratinizing epithelium.

of Langerhans appeared to be normal (pp. 36, 47). There is late atrophy of the sebaceous glands in the rat [275, 276], but in man they and the sweat glands may become blocked early in the deficiency (p. 65).

Air Passages. All the respiratory tract, the nasal sinuses, and Eustachian tubes are involved. This is of special importance in the lungs, where the smaller bronchi become plugged with desquamated cells, thus paving the way for bronchiectasis and infection, both in animals and man (pp. 36, 78). The otitis media, however, which is usual in deficient rats does not occur in children [277].

The Eyes. The conjunctiva undergoes constant and early keratinization followed by œdema and opacity of the cornea and its invasion by blood vessels from the sclera [272, 305]. Dryness or xerosis of the eye is caused by the drying up of the lachrymal secretion due to the involvement of the lachrymal glands. The latter was held at one time to be the primary cause of the changes in the conjunctiva and cornea, but Wolbach and Howe [272] showed that xerosis occurred after keratinization, which is supported by clouding of the cornea in the horse [274] and xerosis in man (p. 72) occurring at the same time as an increased secretion of tears. Ultimately the whole cornea softens, and the condition known as keratomalacia occurs which, aided by infection, leads

to perforation. The changes observed in man, which are broadly the same, are described on p. 72.

The corneal vascularization which follows the keratinization is said by Bessey and Wolbach [278] to be indistinguishable in the rat from that which occurs with a deficiency of riboflavine (p. 316), but Bowles and others [305] after very careful work believe that there is a slight difference: with lack of vitamin A vascularization tends to be more dendritic with a denser collar of blood vessels round the cornea. But if there is really no difference this raises the very interesting question whether the corneal vascularization caused by lack of vitamin A is not really directly due to a local deficiency of riboflavine caused by the absence of ocular secretions, since it appears probable that the cornea is normally supplied with riboflavine not by the limbic blood vessels but by being bathed in the tears and Meibomian secretion, both of these containing large amounts of riboflavine [281]. In any case corneal vascularization is a sign of little value, since it also occurs in rats [305] from a deficiency of tryptophane, lysine, methionine, protein, zinc or sodium and from excess of tyrosine, from poisoning with thallium and from physical or chemical trauma. The rather scant descriptions of the vascularization which occurs in human xerophthalmia appear to have been made when a secondary infection had blurred the picture [279, 280].

Olfactory Epithelium. Loss of smell is a late but constant finding in rats and other animals [277], though the olfactory epithelium appears to be normal [272]. The olfactory nerve endings themselves may be damaged by osseous thickening of the cribriform plate (p. 43). Milas [282] found that the olfactory area in the steer was rich in carotenoids and vitamins A₁ and A₂, which suggests that vitamin A may play a part in smell analogous to that it plays in vision. It would be interesting to know if a deficiency of vitamin A in man causes loss of smell.

Digestive Tract. The mucous membrane of the digestive tract of the rat is not involved, apart from slight changes in the œsophagus, unless the deficiency of vitamin A is very prolonged, when considerable thickening of the mucosa of the forestomach occurs, which does not persist after the deficiency is removed, nor cause the formation of malignant tumours as was once thought [65, 283]. In dogs and rats a deficiency does not alter the secretion of acid in the stomach nor have a definite effect in dogs on the emptying time [284, 285].

In man (pp. 73, 77) severe deficiencies often cause diarrhœa and there is some evidence that vitamin A is important for the function, if not the structure, of the gastric glands and pancreas. Földes and Vajda [286], following up unimpressive German work, report that in twenty cases with deficient or absent hydrochloric acid, twelve improved, both as regards their symptoms and the amount of acid in their test meals, after two or three weeks on 16,000 I.U. of vitamin A thrice daily. The patients who benefited had chronic gastritis, neurasthenia, gastroptosis, diabetes, thyrotoxicosis, or renal lithiasis. The eight patients who did not benefit had gastric carcinoma, pernicious anæmia, or gall stones. One patient in the last group was given bile salts as well as vitamin A, which caused a return of hydrochloric acid to the stomach, even though histamine injections had failed. Herfort [287], however, did not find that vitamin A had any effect in hypochlorhydria, though it did increase the amount of lipase and the tryptic activity of pancreatic juice not only in normal subjects but also in those with various vague intestinal symptoms; in the latter diarrhœa was decreased and the appetite improved. Preliminary work by Seelig [288] suggested that large amounts of vitamin A rapidly removed both the symptoms and radiological or gastroscopic evidence of gastric ulcers, but work by Douthwaite [289] did not confirm this.

Gums and Teeth. In man lack of vitamin A causes the gums to become hyperplastic and keratinized [292], while the developing teeth both in

infants [293] and animals are severely damaged, since the enamel organ being of epithelial origin shrinks, or is replaced by keratinizing epithelium, thus apparently removing from the odontoblasts some controlling influence over their growth. They therefore form poor or deformed dentine, which in prolonged mild deficiencies leads to the formation of odontomas and tooth reduplication in the perpetually growing incisors of rodents [294, 295] : such teeth also lose their colour [296]. Mellanby [295] has shown that in animals lack of vitamin A in the maternal diet can seriously damage the teeth of the young before their birth. It therefore seems probable that vitamin A is the most important vitamin for the structure of the dentine and enamel, in spite of the commonly and quite erroneously held belief that lack of vitamin D is the main cause of dental degeneration (p. 570).

Urinary System. Vitamin A plays an important part in the function of



FIG. 9. Incisor tooth of rat on a vitamin A deficient diet for 170 days followed by 14 days with addition of butter fat. Ameloblast inclusions are seen in the dentine. Newly formed dentine has filled spaces between the folds of dentine and has surrounded the inclusions of ameloblasts. The restoration of odontoblasts is shown and heavy calcification of the dentine on the labial side of the tooth, a usual response to restoration of vitamin A to the diet.

the kidney, which is discussed on p. 47, but lack of vitamin A does not structurally alter the epithelium of the renal tubules, though it causes the usual epithelial changes in the pelvis of the kidney, ureters and bladder in animals ; in man changes are seldom so severe (p. 78). There is, however, one important result of the change in the mucosa, since the new epithelium sheds cells into the urinary tract, so providing a nidus round which salts are deposited to form calculi : this occurs frequently in rats and guinea-pigs.

In man, however, it is probable that lack of vitamin A is not a factor in causing renal calculi, though this has frequently been suggested partly from analogy with animals, partly from observations that patients with renal calculi often have symptoms of a deficiency of vitamin A. Thus Long and Pryah [297] and others [298] have reported that renal calculi are frequently though not always associated with poor dark adaptation, the most chronic cases having the worst adaptation [297]. On the other hand, Jewett and his colleagues [299] compared a group of twenty patients suffering from renal calculi with a group of forty normal people ; both groups had equally good

dark adaptation and similar levels of blood vitamin A. Post-mortems on seventy-eight patients with renal calculi showed no epithelial changes suggestive of a deficiency of vitamin A in either the lungs or urinary tract. It would appear most probable that when urinary calculi and signs of a deficiency of vitamin A occur together it is not the deficiency which has caused the calculi but the calculi which have caused the deficiency, through disturbing the metabolism of the vitamin as a secondary result of the damage they have inflicted on renal function (p. 47).

Genital Ducts and Epithelium. Mason [300] in a very careful study on rats has shown that vitamin A is essential for the germinal epithelium of the testes, its deficiency causing changes unlike those produced either by starvation or deprivation of vitamin E. The earliest changes are sloughing of germinal cells into the lumen of the tubules, with a gradual reduction in the latter's size. As the degeneration becomes more advanced only three or four layers of cells are left lining the tubules, but these still are capable of forming an occasional sperm, and at no time can the testis be so damaged that it cannot return to normal when the vitamin A deficient diet is stopped. These changes are due to a direct effect on the cells themselves and not an indirect one from vitamin A acting on the pituitary, since neither pituitary transplants nor injections of pregnancy urine hastened recovery, and also because the degeneration caused by removal of the pituitary is unlike that caused by a lack of vitamin A. A rather puzzling relationship was also noted between vitamins A and E. When both were deficient the testes sooner showed signs of a vitamin E deficiency than when a deficiency of vitamin E was present alone, though the decrease in the number of cells due to lack of vitamin A might have been expected to decrease the need for vitamin E. It was further found that a vitamin E deficiency when superimposed on an existing vitamin A deficiency did not cause such serious damage as when the vitamin E deficiency occurred alone. Wolbach and Howe [272] also noted œdema outside the basement membrane of the seminiferous tubules and the usual epithelial changes caused by the lack of vitamin A in the mucosa of the epididymis, prostate, and seminal vesicles and, in the female, in the oviducts, uterus and vagina. Similar testicular changes have been produced in mice [611] and bulls [301]. The vaginal changes have been used as a guide when doing biological tests for vitamin A, being among the earliest signs of a deficiency in animals (p. 7), while Hohlweg [302] reports that in female infants the appearance of cornified cells is one of the first results of a deficiency. The part played by vitamin A in reproduction is discussed on p. 47.

Endocrine Glands. The structure, as apart from function (p. 46), of the endocrine glands is said to remain normal; no change beyond decrease in size was noted in the rat's anterior pituitary, thyroid, thymus, parathyroids, suprarenals, islets of Langerhans, ovaries, Graafian follicles, corpora lutea, and interstitial tissue of the testes. In a human infant dying from lack of vitamin A Hassall's corpuscles were found to be enlarged [273].

Skin and Hair. The rough coat of the rat [275, 276] and horse [274] and "toad skin" and absence of sweat in man (p. 67) are due to hyperkeratinization of the epidermis and the atrophy of the hair follicles and the sebaceous and sweat glands, and their blocking by desquamated cells. In man the scalp hair and nails are affected little if at all [262, 303, 304].

The Secondary Result of Changes in the Epithelia due to Lack of Vitamin A : Decreased Local Resistance to Infection

Lowered local resistance to infection is the most important result from the changes in the epithelia brought about by lack of vitamin A. The general defence mechanisms of the body, on the other hand, are not impaired so that the name "anti-infective vitamin" is too broad in its implications; vitamin

A is only "anti-infective" to the extent that when it is given to man or animals suffering from its deficiency it increases the power of the epithelial surfaces to resist local infection by bringing them back to their correct and normal condition.

The importance of vitamin A for the local epithelial defences of the body has been recognized for many years. Among earlier workers Cramer and Kingsbury [316] in 1924 pointed this out very clearly and emphasized that these local defences were not entirely concerned with bacterial infections, since their animals also were heavily infected with intestinal worms, which is supported by vitamin A deficient rats being more susceptible to infection with trichinosis [97], and deficient chicks and turkeys being more susceptible to coccidiosis and trichomoniasis [290]. Green and Mellanby [10] found that rats on a vitamin A deficient diet all died with mucosal infections, and that the addition of vitamin A, as carotene, to the diet afforded a degree of protection against these infections which was proportional to the amount of carotene added [317]. That the value of vitamin A is purely due to its local effects is shown partly by the infective lesions caused by its deficiency being always epithelial, and partly by observations on the relationship of the humoral defences of the body to vitamin A. Thus Gellhorn and Dunn [318] found that the phagocytic index during the early stages of a deficiency might be increased or decreased, but that after a prolonged deficiency it was always low; they suggest that when the index is raised it is due to the normal reaction to infection, and that when it is low after a prolonged deficiency this is due to exhaustion and is not a direct result of the deprivation of vitamin A, though the index being low sets up a vicious circle which further decreases resistance. Torrance [319] observed that vitamin A deficient animals were no more susceptible to bacterial toxins than were normal animals, and it is the antibodies in colostrum and not the vitamin A which protect calves against white scour [336]. In ducks [337] lack of vitamin A has no influence on the course of *Plasmodium lophurae* infections.

Changes in the blood picture due to deprivation of vitamin A are not so severe as to suggest they would seriously decrease resistance to infection. Wagner [320], studying ten men who took an experimental diet nearly devoid of vitamin A, noted a decrease in the hæmoglobin and erythrocytes, degenerate red cells, a leucopenia with degeneration of the myeloid cells, and a marked fall in the thrombocytes, though none of these changes except the latter were very marked. Abbott and others [321] who diagnosed vitamin A deficiency in 84 children, 45 women, and 28 male students by the condition of their skin and conjunctivæ, and by their diets, found a mild leucopenia with a decrease in polymorphs, a relative increase in large lymphocytes, a decrease in small lymphocytes, with an increase in juvenile and degenerate cells. These findings corresponded to those they had previously obtained in vitamin A deficient rats. That the changes in the blood were due to lack of vitamin A is strongly supported by the blood of all the patients returning to normal after they had taken 51,000 I.U. daily for six weeks. Sweet and K'Ang [262], however, in their very extensive study of vitamin A deficiencies among the Chinese found no alteration in either the red or white cells of the blood, and Hennessey [334] in Uganda reported that in prisoners who were deficient in vitamin A the giving of cod-liver oil did not alter the leucocytic response to injections of a bacterial antigen. The bone marrow of vitamin A deficient rats is normal [335].

Clinical work on the whole bears out that vitamin A is only of value for increasing resistance to infection when the patients are on a deficient diet and the infection is chiefly concerned with epithelial surfaces; but observations on man are difficult to interpret since there are few reports which accurately mention whether the patients treated with vitamin A were previously on a good or deficient diet. In the first important clinical trial of vitamin A Green and others [322], having observed that local infections of

the uterus and Fallopian tubes developed in vitamin A deficient rats after parturition, gave 275 pregnant women in the last month of pregnancy—when they were presumably deficient in vitamin A (p. 65)—large supplements of

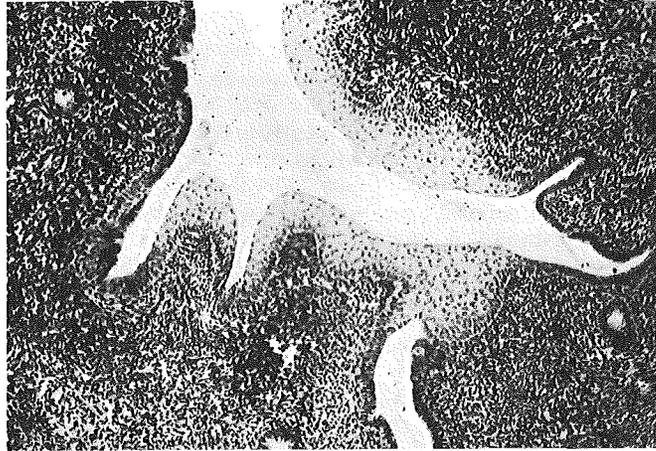


FIG. 10. Uterus of an American infant with epithelium in part replaced by keratinizing epithelium.

vitamin A as "radiostoleum." Only 1.1 per cent. developed puerperal sepsis, as against 4.7 per cent. of 275 women who had had no extra vitamin A.

Donaldson and Tasker [323] in Johannesburg reduced the mortality from pneumonia among native workers from thirteen per cent. in 100 untreated cases to eight per cent. in 200 cases who were treated with extra vitamin A in the form of "radiostoleum" or liver. Orenstein [324] failed to confirm these results.

Ellison [325] found vitamin A of some slight value in measles, though Mackay and others [326] could not confirm this. Sutliff [329] failed to protect children with scarlet fever from developing otitis media by giving them vitamin A. In typhoid fever in children Giraud and Valette [328] state that vitamin A is of great value for preventing hæmorrhage or perforation of the bowel



FIG. 11. Lung of an American infant with bronchiectatic cavity due to keratinizing metaplasia in bronchi. The cavity is partially lined with keratinizing epithelium.

and skin lesions but it has no effect on the cardiac and pulmonary complications or the duration of the disease. Other forms of enteritis might be benefited by vitamin A since its deficiency in animals increases not only the number but also the variety of intestinal bacteria [338].

The "common cold" in its relation to vitamin A has been investigated

by many workers. Again the rule appears to hold good that vitamin A is only of value when it corrects its own deficiency. Thus Wright and others [330] in Canada found no effect at all from giving large extra amounts of vitamin A to twenty of sixty infants all of whom already received a dessert-spoonful of cod-liver oil daily, and Uddströmer [331] likewise observed no benefit from adding 6,000 I.U. of vitamin A daily to diets which were already excellent. Similar results are reported from America [339], and England [340]. Where the diet is reported to have been reasonably good most observers state that the duration of colds, though not their number, is decreased by additional vitamin A [332] and some workers have reported that in poorly nourished people the number of colds is also less [333]. Vitamins A and D given together are stated to have a greater prophylactic value than either given alone [341].

In infections of the skin vitamin A may be of great value, Ryrie [342] reporting that vitamin A or carotene is almost a specific cure for leprosy ulcers when applied locally, and Banyai [343] obtained good results with cod-



FIG. 12. High power of Fig. 10.

liver oil applied to all forms of tuberculous ulceration of the skin, larynx, and pharynx. He also stated that injections of cod-liver oil into tuberculous empyemata, glands, epididymes, and ischio-rectal abscesses were of value, but the benefit was probably due to the oil and not its vitamin A (p. 680). Infants fed on roller dried milk supplemented with vitamin A had a decreased susceptibility to minor skin infections in one very thorough investigation of Mackay's, but she found in a second investigation that extra vitamin A had no effect either on the skin, or the general health and immunity to infection, from which she infers that some dried milk may be adequate in vitamin A, but that supplementing it is wise [327]. Thirty cases of senile vaginitis had their symptoms and the changes in the mucosa improved by Simpson and Mason [345] who gave cod-liver oil by mouth. Skin diseases and vitamin A are discussed on p. 65.

Fœtal Development and Rubella. This subject may be of great importance because of the possibility that human congenital abnormalities are due in some cases to the fœtus receiving insufficient vitamins at critical periods of its development. If this is so it must be remembered that different abnormalities will arise according to the stage of development reached when the

deficiency occurs. Further, such deficiencies must probably be severe to have an effect and also be of short duration if the foetus is not to be altogether destroyed. Such a profound but very brief deficiency could hardly ever be caused by simple deficiencies in the maternal diet, but it could be caused by illness suddenly but briefly altering the vitamin content of the maternal blood. For instance, even slight fever greatly reduces the level of vitamin A in the blood (p. 30), and so it is reasonable to assume that when mothers contract rubella in the first trimester of pregnancy the foetus is exposed during the short period the fever lasts to an acute lack of vitamin A. It is known that rubella during the first trimester may cause congenital cataract and congenital heart disease [306] and congenital deafness [307]; it is also known—and discussed later—that in sows and in rats a deficiency of vitamin A causes farrows and rats to be born with abnormalities of the eyes and also, at least in rats, with cardiac abnormalities. Therefore it is tempting to suggest that it is not the toxin of rubella but its indirect effect on the level of vitamin A in the blood which destroys the nice organization of foetal development. If this be true, then it is valueless to give vitamin A by mouth during rubella, since this will not raise the vitamin A in the blood (p. 30), but large doses of vitamin A *alcohol* (p. 24) should be injected daily in an aqueous suspension (p. 18).

Hale [308] found that farrows born of vitamin A deficient sows were blind, often with no eyeballs, cleft palates, hare lips, extra ear-like growths and misplaced kidneys. Lack of vitamin A also causes congenital blindness in calves [309], while in rats [310] the commonest defect is replacement of the vitreous humour by a fibrous retrolenticular membrane, other frequent abnormalities being colobomas, abnormal structure of the retina, defects of the cornea and other failures of normal development. In rats there is also a high incidence of diaphragmatic hernia, especially in breeds where this is common [311], but cystic fibrosis of the pancreas does not occur [311]. Misplaced kidneys have been reported in farrows [308], while in rats [312] there may be hypoplasia of the renal parenchyma, ectopic kidneys, ectopic ureteric openings, abnormal development of the genital ducts and less commonly horseshoe kidneys and hypospadias, etc. There is also keratinizing metaplasia of the epithelium of the lower uro-genital tract. The cardiovascular defects have been very fully described and illustrated by Wilson and Warkany [313], who state that "*they are of particular interest because they show remarkable similarity to many congenital cardiovascular conditions that occur in man.*" "It is emphasized that present clinical and experimental observations indicate that environmental as well as genetic factors may alter the development of the cardiovascular system." The chief findings in rats were defects in the interventricular and aortico-pulmonary septa, abnormal development of the arch of the aorta and of the arteries arising from it, and abnormalities of the *ductus arteriosus*. Infants have been born with keratomalacia [314] and it has been suggested [315] that *vernix caseosa* is caused by the hyperkeratosis of a deficiency of vitamin A, but this is probably incorrect as there is no relationship between the amount of *vernix caseosa* on newly born infants and the level of vitamin A in their blood [103].

Vision. Vitamin A is essential for scotopic vision, that is, for vision in dim light; it is also probably essential for photopic vision, that is, for vision in daylight, and for the appreciation of colour.

Scotopic Vision or Vision in Dim Light. Even with a mild deficiency of vitamin A the rapidity of dark adaptation in most people and its extent in all people is impaired [346], while a severe deficiency leads to complete night blindness or hemeralopia. The subject is important partly because night blindness is a grave drawback to countrymen or fishermen working in the dusk or by moonlight and to town dwellers during "black outs," partly because poor dark adaptation is a very early symptom of a deficiency which is widely used in nutritional research on vitamin A. For a full discussion on

the physiology of scotopic vision the excellent review by Lythgoe [347] should be consulted ; here only enough can be said to explain the commonly accepted rôle of vitamin A. (Clinical applications are discussed on pp. 60 and 72.)

In bright light vision is carried out by the cones of the retina, while in dim light the rods are used. When illumination is suddenly decreased so that there is only about as much light as is given by a three-quarters full moon, vision is impossible for a moment and then "dark adaptation" occurs, the eyes "growing accustomed to the dark." For about the first six minutes of this adaptation the increasing power of vision is due to an increasing sensitivity of the cones to a poor light, but after this further adaptation, which may not be complete for an hour or even much longer and may lead to an increase in sensitivity of 10,000 fold, is due to vision being carried out by the rods of the retina, these becoming sensitive to illuminations which never can stimulate the cones.

The rods, however, are not directly stimulated by light, but only indirectly through the chemical changes light causes in visual purple or rhodopsin, which is a conjugated protein found in the dark-adapted retina, the prosthetic group being derived from vitamin A [348] ; it is contained in the outer segment of the rod [349]. When light falls upon it, it is converted to vitamin A, through retinene or vitamin A aldehyde, by a complicated series of reactions which have been partially unravelled by Wald and Hubbard [349] : these are of particular interest because they may involve codehydrogenase I and therefore nicotinic acid (p. 339). The improbable possibility that riboflavine is also concerned in these reactions is discussed on p. 64. In the intact eye in the dark the vitamin A which has been formed through exposure to light is again conjugated with protein to form visual purple ; nothing is known about how this occurs except that it probably does not involve the formation of retinene [349].

Since visual purple is thus bleached or destroyed by light the eye contains little after being exposed to bright light, and so has to reform it before it can be utilized by the rods for vision in dim light. On the rapidity with which the visual purple is reformed must depend the rapidity with which dark adaptation, that is, full use of the rods, can occur. Since vitamin A is a necessary part of visual purple any shortage of vitamin A must slow down the formation of visual purple and so slow down dark adaptation. Since even the dim light in which rod vision is used destroys some visual purple, the amount in the retina will depend on the relative rates of destruction and formation. This is the reason for shutting one's eyes for a minute on coming from a brightly lit room to a dark street ; by shutting out all light the visual purple can accumulate more rapidly. In the same way badly fed slaves used to see better in the dawn after a night in the dark than at dusk after a day's work in the light. For centuries poorly fed fishermen have known that a day's exposure to glare from the water often causes sudden night blindness—in other words, prolonged bleaching destroys so much of the visual purple that the vitamin A deficient eye cannot reform it in sufficient amounts to give even poor night vision. On the other hand, dark adaptation cannot be improved beyond the normal, however much vitamin A is taken [350].

Two other factors, besides vitamin A, influence the formation of visual purple. Firstly there must be an adequate supply of oxygen to the retina, and secondly visual purple is regenerated more rapidly—as far as the supply of vitamin A permits—if the retina has been previously exposed for a long period to a bright light. The latter fact may be of importance in clinical work on dark adaptation, though it appears to be generally ignored.

There are many clinical reports that delayed dark adaptation or even severe night blindness can be cured in a few hours with vitamin A, while others state it may take weeks or months. It is possible that the latter were also having to cure some further defect from the vitamin A deficiency, such

as nervous degeneration in the retina, or in the rods themselves since visual purple is an integral part of their structure and so, by its absence, might cause structural damage [351]. There is also every reason to believe that dark adaptation is dependent on fine readjustments in the nervous system of the retina once sufficient visual purple has been formed. Thus while the amount of visual purple during adaptation may be only doubled sensitivity may increase 10,000 fold, which appears to be only explicable if a synaptic rearrangement of the nervous elements of the retina allows each rod and cone to become connected to additional nerve fibres, so allowing for a summation of subliminal stimuli—a theory which is further supported by the decrease in visual discrimination which occurs during dark adaptation.

Photopic Vision or Vision in Daylight and Appreciation of Colour. In sharp contrast to scotopic vision, vitamin A plays no clinically obvious part in photopic vision, since, however great is the deprivation, vision in bright light and the recognition of colours remains unaltered or altered so slightly that it has never been noticed except by Wosika [355], who found some impairment in the recognition of blue. On the other hand, there is no substance in the eye apart from vitamin A which could be used for photopic vision, and there is strong evidence that it is so used. But if this is correct then the cones must so tenaciously retain their vitamin A and preserve it in so self-contained a photosensitive system that none is ever wasted and no fresh vitamin is ever required. In other words, vitamin A must be an integral and immutable part of the structure of the cones, fixed there for ever.

Dartnell [352] among others believes that the visual purple of the rods is probably the mediator of sensations of luminosity not only in dim but also in bright light, the cones thus being left with the sole function of mediating colour sensations.

Ball and Morton [353] have investigated whether vitamin A or retinene can provide the colour receptor substances postulated by Granit's theory of modulators [354], and they have found that broadly speaking this is so, at least *in vitro*.

Vitamin A and the Nervous System. Deprivation of vitamin A causes in animals a primary degeneration of the nervous system and also a secondary degeneration, discussed later, due to pressure from the abnormal or inadequate growth of the skull and spinal column. In man the influence of vitamin A on the nervous system is less certain; it is discussed on p. 74.

Primary degeneration in young deficient rats occurs so constantly that Irving and Richards [72] and Coetzee [73], after very careful work, suggest it should be used as a method of assaying vitamin A, since the difference between the amount of vitamin A which does and does not protect the nervous system is very small. They found degeneration in the medulla was constantly present after seven weeks, and since, of course, this degeneration must have been present for some time before that and since it occurs in rats that show no other sign of deprivation, it must be among the earliest signs of a deficiency. That inanition alone causes no such degeneration has been shown by Aberle [335] and by Wolbach and Bessey [356].

Many other workers, whose investigations were summarized in the second edition of this book, have reported changes in the nervous system of deficient rats and rabbits, but since they did not examine the osseous system there is no knowing whether they were observing a primary or a secondary degeneration. In young rats [72, 73, 356] both forms of degeneration occur, while in dogs, as far as is known, the nervous degeneration is secondary to the osseous changes discussed later. In chicks Wolbach [362] reports injury from osseous pressure, but Adamstone [357] found no evidence of this but only pinpoint areas of primary degeneration in the brain stem, base of the cerebellum, optic chiasma and, rarely, in the cerebrum. In ducklings [358] the apparent overgrowth and so the compression of the lower parts of the spinal cord in the spinal canal is reminiscent of Wolbach and Bassey's rats (Figs. 13, 14), but

the picture is confused by hæmorrhages confined to the upper cervical cord and medulla—in spite of there being least overgrowth and compression here—and also by the formation of true bone in both the white and the grey matter. Later work by the same authors [358] suggests nervous degeneration is the essential lesion.

Internal hydrocæphalus and an increase in the pressure of the cerebrospinal fluid also occur in dogs [364] but its cause is not clear. Against it being due to pressure from the osseous thickening are the observations of Moore and Sykes [366] that in calves papilloedema and the raised pressure decrease rapidly when vitamin A is given. This decrease could not in the time have been caused by a return to normal of the cranial bones. It seems most probable that the pressure of the cerebrospinal fluid is affected by lack of vitamin A because the ependyma is in origin epithelial and thus dependent, like all other epithelia, on an adequate amount of vitamin A. The problem is further complicated by a report [367] that vitamin C also reduces the pressure of the cerebrospinal fluid of vitamin A deficient calves (p. 50).

It appears possible that the nervous degeneration is really due to some unidentified factor which is absent in some experimental diets and not others [361], or even more probably, in view of the protective action of vitamin A [72, 73], that the nervous degeneration only occurs when there is a double

deficiency of vitamin A and an unknown factor. For instance, the work of Wintrobe and others [359] on pigs, which in spite of a simplified diet supplemented with nearly every known vitamin still developed a widespread nervous degeneration, shows how complicated and delicate are the dietetic needs of the nervous system: even copper is important [360].

Vitamin A and Growth of Bone. Our knowledge of the control which vitamin A exerts over the growth and shaping of bones is almost wholly based on Mellanby's work with puppies in England and on Wolbach and Bessey's work with young rodents in the U.S.A., so it is unfortunate that these investigators do not agree with each other: the former, broadly speaking, finds that lack of vitamin A alters the pattern of bone growth while the latter



FIG. 13. Brain of young vitamin A deficient rat showing compression of the cerebellum into the foramen magnum and numerous herniations into the transverse sinuses.

find that growth is largely arrested. But both agree that the nervous system is compressed and damaged by the osseous. Mellanby [363, 364, 365] has shown that vitamin A controls the shape of growing bone and especially its fine moulding by influencing the position and the activity of osteoclasts and osteoblasts. A deficiency of the vitamin does not lead to anarchic activity, but rather to a slowing or stopping of osteoclastic activity and its replacement or reversal to a lesser or greater degree by osteoblastic activity. The result, especially in the cranial bones which are the most studied by Mellanby, is

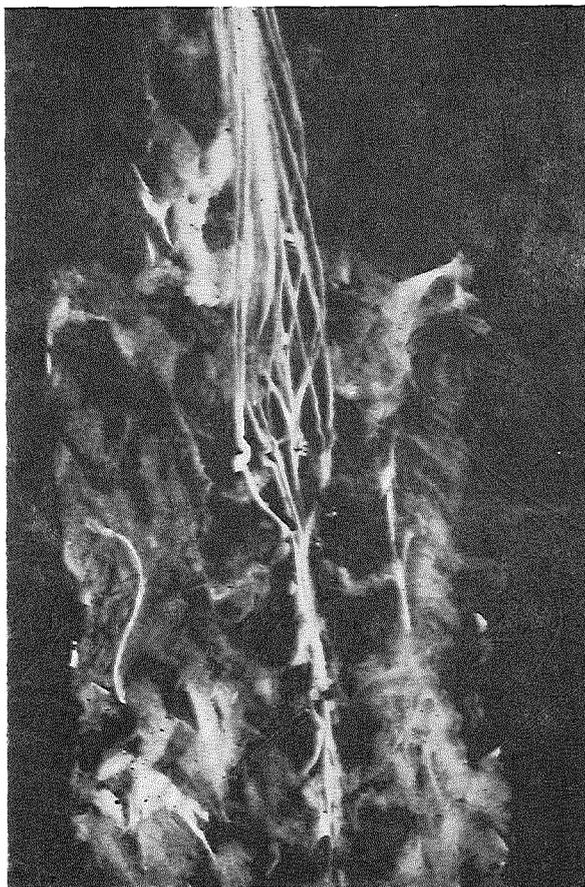


FIG. 14. Nervous system of young vitamin A deficient rat showing the disproportionate growth between the nerve roots and spinal canal, leading to herniations of the former. (The herniations have been dislodged so as to demonstrate them.)

Mellanby, but emphasizes that vitamin A chiefly acts on the osteoblasts which are stimulated by its lack and depressed by its excess.

Wolbach [362] on the other hand believes that vitamin A affects the growth of bone because it is essential for the growth, maturation and degeneration of epiphyseal cartilage cells. Lack of vitamin A arrests this normal evolution of cartilage as does inanition from any other cause, be it due to insufficiency of a perfect diet or to insufficiency of only one essential food. But with all forms of inanition except that due to vitamin A the arrest of endochondral bone formation takes place only as part of the uniform arrest of all bodily growth. With lack of vitamin A on the other hand growth of bone

that there is a general thickening and dysplasia with loss of the fine architecture of the bone. This has the disastrous result that the foramina through which pass the cranial nerves are narrowed and so the nerves are compressed. Among the cranial nerves those which suffer most are the olfactory, auditory and optic, while in the spine compression is worst in the cervical region; the hind brain also suffers. In deficient adult dogs this bone dysplasia with its subsequent nervous changes may not appear for two years. In rats [365] Mellanby has produced the same changes as in dogs, while in calves [366] lack of vitamin A causes stenosis of the optic foramina. When vitamin A is given to deficient puppies there is a return to the normal distribution of osteoclastic and osteoblastic activity which is often intense, apparently being aimed at the restoration of the correct shape of the bones [365]. Irving [638] largely agrees with

ceases *before* that of the rest of the body. This leads to compression of the still growing central nervous system, which is convincingly shown in Wolbach and Bessey's most interesting paper [356], from which we have been fortunate enough to be allowed to reproduce two photographs (Figs. 13, 14). The brain herniates into the venous sinuses and foramen magnum; the spinal nerves herniate into one or more of the intervertebral foramina which they pass before leaving the spinal canal; the spinal nerves are also kinked into large pits on the dorsal surfaces of the vertebral bodies, these pits being apparently due to the nerves eroding the bone as they are forced into the foramina of emissary veins. The spinal nerves which are thus herniated show the classical histological picture of degeneration and regeneration following a crush injury: this strongly suggests that this nervous degeneration is not a primary degeneration due to the lack of the vitamin.

A further effect of lack of vitamin A according to Wolbach [362] is that remodelling ceases though the deposition of bone by the periosteum and endosteum continues normally. It is suggested that remodelling is caused by an agent or "inductor" provided by dying epiphyseal cells acting on "competent" osseous tissue. Since lack of vitamin A stops the evolution of cartilage cells, there can be no "inductor" and so no reaction of "competent" osseous and so no remodelling: this is supported by the effects of excessive amounts of vitamin A, which cause a very rapid evolution of cartilage cells and so, on this theory, an excessive liberation of "inductor" with such great remodelling activity of "competent" tissue that fractures are common owing to the new bone which is laid down not being dense enough to take over the strain imposed by the removal of the old bone. The effect of hypervitaminosis A on bone is discussed further on p. 85.

Vitamin A and the Endocrine System. *The thyroid* among the endocrine glands has been most fully investigated, though it is still impossible to force all observations into the strait-jacket of one rigid theory. Drill [369] has given an excellent review of the literature up to 1943, since when further light has been thrown on the subject, chiefly owing to the use of thiouracil, to the realization that the conversion of carotene occurs in the gut wall and to the use of iodine isotopes. It now seems probable that (a) the thyroid affects carotene metabolism by increasing its absorption from the gut into the gut wall, (b) the thyroid plays no part in the conversion of carotene to vitamin A within the gut wall, (c) the thyroid does not to any great extent directly increase the body's need for vitamin A and (d) vitamin A decreases the effect of thyroxine in stimulating metabolism.

Cama and Goodwin [370] in 1949 showed from work on rats and rabbits that hypothyroidism caused by thiouracil increased the faecal excretion of carotene and did not cause the appearance in the blood of either carotene or vitamin A aldehyde: from this it seems clear that hypothyroidism hinders the conversion of carotene to vitamin A by hindering the absorption of carotene into the gut wall and not by preventing its conversion after absorption into either vitamin A or its possible immediate precursor vitamin A aldehyde (p. 13). Further, desiccated thyroid decreased the excretion of carotene, and when given together with thiouracil nullified the effect of the latter, which shows that the thyroid acts not by stimulating the conversion of carotene to vitamin A but by stimulating the absorption of carotene. Cama and Goodwin [370] have explained that as regards rats and rabbits there are no inexplicable discrepancies between their work and that of others which they review, while their own is clearly confirmed by Johnson and Baumann [371] who investigated the effect of thyroxine and thiouracil on carotene metabolism as judged by liver storage of vitamin A. The latter authors further report that the effect of thyroxine could not be duplicated by dinitrophenol, so that it is not simply the raised basal metabolic rate caused by thyroxine which is the reason for the increased absorption.

Clinically the above work is supported by night blindness in myxœdema

[372], by the low level of vitamin A (which is only slightly raised by giving carotene) in the blood of cretins [373] and also by their susceptibility to respiratory infections. That hyperthyroidism increases the absorption of carotene above the normal appears to be borne out by Moore [184] finding in the livers of patients dying from thyrotoxicosis larger stores of vitamin A than were present in any other human livers.

Some experimental work, however, is not congruous with any of the above clinical and laboratory work. Thus Fasold and Heidemann [375], confirming earlier work, found that the milk of thyroidectomized goats was yellow with carotene but contained no vitamin A, in contrast to that of normal goats which contains no carotene but is rich in vitamin A. Since normal goats do not absorb any carotene into the blood, the effect of lack of thyroxine in these animals must be either to prevent the conversion of carotene to vitamin A or to so damage the lower bowel wall that carotene seeps through it in an area where no conversion of carotene takes place. It has also been stated that thyroidectomized guinea pigs [376] store carotene but not vitamin A in their livers.

Destruction of vitamin A in the body is not increased by thyroxine to any great extent, since besides Moore's findings in man mentioned above, he has shown that in rats given lethal amounts of vitamin A the addition of thyroxine did not have a protective effect by destroying the vitamin, which some workers have reported, but instead hastened the animals' death [377]; while Logaras and Drummond [378] found that the increased metabolism caused by thyroxine and dinitrophenol increased the storage of vitamin A in the liver. However, Heimer and others [386], judging by the rate of depletion of hepatic stores, believe that thyroxine predominantly affects vitamin A indirectly through growth: if either hypo- or hyperthyroidism checks growth then stores of the vitamin are spared. They also have reported that to a very slight extent—masked by the indirect affect on growth—thyroxine itself hastens depletion. This latter action is better demonstrated in chicks [382] where thyroxine and thiouracil respectively increase and decrease the amount of vitamin A necessary for growth.

The damping effect which vitamin A has on the activity of the thyroid was first suggested by McCarrison [379], who found that cod-liver oil delayed the metamorphosis of tadpoles; this has since been confirmed with purer vitamin A preparations. Experiments on animals by Logaras and Drummond [378] and many others [374, 383] have conclusively shown that vitamin A reduces the increased metabolism caused by thyroxine, while Belasco and Murlin [383] have reported that the metabolism of thyroid tissue from animals taking large amounts of vitamin A is decreased compared to that of controls, this decrease being even greater if the animals have been given thyroxine as well. In fact vitamin A and thyroxine far from being antagonistic actually reinforce each other in their action on the thyroid. In severely deficient animals [384] the thyroid is said to be relatively heavier than in normal animals, but it takes up the same amount of radioactive iodine, though the rate of thyroxine synthesis is decreased.

Clinical work on the use of vitamin A in the treatment of goitre is not satisfactory. The Mellanbys nearly thirty years ago noted a clinical improvement in patients with exophthalmic goitre treated with cod-liver oil, which they believed to be due to the iodine in the oil: some fifteen years later German workers claimed that vitamin A itself was of value in simple adolescent goitre [380] and in toxic goitres [381], but the vitamin A preparation "Vogan" which they used contained enough iodine to explain their results. However, the erroneous claim that vitamin A is of value in the treatment of hyperthyroidism still crops up with depressing regularity. For instance, Simkins [385] in 1947 published an account of two cases who when treated with 200,000 to 400,000 I.U. daily apparently responded in a dramatic manner, but both these cases were the type which often recovers spontan-

ously, while Simkins' very extensive review of the literature can but convince any critical reader that vitamin A is valueless in the treatment of thyrotoxicosis.

The level of vitamin A in the blood is no guide to the level of thyroid activity [370].

Fertility is not very dependent on vitamin A. The structural changes which occur in the testis of the rat and bull as a result of a severe deficiency have been described on p. 36, so that here it is only necessary to emphasize that fertile sperm are still produced in small numbers amidst the ruins of the germinal epithelium and that libido persists when bulls, for instance, are too weak to stand [301]. Œstrus in the rat is not stopped by lack of vitamin A, but becomes delayed and irregular [70], while Canon [387] states that in severe deficiencies rats refuse to mate and that with decreasing degrees of deficiency there is mating but no conception; conception, but resorption of the fœtuses; death of the fœtuses; prolonged gestation. The death and resorption of the fœtuses is probably due to the fœtuses themselves being so damaged by lack of the vitamin that they could not continue to develop (p. 39). In cows [388] œstrus remains normal and conception occurs even when deficiency symptoms have developed, though calves are often born dead. The report that excessive amounts of carotene by mouth [389] stop œstrus and a desire to mate in rats appears to receive support from the observation that vitamin A applied directly to the vaginal mucosa prevents œstrogenic cornification [390].

The pituitary has inevitably been held to be the primary gland affected by vitamin A, all other endocrine changes being secondary to this. For instance, it is stated that the amount of thyrotropic hormone in the anterior pituitary is low in rats on a high vitamin A diet, and high in rats on a deficient diet [391]. It has also been found that the factor in the anterior pituitary which stimulates the growth of the female genital system is increased in vitamin A deficient male rats, but Mason [300] has pointed out that this is a purely secondary effect due to the virtual castration of the male rats by the degeneration of the vitamin A deficient testes (p. 36), since deficient female rats showed no such changes in the pituitary's secretion.

The principle of the pituitary which stimulates lactation does not appear to be affected by vitamin A, since Williams and others [392] found that the amount of milk secreted by nursing mothers was not altered by varying their intake of vitamin A. Kepinov [393] in some interesting experiments on starved frogs found that adrenaline did not accelerate the hydrolysis of liver glycogen to glucose unless vitamin A was previously given. Vitamin A apparently stimulates the glycogenic hormone of the pituitary, since it has no effect on frogs after the removal of the latter. That the function of the adrenals themselves may possibly be directly affected is suggested by Moore [123] and Popper [58] observing that they sometimes store large amounts of vitamin A. Wegelin [394] found that vitamin A checked the loss of glycogen from the liver which is caused by thyroxine, but here it seems most probable that the vitamin was directly decreasing the action of the thyroxine (p. 46) and not acting indirectly through the pituitary. The changes brought about in the pancreas and thymus of children by a deficiency of vitamin A appear to be a direct effect on the glands themselves (p. 78), and in rats thymectomy does not alter vitamin A metabolism [395]. The cystic degeneration which occurs in the pituitary of vitamin A deficient cattle [396] is probably a secondary result of compression by the osseous hypertrophy which follows lack of vitamin A (p. 43).

Vitamin A and Renal Function. The relationship of vitamin A to the kidney is not clear, but there appear to be four possibilities which are worth consideration: (a) The kidney merely acts as a storehouse for vitamin A. (b) The kidney destroys or excretes excess of vitamin A. (c) Vitamin A is necessary for the functioning of the kidney and, secondarily, reduces hyper-

tension. (*d*) Impaired renal function allows vitamin A to leak away in the urine and also causes a toxic condition of the body which hinders it in utilizing vitamin A.

(*a*) The kidney is avid for vitamin A, so that at very low levels of intake there is a higher concentration, though not greater total stores, in the kidney of the rat than in the liver [397]. In the male this concentration is considerably higher than in the female, due either to a true sex difference [398] or to the more rapid growth of the male, since growth appears to increase renal stores [399]. At very high levels of intake the renal stores may exceed those found in the liver of animals on normal diets [123].

But the structure of the kidney, the avidity of the kidney for vitamin A,

the effect of growth in increasing renal stores and the effect of vitamin A—discussed below—on renal function, all these point to the kidney actively using vitamin A and not merely storing it passively.

(*b*) However great the excess of vitamin A in the body no vitamin A ever appears in the urine unless the kidney is diseased (p. 30). Of course it is possible that the kidneys excrete the breakdown products of vitamin A (p. 30), but there is little proof that the kidneys destroy vitamin A themselves, as a first step in its elimination, except the observations of Belasco and Murlin [383], who found that renal tissue from rats on a high vitamin A intake had a slightly raised metabolism compared to that from control animals, from which they suggest an increased effort to destroy the surplus vitamin A. The



FIG. 15. Pelvis of kidney of an American infant filled with keratinized epithelium.

high blood level of vitamin A often found in nephritis [98] and nephrosis [226] does not appear to be due directly to the lesion in the kidney but to abnormal hepatic storage (p. 27).

(*c*) Our knowledge of the rather surprising effect vitamin A has on the excretory power of the kidney is chiefly due to Herrin [400], who found that in rats on a vitamin A deficient diet the urea clearance fell by twenty-three to twenty-seven per cent., this being a purely functional effect, since not only was the urine normal, but histologically no structural changes were found in the kidneys. Further work on dogs, extended to include inulin clearance, confirmed the work on rats and also showed that excess of vitamin A raised the urea clearance above normal, though this could not be maintained indefinitely [401]. It was thought the effect was due to increased glomerular

filtration, which has been confirmed by Bing [403]. Experiments on man were even more interesting [402]. When thirteen subjects were given 50,000 to 75,000 I.U. of vitamin A daily two showed no response, four had an increase in their urea clearance of from eleven to fifteen per cent., and seven had an increase of twenty-four to ninety-one per cent. The last group belonged to the type whose body weight fluctuates widely and rapidly. No subject showed any significant change in blood pressure or oxygen consumption. As in dogs the increased clearance could not be maintained, though in some cases it did not return to normal for one hundred and twenty-eight days. We have occasionally found vitamin A a powerful though transitory diuretic in daily doses of 72,000 I.U. by mouth in patients whose weight fluctuates rapidly.

Hypertension is not benefited by vitamin A, though fish oils may have a favourable effect irrespective of their content of the vitamin. Early workers thought that it was the vitamin A in fish oils which sometimes relieved essential hypertension in man [404] and experimental hypertension in animals [405], but later work on rats [406] has shown that the hypotensive action of oils is enhanced by oxidation though this destroys their vitamin, and in dogs [407] the effect of vitamin A concentrates is not related to the amount of vitamin A which they contain. Gounelle and Bonfils [408], moreover, report that one man and three women—whose hypertension could be temporarily improved by injections of fish oil—did not respond after two weeks to daily injections of 200,000 I.U. of crystalline vitamin A acetate dissolved in an inert oil.

(d) There are a large number of observations on man which show that chronic renal disease is associated with low stores of vitamin A in the liver (p. 22) as well as the reports of Long and Pyrah [297] and others [298] that renal calculi are frequently though not always [299] associated with poor dark adaptation, the most chronic cases tending to have the worst adaptation [297]. It is not clear from these papers how much the renal function was damaged, but the most probable explanation of the observations appears to be that the damaged kidney drains away vitamin A (p. 30), or that the excretory power of the kidney being impaired, the resulting early "uræmia" may interfere with the formation of vitamin A from carotene (p. 15), or may interfere with the formation of visual purple in the eye or may cause a toxic amblyopia. In favour of this last view is the failure to cure the night blindness of nephritics with vitamin A [297, 298] and the high level of the vitamin often found in the blood of nephritics [98] and nephrotics [226].

Relation of Vitamin A to Other Vitamins. The balance of evidence, though often scanty, is against vitamin A being directly concerned with the metabolism of any of the other vitamins: some indirect associations, however, have been proved, such as vitamin E protecting vitamin A against oxidation by vitamin F or the essential unsaturated fatty acids (pp. 15 and 23), and vitamin K [415] and possibly vitamin D [416] giving protection against the hypoprothrombinæmia of hypervitaminosis A. On the other hand vitamin A in large doses protects ducks [417] against dicoumarol poisoning. Less certain is the calcifying effect of large doses of vitamin A on the rachitic osteoid tissues caused by lack of vitamin D (p. 85) and the amelioration of hypervitaminosis D by vitamin A (p. 534).

Even more uncertain is the relation of vitamin A to the B complex: for instance it is reported that yeast decreases the hepatic stores of vitamin A in pigs [418], and that in rats [419] the epithelial changes caused by lack of vitamin A are increased by removing a deficiency of aneurine, and the loss of weight caused by lack of aneurine is increased if small amounts of vitamin A are given.

Ascorbic acid or vitamin C, however, is the vitamin whose relationship to vitamin A has caused most research and most uncertainty. Thus it has been claimed that vitamin A deficient rats live longer and gain more weight

if given ascorbic acid [414] but this could not be confirmed by Sharman [420], while Mapson and Walker [421] have proved false claims [367, 422] that the rat needs vitamin A for the synthesis of ascorbic acid. The same appears to be true for the chick [423]. Reports that lack of vitamin A causes low levels of ascorbic acid in the aqueous humour of rabbits [424], and in the blood and cerebrospinal fluid of calves [367] could be better explained by the general impairment of metabolism in sick animals than by postulating a direct relationship between the two vitamins, and the same can be said about the reputed effect of scurvy in reducing hepatic stores of vitamin A in the guinea pig [425].

Vitamin A, Fat and Protein Metabolism. Josephs [151] considers that vitamin A has an important and specific effect on the metabolism of lipids, basing this opinion not only on his own very careful work with rats but also on the investigations of others, which he reviews. Both in man and animals giving large or toxic (p. 81) doses of vitamin A causes a transitory rise in the level of serum lipids, including cholesterol, this rise being greater and persisting longer in animals previously depleted of vitamin A. Conversely, lack of vitamin A has a specific effect in reducing lipid levels. An interesting exception to this effect of vitamin A is found in the dog whose serum lipids remain normal even after poisoning with enormous doses of the vitamin (p. 81). In rats it has been shown that vitamin A has a specific effect in increasing the amount of fat in the body [410, 411].

In illness, as Stannus [409] has pointed out, there is often an increase in the levels of carotene in the blood when there is a lipæmia such as occurs in myxœdema, diabetes or lipid nephrosis and in the latter condition vitamin A also tends to be very high (p. 29).

Protein metabolism is also intimately connected with vitamin A, since paired feeding experiments with young rats [410, 411] show that the vitamin has a specific effect in causing protein retention and appears to be used up during protein metabolism, since replacing most of the fat or carbohydrate by protein in the diets of rats deficient in vitamin A retards growth and hastens death [414]. Meunier and his colleagues [412], after careful work, state that 20 micrograms of vitamin A are as effective as 150 mg. of glycine in protecting rats on a low protein diet against the injurious effects on growth of sodium benzoate. Since the latter is injurious because it causes a loss of glycine from the body owing to its excretion as hippuric acid or benzoyl glycine, it seems that Meunier's claim is correct that vitamin A causes the synthesis of glycine. This is confirmed by similar work carried out with bromobenzene [413].

THE PROVISION OF VITAMIN A IN HUMAN DIETS

Vitamin A and carotene are very far from being one and the same, but propaganda over food during war and the use in advertisements of such phrases as "provitamin-A" and "the amount of vitamin A (as carotene) in this preparation," have spread the erroneous belief that vitamin A and carotene are of equal value in the diet. Actually, unit for unit, more carotene than vitamin A is necessary if all requirements are to be satisfied, and also some stores of the vitamin are to be built up, since the body only utilizes carotene as efficiently as it does vitamin A when there is already a severe deficiency of both (pp. 12, 16). For banking vitamin A against any future shortage carotene is wastefully used compared to vitamin A. Also, if pp. 11 and 16 are read, it will be seen that while vitamin A is nearly always well absorbed, only five per cent. of the carotene in some vegetables may be absorbed by healthy men and, further, other factors, which do not greatly influence the absorption of vitamin A, may adversely influence that of carotene, such as poor general health of the body and of the bowel, the absence of fat and bile, and the taking of liquid paraffin either alone or in an emulsion. So it must be emphasized that if the tables on p. 54 are consulted about

the carotene content of a food, the values given bear little relation to the amount of carotene which will be absorbed. All this means that where possible in health, and certainly in disease, some at least of the vitamin A requirements of the body should be supplied by vitamin A rather than carotene (p. 58). This is especially important for children (p. 12).

Vitamin A itself is found only in a very few foods, of which the commonest are butter, eggs, milk, honeycomb, liver, some fish and fish liver oils. Of these eggs, milk and butter are by far the most widely eaten, and for that reason it is important to realize that their vitamin content is not constant but depends entirely on the diet of the hens and cows. Broadly speaking, the more that eggs and milk are produced by "commercial methods" the less their value. This especially applies to eggs, which when produced on egg farms have only to conform to the naked eye appearance of eggs, since the fact that they are often too deficient in vitamins for any chick to hatch out of them does not here make the farmer feed the hens properly. The old belief that a dark yolk meant a good egg is undoubtedly correct, and explains why people still prefer the "farmyard" egg of their holidays to the pallid yolked egg of their town grocer. Sjollem and Douarh [426] have summed the matter up by saying "where poultry have access to pasture they eat enough grass to bring up the vitamin A content of the yolks to a maximum. This undoubtedly is often not the case with poultry kept to produce eggs for consumption." Of course the colour of the yolk is due to valueless carotenoids (p. 11), but these are a good indication of the content of vitamin A itself, except in the improbable event of the fowls having had very large amounts of cod-liver oil [427, 428], when pallid yolks may be rich in vitamin A.

Cow's milk and butter are excellent sources of vitamin A, their content again being dependent on the diet of the cows. Watson and others [436] found that the vitamin A in milk could be doubled and the carotene trebled by altering the diet, though neither could be increased beyond a certain level which varied with the breed of cow. Oldfield [437] states that "one pint of milk from a pasture-fed cow may be equal in protective value to two pints from a stall-fed cow," and it has been suggested that in England, as in Finland, legislation should be passed to ensure that the diet of cows contains enough carotene to maintain the vitamin A content of their milk at a reasonable level [431]. The colour of milk and butter is not a good guide to the amount of vitamin A present, since vitamin A and carotene do not run parallel to each other in milk, but vary in their proportions with the breed of cow; Jerseys, for instance, have a yellow milk which contains nearly twice the carotene of that from Shorthorns, but only half the vitamin A [433]. Pasteurized milk, dried milk, etc. [432] have the same vitamin A and carotene content as fresh milk, even after storage for several months, but sweetened condensed skimmed milk has none. Goat's milk contains only vitamin A (p. 46) and so is quite colourless, which may in part explain the unreasonable bias against a valuable and sweet smelling food. Vitamin A in milk and in colostrum is in the form of the ester [451].

Human milk has never been investigated with the care which has been lavished on many less important subjects, probably because of the difficulties [452] implicit in its investigation. Kon and Mawson [452] in 1950 reviewed most of the sparse literature while reporting their own studies, based on 2,284 samples of milk. They found that the mean vitamin A content of milk in England is 153 I.U. per 100 ml., the small amount of beta-carotene in the milk carotenoids [452] only contributing the equivalent of about another 3 I.U. In America values of about 180 I.U. seem more common [453, 454]. The season of the year has no definite effect, but the stage of lactation is extremely important, there being a rapid rise [454] during the first three days from an average of 426 to 594 I.U. per 100 ml., figures of over 1,000 I.U. being recorded in America. After this there is a rapid fall during the next six to ten days and then a more gradual decline until the eighteenth week,

when values again rise [452]. Mean English values [452] at three weeks are 230 I.U. per 100 ml. and at eighteen weeks 143 I.U. Milk rich in fat has a high content of vitamin A and *vice versa*, while the level of vitamin A tends to rise with the age of the mother, but is not affected by the number of her previous children. There is considerable variation in the milk of different women, some secreting three to four times more than others [452, 453].

The factors which govern the level of vitamin A have not been fully investigated: it seems probable that a high fat diet causes an increase due to the increased fat of the milk [455], and vitamin A given during lactation causes an increase, but daily doses of 4,000 I.U. during the latter part of pregnancy have no effect [452], due presumably to the liver storing the vitamin, so that the blood level remains constant (p. 27). Given immediately before parturition 240,000 I.U. in oil cause an appreciable increase in the milk content which is still evident after nine days, while 24,000 I.U. daily for the first nine days of lactation causes an increase of about seventy per cent. Leshner and her co-workers [453] report doubling or even quadrupling the vitamin A content with 50,000 I.U. in oil daily, an effect also achieved by a good helping of liver [452]. Other American workers giving a single dose of roughly 140,000 I.U. in an "aqueous dispersion" (p. 18) report rises in the milk of about 855 I.U. after twelve hours, normal levels not being regained for twenty-four hours or more. The latter workers also found that the rise in the milk was almost wholly dependent on the level of the vitamin in the blood, vitamin A given in oil having the same effect as in an "aqueous dispersion" if given in large enough doses to obtain the same blood level. Kon and Mawson [452] on the other hand found from their studies on women not taking extra vitamin A, that the breast actively secretes vitamin A so that the levels in the blood and milk are not related to each other, the greatest difference occurring just after delivery when blood levels are low (p. 28) and milk levels very high. Probably the only way of enhancing the value of vitamin A in milk throughout lactation—apart from keeping the blood flooded with large quantities of the vitamin—is to increase the consumption of fat, since Salmi [457] in Helsingfors found the impoverished war diet caused a fat impoverished milk, while Deem [455] found that extra fat in the diet caused extra fat in the milk which then increases the level of vitamin A [452]. Other factors affecting milk have not been investigated except cursorily [452, 455], though the effect of maternal disquietude on milk yields has been long recognized [458]. Consuming carotene does not enhance the vitamin A value of the milk [452, 459].

Margarine in England is under Government control and consists of four main varieties [460]. "Special" margarine, "Standard" margarine and Kosher margarine are the only kinds which are allowed to be sold to the public by shops and are provided in restaurants: the first two of these by law must contain 450 to 550 I.U. of vitamin A per ounce. The vitamin may all be added as fish liver oil or as fish liver oil and carotene. Kosher margarine—suitable for Jews and vegetarians who refuse to eat fish oils—contains 200 to 300 I.U. per ounce in the form of carotene. This almost negligible amount is due to the impossibility of adding more without making the margarine too red to mimic butter. The margarine permitted to bakeries, cake shops, etc., contains no vitamins. Good butter may, of course, contain about twice the vitamin A of margarine [461].

Butcher's dripping bought in the poorer parts of English towns in July, 1940, when dripping was still both cheap and plentiful, had no vitamin A [440] though it did contain as much vitamin D as summer butter and, of course, had a higher energy value than margarine.

Lard, although containing no vitamin A, contains some substance which has the same biological action as vitamin A. This, however startling, appears certain from the very careful work of Kaunitz and Slanetz [462] on rats in America: lard was as effective as vitamin A not only for growth but also

for the cure of deficiency symptoms. Confirmation comes from France, where Le Gallie [463] has published a series of papers on the results of giving both mice and rats diets devoid of vitamin A but rich in lard.

Fish may contain considerable amounts of vitamin A in their body fat, so that fat fish like eels, halibut, herrings, lampreys, salmon and sardines are valuable, but Pyke and Wright [444] found no vitamin A in twelve brands of tinned salmon and in one "chilled" salmon, so that salmon as usually eaten in England is worthless from this point of view. In England also, Bacharach and others [429] showed in 1942 that the average individual daily intake of vitamin A from herrings either fresh, tinned, bloatered or kippered, was only 6 I.U.—a lamentable fact, as these fish are often cheap and plentiful: the herring fleet could provide three herring a fortnight for everyone [430]. Two fresh herring in the summer months, when they are richest in vitamins (p. 56), would provide roughly 500–1,700 I.U. of vitamin A as well as having considerable antirachitic value (p. 537).

Fish liver oils may be amazingly rich in vitamin A, forming the only natural concentrated medicinal preparations in use. Lovern [430] in 1941, stated that the total amount of vitamin A in fish and fish liver oils which was provided by the English fishing fleet was 1,862 I.U. daily per person in Britain and this could be greatly increased.

Concentrated preparations of vitamin A are available as ordinary cod liver oil, as concentrated fish liver oils, as "aqueous dispersions" (p. 18) of fish liver oils and as synthetic vitamin A. Of all these, ordinary unconcentrated cod liver oil is by far the best: the diets of children and adolescents and even of adults could be greatly improved by their taking daily two teaspoons, which would roughly provide most of the daily requirements of vitamin A as well as most valuable fat (p. 675) and vitamin D (p. 540). Both the English [430] and South African [435] fishing fleets could bring back enough fish liver oils to make an increased consumption possible. The concentrated oils have the great advantage that they can be taken in tasteless capsules, but have the two great disadvantages that the amount of their fat is negligible and that they are so concentrated they can easily cause vitamin A poisoning (p. 81).

Carotene and the provitamin A carotenoids are very widely distributed throughout vegetables and fruits. As a rough rule it may be said that carotene is always present in association with chlorophyll (the green colour of which masks the red of the carotene) and in yellow vegetables and fruits. Thin green leaves, like those of cabbage, spinach and lettuce, are especially rich in carotene, while the bleached stalks of celery and the white hearts of cauliflowers contain little or none; white flour and milled rice are again an example of the loss of valuable carotene with the loss of colour. The carotene content of tomatoes was not found to be altered when the plants were grown in eighty-seven different nutrient solutions, but growing or ripening tomatoes indoors reduces their carotene by over one-quarter [446]. Factors influencing the carotene content of plants, including the importance of boron, have been reviewed by Maynard [447]. The excellent paper by Graves [91] and p. 11 should be consulted for a discussion on the different biological values of carotene from different vegetables: carotene in red or yellow vegetables, like carrots, is very poorly utilized in comparison with that of green leafy vegetables, the latter being thrice as valuable—due possibly to their high content of vitamin E (p. 15).

In some tropical and sub-tropical countries like the Philippines, the Dutch East Indies, Ceylon, India, China, the West Indies and parts of East Africa the problem of child blindness due to lack of vitamin A is so widespread and so serious that Fitzgerald Moore [441] in a practical discussion of the whole problem concludes that the only hope of a solution is reinforcing with vitamin A concentrates the local vegetable oils and fats which are eaten by the inhabitants. Of these arachis oil is the commonest and forms an excel-

lent vehicle for vitamin A. It must be stressed that it is no use introducing alien forms of vegetable fats rich in carotene, like red palm oil, to take the place of the local varieties, because both expense and custom will prevent their use.

Effects of Cookery, Storage, Canning, Freezing, Drying and Dehydration on Carotene and Vitamin A. Domestic cookery causes no appreciable loss of either vitamin A or carotene, since neither are soluble in water nor easily destroyed by heat. The prolonged boiling of milk—though not rapid boiling or pasteurization—and the slow cooking of vegetables in stews is harmful, but there is little loss of vitamin A from butter during cooking and frying [442]. Most fats, however, which are used for frying—especially when they are reheated many times as in “deep fat frying”—develop an “anti-vitamin A” factor when heated which destroys part of the vitamin A activity of foods eaten at the same time [65, 66]. Probably such destruction is not of any practical importance except where foods such as commercial fish and chips are eaten in large amounts by the poor, who are always on the edge of a deficiency of vitamin A. The canning of fish probably destroys some vitamin A [429]. The cold storage of vegetables destroys small amounts of carotene [434], but canning, the ordinary methods of storing apples, oranges and tomatoes, and the domestic ways of preserving fresh vegetables and fruits, do not affect carotene, nor does drying peas and beans, though the slow sun-drying of fruit may be injurious. Dehydration of vegetables followed by reconstitution and cooking may cause a loss of from nil to seventy per cent. of carotene [445]; losses of course will vary greatly with the various processes, such as blanching, to which the vegetables may be submitted [433]. The commercial drying and evaporation of milk and its subsequent storage for a year was found not to reduce its vitamin A or carotene [443]. The “band drying” of eggs causes considerable loss of vitamins A and D, but “spray drying”—the method usually employed—has no injurious effect [448]. Human food is seldom sufficiently rancid to cause any serious loss of vitamin A [64].

AMOUNTS OF VITAMIN A AND CAROTENE IN FOODS

The following figures come from various papers and from the unique tables of Fixsen and Roscoe [449], which should be consulted if fuller figures about fish liver oils and vegetables are required. The figures for the vitamin A content of the flesh of fish are only approximate, since research has been chiefly directed to the amount of vitamin A in the body oils of fish. The amount of vitamin A in the edible part of fish has been calculated from the latter's fat content [450] on the assumption that this fat is the body oil referred to by research workers.

FOOD.	International Units of vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams or roughly 3½ ounces.		
	VITAMIN A.	CAROTENE.	TOTAL ACTIVITY.
ANIMAL PRODUCTS			
<i>Bacon</i>	0		
<i>Beef</i>			
<i>Kidney</i>	1,100		
<i>Steak</i>	60		
<i>Suet</i>	600		
<i>Bone Marrow</i>	800
<i>Butcher's Dripping</i>	0	..	0
<i>Chicken</i>	0		
<i>Heart</i>	0-200		
<i>Lamb</i>	0		
<i>Lard</i>	See p. 52		

FOOD.	International Units of Vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams of roughly 3½ ounces.		
	VITAMIN A.	CAROTENE.	TOTAL ACTIVITY.
<i>Liver</i>			
Calf	52,600-159,800
Pig	12,600- 36,700
Ox	12,700- 41,800
Polar-Bear	1,300,000-		
	1,800,000		
Seal (Arctic)	60,000-1,300,000		
Seal (Arctic) Oil	3,000,000-		
	60,000,000		
Whale (Antarctic) Oil	500,000-		
	15,000,000		
Pork	0		
DAIRY PRODUCTS			
<i>Butter.</i> American	1,203-1,974	164-419	3,500
Danish. Nov.-Jan.	3,380-1,270
Feb.-Apr.	1,080-1,690
May-July	1,840-3,610
Sept.-Oct.	4,180-5,670
English	800-2,000
New Zealand	2,400
Scottish. Nov.-Jan.	2,410-1,140
Feb.-Apr.	1,220-1,530
May-July	2,560-2,950
Aug.-Oct.	2,990-2,200
Stall-fed cows	800-2,900
Goat	900		
<i>Cheese.</i> Camembert	3,610
English Cheddar	5,500
Cottage	195- 729
Cream	2,110-2,320
Danish	2,400-3,500
Kraft. American	2,160
English	3,070
Swiss	1,970
Parmesan	900
Roquefort	2,500-4,000
<i>Eggs.</i> Duck. Whole	1,800		
Yolk	2,900		
Hen. Whole	420-1,100		
White	0		
Yolk	130-8,800		
Spray Dried	As Fresh		
<i>Margarine.</i> Vitaminized	192	..	See p. 52
<i>Milk.</i> Holstein. Summer	151	55	
Winter	101	43	
Guernsey. Summer	85	170	
Winter	51	78	
Irradiated	Unchanged	Unchanged	
Pasteurized	Unchanged	Unchanged	
Condensed. Full cream			
sweetened	144-442
Unsweetened	128-355
Dried. Skimmed	0
Whole	384-1,090
Colostrum	900-2,756
Ewe	20		
Goat	133-224	1	
Sow	90		
Colostrum	247		
Woman	See p.		
FISH			
Bloaters	28		
Carp	1,020		
Cod. Flesh	0		
Liver	60,000		
Roe	0		

FOOD.	International Units of vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams or roughly 3½ ounces.		
	VITAMIN A.	CAROTENE.	TOTAL ACTIVITY.
FISH—continued.			
Eel	660-18,500		
Hake, liver	2,300-92,000		
Herring			
Canned, average	28		
" with Tomato	98-210		
"	53-105		
Fresh, English			
Mar.	88		
Aug.	753		
Sept.	193-238		
Dec.	35-98		
Kippers	28		
Mullet	430-1,075		
Oysters	140-420		
Salmon, Fresh	4-120		
" Canned	0		
" Chilled	0		
Roe	0-2,000		
Sardines	4,850-54,000		
<i>Fish liver oils</i>			
Cod (B.P.)	60,000		
Cod (Ministry of Food Cod Liver Oil Compound)	100,000		
Cod (retail)	40,000-400,000		
Halibut	2,000,000-		
.	36,000,000		
Tunny	512,000-8,000,000		
VEGETABLE PRODUCTS			
<i>Cereals</i>			
Maize	10-900	
Rice. Brown	34	
" Milled	0	
Wheat whole flour	102-456	
Wheat whole flour bleached	50-76% loss	
Wheat 85% extraction	50-300	
" 70% "	0	
Semolina	291	
Spaghetti	52	
Vermicelli	0	
<i>Fruits</i>			
Apple	50-90	
Apricot. Fresh	1,800-2,300	3,000
Sundried	5,100	
Banana	17	
Black currant	300-500
Blackberry	100
Cherry	15-800	
Date (preserved)	600	
Fig	80
Gooseberry	380	
Grape	15	
Guava	200
Orange juice	300-400	
" flesh	0-22	
Pear, English	80	
Palm fruit flesh	3,260	
Peach. White	5-100	
Yellow	880-2,000	
Dried	3,000	
Tinned	450	
Peppers (<i>Capsicum annuum</i>) Green	110-1,080	
Red	3,390-37,700	
Pineapple. Fruit	60-160	
Tinned juice	50

FOOD.	International Units of vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams or roughly 3½ ounces.		
	VITAMIN A.	CAROTENE.	TOTAL ACTIVITY.
<i>Fruits—continued</i>			
Plum	0-230	
Prune, dried	1,600-2,500	
Raspberry	130	
Red currant	120	
Tangerine	690	
<i>Nuts and Oily Seeds</i>			
Brazil Nuts	10	
Chestnuts	80	
Ground Nuts	63	
Hazel Nuts	100	
Olive Oil	0	
Red palm oil		
African	110,000-306,000	
Burmese	44,000-56,900	
Malayan. Unripe	24,000-60,000	60,000
Ripe	66,000	190,000
Over-ripe	62,000	160,000
Walnuts	50	
<i>Vegetables</i>			
Artichokes. Globe	200-400	
Beans. French	221-400	
Runner	600
Soya	450-970
Soya flour, Low fat	70	
Full fat	140	
Beetroot	0	
Broccoli leaves	12,000
Brussels sprouts	12,000
Cabbage	900
Carrot	2,000-9,600	
"	1,900
Cauliflower heads	38	
Cucumber	0	
Kale	7,500-20,000	
Leeks	1,000	
Lentils	53-450	
Lettuce	1,500-2,400	3,000
Lucerne, dehydrated	3,000-6,000	
Marrow	0	
Onion	25	
Parsley	5,000-30,000	
Parsnip	30	
Pea	139-680	
Dried, Green	530	
Split	370	
Potato	28-56	
Radish	3	
Rhubarb	100	
Spinach. Fresh	2,630-6,500	
Tinned	12,230
Sweet potato. Brown	10	
White	0	
Tomato. Whole, green	170	
" ripe	400	
" ripened indoors	170-270	
Flesh	14,160-35,640	
Juice	320-590	
Tinned	4,280
Turnip. Greens	10,000-20,000	
White	0	
Yellow	20	
Watercress	12,000

FOOD	International Units of vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams or roughly 3½ ounces.		
	VITAMIN A.	CAROTENE.	TOTAL ACTIVITY.
MISCELLANEOUS			
Chocolate. Milk	480
Sweetened	36
Unsweetened	60
Cocoa powder	26	
Fungi	0
Honeycomb	4,096
Marmalade	5	
Mushrooms	0
Yeast	0

HUMAN REQUIREMENTS OF VITAMIN A AND CAROTENE

In Health. The latest and now widely accepted broad statement on the requirements of vitamin A was made by the U.S.A. Food and Nutrition Board and the National Research Council [464] in 1948 : these requirements are "based on the premise that approximately two-thirds of the vitamin A value of the average diet . . . is contributed by carotene and that carotene has half or less than half the value of vitamin A." Adults of both sexes, whatever their employment, and children over the age of thirteen are said to require daily 5,000 I.U., which should be increased during the latter half of pregnancy to 6,000 I.U. and during lactation to 8,000 I.U. Children below the age of thirteen require 4,500 I.U., below the age of ten 3,500 I.U., below the age of seven 2,500 I.U., below the age of four 2,000 I.U., and below the age of one 1,500 I.U. These figures, as will appear from the following discussion, are parsimonious except for children, because carotene is such an unreliable source of vitamin A (p. 12).

With a diet containing plenty of the other vitamins (p. 49) and with no liquid paraffin or paraffin emulsions used as aperients (p. 12), the requirements of vitamin A are only dependent on the weight of the body and not on age or physical activity (p. 31).

The correct assessment of vitamin A requirements we believe has been given by Guilbert, Howard and Hart [147], who in 1940 not only finally reviewed their own work, but also that of others on human requirements.

"The minimum (daily requirements of vitamin A or carotene) for significant storage, optimal dark adaptation and reproduction" are for vitamin A itself 60 I.U. per kilogram of body weight, and for carotene (provitamin A) 200 I.U. ; that is for a man of 11 stone, or 70 kg., 4,200 I.U. and 14,000 I.U. respectively. For "normal growth, freedom from clinical symptoms, and little or no storage," 20 I.U. of vitamin A and 40 I.U. of carotene daily per kilogram are necessary ; that is for a man of 11 stone 1,400 I.U. and 2,800 I.U. respectively. Confirmation of these requirements is provided by the Medical Research Council's investigation [93] carried out on human volunteers : the conclusion reached was that 1,300 I.U. of vitamin A itself is the minimum protective dose, which is almost identical with the figure given above. Only 2,500 I.U. are recommended, however, "to cover individual variations and to leave a margin of safety" ; this is a low figure compared to Guilbert's, apparently due to less importance being attached to the building up of reserves. The requirements for carotene are so bedevilled by all the factors which influence its absorption (p. 15) that it is difficult to compare the Medical Research Council's figures with those already given but broadly speaking they are in agreement. Wagner [320], from carefully controlled experiments on ten men, has stated that 2,000 I.U. of vitamin A or 4,000 I.U. of pure β -carotene are the daily minimum for preventing impaired dark adaptation.

Lewis and Haig [465], from dark adaptation studies, originally put the daily minimum requirements of infants at 18–20 I.U. of vitamin A per kilogram, but this was later raised to 100–200 I.U. by Lewis and Bodansky [466], who based their observations on the level of vitamin A in the blood, though as this in infants has little significance (p. 25) the smaller amounts recommended by Guilbert, Howard and Hart to allow both for current needs and storage, should be aimed at.

Pregnancy and lactation, which are a common cause of a deficiency of vitamin A in all countries (p. 65), increase the maternal need for vitamin A because the weight of the child both before and after childbirth should from this point of view be added to that of the mother; in fact her needs are roughly that of an 11-stone man, instead of a 10-stone woman. By taking a diet rich in vitamin A she not only reduces the risk of puerperal fever (p. 37), but also increases the stores of vitamin A in the liver of the foetus and child (p. 19), thus giving him the most important vitamin for good dental development (p. 34). It is important to give vitamin A rather than carotene during lactation because the former influences the vitamin A content of the milk, far more than the latter (p. 52). The milk of well-fed mothers contains ample vitamin A both for the infant's immediate needs and for increasing the low stores which are present at birth (p. 21), providing about 1,100 I.U. of vitamin A itself (p. 51). The maternal diet should be rich in fats, as there is experimental evidence that this aids the transfer of vitamin A both to the foetus (p. 19) and to the milk (p. 52). When breast feeding is impossible, cow's milk or dried full-cream milks give enough vitamin A, especially if the child is given cod-liver oil as well. Sweetened condensed skimmed milk containing no vitamin A, is fortunately seldom bought in England, but in the tropics, where this waste product of civilization is widely sold to the natives, its consumption causes much widespread xerophthalmia and permanent blindness in children (p. 72).

It is important to build up reserves against any decrease in consumption such as tends to occur during the winter and during illness; a high intake has the further advantage of being a safeguard against any unrecognized condition of the body which may hinder absorption or the conversion of carotene into vitamin A (p. 13). Children especially need vitamin A itself, as they utilize carotene very badly (p. 12). There is also some evidence that there may be an inherited or familial need for unusually large amounts of vitamin A [467, 468], and, at least in animals, ample supplies throughout life are most valuable (p. 32). Adequate reserves have been found in most livers recently examined in England [93], though there is still an appreciable number in which the reserves are too slight to tide over any prolonged deficit. In passing it is interesting to ponder on whether increased storage, such as apparently occurred during the war [93], indicates better or worse nutrition: an increased consumption of valuable foods or an increase in eating vegetables enforced by scarcity of meat, etc.

For workers who have to use their eyes for matching colours (p. 42) it has been found that the provision of extra vitamin A lessened eye-strain and improved the health [470], so that it might even pay employers to provide free supplements of vitamin A to their workpeople. Clarkson [469] reports that in foundrymen the removal of particles of metal embedded in the cornea is made easier and fragmentation, flaking and splitting of the cornea while this is being done is prevented by the provision of extra vitamin A and, further, the men report their condition immediately instead of delaying over doing so.

General physical fitness and health in normally nourished people is not improved by an increased consumption of vitamin A. Bronsby and others [340] gave one half of 1,242 children between the ages of five and fourteen a capsule of arachis oil and the other half a capsule containing 4,000 I.U. of vitamin A and 600 I.U. of vitamin D. The capsules were given every school

day for nine months. No effect was produced on growth, nutritional status, muscular strength, the teeth, the gums or the incidence of illness. In 214 men doing very heavy manual work, neither the weight, blood pressure, hæmoglobin, frequency of illness nor output were altered. Jenkins and Yudkin [471] gave 178 children, aged eleven to twelve, 5,500 I.U. of vitamin A and other vitamins every school day for a year and found no alteration in the pulse rate and vital capacity or in breath-holding and the 40 mm. endurance test.

In Disease. As a general rule it may be stated that most pathological conditions of the body interfere with the absorption of carotene from the bowel, and also with the absorption and metabolism of vitamin A itself. Therefore in all chronic illnesses the diet should provide vitamin A itself in larger amounts than those normally required, or fish liver oil concentrates should be given.

The particular aspects of vitamin A metabolism germane to diabetes, thyroid diseases, infections, steatorrhœa, nephritis, hepatitis, pregnancy, etc., have already been described in earlier sections of this chapter.

METHODS USED FOR RECOGNIZING HUMAN VITAMIN A DEFICIENCIES

Four methods have been employed for determining whether a patient has a deficiency of vitamin A: Clinical examination, slit-lamp microscopy of the conjunctiva which is valueless, estimation of vitamin A in the blood already discussed on p. 24, and measurement of dark adaptation. Though the last three of these are not generally feasible, being essentially methods used in academic medicine or in painstaking nutritional surveys, this does not matter in clinical medicine since xerophthalmia and keratomalacia, the only conditions urgently needing vitamin A, must be treated without waiting for scientific corroboration of the obvious clinical diagnosis.

Clinical Examination. The clinical signs of a deficiency of vitamin A are described on p. 64, so that all that need be said here is that a clinical diagnosis is only possible when the deficiency is already moderately severe, though some workers claim that a hesitant diagnosis can be confirmed by the finding of keratinized cells in the conjunctiva, respiratory tract and urine [228, 262].

Slit-Lamp Microscopy. Kruse [472] in 1941 aroused considerable interest by his claim—now known to be wholly incorrect—that biomicroscopy of the conjunctiva of adolescents and adults revealed certain subepithelial opacities which were among the earliest detectable signs of a deficiency of vitamin A. Berliner [473], however, pointed out that the opacities were only the common presenile or senile alterations which occur in the sub-epithelial layers of the conjunctiva, and the “spots” Kruse described only the common pingueculæ. It would also be most surprising that a lack of vitamin A should affect subepithelial but not epithelial tissues. Further investigations have demonstrated the frequency and variety of these conjunctival opacities both in normal adults [93, 239] and children [93, 474], and the impossibility of either causing them by vitamin A deficient diets prolonged for over two years [93] or of curing them with large doses of vitamin A given daily for two years [239].

Dark Adaptation. A slight decrease in the *final* degree of dark adaptation of which the fully dark adapted eye is capable and also, though not so constantly, a slight delay in the rate of early dark adaptation are generally considered to be among the earliest signs or symptoms of a deficiency of vitamin A, though the patient himself is seldom aware of these slight disabilities.

Measuring the extent and rapidity of dark adaptation has therefore been extensively used in nutritional research and surveys to unmask mild defi-

ciencies of vitamin A, though it is an investigation which is not feasible in general practice since it requires both apparatus and experience. Various adaptometers have been constructed [93, 320, 475, 476, 477, 479] and the rotating hexagon, described by Livingstone [478] for rod scotometry, promises, when its uses have been more fully explored, to be of great value in investigating further aspects of the rôle of vitamin A in night vision and in diagnosing early deficiencies [93].

The papers by Harris and Abbasy [481], Yudkin, Robertson and Yudkin [477], Godding [479] and Craik [480] should be read for discussions on what instruments and what techniques give the most reliable results. The main differences in technique among different workers are the time the eyes are exposed to a preliminary bleaching of their visual purple (p. 41); the use of a fixation point during testing rather than allowing the eyes to move and so select their own most sensitive retinal area, thus avoiding any area which would give a fallacious impression of impaired adaptation due to congenital deficiency of the rods or localized pathological changes [478], though this, on the other hand, has the drawback that such instruments are less sensitive; the distance of the eyes from the test object; the size and nature of the test object; and, most important of all, whether the whole curve of dark adaptation is plotted: this is discussed later.

The broad principles of testing dark adaptation are roughly the same in all instruments. The eyes are first exposed to a bright light to cause bleaching of the visual purple after which the light is extinguished and the patient is shown small illuminated test objects. As the eyes become more adapted to the dark the illumination of the test objects can be reduced without their becoming invisible. From the different degrees of illumination of the test objects and from the time taken for the test objects to become visible with these different illuminations, a curve of dark adaptation can be constructed (Fig. 16). Children under eleven or those who are mentally dull or deaf or whose vision is bad, are unfit for the test [481].

Early investigations on vitamin A deficiency were often done by measuring dark adaptation after the subjects had been only a few minutes or even seconds in the dark. But this measurement of the initial rate of adaptation may give completely fallacious results, since when the initial rate is rapid, the final threshold or degree of adaptation may be low and it is the latter, not the former, which is *invariably* affected, if vision is affected at all, by lack of vitamin A [477]. The dark adaptation curves of two subjects may even cross as late as twenty minutes after the start of dark adaptation, so readings taken during the first twenty minutes of adaptation may give completely erroneous information about the relative state of the vitamin A nutrition of two subjects. The curves (Fig. 16) constructed by Yudkin, Robertson and Yudkin [477] clearly show the various types of curve which may be encountered, the different effects which vitamin A may have on the curves and the paramount importance, if only one reading is to be used for assessing dark adaptation, of taking the reading when dark adaptation is virtually *complete*. This in normal men occurs after thirty to forty minutes but in deficient men may even be delayed for eight hours [93]. Dow and Steven [476], Hecht and Mandelbaum [482], and Basu and De [483] have also emphasized the importance of plotting the whole curve of dark adaptation and measuring its final extent. Complete proof that impaired dark adaptation is caused by lack of vitamin A, and not by any of the other conditions mentioned below, must rest on the final threshold being raised by vitamin A. Large amounts, such as 100,000 I.U. daily for ten days or longer, should be given, and blood vitamin A estimations also carried out to make sure that the vitamin is being absorbed and is circulating in the blood [214].

The validity of the dark adaptation test for showing mild degrees of a deficiency of vitamin A is now generally acknowledged. Thus Harris and Abbasy [481] found that impaired dark adaptation was common in children

whose diet was estimated to be low in vitamin A while it was rare in well nourished children. Steel [484] found that even when using a simplified test he obtained consistent readings which, when low, were improved by vitamin A. Both these important findings have been confirmed by Yudkin and others [477] who in a like manner reproduced curves on different occasions for the same subject with little variation, and improved adaptation with

Dark-Adaptation Curves and the Various Effects of Vitamin A on Dark-Adaptation

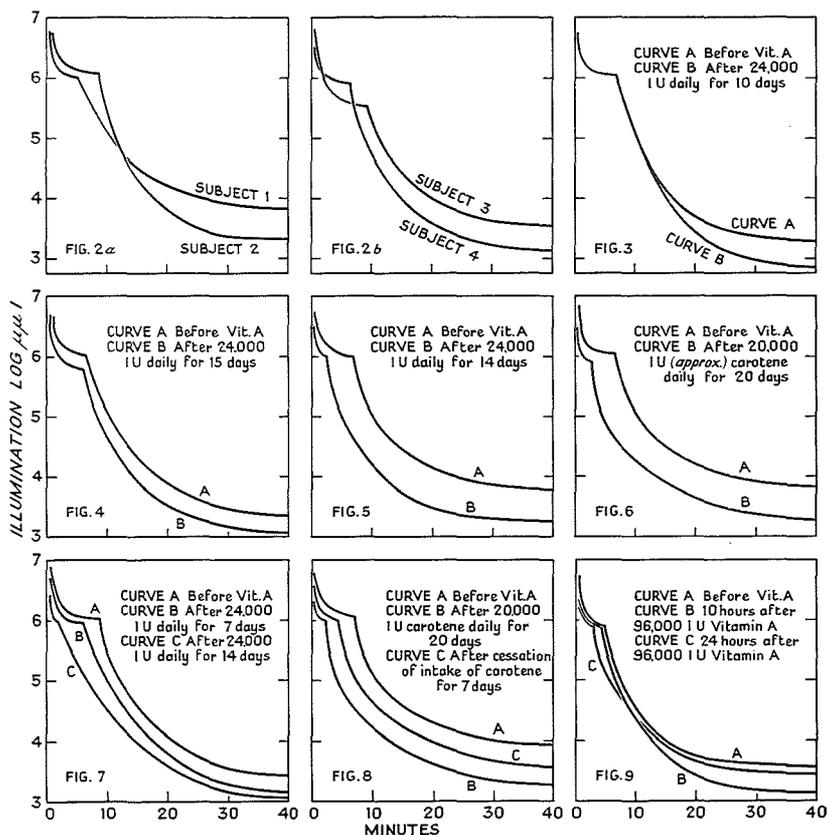


FIG. 16.

Figs. 2a and 2b. Individual differences in curves of dark-adaptation.

Fig. 3. Change in rod threshold only.

Fig. 4. Change in cone threshold and rod threshold.

Fig. 5. Change in rod threshold and transition time.

Fig. 6. Change in cone threshold, rod threshold and transition time.

Fig. 7. Gradual improvement with continued administration of vitamin A.

Fig. 8. Improvement after administration of carotene; relapse after cessation.

Fig. 9. Transitory changes produced by one large dose of vitamin A.

Figs. 3-9 illustrate various effects of vitamin A on dark-adaptation.

vitamin A. Cowell [224] reports that night blindness occurs when the level of vitamin A in the blood is below 70 I.U. rather than between his normal of 100 to 300 I.U., and in Pett and Le Page's patients [225] dark adaptation improved as the blood vitamin A rose from 76 to 133 I.U. in the four hours following the consumption of a vitamin A concentrate; others have noted an improvement within one to two hours [467], ten hours [477] and two weeks [93], the dose in the last experiment being only 1,300 I.U. daily. In the Medical Research Council's investigation [93] dark adaptation was

impaired when blood levels fell below about 50 I.U., a figure congruous with the findings mentioned above since earlier techniques tended to give too high blood levels. Josephs [485] also has confirmed that there is a correlation between the level of vitamin A in the blood and dark adaptation. However, Hecht and Mandelbaum [482] found that while dark adaptation was often promptly impaired by a diet deficient in vitamin A, it took as long as six to twelve weeks for it to return to normal, in spite of large doses of vitamin A. This puzzling observation may possibly be explained by an instrument having been used which had a fixation point, which might mean that an area of the retina in which recovery from deprivation of vitamin was slow, or where the retina was damaged [351, 478], was being stimulated [477, 478]. Most workers who deny, like Caveness [486], the value of estimating dark adaptation have used a biophotometer, the fallacies of which have been pointed out by Harris and Abbasy [481].

Robertson and Yudkin [487] have shown that dark adaptation decreases with increasing age. Therefore in nutrition surveys based on dark adaptation studies allowances must be made for the age of the subjects.

The season of the year has an effect on dark adaptation in men on deficient diets, a slight but definite deterioration occurring in the winter [93], which is reminiscent of McCance's observations on seasonal fluctuations in the response to vitamin D (p. 528).

Other changes which may be caused by lack of vitamin A are an alteration in the cone-rod transition time [93, 447, 482] which is shown in Fig. 16 and alterations in the rod visual fields, which can be shown by rod scotometry [93, 478].

Differential Diagnosis of Impaired Dark Adaptation. It must be remembered that other conditions apart from deprivation of vitamin A may impair dark adaptation, such as congenital night blindness, retinitis pigmentosa, or a detached retina [488]; starvation with a low blood sugar [489]; deficient oxygenation of the blood [478, 489]; lack of sleep, though not physical fatigue [492]; and also hysteria and possibly lack of other vitamins, both of which are discussed below.

The nystagmus of coal miners is not in itself a grave drawback, but miners often add to it—as Culpin [490] has pointed out—functional disabilities, among which may be night blindness. Kellett [491] in 1939 investigated the diets of Durham coal miners and could find no proof that lack of vitamin A was the cause of nystagmus, the men insisting on about 14 ounces of butter a week whatever their incomes, margarine being virtually never accepted as a substitute: thus whether or no the men had nystagmus, they obtained roughly 1,140 I.U. daily from butter alone. Campbell and Tonks [498], to clinch matters, showed in 1948 that nystagmus is not associated with low levels of vitamin A in the blood, though they reported that miners have a raised threshold for dark adaptation which is not due to lack of vitamin A. Campbell [495] in 1941 reviewed most of the relevant literature.

A further type of purely functional night blindness may develop among soldiers who have to carry out dangerous duties in the dark. This was recognized in England in 1917, but was not publicly reported at the time, because it was commoner in the German than English armies, leading to wastage of men in the former, where its cause was not understood [490]. In 1941 Wittkower and his collaborators [496] examined fifty-two soldiers complaining of night blindness. Most were found to have severe psychological disorders not, in origin, due to fear of fighting. The surprising and to us unjustifiable conclusion drawn from this examination is that "most cases of night blindness seen in this country are probably of psychological origin." It seems more probable that only the psychopath and malingerer spontaneously complain of night blindness: normal people are seldom worried by the condition. Harman [497] has discussed how to recognize the malingerer.

The importance of vitamins, other than vitamin A, for dark adaptation

is uncertain, but the work of Harris and Abbasy [481], of Stewart [492], and of Wald and Steven [499] suggest that ascorbic acid is important. This is very definitely denied by Yudkin [500] after a careful study of six subjects with poor dark adaptation, one subject with severe night blindness and a deficiency of ascorbic acid, and five subjects with low normal adaptation. Nicotinic acid, though an essential ingredient of the visual purple cycle (p. 41), has not been reported to be of clinical importance in dark adaptation. A paper by Kimble and Gordon [493] stresses as well the value of riboflavine. This latter paper has been frequently quoted apparently without having been read, because it is generally believed to state that riboflavine is in some cases necessary for the formation of visual purple in the eye itself. All that is really stated is that in a few patients who had both poor dark adaptation and a low blood vitamin A, large doses of vitamin A had no effect on either until riboflavine was given, when both became normal. In other words, riboflavine appeared necessary for the absorption or mobilization of vitamin A, but there is no proof that once there was an adequate supply of vitamin A in the blood the eye itself could not utilize it normally. Dr. Pollak has very kindly shown us convincing and well-controlled complete dark adaptation curves which leave little doubt that riboflavine alone does in some cases improve dark adaptation, though he has not yet investigated if this is due to a direct action on the retina or to an indirect action through raising the level of vitamin A in the blood. Morton [504] in 1944 reviewed the evidence in favour of riboflavine and not vitamin A being the chromophoric group of visual purple.

Having excluded all the above factors which, apart from lack of vitamin A, may cause poor dark adaptation, there still remain various physiological conditions which Phillips [494] has shown will produce individual differences in dark adaptation. A large pupil in the light retards dark adaptation, while a large pupil in the dark hastens it. Dark adaptation is always slow in old age irrespective of the size of the pupil, and, though general colouring has no effect, heavy pigmentation of the retina favours more rapid adaptation.

Alcohol and amphetamine may, according to Yudkin [500], improve dark adaptation, even when they cause no increase in the blood vitamin A. The improvement is transitory, is beyond that obtainable with vitamin A, and is apparently not related to the mental effect of the drugs. No such effect was obtained by the Medical Research Council [93] with alcohol, though it and maybe other drugs probably raise the level of vitamin A in the blood (p. 28). Only a few negative observations have been made on the effect on dark adaptation of thyroid, dinitrophenol, caffeine, bromide, strychnine, phenobarbitone and morphia [247, 500, 502, 503].

HUMAN DISEASES DUE TO A DEFICIENCY OF VITAMIN A

Many people in England and America take so little carotene or vitamin A that they either have definite symptoms of a deficiency, or are so close to a deficiency that they have no stores in their bodies (p. 19) to carry them through periods when vegetables, fish, eggs and milk are costly, as in the winter, or when the amounts of these which can be eaten or bought are reduced by rationing, old age, ill-health or unemployment. Figures based on what percentage of the population has poor dark adaptation or skin changes give too rosy a picture of their nutritional condition, as a recognizable deficiency only occurs when the body has already broken down from lack of the vitamin, this breakdown being preceded by a period when the functions of the body are at best being carried on under an extra strain.

Changes in the skin and poor dark adaptation are the earliest signs of a definite deficiency which can be diagnosed. Judging by poor dark adaptation, more than half the children from working-class homes in England were found to be deficient by Harris and Abbasy [481] in 1939, while America in 1940 had a similar or even worse percentage [467]. In some parts of the

tropics a deficiency must be almost universal, since Fitzgerald Moore [441] reports that in the Philippines nearly one-third of the children attending a hospital before the war had xerophthalmia, and this has become even worse since the Japanese invasion [505]; while in the Dutch East Indies, with a child population of half a million, nearly four thousand children between the ages of twenty-one months and fifteen years were blind, apparently from lack of vitamin A [441]. Both in Africa and India skin changes due to lack of vitamin A were present in eighty per cent. of some groups of children [506, 507].

In English adults a deficiency is apparently less common than in children, Harris and Abbasy [481] in 1939 finding only slightly impaired dark adaptation in half of thirty-eight working-class mothers, and in three of twenty-five middle-class males, though in 1948 about one-fifth of university students in London were affected [512]. But in America the figures appear to be higher, over one-third of one-hundred and sixty-two medical students being found to be affected [467] in 1940, and half the adults attending a hospital outpatient department [508] in 1938. Pregnancy has been and probably still is commonly associated with lack of vitamin A in England [224, 484], America [392], Holland [224], Germany [509] and the tropics [510].

The effects of lack of vitamin A on (a) the skin, (b) the eyes, (c) the nervous system and (d) other tissues will be considered separately.

The Effect of Lack of Vitamin A on the Skin. (*Toad Skin or Phrynoderma, Shark Skin, Keratosis Pilaris, Ichthyosis Follicularis, Lichen Pilaris, Lichen Spinulosus, Darier's Disease, etc.*) In England skin changes due to lack of

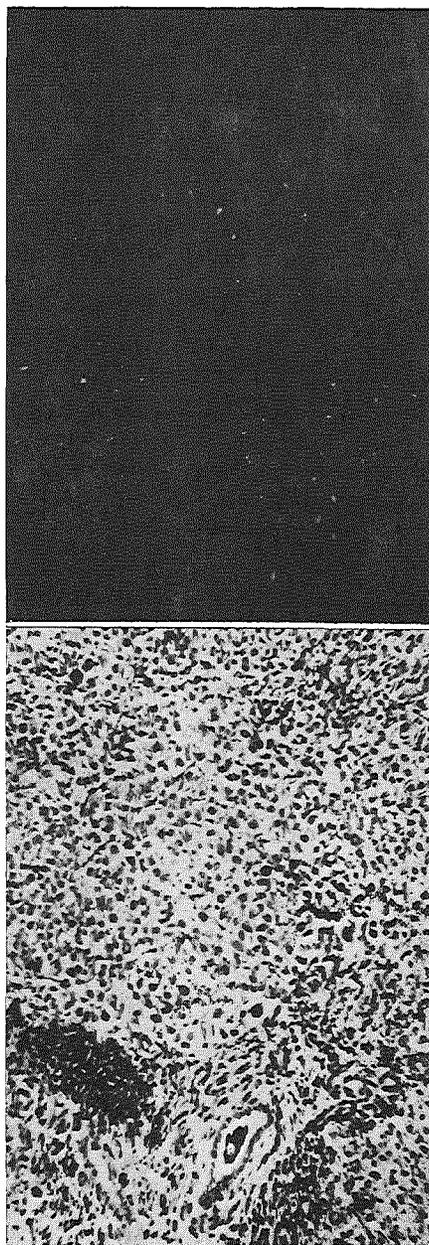


FIG. 17. Photomicrographs of a biopsy specimen from the liver of a patient with disease of the gallbladder with jaundice of short duration and without clinical signs of damage to the liver. A, routine histologic examination; extensive lymphocytic infiltration in the enlarged periportal field. No visual damage to the parenchyma. B, examination with fluorescence microscope; irregular vitamin A fluorescence is imparted almost only by Kupffer cells. The bile casts in the enlarged bile capillaries appear black. (See also Figs. 3 and 5.)

vitamin A probably do not occur or occur only as an extreme rarity. The folliculosis or follicular keratosis (Figs. 18 and 22) in children ascribed to lack of vitamin A by Pemberton [515] and other observers during the late thirties and early forties is now known to be a normal skin condition found in up to eighty per cent. of children. Stannus [513] from his very extensive experience gained in nutrition surveys of English children during the war, has described the condition in detail and reviewed the evidence about its cause. It appears to be the customary reaction of the skin to exposure to the weather, to the trauma of clothes and to dirt, being most pronounced on the fronts of the thighs above the knees where the bare skin is chafed but not protected by "shorts" and skirts. Unlike the true toad skin caused by a deficiency of vitamin A, it is common before puberty and increasingly rare later and, moreover, its papules never grow so large nor spread so widely.

The skin changes which do occur as a result of lack of vitamin A were described by Nicholls [507] in India in 1933, who first used the name toad skin or phrynoderma, and by Lowenthal [511] in the same year in Africa, who made his observations without knowing that the importance of vitamin A for the skin had already been recognized two years before in China by Frazier and Hu [303]. Pallister [514] in 1940 found toad skin common in Malaya.

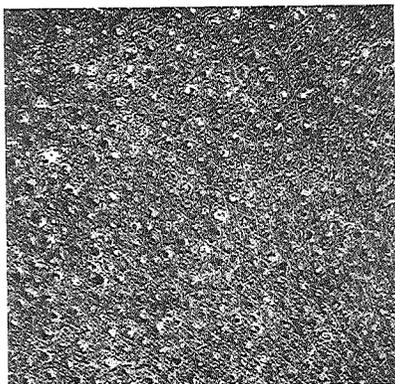


FIG. 18. Life-size skin print showing mild folliculosis on the front of the thigh of an English schoolgirl. Skin prints, in contrast to photographs, enable records of the condition of the skin to be made rapidly, easily and cheaply. Prints should be viewed with a reading-glass giving five magnifications. This figure does not do justice to the technique first described by Dr. Hugh S. Stannus in *The Lancet*, 1944, *ii*, 359.

There is, however, still some uncertainty as to whether toad skin is due to a simple deficiency of vitamin A or whether some other factors are also involved. In favour of a deficiency of vitamin A being the only cause is the work of Lowenthal [511], who cured two of his cases with vitamin A alone, and practically all the rest with cod-liver oil, while Lehman and Rapaport [467] cured their cases with halibut-liver oil. Pallister [514] in Malaya noted an association between toad skin and Bitot's spots. Steffens and others [516] produced typical changes in the skin of a man by a vitamin A deficient diet, and Nichols [507] and Lowenthal [511] have noted a close

association between skin changes and night blindness or xerophthalmia, this association being apparently far commoner after than before adolescence.

Against these observations, however, must be put those of Aykroyd and Rajagopal [517] and Rao [521] who did not find any close correlation between toad skin and xerophthalmia, or between the former and a diet deficient in vitamin A during a very extensive investigation of Indian schoolchildren. Frazier and Hu [303] and Sweet and K'Ang [262] found no correlation at all between the condition of the skin and eyes, so that they decided that in children the eyes but not the skin were affected by a deficiency of vitamin A, while after adolescence the skin chiefly suffered. This is confirmed by Frazier, Hu and Chu [304] who showed that in young children the skin is generally only xerotic and atrophic, follicular hyperkeratosis seldom occurring before adolescence (Fig. 70). This has been the experience of many other workers [505, 545, 546]. The problem is still further complicated by the descriptions given by Fox [519] and Wiltshire [518] of the early skin changes in scurvy (p. 70) which appear to be almost identical with those of toad skin. The position appears to be that lack of vitamin A alone can cause toad skin, but

that there is often some other factor which alters the reaction of the skin so that it is more sensitive to a deficiency of vitamin A. This ancillary factor may be either a second food deficiency [521], or the stage of sexual development [304], or a familial need for abnormally large amounts of vitamin A [467, 515], or a racial susceptibility such as is apparently shown in India [507, 517] and Africa [506], but not in China [262, 303, 304]. Whatever the cause the reaction of the skin varies so much that sometimes changes occur before there

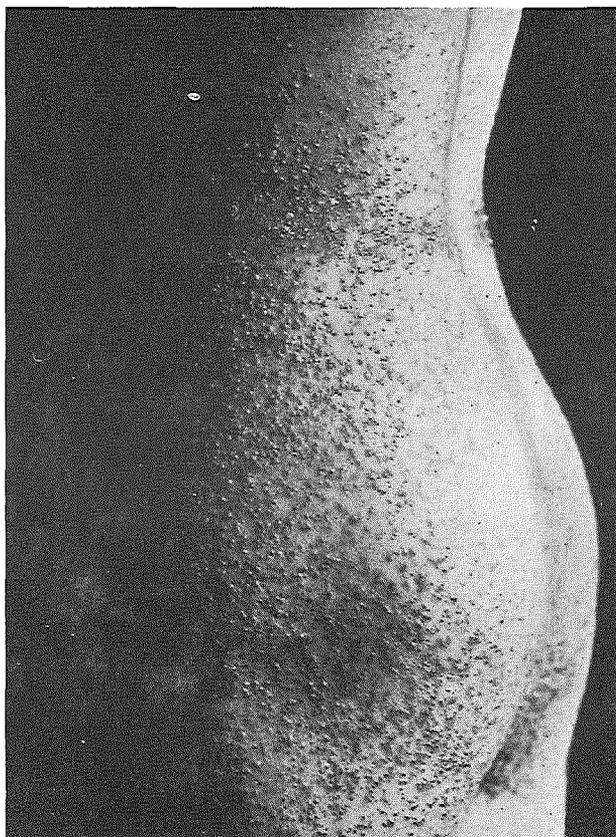


FIG. 19. Diffuse involvement of hair follicles due to vitamin A deficiency in a fourteen-year-old Chinese boy: his skin is also shown in Figs. 20 and 21. Hyperpigmentation of the conjunctiva was the only ocular sign of vitamin A deficiency.

is any obvious involvement of the eye [303, 304, 508, 517] or even slight impairment of dark adaptation [516], while in other cases the eyes may be seriously damaged while the skin apparently remains normal.

The insidious onset of a dry rough skin, especially in those areas where the papular eruption occurs later, is the first cutaneous symptom of a deficiency of vitamin A. Loewenthal [506], and Frazier and Hu [303] stress this early symptom, which has been noted at all ages from infancy to old age and in both sexes. There is an increase in the spring [262] after the deficient winter diet. The dry skin may be followed by a sudden local eruption which often spreads rapidly over the fronts and sides of the thighs, and the

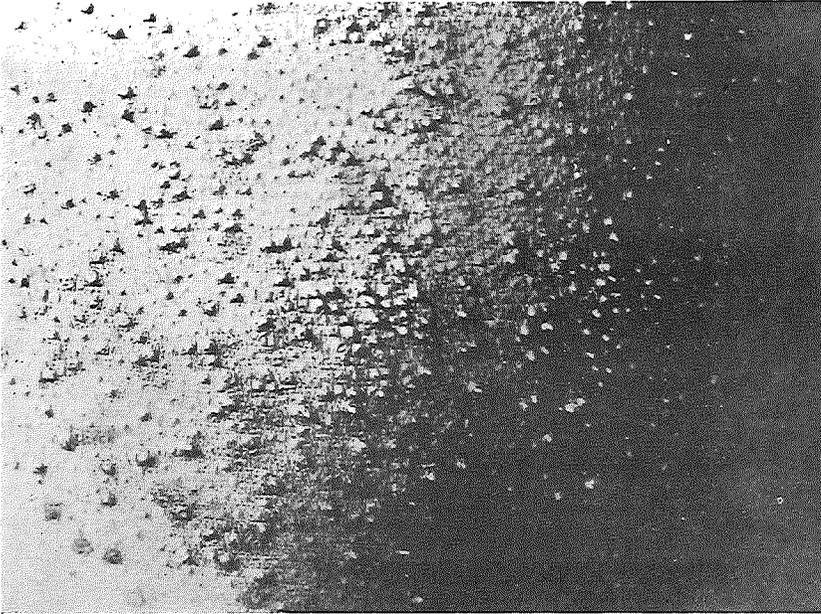


FIG. 20. Follicular hyperkeratosis, with projecting horny spines, before treatment. (See also Figs. 19 and 21.)

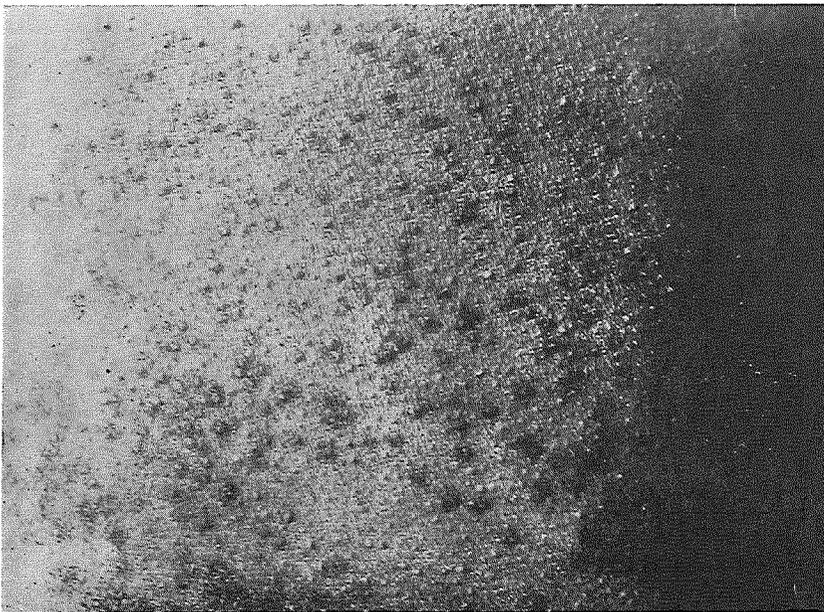


FIG. 21. The same case shown in Figs. 19 and 20 after five weeks' treatment with a vitamin A concentrate and cod-liver oil, which provided about 63,000 I.U. daily.

posterior and lateral sides of the forearms just below the elbows, and the fronts of the arms and shoulders. Some observers report that the eruption generally spares the front of the chest [507], the groins and axillæ, and the backs of the hands and feet [303, 304], but others state that it may ultimately cover the whole body apart from the face, which is seldom involved [507, 521] though "black-heads" are common [262, 303, 304] and may in Europe be the dominant symptom. The scalp is not affected, but the hair may be dry and brittle and the nails may have transverse or longitudinal ridges [262], though generally the hair and nails are normal [303, 304]. Increased pigmentation [262] both of the papules and the skin, which has been likened to argyrosis by Mu and others [262], is sometimes seen in coloured patients,

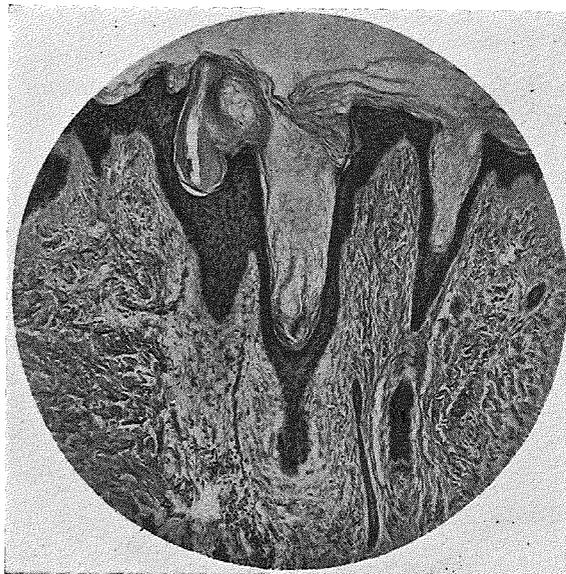


FIG. 22. Follicular hyperkeratosis in a boy aged fourteen. (Great Britain.) Note the plugging of the hair follicle with keratinized material; the absence of the sebaceous gland; the increased keratinization of the superficial layers of the skin; and the infiltration of small round cells near the base of the follicle.

being analogous to the scleral pigmentation (p. 74). Itching has been reported to be present [507, 511] and absent [521].

The eruption consists of dry horny round or oval sharply defined papules, varying in diameter from that of a pin's head to as much as a quarter of an inch [511]. The size of the papules increases with the duration of the deficiency, in the early stages being more easily felt with the fingers than seen, while later the skin looks from a distance as if many split lentils had been stuck upon it. Each papule is formed by hyperkeratosis of the pilosebaceous follicles, and has a hard keratinous core which can be picked out, leaving a small pit. Often broken or coiled up unerupted hairs are found either projecting through the papule or imprisoned beneath. The papules seldom, if ever, undergo pustulation [262, 507, 511, 521], though Young [523] believes that the skin is more susceptible to fungus infections. Rao [521] and Frazier and others [303, 304] have reported skin changes in keratomalacia which are typical histologically of phrynoderma apart from the papules (Fig. 70).

Microscopical examination of the skin in phrynoderma shows that the papules are composed of masses of keratinized cells which have been shed from

the pilosebaceous follicles, becoming compressed in their centres into horny amorphous plugs [511, 521]. These block the hair follicles and the sebaceous glands which then tend to atrophy. There is also hyperkeratinization of the epidermis, especially round the papules, and a thickening of the stratum corneum and sometimes an increase in the pigment cells and a mild lymphocytic infiltration near the base of the follicle. The sweat glands are not markedly changed, but do not appear to be secreting, and they are often plugged with keratinous material. Vitamin A, in spite of its importance for the skin, is not present in the epidermis [58].

The differential diagnosis is from acne and from early scurvy. In acne the skin is greasy instead of dry, the eruption is mostly limited to the face and front and back of the chest, unlike that of toad skin, and it mainly occurs during adolescence and early adult life. Pustulation of the papules is the

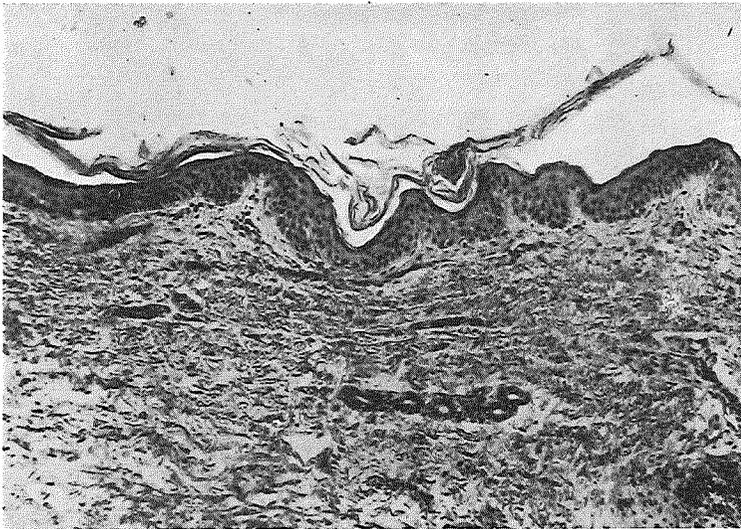


FIG. 23. Showing the atrophic condition of the epidermis of a Chinese child approximately three years of age who had generalized xerosis of the skin but no obvious follicular keratosis. There was pronounced xerosis of the conjunctiva, which was wrinkled and leathery, and early keratomalacia. The child had had diarrhoea for a month. The section is from the abdominal wall.

rule rather than the rare exception, generally leaving behind small scars which are never seen when uncomplicated cases of toad skin are cured.

The earliest sign of scurvy, according to Wiltshire [518] and Fox [519] is a skin eruption which is identical with that caused by lack of vitamin A, since the skin is dry and the papules are formed in the mouths of the pilosebaceous follicles by masses of keratinized epithelium in which or under which are broken or coiled up hairs. But these papules appear to be rubbed off easily and leave behind them pink follicles which have not been described in toad skin, and also their distribution is slightly different, being more confined to the legs. The perifollicular hæmorrhages which would confirm a diagnosis of scurvy only appear some weeks after the papules.

Chronic ulcers of the skin are a very rare complication of phrynoderma, and when they do occur only heal with vitamin A [262, 524].

Treatment with vitamin A is entirely successful. The first sign of recovery is a return of sweating so that the skin within two or three weeks no longer

feels dry [303, 304, 467], though it does not return to normal for two to nine months, the shorter period being on very high doses of vitamin A, such as 100,000 I.U. a day. - Concentrated preparations or even injections should be used when diarrhoea is present [262], or aqueous dispersions (p. 18) should be of value. The keratotic plugs in the follicles are reported to be extruded as tiny rice-like bodies, but by remaining partly adherent to the skin they give to it a shaggy appearance [467]. Ultimately the epidermis and the follicles return to normal and new hairs develop.

Darier's Disease. Since Peck and his collaborators [527] in 1941 suggested that Darier's disease was due to a deficiency of vitamin A, many further papers have appeared on the subject [528, 529, 530, 534, 536, 537, 538].

A few cases respond to large doses of vitamin A, more do not. Therefore in so intractable a disease it is reasonable to try vitamin A, though most unwise to promise any improvement. Either of the two genetic types of the disease may respond, Carleton and Steven [529] curing a mother but not her son and a brother but not his sister, while Lissia [537] failed to cure three familial and one idiopathic case. The level of vitamin A in the blood is just within normal limits [529, 534, 536] or very low [527, 536] and is raised and maintained in most cases only by continuous and large doses of the vitamin [527, 536], though even these may fail to cause a rise [536]. Hepatic function is often impaired [536] as may be dark adaptation [527], though both may be normal [529, 536]. The doses of vitamin A generally given by mouth have ranged from 100,000 to 300,000 I.U. daily and should be continued for at least two months before abandoning them as useless. Occasionally large doses cause an exacerbation, the papules becoming bullous [528].

Tylosis or Hyperkeratosis Congenitalis Palmaris et Plantaris or Mal de Meleda. Brunner and Fuhrman [539] successfully treated one patient with this rare and reputedly incurable hereditary condition, giving oral daily doses of 300,000 of vitamin A. When treatment was stopped there was a relapse, followed again by recovery when treatment was restarted. Smaller doses were less effective. Porter and Haber [540] cured the keratosis on the palms of their patient and improved the soles by daily doses of 100,000 to 200,000 I.U. for six months: the palms were cured after three months and the improvement persisted six months after treatment had stopped. Porter [605] later reported improvement in three of six cases.

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FIG. 24. Keratomalacia in a Danish infant (see p. 2). The disease arose after two months' feeding with oatmeal. After treatment with cod-liver oil the right eye improved and became almost normal. The left cornea is largely necrosed.

Ichthyosis. Rapaport and others [531] in 1942 reported six cases of ichthyosis all of which were very considerably improved by vitamin A, and also gave references to similar results obtained by two other workers. The vitamin was given orally in daily doses of 60,000 to 200,000 I.U. or intramuscularly two or three times a week in doses of 100,000 I.U. Some cases responded only to the former method of administration and some only to the latter. Improvement occurred within a few weeks, but relapses, especially in the winter, were common if the treatment was stopped. All five cases who were tested had poor dark adaptation and in five cases there was a family history of ichthyosis. The level of vitamin A in the blood is low [538]. Porter [605] reports improvement in one case out of nine.

Eczema. The absorption of vitamin A in infantile eczema is said to be impaired [532] and the level in the blood low [167]. Gross [533] reports curing eighteen of twenty-four cases of nummular eczema with 75,000 U.S.P. units daily. Leitner and Moore [538] found the average level of vitamin A in the blood of twenty-six adult patients was higher than in control patients. (See also vitamin F, p. 677).

Other Skin Conditions. Many other skin conditions have been treated by vitamin A, but the review by Carleton and Steven [529] and investigations on the level of vitamin A in the blood of patients with thirty-two different skin conditions [534, 538] leave little doubt that vitamin A will benefit none, other than those already discussed, with the possible exception—when given in doses of 100,000 to 500,000 I.U. daily—of *pityriasis rubra pilaris* [535, 541], *pachyonychia* [540, 605], *acne vulgaris* [542, 543], *senile keratoses* [543] and *lichen ruber planus* and *psoriasis* [544].

The Effect of Lack of Vitamin A on the Eyes. The earliest detectable result of a lack of vitamin A on the eyes is a slight impairment of dark adaptation, the underlying physiology of which has been described on p. 40, and the methods for its detection and its differential diagnosis on p. 60, so that here it is only necessary to point out that the condition is seldom noticed, especially by those living in well-lit towns, until it has become very pronounced. Few patients with mild night blindness are sufficiently observant to realize that their twilight vision is better in the early morning than in the evening, though this valuable diagnostic point was noticed years ago in badly fed slaves [488]. Sometimes, however, as in poorly fed Newfoundland fishermen [1], night blindness comes on suddenly after a long day in very bright sunlight, so that there is complete blindness in the evening dusk though normal people are seeing perfectly. Sudden changes in diet, such as that of Orthodox Russians during the Lenten fast, may also precipitate frank night blindness [525].

Infants and young children are far more liable than are older children and adults to keratomalacia and permanent blindness due to lack of vitamin A. The first change in the eye which can be observed clinically is a drying or xerosis of the eye, known as xerophthalmia, which is generally accompanied by photophobia. In early cases the condition may be unmasked by holding the eye open for a minute or two when the lustre is lost through the rapid drying of the eye. This is due to the metaplasia of the conjunctival epithelium stopping the secretion of the mucous cells, which can be confirmed by finding keratinous cells in gentle scrapings of the eye [262]. Wrinkling of the conjunctiva can also be seen at an early stage. Later the conjunctiva may become thick and leathery from the gross keratinization. (The diagnostic value of slit-lamp examinations of the conjunctiva is discussed on p. 60.)

Once xerosis is present no further changes may occur, or the whole eye may be rapidly destroyed. The patient suddenly complains of a feeling of a grain of sand in the eye, which is followed by photophobia, lacrymation, inflammation, and a sticky discharge. These symptoms are due to the dry thickened conjunctiva wrinkling up, which gives the sensation of a foreign body in the eye, while the discharge is caused by a secondary infection [262].

The condition is now serious, since the stroma of the cornea may become oedematous, necrotic, and weak, so that keratomalacia occurs. The first sign is a spreading opaque white spot in the cornea which can grow so rapidly that the sight may be lost in a few hours. At the same time secondary infection may lead to ulceration and finally perforation of the weakened cornea, with destruction of the eye. This fulminating course is commonest in infants [468], even having been present in one of Maxwell's [314] at birth, but it can occur at any age [279, 280].

One-quarter of Sweet and K'Ang's cases [262] and of Blegvad's [468] had one eye involved for several weeks before the other.

The vascular changes which occur in the cornea and their possible relation to a local secondary deficiency of riboflavine are discussed on p. 34.

De Haas and Meulemans [510], investigating vitamin A in the blood of children suffering from xerophthalmia, found none in six and 4, 6 and 8 I.U. per 100 c.c. in three and 22 I.U. per 100 c.c. in one with perforation of the cornea.

Recovery, apart from scarring of the cornea, is always possible if perforation has not taken place. The treatment of acute cases in infancy is to give urgently vitamin A. Aqueous suspensions (p. 18) when available should be used: a first dose of 100,000 I.U. being followed daily by a 10,000 I.U. for week, after which cod-liver oil in normal amounts of about two teaspoons should be sufficient. Four to six times these quantities are needed by older children and adults. When only cod-liver oil is available as much must be given as the patient can tolerate, while highly potent fish liver oils should be used in doses about fifty per cent. higher than those given in aqueous suspensions. Injections act too slowly (p. 27) to be as valuable as oral treatment.

Local treatment of the eye should be confined to saline irrigations when there is discharge, given with great gentleness by the doctor himself or a nurse of experience, since the weakened cornea may easily be ruptured [546]. Sulphonamides and antibiotics are not necessary, nor are local applications of cod-liver oil of value since Rötth [189] found that in rats with xerophthalmia cod-liver oil placed only in one eye cured the other just as quickly, while the general condition of the animals improved, which shows that the effect of vitamin A was not local but general through its absorption into the body.

In infants [468] bronchopneumonia, due to infection of the metaplastic epithelium of the respiratory tract, is the usual cause of death. At all ages [280, 505, 545, 546] gastro-intestinal disorders and intestinal worms, being both a cause and consequence of lack of vitamin A, often precede or follow xerophthalmia.

Prolonged mild deficiencies of vitamin A lead to the formation of "Bitot's" spots which were first described by Bitot [549] in 1863 in patients in the foundling hospital in Bordeaux: "*Un assemblage de points d'un blanc éblouissant, produisant comme une tache nacréée, ou argentées à côté de la cornée transparente.*" The "taches" fluctuated in size with the degree of night blindness, being larger when it was most severe. They were always placed just lateral to the cornea on the equator of the eye, generally being in the shape of a triangle whose base was slightly concave and about 5 mm. in

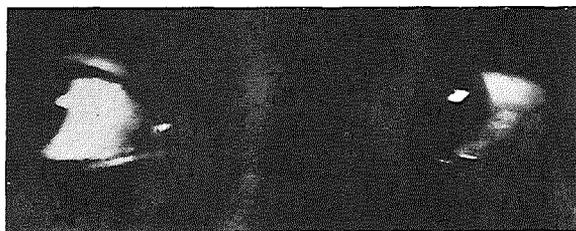


FIG. 25. Bitot's spots in a Singhalese child. The left eye shows thickening, pigmentation, and white striated patches of the temporal bulbar conjunctiva. This was also present in the right eye. The nasal bulbar conjunctiva was free from these changes.

width, while the sides of the triangle were about 8 mm. Sometimes, however, they were round or oval, and might also be composed of fine lines as well as dots. Very occasionally a few scattered dots were seen to the inner side of the cornea. Bitot also pointed out that the "taches" were a definite change in the conjunctival epithelium itself, which might have either a rough or striated surface. Nicholls and Nimalasuriya [550] from extensive observations on several hundred cases in children in Ceylon have confirmed this picture, though night blindness was not usually present. They state that the first changes are a slight thickening and pigmentation of the scleral conjunctiva, which is followed by a heaping up of epithelial cells which stand out white against the pigmented background, and are generally striated, looking like "a dab of chalk paste striated with a pin." They are only seen on the inner side of the cornea in two per cent. of cases; they never ulcerate; never occur over the cornea itself, though generally approaching to within 1 or 2 mm. of its edge; and mostly appear in only one eye. May and Wolffe [526] reported that in their English infant the triangular Bitot's spots were on each side of the cornea and appeared to be covered with foam, and Aykroyd and Rajagopal [517] describe the Bitot's spots in their Indian children as yellowish foaming patches. Nicholls [550] suggests that this foamy appearance is due to a very rapid and loose piling up of epithelial cells which does not occur in communities where a chronic deficiency of vitamin A for generations has led to a more chronic and slow reaction to the deficiency. The increased general pigmentation of the sclera—also noted by Mu [520]—probably only occurs in dark races, and its diagnostic value before Bitot's spots appear is still uncertain. Early conjunctival changes only visible with a slit-lamp are discussed on p. 60.

Treatment is with large doses of cod-liver oil or vitamin A concentrates, recovery generally being slow [517, 550], as it is in phrynoderma, though it may only take two weeks in young children [505].

Blumenthal [554] describes a very peculiar keratitis which he has observed frequently in the South African Bantu. He ascribes it to malnutrition and especially to lack of the vitamin B complex. In the uncomplicated form in children "the cornea dissolves away quietly and insidiously at one small point," followed by a prolapse of a knuckle of iris without pain, discomfort, inflammation or infection. In adults the central or whole cornea may soften and expand, with or without compensatory thickening and rupture. Recovery especially in children is dramatic and complete when "mixed vitamins" are given.

Asthenopia is stated to be often caused by lack of vitamin A, patients continuing, in spite of the usual treatments, to complain of a dislike of bright lights, headaches and difficulty in seeing while driving or at theatres, or fatigue whenever they use their eyes. Impaired dark adaptation is present. Cordes and Harrington [551] gave 30,000 I.U. of carotene daily for a month with complete relief of all symptoms in seventy-nine per cent. of eighty-two cases between the ages of thirteen and seventy-three, though as some of their patients became yellow during the treatment vitamin A should be used if such large doses are really necessary. Similar results have been reported by Vanzant [552], and we have found 48,000 I.U. of vitamin A daily very effective. Follicular conjunctivitis in children has been stated to be due to lack of vitamin A and to respond rapidly to 13,000 I.U. daily [553].

The Effect of Lack of Vitamin A on the Nervous System: Lathyrism. The experimental work discussed on p. 42 shows that deprivation of vitamin A causes degeneration of the nervous system in animals, though the degeneration is surprisingly severe before there are any clinical symptoms. In man no neurological symptoms are generally associated with those conditions, such as keratomalacia, where they would be expected if the central nervous system were affected by lack of vitamin A. There is, however, the possibility that in man, as in animals, the degeneration gives such tardy symptoms

that they seldom occur before the deficiency has been remedied or death occurs, unless the degeneration is accompanied by a second deficiency (p. 43). This is supported by Nicholls [555], who in Ceylon constantly found degeneration of the spinal cords of children dying with symptoms of a vitamin A deficiency, though he also found it in two children who had not been clinically deficient. He also points out that pregnant women in Ceylon often have transitory neurological disturbances which he is inclined to attribute to lack of vitamin A, since this is very common while beri-beri is rare. He previously reported [507] that prisoners with phrynoderma and eye signs generally had neuritis and diarrhoea; the latter condition may have caused or increased a deficiency of vitamin B₁ or both may have been further symptoms of lack of vitamin A (p. 77). In the same way as phrynoderma may require both lack of vitamin A and some other factor for its development (p. 67), Mellanby [361], from his work on puppies, has suggested that vitamin A is necessary for the health of the nervous system in man, its lack, however, only becoming important when a secondary deficiency or some toxin is present: so that beri-beri is accentuated by lack of vitamin A and gangrene in ergotism occurs alone when vitamin A is plentiful, but when it is not convulsions also appear, because the nervous system needs vitamin A to protect it against the ergot. Mellanby also suggested that lathyrism is a disease not only due to toxins which are always present in the germ of grain but also to lack of vitamin A, since puppies on a high cereal vitamin A deficient diet developed a more serious degeneration of the cord when they were also given rye germ and, in two cases, wheat germ or dried beans.

Lathyrism is a disease which in the past has been ascribed to the eating of a vetch of the *Lathyrus* family during periods of famine, either deliberately in areas where it is used as cattle food, or by mistake where it has grown among the corn. But the published accounts of the disease give such varying symptoms, and in several instances so definitely rule out the possibility of any kind of *Lathyrus* being the cause, that it appears certain that lathyrism is not one disease but a group of rather similar diseases, only sharing in common a background of famine and some form of paralysis of the legs.

The only cause common to all outbreaks of lathyrism is a deficiency of food which apparently weakens the resistance of the lower segments of the spinal cord to various toxic agents; though with proper nutrition these would be harmless. The particular symptoms of each outbreak depend on what particular toxic agent is present; these have been extensively studied, while some work has also been done on what particular ingredient of the diet is deficient. Considering the latter first, the importance of vitamin A is stressed by Young [557], who not only found night blindness common in a village suffering from lathyrism, but also noticed that the disease did not occur in neighbouring villages where the diet contained as much *Lathyrus* but more vitamin A, fish and meat; while Shah [558] has reported great improvement in patients when vitamins A and D were given. Apart from night blindness no deficiency diseases have been reported as occurring with outbreaks of lathyrism, so that it seems improbable that lack of any vitamin, apart from vitamin A, is a factor. The nervous degenerations caused by vitamin E (p. 647) and those due to lack of some unknown factor reported by Wintrobe and others [359] do not, it is true, give any except neurological symptoms, but they are in essence progressive while lathyrism is a disease which never progresses beyond the initial paralysis. But, of course, lack of other substances in the diet apart from vitamins may be important, which is suggested by Basu and others [559], who found that the seeds of *Lathyrus sativus*, which often form the staple food in famine villages, are a very poor source of protein, being especially deficient in tryptophane. Minchin [560] believes that some protein deficiency may be the important factor, while McCarrison's experimental work with pigeons [561] on the effect of manurial conditions on the nutritive value of millet and wheat suggests that in areas

where husbandry is poor grain may not only be less nutritious, but even toxic.

Of the factors which actually injure the already debilitated nervous system, and so are the immediate precipitating cause of lathyrism, some toxic substance in vetches of the *Lathyrus* family has for long been postulated. As early as 1770 an epidemic in France causing paralysis of the legs was thought to be caused by eating vetches, and a similar outbreak was seen in England in 1785, while in 1840 *Lathyrus cicera* was reported to cause paralysis when fed to rabbits [562]. In 1882 *Lathyrus cicera* or *clymenum* was held responsible for an outbreak in Syria which affected 1,200 people [563], and in the long French review of recent lathyrism in Syria *Lathyrus sativus* is held by Trabaud and his colleagues [563] to be the cause of lathyrism even when only a few seeds have been eaten. But they found this vetch harmless for the lower apes, camels, cows, fowls, rabbits and dogs ; as did Basu [559] and others [564] for rats, and Anderson [565] for monkeys and other animals. Lewis and others [567] investigated eight species of *Lathyrus* : some were toxic for rats and mice, but a disease similar to lathyrism was not produced. None of these experiments, however, are really conclusive, since the animals were not on a famine diet analogous to the diets taken by men prior to developing lathyrism, nor was the vetch grown in the same soil as that around villages where lathyrism occurs. On the other hand, Anderson and others [565] showed that the seeds of a weed, *Vicia sativa*, and also an alkaloid extracted from them, affected the nervous system of monkeys and other animals, while Shah [558] investigating an outbreak of lathyrism found that seeds of *Vicia sativa* but not of *Lathyrus* had been eaten mixed with the corn. Young [557], however, reports that his cases had eaten *Lathyrus* but little or no *Vicia sativa*, and Minchin [550] describes " lathyrism without *Lathyrus*," as does Gopalan [566], while Spillane [568] saw a condition like lathyrism in Japanese prisoner of war camps where there was starvation but no *Lathyrus* had been eaten.

To sum up : it seems that lathyrism is due to lack of vitamin A and a poor diet paving the way for toxic agents to attack the nervous system, these toxic agents not always being the same and therefore not always causing identical damage and symptoms. Probably they are sometimes alkaloids found in the seeds of *Vicia sativa*, sometimes alkaloids present in the seeds of various kinds of *Lathyrus* grown in particular soils, sometimes no clue is given as to their nature, so that one is forced to consider whether lathyrism may be due to an infection by some organism which is only pathogenic when the diet is impoverished.

Men are generally reported to be affected far more frequently than women [557, 560, 564], though not always [563], and the condition occurs at any age [557, 563], though mothers do not transmit the disease by suckling their children [563].

The clinical picture of lathyrism varies in every outbreak. Minchin [560], whose patients had eaten no *Lathyrus*, found that the onset of the paralysis of the legs may be sudden or slow ; McCarrison [564] says the incubation period on bread containing *Lathyrus* is two to six months, while Trabaud [563] states that four days to four weeks after eating *Lathyrus* a tingling starts in the legs, which progresses to a tremor that is present at rest but changes to a spastic stiffness on walking. The patients may never be ill in themselves [560, 563], but Young [557] noted that there is a previous fever and Shah [558] that the condition begins with gastro-intestinal symptoms.

Shah [558] reports that his cases had both spastic paralysis of the legs and sensory impairment and that occasionally the arms were involved, which also occurred in a few cases of Minchin's [560], but all other reports emphasize that there is a pure upper motor neurone involvement of the legs alone, so that the patients walk as if balancing along a rail [557] without any loss of their sense of position. The condition of the reflexes is very puzzling, since

Minchin [560] observed that while the legs were spastic with extensor plantar responses, the cremasteric and abdominal reflexes remained normal, even in some cases where the arms were affected. Trabaud [563] found completely normal reflexes, including the plantar responses, though there was spasticity and clonus of the legs. The cerebrospinal fluid in new cases gives a paretic curve [560] and in old cases is normal [563].

Trabaud suggests that the damage is localized in the pyramidal tracts to the lower limbs because of changes in chronaxie. Minchin [560] noted that the bladder was occasionally affected, but other observers have not reported this.

Sexual impairment has been noted by earlier writers, but it is not mentioned in recent English reports from India, while the French observers [563] point out that lathyrism is an ideal disease for the Eastern male, since while it prevents him from working it in no way hinders him from enjoying those pleasures which are necessary for continuing his family tree. Neither pregnancy nor lactation are affected [563].

The disease is never progressive after a few days or weeks, and is generally

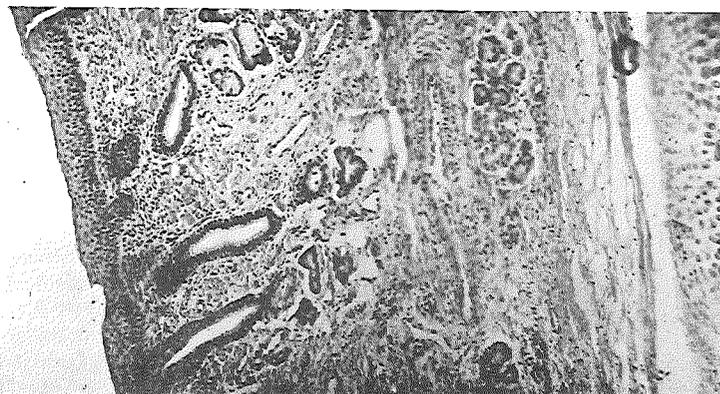


FIG. 26. Trachea of an American infant showing stratified keratinizing epithelium.

considered incurable, though Shah [558] reports great improvement with cod-liver oil.

Other Effects of Lack of Vitamin A. Frequent respiratory infections [262, 468] and diarrhoea [262, 280, 468, 505, 524, 545, 546] are the only symptoms, apart from those of the eyes and skin, which are frequently reported as occurring with a deficiency of vitamin A. Neither of these are of much value in diagnosis, though the cough is typical, being unproductive because of the blocking of the mucous glands by desquamated epithelia, and its cause can sometimes be confirmed by examination of nasal scrapings for metaplastic changes in the cells [262]. Children with xerophthalmia usually die of bronchopneumonia [468]. The diarrhoea has been cured by Pillat [524] and Sweet and K'Ang [262] with fats and vitamin A.

Children with mild chronic deficiencies are lively and active [549, 550] and have been described as podgy [524], but they are small for their age and have a high death-rate [550].

Post-mortem examinations in man (Figs. 8, 10, 11, 12) emphasize that there is a great individual variation in which epithelial surface is most affected by lack of vitamin A, as indeed has been seen clinically by the frequency of either the skin (p. 65) or the eyes (p. 72) being damaged separately. This means that the diagnosis of a deficiency need not be discarded because all

the typical changes are not present. Sweet and K'Ang [262] have carried out the largest number of post-mortem examinations of patients dying with a definite deficiency of vitamin A. Of seventeen cases eight had metaplasia of the epithelium of the larynx or trachea, which sometimes even involved the small bronchi and the mucous glands, whose ducts were blocked by the desquamated epithelium. In five cases the epithelium of the œsophagus was affected, though the rest of the digestive tract was normal, apart from the pancreatic ducts in one case. Changes were observed in the renal pelves of three cases, but the ureters and bladders were normal, and so were the prostates, except one, of ten males. Of seven females there were changes in the uterine mucosa of one. Hæmosiderosis was present in the liver and spleen of half the cases, but this was not regarded as being due to lack of vitamin A. In Wilson and Du Bois' child [273] the respiratory tract was extensively involved and so was the pancreas, the ducts being blocked by the shed cells and the acini cystic. The islets of Langerhans were normal, but in the thymus Hassall's corpuscles were enlarged. There was metaplasia of the renal pelvis, which was also noted in Boyle's infant [293] together with tracheal changes and defective tooth formation.

The relationship of vitamin A to other diseases and the part which it plays in the normal physiological function of the various systems of the body have been discussed in the earlier sections of this chapter.

CAROTINÆMIA, XANTHOSIS CUTIS AND HYPERVITAMINOSIS A

Carotinæmia. This is a word which is loosely used to mean excess of carotene in the blood, though what constitutes an excess is unknown. Normal values are roughly 50 to 240 micrograms per 100 ml., depending on the diet [569]. When the carotene rises beyond a certain level in the blood, which varies with the individual, xanthosis cutis, or yellowness of the skin, occurs and as this is the symptom which draws attention to the excess of carotene in the blood, carotinæmia is increasingly used as a synonym for xanthosis cutis. This is incorrect as many patients with lipoid nephrosis or nephritis have a carotinæmia which does not discolour their skin though it would do so in normal people. The explanation of this is obscure: it may be due to the glands of the skin failing to secrete carotene owing to their dysfunction caused by the nephritis or to the carotene being anchored in the abnormal nephritic blood.

Carotinæmia without xanthosis cutis is probably a harmless condition, though Clausen [97] in a very thorough investigation showed that both very high and very low levels of carotene in the blood appeared to decrease slightly the resistance to respiratory infections. In animals Davies and Moore [123] could not produce a toxic state by giving huge amounts of carotene, although Sherwood and others [389] report that doses of carotene equal to about 1,500 I.U. of vitamin A stop œstrus and libido in rats.

Xanthosis Cutis. Stannus [409] in 1929 gave an extremely valuable review of clinical reports about this condition up to that date, while Joseph's paper [570] in 1944 should be read for its bibliography and summary of later clinical and experimental work.

The causes of the high level of carotene in the blood which leads to xanthosis cutis are pathological or dietetic; the former, which are mostly discussed elsewhere, include metabolic diseases, such as myxœdema (p. 50), tuberculosis [409], disturbances in lipoid metabolism (p. 50) and, possibly, failure to oxidize carotene [409, 570]. The dietetic cause of xanthosis cutis is simply the unduly high consumption of vegetables containing carotene such as happened in England in 1942 when the Ministry of Food, owing to a glut of carrots, advertised their virtues in every newspaper and even commissioned Walt Disney to draw the carrot family. In 1943 the carrot fly ruined the crop and propaganda and xanthosis cutis both declined.

The amount of carotene in the diet which causes xanthosis cutis varies with the individual. Two to three pounds of raw carrots weekly had been eaten by Thomson's patient [574] for eleven months, while an extra four pounds weekly for seven months was the smallest quantity which had been consumed by Almond and Logan's five patients [573]; this amount or its carotene equivalent in spinach had no effect on the colour of the skin after roughly two and a half months in Hoch's three experimental subjects [571], though double this amount did so within nineteen and twenty days in two subjects. Hoch noticed that the steady uniform rise in the blood carotene

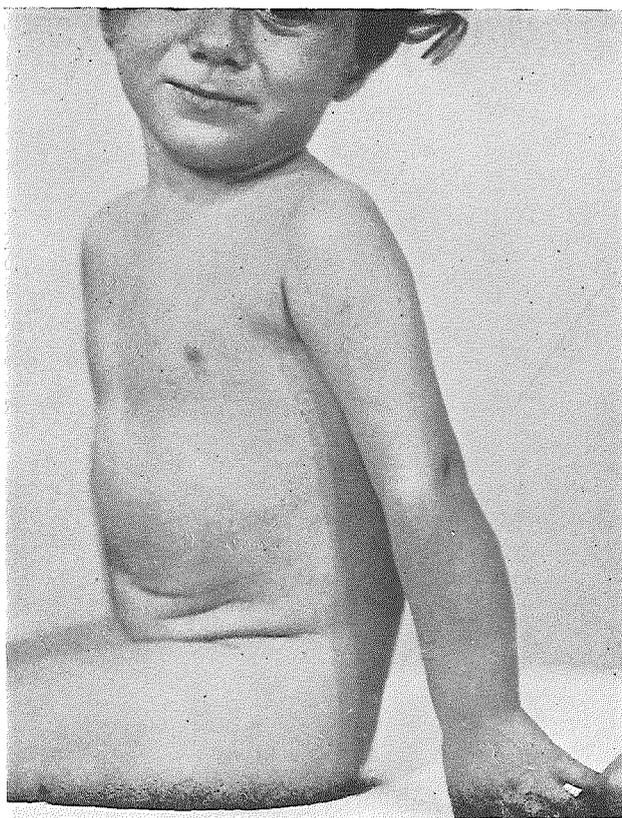


FIG. 27. Swellings in forearms and fissuring of lips of child of twenty-one months who had taken 200,000 to 300,000 I.U. of vitamin A daily. Blood contained 330 I.U. per 100 ml. of plasma.

was checked when xanthosis cutis developed, presumably due to its storage in the skin. The level of vitamin A was raised to the upper limits of normal—126 to 155 I.U. per 100 ml.—but not beyond.

The level of carotene in the blood at which xanthosis cutis develops differs widely in different people. Hoch [571] found in one experimental subject it was 375 micrograms and in another 470 micrograms per 100 ml. In cases of established xanthosis cutis the levels have been between 220 and 610 micrograms per 100 ml. [570], the former figure being within normal limits [695].

The curious canary yellow colour of the skin, which in severe cases becomes a deep orange, appears first in the palms of the hands and naso-

labial folds [409, 570, 573] and in other areas where sweating is most marked or the horny layer of the skin is thickest, such as the forehead, axillæ and groins or the soles of the feet and knuckles. The nails may become dark and flecked with brown, and darkening of the eyebrows, but not of the hair of the scalp has been reported [409]. The mucous membranes and gums are not affected. This distribution of the colour is due to the staining of the horny layers of the skin by the carotene excreted by the sebaceous glands [570, 573]. The conjunctivæ are never discoloured and the urine and fæces are normal, which should prevent the usual mistake of diagnosing xanthosis

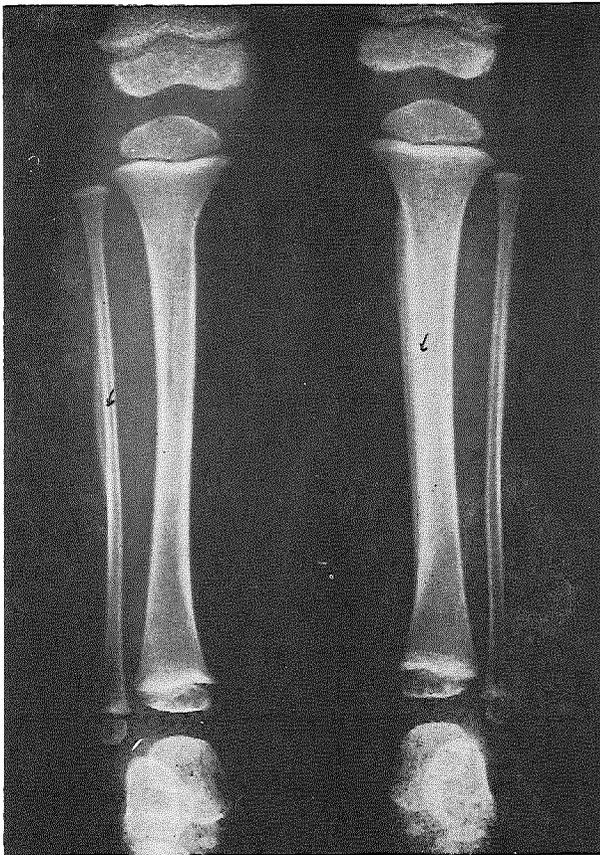


FIG. 28. Hyperostoses in the tibia and fibula of a girl of twenty-five months who had taken daily 150,000 I.U. of vitamin A for several months.

cutis as jaundice [573, 574]. Sucklings develop xanthosis cutis when their mothers suffer from this condition [573, 574], and also infants and children [409] when fed on carrots from necessity during war [409, 575], or because of the foolish belief that carrots are good for children (p. 12). The colour of the skin may fade away within a little over two weeks of resuming a normal diet or may persist for many months.

Xanthosis cutis, when not a symptom of disease, is generally a harmless condition both in infants [570, 573, 574] and adults [570, 573], though Henschen [572] reports a woman who, after eating two pounds of carrots daily, often scavenged from garbage, for six months developed a secondary anæmia and enlargement of the liver and spleen, which took many months

to disappear after her diet became normal. Josephs [570] believes that high blood lipoids and cholesterol and a lowered basal metabolic rate are usual in chronic xanthosis cutis while weakness, loss of weight, leucopenia and a low blood pressure may also occur.

Hypervitaminosis A in Man. *Acute poisoning* by vitamin A has only been reported in Arctic explorers who have obstinately eaten the liver of the polar bear or the seal in spite of this being forbidden by religion to the Eskimo and being shunned by his dogs and even the greedy raven. Rodahl [577] gives a very interesting historical account of outbreaks of poisoning in Arctic

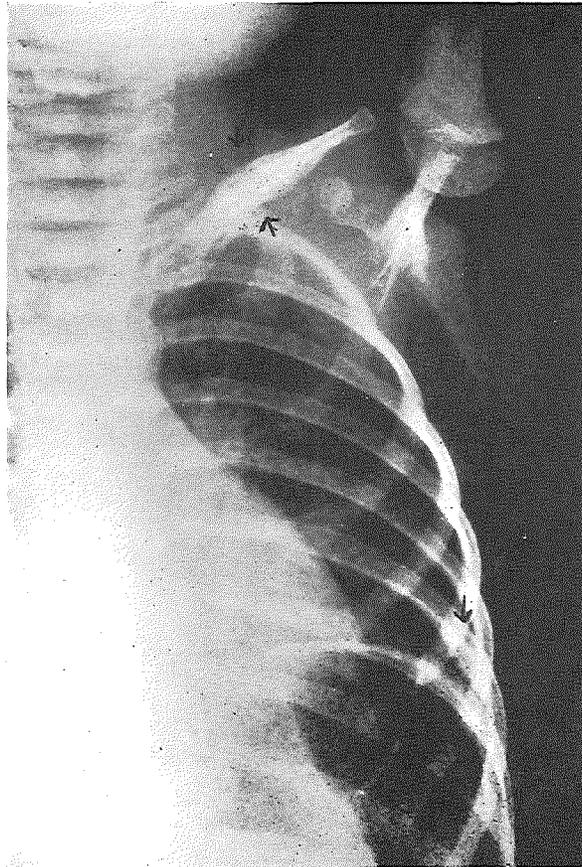


FIG. 29. Hyperostoses in the clavicle and ribs of the patient shown in Fig. 28.

expeditions and also the steps which he has taken to confirm earlier work by himself and Moore [576] that the poisoning is solely due to the enormous amounts of vitamin A which may be eaten in a meal of seal [578] or bear [576] liver : three-quarters of a pound often containing 7,500,000 to 8,000,000 I.U. The most constant symptom of acute poisoning is a desolating headache which comes on a few hours after the liver is eaten ; nausea, vomiting, diarrhoea and an indescribably bad taste in the mouth are also common together with drowsiness, sluggishness, irritability and an intense desire to sleep. Less usual symptoms are disturbances of vision, diplopia, flames before the eyes, dizziness, cardiac weakness, and tonic and clonic attacks of cramp. In severe cases peeling of the skin round the mouth starts after

twenty-four hours and may remain confined to the face, or the whole skin from head to foot may be involved. Getz [236] reports that 2,000,000 I.U. in a single dose may give a dull headache while Rodahl and Moore [576] mention a man who took about 6,000,000 I.U. daily for five days—that is four to five ounces of halibut-liver oil. He became severely ill, his main symptom being giddiness. The oil was stopped and he recovered within ten days.

Chronic poisoning in infants and young children [570, 579–584, 604] is a rare but serious condition which must be remembered now that concentrated preparations of vitamin A are freely available to the public, generally being sold with no warning by the makers that they are toxic: a serious example of commercial irresponsibility.

First described by Josephs [570] in 1944, chronic poisoning by vitamin A

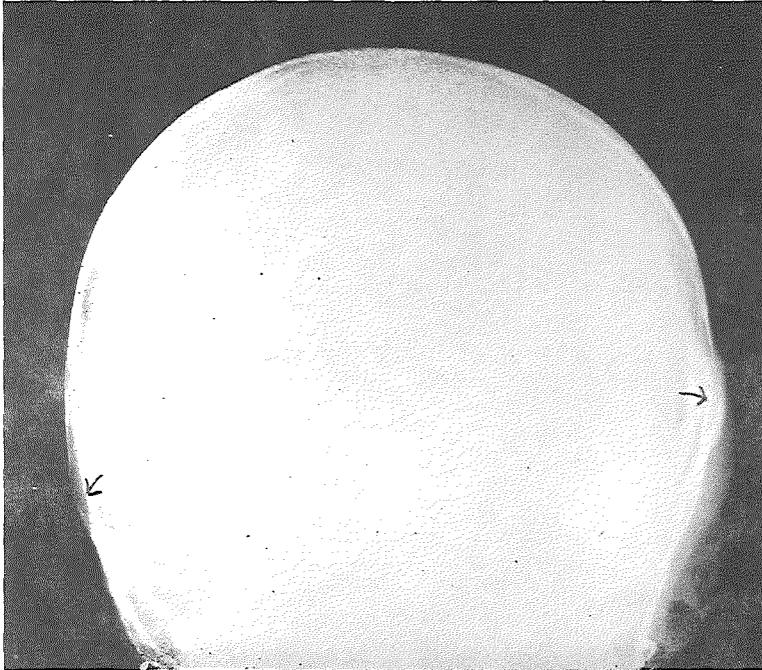


FIG. 30. Hyperostoses of the skull of a boy of twenty-eight months who had taken 250,000 I.U. of vitamin A daily for several months. The blood contained 1,040 I.U. per 100 ml. of plasma.

has become increasingly common in the U.S.A., as many as twelve cases being reported in 1950. The history in every case has been that the mother has given some concentrated preparation of vitamin A in enormous doses because she has not realized that the dose is measured in drops and not in the traditional teaspoon of cod-liver oil. Of course this is regarded by her as a normal part of her child's upbringing, so she does not mention it in giving the child's history, it only being discovered by direct questioning after the diagnosis has been made. Infants vary greatly in their resistance to vitamin A: one infant [570] took 500,000 I.U. daily for four months and remained clinically normal with only 110 I.U. per 100 ml. in his blood, while another infant [579] developed the full picture of poisoning after 75,000 I.U. daily for six months. A child who took 250,000 I.U. daily for nearly three years remained in moderately good health though he was left with a permanent enlargement of his liver and other abnormal physical signs [570].

The earliest and almost constant effect of excess of vitamin A is a dry rough itching skin with dry coarse sparse hair on the scalp, eyebrows and eyelashes. Growth and appetite remain normal though there may be some loss of weight. There is no constipation or polyuria. The child may be irritable and unhappy but this is a late symptom probably caused by the onset of pains in the limbs and feet, which are the usual reason for the limping child being brought to a doctor.

On examination the salient findings are very tender firm deep swellings in the forearms, and less constantly in the legs, feet and hands. Such swellings are not hot or discoloured or attached to the skin. Caffey [579], from very careful radiological examinations of seven children—some of whose pictures

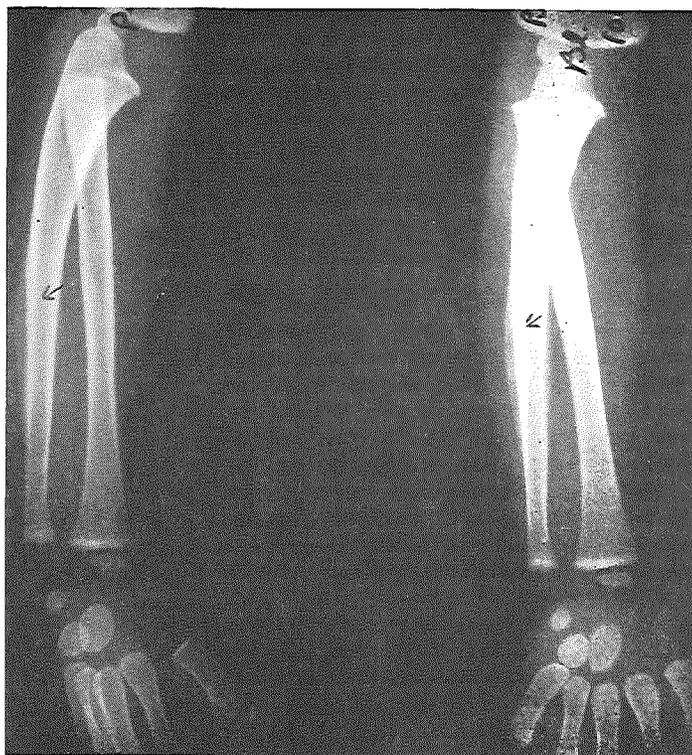


FIG. 31. Hyperostoses of the ulna of the patient shown in Fig. 30.

he has most kindly allowed us to reproduce (Figs. 27–32)—has shown that these swellings are cortical hyperostoses which have “a shell-like appearance with a zone of diminished density between the subperiosteal thin layer of bone and the external surface of the old cortex. After withdrawal of vitamin A this intermediate clear zone disappears: the hyperostoses then shrink on to the old cortex and fuses with it. These solid sclerotic cortical thickenings are in turn gradually resorbed from within but remain visible for many months after complete clinical recovery.” The constancy with which the ulnæ and metatarsals are involved—possibly due to their being exposed to trauma—is an important diagnostic point.

Other, but inconstant findings, are an enlarged liver, a raised sedimentation rate, an increase in serum alkaline phosphatase and lipoids, a decrease in serum protein and a moderate leucocytosis and anæmia. The temperature and urine are normal though the latter has been reported to contain small

amounts of vitamin A. The level of vitamin A in the blood has varied in different infants from 400 I.U. to 3,743 I.U. per 100 ml.

Recovery when the vitamin is no longer given is surprisingly rapid: within a week the child is no longer terrified of being touched and can run without pain. The level of vitamin A in the blood, however, may remain raised for months and the liver, rarely, remains enlarged.

The differential diagnosis from infantile cortical hyperostosis should not be difficult since this condition always appears before the fourth month of life and involves the face and jaw, while vitamin A poisoning has never been reported during the first year nor has it ever affected the face and jaw. Further points of difference are that in infantile cortical hyperostosis the temperature is usually raised, the metatarsals are but rarely involved and the level of vitamin A in the blood is normal. Other conditions such as



FIG. 32. Hyperostoses of the metatarsals of the patient shown in Fig. 30.

scurvy (p. 70), poisoning with vitamin D (p. 578), tuberculosis, syphilis and rheumatic fever are too unlike vitamin A poisoning to cause confusion if the possibility of the latter is borne in mind.

In adults and older children only one doubtful case [603] of chronic vitamin A poisoning has so far been reported, though Spiesman [341] states that only 40,000 I.U. daily may cause general malaise with loss of weight and appetite. But one of us often gives 144,000 I.U. daily for many weeks with no ill effects apart from an occasional and transient diuresis at the beginning of treatment, and doses of 300,000 I.U. daily for several months appear to be harmless for older children [467].

Hypervitaminosis A in Animals. Rodahl [585] in 1950 published a most comprehensive monograph on hypervitaminosis A which includes not only a complete bibliography and summary of the work of others but also a detailed account of his own work on rats, guinea pigs, mice, rabbits and cockerels. More recently Wolbach and his collaborators [587] also studied the condition in dogs. In passing it is of interest that polar bears [577] are often found with septic wounds, various bony deformities and abnormalities and also

partially united fractures with large amounts of callus, all of which could be caused by chronic vitamin A poisoning from the bear's habit of eating a whole seal at a meal, which would provide from 30 to 100 million I.U. of vitamin A.

The symptoms of acute poisoning in rats are general malaise, a staring coat, drowsiness, muscular weakness and reduced activity: even with a single dose as high as 1,500,000 I.U. death does not occur and no changes are found in the bones or internal organs.

The symptoms of chronic poisoning come on after several days on doses of 200 to 500 I.U. per gram of body weight. There is impaired appetite, weakness, loss of hair, soreness of the skin, swelling of the eyelids and exophthalmos, limping and spontaneous fractures. Biopsies show in most animals enlarged adrenals, degeneration of the renal tubules and general hyperæmia with extensive macroscopic and microscopic hæmorrhages throughout all the organs. This hæmorrhagic tendency is in part due to the hypoprothrombinæmia which is caused by hypervitaminosis A and can be prevented by giving vitamin K [415]. On the other hand hæmorrhages may occur with a normal prothrombin time and in spite of vitamin K [585].

The effect of vitamin A on ossification has already been discussed on p. 43, so that here it is only necessary to add that Fell and Mellanby [586, 609] have shown by *in vitro* experiments on the foetal bones of mice that hypervitaminosis A acts directly on the bone itself and not by any indirect effect. Maddock and Wolbach [588] claim that in rickets large amounts of vitamin A cause rapid repair of the metaphysis and resumption of the calcification of the cartilage matrix and osteoid tissue, but Rodahl [585] found that far from vitamin A aiding calcification in rickets it decreases it and is also more injurious than it is to normal animals. Though scurvy in guinea pigs produces lesions very similar to those caused by hypervitaminosis A, yet only slight protection against excess of vitamin A is given by ascorbic acid and lack of ascorbic acid only slightly increases the toxicity of vitamin A.

Fertility in hypervitaminotic rats is reduced, though there are no pathological changes in the genital organs. Normal young may be produced which have very high hepatic stores of vitamin A; these young if suckled by their own mothers develop hypervitaminosis A and die.

VITAMIN A₂

Vitamin A₂ is chiefly found in fresh-water fish and is probably of little more than theoretical importance, since it appears to be made from the same vegetable precursors as vitamin A₁ and to have the same biological functions. Biological tests show that the pure vitamin [589] has a vitamin A activity of 1,300,000 U.S.P units per gram [590] or about forty per cent. of the activity of crystalline vitamin A₁.

Its structure, according to Morton and others [591], is the same as that of vitamin A₁, except that it has an extra double bond situated in its ring. Shantz [589], however, after reviewing the evidence is dubious of this and all the other formulæ which have been suggested.

The absorption spectrum of vitamin A₂ gives two bands with maxima at 350 and 288 millimicrons, and with antimony trichloride a band at 693, with subsidiary bands at 660 and 635-640 millimicrons [149], the latter, according to Lederer and Rothman [592], causing difficulty in the estimation of vitamin A₁ by overlapping its band at 620 millimicrons. Cyclized vitamin A₂ gives bands at 391, 369, 349, 334 millimicrons which are almost identical with those of cyclized vitamin A₁ [149, 593], but the latter is not so well absorbed by alumina [593]. Cyclization does not alter the antimony trichloride spectrum of vitamin A₂ [593]. Shantz [589] using a purer preparation of vitamin A₂ gives very slightly different values to some of the above.

Vitamin A₂ is found in all fish liver oils, but those of fresh-water fish contain most [149]. In the latter there appears to be a fixed ratio between

vitamin A₁ and vitamin A₂, which is dependent on whether the fish is carnivorous, omnivorous, or migratory, but is not affected by age, sex, weight, or season [594]. Mammals, including man, birds and reptiles [595] do not store vitamin A₂ in their livers unless their diets, like that of the seal and otter which live on fresh-water fish, are very rich in the vitamin [599, 600]. Rats and frogs have been found to store vitamin A₂ when it has been given in large quantities [594], and Milas [282] has reported finding it in the olfactory mucosa of the steer. The precursors of vitamin A₂ are probably the same as those of vitamin A₁, since Morton and Creed [596] showed that perch after being fed on leaf carotene formed both vitamins. Vitamin A₂ is probably not converted in the body to vitamin A₁ [590].

The functions of vitamin A₂ are apparently the same as those of vitamin A₁, since vitamin A₂ is biologically active when fed to rats [590, 599, 600], though it is more toxic [601] and less efficiently stored [590]. Wald [597] found that it entirely replaced vitamin A₁ in the visual purple cycle of fresh-water fish. In migratory fish both vitamins are present in the eye, the proportion of vitamin A₁ being greatest in those fish which spawn in salt water [5-8]. Fluorescence microscopy is able to differentiate between the two vitamins in the tissues and examination under an ultraviolet lamp is an excellent way of separating livers which contain one or the other vitamin, vitamin A₂ giving a brownish-orange instead of a brilliant yellow fluorescence [58].

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CHAPTER II

THE VITAMIN B COMPLEX

UNTIL 1926 it was generally believed that "vitamin B" was a single entity. In that year Smith and Hendrick [1] showed that it consisted of two factors, a thermolabile anti-neuritic factor and a thermostable growth promoting factor. After the dual nature of vitamin B had been demonstrated the American Society of Biological Chemists decided to call the factors *vitamin B* and *vitamin F* respectively. In England the names vitamins B_1 and B_2 were suggested. Vitamin B_1 , or aneurine, was first isolated in 1926; its identity and synthesis took another ten years. It soon became evident that vitamin B_2 , the thermostable factor, was a complex.

Considerable confusion has resulted over nomenclature. Thus the name vitamin B_2 was subsequently given to the vitamin now known as riboflavine, which is also called lactoflavine on the Continent, and was formerly known as vitamin G in America. The situation has been further complicated by the fact that workers in different laboratories have discovered factors independently and each group has given the factor its own name. For example, pyridoxine has been successively known as factor Y, factor I, factor H, adermin and vitamin B_6 .

When the B vitamins were isolated as chemical compounds many were given chemical names indicating their nature, e.g. pantothenic acid, riboflavine, pyridoxine, etc., and these are used in the literature instead of the older names. The term "vitamin B complex" now refers to all the vitamins split off from the original "vitamin B" and identified chemically or by



FIG. 33. A case of vitamin B deficiency in a London woman before treatment. The photograph shows angular stomatitis and a red fissured tongue.

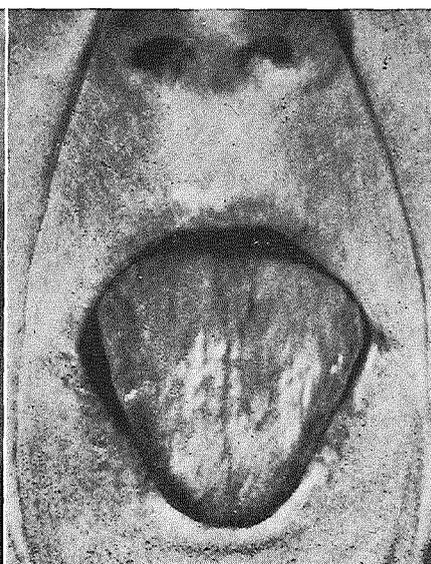


FIG. 34. Same case as in Fig. 33 after treatment with the vitamin B complex.

VITAMIN B DEFICIENCY



FIG. 35. A case of vitamin B deficiency in a London woman ; before treatment. Presenting complaints : depression ; insomnia ; memory impairment ; red cracked lips ; glossitis ; dermatitis on exposed parts, flexures and vulva ; photophobia. Poor dietary history.

FIG. 36. Same case as Fig. 35 after several weeks' treatment with the vitamin B complex.

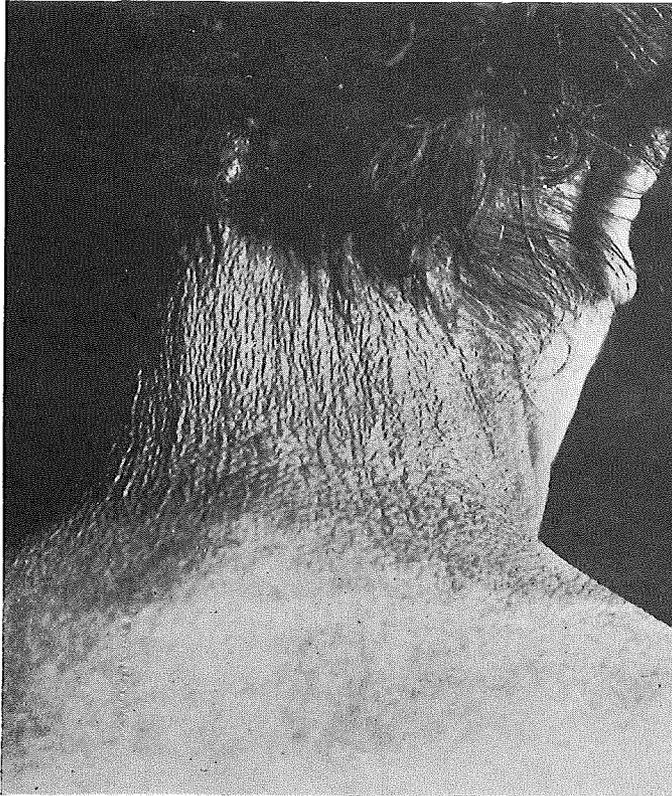


FIG. 37. A case of vitamin B deficiency in a London woman ; before treatment. Same case as Fig. 35 showing scaly pellagroid dermatitis at back of neck.



FIG. 38. Same case as Figs. 35 to 37. After treatment with the vitamin B complex.

their biological effects. A B vitamin has been defined as an organic substance which acts catalytically in all living cells and which is essential for the nutrition of higher animals [2].

The components of the vitamin B complex are :

Vitamin B₁, Aneurine or Thiamine (see p. 183).

Riboflavine, formerly Vitamin B₂ (see p. 285).

Nicotinic Acid (see p. 333).

Vitamin B₆.

Pantothenic Acid.

Biotin.

Folic Acid.

Vitamin B₁₂.

Folinic Acid or the Citrovorum Factor.

In addition there are several substances of somewhat doubtful status as B vitamins. They are :

Inositol.

Choline.

Para-Aminobenzoic Acid.

“ Vitamin B₁₃. ”

“ Vitamin B₁₄. ”

VITAMIN B₆

Isolation and Chemistry of Vitamin B₆. In 1934 György reported the existence of a factor distinct from the water-soluble factors known at that time, lack of which caused dermatitis or acrodynia in rats. It was called

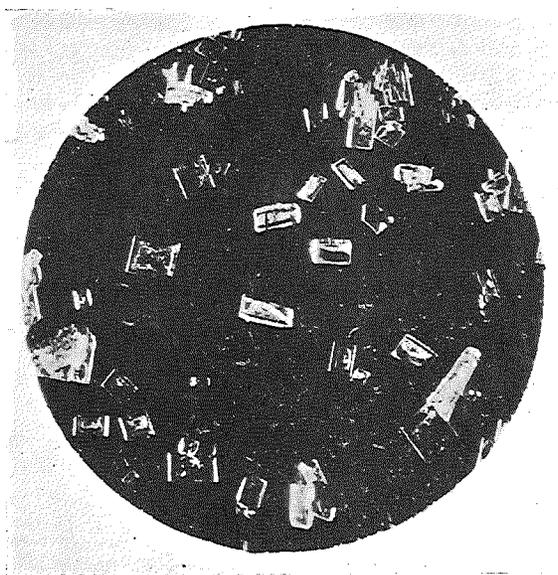
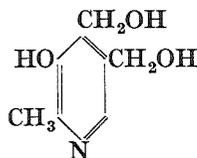


FIG. 39. Crystals of Vitamin B₆ (Pyridoxine).

vitamin B₆ and was later shown to be identical with the factor Y of Chick and Copping [63], the antidermatitis factor of Hogan and Richardson [64], the “ vitamin H ” of Boohar [65] and the factor I of Lepkovsky, Jukes and Krause [66]. The vitamin was isolated in 1939 by a number of investigators [69-71], and its structure determined in the same year [72-75]. It is 2-methyl-3-hydroxy-4 : 5-dihydroxymethyl pyridine,



The compound was synthesized in 1939 by Harris and Folkers [76] in America and by Kühn [77] and his co-workers in Heidelberg.

Since two other compounds related to pyridoxine, pyridoxal and pyridoxamine, show vitamin activity it is suggested that the term vitamin B₆ be used to signify the group.

Kühn suggested the name adermin in 1938, and in the following year György and Eckhardt [79] proposed that it should be called pyridoxine, a name which was adopted in 1940 by the Council on Pharmacy and Chemistry of the American Medical Association [80].

Pyridoxine forms colourless crystals, M.P. 206–208° C. (with decomposition), soluble in water and alcohol, stable to heat and alkali, but not to light, especially ultra-violet. It is more susceptible to the action of light in neutral and alkaline media [186]. The pH of a one per cent. solution of the hydrochloride is 2.44.

Chemical methods are generally inapplicable for assaying pyridoxine because they estimate pyridoxal and pyridoxamine which also have vitamin activity (p. 105). All three give different colours when treated with diazotized sulphanilic acid [3, 139]. Melnick [4] has modified the method for estimating pyridoxine only, by using borate. Microbiological methods depending on the stimulation of the growth of yeast and bacteria (*Streptococcus faecalis* and *Lactobacillus casei*) are also used. The yeast *S. carlsbergensis* estimates total pyridoxal, pyridoxamine and pyridoxine; *S. faecalis* estimates pyridoxamine and pyridoxal; and *L. casei* estimates pyridoxine only. It is thus possible by using these organisms to estimate all three of the B₆ vitamins [5–8]. A biological method involving the growth response of rats has been employed, but this estimates not only pyridoxine, but also pyridoxamine and pyridoxal [9, 10].

Distribution of Vitamin B₆ in Foods. Vitamin B₆ appears to be widely distributed in foods. It is mainly present as pyridoxal and pyridoxamine in hydrolysed foodstuffs; very little pyridoxine is present. In plant material pyridoxine occurs commonly with pyridoxal and pyridoxamine. Yeast, liver, cereal polishings, cereals and pulses are particularly good sources. There is an increase of this vitamin in cereals on germination. Fish is a moderately good source; vegetables and milk contain little.

Vitamin B₆ Content of Foodstuffs [5]

	Micrograms per gm.
Apples	0.26
Bananas	3.2
Bean, dried	5.5
Beef, muscle	0.77
liver	1.7
heart	1.2
Beets	1.1
Cabbage	1.2
Carrots	1.2
Cauliflower	0.20
Cheese	0.66
Chicken, leg	0.25
breast	1.3
Chocolate	0.23

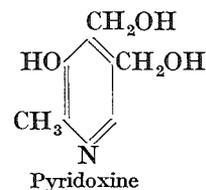
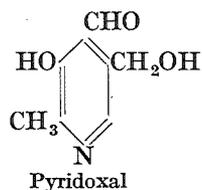
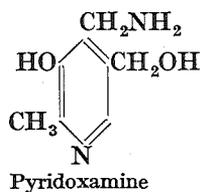
Vitamin B₆ Content of Foodstuffs [5]—continued

	Micrograms per gm.
Corn meal, white (maize)	0.54
Eggs	0.22
Grape-fruit	0.09
Halibut	1.1
Lamb, leg	0.81
Mackerel	2.1-2.7
Marmite	4.0
Milk, new	0.06
dried	0.50
Molasses	2.7
Mushrooms	0.45
Mutton, shoulder	0.18
Onions	0.63
Orange	0.8
Oyster	0.33
Peas, fresh	0.79-1.9
dried	3
Peanuts, roasted	3
Pork, loin	0.86-2.7
bacon	0.29-1.0
ham	0.19-1.7
Potatoes	2.2-3.2
Raisins	0.94
"Royal jelly"	2.4
Salmon	4.5
Sardine	1.6-2.8
Spinach	0.83
Strawberries	0.44
Tomatoes	0.6
Turnips	1.1
Veal	0.56-1.3
Wheat, whole	4.2*
National wheat flour (85%)	3.1*
white flour	1.8*
wheat germ	6-17.5
Yeast (dried)	3.0-4.0

* Ministry of Food figures [371].

PHYSIOLOGY AND FUNCTIONS OF VITAMIN B₆

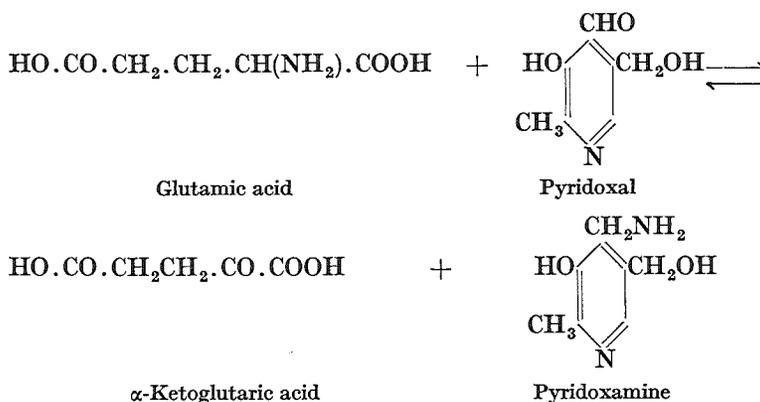
The term vitamin B₆ actually includes a group of compounds. In assaying sources of pyridoxine with *Streptococcus faecalis* R Snell, Guirard and Williams [17] found that some substances contained several hundred to several thousand times as much pyridoxine as could be accounted for by other means. This "pseudo pyridoxine" was found to have properties similar to pyridoxine. Later Snell [18] showed that "pseudo pyridoxine" consists of two derivatives of pyridoxine, pyridoxal or pyridoxine aldehyde, and pyridoxamine, the amine of pyridoxal.



Harris, Heyl and Folkers [20] determined the structure and synthesis of these compounds. The work of Gunsalus and his co-workers [22] has clearly demonstrated that pyridoxal phosphate is the coenzyme for amino-acid decarboxylase (which removes the carboxyl group from amino acids):

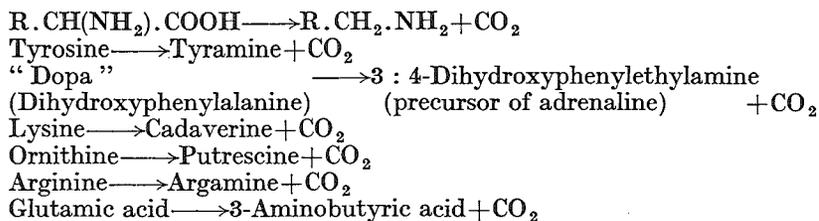


and for transaminase, an enzyme catalysing transamination. The latter is the biological process of transferring amino groups from appropriate amino-acids to keto acids or compounds with carbonyl groups. The following scheme has been suggested but recent work by Gunsalus and his co-workers [22] does not confirm this.



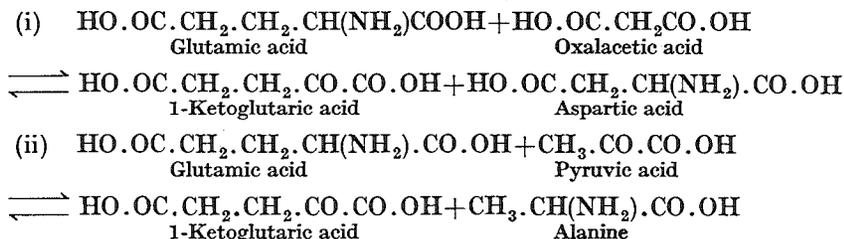
Only pyridoxal phosphate, and neither pyridoxamine phosphate nor pyridoxine phosphate, is active as a coenzyme [23]. Although the final proof of structure is lacking the active form of pyridoxal phosphate appears to be the alcoholic or 5-phosphate [23].

Reactions Catalysed by Pyridoxal Phosphate. 1. Amino-Acid Decarboxylation. This is probably an important means by which bacteria can metabolize some amino-acids. Pyridoxal phosphate has been conclusively shown to catalyse the following decarboxylations:



This has been shown only in bacterial enzyme systems. It is not known whether pyridoxal phosphate functions in the decarboxylation of other amino-acids in the mammal.

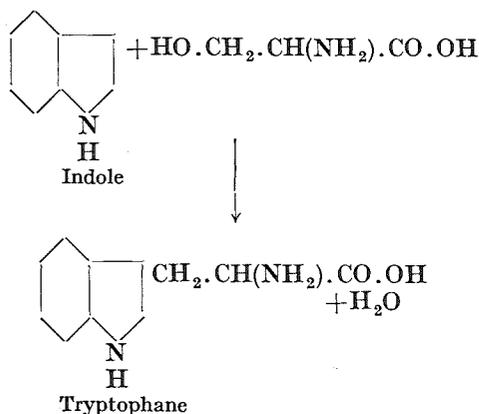
2. Transamination. Pyridoxal phosphate is a coenzyme for the glutamic acid—*aspartic acid* and the glutamic acid—*alanine* systems:



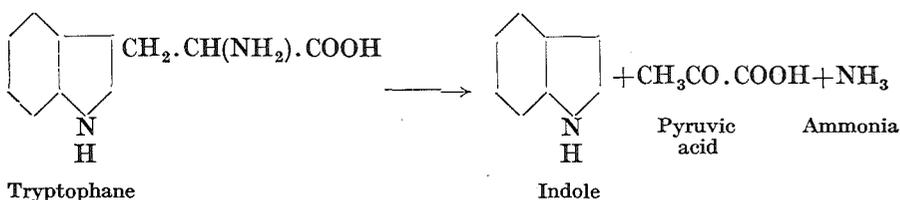
Another transaminase catalysing a glutamic-cysteic acid system is known.

The rate of transamination in the tissues of animals deficient in vitamin B₆ is considerably diminished [694].

3. **Synthesis and Degradation of Tryptophane.** Pyridoxal phosphate catalyses the synthesis of tryptophane from indole and serine [27].



The formation of indole from tryptophane by *B. coli* proceeds as follows [27] :



Vitamin B₆ and Amino-Acid and Protein Metabolism. The metabolism of tryptophane in animals and man is known to be altered profoundly in pyridoxine deficiency, large amounts being excreted as xanthurenic acid [29, 45], the amount of the latter excreted being proportional to the quantity of tryptophane in the diet [106]. Reid and his co-workers [41] studied the excretion of xanthurenic acid in pyridoxine deficient rats to determine the pathway of tryptophane to xanthurenic acid. Of the various compounds fed to rats only L-tryptophane and kynurenine appeared to yield xanthurenic acid, the administration of which was diminished by administering vitamin B₆. The metabolic pathway is apparently : tryptophane → kynurenine → xanthurenic acid. Pyridoxal may play a part in the metabolism of tryptophane, possibly by hydrolytic removal of the side chain. The possibility exists that the further metabolism of xanthurenic acid requires vitamin B₆, and that in the presence of the latter kynurenine is metabolized without the formation of xanthurenic acid as an intermediate to nicotinic acid [49] or to anthranilic acid and alanine [107]. Tryptophane, or casein, which contains considerable quantities, if administered in large amounts to the rat or mouse accentuates a deficiency of vitamin B₆ [28]. High protein diets also accentuate a vitamin B₆ deficiency, probably because of the increased intake of methionine and other thioamino-acids rather than of tryptophane [106]. Vitamin B₆ can prevent the depression of growth due to excessive doses of methionine [705].

Rats suffering from a prolonged vitamin B₆ deficiency are unable to convert tryptophane to nicotinic acid [42]. There is no direct evidence that

vitamin B₆ plays a role in the conversion of tryptophane to nicotinic acid in man ; it is involved, however, in its conversion into indole acetic acid-like compounds [46]. The vitamin B₆ deficient monkey shows deranged tryptophane metabolism and increased excretion of xanthurenic acid [154].

The observations of Sadhu and Brody [332] suggest that the specific dynamic action of an amino-acid depends not only on its nature but also on the presence of vitamin B₆ which functions as a prosthetic group in the catalytic system for transamination and on the presence of α -ketonic acids, such as pyruvic acid, which function as amino-acceptors.

There is an increased excretion of urea, ammonia, uric acid and creatinine in vitamin B₆ deficient animals and a lowered blood urea [56]. A lowered blood urea has also been observed in patients suffering from hyperemesis gravidarum, and this has been returned to normal levels by giving vitamin B₆ [78]. It has therefore been suggested that a vitamin B₆ deficiency or insufficiency occurs in the vomiting of pregnancy, although it is equally possible that metabolic changes occur associated with a low blood urea.

Recent work has linked vitamin B₆ to reactions involving the "unnatural" D-amino-acids. Some lactic acid bacteria show increased ability to utilize D- in place of L-amino-acids when grown in a medium rich in vitamin B₆ [81]. Armstrong and her co-workers [82] have shown that in the vitamin B₆ deficient rat the administration of D-amino-acids causes the excretion of a large amount of dietary nitrogen that would otherwise be utilized.

The sparing action of alanine on the vitamin B₆ requirements of some organisms was formerly presumed to be due to its utilization in the synthesis of pyridoxal [86]. It is now known that alanine is not a precursor of vitamin B₆, but that it is a product of its catalytic activity or can spare some metabolite produced by the activity of vitamin B₆ [90].

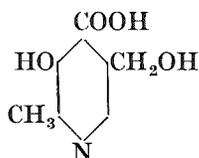
Cerecedo and his associates [126] conclude that in vitamin B₆ deficiency there is an abnormal metabolism of cystine and methionine. Pyridoxal phosphate is a co-factor in enzyme systems essential for the biosynthesis of cysteine [703]. There may be a connection between the integrity of the epidermis and the normal metabolism of cystine and methionine. This could explain the dermatitis occurring in vitamin B₆ deficient animals.

The ability to convert protein to carbohydrate is diminished in dogs on a diet deficient in vitamin B₆. This is shown by a decreased dextrose-nitrogen ratio in phloridzinated animals on a high protein diet ; the ratio returns to normal when the animals return to a diet adequate in vitamin B₆ [95]. An increase in the protein intake in the diet of the rat increases the requirement of vitamin B₆ [19].

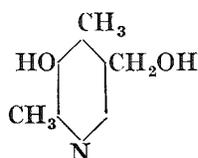
Fat Metabolism. Some of the early observations on vitamin B₆ deficiency related this vitamin to fat metabolism, particularly of the unsaturated fatty acids [86, 96]. There is no biochemical explanation of this in terms of the reactions catalysed by pyridoxal phosphate. If rats are kept on diets deficient in fat or vitamin B₆ they develop an acrodynia-like syndrome (p. 110) which is relieved both by ethyl linoleate, an ester of one of the essential fatty acids, or by vitamin B₆ [87, 118]. Very large doses of the essential fatty acids can also relieve the condition [121]. Gavin and McHenry [96] noted that the administration of vitamin B₆, aneurine, riboflavine and choline to rats led to an increase in body fat. The same workers observed that pyridoxine is essential for the synthesis of fat from protein [163].

Adrenal Cortical Damage and Water Metabolism. Pyridoxine deficiency produced by diets containing no pyridoxine or by feeding pyridoxine antagonists produces adrenal cortical damage, which is reflected in a disturbance of water metabolism [693].

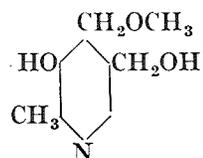
Analogues and Antagonists. A metabolite of pyridoxine, pyridoxic acid, or 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine, occurs in human urine [85]. It is the chief metabolic product of either pyridoxine, pyridoxal or pyridoxamine.



Pyridoxic acid

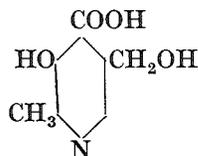


Desoxypyridoxine

Methoxy-
pyridoxine

As with other vitamins several analogues of vitamin B₆ are known that are antagonistic to its action. Desoxypyridoxine or 2:4-dimethyl-3-hydroxy-5-methylol pyridine [104] administered to the chick, rat, mouse, monkey, dog and man produces symptoms of vitamin B₆ deficiency. It also interferes with reproduction in the rat [123]. Almost one hundred per cent. mortality occurs in chick embryos treated with 1 mg. of the compound; this effect is prevented by simultaneous injection of vitamin B₆ into the egg [105]. At least eight other analogues inhibit vitamin B₆ activity. The most active of these is methoxypyridoxine, or 2-methyl-3-hydroxy-4-methoxymethyl-5-hydroxymethylpyridine [107], which produces deficiency symptoms in the chick, dog and monkey. Approximately four molecules of this analogue counteract the response to one molecule of pyridoxine.

Absorption, Storage and Excretion. On most natural diets most of the vitamin B₆ is ingested as pyridoxal or pyridoxamine [129]. It is rapidly absorbed from the digestive tract of man, dog and the rat. Little is known about the destruction of vitamin B₆ in the tissues. Small amounts of pyridoxine, pyridoxal and pyridoxamine may be excreted in the urine, but the major product of metabolism is 4-pyridoxic acid, or 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine [341].



Pyridoxic acid

This is the chief metabolic product of pyridoxine, pyridoxal or pyridoxamine [131]. Much of the earlier studies on the excretion of vitamin B₆ were made before it was appreciated that it occurs naturally as a complex and that it may be excreted in any of the four forms just mentioned. The method used for assay was not specific for pyridoxine and all the material estimated by this method was considered to be pyridoxine. Although the recovery of ingested vitamin B₆ in the rat was fifty to seventy per cent. [122], only ten to twenty per cent. of the dose was recovered when pyridoxine was fed to dogs or to human subjects [120]. Using microbiological assay methods by means of which pyridoxine, pyridoxal and pyridoxamine could be estimated [129], Rabinowitz and Snell [131] studied the excretion products of three male subjects on a normal diet after administering the different forms of vitamin B₆. Regardless of whether pyridoxine, pyridoxal or pyridoxamine were given the chief excretion product was pyridoxic acid. Pyridoxal gave rise to significantly larger quantities of this compound than did pyridoxine or pyridoxamine. Neither pyridoxal nor pyridoxamine were converted to pyridoxine. When pyridoxamine was administered both pyridoxal and pyridoxamine were excreted in approximately equal amounts and the administration of pyridoxine greatly increased the amount of pyridoxal and pyridoxamine excreted. The excretion of all products was at a peak

at two to five hours after administration of the compound and returned to normal values after eight to twelve hours. The highest recovery was seventy per cent., when pyridoxal was given; forty-five per cent. of the pyridoxine was recovered, but only thirty-one per cent. of the pyridoxamine. All forms of vitamin B₆ give rise to pyridoxal, which suggests that this is the form used in metabolic processes (i.e. as pyridoxal phosphate). A major portion of each form is eventually oxidized to 4-pyridoxic acid.

According to Linkswiler and Reynolds [681] vitamin B₆ is synthesized by man. It is presumably synthesized by the tissues and not intestinal organisms as excretion is greater than the intake even when sulpha drugs are administered to sterilize the gut. According to Lossy, Goldsmith and Sarett [704] the excretion of vitamin B₆ in man does not seem to depend on the general level of nutrition.

Requirements. Vitamin B₆ is known to be an essential vitamin for many species. It is probably essential for man since deficiency symptoms have been produced in human subjects by administering the antimetabolite desoxy-pyridoxine (p. 109), and these symptoms have been made to disappear rapidly by giving pyridoxine [84]. Probable requirements, calculated from those of different animal species, may be of the order of 1.5 to 2 mg. daily. The requirements are increased in experimental hyperthyroidism [162].

Vitamin B₆ Deficiency Symptoms in Animals. *Skin Lesions.* Rats fed on diets deficient in vitamin B₆ develop rat acrodynia, characterized by a symmetrical dermatitis affecting first the paws, then the tips of the ears and nose, which become red, swollen and cedematous. The matted fur on the backs of the hind paws desquamates, leaving a denuded, pale pink, glistening skin. Sometimes there is fissuring or ulceration at the corners of the mouth and over the tongue [26, 62]. Other species show deficiency symptoms but only the rat suffers from acrodynia. It is considered by some workers that rat acrodynia is not a specific sign of vitamin B₆ deficiency as they have failed to repeat these observations; others have prevented it by administering the essential fatty acids (p. 674).

Nerve Lesions. As far back as 1938 Chick and her co-workers [89] observed that rats kept on diets deficient in vitamin B₆ for periods of more than four months suffered from epileptiform convulsions, which could be prevented by administering vitamin B₆. Similar convulsions have also been reported in dogs [92] and pigs [128, 194]. These convulsions can be prevented by glutamic acid, an amino-acid taking part in certain biological transaminations and decarboxylations, for which vitamin B₆ is a necessary enzyme. It is also known that glutamic acid is essential for the metabolism of brain tissue, so that it is possible that the epileptiform convulsions occurring in vitamin B₆ deficient animals may be due to a derangement of a glutamic acid metabolism.

Wintrobe and his associates [193-195] and Swank and Adams [187] investigated the pathological changes in the nervous system of pigs suffering from vitamin B₆ deficiency. The animals developed stiffness of the hind legs eventually resulting in ataxia, with loss of tendon reflexes and failure to respond to painful stimuli; motor activity was not impaired. Degenerative changes were observed in the peripheral nerves, spinal roots, posterior root ganglia, sensory ganglia and in the posterior columns of the spinal cord; the brain stem and brain showed no changes. At the time the possible relation of these lesions to those seen in the nervous system in pernicious anaemia was commented upon. It is now known that the latter is in no way associated with a deficiency of vitamin B₆. Davenport and Davenport [141] have shown that vitamin B₆ deficiency decreases the electrical convulsion threshold which rises in deficient animals, but not in normals, after the administration of vitamin B₆. Glutamic acid also increases the threshold but tryptophane, which intensifies vitamin B₆ deficiency, lowers it. In severe deficiency pyridoxine causes only a slow rise in the electrical convulsion

threshold unless glutamic acid has been administered previously. These facts suggest that maintenance of transaminase activity is critical for a high electrical convulsion threshold.

Hæmatological Changes. Vitamin B₆ deficient animals become anæmic. This has been demonstrated in the dog, pig and monkey [92, 93, 112, 140, 153, 164, 194, 736]. A mild anæmia also develops in the deficient chick [145], accompanied by a decreased clotting time, hyperprothrombinæmia and a small spleen. The anæmia in dogs and swine is microcytic and slightly hypochromic with a considerable fall in hæmoglobin, which may fall as low as 1.4 grams per 100 ml., and in the packed cell volume. The Price-Jones curve is shifted to the left and an irregular reticulocytosis may appear. The bone marrow is hyperplastic. Hæmosiderosis of the spleen, liver, and bone marrow has been reported [194]. Elevated plasma iron levels have been observed in dogs [93, 164]. In the vitamin B₆ deficient pig, iron is absorbed but not utilized for hæmopoiesis [331]. The anæmia in deficient animals responds to treatment with vitamin B₆, although some investigators maintain that this vitamin alone is insufficient [112]. It does not respond to iron. In human subjects kept on diets deficient in vitamin B₆ there was no evidence of anæmia [84].

Anæmia due to a deficiency of vitamin B₆ has some of the features of Mediterranean anæmia (thalassæmia, Cooley's anæmia). In both the red cells are hypochromic and microcytic, the serum iron is raised, and iron-containing pigment is found in the tissues. Vitamin B₆ is of no value in the treatment of human anæmia of nutritional origin, or the anæmia of nephritis, aplastic anæmia, pernicious anæmia or the anæmia of infection [150].

Various changes in the white cells have been recorded. In the dog and monkey there is a leucocytosis, an absolute increase in the number of circulating neutrophils and an absolute decrease in the number of leucocytes [153, 161].

Vitamin B₆ deficiency in the mouse results in agranulocytosis and lymphopenia [166]; this also occurs in the mouse with transplanted leukæmia. In the monkey vitamin B₆ deficiency produced by administering desoxy-pyridoxine causes atrophy of the thymus, lymph nodes and bone marrow, leucopenia and lymphopenia [419]. The efficiency of phagocytosis by the leucocytes in the blood of animals deficient in vitamin B₆ is seriously impaired [345].

The anæmia of vitamin B₆ deficiency is not due to interference with tryptophane synthesis (p. 107), because anæmia can be produced by a deficiency of this amino-acid and it does not resemble that due to vitamin B₆ deficiency [150].

A high incidence of poikilocytosis in certain dairy cattle in Michigan has been observed [191]. It is stated to disappear by feeding supplements of yeast or vitamin B₆ [192].

Cardiovascular Changes. In the rhesus monkey vitamin B₆ deficiency produces widespread arterial lesions resembling those of human arteriosclerosis [154, 156]. In both there is accumulation of a mucoid metachromatic material in the intima and associated proliferation of cells and deposition of collagen and elastic fibres in the mucinous matrix (Figs. 40 and 41). The close resemblance between the pathology in the two conditions does not prove, however, that vitamin B₆ deficiency is necessarily concerned in the genesis of human arteriosclerosis.

Other Changes. Corneal vascularization [180], hæmaturia [219], fatty degeneration of the liver [194], degenerative changes in periodontal structures [216], and poor breeding performance [182] have been described in vitamin B₆ deficient animals. Lesions of the glomeruli and kidney tubules, resulting in hæmaturia, occur in pyridoxine deficient rats [695]. Complement titre and the formation of specific immune bodies (γ -globulin) are disturbed during the early stages of vitamin B₆ deficiency in rats [179].

Vitamin B₆ deficiency has been produced in rats by feeding excess of aneurine and flour of high extraction rate (seventy per cent.) [184, 185].

Human Vitamin B₆ Deficiency. Hawkins and Barsky [208] attempted to produce vitamin B₆ deficiency in human volunteers by feeding diets deficient in the vitamin, but the period of deprivation was only two months and was probably not long enough. The only changes observed were a rise in the total white cell count and a decrease in neutrophils. The mental confusion and depression observed could have been due to the monotonous diet. Gellhorn and Jones [188] made similar attempts to produce a deficiency by administering desoxypyridine to six patients with lymphosarcoma and acute leukæmia. Two patients had epileptiform convulsions, but there was no true evidence of vitamin B₆ deficiency as there was no abnormality of tryptophane metabolism as measured by the xanthurenic acid excretion [189].

Mueller and Vilter [190] succeeded in producing deficiency symptoms in eight subjects given doses of 60 to 150 mg. of desoxypyridoxine and a diet poor in the vitamin B complex. Seborrhœa-like skin lesions developed about the eyes, nose and mouth, and erosions appeared about the mouth resembling ariboflavinosis. Glossitis and stomatitis resembling the lesions of nicotinic acid deficiency (sore, swollen, red tongue and buccal mucous membrane) were also observed in some of the patients, one of whom developed nausea, vomiting, weakness and dizziness. These symptoms could well have been due to the toxic effect of the desoxypyridoxine. The lesions did not respond to riboflavine, nicotinamide or aneurine, but disappeared within three days of administering pyridoxine. The ratio of desoxypyridoxine to pyridoxine was approximately 1 : 1. The only change of note in the hæmatogram was a mild, absolute lymphopenia. Hypochromia, siderosis and the other changes seen in animals suffering from vitamin B₆ deficiency (p. 110) were not observed nor were xanthurenic acid or kynurenine found in the urine of any of the patients unless large quantities of tryptophane were administered [735].

Toxicology and Pharmacology. Vitamin B₆ is relatively non-toxic even in large amounts [111]. Rats tolerate doses up to 1 gram per kilogram of body weight ; above this dose convulsions appear. The lethal dose for this animal is 4 to 6 gram per kilogram by mouth. The repeated administration of 100 mg. per kilogram of body weight is well tolerated. Toxic effects have never been reported in man.

The effect on the blood sugar in man is variable. Doses of 20 to 800 mg. intravenously may produce a rise or a fall in normal subjects [119]. Even minute amounts of vitamin B₆, e.g. 0.00001 to 1.0 mM/litre significantly increase the total work output of perfused muscle.

Antibody formation is defective in animals on diets deficient in vitamin B₆ [179, 197] and there is evidence that susceptibility to infection, e.g. pneumonia, is increased [205].

THERAPEUTIC USE OF VITAMIN B₆

Vitamin B₆ has been used clinically in a wide variety of conditions, but on the whole the results do not suggest that the vitamin has any therapeutic action. A deficiency syndrome has not been observed, except in human subjects purposely deprived of the vitamin.

Deficiency States. Spies and his colleagues [108, 109] state that 50 mg. of vitamin B₆ given parenterally produces considerable subjective improvement in patients suffering from deficiency diseases such as pellagra and beriberi. They observed relief of symptoms and increased muscular power.

Blood Diseases. In some respects vitamin B₆ deficiency anæmia resembles pernicious anæmia and thalassæmia (Mediterranean anæmia). Vilter and his co-workers [110] state that vitamin B₆ produces a reticulocytosis in nutritional macrocytic anæmia and in pernicious anæmia. Kark [94], however, failed to observe any improvement in six anæmic patients receiving vitamin

VITAMIN B₆ DEFICIENCY IN THE MONKEY

FIG. 40. Vitamin B₆ deficiency in the monkey. Thionine stain. $\times 160$. Showing cellular proliferation of the thickened intima of an iliac artery. The dark greyish matrix in the intima and in the media is a mucoid material showing metachromatic staining. There is also degeneration of part of the internal elastic membrane.

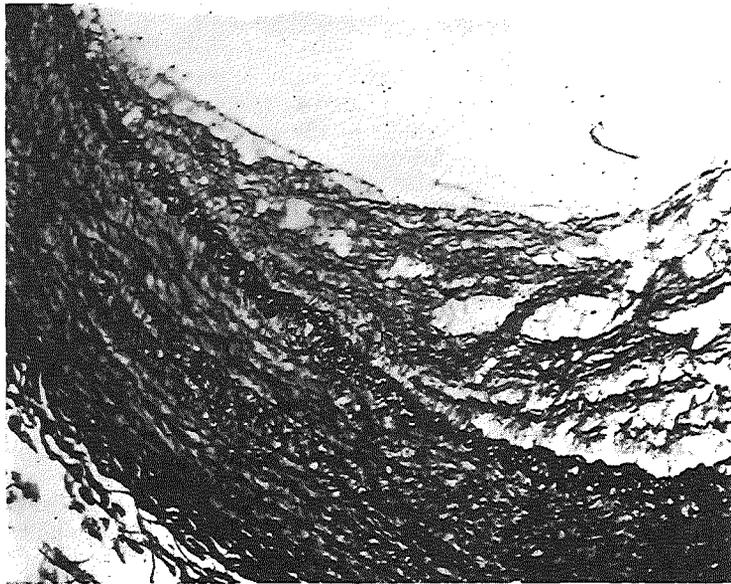


FIG. 41. Vitamin B₆ deficiency in the monkey. Weigert-Van Gieson stain. $\times 80$. Abdominal aorta showing newly formed elastic tissue in the thickened intima. The changes in Figs. 40 and 41 are arteriosclerotic and bear a close resemblance histologically to those seen in human arteriosclerosis.

B₆. It is generally considered that vitamin B₆ is of no value in the treatment of pernicious anaemia or Mediterranean anaemia [150].

Following a report of the occurrence of a leucocytosis in cases of anaemia treated with vitamin B₆, Cantor and Scott [209] treated three patients with granulocytopenia (agranulocytosis) due to drugs with 200 mg. of vitamin B₆ intravenously daily with apparently beneficial results. Recovery was said to have occurred in forty-eight hours. Fishberg and Vorzimer [210] claimed that vitamin B₆ was a powerful leucopoietic stimulant in a case of granulocytopenia due to thiouracil. These observations have not been confirmed. Knutson and his colleagues [211] treated eight cases of granulocytopenia due to benzene and one due to amidopyrine, but could observe no beneficial action. Menten [212] treated children suffering from granulocytopenia due to sulphonamides with folic acid and vitamin B₆ without effect. Negative results were also obtained by Bandli [213]. Death or recovery occurs in granulocytopenia and death is due to the sepsis which follows it. The concomitant use of antibiotics, such as penicillin, has done much to reduce the mortality from the disease.

Neuromuscular and Nervous Diseases. Spies and his associates [108, 109] noticed an increase in muscular strength in patients given vitamin B₆ parenterally. Accordingly Antopol and Schotland [114] administered it to patients with pseudohypertrophic muscular dystrophy in doses of 100 to 500 mg. weekly and reported considerable improvement in muscular power. Spies [109] claimed to have obtained improvement in patients with epilepsy, amyotrophic lateral sclerosis and myasthenia gravis after treatment with the vitamin. Rosenbaum, Portis and Soskin [113] also reported relief of muscular weakness in patients suffering from such differing conditions as neurasthenia, hyperthyroidism, ulcerative colitis and general malnutrition. Stone [214] has treated 169 patients suffering from neurological conditions with vitamin B₆ intrathecally in doses of 10 to 50 mg. often with aneurine at the same time. Among the diseases treated were poliomyelitis, general paralysis, tabes, disseminated sclerosis, Sydenham's chorea, neuritides of varying aetiology, and meningomyelomalacia. Stone claims that the patients experienced subjective improvement, increase in muscle strength, improvement in gait and relief from pain when present. Investigations by a number of other investigators have failed to confirm the results of these workers [243-250].

Spies [109] has used vitamin B₆ in eleven selected cases of Parkinsonism of at least four years' duration, eight of the cases being arteriosclerotic and three post-encephalitic. In the latter considerable improvement was reported within a few minutes; rigidity was significantly decreased and the patients walked without their usual stiffness. Two of the arteriosclerotic patients showed definite improvement, five were unchanged and one was considerably worse. From a study of forty-six cases Jolliffe [115] concluded that no improvement occurred in postencephalitic cases and little or no improvement in patients hospitalized for over three years, but that a dramatic improvement resulted in approximately twenty per cent. of cases of non-postencephalitic Parkinsonism that had been helpless for less than a year. All patients received 50 to 100 mg. of vitamin B₆ intravenously. Jolliffe [200] subsequently extended the series to ninety cases and reported permanent improvement in nine, i.e. ten per cent. Baker [142] has also observed improvement in eight cases out of nineteen and Meller [171] in nine cases out of ten receiving a similar dosage. They both state that in idiopathic paralysis agitans vitamin B₆ therapy decreases pain and rigidity. Three cases of paralysis agitans were also successfully treated by Rudesil and Weigand [172] with doses of 50 to 100 mg. of vitamin B₆ daily for five to seven months. They noted subjective and objective improvement in rigidity, tremor and strength. Zeligs [143], however, treated fifteen cases and Barker and his colleagues [173] ten cases of Parkinsonism with daily intravenous injections of 50 to 100 mg. of vitamin B₆ and observed no beneficial effects whatsoever. Loughlin [201] also failed

to observe any benefit in twelve chronic cases. The reported material is still too meagre for final appraisal, but even accepting Jolliffe's latest report it would appear that vitamin B₆ therapy is only effective in ten per cent. of patients with paralysis agitans, a low figure that makes its use hardly worth while. The introduction of such efficient drugs as the antihistamines, artane and kemadrin has now made these observations of historical interest only.

Vilter, Aring and Spies [116] describe a case of arsenical peripheral neuritis treated with vitamin B₆ in doses of 20 mg. intravenously. Improvement occurred and the patient relapsed when treatment was withdrawn.

Schwartzman and his colleagues [170] treated three cases of Sydenham's chorea with 9 to 60 mg. of vitamin B₆ daily and reported good results. Improvement was rapid and progressive. This was confirmed by Kost [97].

As vitamin B₆ deficient animals suffer from epileptiform convulsions, Fox and Tullidge [168] treated a number of epileptics with vitamin B₆ in doses of 20 to 100 mg. daily for several weeks. No improvement was noted.

Skin Diseases. Infantile seborrhoea has been treated with vitamin B₆, but the results obtained are not convincing [169]. Jolliffe [203] treated a number of patients suffering from adolescent acne with oral doses of 50 to 250 mg. of vitamin B₆ daily. He reports that of thirty-seven patients, nine were cured and nineteen improved; in a control series of thirty-five, only seven improved. Wright and his colleagues [204] described the treatment of an unspecified number of patients suffering from seborrhoeic dermatitis and eczematous eruptions with 20 to 100 mg. of vitamin B₆ daily. The patients were stated to show a rapid response; no details, however, are given.

Vomiting. Vitamin B₆ has been used in the treatment of nausea and vomiting in pregnancy and resulting from irradiation. Many of the observations on the use of vitamin B₆ in the treatment of nausea and vomiting of pregnancy have been uncontrolled [206, 207, 342]. Treatment consisted of injections of 50 to 100 mg. daily or smaller doses more frequently. Hessel-tine [174] controlled his observations with injections of normal saline, which he stated gave better results than vitamin B₆. Dorsey [178] claims that excellent results are obtained by the combined use of vitamin B₆ and adrenal cortical extract. No controls were used nor were the results compared with any other form of treatment. The influence exerted by psychic factors, sedation and bed rest in the treatment of the vomiting of pregnancy cannot be overemphasized. Owing to the lack of controls and the tendency for the condition to improve spontaneously such reports should be treated with considerable reserve.

Bergmann [196] claimed similar beneficial results in the prevention of post-anæsthetic nausea and vomiting with 100 mg. vitamin B₆ given post-operatively. Only twelve cases were presented. Hill [199] and Kernis and Stodsky [202] failed to confirm this on a study of 120 patients.

Many therapeutic measures have been tried in the treatment of radiation sickness. The presence of malnutrition and avitaminosis seems to increase lack of tolerance to radiation therapy. There have been several reports on the use of vitamin B₆ in the prevention and treatment of radiation sickness [215, 218, 220, 224, 230, 239]. Some of these have been well documented and controlled and the total number of patients treated is well over 500. Wells and Popp [239] obtained the best results by giving 100 to 200 mg. intravenously before each treatment, larger doses being given when treatment was directed to the abdomen, thorax or pelvis. In most instances it was found that symptoms disappeared or were definitely relieved in ninety per cent. of the cases. Control tests were done to eliminate the possibility of psychic effects. Nabarro [270], however, failed to observe any beneficial effect from treating patients subjected to irradiation with vitamin B₆.

Patients suffering from neoplastic diseases, such as Hodgkin's disease and leukæmia, treated with nitrogen mustards and other mitotic poisons

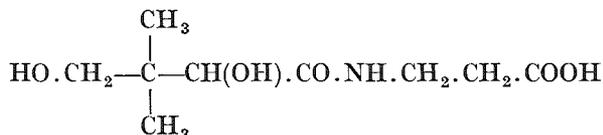
invariably experience nausea and vomiting. According to some workers this can be prevented by administering vitamin B₆ in doses of 100 mg. parenterally one and a half hours after the administration of the nitrogen mustard [250, 251].

Goldfeder and his co-workers [237] investigated the effect of vitamin B₆ on radiation injury, resulting from X-ray exposure, in mice. It had no appreciable effect on the hæmopoietic system, but given before and after irradiation it significantly prolonged the survival rate of the animals as compared with irradiated but untreated controls.

Other Conditions. Vitamin B₆ is stated to have an anti-histamine like action. In man the skin reaction to histamine is slightly reduced by previous administration of the vitamin [217].

PANTOTHENIC ACID

History, Isolation and Chemistry. The discovery of pantothenic acid had its origin in 1901 in the discovery of *bios*, a hypothetical substance, now known to be a mixture, necessary to the reproduction of yeast. In 1930 a pellagra-like dermatitis occurring in chicks on restricted diets was described [47], and it was subsequently shown that this can be prevented by feeding pork liver and by a factor in liver extract [48], which was subsequently termed the "filtrate factor." Nutritional achromotrichia (greying of the fur) of rats fed diets deficient in one or more of the B vitamins has been observed by several investigators. Morgan and his co-workers [22] were the first to observe that the active substance which prevents and cures this greying of hair in rats on diets deficient in the B vitamins is present in the filtrate factor. A collateral line of research was begun by Williams [256] who found that a naturally occurring compound of unknown composition stimulates the growth of yeast, and to this compound he gave the name "pantothenic acid" on account of its ubiquitous distribution (Greek πανταρος, everywhere). In 1939 Williams [50] isolated the compound and with Major [51] determined its structure in the following year. It was found to be 1-hydroxy-2:2-dimethylbutyryl-β-alanine.



The identity of the chick anti-dermatitis factor and pantothenic acid, which had been suggested because of their similarity in chemical behaviour [53], was confirmed biologically by Jukes and others [55]. In 1940 pantothenic acid was synthesized by several groups of investigators in America, Switzerland and Germany [257-259]. Macrae and his co-workers [340] showed that the filtrate factor contains pantothenic acid, and subsequently the latter was shown to be identical with the anti-grey hair factor of former investigators [91, 133].

Pantothenic acid shows both acidic and basic properties. It is readily soluble in water and acetic acid, slightly soluble in ether, but almost insoluble in the other fat solvents (benzene, chloroform). In a pure condition it is a yellow viscous oil. It is unstable, being sensitive to heat and to changes in the pH of the medium and is easily hydrolysed. Pantothenic acid forms a well-defined, stable calcium salt, which is the form in which it is marketed.

Distribution. Pantothenic acid is widely distributed in foodstuffs. Yeast, liver, kidney, wheat bran, and peas are the best common sources; royal jelly contains about six times as much as yeast [260]. Pantothenic acid is synthesized by certain moulds, bacteria and green plants, and it also appears to be synthesized in the gut of ruminants. Grains are good sources of panto-

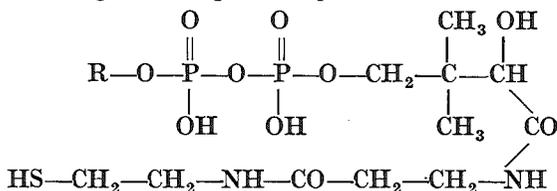
thenic acid, but in the case of wheat about fifty per cent. is removed in the milling process. A considerable increase in the pantothenic acid content of cereals occurs on germination. About eighty per cent. of the pantothenic acid in foodstuffs is in the bound condition, from which it can be liberated by enzymic digestion with diastase and papain or by hydrolysis with acid or alkali.

The distribution of pantothenic acid in various foodstuffs has been determined by Waisman, Mickelsen and Elvehjem [59], using a biological technique. The stewing of meat reduces the pantothenic acid potency to one-third, probably through loss in the cooking-water. In the cooking, dehydration and curing of meat about three-quarters of the pantothenic acid is retained [355]. Snell [60, 290] and his co-workers have elaborated a biological method of assay depending on the growth response of *Lactobacillus casei*. Methods depending on the growth rate of *Neurospora* [67] and *Lactobacillus arabinosus* [288, 155] have also been devised [67]. Schindt [222] and Neilsen, Hartelius and Johansen [272] employ *Streptobacterium plantarum*.

Pantothenic Acid Content of Foodstuffs

	Micrograms per gm.
Apples	0.60
Apricots (canned)	0.95
Artichoke	4.0
Asparagus	1.65
Bananas	0.7-1.8
Barley	10
Beans, dried	8.3
baked (canned)	0.55-0.85
Beef, muscle	4.9-15
liver	40-76
heart	20
brain	18
Beets	1.1
Bread, whole wheat	5.7
white	4.6
Broccoli	11-14
Cabbage	1.8
Carrot	2.0-2.5
Cauliflower	9.2
Cheese	1.3-9.6
Chicken	5.3-6.2
Chocolate	1.9
Corn	3.1
Eggs	8-48 Av. 27
Egg yolk	50-100 Av. 63
Grapefruit	2.9
(canned)	1.2
Halibut	1.5
Kale	3.0
Lamb, leg	6.0
Lettuce	1.1
Mackerel	3.1-4.7
Maize	3.1
Marmite	62
Milk	1.3-4.7 Av. 2
skim	2.1-4.3
Molasses	2.6
Mushrooms	17

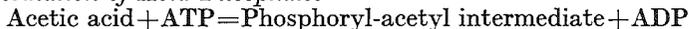
Functions and Physiology. Coenzyme A. Pantothenic acid is the prosthetic group of a coenzyme concerned in acetylation, known as coenzyme A. In 1942 an enzyme was discovered in brain tissue necessary for the synthesis of acetylcholine from acetic acid, choline and adenosine triphosphate [103]. A few years later it was shown that an enzyme in liver acetylates sulphonamides and other amides [135], and a coenzyme was isolated and shown to be a necessary component of the system which acetylates choline. Coenzyme A is a derivative of pantothenic acid and adenosine and has been tentatively assigned the following formula [159, 346] :



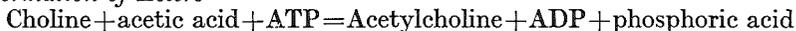
R is an adenosine residue. According to King and Strong [483] coenzyme A is a phosphate, not a pyrophosphate. Coenzyme A probably brings about acetylations by accepting an acetyl group at its terminal—SH, the resulting thioacetyl coenzyme transferring its "energy-rich" acetyl group to substrate molecules. Plasma pantothenic acid is in the form of the free vitamin, whereas that in the red blood cells is in the form of coenzyme A. Although all the reactions catalysed by coenzyme A involve a common substrate they result in the formation of a variety of compounds including amides, esters, anhydrides and compounds produced by the condensation of an acetate radical with keto acids or acid phosphates.

Reactions in which Coenzyme A is Co-factor *in vitro*.

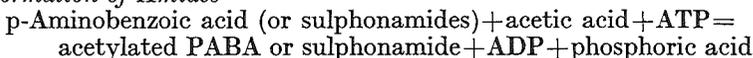
Formation of Acid Phosphates



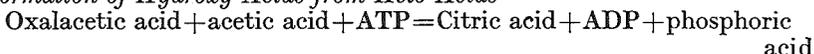
Formation of Esters



Formation of Amides



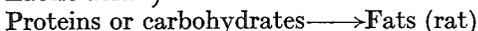
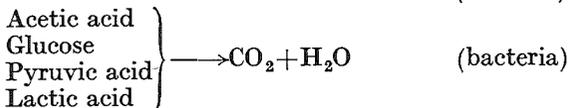
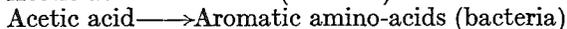
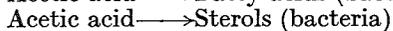
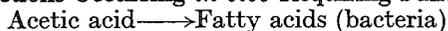
Formation of Hydroxy Acids from Keto Acids



Condensation of Acetic Acid



Reactions Occurring *in vivo* Requiring Pantothenic Acid



The catalytic function of coenzyme A in citric acid synthesis has been confirmed in experiments with cell free extracts of yeast and bacteria [234]. The reaction in which oxalacetic acid is converted to citric acid is the one which initiates the cycles by which both carbohydrates and fatty acids are metabolized aerobically. When carbohydrate metabolism furnishes the acetyl group, aneurine and pantothenic acid are required. When fatty acids or alcohol are the source pantothenic acid only is needed to initiate the reaction. The synthesis of fats from carbohydrate or protein involves

processes in which reactive acetyl molecules condense under the influence of coenzyme A. Pantothenic acid also functions in the pyruvic acid metabolism of the organism *Proteus morgagni* [265]. Pantothenic acid stimulates the oxidation of those amino-acids that are converted to pyruvic acid [255]. Protein has a pantothenic acid sparing action in the rat [254].

Pantothenic Acid Deficiency. Pantothenic acid is essential for the adequate nutrition of a number of species, including rats, dogs, mice, chicks, hogs and monkeys. It is not known whether it is essential for human nutrition as no definite deficiency symptoms have ever been described in man.

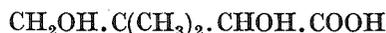
Rats maintained on a diet deficient in pantothenic acid develop a deficiency syndrome characterized by scant, coarse fur, inflammation of the nose, staining of the fur with porphyrin, failure to grow, hæmorrhage and necrosis of the adrenals and gastric ulceration [58, 294]. Anæmia and leucopenia also occur [125, 275], and inflammatory changes in the lungs have been described [278]. Corneal vascularization has been observed [292]. Dogs on deficient diets show a fatty degeneration of the liver, a rise of blood sugar, convulsions and coma, anæmia, gastritis and enteritis, and diminished carbohydrate and protein digestion and absorption [183, 231]. Pantothenic acid is also essential for the growth of young pigs, in which deficiency of the vitamin results in an ulcerated gastro-intestinal tract, emaciation, incoordination (goose-stepping gait), loss of hair, rhinorrhœa, a scaly dermatitis, and degenerative changes in the peripheral nerves, posterior roots, and the posterior columns of the spinal cord [271, 296]. The animals also suffer from a normocytic anæmia. In the monkey the pantothenic acid deficiency syndrome shows lack of growth, ataxia, greying and thinning of the fur, anæmia, diarrhœa and cachexia [279]. Chicks suffering from pantothenic acid deficiency develop dermatitis and degenerative lesions of the spinal cord [123, 269]. In growing mice pantothenic acid is essential for the growth of the skeleton, deficiency causing inhibition of growth and endochondral ossification [295].

Pantothenic acid deficiency in the experimental animal reduces the phagocytic power of the blood [345], and antibody response [268], but the animals are less susceptible to certain infections, such as pneumococcal pneumonia and poliomyelitis [151, 227], presumably because the organisms concerned require pantothenic acid as a growth factor.

Nutrition of Micro-organisms. Pantothenic acid is essential for the nutrition of a large number of micro-organisms, both pathogenic and non-pathogenic, and some organisms have been used for the microbiological assay of the vitamin (p. 117). It is essential for the growth of *Streptococcus hæmolyticus*, *Diplococcus pneumoniae*, *Proteus morgagni*, *Clostridium tetani*, *C. Welchii*, certain strains of *Corynebacterium diphtheriae*; some species of *Pasteurella*, *Brucella* and *Shigella paradysenteriae*. The malarial parasite *Plasmodium lophuræ* requires pantothenic acid for growth [274].

Many organisms, e.g. *E. coli*, can synthesize pantothenic acid from β -alanine, and this would appear to be the source of the vitamin in the intestine. The excretion of pantothenic acid in the urine and fæces exceeds the intake in the diet, indicating that bacterial synthesis occurs in the gut [282]. In ruminants this pantothenic acid synthesized by bacteria is probably utilized, but it is not certain whether it is in man.

Pantothenic Acid Antagonists. Many compounds are known that interfere with the synthesis of pantothenic acid by micro-organisms or its utilization by animals. Sulphapyridine will produce achromotrichia or lack of pigmentation in rats by producing an induced pantothenic acid deficiency [280]; salicylates will also prevent the growth of bacteria utilizing pantothenic acid [160], probably by interfering with the synthesis of pantoic acid,



which when coupled with β -alanine forms pantothenic acid.

A number of analogues of pantothenic acid have been prepared that can act as antagonists to the vitamin, blocking its participation in essential enzyme actions. One of the first of these to be prepared was pantooyltaurine, $\text{CH}_2\text{OH} \cdot \text{C}(\text{CH}_3)_2 \cdot \text{CHOH} \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2\text{CH}_2\text{SO}_2\text{OH}$, which inhibits the utilization of pantothenic acid by organisms such as *S. haemolyticus*, *C. diphtheriae* and *D. pneumoniae* [271], and also by animals [273]. A large number of pantothenic analogues were prepared when it was discovered that the malarial parasite *P. lophurae* requires pantothenic acid as a growth factor [274]. Panthenol, the alcohol of pantothenic acid, is almost as active as pantothenic acid. Phenylpantothenone, $\text{CH}_2\text{OH} \cdot \text{C}(\text{CH}_3)_2 \cdot \text{CHOH} \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2\text{CH}_2\text{COC}_6\text{H}_5$, is a pantothenic acid antagonist, preventing its utilization by bacteria [236]. ω -Methyl pantothenic acid produces a pantothenic acid deficiency in mice [691]. Many other antagonists have been prepared, the most active being those in which the pantoic acid part of the molecule is coupled to a suitable amino-acid, amino-ketone, amino-alcohol or amine.

Absorption, Storage, Excretion of Pantothenic Acid. Pantothenic acid is absorbed from the gastro-intestinal tract. In human blood it is stated to be present in quantities carrying from 3.7 to 40 micrograms per 100 ml. [44, 158, 181]. Following the intravenous injection of sodium or calcium pantothenate the blood concentration rises by about fifty per cent. in three hours in normal subjects, but not in patients suffering from deficiency diseases [61]. Most of the pantothenic acid is present in blood in the combined form as it is precipitated with protein precipitants. When pantothenic acid is injected into human subjects there is a rise of blood riboflavine as well, and conversely administration of riboflavine causes a rise in blood pantothenic acid. Pantothenic acid is partly destroyed in the body, that escaping destruction being excreted in the urine or stored.

The pantothenic acid content of different human tissues has been estimated by Nielsen and others [272] who give the following figures in micrograms per gram of dry material: muscle, 4; liver, 40; kidney, 30; spleen, 20; nerve, 3; pancreas, 7; adrenals, 5; and stomach, 10.

Pantothenic acid is excreted in the sweat, milk and urine. The amount excreted in the sweat is stated to vary from 3.8 to 30 micrograms per 100 ml. [262, 263]. Human milk contains 48 micrograms per 100 ml. on the first day, increasing to 245 micrograms by the fourth day and finally averaging about 200 to 250 micrograms per 100 ml. [266, 297]. According to Coryell *et al.* [312] with intakes of 6.0 to 9.5 mg. of pantothenic acid daily, thirty-two to eighty-nine per cent. is excreted in the urine. Cow's milk contains similar amounts to human milk.

The average daily excretion of pantothenic acid in man varies from 1.5 to 7 mg., or 70 to 600 micrograms per 100 ml. of urine [176]. Sarett [147] states that the normal excretion averages 3.5 mg. daily and Denko *et al.* [223] give the figure 2.68 to 3.46 mg.; about 0.89 to 3.66 mg. is excreted in the faeces. After ingestion of 100 mg. the excretion rises to 18.5 mg., and to 38.5 mg. after the intravenous administration of this amount. Schmidt [222] gives the daily urinary excretion of pantothenic acid as 2.72 ± 0.61 mg. in men, 2.63 ± 0.6 mg. in women and 2.05 to 2.86 mg. in children. He attributes the higher values of other authorities to failure to sterilize the urine, which acts as a growth medium for bacteria synthesizing pantothenic acid. Injection of 25 mg. of pantothenol, the alcohol corresponding to pantothenic acid, increases the excretion of pantothenic acid to from 4.5 mg. to 6.7 mg. in normal young subjects and less than this in elderly persons. In the newborn child the excretion ranges from 99 to 213 micrograms [228]. Schmidt [222], Gershberg and others [241] investigated the excretion of pantothenic acid by patients suffering from various diseases (gastro-intestinal, coeliac disease, achylia, aplastic anaemia, hepatitis, pneumonia, Addison's disease, diabetes) but found that it was within normal limits, except in pernicious anaemia, in which

there was diminished excretion. The excretion of pantothenic acid is not increased after the injection of β -alanine, suggesting that the human organism is not capable of synthesizing pantothenic acid [240]. Sulphonamides, such as sulphathalidine, have no effect on the excretion of pantothenic acid by normal adults [357].

Pharmacology. The toxicology of pantothenic acid was investigated by Unna and Greslin [136]. The LD_{50} following subcutaneous injection is 2.7 grams per kilogram in mice and 3.4 grams in rats. The daily administration of calcium pantothenate over a period of six months to monkeys, dogs, and rats (50 to 200 mg./kilo) failed to produce any toxic manifestations or pathological changes in the organs. Blood pressure, respiration and heart rate were uninfluenced by doses of 10 to 50 mg. per kilogram and a ten per cent. solution was not irritant to the rabbit conjunctiva. Spies and his co-workers [61] administered 100 mg. intravenously to human subjects without producing any side effects. They observed that the administration of riboflavine causes an increased excretion of pantothenic acid. It is stated that the intravenous injection of 100 mg. calcium pantothenate causes a rise of blood sugar in normal and diabetic subjects [157].

The total work output of the frog gastrocnemius muscle is significantly increased by perfusion with 0.01 mille mols. of calcium pantothenate per litre [367]. The effect is probably due to vasomotor changes and it does not follow that a similar result would be obtained in the intact animal or in man.

Requirements of Pantothenic Acid. The daily requirement of pantothenic acid needed to prevent achromotrichia in rats is 50 to 100 micrograms [91, 133]. The requirements of other species have been calculated but those of man are entirely speculative. It has been assumed that it is an essential constituent of human diet but absolute proof is lacking. It has been estimated that an average American diet contains 4.5 mg. of pantothenic acid per 2,500 calories. Human excretion figures suggest that the daily requirement for man might be about 10 mg. This figure, however, is entirely speculative, those figures obtained from dietary studies being more reliable. A good mixed diet *must* contain all the vitamins and in required amounts for man.

Clinical Studies on Pantothenic Acid. The production of nutritional achromotrichia in animals by dietary means and the restoration of normal colour by feeding pantothenic acid led to the hope that it might be effective in the treatment of human grey hair. There are two fallacies here; one is that similar symptoms do not necessarily have a common cause; the other is that the findings from animal experiments cannot be transferred to human conditions. It has never been shown that dietary deficiency is a factor in the production of grey hair in the human adult. Widespread publicity was given in the popular press to the possibility of pantothenic acid curing human greyness. Subsequent investigation proved that no authenticated case of human greyness has responded to treatment with pantothenic acid [276, 277, 366]. Schmidt [692] observed no difference in the excretion of pantothenic acid in patients with grey hair and baldness and normal individuals. Nicholls [198] and Hughes [358] believe, however, that greying of hair can occur in native children in the tropics owing to malnutrition. Hughes found achromotrichia widespread among malnourished children in Lagos, usually associated with hypopigmentation, and he believes that it is due to pantothenic acid deficiency, since the administration of the vitamin seemed to restore the normal colour. As a good diet was administered at the same time it is difficult to ascribe the greyness solely to a deficiency of pantothenic acid.

The "burning feet" syndrome, although previously described in the literature on deficiency diseases, has recently received considerable attention on account of its frequent occurrence in Japanese prison camps in the last

war (see p. 320). According to Gopalan [359] the daily injection of 20 to 40 mg. of calcium pantothenate produced spectacular improvement in patients suffering from this syndrome. A similar syndrome was observed by Paraita [360] in the Spanish Civil War of 1936-39 and although pantothenic acid was not discovered then, Paraita accepts the conclusions of Gopalan that this syndrome is due to a deficiency of the vitamin B₂ complex, particularly pantothenic acid. This view is also accepted by Smitskamp [361].

Cases of glossitis and cheilosis are stated to have healed following the administration of pantothenic acid after treatment with other members of the vitamin B complex had proved ineffective [364, 365]. Doses of 150 to 300 mg. daily were used.

Panthenol, the alcohol of pantothenic acid, has been suggested as an adjunct to the pituitary adrenocorticotrophic hormone and cortisone in the treatment of acute disseminated lupus erythematosus and alone in the treatment of subacute lupus erythematosus and chronic discoid lupus erythematosus [368, 738]. Welsh [732] claims that vitamin E and pantothenic acid, or its alcohol, are also effective.

BIOTIN

History, Isolation and Chemistry. The discovery of biotin, also known as vitamin H and coenzyme R, had its origin in the casual observation that a high concentration of egg white in experimental diets is toxic; some years

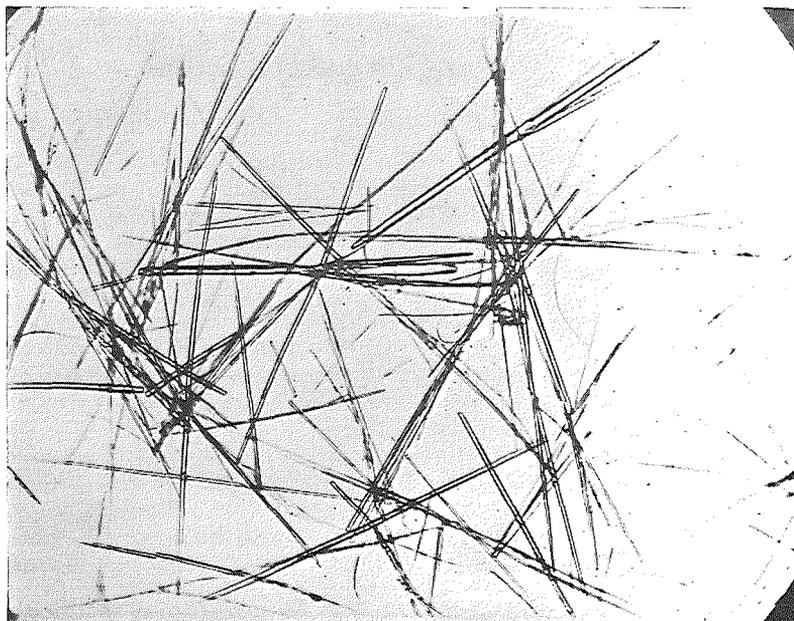
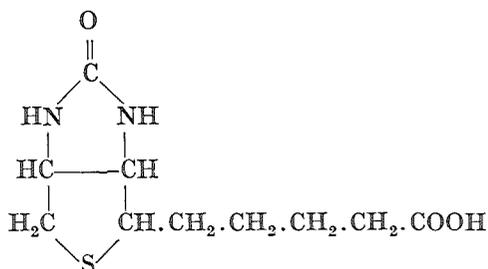


FIG. 42. Crystals of biotin. Magnification $\times 150$.

later certain foods, notably liver and yeast, were discovered which contained an organic substance capable of protecting rats against the toxic effects of egg white, or "egg white injury." The protective factor was called "the protective factor against egg white injury" and also "vitamin H" by György in 1931. It was extensively studied by György and his co-workers [10-16] between 1931 and 1940. In another laboratory attempts were being made to resolve "bios," a yeast growth factor, and this resulted in the isola-

tion in 1936 of a crystalline substance from egg yolk by Kögl and Tönnes [281], who named it biotin. About a milligram of active material was obtained from a quarter of a ton of dried egg yolk. Independently in yet another laboratory attempts were being made to isolate a compound, essential for the growth of *Rhizobium*, a nitrogen-fixing organism; this was called "coenzyme R" [474]. It was not realized by these various laboratory groups that they were dealing with the same entity. In 1940 György and his associates [16] announced the identity of biotin with vitamin H and coenzyme R. Biotin was subsequently isolated from liver [146], its structure established by Du Vigneaud and his co-workers [283] in 1942, and its synthesis achieved in 1943 by Harris and his colleagues [284] in the Merck laboratories in the United States.

Biotin is water- and alcohol-soluble, but relatively insoluble in the fat solvents. It crystallizes in long thin needles (Fig. 42), melting at 232–233° C. with decomposition. It is heat stable, and not decomposed by acids or alkalis, although it is inactivated by hydrogen peroxide and rancid oils and fats. Biotin is a bicyclic urea derivative containing sulphur in a thiophene ring. It is D-2' keto-3 : 4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid,



A series of papers has been published by Kögl and his co-workers which state that the biotin isolated from egg yolk is not the same as that isolated from liver [362]. It has a m.p. of 220° C. The two compounds, which differ in physical and chemical properties, have been designated α -biotin and β -biotin, respectively. The formula above is that of β -biotin obtained from liver.

No chemical or physical methods are known for the estimation of biotin. The bioassay, based on the cure of egg white injury in rats and chicks is time consuming, and has been replaced by microbiological methods depending on the growth response of yeast [287], *Lactobacillus helveticus* [290], *L. arabinosus* [288] and other organisms [285].

Units and Distribution. Until the isolation and identification of biotin, biological units were employed to denote the potency of biotin-containing material. The "rat unit" of biotin is the daily dose of a standard preparation which will cure egg white injury produced in rats on a special diet; this corresponds to 0.1 micrograms of biotin or 10,000 units per milligram of biotin [293]. The "yeast growth" unit [281] is the amount of biotin producing a one hundred per cent. increase in cell growth of a specified strain of yeast under certain conditions; this corresponds to 27,000 yeast growth units per milligram [293].

Biotin is found in foodstuffs containing other members of the vitamin B complex, particularly yeast, liver, kidney, and other offal, light chicken meat, eggs, peas, cocoa and cereals. An increase in the biotin content of cereals occurs on germination. Biotin is present in most foodstuffs in a bound form, from which it is liberated in the intestine by enzymic hydrolysis [291]. An average of seventy-seven per cent. of the biotin is retained in meat after cooking [329]. The biotin content of some common foods is given in the following table :—

Biotin Content of Foodstuffs

	Micrograms per gm.
Apples	0-009
Bananas	0-044
Beans, dried	0-098
Beef, muscle	0-026
Beets	0-027
Bread, whole wheat	0-019
white	0-011
Cabbage	0-024
Carrot	0-025
Cauliflower	0-17
Cheese	0-036
Chicken	0-054-0-098
Chocolate	0-32
Corn	0-058
Eggs	0-090
Grape-fruit	0-030
Halibut	0-08
Lamb, leg	0-021
Lettuce	0-031
Mackerel	0-03-0-18
Maize	0-058
Marmite	0-001
Milk, cow	0-011-0-037
human	0-038-0-08
Molasses	0-091
Mushrooms	0-16
Mutton, shoulder	0-027
Onions	0-035
Oranges	0-019
Oyster	0-087
Peas, fresh	0-035
dried	0-18
Peanuts, roasted	0-34
Pork, muscle	0-02-0-046
bacon	0-075
ham	0-04-0-06
Potatoes	0-006
Raisins	0-031
"Royal jelly"	1-7-4-1
Salmon	0-15
Sardine	0-04-0-24
Spinach	0-069
Strawberries	0-040
Tomatoes	0-040
Tuna fish	0-03
Turnips	0-021
Veal	1
Wheat, whole	0-081
flour, white	0-02-0-047
Yeast (<i>Torula utilis</i>)	1-33
(Brewers')	0-071

Avidin and Biotin Inhibitors. The syndrome produced in rats by feeding large quantities of raw egg white, first described in 1927 by Boas [286], is in effect the syndrome of biotin deficiency. There exists in egg white a basic protein that combines with biotin and renders it unavailable to the body, forming a complex with it that is not utilized [307]; egg white also inhibits

the utilization of biotin by micro-organisms [308]. The isolation and properties of this protein in egg white, which has been called avidin, has been described and its analysis effected [149, 309]. It is a glycoprotein and the complex it forms with biotin cannot be broken down by proteolytic digestion, only by heating or irradiation. This explains why raw egg white inactivates biotin, whereas cooked egg white does not. The combination between biotin and avidin is stoichiometric and the biotin cannot be separated by dialysis. It is peculiar, however, that although the avidin-biotin complex cannot be broken down by digestion, the biotin is utilized if the complex is injected [311]. Biotin deficiency can be induced in animals and in man (p. 129) by feeding raw egg white.

A number of analogues of biotin have been prepared that act as physiological antagonists to biotin, preventing its utilization by animals and micro-organisms. Biotin deficiency can in fact be produced by administering such compounds. They act not by combining with biotin like avidin, but by blocking essential enzyme mechanisms in which biotin plays a part. Desthio-biotin, in which the sulphur atom of biotin is replaced by hydrogen, is a biotin inhibitor [306]. Biotin sulphone [475], although a growth factor for yeast, is a biotin inhibitor for *Lactobacillus helveticus*, *L. arabinosus* and *Staph. aureus*. Some other analogues are known with biotin-activity, although less than that of the parent substance, e.g. oxybiotin or O-heterobiotin, in which the sulphur atom is replaced by oxygen [177].

Physiology and Functions of Biotin. Biotin is essential for the growth of many bacteria, yeasts and fungi, although some organisms are capable of synthesizing it [298]. Bacterial synthesis occurs in the gut of animals [299] and also in the human gut as the combined excretion in urine and faeces is greater than the intake [301]. The vitamin appears to be related to the fundamental process of growth since the biotin content of embryo tissues and tumour tissue is very high [148].

Biotin has been shown to activate the reversible deamination of aspartic acid, and the deamination of serine and threonine [313]. Since biotin restores the ability of *B. coli* to produce carbon dioxide from aspartic acid, malic acid and oxaloacetic acid it has been concluded that it is concerned with the coenzyme of oxaloacetic decarboxylase [313]. By analogy with other vitamins it was suspected that it does not function in these reactions as such but is converted into a coenzyme form. Lichstein [314] has shown that biotin probably exists in a coenzyme form in natural materials. It has been shown that the failure of certain micro-organisms to grow on a biotin-deficient medium is due to their inability to condense carbon dioxide with pyruvic acid to form oxalacetic and aspartic acids. Using radio-active carbon (C_{14}) as a tracer element Lardy and his co-workers [315] have shown that biotin may be associated with the fixation of carbon dioxide in several different enzyme reactions in animals as well as micro-organisms. Thus in presence of biotin *L. arabinosus* fixed C_{14} into cellular aspartic acid; rats were better able to fix C_{14} into adenine, guanine, arginine, aspartic acid, citric acid, and bone carbonate if they were supplied with adequate biotin.

Biotin may be concerned with the oxidation of pyruvic acid and lactic acid, since carbon dioxide production is markedly increased in systems in which tissues are respiring in solutions containing pyruvate acid lactate as substrate [369]. This has been confirmed using radio-active carbon (C_{14}) as a tracer element [369].

It has been suggested that biotin plays a part in fat metabolism as fatty livers are produced in animals by feeding biotin [305]. There may also be a connection between biotin deficiency and pantothenic acid, since symptoms of biotin deficiency induced in rats by administering succinylsulphathiazole are aggravated in the presence of a deficiency of pantothenic acid [318]. Biotin appears to be necessary for the maintenance of normal creatine levels in the muscle of the rat [320].

Metabolism, Absorption and Excretion. Biotin is absorbed from the gastro-intestinal tract and freed from the bound form in which it occurs in foodstuffs. The amount excreted daily by human subjects on a normal diet varies from 11 to 183 micrograms a day [301, 322, 324]. It is not influenced by disease and even during periods of starvation is not abnormally low, although it does depend on protein intake [285]. Sydenstricker [322] noted that the excretion fell to 3.5 to 7.3 micrograms daily on a biotin-deficient diet. The average diet may supply from 4 to 170 micrograms of biotin daily; the amount is increased if liver is included. The excretion may increase from 245 to 357 micrograms after a tolerance dose of 500 micrograms of biotin by mouth [267]. Biotin is also excreted in the faeces to the

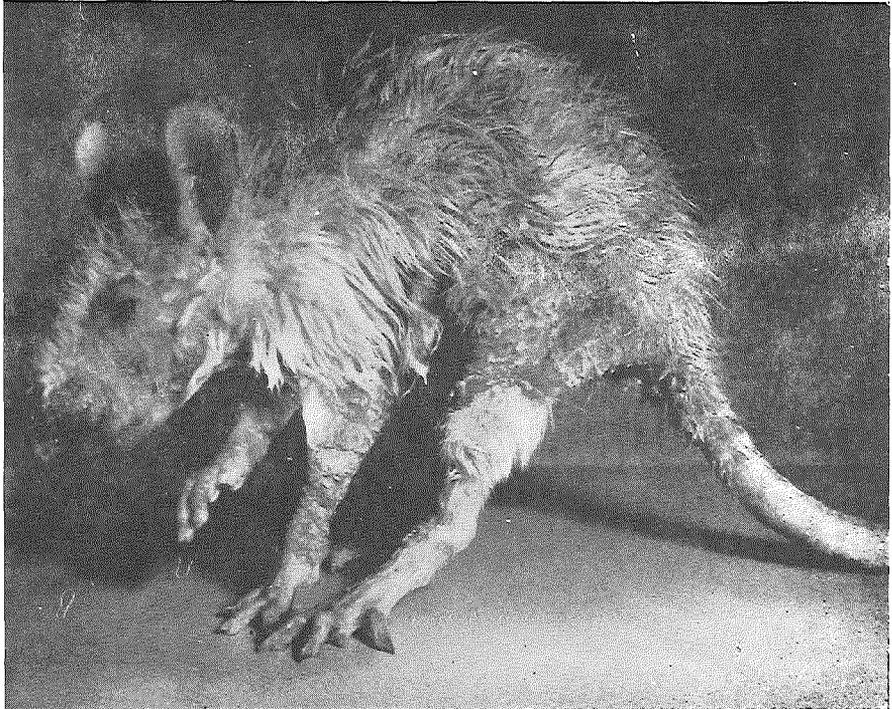


FIG. 43. Biotin deficiency in a rat. This animal, age eighty-five days, received a diet containing thirty-five per cent. uncooked egg white since the age of twenty-one days, and exhibits typical acute, exfoliative, desquamative dermatitis, accompanied by loss of hair and abnormal posture. The dermatitis is particularly severe about the flanks, fore feet and head.

extent of about 322 to 393 micrograms daily, and as this is greater than the intake intestinal synthesis and absorption must occur [68]. It has been shown that biotin can be absorbed from the colon [267]. This intestinal synthesis is inhibited by large doses of non-absorbable sulphonamides such as sulphasuxidine, although other sulpha drugs in normal doses do not seriously interfere [267]. Several organisms are probably responsible for the synthesis; the following are known to produce it: *B. coli*, *B. proteus vulgaris*, *B. faecalis alcaligenes*, *B. mesentericus*. Only small amounts are excreted by human subjects on diets containing large amounts of egg white [301]. The exact nature of the excretion product is not known; some of the biotin present does not combine with avidin [301]. The excretion of biotin is said to be low in patients with seborrhœa [267, 330]. The amount excreted in milk is low, particularly just after parturition. In mature milk

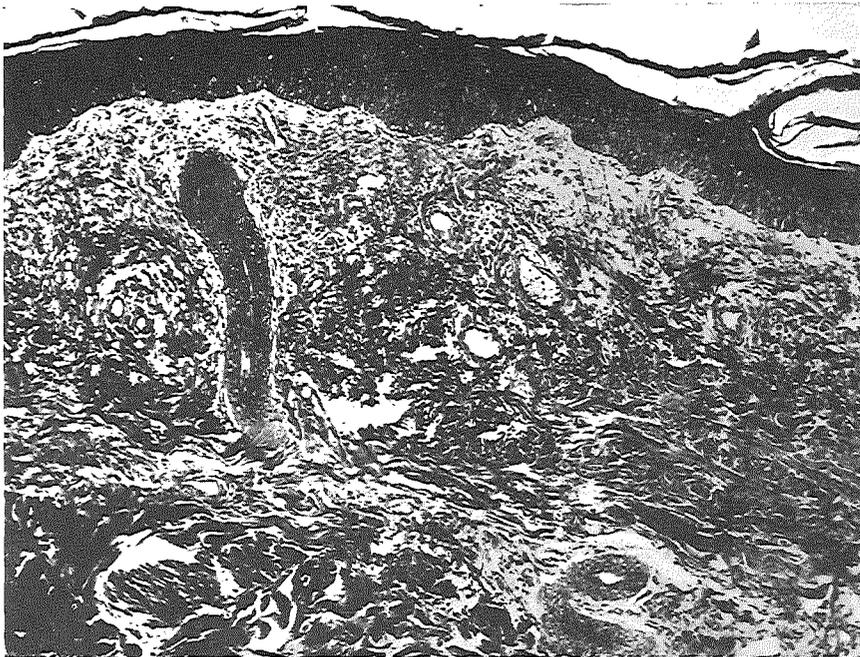


FIG. 44. Section of Skin from Case of Suspected Human Biotin Deficiency. It shows loose sheets of keratinized epithelium, absence of rete pegs and cellular infiltration around the hair follicles, sweat glands and blood vessels. The corium is thickened, hair follicles atrophic and sebaceous glands absent. See text, p. 130.

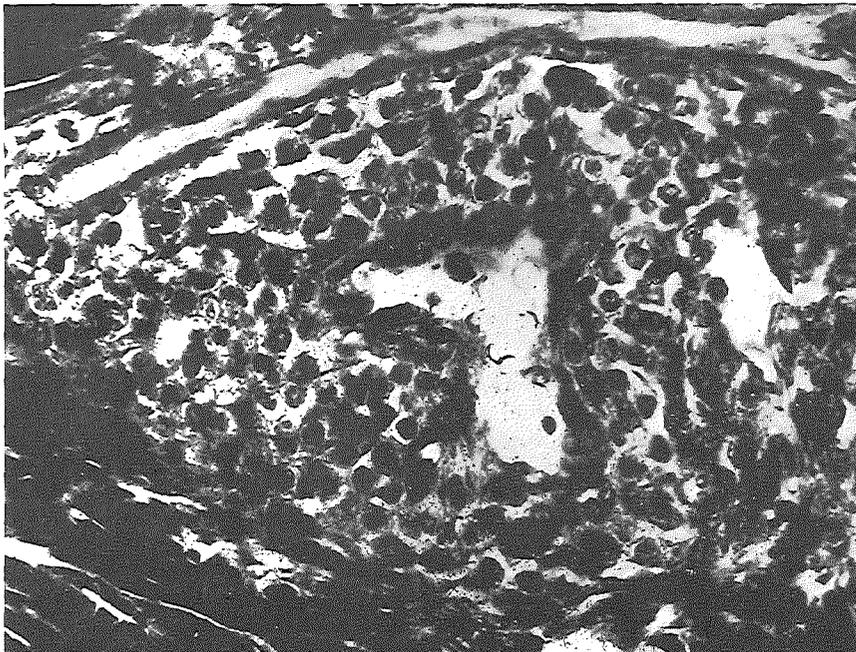


FIG. 45. Section of Skin from Case of Suspected Human Biotin Deficiency. It shows a marked cellular reaction around the small blood vessels in the corium. See text, p. 130.

the concentration has been estimated to be two to fourteen per cent. of the intake, and varies from 0.9 to 11.2 micrograms in twenty-four hours [328].

Pharmacology [335, 337]. Biotin is non-toxic even in large amounts. A single intravenous injection of 1 gram per kilogram produces no toxic effects in mice. It produces no significant signs of irritation applied to the cornea or injected intramuscularly or intradermally. In the anaesthetized animal it has no effect even in large doses, on blood pressure, respiration, hepatic function, renal function, metabolic rate or intestinal circulation. It has no effect on the regeneration or healing of tissue.

Effects of Biotin Deficiency in Animals. Biotin deficiency can be produced in animals by feeding diets containing much raw egg white or by administering sulphonamides.

In rats biotin deficiency is characterized by a generalized erythematous, scaly, greasy pruritic dermatitis, arrest of growth, an abnormal posture and spastic gait (Fig. 43), and a typical atrophy of fur around the eyes, producing the condition known as "spectacle eye." Degenerative changes have also been described in the thymus, testes and epididymis, skin and muscles [316], and alopecia may occur [370]. When biotin deficiency is induced by feeding sulphaguanidine or succinylsulphathiazole granulocytopenia, leucopenia and anaemia develop as well [303, 304]. Hyalinization and necrosis of voluntary muscle, and sclerosis and calcification of the blood vessels also occur. The stress of lactation may produce a mild biotin deficiency in the rat; the rat requires biotin for gestation and lactation [338].

In the mouse, biotin deficiency causes all the symptoms described in the rat, and alopecia and greyness in mice with dark fur [343]. It is essential for reproduction and lactation in mice [344].

Cattle do not need an exogenous supply of biotin, which is synthesized by bacteria in the rumen [319].

Biotin deficiency in the chick results in dermatitis and perosis [317]; the vitamin is necessary for normal embryonic development of the egg [299]. The pig with biotin deficiency suffers from alopecia, spasticity, cracks in the skin of the extremities, and a dry rough dermatitis [300]. In the monkey biotin deficiency is characterized by thinning of the fur and pigmentary changes in the hair [347]. The biotin deficient puppy suffers from a progressive paralysis [349]. This is probably due to a secondary potassium deficiency, since it is reversed by potassium.

Biotin and Infection. It has been claimed that biotin activates lysozyme, the lytic enzyme in tears, mucus, sputum and body fluids that digests bacteria [374]. This, however, has been disputed [375]. A deficiency of biotin increases the severity of infection with *Plasmodium lophurae* in chicks and infection of rats with *Trypanosoma lewisi* [377, 380]. Biotin deficient rats are more susceptible to infection with *Salmonella typhimurium* [356].

Biotin Deficiency in Man. Sydenstricker and his colleagues [175] have reported a deficiency syndrome in four volunteers kept on a diet poor in biotin, lack of which was accentuated by including large quantities of egg white in the diet. Vitamin supplements were added so that the diet was adequate in all other respects. At the beginning of the experiment all the volunteers were in good condition and free from symptoms and signs of avitaminosis. During the third and fourth weeks all developed a fine, scaly dermatitis, which disappeared spontaneously. After the seventh week one volunteer developed a maculosquamous dermatitis of the hands, arms and legs. During the seventh and eighth week all showed a striking greyish pallor of the skin, which was interpreted as a sign of vasoconstriction. Eventually all the volunteers developed a definite atrophy of the lingual papillae, which was either general or patchy with the production of a "geographical" tongue. The tongue remained pale, and in no way resembled that seen in ariboflavinosis (p. 312) or pellagra (p. 358). By the ninth and tenth week all showed dryness of the skin of the extremities, with a tendency to fine branny desquamation.

After the fifth week many of the symptoms associated with aneurine deficiency (p. 239) began to appear—mild depression to extreme lassitude, muscle pains, hyperæsthesia, localized paræsthesiæ, anorexia and nausea. Blood examinations showed a fall in hæmoglobin percentage, the number of erythrocytes and volume of packed red cells; these changes occurred in spite of an adequate iron intake. There was a striking rise of bile pigments and cholesterol in the blood. The urinary excretion of biotin fell to 3.5 to 7.3 micrograms in twenty-four hours. Treatment with an injectable biotin concentrate, 75 to 300 micrograms daily, resulted in relief of signs and symptoms in three to five days. The minimal amount of biotin for prompt relief was 150 micrograms daily. The urinary excretion rose from 3 to 7 micrograms to an average of 55 micrograms of biotin daily.

A case of suspected biotin deficiency has been reported by Williams [321]. The patient was an old retired Italian labourer, who suffered for years from an exfoliative dermatitis and mild conjunctivitis. There was nothing in the history of the case to account for the rash, but the dietary history was significant. Since adolescence the patient had a passion for raw eggs, the consumption of which ran into six dozen weekly. The whites of twelve eggs contain sufficient avidin to bind 230 micrograms of biotin [322]. Therefore, it would seem that little if any biotin would be absorbed from the gastrointestinal tract. His choice of foods was narrow, and excluded good sources of biotin; the diet included one to four quarts of wine daily. The skin lesion, which was studied by biopsy, did not correspond to that due to a deficiency of nicotinic acid, riboflavine, pantothenic acid or pyridoxine, but closely resembled that seen in animals with biotin deficiency. Half the total surface of the body appeared normal, but the entire face, ears, shoulders, dorsum of forearms, hands and lower legs were a fiery red and covered with scales. Skin biopsy showed loose sheets of keratinized epithelium, flattened rete pegs, and cellular infiltration around the hair follicles, sweat glands and blood vessels (Figs. 44 and 45). The epidermis showed hyperkeratinization, and parakeratinization, and the corium was thickened, due to interstitial cedema and hypertrophy of the collagen fibres. Sebaceous glands were absent, hair follicles atrophic, and sweat glands and ducts dilated. The macroscopic and microscopic changes of the skin were compatible with a diagnosis of biotin deficiency, although not pathognomonic of the condition as seen in animals. Before treatment the serum biotin was low. After the patient had been hospitalized for a fortnight on a liberal diet and given injections of biotin methyl ester, the dermatitis largely disappeared, the serum biotin returned to normal levels, and the patient's general condition improved.

Brown [221] described three cases of infants who had a mild skin lesion after the administration of sulphonamides for respiratory infections. This lesion resembled those described by Sydenstricker in volunteers fed on raw egg white (p. 129). The lesions were aggravated by administering egg white and improved by biotin methyl ester. According to Berger [330] the excretion of biotin in infants with seborrhœa is lower than normal; the effect on the seborrhœa of administering biotin is not mentioned. Oppel [267] also recorded a low response in patients with seborrhœa to a tolerance test with biotin, but he was unable to observe any beneficial effects from administering biotin.

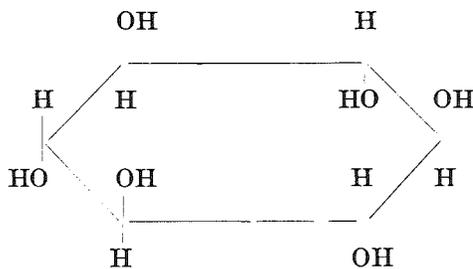
Nutritional deficiency in children is sometimes associated with changes in the texture and colour of the hair (p. 363). Chavarria and his co-workers [225] have noted that in Costa Rica undernourished infants and young children often have depigmented hair. The administration of biotin was thought to accelerate the growth and return of the hair to its normal colour, although the authors are careful to point out that the observations were uncontrolled and not fully proven.

Biotin and Cancer. If butter yellow (*p*-dimethylaminoazobenzene) is

fed to rats hepatic tumours are produced, although their formation is delayed by diets rich in protein or some members of the vitamin B complex. This protective effect is prevented by administering biotin [334]. Tumour tissue, including carcinomata, also contains abnormally high concentrations of biotin [289]. It was therefore considered that biotin might be essential for the growth of neoplastic tissue and that by diminishing the biotin intake of cancer patients the growth of existing tumour tissue might be inhibited. Kensler and his co-workers [37] did not note any beneficial effect in mice with mammary carcinoma fed a diet rich in egg white, and Kline and his colleagues [35] failed to observe a decrease in liver tumours in affected rats kept on diets deficient in biotin. The inoculation of fragments of sarcoma 37 or sarcoma 180 into mice already suffering from biotin deficiency is followed by tumour growth, occurring at the expected rate [39]. Rhoads and Abels [323] gave large quantities of egg white to two cancer patients in the hope of arresting the growth of the neoplasm. There was no improvement in their clinical condition and there was no evidence of biotin deficiency although the egg white contained sixteen to forty times enough avidin to bind the biotin of the diet. Kaplan [333] treated a group of ten cancer patients maintained on diets low in biotin with the whites of thirty-six to forty-two eggs daily, but no beneficial effect on the disease was noted, although it was considered that the general condition of the patients was markedly improved.

INOSITOL

In 1940 Woolley [138] showed that inositol was essential for the growth of hair in the mouse; deficiency leads to alopecia. Since then it has been shown to be required for the nutrition of several other species. Although inositol has been known to be a cyclohexanehexanol since 1887 its configuration has only more recently been established as meso-cyclohexanehexanol with the following configuration :



Meso-inositol

Inositol is widely distributed in large amounts in most animal and plant tissues, fruits and cereals being good sources. Yeast and crude liver extracts contain a considerable amount. In animal tissues it appears to be combined with a protein, whereas in the plant it is present as phosphoric esters, the commonest being the hexaphosphate or phytic acid. The mixed calcium magnesium hydrogen salt of this is known as phytin, and it occurs in a number of cereals. Phytic acid precipitates calcium ions to form the insoluble calcium salt, the calcium and phosphorus of which cannot be utilized. The phytic acid of cereals not only renders much of the calcium unavailable but also combines with the calcium of other foods, e.g. milk, and prevents its absorption. Foods containing much phytic acid, e.g. oatmeal, are therefore rachitogenic (p. 527). Phytic acid can also combine with iron to form an insoluble salt and so prevent its absorption from the gut.

Inositol deficiency in mice and rats produces alopecia and loss of weight [138]. Dogs kept on diets deficient in inositol and pantothenic acid show

increased gastric emptying time, pylorospasm, segmentation in the large and small intestine and gastro-intestinal hypertonicity and hypomotility [144]. Deficiency syndromes have also been produced in the guinea-pig, pig and fowl.

The production of fatty livers in rats by feeding a beef liver fraction can be prevented by inositol, which has a lipotropic effect [484]. In pigs and rats the symptoms produced by administering sulphathalidine or sulphasuxidine can be relieved by inositol [252, 253].

The function of inositol is unknown. It is possible that it is not a prosthetic group of an essential enzyme system, like the other B vitamins, but an essential component of living tissue. It is uncertain if it is essential for human nutrition as no deficiency syndrome due to lack of the vitamin has been described. The probable daily intake in man is about 1 gram daily [339]. It is present in the blood to the extent of 0.37 to 0.76 mg. per 100 ml. [372] and about 12 mg. are excreted in the urine in twenty-four hours [379]. It has been claimed that the administration of inositol to patients with gastro-intestinal cancer reduced the fatty infiltration of the liver [288]. Lupton and his co-workers [21] investigated the lipotropic effect of inositol in human atheromatosis, but they were unable to observe any beneficial effect after administering it for six months.

An antimetabolite for inositol is known. It is lindane ("gammexane"), the γ -isomer of 1 : 2 : 3 : 4 : 5 : 6-hexachlorocyclohexane, which is used as an insecticide. It may act by a process involving interference with the metabolism of inositol [348].

CHOLINE



There has been some hesitation about accepting choline as a member of the vitamin B complex. Admittedly it is essential for nutrition, but it is required in such relatively large amounts (35 to 100 mg. per kg. body weight) that it is probably a structural component of the body tissues rather than a biochemical catalyst. Lack of it can produce a deficiency syndrome but so can lack of protein or essential amino-acids.

Choline in Foodstuffs

Food	Choline content in mg. per gram.
Asparagus	1.28
Barley	1.39
Bread, white	0.625
black	0.565
Brussels sprouts	1.03
Butter	0.4
Cabbage	2.51
Carrot	0.95
Cheese	0.48-0.56
Cod	2.0
Egg white	negligible
yolk	17.13
Flour, white	0.52
Ham	0.88
Herring	1.27
Kidney, pig	2.56
lamb	3.6
beef	3.33
chicken	2.23
Leek	0.095

Choline in Foodstuffs—continued

Food	Choline content in mg. per gram.
Liver, pig	5.52
beef	6.3
calf	6.5
chicken	3.42
Milk	0.147
Muscle, veal	0.7-1.4
lamb	1.1
pork	0.75-1.0
beef	0.9
Oats	0.94
Onion	Nil
Parsley	0.16
Peas	2.63
Pork chops	0.77
Potato	1.06
Rice	1.07
Salmon	1.81
Soya bean	3.0
Spinach	2.38
Trout	0.87
Turnip	0.94
Wheat	0.92
bran	1.43
germ	4.1

Choline Deficiency. The importance of choline as a food factor was first suggested several years ago by Best [31], who showed that in rats the addition of choline to a diet high in fat prevents the deposition of excess fat in the liver. Choline is, therefore, said to be a lipotropic factor; betaine, the anhydride of choline, and the amino-acid methionine also appear to have a lipotropic action. The exact mechanism of the lipotropic action of choline is unknown, but it is assumed that dietary choline increases the phospholipid content of the liver and this promotes the transport of fatty acids, as phospholipids, from the liver to other tissues, or promotes the utilization of fatty acids in the liver itself [30]. A diffuse nodular cirrhosis of the liver occurs in a number of species kept on diets deficient in choline [235], and at the same time hæmorrhagic degeneration of the kidneys has been observed, the lesion occurring in the cortex of the organ [32-34]. A chronic choline deficiency produces destruction of the renal parenchyma in the rat [384]. Hypertension also occurs in choline deficient rats [385]. Not only is choline essential for the metabolism of neutral fat, but also for that of cholesterol [238]. Choline also prevents the formation of fatty livers in rats on diets deficient in aneurine. This excess fat is due to synthesis from carbohydrate sources.

Choline is able to prevent specific dietary hepatic injury in rats, and protects against liver damage by toxins such as chloroform and carbon tetrachloride [25, 36, 383]. A considerable reduction in phagocytic activity has been observed in rats fed diets deficient in choline [345].

Choline deficiency in animals produces a hæmorrhagic tendency. The similarity between the enlarged hæmorrhagic kidney and ocular hæmorrhages in choline deficient animals and hypertensive retinopathy in man has been pointed out. However, it is unlikely that choline deficiency exists in man, as lecithin, of which choline is a component, is widely distributed in animal and vegetable foods.

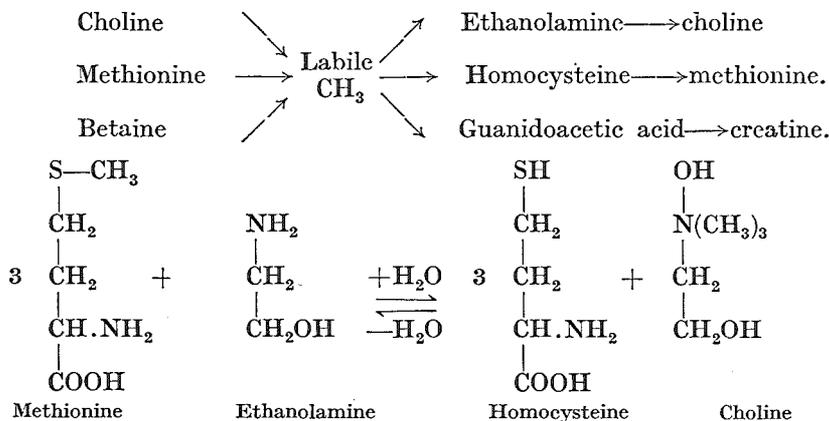
Lowry and his co-workers [43] produced cirrhosis of the liver in rats resembling Laennec's cirrhosis in man. Choline both prevented and cured

the condition, although, as would be expected, the fibrous tissue persisted. The liver cells regenerated and the gross appearance of the liver improved. Similar findings are reported by Fouts [226]. Best and his co-workers [386] noted that choline prevents liver damage in rats caused by feeding excessive quantities of sugar and alcohol. Milk is not a very good source of choline (1.07 mg. per gram), and Rao [229] has suggested that the infantile cirrhosis seen in Hindu children (a variety of portal cirrhosis) may be caused by a diet of cows' milk and a *B. coli* infection. Recent clinical studies also point out the importance of a high protein diet in the treatment of patients with hepatic lesions.

Functions of Choline. Choline is essential for the normal nutrition of the chick and for egg production [232]; for the prevention of perosis or slipped tendon in some birds [233]; for the lactation and normal nutrition of the rat [235]. Generally speaking the young growing animal needs more than the adult. The choline requirement of the dog is about 35 mg. per kilo of body weight daily [242]; that of the chick is 75 mg. daily [381].

In addition to the above-mentioned functions choline is utilized in the animal organism for the formation of acetylcholine.

The methyl groups of choline and the other lipotropic factors, betaine and methionine, play a part in the metabolic process known as transmethylation, which is concerned with the shifting of specific methyl groups as such from one metabolite to another [382]. The hypothesis has been advanced that methyl groups in a utilizable form are indispensable in the diet because the animal organism cannot itself generate the methyl groups for the essential methylations. Thus methionine is essential for the growth of young rats. Growth occurs, however, if they are fed a diet containing choline and homocysteine, because a labile methyl group is supplied by the choline and donated to the homocysteine which is thereby converted to methionine. Conversely, choline can be formed by the methylation of ethanolamine, methionine acting as a methyl donor [382]. Another important physiological methylation is the conversion of guanidoacetic acid into creatine.



Clinical Uses of Choline. Very little choline is excreted in the urine or faeces. The intake in the average human diet is about 650 mg. daily. It can to some extent be replaced by methionine.

Choline has been used in the treatment of hepatic cirrhosis in man. In 1937 Patek and Post [38, 40] stated that a diet containing a large amount of the vitamin B complex had a favourable effect on the survival rate in patients with hepatic cirrhosis. Since then several papers have appeared on the beneficial effects of choline on human hepatic cirrhosis, particularly when associated with fatty infiltration [387-389]. The results have not been conclusive because controlled studies are difficult to make. The results in

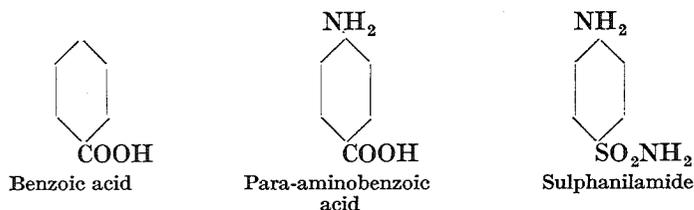
advanced fibrotic cirrhosis have been disappointing. The normal human diet contains a considerable quantity of choline and there is no evidence that a deficiency state due to choline deficiency exists in man. Many workers have given doses as high as 6 grams a day, which is approaching the toxic level; the danger is a sudden flooding of the circulation with choline and consequent depression of the heart and blood pressure. Herrmann and Rockwell [390] claim that the beneficial effects they observed might have been due to improved myocardial efficiency as the choline is converted to acetylcholine. Choline and methionine have been used in the treatment of infectious hepatitis with conflicting results [391-394]. Williams, Cayer and Cornatzer [421] have shown that in patients with fatty infiltration of the liver the rate of phospholipid turnover, measured by administering fats containing radioactive P_{32} , is higher following large doses of choline or methionine.

Moosnick, Schleicher and Peterson [395] reported that the administration of choline chloride resulted in the hæmatological remission of a case of pernicious anæmia refractory to parenteral liver therapy. Davis and Brown [396] failed to observe any beneficial effect in a case of pernicious anæmia that subsequently responded to parenteral liver therapy, in one of nutritional megaloblastic anæmia, in a case of megaloblastic anæmia of pregnancy and in two cases of megaloblastic anæmia associated with the sprue syndrome. A significant response to choline occurred in two cases resembling Addisonian pernicious anæmia which were refractory to parenteral liver extracts. Davis and Brown concluded that choline has no direct erythropoietic activity, but that under certain circumstance it may potentiate the effect of liver extracts. Watson and Castle [397] noted that choline increased the red cell and hæmoglobin values in a patient with hepatic cirrhosis and macrocytic anæmia.

PARA-AMINOBENZOIC ACID

There is some doubt about the status of para-aminobenzoic acid (PABA) as a vitamin. It is an integral part of one of the other B vitamins, folic acid, and is essential in animal, and possibly in human nutrition, although in the latter this has never been demonstrated. It has not been conclusively shown that PABA is required nutritionally in addition to folic acid or that it has catalytic functions independent of folic acid. PABA is unique in that it is a "vitamin within a vitamin" [398].

PABA was first synthesized in 1863 by Fischer, although its claim to be a member of the vitamin B complex dates from only 1940. In that year Woods [399] and Fildes [400] suggested that a sulphanilamide antagonist in yeast was PABA and that it was an essential metabolite for the growth of bacteria. Woods and Fildes advanced a hypothesis to explain the antagonism of PABA and the sulphonamides, based on similarity of structure.



In 1941 Ansbacher [401] stated that PABA is essential for the normal pigmentation of the rat and is a growth factor for the chick. This was challenged by other workers, but Martin [402] reconciled the opposing views by showing that PABA prevents achromotrichia only by altering the intestinal flora, thus favourably influencing the bacterial synthesis of folic acid, which is the factor preventing achromotrichia.

The occurrence of PABA in foodstuffs is correlated with that of folic

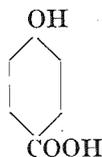
acid, of which it is a constituent. Cereals contain from 0.3 to 1.0 micrograms per gram; vegetables from 0.18 to 0.6 micrograms per gram; ox liver 2.5 micrograms per gram; dried egg 0.2 to 0.36 micrograms and milk 0.15 micrograms per gram. Yeast is the richest source and contains from 4 to 100 micrograms per gram. Much of the PABA in foods is "bound" with proteins, amino-acids or polypeptides.

Absorption and Excretion of PABA. PABA is absorbed rapidly from the human intestine, absorption being complete within eight hours, but no more than fifty per cent. of a dose of PABA given by mouth is absorbed [403]. By administering PABA containing the radioactive isotope N_{15} Lustig, Goldfarb and Gerstl [404] showed that there was no storage or utilization of the compound. Nineteen hours after injection only traces were detected in the organs, but eighty-two per cent. was found in the excreta. Most of the PABA excreted is in the conjugated form, i.e. acetylated as *p*-acetylaminobenzoic acid. PABA is synthesized by the bacteria in the human gut because the faecal excretion is considerably in excess of the dietary intake [405]. It is not known whether synthesized PABA is absorbed. It can be absorbed from the large intestine [403], and is probably absorbed from the bacteria that synthesize it. PABA is excreted in human sweat, which contains 0.0024 micrograms per gram [406].

PABA Deficiency in Animals. There is some doubt whether lack of PABA *per se* produces deficiency symptoms in animals. Those reported—grey hair in rats and mice [401], and failure of reproduction and lactation [407]—are probably indirect. Any action it may have is believed by most workers to be on the intestinal micro-organisms rather than on the animal. The quantities required to remove the effects of a so-called deficiency are far greater than those ingested in the animal's normal diet, or those that can be assimilated or utilized [408]. The small amount of PABA present in various animal tissues and fluids and finally excreted in the urine are probably derived from bacterial growth in the intestine and not from the food. Sulphaguanidine inhibits the growth of intestinal bacteria and if this is administered to human subjects there is a marked drop in the excretion of PABA, only traces being excreted [408]. It can therefore be considered that it originates in the intestine during the growth and multiplication of the bacterial flora. PABA is known to stimulate the growth of bacteria in the gut, and these bacteria produce several other vitamins, in particular folic acid. This can, in fact, cure deficiency symptoms said to be due to PABA [409].

Antibacterial Action of PABA. Although a growth factor for many micro-organisms, PABA in a concentration of 1 in 1,000 inhibits the growth of *M. tuberculosis*. The severity of infection with this organism is diminished and the survival time increased in guinea pigs given 100 mg. of PABA daily [410]. On the other hand, the survival time of mice infected with typhoid is diminished by feeding PABA [410].

When PABA is incorporated in the diet to the extent of three per cent., it is remarkably effective in suppressing typhus infection in mice [411], and some rickettsial infections of chick embryos when injected into the yolk-sac. This suggested its use in the treatment of some rickettsial infections in man (p. 142). The anti-bacterial and anti-rickettsial action of PABA can be inhibited by para-hydroxybenzoic acid [560],



Pharmacology and Toxicology. The LD_{50} by the oral route in mice, dogs and rats respectively is 2.85, 1.3 and 7.6 grams per kilogram [412]. Oral

doses much in excess of 1 gram/kg. in dogs cause death following acute gastro-enteritis and hæmorrhage into the small intestine.

A number of toxic manifestations have occurred following the administration of PABA to human subjects. Drug fever, dermatitis medicamentosa, nausea, vomiting and leucopenia may occur after its administration [414], and myocardial, renal and hepatic damage have been reported [559]. Toxic hepatitis has been stated to occur after its administration [413].

PABA diminishes the toxicity of organic arsenicals such as acetarsone, neoarsphenamine, and tryparsamide [433] and also some organic bismuth preparations, e.g. sodium bismuth tartrate [434]. Clinically it does not prevent nitritoid crises, gastro-intestinal reactions or optic nerve injury produced by tryparsamide [352]. The effect is not specific as it is produced by other organic acids such as benzoic, phenylacetic and phenylpropionic acids [351].

The oral administration of PABA in man interrupts or greatly depresses the conjugation of ingested salicylate with glycine, so that only very small quantities of salicylic acid appear in the urine. It also lowers the *pH* of the urine and thus decreases the renal clearance of free salicylate; and it causes a decreased urinary excretion of salicylate and a rise in the plasma salicylate [415]. It has therefore been proposed to administer PABA to increase the plasma salicylate without producing the symptoms of salicylism in the treatment of rheumatic fever and similar conditions without pushing the dose of salicylic acid. From a clinical standpoint the combination of salicylate and PABA has little advantage over the administration of salicylates alone in the treatment of rheumatoid arthritis [482].

Clinical Uses of PABA. Grey Hair and Skin Conditions. After it was discovered that deficiency of PABA in the black or piebald rat resulted in greying of the fur (p. 136) clinical interest centred around the compound in the hope that it might cure grey hair in humans. A claim was made by Sieve [152] that it was effective for this purpose, particularly in subjects with premature greyness, but subsequent investigations by others did not confirm this [417, 418]. Recently, however, Zarafonitis [423] claims that massive doses of PABA, e.g. 6 to 48 grams daily, given for long periods do darken grey hair. He recorded this during observations on the treatment of other conditions with PABA. A possibility that cannot be excluded is the excretion into the hair of a *p*-phenylenediamine-like compound (which is a hair dye), derived from PABA, and acting as a dye. In this case the effect, as in dyeing, would not be permanent. A warning is necessary against the continuous ingestion of PABA. Although its acute toxicity is low, it can cause leucopenia in man [414].

A claim has also been made that PABA, in the form of its monoethanolamine derivative, is effective in the treatment of vitiligo [353, 373]. This was based on its suspected ability to influence melanin formation. Further investigations have failed to substantiate this [354].

It is stated that PABA protects the skin against sunburn, and ointments containing it have therefore been used for this purpose [327]. Rothman and Henningsen [422], using mercury arc lamps, found that human skin with a layer of 0.03 mm. of fifteen per cent. PABA cream required fifty to a hundred times longer exposure to produce a threshold erythema than a control cream containing a plain vehicle. Shaw [424] has recommended topical application of five to ten per cent. solution of PABA in seventy per cent. alcohol, and Sulzberger and Bayer [425] have reported good results in the prevention of sunburn applying PABA in ten per cent. alcoholic solution and in oil-in-water emulsion creams. Shaw claims that patients who were extremely sensitive to sunburn were able to expose themselves for eight to twelve hours to the Florida sun without discomfort. PABA was, however, ineffective in photosensitization dermatitis.

Zarafonitis and his co-workers [426-429] describe the beneficial effect of

TREATMENT OF LUPUS ERYTHEMATOSUS WITH PABA

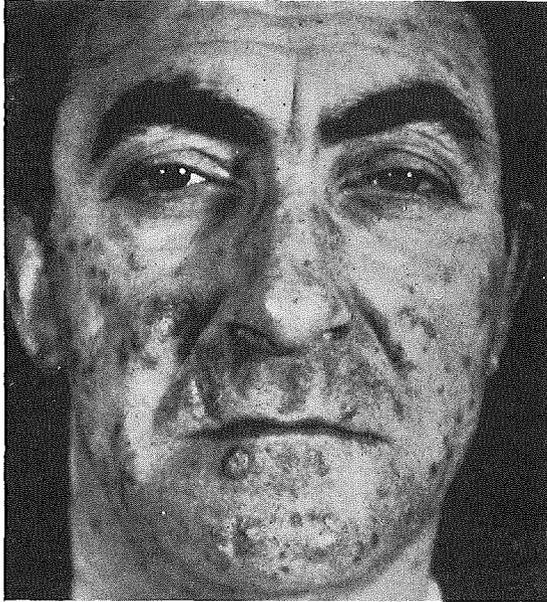


FIG. 46. Patient with Lupus Erythematosus before treatment with PABA. He had previously had treatment with bismuth, gold and local therapy with little success.



FIG. 47. Patient with Lupus Erythematosus. Same as in Fig. 46. After five weeks' treatment with PABA.

TREATMENT OF LUPUS ERYTHEMATOSUS WITH PABA

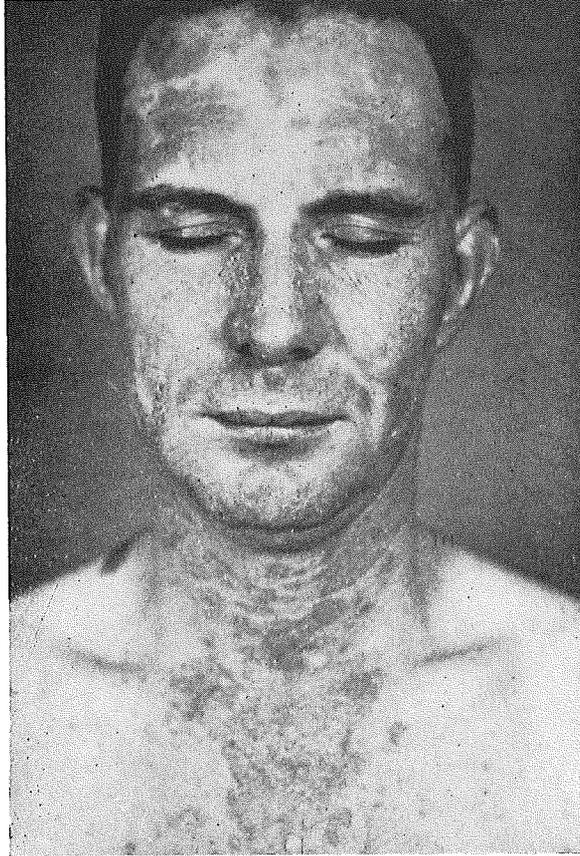


FIG. 48. Patient with Lupus Erythematosus before treatment with PABA.

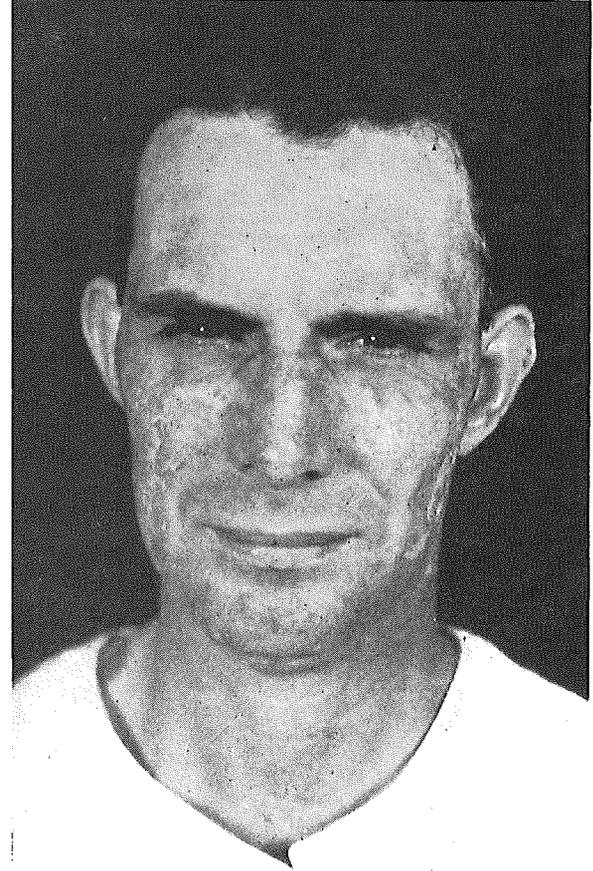


FIG. 49. The same patient as in Fig. 48 after ten weeks' treatment with PABA.



FIG. 50. Patient with Dermatitis Herpetiformis before treatment with PABA

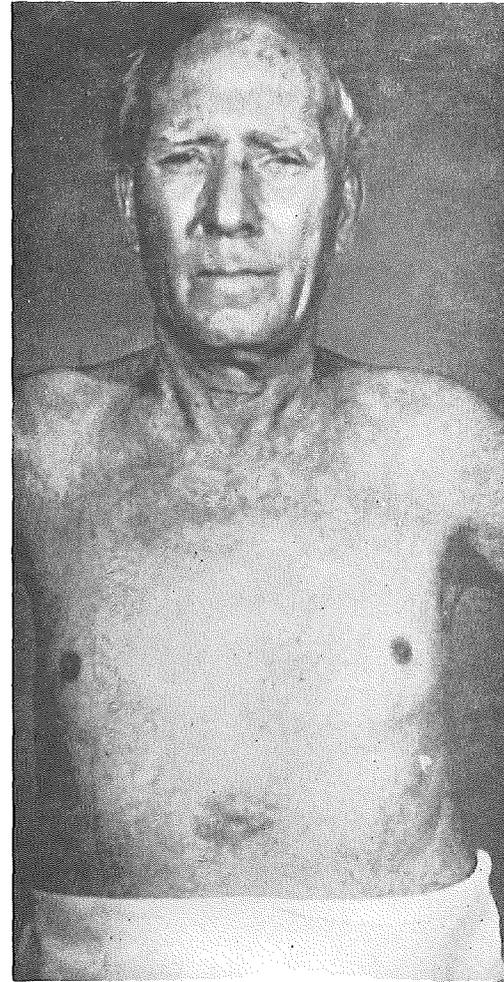
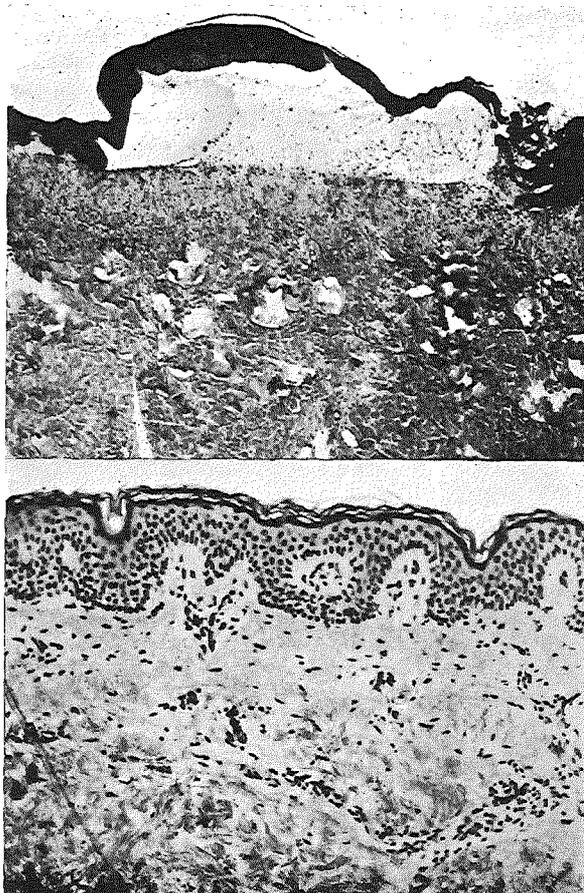


FIG. 51. Same Patient with Dermatitis Herpetiformis as in Fig. 50. Five months after treat-

PABA or its sodium salt in the treatment of a number of skin conditions, including lymphoblastoma cutis, lupus erythematosus, scleroderma, pemphigus and dermatitis herpetiformis. All these disorders are of unknown pathology and the mode of action of PABA in their treatment is quite unknown. The PABA was administered in doses of 1 to 4 grams (10 to 40 ml. of ten per cent. solution of the sodium or potassium salt) at intervals of two to three hours. Loewenthal [696] states that PABA is effective in a dosage of 1.5 to



FIGS. 52 and 53. Upper figure is photomicrograph of skin of a patient with dermatitis herpetiformis shown in Fig. 50. The section, which shows hyperkeratosis, is through a bullous lesion. Lower figure is photomicrograph of skin of same patient as in Figs. 50 and 52 after five months' treatment with PABA. There is only a slight degree of hyperkeratosis and lymphocytic infiltration and no bullæ.

4 grams four times daily in suppressing the manifestations of eczema and atopic dermatitis. Any effect produced is certainly a pharmacological one and not the result of remedying a deficiency.

Leukæmia. Following the observation that the administration of PABA to patients with rickettsial diseases (p. 142) causes leucopenia, it has been used in the treatment of leukæmia to reduce the white count. Zarafonitis and his co-workers [430] administered large doses of the sodium salt (2 to 4 grams every two hours day and night) to five patients with chronic myelogenous leukæmia; a striking lowering of the leucocyte count

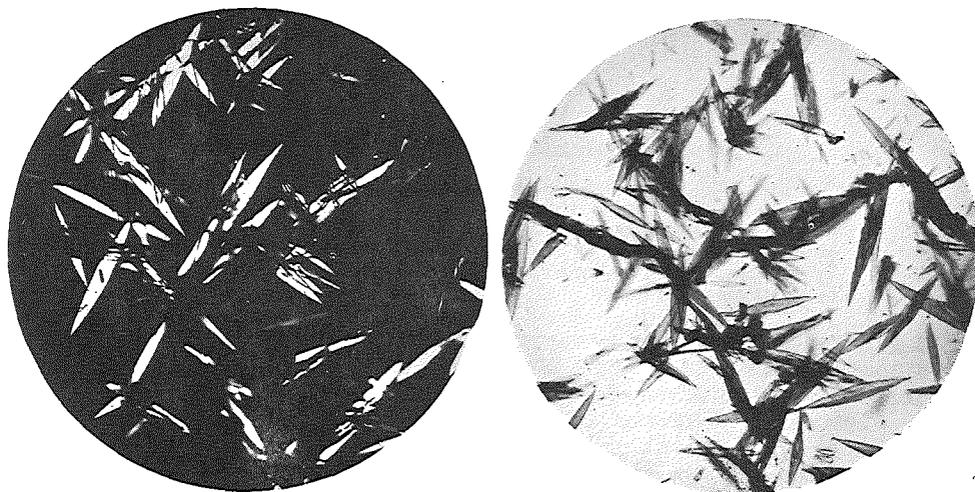
was observed. A less definite fall in the white cell count in two patients with chronic lymphatic leukæmia was observed. There was a prompt rise in the white cell count after stopping the administration of PABA. This effect on the white cell count in leukæmia was confirmed by May and Vallance-Owen [431], who also noted a fall in the hæmoglobin level. Bichel [432], however, using a much smaller dosage, observed a rise. The possible explanation is that the division of myeloid cells, like bacterial multiplication, is stimulated by low concentrations of PABA and depressed by high concentrations.

PABA in Rickettsial Infections. PABA and its sodium salt have been found to exert a depressant action on the metabolism of rickettsiæ. It is reported to be of value in the treatment of Rocky Mountain spotted fever [435-443], epidemic and endemic (murine) typhus [444-448] and scrub typhus (tsutsugamushi) [449-451]. It has not proved uniformly successful in the treatment of all rickettsial infections. Its use in this connection is now of historic interest as it has been replaced by the antibiotics aureomycin, chloramphenicol and terramycin, which are the agents of choice for the treatment of rickettsial infections. Since PABA may also cause leucopenia and has an inhibitory effect on sulphonamides, its therapeutic use in large doses—2 to 3 grams every two hours has been given in the treatment of rickettsial infections—is not recommended. Blood levels of 40 to 50 mg. were aimed at in the treatment of rickettsial infections; blood levels above 60 mg., however, may be dangerous because of the production of hepatic and renal lesions [420].

PABA in Arthritis. Wiesel, Barritt and Stumpe [697] claim that although PABA has no effect in controlling the manifestations of rheumatoid arthritis, when given with cortisone it potentiates the latter and enables a smaller dose to be given for the control of symptoms.

FOLIC ACID GROUP

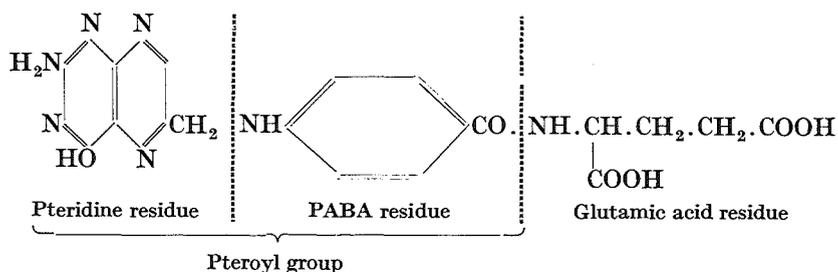
History. The discovery of the compounds known collectively as "folic acid" had its origin in the observation of Mitchell, Snell and Williams [416] that spinach contained an acid nutrilit called by them folic acid, required for the growth of *Streptococcus lactis R* (later shown to be a strain of *S. faecalis*) and also potent for *Lactobacillus casei E* (*L. helveticus*). For this



Figs. 54 and 55. Crystals of Folic Acid from Horse Liver. Polarized light on left. ($\times 85$)

reason it also became known as the "*L. casei* factor," which was subsequently obtained from liver by Stokstad [452]. Earlier, in 1938, Stokstad and Manning [453] showed that chicks required a growth factor which was tentatively named "factor U." In the next year Hogan and Parrott [454] reported another factor essential for the nutrition of the chick; this they named vitamin Bc. Comparison of these factors suggested that they might be identical and that *L. casei* factor might be the same as vitamin M, which had been shown by Day [455] to be necessary for the nutrition of the monkey. In 1944 Mitchell, Snell and Williams [456] concentrated folic acid from spinach, obtaining a product 137,000 times as active as the original product. Others obtained folic acid in crystalline form [457]. The situation was clarified by the successful synthesis and chemical identification of the *L. casei* factor by Angier [458] and the Lederle laboratory group in 1945.

The *L. casei* factor was shown to be N-[4-[(2-amino-4-hydroxy-6-pteridyl)-methyl]-amino]-benzoyl]-glutamic acid :



Variation in the numbers of glutamic acid molecules attached to the pterin-p-amino benzoic acid residue explains the existence of the different "folic acids." Folic acid or liver *L. casei* factor or vitamin Bc obtained from liver is pteroylglutamic acid; *L. casei* factor obtained by fermentation is pteroyltriglutamic acid ("teropterin"); vitamin Bc conjugate, obtained from yeast, is pteroylheptaglutamic acid. *Streptococcus lactis R* (S.L.R.) factor, or rhizopterin, is not a glutamic acid derivative, but is pteroyl formate.

Estimation of Folic Acid. The chief difficulty in assaying folic acid arises from the differences in the response elicited by the different forms of the factor. In determining the folic acid content of foodstuffs a biological method of assay using an animal is desirable. Such a method does not exist so that only microbiological methods are available. The material is first digested with an enzyme such as takadiastase, pancreatic extract, or vitamin Bc conjugase from hog kidney, or broken down by autoclaving. The total folic acid is then assayed microbiologically using *S. faecalis* or *L. helveticus* [459, 460]. Chemical methods of assay are available, but they are not applicable to foodstuffs or body fluids.

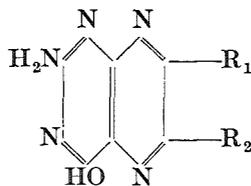
Occurrence in Foods. Folic acid is present in many foods partly as free folic acid (pteroylglutamic acid or PGA), but mainly in the conjugated form, i.e. with several glutamic acid residues. Fresh, deep green, leafy vegetables and liver are very rich sources; fresh green vegetables, cauliflower and kidney are rich sources; beef and wheat cereals intermediate; and root vegetables, tomatoes, cucumbers, light green leafy vegetables, bananas, pork, ham, lamb, cheese, milk, corn, rice and many canned foods are poor in folic acid.

Losses on cooking and on storing food at room temperature are fairly high; refrigerated food retains considerable folic acid, e.g. up to two weeks. Meat retains from eight to forty-six per cent. of folic acid on cooking and ham about sixty-five per cent. when cured. According to Girdwood [499] the average diet contains 0.6 mg. daily before cooking and 0.15 mg. after cooking; the daily intake is not likely to be more than 1 mg.

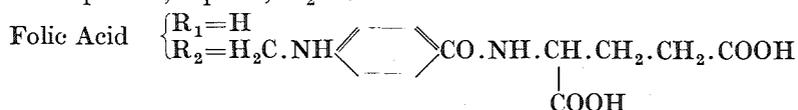
DISTRIBUTION OF FOLIC ACID IN FOODSTUFFS [461]

	Range	
	<i>S. faecalis</i> micrograms/100 gm.	<i>L. casei</i> micrograms/100 gm.
Apples	1	—
Asparagus	48	44
Bananas, green	13	21
ripe	7	15
very ripe	4	8
Beans	43-59	40-41
Beef, muscle	15-33	10-11
brain	11-13	12
liver	44-150	56-105
kidney	30-43	35-56
Broccoli	54	83
Cabbage	12	—
Carrots	11	13
Cauliflower	10-17	45-62
Chard	86-125	87-142
Cheese	5	—
Corn cereals	6	—
Cucumber	5	7
Lamb, muscle	8.2-12	6.4-11
Lettuce	7-8	16
Milk, human	0.071	16
Parsley	88	—
Peas	59	50
Pork, ham	6.7-13.5	5.6-13.8
loin	6.9	—
liver	63-84	—
Potatoes	5	—
Radish	10	11
Rice cereals	11	—
Spinach	76-89	80-114
Tomatoes	8-12	10-12
Veal, muscle	14-33	14-22
liver	30-52	48-51
kidney	36	47
Wheat cereals	29	—

Compounds Physiologically Related to Folic Acid. Xanthopterin. In 1937 Tschesche and Wolf [463] showed that anaemia produced in rats by feeding goats' milk could be cured by the administration of xanthopterin, the yellow pigment in the wings of the brimstone butterfly. Like folic acid, xanthopterin contains the pterin nucleus :

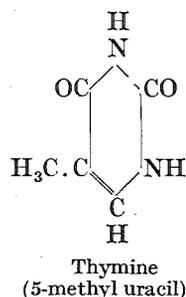
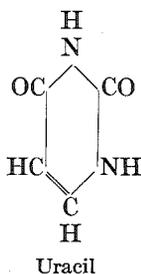


Xanthopterin, $R_1=H$; $R_2=OH$



There have been conflicting reports on the value of xanthopterin in the cure of nutritional cytopenia and anæmia in monkeys suffering from nutritional deficiency attributed to folic acid (p. 148). Rat liver is capable in synthesizing folic acid from xanthopterin [261], which is probably a precursor of folic acid. Like folic acid xanthopterin has hæmopoietic properties, but is far less active.

Uracil and Thymine. In 1944 Stokes [464] found that thymine or 5-methyl uracil could replace folic acid in the nutrition of certain bacteria, although a much larger amount of thymine was required. Massive doses of thymine can also partially substitute for folic acid in the treatment of nutritional macrocytic anæmia, pernicious anæmia and sprue [465-467]; but 15 grams of thymine are needed to obtain the effects produced by 10-20 mg. of folic acid. Thymidine (desoxyribose thymine) also produces a hæmopoietic response in pernicious anæmia [468].



It is possible that thymine might act as a precursor of folic acid or stimulate its production in the intestine. In the rat thymine cannot substitute for folic acid [469].

Folic Acid Antagonists [739]. 7-methyl folic acid [470], 4-amino-folic acid [471], pteroyl aspartic acid [472], and 4-amino-pteroyl aspartic acid [668] are folic acid antagonists, i.e. they can competitively interfere with the synthesis or utilization of folic acid in micro-organisms and in some animals. Their effects can be reversed by large amounts of folic acid. They cannot antagonize folic acid in all species. Thus pteroyl aspartic acid acts as a folic acid antagonist in the chick, but not in the rat [472]. There is considerable evidence that for folic acid activity there must be at least one glutamic acid residue attached to the pteridine fraction; if the glutamic acid is replaced, e.g. by aspartic acid, the compound acts as a folic acid antagonist. 4-amino-folic acid or aminopterin is a folic acid antagonist in rats, chickens, dogs and some micro-organisms. It is of some interest as it has been used clinically in the treatment of cancer and leukaemia (p. 152). Certain antimalarial drugs such as proguanil (paludrine) and 2 : 4-diamino-5-*p*-chlorophenoxypyrimidine (daraprim) are folic acid antagonists [666]; conversely folic acid inhibits the antimalarial action of paludrine (chlorguanide) [667].

Physiology and Functions. Without folic acid the living cell cannot divide, but is halted in metaphase [462]. This property underlies the use of folic acid antagonists, such as aminopterin, in the treatment of leukaemia and cancer (p. 152).

Although folic acid produces a hæmatopoietic response in pernicious anæmia, nutritional macrocytic anæmia and anæmia of pregnancy and sprue, it is not identical with the anti-pernicious anæmia factor present in liver. Indeed, liver extract contains very little pteroylglutamic acid and the small amounts present are not correlated with anti-pernicious anæmia activity. It is not the extrinsic anti-PA factor of Castle, which is almost certainly vitamin B₁₂ (p. 156). There is some evidence that pteroylglutamic acid plays a part in the normal production of red blood cells, including the

maturation of megaloblasts into normoblasts, because this process is inhibited by folic acid antagonists even in the presence of liver [473]. Neither pteroylglutamic acid nor its conjugate have any direct action on primitive erythrocytes *in vitro*, although potent liver extracts cause them to mature. Nor has folic acid any direct action on bone marrow cells [481] like vitamin B₁₂. It is probable that folic acid must be converted to folinic acid (p. 160) to show hæmopoietic activity. Although folic acid is at first effective in the treatment of pernicious anæmia, the anæmia tends to relapse and the dose has to be increased in time; it does not relieve the neurological manifestation of the disease. Pteroylglutamic acid (folic acid), pteroyltriglutamic acid (teropterin) and pteroylheptaglutamic acid (vitamin B_c conjugate) all show hæmatopoietic activity in pernicious anæmia.

The observations of Vilter and his co-workers [476] support the view that folic acid facilitates the formation of thymine and other essential purines and pyrimidines from intermediary compounds such as uracil. They state that thymine will induce a complete hæmopoietic response in pernicious anæmia patients, particularly in the presence of choline and methionine, which supply methyl groups for the synthesis of thymine. It has been suggested by Vilter that folic acid, liver extract and vitamin B₁₂ are essential for the formation of nucleic acid and nucleoprotein and that the megaloblast common to pernicious anæmia and macrocytic anæmia is a primitive erythroblast with an abnormality in the metabolism of nucleoprotein. The so-called maturation arrest in all marrow elements occurs because of this abnormality which may be induced by a deficiency of folic acid or vitamin B₁₂. Thymine is required in amounts at least 5,000 times as much as folic acid, which probably functions as a coenzyme. Thymine is necessary for the formation of thymidine (the reaction being probably catalysed by vitamin B₁₂) which is necessary for the formation of nucleic acid and nucleoprotein. Studies with thymine antagonists do not support this view that folic acid is an activator for the biosynthesis of thymine [477]. Thymine and the purines will not substitute for folic acid in the folic acid-deficient rat [469].

The metabolism of tyrosine and phenylalanine is abnormal in folic acid-deficient animals [478] and this metabolic abnormality, which is also observed in scorbutic guinea pigs and infants, may be reversed by folic acid [479, 480]. Folic acid also appears to function in methylating processes; it is involved in the conversion of choline to betaine and in transmethylation from betaine to methionine [503].

Pharmacology. Folic acid (pteroylglutamic acid) has a low acute and chronic toxicity and shows an almost complete absence of side reactions even in massive doses. The LD₅₀ for intravenous administration is 600 mg. per kilogram of body weight for the mouse, 500 for the rat, 410 for the rabbit and 120 for the guinea pig [485]. Such large doses produce convulsions and renal damage, owing to precipitation in the kidney tubules. In doses up to 100 mg. per kilogram of body weight folic acid has no effect on the respiration and causes only a temporary rise of blood pressure in the dog.

In man, folic acid produces euphoria, increased appetite, decreased basal oxygen consumption in pernicious anæmia patients (no change in normal subjects), an increase in the percentage of total calories derived from carbohydrate, an increase in specific dynamic action, an increase in faecal nitrogen, and an increased nitrogen retention [486]. The results suggested that in patients with pernicious anæmia, cirrhosis of the liver and idiopathic steatorrhœa folic acid increases the absorption of nitrogen from the gastro-intestinal tract and decreases the deamination of amino-acids by the liver. Folic acid increases human serum cholinesterase activity [677].

Xanthopterin is virtually non-toxic given orally. The intravenous LD₅₀ is 50 mg. per kilogram for the mouse.

Absorption, Storage, Excretion. Both free and conjugated folic acid can be utilized by normal subjects. Pteric acid (p. 143) is poorly absorbed in

man (fifteen to forty-six per cent.) and only about one per cent. is converted into pteroylglutamic acid [491]. It was formerly thought that the pernicious anæmia patient could not utilize the conjugate (pteroylheptaglutamic acid). Bethell and his co-workers [487], however, showed that it could be utilized and that the variation in response of the conjugate depends on the presence or absence of conjugase inhibitors, which prevent the hydrolysis of the conjugase to free folic acid (pteroylglutamic acid). The folic acid present in blood is mainly conjugated; whole blood contains only 0.85 micrograms per ml. of free folic acid and 20 to 40 micrograms after enzymic treatment which breaks down the conjugate [488]. In general the ability to utilize folic acid conjugate and hydrolyse it to free folic acid depends on the amount of conjugase-inhibiting substances present in the food. In the absence of conjugase inhibitors the absorption and excretion of folic acid conjugate is comparable with that of free folic acid [489]. Human blood contains folic acid conjugase which hydrolyses the heptaglutamate to pteroylglutamic acid [494].

The average daily excretion of folic acid in man, estimated microbiologically with *S. faecalis* B, varies from 2 to 5 micrograms [405, 489]. The faecal excretion is much greater—200 to 500 micrograms daily—and is in fact greater than the intake, suggesting that in normal subjects intestinal bacteria synthesize folic acid [405]. Patients with nutritional macrocytic anæmia and pernicious anæmia are presumably unable to utilize folic acid synthesized in the intestine. It is uncertain whether normal subjects can utilize the folic acid synthesized by intestinal bacteria. Grundy and his co-workers [492] found that the administration of phthalylsulphathiazole to human subjects on carefully controlled diets diminished the faecal excretion by about ninety per cent., although the urinary excretion was not diminished accordingly as would be expected if the faecal folic acid were absorbed.

The faecal excretion of folic acid increases as the intake increases, varying from fifteen to seventy-five per cent. of the intake. After a 5 mg. oral dose fifty per cent. is excreted [493].

Folic acid is excreted in the sweat, the amount being greater than that excreted in the urine under conditions of profuse sweating [495]. In the rat requirements are increased during lactation [496] and by feeding thyroxine [497]. Folic acid passes the placental barrier [678].

Requirements. Folic acid is required by the monkey, chick, fox, mink, dog, rat, guinea pig, turkey, mosquito and certain micro-organisms. The requirements in chicks can be diminished by feeding aureomycin which probably acts by destroying those intestinal bacteria that compete with the host for folic acid present in the bowel [699]. An experimental folic acid deficiency has never been demonstrated in man, but in view of the activity of the vitamin in nutritional macrocytic anæmia, sprue, and in pernicious anæmia it would seem that it is essential for man, indirectly if not directly. The human requirement is purely conjectural and is based on the amount required to prevent a hæmatological relapse in patients with pernicious and other anæmias. This would appear to be 5 to 10 mg. daily, although Davison and Girdwood [490] consider the requirement to be about a tenth of this, i.e. 0.5 to 1 mg. daily. It may be even less than this, viz. 0.2 to 0.4 mg. daily, as there is no increased excretion when amounts less than 0.2 mg. of folic acid are administered, and only a ten per cent. increase when 0.4 mg. is given to normal subjects [498]. According to Girdwood [499] the daily intake is not likely to be more than 1 mg. and more likely 0.15 mg. if most of the food consumed is cooked. If there is a deficiency of ascorbic acid in the diet the amount of folic acid required to prevent megaloblastic anæmia in the infant is increased [606].

Folic Acid Deficiency. Deficiency in animals produces anæmia, leucopenia and sometimes thrombocytopenia. Oral lesions, diarrhœa and atrophy of the intestinal tract [701] may also result in some species.

In chicks folic acid is essential for the prevention of anæmia, for growth and for normal feathering. In its absence macrocytic hyperchromic anæmia, leucopenia and thrombocytopenia develop. Folic acid also prevents the perosis resulting from diets deficient in manganese, biotin and choline.

In the monkey folic acid deficiency is characterized by loss of weight, leucopenia, anæmia, hæmorrhagic diarrhœa, gingivitis, necrosis of the mouth and gums. The anæmia of folic acid deficiency in the monkey can be corrected with vitamin B₁₂ and ascorbic acid, which may stimulate synthesis of folic acid in the body [698].

Folic acid cures the achromotrichia, anæmia, leucopenia and granulocytopenia caused by administering sulphonamides to rats. In the rat folic acid deficiency causes a pancytopenia and maturation arrest of the red and white blood cells at the level of the stem cell, progressing to total aplasia [701]. In mice folic acid appears to be necessary for lactation and for the maturation of immature blood cells.

Swine deficient in folic acid become anæmic, listless and weak, their hair is shed and becomes lustreless, and they suffer from diarrhœa, leucopenia and neutropenia [501].

The Significance of Folic Acid in Human Nutrition. The diet normally contains pteroylglutamic acid and/or its conjugates and the urine of both normal subjects and patients with pernicious anæmia contain it. It is not the extrinsic factor of Castle (which is vitamin B₁₂) because it cannot maintain patients with pernicious anæmia free from neurological complications [502]. It will convert a megaloblastic bone marrow to the normoblastic state but it cannot prevent the onset of the neural disturbances characteristic of untreated pernicious anæmia; it has in fact been said to accelerate their onset [502]. Megaloblastic anæmia is believed to occur if the diet is deficient in folic acid conjugates and vitamin B₁₂. Although folic acid produces an initial response in the treatment of megaloblastic anæmias both folic acid and vitamin B₁₂ must probably be present in the body for normoblastic blood formation. If a test dose of pteroylglutamic acid is given pernicious anæmia patients they excrete less than do controls, suggesting that there is defective absorption, increased utilization or increased destruction of the compound in such patients [500]. Folic acid may be concerned with the oxidative metabolism of tyrosine [710].

Therapeutic Uses of Folic Acid. Folic acid produces a hæmopoietic response in many types of megaloblastic anæmia, including Addisonian pernicious anæmia [504], the megaloblastic (pernicious) anæmia of pregnancy [521, 523], the anæmia of sprue and pellagra, megaloblastic nutritional anæmia [524, 525], the megaloblastic anæmia of infancy [562], and that following gastrectomy [561]. The pernicious anæmia of pregnancy in this country responds completely to folic acid and usually not at all to vitamin B₁₂ [563]. Folic acid is ineffective in anæmias due to iron deficiency, the leukæmias, and those associated with hypoplasia or aplasia of the bone marrow. Although leucopenia and thrombopenia have been produced in animals deficient in folic acid, it has not proved successful in the treatment of patients suffering from these hæmatological conditions. The effective daily dose for optimal blood regeneration varies from patient to patient in the same way as experience has shown to be the case with liver extracts. Treatment is usually started with an oral dose of 10 to 20 mg. for fourteen days and if there is a satisfactory response the dose can be reduced to 10 mg. and finally to 5 mg. daily; a dose as low as 1 mg. may be effective in some cases [490], whereas some cases may require 20 mg. or more [504]. Large doses given parenterally or orally are unnecessary and wasteful unless the blood picture warrants it. In the majority of cases the maintenance dose is between 2.5 and 5 mg. daily.

Folic acid should never be given as maintenance therapy for the treatment of pernicious anæmia, because it fails to prevent the onset of subacute combined

FOLIC ACID IN THE TREATMENT OF SPRUE

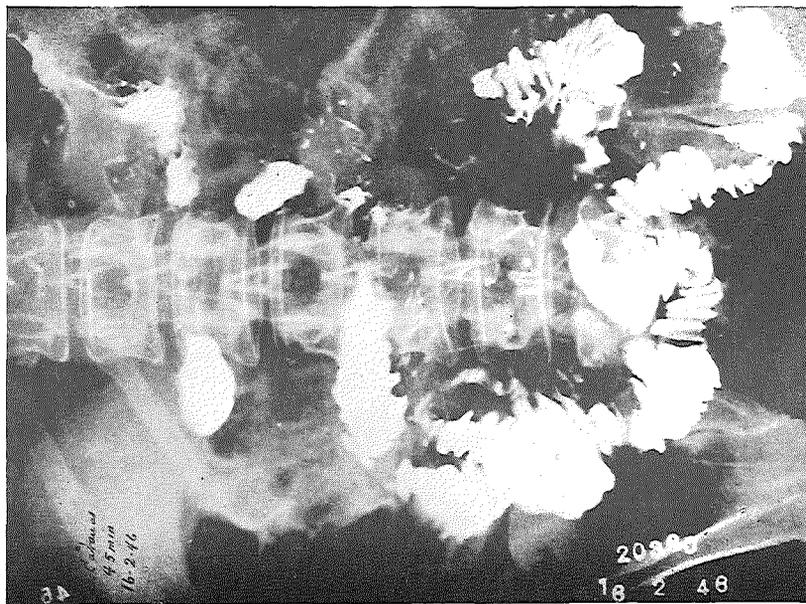


FIG. 56. Patient with Sprue. Three-quarters of an hour after a barium meal, showing a broken barium column, isolated segments and "stack of coins" and "wheel" effects.

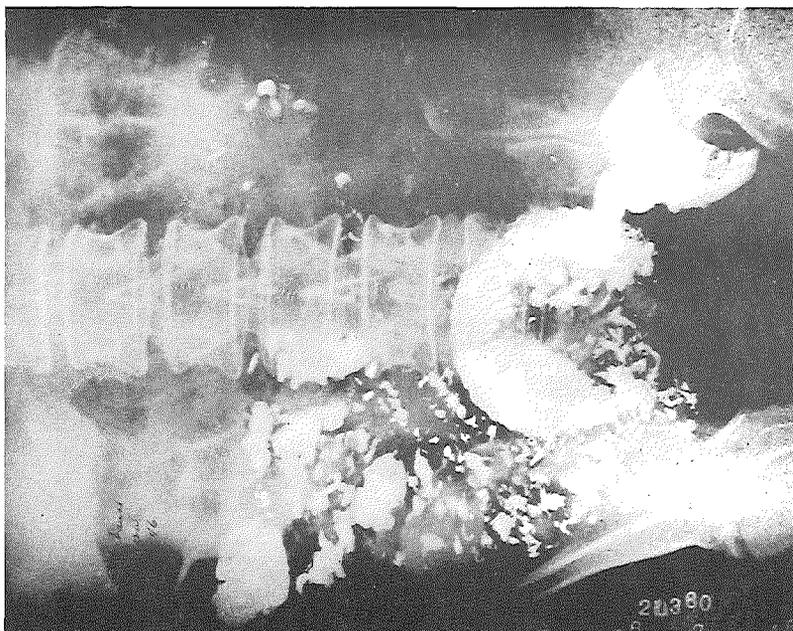


FIG. 57. Same patient as in Fig. 56, one hour after a barium meal, showing "mottlage" of barium.



FIG. 58. Patient with Sprue. The same as in Figs. 56 and 57, after five weeks' treatment with folic acid. The alimentary tract shows normal radiological appearances.

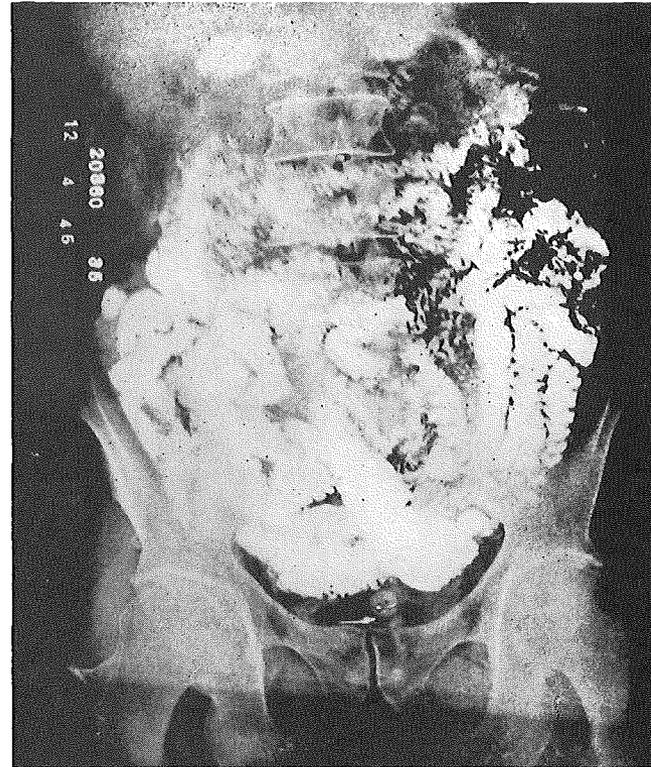


FIG. 59. Same patient as in Fig. 58. The barium meal has passed on further. The radiological appearances are normal (Dr. Spies' case).

degeneration. The therapy of choice is liver or vitamin B₁₂. Before the introduction of vitamin B₁₂ the use of folic acid was justified in patients sensitive to liver while they were being desensitized. There is no justification for its use in pernicious anæmia now. Although folic acid is at first effective in the treatment of pernicious anæmia, the anæmia tends to relapse and the dose has to be raised.

In patients with sprue, idiopathic steatorrhæa and cœliac disease hæmatological improvement usually occurs if the condition is associated with a megaloblastic anæmia. Evidence of the value of folic acid in improving the hæmatological and relieving the gastro-intestinal symptoms of these conditions is, however, conflicting. Spies and his colleagues [504-507] in the United States and Cuba report not only a striking hæmatological response but also the disappearance of the oral and gastro-intestinal symptoms in patients with tropical sprue treated with folic acid. A remarkable improvement in the radiological appearances of the gastro-intestinal tract was also described, including the disappearance of mucosal œdema and intestinal segmentation, spasm, dilatation and hypomotility (Figs. 56 to 59). Darby, Jones and Johnson [508] also report a striking all round improvement in cases treated in the United States. Darby and others [514] noted a return to normal glucose tolerance, improved vitamin A and tocopherol absorption, an increased prothrombin concentration and a decrease in fæcal fat. On the other hand, Davidson and his co-workers [508-510] in Edinburgh, whilst they confirm the dramatic control of diarrhœa and the rapid clinical improvement, consider the hæmatological response very disappointing. They observed no beneficial effects from giving folic acid to patients with cœliac disease or idiopathic ulcerative colitis. Weir and Comfort [512] of the Mayo Clinic also consider the response of non-tropical sprue to treatment with folic acid disappointing; no general improvement occurred that could not be accounted for by improved diet and rest, and macrocytosis persisted. Ferguson and Calder [512] record similar findings. There was no evidence of improvement in fat absorption, biochemical findings, blood pressure or intestinal pattern in any patient with non-tropical sprue treated with folic acid. Whilst Fox [515] agrees that folic acid produces a beneficial effect in sprue, he states that it does not produce remission in all cases, and that it is inferior to liver extract in its effect on the blood picture.

Evidence of the value of folic acid in the treatment of cœliac disease is slender. Brody and Gore [516] and Thomson, Dalton and Wilson [517] noted rapid clinical and hæmatological improvement in three cases. So did Tegelaers and Weyers [518], but their cases received yeast in addition. Davidson and Girdwood [511] and Wilkinson [519] obtained an unsatisfactory response in their cases. Carruthers [520] claimed to have obtained good results in six patients with severe recurrent chronic diarrhœa treated with folic acid; normal stools were obtained in two to five days. The ætiology of the diarrhœa in these cases was obscure.

Israels and Sharp [522] treated five patients suffering from idiopathic steatorrhœa (non-tropical sprue) with folic acid. All patients responded promptly and fully to folic acid given by mouth or parenterally and reasonable blood counts were maintained; the patients were refractory to parenteral liver therapy.

Granulocytopenia. Granulocytopenia produced in rats by feeding purified diets and sulphonamides or thiourea can be both prevented and cured by folic acid [526-528]. Granulocytopenia and leukopenia produced by administering thiouracil and propylthiouracil to rats can also be prevented by the simultaneous administration of folic acid or liver [529]. Menten and Graff [530] have treated children suffering from granulocytopenia following the administration of sulphonamides with folic acid in high doses (150 mg. daily). Thirteen out of twenty-two children showed a rise in the granulocyte to normal levels after seven to ten days; unfortunately no figures are given to

show the rate of recovery of those children whose sulphonamide was stopped and no other treatment given. Black and Stanbury [537] and Waelsch [538] each described two cases of drug granulocytopenia that improved after treatment with folic acid, but spontaneous remission could not be excluded.

Irradiation Sickness. Goldfeder and his associates [531] state that folic acid protects mice against the fatal anæmia and leucopenia produced by X-radiation. This has not been confirmed in the cat [532] or the pig [533]. In man the evidence is conflicting. Davis [534] reports that folic acid in doses of 75 to 150 mg. three times daily diminished the depressant effects of radiation therapy on the bone marrow of sixty-nine patients with lymphoblastoma treated with radiation therapy. Jacobson [535], however, failed to observe any benefit in patients subjected to radiation after treatment with folic acid. Cornatzer and his co-workers [536] state that both folic acid and vitamin B₁₂ have a protective effect on the hæmopoietic system of animals treated internally by radio-active phosphorus, P₃₂.

Folic Antagonists in the Treatment of Leukæmia and Malignant Disease. Folic acid itself is without effect in the treatment of acute and chronic leukæmia [519]. In 1944 Leuchtenberger and others [539] had found that a folic acid concentrate produced regression of sarcoma 180 transplanted into mice, although the work was not confirmed. It was subsequently shown that the concentrate contained pteroyltriglutamic acid (teropterin), and pteroyldiglutamic acid (diopterin). These compounds have since been tried for the treatment of human malignant disease and allied conditions such as acute leukæmia and Hodgkin's disease. Appetite and well-being improve but there is little evidence that these folic acid derivatives have any effect on the disease process [540].

It has been found that pteroylglutamic acid or its conjugates intensify the leukæmic process in the leukæmias [541]. Folic acid antagonists have therefore been tried for the treatment of these diseases. The principal compounds that have been employed are 4-aminopteroylglutamic acid (aminopterin), 4-aminomethylpteroylglutamic acid (A-methopterin), and 4-aminopteroylaspartic acid (amino-an-fol). These inhibit nucleic acid synthesis [542] and also neoplastic growth [543, 546]. When added to human leukæmic leucocytes *in vitro* aminopterin produces marked inhibition of mitosis in low concentrations [544]. The clinical use of such compounds is fraught with danger as they interfere with the nuclear mechanism and are toxic to normal as well as to malignant cells. In minute doses, e.g. 0.003 mg., they inhibit the growth of the chick embryo [545]. Their clinical use in the leukæmias dates from 1949, when it was hoped that in the folic acid antagonists one had a weapon for the effective treatment of this group of diseases. Subsequent investigations [547-556] have shown that remissions do occur, but they are only temporary, often incomplete, and seldom of more than a few weeks' duration. Folic antagonists are not curative in the leukæmias. Improvement occurs more readily in children, and rarely in adults, the lymphoblastic form responding to treatment more frequently than the myeloblastic; improvement in the monocytic form rarely occurs. At best this form of treatment offers a child with acute lymphoblastic leukæmia a twenty-five per cent. chance of a few weeks' remission. It is necessary to supplement treatment with blood transfusions and antibiotics. Toxic reactions, which occur in nearly every case, include a greatly increased tendency to bleeding (this may be fatal), stomatis, diarrhœa, vomiting and alopecia. In addition to hæmorrhage into the skin and mucosæ and bleeding from mucosal surfaces, hæmorrhage into the lungs and middle ears may occur. These hæmorrhagic complications have been responsible for many deaths. Although the results obtained with folic acid antagonists in the treatment of the leukæmias are disappointing, they are of considerable theoretical interest and may furnish a lead in the discovery of a new series of drugs of real value in the leukæmias and malignant disease.

Folic Antagonists in the Treatment of Arthritis and Psoriasis. Aminopterin is a potent inhibitor of connective tissue proliferation [557]. Administration of the folic acid antagonists to patients with rheumatoid arthritis results in a remission of symptoms and signs of articular and periarticular inflammatory action [558]. This is understandable as this form of arthritis is characterized by very pronounced connective tissue proliferation. Aminopterin has no effect on the disease process since the sedimentation rate and gamma globulin are unchanged. The effect of aminopterin in rheumatoid arthritis is not mediated through the adrenal cortex, like cortisone or ACTH, but appears to be due to inhibition of mesenchyme. The effect of aminopterin is not confined to mesenchymal derivatives. Striking remissions of the lesions of psoriasis, lupus erythematosus and atopic eczematoid dermatitis have been reported [558]. The toxic effects of aminopterin limit its use as a practical therapeutic agent in these diseases.

THE VITAMIN B₁₂ GROUP

History. The history of vitamin B₁₂ begins with the discovery of Minot and Murphy in 1926 that liver is curative in pernicious anæmia. Various active concentrates were obtained from liver from time to time but progress was hampered by the lack of a suitable assay technique, all assays having to be made on pernicious anæmia patients in relapse. It was the introduction of a microbiological method of assay of the hæmopoietic principle of liver that speeded up the testing of highly active liver concentrates. In 1947 Shorb [564] observed that there is a linear relation between the content of the *Lactobacillus lactis* Dorner growth factor of liver extracts and their potency in curing pernicious anæmia patients in relapse. In the following year Riekens and his co-workers [565] in America and Lester Smith [566] in England independently announced the isolation of the anti-pernicious anæmia factor in pure crystalline form from liver concentrates. It was called vitamin B₁₂ and later cyanocobalamin. Particular credit must be given to Lester Smith because he was unaware of the work of Shorb and all his preparations had to be assayed clinically. Clinical studies by West [567] and Ungley [568] showed that this crystalline substance was not only active in pernicious anæmia in relapse, but was also effective in the treatment of subacute combined degeneration associated with pernicious anæmia. The daily clinical requirement was of the order of only 0.5 to 1.0 microgram. This effective concentration is so small that it has been suggested that a single molecule is concerned in reaction with each of the red blood cells.

Several derivatives of vitamin B₁₂ have been prepared, e.g. vitamin B_{12a}, vitamin B_{12b}, vitamin B_{12c}, and vitamin B_{12d} [569, 590]. The *a*, *b* and *d* compounds are probably identical [731]. Vitamin B_{12c} contains a nitrite group co-ordinated with cobalt [590]. Another form of vitamin B₁₂ has been obtained from rat fæces [718].

There is now little doubt that the extrinsic factor of Castle is vitamin B₁₂ itself. When administered orally in pernicious anæmia the pure vitamin is ineffective, except in very large doses, but small doses are strongly potentiated by normal gastric juice [572].

Chemistry. The chemistry of vitamin B₁₂ is reviewed by Lester Smith [729]. It was originally isolated from liver. After Riekens and his group [573] isolated it from the metabolism fluid of *Streptomyces griseus* it was produced on a commercial scale as a by-product in the manufacture of streptomycin. This has resulted in a considerable lowering of manufacturing costs. The original yield from liver was only 15 mg. from 1,000 kg. of liver. A crystalline product similar to vitamin B_{12b} has been obtained from cultures of *Streptomyces aureofaciens*, which is used in the production of aureomycin [569].

Vitamin B₁₂ crystallizes in small dark red needles. Shortly after its

discovery it was shown that cobalt in the form of a co-ordination complex was an essential constituent of the molecule [574]. It also contains a cyanide group co-ordinated with the cobalt atom, and for this reason it has been given the name cyanocobalamin in the British Pharmacopœa. Vitamin B_{12b} differs only in the absence of this cyanide group and is readily converted into

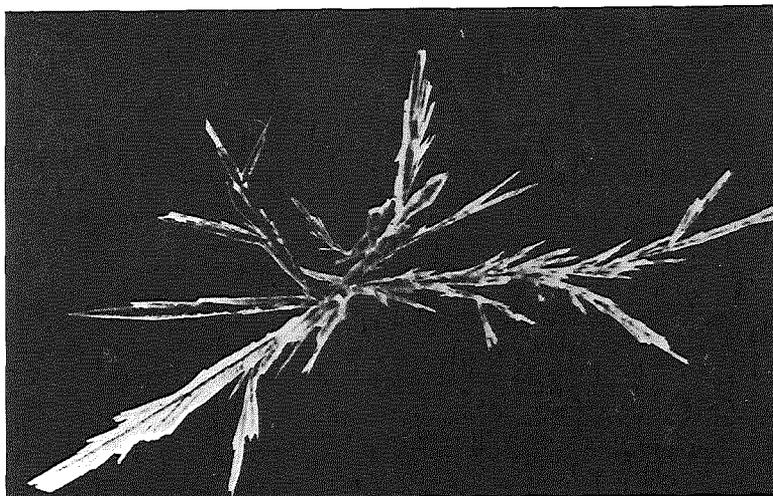
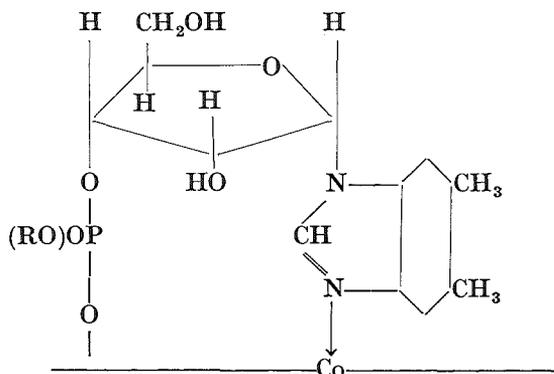


FIG. 60. Crystals of Vitamin B₁₂ obtained from *Streptomyces griseus* cultures.

vitamin B₁₂ by treatment with potassium cyanide [575]. The vitamin B₁₂ molecule contains a 5 : 6 dimethylbenzimidazole residue [576], phosphoric acid, ribose, 1-amino-2-propanol [577] and a cobalt-containing acid with the formula C₄₃H₃₅O₄N₉Co. The probable molecular weight is 1,300. It probably contains the following structure, with the cobalt in the form of a co-ordination compound :



Vitamin B₁₂ appears to be stable in the solid state, and in solution at neutral or slightly acid pH values. It is slowly inactivated in solutions of strong acids or alkalis, but withstands autoclaving for fifteen minutes at 121° C. If kept under sterile conditions in isotonic solution it can be stored at room temperature for more than a year without loss of activity.

Assay of Vitamin B₁₂. The original assay method of Shorb [564] using the growth of *L. lactis* Dorner has not given satisfactory results in the hands of other workers. The addition of tomato-juice and Tween 80 is said to be

necessary in the growth medium [578]. More satisfactory results are said to be obtained using *Lactobacillus leichmannii* as the test organism in conjunction with paper chromatography, the paper strips either being laid on agar plates on which the bacillus has been sown, or cut into strips, each strip being assayed separately [579-581]. The alga *Euglena gracilis* has also been used [586].

Animal assays have also been used. One is based on the increase in weight of chicks from hens deficient in vitamin B₁₂ [582], another is based on the growth of vitamin B₁₂ depleted rats [583]. A colorimetric assay method has been devised, depending on the formation of a blue to purple product after acid hydrolysis and its extraction with a mixture of light petroleum and *n*-octyl alcohol [584]. Another chemical method depends upon the liberation of cyanide on exposure to light [571].

Units. The amount of vitamin B₁₂ is expressed in weight (micrograms)

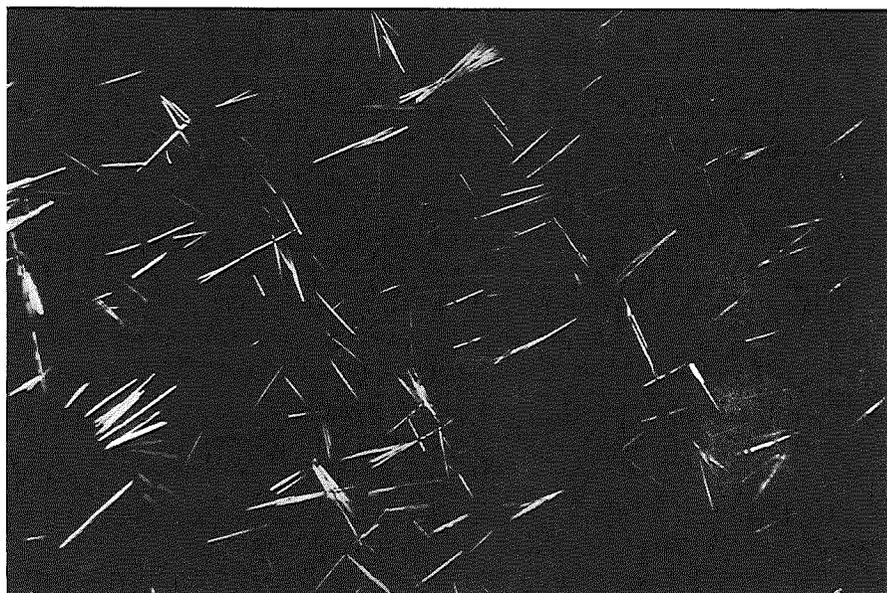


FIG. 61. Crystals of Vitamin B₁₂ obtained from Liver.

of active substance. One microgram of the pure vitamin is stated to have the activity of 1 U.S.P. unit of anti-pernicious anæmia activity, although this may be an overestimate; according to Jones, Darby and Totter [591] it is only a half to a third as potent as this.

Occurrence of Vitamin B₁₂. In view of the doubtful specificity of the methods of assaying the vitamin B₁₂ group, figures giving their distribution in foods must be accepted as provisional only. The richest known sources are liver, kidney, and the nutrient medium in which *Streptomyces griseus* is grown. Meat, milk, cheese, eggs and fish are good sources, while cereals and yeast are poor sources [585]. The tissue fluid of fish contains a cobalt pigment which yields a hæmopoietically active substance on treatment with potassium cyanide [717]. Vitamin B₁₂ is also present in animal dung, muscle and bacterial cultures [717]. The vitamin B₁₂ content of some foods is on p. 156.

Functions of Vitamin B₁₂. Vitamin B₁₂ is as effective as liver in controlling all the manifestations of pernicious anæmia. Not only does it convert megaloblastic erythropoiesis to a normoblastic type [591] but it also prevents the onset of subacute combined degeneration of the cord [592],

	Micrograms per 100 gram.	
	Rat growth	<i>L. leichmannii</i>
Alfalfa meal	—	4
Beef	2-5	4
Cheese	—	—
Dried milk	2-3	—
Egg yolk	2-8	—
Fish meal	—	9-10
Herring	15	—
Kidney	35-50	—
Liver	35-50	—
Liver extract	—	20-2,200
Milk	—	9-10
Mutton	5	4
Pork	1-3	4
Soya bean	—	1
Veal	4	4
Wheat	—	0.7

and relieves the symptoms of glossitis [593]. Vitamin B₁₂ is active parenterally and also orally in much larger doses (thirty to eighty times), or when administered with gastric juice or some other known source of intrinsic factor [594]. Five hundred ml. of normal gastric juice is probably needed to ensure the adequate absorption of 50 micrograms of vitamin B₁₂. The effectiveness of liver and hog stomach preparations when given orally in pernicious anæmia may be explained by their content of vitamin B₁₂ and intrinsic factor. Ternberg and Eakin [596] have shown that the non-dialysable, heat labile component of normal gastric juice unites with vitamin B₁₂; it is less abundant than normal in the gastric juice of pernicious patients, and may be Castle's intrinsic factor. A simple view of the ætiology of pernicious anæmia would be that, as a consequence of atrophic gastritis, the intrinsic factor is produced in sufficient quantity and that the vitamin B₁₂ of the food fails to be modified by it and therefore absorbed. There is experimental evidence to support the view that the intrinsic factor promotes the absorption of vitamin B₁₂ by forming a readily absorbed peptide complex [674, 720]. The fæces of pernicious anæmia patients in relapse contain large quantities of vitamin B₁₂, which is apparently not available [600], although extracts of these fæces are active if injected into other patients with untreated pernicious anæmia.

Vitamin B₁₂ can be utilized locally by the bone marrow cells without any change by intrinsic, or any other factors, which presumably therefore aid its absorption. Direct instillation of the vitamin into the bone marrow cavity of a patient with pernicious anæmia in relapse corrects a qualitative abnormality in cellular ribonucleic acid [596]. Folic acid is not used locally by bone marrow cells within forty-eight hours; it is probable that it must first be converted into an active hæmopoietic substance by enzyme activity elsewhere in the body. Both folic acid and vitamin B₁₂ are probably involved in chemical reactions leading to the formation of nucleic acids in the living cell [597, 742]. Vitamin B₁₂ appears to be necessary for the formation of ribosides, such as thymidine, from purines and pyrimidines. Thymidine replaces vitamin B₁₂ for the growth of *L. lactis* [597] and in large amounts for *L. leichmannii* [598], but it has no erythropoietic effects in patients with pernicious anæmia [599].

In the mammal a deficiency of vitamin B₁₂ has a deleterious effect on growth [603], even if the protein intake is high, and it has been suggested that the vitamin plays a fundamental role affecting the capacity of the normal mammal to utilize protein [601]. According to Henry and Kon [602], this effect may not be specific; aureomycin can replace vitamin B₁₂ as a growth factor in the rat [690]. Pigs fed 10 micrograms of vitamin B₁₂ daily

show a gain in weight greater than that of controls not receiving the vitamin [604]. Concentrates of vitamin B₁₂ obtained as by-products in the manufacture of antibodies are being widely used as feed supplements for livestock. A deficiency syndrome characterized by failure to gain weight has been produced in the human infant fed on a purified ration [605]. The factor responsible is soluble and present in milk; it may be vitamin B₁₂. A cautious attitude must, however, be assumed to the use of vitamin B₁₂ to augment the growth of healthy infants [606]. The vitamin is essential for reproduction and lactation in the rat [687]. In the chick it spares pantothenic acid [725].

There is some evidence that vitamin B₁₂ plays a role in the metabolism of the amino-acids methionine and tyrosine. It protects weanling rats against kidney hæmorrhage induced by diets low in methionine and choline [607], and has a lipotropic effect in rats receiving a diet low in protein and fat, and in choline and methionine [608]. It is choline sparing in the rat [722] and appears to be essential for the utilization of methionine or betaine in the biosynthesis of choline [722]. The vitamin is probably necessary for the transformation of homocystine into methionine [609, 610] and glycine to serine [744]. Vitamin B₁₂ may function in enzyme systems involved in the synthesis and utilization of the labile methyl group and in the process of transmethylation, e.g. the transformation of glycine to choline and homocystine to methionine [611]. The concept of transmethylation supposes the need for preformed methyl groups in the diet which can be transferred from one compound to another to form essential metabolites from precursors existing in the diet or the body (see p. 134). A defect in methylation is seen in dogs deficient in vitamin B₁₂ and results in fatty degeneration of the liver [726].

An aberrant tyrosine metabolism occurs in untreated pernicious anæmia, in which the blood phenol level is high and the urinary hydroxyl phenolic acids are increased. The administration of vitamin B₁₂ to pernicious anæmia patients causes a fall in the excretion of these acids [612].

In the animal vitamin B₁₂ protects against the toxic effects of thyroxine [613]. Wayne and his co-workers [614] conclude that in doses up to 100 micrograms it has no significant effect on human thyroid function.

Absorption, Storage and Excretion of Vitamin B₁₂. Vitamin B₁₂ is poorly absorbed from the gastro-intestinal tract. In man the major portion is excreted in the fæces and practically none in the urine, which appears to have little vitamin B₁₂ activity when tested microbiologically [615]. The daily excretion in normal subjects is about 0.15 micrograms and about a third of this in pernicious anæmia patients in relapse [745]. Even when massive doses, e.g. 10,000 micrograms, are given orally to normal subjects or pernicious anæmia patients practically none is excreted in the urine [688]. The vitamin is synthesized by bacteria in the gut; the administration of cobalt and aureomycin increase the amount, the latter acting presumably on bacteria that compete with the vitamin synthesizing bacteria [686]. 1 : 2-dichloro- : 5-diaminobenzene, inhibits the bacterial synthesis of vitamin B₁₂, probably blocking the utilization of an essential metabolite. 1 : 2-dimethyl-4 : 5-diaminobenzene, which forms a part of the vitamin B₁₂ molecule, on the other hand, stimulates bacterial synthesis [682]. 1 : 2-dichloro-4 : 5-diaminobenzene is an antimetabolite of 1 : 2-dimethyl-4 : 5-diaminobenzene [683]. In the rat given vitamin B₁₂ containing radioactive cobalt about five per cent. appears in the urine over a period of three days, most being excreted in the first day [616]. Small amounts of vitamin B₁₂ given by mouth are only effective in man if accompanied by a source of intrinsic factor [572]. Ungley [617] has made a careful study of the absorption of vitamin B₁₂ in pernicious anæmia patients. He has shown that an oral dose twenty to forty times that effective parenterally is needed for an adequate response; Spies [619] gives a factor of 50 and other authorities

give the figure 200 [688]. According to Ungley some vitamin B₁₂ can apparently be absorbed without combining with the intrinsic factor. At least 100 ml. of *normal* gastric juice is needed to ensure an adequate response from 1 to 2 micrograms of vitamin B₁₂ given orally; the equivalent volume of pernicious anæmia gastric juice would be enormous and certainly beyond the capacity of the atrophic stomach of the pernicious anæmia patient. Doses of 3,000 micrograms are effective given by mouth alone so that some must be absorbed. Ungley showed that vitamin B₁₂ is not absorbed from the buccal mucosa or directly from the intestine. According to Ungley, the intrinsic factor probably does not act by protecting vitamin B₁₂ from destruction by bacteria in the gastro-intestinal tract because absorption is not facilitated by sterilizing the latter with antibiotics. If vitamin B₁₂ is injected into the human subject ninety to ninety-five per cent. of the dose promptly appears in the urine [688]; half is excreted within three to five hours and most of it within twelve hours. The figures are the same for normal subjects and pernicious anæmia patients. It is therefore certain that the fate of orally administered vitamin B₁₂ differs from that of the injected vitamin. The failure to appear in the urine when given orally suggests that it is largely unabsorbed or that after absorption it is combined in such a form that the kidney cannot excrete it (Ungley).

The evidence to date suggests that failure of absorption of vitamin B₁₂ occurs in pernicious anæmia rather than its destruction in the intestine or its preferential utilization by the bacterial flora of the gut. Chronic intestinal disorders may lead to impaired absorption of vitamin B₁₂ and so result in macrocytic anæmia.

The vitamin B₁₂ content of human blood is negligible [624]; according to Conley and his co-workers it is less than 3.5 millimicrograms [688], and according to others [745] it is 100 to 720 millimicrograms. The level is lower in patients with pernicious anæmia in relapse [745]. Eight hours after the intramuscular injection of a solution containing 1 mg. of vitamin B₁₂ none is found in the blood; most is found in the urine [620]. Fifteen minutes after the injection of 1 mg. about 30 micrograms of vitamin B₁₂ is found in a millilitre of whole blood and 50 to 80 micrograms in a millilitre of plasma. After the intramuscular injection of doses from 20 to 75 micrograms from seventy to ninety per cent. is retained in the tissues; the bulk of the remainder is excreted within eight hours [743]. Others have reported an excretion of fifty-three to sixty-eight per cent. after parenteral doses of 84 to 210 micrograms [746]. The vitamin does not diffuse into the red cells [615]. About 0.5 per cent. of an oral dose of 0.89 mg. is found in the liver and kidneys. The amounts found in other organs are negligible [616, 621]. The daily excretion in the stools is of the order of 5 micrograms [621].

Toxicology. Vitamin B₁₂ is virtually non-toxic even in a dose ten million times the therapeutic one. Mice tolerate 1,600 mg. per kg. without any ill-effects [676].

Requirements. The vitamin B₁₂ requirements of the healthy individual are quite unknown. The daily intake in the food may be of the order of 2 to 10 micrograms daily, depending on the diet. A daily intake of 1 microgram, provided it is absorbed, is probably sufficient to maintain a normal blood picture. Considerable vitamin B₁₂ is synthesized by the bacterial flora of the lower bowel; it is not known whether an exogenous source is necessary. The fæces of pernicious anæmia patients have vitamin B₁₂ activity [600, 622], and an extract from them produces a hæmopoietic response if injected into another pernicious anæmia patient [623].

Clinical Use of Vitamin B₁₂. Generally speaking, vitamin B₁₂ is efficacious in those forms of megaloblastic anæmia that respond to injections of refined liver extract. Although sensitivity to the pure vitamin has not been reported it can occur with vitamin B₁₂ concentrates made from liver or streptomycin culture broth [625]. Reactions are more likely to occur in subjects sensitized

to antibiotics prepared from moulds [728]. The clinical aspects of the subject up to 1951 are well reviewed by Ungley [730].

Pernicious Anæmia. Numerous accounts have now been published of the efficacy of vitamin B₁₂ in the treatment of pernicious anæmia, following the original reports of West [567], Spies [587] and Ungley [568]. It is effective in pernicious anæmia patients with or without subacute combined degeneration of the cord. Probably 1 microgram daily is sufficient for maintenance. In uncomplicated cases the initial dose should be 15 to 20 micrograms parenterally once or twice a week, that is about 3 micrograms daily, until remission occurs, then a maintenance dose equivalent to 1 mg. daily, i.e. 15 mg. every other week. Ungley [627] recommends 40 to 80 micrograms initially and then 20 micrograms weekly for three months and 30 to 60 micrograms every three weeks thereafter. Much larger doses have been given by mouth, although thirty to sixty times the parenteral dose is needed for a response. In pernicious anæmia with subacute combined degeneration of the cord 15 to 30 micrograms parenterally once or twice a week should be given until remission occurs, then a maintenance dose of 15 micrograms every other week. An initial dose of 200 to 300 micrograms parenterally in pernicious anæmia is suggested by some workers.

The Sprue Syndrome. Spies and his co-workers [628] in the States have treated a number of cases of tropical sprue with vitamin B₁₂. They report a prompt clinical and hæmatological improvement, characterized by an increase in the reticulocytes, a subsequent rise in the blood cells and hæmoglobin and considerable improvement in the gastro-intestinal symptoms and radiological appearances. Fifteen to 30 micrograms parenterally once or twice a week is stated to induce remission and 15 micrograms weekly thereafter to prevent relapse. It is also active sublingually [748]. The results of Spies and his co-workers cannot necessarily be applied to tropical sprue as known to most British workers; their cases were taken from a very under-nourished population. No well-documented British reports are available at the moment. Folic acid is stated to potentiate the hæmopoietic effect of orally administered B₁₂; daily doses of 1.67 mg. of folic acid and 25 micrograms of vitamin B₁₂ produce an optimal response in sprue [737]. Given by mouth alone, from 150 to 200 micrograms of vitamin B₁₂ daily for two to three weeks or more are necessary to produce a clinical response [741]. Tuck and Whittaker [629] and Israels and Sharp [630] failed to observe any response to vitamin B₁₂ therapy in megaloblastic anæmia associated with idiopathic steatorrhœa. Cooke, Peeney and Hawkins [631] obtained varied hæmatological responses in the latter condition treated with vitamin B₁₂.

Tropical Nutritional Anæmia. Spies and his colleagues [618] and Jones, Darby and Trotter [591] obtained a hæmatological response in cases of nutritional macrocytic anæmia treated with vitamin B₁₂ in America and Cuba, and Patel [632] reported satisfactory results in India. A single dose of 15 mg. is said to produce a favourable initial response, but it may be necessary to repeat this at two-week intervals to prevent relapse. It should be pointed out that nutritional anæmias in different parts of the world may present different hæmatological pictures and the ætiology may be due to a combination of many different factors such as malnutrition, infection and infestation with helminths and parasites. Vitamin B₁₂ is ineffective in the treatment of anæmia due to *Diphyllobothrium latum* (fish tapeworm) [685], probably because the tapeworm absorbs the vitamin.

Megaloblastic Anæmia of Pregnancy and the Puerperium. Although the megaloblastic anæmia of pregnancy and the puerperium respond dramatically to the administration of folic acid, according to most workers vitamin B₁₂ is ineffective in treatment [633-637].

Megaloblastic Anæmia of Infancy. Infants fed on proprietary brands of milk powder sometimes develop this anæmia, which is rare in this country, but has been reported in the United States. It responds to folic acid (p. 148),

but its response to vitamin B₁₂ is variable [638-640]. The effectiveness of vitamin B₁₂ is possibly enhanced by ascorbic acid [640].

Megaloblastic Anæmia after Gastrectomy. The megaloblastic anæmia that sometimes follows total gastrectomy is stated to respond to vitamin B₁₂ [641]. The clinical response is apparently good but the hæmatological response is suboptimal compared with that expected in pernicious anæmia. The macrocytic anæmia of rats produced experimentally by operations on the bowel responds to treatment with vitamin B₁₂ [712].

Leucopenia. Vitamin B₁₂ has no effect on the leucopenia induced by X-radiation [646].

Neurological Conditions. Vitamin B₁₂ is effective in controlling the lesions of the central nervous system that result from the continued treatment of pernicious anæmia with folic acid [642].

Bean, Franklin and Sahs [643] state that painful nutritional neuritis is promptly relieved by the injection of 15 micrograms of vitamin B₁₂. They do not consider the results due to analgesia. Spies and Stone [644] treated five cases of amyotrophic lateral sclerosis with vitamin B₁₂. Stiffness and muscle cramps disappeared and the patients felt stronger. The neurological lesions associated with diabetes are stated to be relieved by doses of 15 to 30 micrograms daily, reduced to once or twice weekly [721].

Vitamin B₁₂ is of no benefit in the treatment of disseminated sclerosis [645].

Growth of Children. Following the observation that vitamin B₁₂ stimulates animal growth (p. 157) its effect on stimulating the growth of children was studied. Wetzel and his colleagues [647] considered that the administration of 10 micrograms daily produced a statistically significant growth response and increased physical vigour and appetite. Chow [615] states that vitamin B₁₂ given to both ill and healthy children produces a gain in weight greater than that of controls. Rascoff and his co-workers [689] observed no significant gain in weight in normal and premature infants given vitamin B₁₂; Downing [679] noted no beneficial effect in premature infants and Chinnoek and Rosenberg [706] none in normal infants. The Council of Foods and Nutrition of the American Medical Association [606] adopts a sceptical attitude towards the results of these experiments and warns that the conclusions must be accepted with reserve.

The Animal Protein Factor. For many years it has been known that a water soluble factor, known as the "animal protein factor" (APF), is required for the survival and early growth of chicks and for the hatchability of eggs. It is present in cow manure, hen fæces, fish meal and the alcohol soluble fraction of liver. Subsequently it was shown that APF is required for the growth of the mouse. It may be transmitted from the mother to the young during gestation and lactation. It was considered that APF is the same as vitamin B₁₂ since it is active in the treatment of pernicious anæmia [669, 670]. The two are not identical. For example, APF can be replaced by a mixture of vitamin B₁₂ and aureomycin or other antibiotics [680]. Stokstad and his co-workers [669] found that a micro-organism from hen's fæces could produce APF in suitable media and that concentrates of the APF produced a hæmopoietic response in pernicious anæmia. Vitamin B₁₂ can replace APF as a growth factor for chicks [671], and APF will give a growth response resembling that of vitamin B₁₂ with *Lactobacillus leichmannii* [672]. APF is effective in the treatment of the megaloblastic anæmia of pregnancy [727]. In later investigations Stokstad and his co-workers [673] considered that APF is vitamin B₁₂ and another unidentified factor.

THE CITROVORUM FACTOR (FOLINIC ACID, LEUCOVORIN)

In 1948 Sauberlich and Baumann [648] showed that liver, liver extracts, yeast extracts and commercial preparations used in the treatment of per-

pernicious anæmia contain a growth factor for the organism *Leuconostoc citrovorum*. This factor is not folic acid, because it will promote the growth of some bacteria, while folic acid will not ; and it is not vitamin B₁₂ because it can be separated from the latter by electrolysis [649]. Sauberlich and Baumann [650] later prepared a concentrate of the citrovorum factor that was much more active than folic acid, and Sauberlich [651] showed that, in animals and man, the urinary excretion of the factor varied with the folic acid intake. Intestinal synthesis is probably not a factor in the production of the citrovorum factor because the urinary excretion is not diminished by suppressing the growth of the intestinal bacteria with sulphonamides [651]. These observations suggested that folic acid might be converted into the citrovorum factor, or else stimulate its excretion. Nichol and Welch [652] showed that liver slices from folic acid deficient rats synthesized the citrovorum factor if incubated with folic acid in the presence of ascorbic acid,

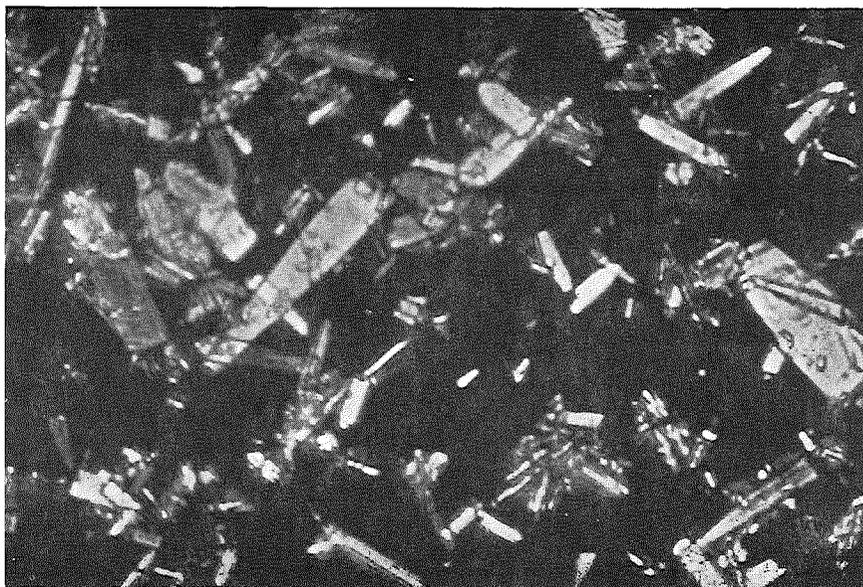
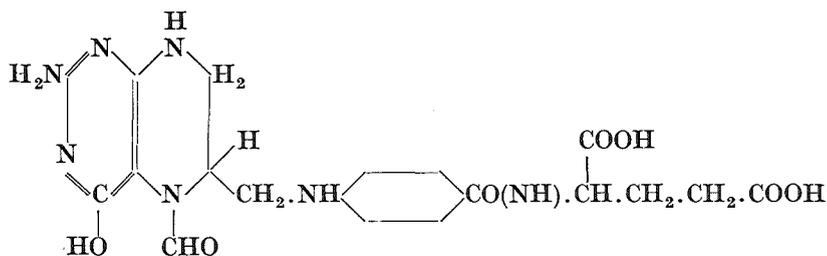


FIG. 62. Crystals of the Calcium Salt of Leuovorin (Folinic Acid, the Citrovorum Factor).

thus proving that folic acid is essential for the formation of the factor. Vitamin B₁₂ and ascorbic acid probably play some part in the conversion of folic acid into the citrovorum factor [684, 709]. The observations of Spray and Witts [734] suggest that in pernicious anæmia there may be a defect in the conversion of folic acid to the citrovorum factor. Cortisone can replace citrovorum factor in the growth of *L. citrovorum* [715].

Another group of workers had meanwhile shown that crude liver extracts are many times more active than folic acid itself in inhibiting the action of folic acid antagonists such as methylfolic acid [653]. From these liver extracts a potent concentrate was obtained and from it was isolated a substance which, on the basis of structure and functional relationship to folic acid, was called folinic acid. Analyses revealed the presence of at least two other factors of what was termed the folinic acid group. Further biological and chemical studies suggested the identity of the citrovorum factor and folinic acid. The synergistic action of folinic acid and thymidine (p. 145) was observed by Bond and his colleagues [653], who considered that it might be

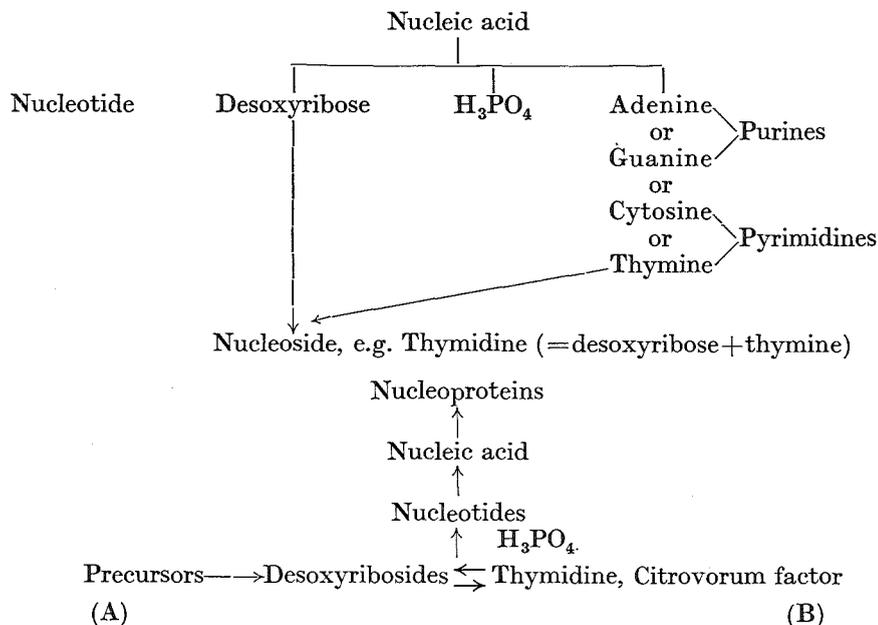
involved in the synthesis of thymidine. The same group later reported the synthesis of a substance, similar if not identical with folic acid, by reducing formylfolic acid in the presence of ascorbic acid [654]. This was shortly followed by the synthesis and isolation in crystalline form by Brockman and his co-workers [655] of a compound which appears to be identical biologically with the citrovorum factor [716]. It is formyl tetrahydrofolic acid [707], also known as leucovorin :



Folinic acid or leucovorin

It is unstable in the region of $pH2$ [714].

Vitamin B₁₂, Folic Acid, Thymidine, the Citrovorum Factor and Nucleic Acid Synthesis. It is believed that vitamin B₁₂, folic acid, thymidine and the citrovorum factor all function in the biosynthesis of the nucleic acids. The latter are essential for the synthesis of nucleoproteins and red and white blood cells. The primary defect in pernicious and related anæmias may well be the inability to synthesize certain nucleosides (particularly thymidine), which are integral parts of the nucleic acid molecule, from parent purines and pyrimidines.



Jukes, Broquist and Stokstad [656] have shown that *Lactobacillus leichmannii* can carry out step B but not step A, and conversely *Leuconostoc citrovorum* can carry out step A but not step B. They found that on purified media *L. citrovorum* had vitamin B₁₂ activity, while *L. leichmannii* had citrovorum factor activity. Folic acid and the citrovorum factor

may be concerned with the "shuttling" of a formyl group in the formation of a pyrimidine (thymine) and its derivative (thymidine); the formation of purine derivatives; and the utilization of glycine in a number of reactions. The ability of mice to incorporate formate containing radio-active carbon (C^{14}) into the purines of nucleic acid is profoundly depressed by the administration of aminopterin, a folic acid antagonist [711].

The citrovorum factor is lipotropic and choline sparing and is essential for the utilization of methionine and betaine in the biological synthesis of choline [722].

Hæmopoietic Activity of Citrovorum Factor. May, Sundberg and Schaar [657] treated megaloblastic anæmia of dietary origin in scorbutic monkeys with crude folic acid. Fifty micrograms produced a prompt response, comparable with that produced by 15 mg. of folic acid. Spies and his colleagues [658] and others [708, 723, 747] have shown that, in adequate amounts, the citrovorum factor is an effective hæmopoietic substance in patients with pernicious anæmia, nutritional macrocytic anæmia and tropical sprue in relapse. Davidson and Girdwood [659, 724] have produced a hæmatological and clinical response in patients with pernicious anæmia and the megaloblastic anæmia of pregnancy with 12 to 16 mg. of synthetic citrovorum factor. Five to 15 mg. daily intramuscularly is effective in pernicious anæmia according to Hausmann and Mulli [733]. This is to be contrasted with the dose of vitamin B_{12} , which is a thousand times less.

Citrovorum Factor and Folic Acid Deficiency. The citrovorum factor is more effective than folic acid in reversing a condition of folic acid deficiency in animals produced by aminopterin (p. 145) or by sulphadiazine [661]. It is of interest that aureomycin also overcomes the toxic effects of aminopterin. Clinically synthetic citrovorum factor is effective in reversing the toxic manifestations of amethopterin and aminopterin, folic acid antagonists used for the treatment of leukæmia and other neoplastic diseases [662, 719]. Schoenbach, Greenspan and Colsky [662] treated two patients with leucopenia and ulcerative mouth lesions following therapy with amethopterin and aminopterin for metastatic neoplasms. The lesions healed and the leucopenia disappeared although previous treatment with high doses of folic acid had been ineffective.

Vitamin B_{13} and Vitamin B_{14}

Vitamin B_{13} is an unidentified growth factor for rats isolated from distiller's dried solubles by Novak and Hauge [663] in 1948. It may be identical with the animal protein factor (p. 160) or vitamin B_{12} .

Vitamin B_{14} is a crystalline compound isolated from urine that is extremely active in stimulating the proliferation of new cells in bone marrow cultures [664]. It is effective in curing experimentally induced anæmia in rats and is inhibited by folic acid antagonists such as methyl folic acid. Enzymes from milk, liver and gastric juice act on xanthopterin, folic acid and pteroyl-triglutamic acid (teropterin) to form vitamin B_{14} , or at any rate related substances with identical activity [665].

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CHAPTER III
ANEURINE
(VITAMIN B₁, THIAMIN)

HISTORY

MODERN beriberi, a disease partly due to aneurine deficiency, dates from the introduction of steam-powered rice mills in the nineteenth century. When beriberi became prevalent in the East it was confined to those persons whose diets consisted largely of highly milled or polished rice from steam mills. In the same regions, those natives who ate rice ground in primitive mills largely escaped. The conquest of beriberi started from 1882, when Takaki [5], the Director-General of the Medical Department of the Japanese Navy, practically eradicated the disease among Japanese sailors by supplementing the diet, which consisted mainly of rice, with fish, vegetables, meat and barley. Steps were also taken to deal with the disease in the Dutch East Indies, where Eijkman [6] showed in 1897 that a paralytic condition in fowls, *polyneuritis gallinarum*, which closely resembled beriberi in its polyneuritic symptoms, could be produced by a diet consisting of polished rice. When the fowls were fed on unpolished rice they did not develop the disease, which was cured in afflicted birds by the administration of rice polishings. Grijns [7], Eijkman's successor in the East Indies, concluded in 1901 that both beriberi and avian polyneuritis resulted from the lack of a certain dietary factor or factors present in rice bran. His work focussed attention on beriberi as a deficiency disease, but this view was not seriously considered until Fletcher [8], Fraser and Stanton [9] confirmed it by their studies on beriberi in the Malay States between 1905 and 1910. Fletcher, working in an asylum at Kuala Lumpur, separated the patients into two groups, one of which was supplied with polished rice, the other receiving brown rice. In the first group thirty-six out of 120 developed beriberi, and eighteen died from the disease; in the second group only two out of 123 developed the disease and there were no deaths. Fraser and Stanton took 300 healthy labourers into a railroad labour camp. To half of them the customary polished rice was given as a staple article of diet, the rest receiving the unpolished grain. In three months time beriberi was rife amongst the white rice group, while those on unpolished rice were practically free from the disease. Later the rice rations were reversed in the two groups, with the result that the disease disappeared in the first group and an epidemic of beriberi broke out in the second.

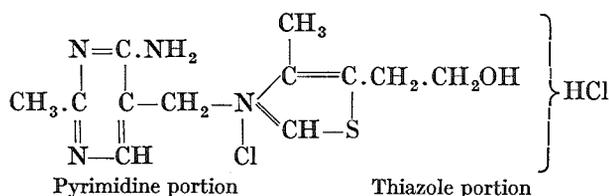
In 1911 Funk [18], of the Lister Institute, succeeded in obtaining a concentrate from rice polishings which was capable of curing polyneuritis in pigeons in doses of 20 mg. He put forward the theory that not only beriberi, but also scurvy, pellagra and possibly rickets were due to the absence of certain specific factors from the diet which he termed "vitamines." In the meantime Osborne and Mendel [10], in America, had shown that butter contained a growth-promoting factor essential for the development of rats (1910-13). This factor, now known as vitamin A, was discovered independently by McCollum and Davis [11] in 1913 in egg yolk as well as in butter and cod-liver oil. These two investigators further discovered that crude milk sugar used in the rats' diets contained another essential growth-promoting factor, which was also found to be widely distributed in a number of foods, including milk, yeast, rice polishings, and wheat embryo. In 1915 McCollum and Davis [12] concluded that: "There are necessary for normal nutrition during growth two classes of unknown accessory substances, one soluble in

fats and accompanying them in the process of isolation from certain foodstuffs and the other soluble in water, but not apparently in fats." The growth factor present in butter and cod-liver oil was called "fat-soluble A," owing to its solubility in fat solvents, and the other growth factor present in milk, yeast, rice polishings and wheat embryo, was termed "water-soluble B." It was soon realized that "water-soluble B" and the anti-beriberi or anti-neuritic factor had similar properties and distribution, and it was therefore assumed that they were identical. The two systems of nomenclature were therefore combined and the factor renamed *vitamine B*, and in 1920 the terminal *e* was omitted. Six years later Jansen and Donath [25] obtained 100 mg. of pure anti-neuritic factor from 3 kg. of rice polishings. At the same time Smith and Hendrick [15] showed that vitamin B consisted of a heat labile antineuritic component and a heat stable component and these were renamed *vitamin B₁* and *vitamin B₂*. The resolution of the latter into a number of other components is described on p. 103. In 1935 Jansen suggested for pure vitamin B₁ the name *aneurine*, a word derived from *a*(nti-poly)-*neur*(itis) *vitamin*. Williams in America proposed the alternative name *thiamin*. Aneurine is the name accepted by the British Pharmacopœia for vitamin B₁.

In 1932 Windaus and his collaborators [32] isolated aneurine from yeast and determined the correct empirical formula of the vitamin. Further work in 1934 by Windaus, Tschesche and Grewe [33] in Germany, and by Williams [40] and his school from 1934 to 1936, led to the elucidation of the chemical structure of the vitamin. The final chapter in its history was written in 1936 by Williams and his co-workers [35], who brought their work to a brilliant conclusion by its synthesis. An alternative synthesis was published later by Todd and Bergel [717].

CHEMISTRY OF ANEURINE

Aneurine, or 2-methyl-5-(4-methyl-5-β-hydroxyethyl-thiazolium chloride) methyl-6-aminopyrimidine hydrochloride, has the following structure :



Aneurine is a colourless crystalline substance containing a molecule of water of crystallization and melting at 248°–250° C. It is sensitive to ultra-violet light of less than 290 mμ, with maximal photochemical decomposition at 255 mμ at pH 7. The effect of heat is important on account of the possibility of the destruction of the vitamin in the ordinary processes of cooking and canning. The rate and extent of destruction is markedly increased by the presence of water and a rising pH. In the dry condition it is stable at 100° C. for twenty-four hours, even in contact with air. The effect of heat on aneurine depends not only on the pH but on the electrolyte system. Thus at pH 5.4 for an hour one hundred per cent. destruction occurs in the presence of borates, fifty-seven per cent. in unbuffered aqueous solution, ten per cent. in acetates, and three per cent. in phosphates [50]. The destruction by heat is a function of time, temperature, pH, and the presence of electrolytes. Meat curing ingredients (sodium chloride, nitrate and nitrite, cane sugar and glucose) have no significant effect in accelerating destruction during heating [50]. Copper catalyses the rate of destruction of aneurine, but iron, aluminium, zinc and tin do not [30]. This is of some significance as these metals are present in cooking utensils.

Destruction due to heating in the ordinary process of cooking is not very great, provided the cooking temperature is not much above 100° C., the reaction is not alkaline, and the heating is not too prolonged. Considerable inactivation occurs in pressure cookers. The presence of other components in the foodstuff under consideration may facilitate the decomposition of the vitamin. The destructive action of sulphites is of some importance, since these are used in the preservation of fruit pulp and juices. The sensitivity of aneurine to sulphites, which inactivate it, depends on the *pH* of the medium. Thus decomposition is instantaneous at *pH* 6; at *pH* 5 about eight to twelve hours is required for complete decomposition; at *pH* 3 there is relatively little destruction even after a period of months. Aneurine is fairly resistant to heat in faintly acid or acid media. Below *pH* 5.0 aqueous solutions are fairly stable to boiling [30]. In sealed tubes at 100°-125° C., when the pressure is considerable, 0.1 per cent. solutions at *pH* 3-6 are unaffected for

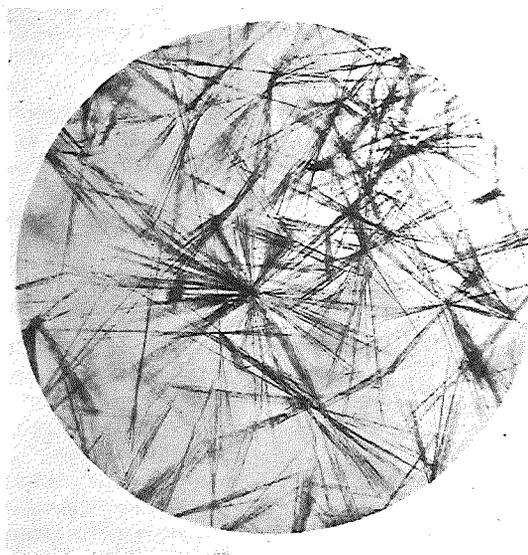
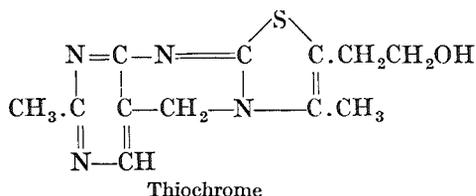


FIG. 63. Crystals of Vitamin B₁ Hydrochloride.

short periods. This is of importance in sterilizing solutions for parenteral injection. Such solutions (*pH* about 3.5) may be heated for half an hour at 100° C. or twenty minutes at 120° C. with little loss of potency. Aneurine is more stable in solution in the presence of five per cent. glucose [551]. Unless anti-oxidants are used, solutions of aneurine in ampoules suffer slow deterioration, some sixty per cent. decomposing after twelve months even when the ampoule is filled under nitrogen [552].

Aneurine is not oxidized by atmospheric oxygen under ordinary conditions. The nitrate is more stable than the hydrochloride [588]. When subject to mild oxidation with potassium ferricyanide in alkaline solution it is oxidized to thiochrome [729, 742],



which shows an intense blue fluorescence under ultra-violet light, a reaction employed in the estimation of aneurine (p. 241). Thichrome is devoid of any antineuritic action.

UNITS OF ANEURINE

In 1931 the Health Organization of the League of Nations [419] adopted an international standard for aneurine, consisting of an acid clay adsorbate of the vitamin from rice polish extract, prepared in a standard manner described by Jansen and Donath. An International Unit was defined as the antineuritic activity of 10 mg. of the adsorbate. The International Unit is still retained in much of the literature, and until 1938 was generally considered to be equivalent to 2 micrograms of pure aneurine (i.e. 500 I.U. \equiv 1 mg.). In that year the International Conference on Vitamin Standardization recommended that the International Unit be defined as possessing the antineuritic activity of 3 micrograms of pure aneurine hydrochloride (i.e. 333 I.U. \equiv 1 mg. or 333,333 I.U. \equiv 1 gram). The Permanent Commission on Biological Standardization of the Health Organization of the League of Nations adopted this recommendation. The International Standard now consists of pure crystalline aneurine hydrochloride, which is kept at the National Institute for Medical Research, Hampstead. The mean value of nine laboratories using the rat growth method was 317.2 I.U. per milligram of aneurine hydrochloride with a molecule of water of crystallization. This is the value, now approximated to 320 I.U., accepted by the British Pharmacopœa.

DISTRIBUTION OF ANEURINE IN FOODS

Aneurine is widely distributed in raw untreated foodstuffs, the richest sources being whole cereals, yeast, pork and pulses. The cereals rank first as the most important source of aneurine in human diets. On account of their cheapness and high calorie value they are consumed by most people. Refined cereals and flours, however, suffer considerable loss in their aneurine content because the germ and bran are largely removed in the milling. The greater part of the aneurine is concentrated in the scutellum, the shield-like tissue of the germ lying between the embryo and the endosperm [590]. There is an increase in the aneurine content of cereals on germination, e.g. from 7 to 9 micrograms per gram in the case of wheat.

White flour contains relatively little aneurine in comparison with wholemeal flour. It contains only 45 to 90 micrograms of aneurine per 100 grams, i.e. about a fifth to a tenth of that originally present in the whole wheat. The production of white flour by roller mills removes a great deal of aneurine; little was lost in the now obsolete process of stone grinding. "Germ bread" (e.g. Hovis), a proprietary foodstuff prepared from three parts of white flour and one of wheat germ, is approximately equal to wholemeal bread in its aneurine content. Stoneground flour contains the whole germ and endosperm and the inner layers of the pericarp, which give a cream-coloured flour and not a brown one. "Peeled wheat" is prepared by a flotation process which removes only the thin epidermis and leaves ninety-eight per cent. of the wheat berry. It has a high content of aneurine and other members of the vitamin B complex [17].

In 1940 the Ministry of Food arranged for the fortification of white flour with aneurine to bring its content up to 0.083 mg. per 100 grams of bread. Owing to the lack of shipping space the Ministry forbade the milling of white flour after March 23rd, 1942, and the sale of white bread after April 6th, 1942, and replaced white flour, which only contains seventy-three per cent. of the wheat berry, by flour of eighty-five per cent. extraction. This flour, which was not fortified with aneurine, was known as National flour in England and con-

tained an average of 300 to 400 micrograms of aneurine per 100 grams. The loaf made from this contained 240 to 255 micrograms per 100 grams. On October 1st, 1944, a flour of 82.5 per cent. extraction was introduced by the Ministry of Food. In the early part of 1945 the extraction was lowered to eighty per cent. to satisfy the public. In the United States the millers may enrich white flour if they desire. Such flour must be labelled "enriched" and satisfy the following specification: Each pound must contain not less than 1.66 mg. and not more than 2.5 mg. of aneurine (or 0.47 to 0.7 g. per 280 lb. sack); not less than 1.2 mg. and not more than 1.8 mg. of riboflavine; not less than 6 mg. and not more than 9 mg. of nicotinic acid or its amide; and not less than 6 mg. and not more than 24 mg. of iron. Vitamin D may be added between the limits of 250 and 1,000 U.S.P. (International) units, and calcium between 500 and 2,000 mg. per pound of flour. "Enrichment" was compulsory during the last war; the order expired in October, 1946, but since that date many States have made "enrichment" compulsory again.

Legumes and nuts are important sources of aneurine. Eggs contain a fair amount, although the content depends on the diet of the bird. Meat, apart from pork, is not a rich source, although it may supply up to one-third of the daily needs when it forms 10 per cent. of the diet [194]. Liver extracts may contain considerable aneurine.

Yeast is an exceptionally potent source of aneurine, and is frequently used as a dietary supplement when large quantities of the vitamin are required. Marmite is a commercial yeast extract. Brewer's yeast is more active than baker's yeast.

Milk is a poor source of aneurine, an average sample of raw cows' milk containing about 40 to 50 micrograms per 100 c.c. Pasteurization of milk results in a loss of ten to twenty per cent. of the aneurine content, and commercial sterilization destroys some twenty-six to forty-five per cent. [59]. If the milk is concentrated by evaporation or drying the loss is greater still, e.g. thirty to fifty per cent., although in the presence of sugar, as in sweetened condensed milk, the loss is only ten per cent. [63]. Human milk is also poor in aneurine, the content of which gradually increases until the twelfth week and then remains constant [759]. At beginning of lactation human milk contains 3.4 ± 1.45 micrograms of aneurine per 100 ml.; by the second week it rises to 10.9 ± 3.46 micrograms; in the third week and subsequently it rises to 13.8 ± 4.47 to 17.9 ± 3.13 micrograms [674]. It can be increased to 24 to 30 micrograms by giving supplements of aneurine. On a daily intake of 1.5 mg. of aneurine the daily excretion in the milk is approximately 20 micrograms per 100 ml. [204].

Effect of Cooking, Canning, Freezing and Drying on Aneurine. Many of the vitamin assays are based on the raw foodstuff, but by the time the latter is prepared for the table it may have suffered some loss in its aneurine potency. The chemical principles involved in the destruction of the vitamin by cooking have been previously discussed (p. 184). Generally speaking, the degree of inactivation is directly proportional to the temperature, duration of heating, and alkalinity of the medium. For all practical purposes there is little inactivation in acid or neutral solutions heated to 100° C. or just over for one hour. Higher temperatures and increased alkalinity (increased pH) hasten the rate of destruction; thus fifteen per cent. of the aneurine in yeast is destroyed when it is heated for two hours at 130° C.

The destruction of aneurine when foods are cooked in the ordinary way is not appreciable. In the case of peas moderate amounts of soda do not seem to cause excessive destruction of aneurine [64]. The average loss in the boiling of vegetables is about twenty to thirty per cent. [205]. This is reduced to ten per cent. by cooking with as little water as possible and retaining much of the steam. Much aneurine is dissolved out in the cooking water (twenty to thirty-five per cent.) and may be lost unless incorporated in soups and stews. Root vegetables, presenting less surface, suffer less. Potatoes

suffer less loss if cooked in their skins, retaining about ninety per cent. of their aneurine. A considerable quantity resists aqueous extraction in starchy vegetables owing to adsorption on the starch grains. The loss is high if potatoes are steamed and the cooking liquid rejected, as a greater part of the aneurine is washed out [27]. The more succulent vegetables ("greens") do not retain so much of their aneurine on boiling. The widespread practice of using baking soda in cooking is to be condemned since it destroys much of the aneurine. In cooking rice all the water should be evaporated. Running water over the cooked rice to separate the grains washes out much aneurine. As much as sixty per cent. of the vitamin may be lost in this way [26]. Pressure cooking, although it may be rapid, causes considerable destruction of aneurine if prolonged.

In the baking of bread the loss of aneurine is variously reported from twelve to thirty-five per cent. [65, 66]; it increases with the time of baking, e.g. seventeen per cent. at twenty minutes and thirty-three per cent. at forty minutes. An average figure under normal conditions of large scale baking is twenty per cent. Dawson and Martin [65] give the following figures for the percentage losses of aneurine in the baking of various breads: white bread, eight to twenty-two; National loaf, twenty-seven; wholemeal ("brown" bread), thirty-five; germ bread, nineteen. A loss of up to thirty per cent. can occur when baking soda is used [646] although normally little destruction is stated to occur [661]. When bread is toasted considerable destruction occurs in the outer part of the bread, although the total loss is not great. Toasting wholemeal bread for seventy seconds causes a drop in the aneurine content from 3.36 micrograms per gram to 2.29 micrograms per gram [49]. The cooking of oatmeal results in very little loss—five to ten per cent. in half an hour [19].

The loss of aneurine that occurs in foods cooked in restaurants and cafeterias has been studied by a number of investigators, particularly in America. Losses varying from sixteen to seventy-five per cent. have been reported [48]. Nagel and Harris [48] compute that twenty per cent. is lost in the cooking, twenty-five per cent. in the cooking water, and twenty-five per cent. between the time of cooking and serving. In the cooking of meat it has been estimated that there is from thirty to nearly sixty per cent. loss on roasting and boiling and fifty per cent. on braising [202]; drying, being a quick process results in less destruction, e.g. ten to forty-five per cent [194]. The loss in pressure cooking is approximately the same as in roasting [615], although if prolonged it may be considerable [29].

In the process of canning there should be little loss of aneurine due to the processing *per se* [67]. Any loss in potency usually occurs during the preparation of the food before it is put into the cans. In the blanching of fruit before canning thirty-six to ninety-nine per cent. of the aneurine is retained [553, 557]. As a rule fruits are processed at 100° C. or a little more, vegetables at 112°–115° C., and meat and milk at 117°–120° C. Provided the medium is not alkaline there should be no appreciable destruction of the aneurine if the heating is limited to half an hour. It is understood, of course, that the juices as well as the solid food are consumed, as much of the vitamin (thirty per cent.) is in the former. Arnold and Elvehjem [202] report a loss of up to twenty per cent. in tinned meat, Rice and Robinson [906] thirty per cent. in canned ham, and Mayfield and Hedrick [1] approximately seventy per cent. in tinned beef. According to the latter workers losses are greater (eighty-five per cent.) in glass jars. In the canning of vegetables losses of thirty-four to seventy-four per cent. of aneurine may occur [898]. The loss in canning is increased on storage and may be as much as forty-five per cent. after a year [23], although if the pH is suitably adjusted (e.g. 4.5 or less) there is said to be no loss of aneurine in the storage of canned fruits at room temperature [554]. In the preparation of corned beef losses of fifty to eighty per cent. may occur [1].

Foods preserved by freezing suffer little or no loss in their aneurine content [67, 69, 70]. The losses that do occur are due to the preliminary blanching or cooking rather than to refrigeration. Thus in the processing losses of five to twenty-five per cent. may occur [22]. It should be remembered that in the thawing process cell membranes are ruptured, and that when frozen foods are cooked aneurine and the other water-soluble vitamins are more readily extracted by the cooking water than from the fresh product.

Dehydrated vegetables and meat were introduced as a wartime measure to save space in transport. The loss of aneurine in dehydration is never more than about fifteen per cent. in vegetables, and most of this occurs during the blanching and not from the actual drying [21]. In dehydrated meat the loss is from twenty-five to thirty-five per cent. [906], although figures as high as fifty per cent. are quoted [942].

In the preservation of vegetables by salting and brine preservation appreciable amounts of aneurine are retained [913]. Considerable loss may occur in foods sulphited at a high pH. As the legal maximum of sulphite is less than 4,000 p.p.m. and this would be diluted with other foods on ingestion, there is not much danger of sulphited food causing aneurine deficiency [659].

Aneurine Content of Foods. The aneurine content of some of the more important foodstuffs in the raw and cooked states is given in the following table.

ANEURINE CONTENT OF FOODS

Foodstuff	Description	Micrograms of An urine per 100 grams or 3½ oz.
VEGETABLE PRODUCTS		
<i>Breads</i>		
Maize	240
Rye	Whole grain	240-500
	Germ	225
	Bread	160
Wheaten bread	Whole grain (wholemeal)	225-450
	White bread (73% extraction)	45-90
	" " baking powder	31-63
	" " with malt	81-105
	" With germ " brown bread	240-270
	" Without germ " " "	195-240
	Bran bread	150
	" Germ " bread	240-510
	Milk bread	90
	Ministry of Food loaf (85% ex- traction (1942-44))	240-255
	" Enriched " (U.S.A.)	240-400
	Doughnuts	280
<i>Cereals and Cereal Products</i>		
Barley	Whole grain	500
	Germ	4,200
	Pearled	120
Buckwheat	610
Corn, sweet	150
Maize	Whole grain	135-180
	Germ	1,380
Oatmeal	Whole grain	540-810
	" Breakfast "	420-810
Rice	Whole	60-290
	Polished	50
	Bran	1,680-2,280
Rye	Whole	470-500
	Germ	2,250
Sorghum (Kaffir corn)	Black	270
	White	240
	Seed husk	735

Foodstuff	Description	Micrograms of Aneurine per 100 grams or 3½ oz.
<i>Cereals and Cereal Products—continued</i>		
Wheat	Whole grain	540-1,080
	White flour	60-90
	Peeled wheat flour	580
	"Germ" flour	360-390
	Bran	1,440
	Germ (commercial)	1,800-3,750
	Middlings	1,350-1,675
	Stone ground	270-330
	" " "white"	270-330
	85% extraction (National Wheat-meal 1942-44)	300-400
	80% extraction	231-246
	"Enriched" (U.S.A.)	440-550
	Self-raising	20
<i>Prepared Proprietary Cereal Foods</i>		
Allbran	—	370-520
Bemax	—	2,625
Cerevim	Vitamin concentrate added	2,100
Corn flakes	Kellogg's	390-450
	(Vitamin concentrate added)	
	Post's	280-400
	(Vitamin concentrate added)	
Cream of rice	—	160
Cream of wheat	Vitamin concentrate added	410-680
Force	—	40
Grape nuts	Post's	810
	(Vitamin concentrate added)	
Oats	Quaker	580-700
Rice Krispies	Kellogg's	450
Shredded wheat	Kellogg's	190-230
Soya wheat	—	710
<i>Fruits</i>		
Apple	Raw	30-120
Apricot	—	30-45
Avocado	—	90-120
Banana	—	50-160
Blackberry	—	30-45
Black currant	—	30
Cherry	—	50
Gooseberry	—	150
Date	—	75
Fig	Fresh	60-75
	Dried	45-66
Grape	—	50
Grapefruit	Juice	50-75
Guava	—	45-150
Lemon	—	40-60
Lime juice	—	30
Loganberry	—	33
Melon	—	30-60
Orange	Juice	70-92
	Marmalade	20
Peach	Fresh	40
	Canned	76% retention
		20-45
Pear	—	80-90
Pineapple	—	50-150
Plum	—	100-180
Prune	Dried	150
Raisin	Dried	150
Raspberry	—	20-30
Redcurrant	—	45
Strawberry	—	30
Tangerine	—	70-120
<i>Nuts</i>		
Almond	—	255
Brazil	—	500-1,180
Cashew	—	150
Chestnut	—	240-270
Cob	—	228-660

Foodstuff	Description	Micrograms of Aneurine per 100 grams or 3½ oz.
<i>Nuts—continued</i>		
Coconut	Dried	trace
	Fresh	30-60
Hazel	—	400-600
Hickory	—	600
Peanut		694-1,050
	Roasted	300-400
	Butter	200-300
Pecan	—	500
Walnut	—	330-480
<i>Vegetables</i>		
Artichoke	—	75-180
Asparagus	—	180
	Canned	67% retention
Bean, baked	—	120
butter	Dried	480
haricot	—	156-400
	Cooked	120-180
green	—	80
kidney	—	210
string	—	198-450
runner	—	75-225
Beetroot	Boiled	10-30
Broccoli	—	130
Cabbage	—	30
	Dehydrated	410-590 (42-76% retained after preparation)
Carrot	Raw	60-70
	Canned	33
Cauliflower	Raw	100-150
	Cooked	90
Celery	—	30
Chives	—	120
Cress	—	90-150
Cucumber	—	30-40
Eggplant	—	45-70
Endive	—	50-75
Garlic	—	150
Kale	—	150
Kohlrabi	—	50
Leek	—	80-150
Lentil	—	120-630
Lettuce	—	60-75
Mango	—	60-90
Marrow	—	30-60
Mushroom	—	60-120
Okra	—	120
Onion	Stewed	30
Parsley	—	80
Parsnip	—	80-120
Pea	Fresh	400-800
	" cooked	200
	Dry	550-590
	" cooked	45-135
	Canned	40-70% retained
Peppers, green	—	30-70
" red	—	30-70
Potato	Raw	90-180
	Dehydrated	250
	Chips	350
	Peeled and boiled	90
Pumpkin	—	50
Radish	—	40-60
Rhubarb	—	10-25
Soya bean	—	470
	Dried	1,140-1,200
Spinach	Raw	50-120

Foodstuff	Description	Micrograms of Aneurine per 100 grams or 3½ oz.
<i>Vegetables</i> —continued		
Sprouts	—	110-180
Sweet potato	—	100-140
Tomato	Pulp raw	70
	Canned	89-100% retained
Turnip	Raw	60
Watercress	—	120
Mushrooms	—	110
DAIRY PRODUCTS		
Butter	—	0-120
Cheese, Cheddar	—	24-40
Milk	Cream	10-30
	Cow's, fresh, whole	41-48
	„ pasteurized	30
	„ „ „ boiled	24
	„ whole, dried	300
	Goat's milk	60
	Human milk	5-36 (see p. 187)
	Buttermilk	Av. 20
	Condensed, sweetened	40
	Evaporated	50
	Evaporated	50
Eggs	Hen's, yolk, raw	300-420
	„ „ „ boiled 5 mins.	300-420
	„ white	trace
	„ dried	350
	„ whole	150
	Duck's, yolk, raw	300
FISH		
Cod	Muscle	40-90
	Liver	270
	Roe	900-1,800
Clam	—	25
Crab	—	90-140
Dogfish	Liver	210-540
Haddock	—	15-120
	Liver	750-2,190
Halibut	Fried	90-120
Herring	Whole	120
	Roe (soft)	30
	Smoked	111
Lobster	—	150
Mackerel	Muscle	26-58
	Roe	600
Oyster	—	180-300
Prawn	Boiled	<90
Salmon	—	30-120
	Canned	24-45
Sardines	Tinned	7-15
Tuna	—	40
Whiting	Muscle	90
Average lean fish	—	90-180
MEAT		
Beef	Average lean, raw	90-300
„	„ „ „ cooked	72-240
„	Dried	180-600
„ brain	—	168
„ corned	—	50
„ heart	—	675
„ kidney	—	250
„ liver	Raw	450
	Cooked	198-450
„ pancreas	—	318
„ tongue	—	220
Veal	—	180
Chicken	Muscle, roast	90-150
Duck	—	360

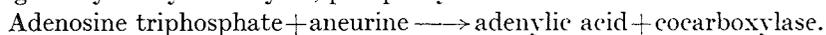
Foodstuff	Description	Micrograms of Aneurine per 100 grams or 3½ oz.
MEAT—continued		
Goose	—	150
Lamb	Lean, raw	200-280
„	„ roast	140
„ liver	—	300
„ heart	—	600
„ kidney	—	300
„ tongue	Braised	60
Pork	Lean, raw	960-1,200
„	„ cooked	900
„	Salted	360
„ ham	Boiled, smoked	660-1,070
„ kidney	—	1,020
„ liver	—	270-468
„ bacon	—	840-1,440
„ brains	—	180
„ lard	—	150
Rabbit	Stewed	90
Turkey	Cooked	120-150
MISCELLANEOUS		
Beer	—	9
“Bemax”	—	2,625
Chocolate	Milk	50
Cocoa	—	75
Coffee bean	Ground	900
Honey	—	0-5.5
Ice cream	—	24-40
Jam	—	20
Macaroni	—	130
“Malt”	Extract	300-475
“Marmite”	—	2,400-3,000
Mayonnaise	—	40
Molasses	Cane	2,100
„	Beet	360
Spaghetti	—	45
Tapioca	—	130
Yeast	Baker's	930-6,000
„	Brewer's	7,500-24,000
„	Extract	2,000-20,000
„	“D.C.L.” (Distillers Co. Ltd.)	90,000
„	<i>Torulopsis utilis</i>	2,000

THE PHYSIOLOGY OF ANEURINE

Aneurine and Carbohydrate Metabolism. The essential role played by aneurine in carbohydrate metabolism was first demonstrated by Peters and his co-workers at Oxford [78, 79]. The vitamin has been shown to catalyse the decarboxylation and carboxylation of pyruvic acid, an intermediary degradation product of carbohydrate metabolism, both in alcoholic fermentation and in tissue metabolism. In anaerobic fermentation by yeast pyruvic acid is decarboxylated to acetaldehyde and carbon dioxide :

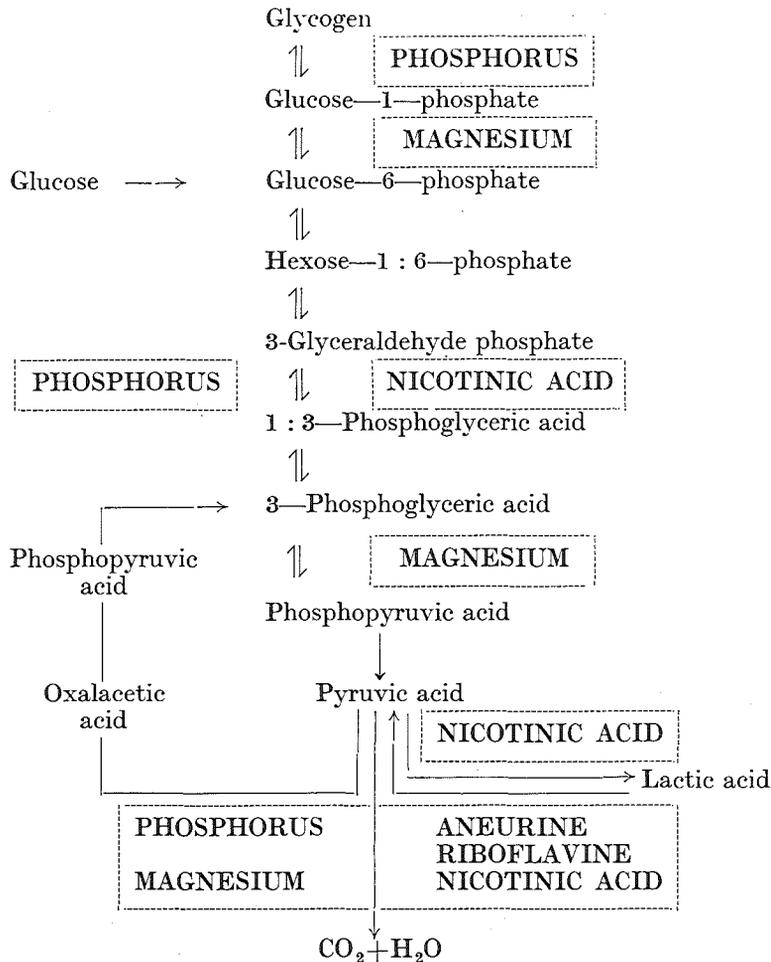


Yeast contains an enzyme, cocarboxylase or phosphothiamin, catalysing this reaction. In 1937 Lohman and Schuster [90] isolated pure cocarboxylase from bottom yeast and identified it with aneurine pyrophosphate. The phosphorylation of aneurine to cocarboxylase is probably accomplished through the agency of adenylic acid and adenosinetriphosphate, the reaction being catalysed by an enzyme, phosphorylase :



Glucose is not directly oxidized in the body. There is a progressive phosphorylation and hydrogen transfer through an intermediate series of

compounds until pyruvic acid is formed. The successive stages in its oxidation (glycolysis) are probably as follows :



The various steps in the breakdown of glucose are catalysed by enzymes which are activated by coenzymes. The enzymes are synthesized in the body, but the coenzymes, or at any rate their precursors, can only be made from dietary sources. Among the coenzymes essential for the degradation of glucose are :—

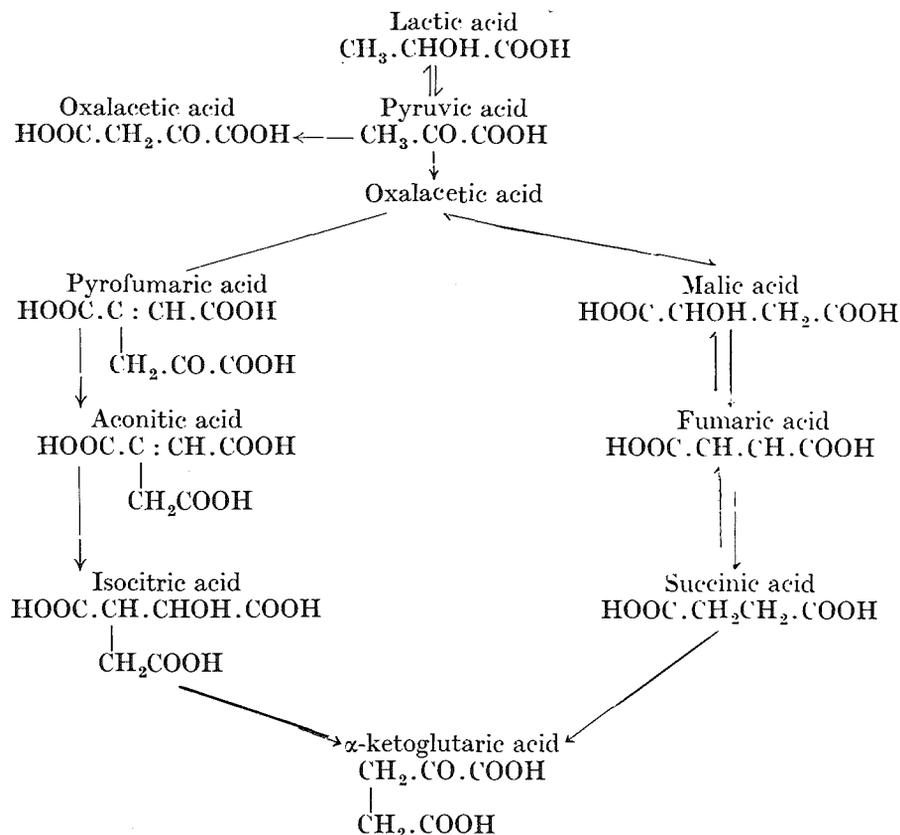
1. Adenosine triphosphate, derived from adenylic acid, which is a phosphate donor and acceptor.
2. Diphosphopyridine nucleotide (codehydrogenase I) and triphosphopyridine nucleotide (codehydrogenase II), of which nicotinic acid is the precursor. These coenzymes are hydrogen transporters.
3. Magnesium.

The oxidation and decarboxylation of pyruvic acid requires the enzyme-coenzyme system carboxylase (protein-aneurine pyrophosphate-magnesium) and cocarboxylase, or aneurine pyrophosphate. There are other factors essential for the oxidation of pyruvic acid, including flavoprotein (p. 294), codehydrogenases I and II, adenosine triphosphate and the cytochrome system.

Pyruvic acid is not oxidized directly to lactic acid, but is probably first carboxylated to oxalacetic acid, which in turn is utilized in two cycles, the

citric acid and succinic acid [81]. The enzyme catalysing this reaction is carboxylase. The rate of pyruvic acid oxidation is controlled by the presence of aneurine. *In vitro* the synthesis of citric acid from pyruvic acid in the presence of kidney tissue is accelerated by aneurine, and *in vivo* aneurine deficiency in rats results in a decreased urinary excretion of citric acid [14, 89].

THE TRICARBOXYLIC ACID CYCLE



This is known as the tricarboxylic acid cycle. Some of these reactions have been shown to occur in bacterial metabolism, and in isolated animal tissues, such as brain, liver and kidney, but the exact pathways of carbohydrate metabolism in man have not been fully elucidated.

Aneurine becomes phosphorylated to cocarboxylase when added to many animal tissues. According to Goodhart and Sinclair [94] the white blood cells originating in the bone marrow convert aneurine into cocarboxylase, which is then probably combined with a protein. The circulating form of aneurine is the free substance or its monophosphate, not cocarboxylase, which is probably formed mainly in the liver. At any rate there is a massive synthesis of it in the liver after the injection of aneurine. Cocarboxylase in the liver is hydrolysed when necessary to replenish the blood aneurine. The kidney also phosphorylates the vitamin, the process being probably essential for its reabsorption in the kidney tubules. It is possible that insulin plays a part in the phosphorylation of aneurine; injection of insulin is followed by an increase in cocarboxylase and a fall in blood inorganic phosphate [561].

Since the liver and kidney possess the power of phosphorylating aneurine to cocarboxylase, it would be expected that some disturbance of phosphoryl-

ation might occur in hepatic and renal disease. This has been demonstrated by Williams and Bissell [914]. They found that the injection of 15 mg. of aneurine intravenously caused a rapid increase in cocarboxylase and free aneurine in the blood of normal subjects. The aneurine level rapidly returned to normal, but the cocarboxylase remained elevated for an hour or so. In patients with advanced hepatic cirrhosis there was an immediate rise in the free aneurine in the blood, but the increase in cocarboxylase was considerably less than in normal subjects. In cases of nephritis the changes were intermediate between those in the normal subjects and those with hepatic disease. Davis and Bauer [936] have also shown that there is some degree of aneurine deficiency in patients with hepatic disease, as shown by elevated blood pyruvic acid levels (p. 242). They did not, however, find a raised blood pyruvic acid in patients with renal disease.

Through its effect on the oxidation of pyruvic acid cocarboxylase may influence the various phases of carbohydrate metabolism, since the oxidation of pyruvic acid causes the storage of a considerable amount of energy as adenosine triphosphate. It may be indirectly concerned in the synthesis of glycogen from glucose and the conversion of fructose to glucose, reactions in which phosphorylation of the sugar are essential. Cocarboxylase would also appear to be essential for the synthesis of carbohydrate from lactic and pyruvic acids. It has been shown that the synthesis of carbohydrate from pyruvic acid is decreased in kidney slices of aneurine deficient rats and restored to normal by the addition of aneurine.

Many investigations have been carried out to see if increased quantities of pyruvic acid and other intermediate products of carbohydrate metabolism can be detected in the blood of animals and human beings suffering from aneurine deficiency. The presence of pyruvic acid in the blood can be demonstrated by an increase in the bisulphite binding power (B.B.S.) of the blood. Pyruvic acid contains a ketonic group (CO) and therefore combines with sodium bisulphite. The test, not being specific for pyruvic acid, is given by other substances containing an aldehyde or ketone group, e.g. methyl glyoxal, which is also an intermediate product of carbohydrate metabolism. Pyruvic acid is best estimated not with sodium bisulphite but by means of its reaction with 2 : 4-dinitrophenylhydrazine [2, 4]. It has been found in abnormal amounts in the blood of animals suffering from aneurine deficiency and the level returns to normal after treatment of the latter [83]. This has also been demonstrated in human beings suffering from beriberi and related deficiency diseases [84, 85]. Light muscular exercise in patients suffering from aneurine deficiency causes the level of blood pyruvate to rise still further [86]. The blood pyruvate rises after exercise in untrained normal persons, but the blood lactate does not [102], in contrast to patients with beriberi and suffering from aneurine deficiency, who show both a raised blood lactate and pyruvate level. Blood lactate and pyruvate are increased after severe exercise and at high altitudes [82] and in toxic, infective and hæmolytic states in infants [13]. Chesler, Homburger and Himwich [919] noted a high post-absorptive blood sugar, a rise in the blood lactic and pyruvic acids, and a lowering of the lactic acid pyruvic acid ratio in aneurine deficiency. There is considerable difference of opinion as to whether the blood pyruvate level is of value for the biochemical detection of aneurine deficiency (p. 242).

Statements on the effect of aneurine on the fasting blood sugar and on insulin hypoglycæmia are conflicting [98, 100, 101, 105, 106]. In the normal human subject and in the diabetic it probably has no effect [555, 670, 754]. Magyar and Resofski [16] found that aneurine had no effect on the arterio-venous difference in blood sugar (which may be taken as a measure of the degree of utilization of glucose by the tissues). They state that aneurine facilitates the diffusion of insulin into the cells of the tissues. In a human subject suffering from aneurine deficiency a rise in blood sugar and diminished glycogen storage in the liver is stated to occur [330]; according to

Tonutti and Wallraff [106] glycogen is absent from the livers of aneurine deficient rats and is restored by injecting aneurine and glucose. There is a decreased glucose tolerance in aneurine deficiency in man and animals [327, 330]. Aneurine also influences the absorption of glucose from the intestine; the rate is decreased in aneurine deficiency [20, 730]. The specific dynamic action of glucose is increased by aneurine; the specific dynamic action of a diet high in carbohydrate and containing adequate aneurine is twice as great as that of a similar diet lacking in aneurine [28].

The most striking lesions in animals and human beings suffering from a pure aneurine deficiency are in the nervous system, the cells of which utilize only carbohydrate for their energy [175]. It is supposed that lack of aneurine results in inefficient metabolism of carbohydrate throughout the tissues, including the nervous system. The oxidation of carbohydrate by nerve tissue is not only depressed in aneurine deficient subjects [105], but also by alcohol, anæsthetics and narcotics, which are stated to increase the aneurine requirements of the organism considerably [678]. It must not be supposed that aneurine functions specifically in nervous tissue only; it affects metabolism in general.

Aneurine and Fat Metabolism. In animals a diet poor in aneurine and rich in carbohydrate brings on the symptoms of aneurine deficiency more rapidly than a diet rich in fat or protein [141, 142, 287]. If the animals are offered a choice of diet they eat fat in preference to carbohydrate [225]. When fat is substituted isocalorically for carbohydrate in the diet of rats suffering from aneurine deficiency, there is a decrease in bisulphite-binding substances in the urine [581]. The aneurine requirements of an animal are less on a diet rich in fat than on one containing much carbohydrate. Yudkin [677] has shown that not only do fat and protein spare aneurine, but that in the complete absence of carbohydrate rats can dispense with aneurine altogether. The rôle of aneurine in the metabolism of carbohydrate renders this aneurine-sparing action of fat and protein intelligible since the vitamin is needed for the oxidation of carbohydrates, but not for that of fats or protein. Increased consumption of fat does not cause a rise in the blood pyruvate [756].

According to McHenry [114] the presence of choline is necessary for the aneurine-sparing action of fat. Evans and his collaborators [112] have arranged fats according to their aneurine-sparing action, the efficiency of the fat depending on the length of the fatty acid chain, maximum protection being reached with fatty acids containing eight carbon atoms. McHenry and Cornett [34, 113] believe that aneurine is essential for the synthesis of fat from carbohydrate; other components of the vitamin B complex can augment the synthesis [478] and severally they determine the quality of the fat, that laid down under the influence of aneurine containing less unsaturated acids and with longer carbon chains. They believe the aneurine-sparing action of fat is due to the fact that less aneurine is consumed for fat synthesis and more is available for other functions.

In man the results are equivocal. Thus Widenbauer and Wieland [916] state that there is increased utilization of aneurine following the consumption of high carbohydrate diets and Wang and Yudkin [167] record a reduced excretion of aneurine in subjects receiving an increased intake of carbohydrate. On the other hand, Cahill [917] could find no difference in the urinary excretion of aneurine during alternate periods of high fat and high carbohydrate intake. Reinhold, Nicholson and Elsom [918] state that they were unable to find any evidence for the aneurine-sparing action of fat in man; in fact, the urinary excretion of aneurine was decreased in five out of six subjects when the fat intake was increased. Their observations confirm the view that the amount of carbohydrate in the diet is an important factor in determining the daily requirements of aneurine. These conflicting results are probably due to the difficulty of keeping human diets constant in one factor while varying another.

The isocaloric replacement of carbohydrate by ethyl alcohol and an adequate diet in other respects is stated by Butler and Sarett [14] to result in an increased excretion of aneurine, and they suggest that alcohol, like fat and protein, has an aneurine-sparing action. This has been confirmed in the rat [669]. Alcohol, like fat and protein, requires less aneurine for its metabolism than carbohydrate. Sulphadiazine also has an aneurine-sparing action in the rat, probably by interfering with a catalytic mechanism resulting from the inhibition of thyroxine synthesis [650].

Aneurine and Protein Metabolism. An adequate supply of aneurine improves nitrogen retention in the rat [567]; on an aneurine deficient diet the nitrogen balance becomes negative [576].

Aneurine and the Endocrine System. Thyroid. Most of the studies on the relationship between thyroid function and aneurine are experimental, although a few clinical studies have been made. The literature is well reviewed by Drill [279] and Blaizot [117]. Experimental observations can be divided into: (a) the effect of aneurine deficiency on the thyroid gland, (b) the effect of the administration of aneurine on animals given desiccated thyroid or thyroxine or the thyrotropic hormone of the anterior pituitary gland. The literature on the effect of aneurine deficiency on the thyroid gland is conflicting. Creatinuria occurs in animals deficient in aneurine, but this does not result if the animal is first thyroidectomized [117]. After prolonged aneurine deficiency structural changes in the thyroid gland have been described, characterized by an increase in colloid [115] and by hyperplasia [239], followed by atrophy of the gland as the deficiency was prolonged. Other investigators [240] state that no changes in the thyroid gland can be detected in animals suffering from aneurine deficiency, and attribute the increased colloid formation to iodine deficiency. Similarly it is variously stated that injections of aneurine stimulate the thyroid and also have no effect on the gland [116, 432]. Hyperthyroidism increases the requirements of aneurine (p. 210), but the effect is not specific as hyperthyroidism results in increased metabolism, which is known to increase the requirements of a number of vitamins, including aneurine and ascorbic acid.

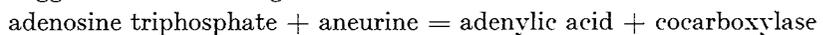
The loss in weight and anorexia produced in animals by feeding thyroid gland or thyroxine can be corrected by the administration of aneurine [118-120, 644]. Doses of 100 micrograms of the latter can annul the effect of 0.2 mg. of thyroxine in the experimental animal. Experimental hyperthyroidism is accompanied by a fall in tissue cocarboxylase [120]. Drill and Sherwood [118] showed that the effect of aneurine in preventing the loss in weight of hyperthyroid dogs is due to the increased caloric intake which it produces. It also appears that not only aneurine but other members of the vitamin B complex are needed for the recovery of lost weight in hyperthyroid animals [278]. Liver function, including glycogen storage, is also depressed in hyperthyroid dogs, and this is intensified by removing the vitamin B complex from their diet. A diet rich in the vitamin B complex delays but does not prevent the onset of damaged liver function [295]. Hyperthyroidism decreases the amount of aneurine in various rat tissues, particularly the liver. Summarizing the animal work it may be said that in hyperthyroid animals there is an increased demand for aneurine, which if not supplied results in a depletion of the body stores of the vitamin, with resulting anorexia, loss of weight, decreased stores of glycogen and diminished hepatic function. These changes can be prevented by aneurine and the B complex.

A number of clinical observations on the subject have been made. There is an excessive urinary excretion of aneurine in thyrotoxic patients [296], and Frazier and Ravdin [121] as well as Means [297] have pointed out that such patients often show symptoms suggestive of aneurine deficiency. Williams and his co-workers [296] observed that the blood cocarboxylase was below normal and the blood pyruvate and lactate elevated in thirty-four

of forty patients with thyrotoxicosis. The intravenous injection of glucose also resulted in a blood pyruvate level higher than normal. Williams and Kendall [298] state that patients given thyroid tolerate it better if aneurine is given as well. From tests on normal volunteers they concluded that the thyroid hormone is less effective in stimulating metabolism in a state of aneurine deficiency. Two normal subjects were given approximately 0.5 gm. of desiccated thyroid daily and placed on diets containing variable quantities of aneurine. When the diet was adequate in the vitamin the B.M.R. rose to + 25 per cent. : it fell to between - 8 and + 11 per cent. when the vitamin was restricted, and rose to + 25 to + 30 per cent. when aneurine was again provided in adequate amounts.

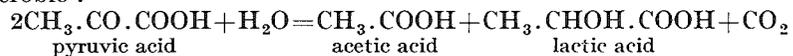
Aneurine and Acetylcholine. Aneurine is an essential factor in the transmission of peripheral nerve impulses. It augments the activity of acetylcholine at nerve endings by inhibiting the formation of cholinesterase, an enzyme that hydrolyses acetylcholine and inactivates it [122, 126, 606, 39]. In the isolated gut and heart aneurine augments the action of acetylcholine [123, 771]. Glick, Antopol and others [124] showed that it inhibits the action of cholinesterase in animal sera, but they point out that it is only effective in a concentration greater than that found in the tissues. They also observed that the blood cholinesterase is increased in pigeons with aneurine deficiency. Aneurine itself has no effect on smooth muscle, but acetylneurine, the acetyl ester, like acetylcholine, causes it to contract [147]. During nerve stimulation both aneurine and acetylcholine are formed [885], and it has been suggested that it is not the vitamin itself but the acetyl ester that is liberated. Aneurine is formed in heart muscle on stimulating the vagus [647]. If acetylneurine, like acetylcholine, is a chemical intermediary in the propagation of the nerve impulse one would expect it to be rapidly removed from the site of action. Acetylcholine is rapidly hydrolysed at the nerve ending by cholinesterase. Actually the enzymatic hydrolysis of acetylneurine by serum and brain extracts is very slow [124]. In the presence of pyruvic acid and potassium ions aneurine effects the synthesis of acetylcholine in brain tissue [128]. Most of the aneurine of nerve tissue is in the myelin sheath and this is the storage battery in which acetylcholine formation takes place. In Wallerian degeneration of nerve there is a marked disappearance of aneurine [527].

By alkaline oxidation of nerve fibres aneurine can be demonstrated in the myelin sheath by the fluorescence of the resulting thiochrome [923]. In degenerated nerve a diminution in the aneurine content of the myelin sheath can be demonstrated in twenty-four hours. The acetylcholine content of nerve tissue decreases in the aneurine deficient animal [924]. Aneurine may play a part in the synthesis of acetylcholine [36, 37, 39]. Von Muralt [39] has suggested the following scheme :



This mechanism may be essential for the formation of acetylcholine. The breakdown of adenosine triphosphate results in the phosphorylation of aneurine to cocarboxylase, which catalyses the anaerobic and aerobic decarboxylation of pyruvic acid.

Anaerobic :



These reactions provide the acetic acid for the acetylation of choline, which is formed from the dephosphorylation of nerve phosphatides. Adenosine triphosphate and cocarboxylase act as phosphate donor and acceptor, and are associated with the breakdown of glucose, acting as energy transmitters.

Reproduction. Aneurine plays some part in the reproductive mechanism of the rat, since the fertility of this animal is seriously impaired if it is deprived

of the vitamin [757]. Disturbances of lactation also result. Mice kept on diets deficient in the vitamin are more susceptible to infection [758].

Sure [189] reports that exceptionally large doses of aneurine administered to rats produce partial sterility in the first generation and diminished lactation in the third generation. A similar effect was recorded by Perla [190], who found that feeding rats with excess aneurine resulted after one generation in interference with lactation, loss of maternal instinct, cannibalism and progressive loss of fertility. These symptoms were prevented by giving 2 mg. manganese chloride daily to the animals.

Anoestrus is also produced in rats suffering from aneurine deficiency [757], although it is claimed that this effect is due to concomitant inanition depressing the functions of the anterior pituitary gland [941]. It has been reported that the male and female sex hormones and vitamin D delay the symptoms of aneurine deficiency [299]. Large doses of aneurine are stated to depress the activity of the anterior pituitary gland, as shown by diminished excretion of progesterone [300].

Aneurine and Phagocytic Function. Studies in vitamin deficiencies have shown that adequate amounts of most vitamins are essential for normal resistance to infection. Careful quantitative studies of variations in phagocytic power in different nutritional conditions have been made by Cottingham and Mills [939]. They find that the phagocytic activity of the peritoneal fluid in mice is diminished by eighty per cent. if the animals are suffering from a mild degree of aneurine deficiency. When this is severe phagocytosis cannot be demonstrated. Reduction in phagocytic power was also observed in mice suffering from a deficiency of other vitamins (pp. 120, 297, 420).

Aneurine and Mineral Metabolism. Perla and Sandberg [129, 130] believe that there is a metabolic interdependence of the vitamin with manganese, the latter acting as an oxidative catalyst in the utilization of aneurine in the tissues. Perla [190] also observed that aneurine deficiency caused an increased retention of manganese in rats, and that the toxic manifestations of an excess of aneurine in the diet could be prevented by small doses of manganese. The results of studies on iron and copper were not sufficiently constant to be reported. Manganese in minute amounts stimulates the carboxylase system (p. 194) [763].

There appears to be some relationship between aneurine and zinc, which like manganese can replace magnesium in the carboxylase complex [764]. In beriberi the zinc content of the blood, nails and skin falls to half the normal values [765]. There would seem to be some correlation between the aneurine and zinc content of foodstuffs.

Relationship to Other Vitamins. The phosphorylation of aneurine is presumed to occur through the agency of adenylic acid (pp. 193, 199), and the oxidation of pyruvic acid is stated to require not only aneurine but pantothenic acid and biotin. In animals suffering from aneurine deficiency there is a pronounced disturbance of riboflavine metabolism, the riboflavine content of the tissues falling considerably, mainly because of poor absorption [766]. There is, however, an increase in the riboflavine content of the liver in animals deficient in aneurine. Clinically it has been shown by Sydenstricker [767] that in cases of nutritional deficiency resulting from lack of the whole vitamin B complex the administration of massive doses of aneurine may precipitate symptoms of a deficiency of one of the other members of the vitamin B complex, e.g. nicotinic acid or riboflavine. The administration of large doses of aneurine, e.g. 10 to 80 mg., over a period increases the urinary riboflavine excretion in man [768] and decreases the excretion of nicotinic acid [597]. Large doses of aneurine given to rats precipitates a deficiency of pyridoxine [38]. Vitamin A is stated to act antagonistically against aneurine, since the symptoms of a deficiency of the latter are intensified by giving vitamin A. In the rat, however, a deficiency of vitamin A results in an increase of blood pyruvic acid, which is a manifestation of aneurine deficiency, and which

responds to the administration of aneurine [769]. A possible physiological relationship exists between ascorbic acid and aneurine. It is said that the onset of scurvy on a minimal intake of the former is delayed by small amounts of aneurine, and that the antineuritic action of the latter is increased by ascorbic acid [671]. The oral lesions in dogs deficient in aneurine have been relieved by giving ascorbic acid [778]. The biosynthesis of the latter in the rat is influenced by aneurine [39]. Aneurine deficiency is stated to be associated with a prolonged prothrombin time [595].

Absorption of Aneurine. Aneurine is absorbed to a limited extent and mainly from the upper portion of the small gut. It is doubtful whether it is normally absorbed from the large gut. According to Schroeder and Liebich [132] it was not absorbed when administered through a cæcostomy opening, and Alexander and Landwehr [951] could not detect its absorption when physiological amounts, e.g. 2 mg., were administered in an enema. On the other hand, Najjar and Holt [780] did observe absorption from the large gut but only when large quantities (50 mg.) were given in an enema. It may be re-excreted into the gastric juice after absorption [52]. The maximum quantity that can be completely absorbed when given by mouth is 2 to 5 mg. [41, 42, 51]; if quantities larger than this are given the excess is found largely in the stools or destroyed in the lower bowel. In the aged absorption is apparently extremely limited throughout the gastro-intestinal tract [781]. Supplements of 1 mg. taken three times a day are apparently completely absorbed as the aneurine content of the faeces is not increased. The maximum amount completely absorbed and therefore the maximum economic intake of the vitamin by mouth is about 5 mg. daily. Doses of more than this which are often prescribed, particularly in "tonics," are therefore wasted. According to Friedemann and his co-workers [42] the maximum quantity destroyed in the intestinal tract and tissues is about 4 mg. daily in persons with normal intestinal motility; the colon is the site of greatest destruction. According to Alexander [58] 10 mg. of aneurine is the maximal amount of the vitamin that the body can metabolize daily. It is apparently not absorbed per rectum [779].

Absorption appears to be largely confined to the upper gastro-intestinal tract because it is influenced by food intake, less being absorbed on an empty stomach than after a meal [51]. This may be due to lower stability in the alkaline secretion of the duodenum. *In vitro* tests show that aneurine is stable in gastric juice over a range of pH 1.5 to 8.0, but not in bile, pancreatic juice and suspension of antacids [43]. Rafsky and Newman state that the presence of hydrochloric acid in the gastric juice is not essential for its absorption [55], although it is also claimed that achlorhydria may impair it [44, 237]. Antacids such as magnesium trisilicate adsorb aneurine and prevent its absorption, but kaolin does not [43, 44]. If live yeast is taken by mouth, not only is very little aneurine absorbed from the yeast cell (about seventeen per cent.) but the latter actually withdraws aneurine from the food in the gut [45, 46]. Yeast if given as a dietary supplement should therefore be heated first to kill the cells; it is not sufficient to dry it.

Absorption of aneurine may be diminished in gastro-intestinal disturbances such as vomiting, diarrhoea [133], ulcerative colitis and neoplastic disease. Short circuiting operations of the intestine [135], internal and external fistulae and strictures, and in fact any pathological condition of the gastro-intestinal tract, may lead to diminished absorption. Aneurine is imperfectly absorbed by patients with hepatic disease [726] and achlorhydria [259], but the latter may be associated with dyspepsia and not directly concerned with absorption [688].

Aneurine is probably not phosphorylated before absorption from the intestines [52] but is absorbed in the free state [640]. Absorption probably occurs by simple diffusion because the amount absorbed is roughly proportional to the intake [42], and because the initial rapid absorption is followed

by a much slower rate of absorption, presumably after diffusion equilibrium between the lumen of the gut and the intestinal mucosa has been established [53].

The phosphorylation of aneurine occurs in all nucleated cells [94], those of the liver and kidneys being particularly active. After aneurine is absorbed from the gut it reaches the bloodstream and is carried to the liver and kidneys where it is phosphorylated to cocarboxylase [763]. The same organs can dephosphorylate cocarboxylase and supply the free vitamin to the blood [91]; this is transported in the plasma to other tissues [94], which rephosphorylate it [763], or else it is excreted in the urine.

Storage of Aneurine. The body is unable to store aneurine for any length of time. When the subject is placed on an aneurine deficient diet, losses of aneurine from the tissues are not equal. Depletion occurs most rapidly from the muscles and most slowly from the brain, nervous system, heart and liver. The aneurine in skeletal muscle is intracellular and extracellular, mostly the former. The latter is freely diffusible and in equilibrium with that of the plasma [903]. The concentration of intracellular aneurine is about 50 micrograms per 100 grams. When the aneurine intake is increased a point of saturation is reached, and the tissues do not store any further aneurine in spite of increased intake [140]. The larger part is then stored in the muscles. If more than the optimum needs of aneurine are ingested, the excess is metabolized in the body or excreted in the urine. Aneurine is present in the cerebrospinal fluid (range 0.01 to 6.5 micrograms) [409].

The total amount of aneurine in the body of a well-nourished person is about 25 mg. [785]. The richest tissue is heart muscle (2 to 3 micrograms per gram) followed by brain, kidney and liver (1 microgram per gram), and skeletal muscle (0.5 microgram per gram) [139, 785].

The blood level range of aneurine is 4 to 10 micrograms per 100 ml. whole blood [903]. Using different methods of estimation a range of 2 to 17 micrograms has been reported [150-153, 163, 177, 417, 714, 723, 832]. It is present in the red and white cells mainly as cocarboxylase (3 to 12 micrograms per 100 mg. with an average of 7 micrograms); free aneurine is present mainly in the plasma (0.5 to 2 micrograms per 100 ml.) [903]. The blood aneurine varies widely at a given intake even in the same individual and is not related to the rate of excretion [177]. The concentration in the tissues is about twenty times that in the blood. The leucocytes and platelets contain four to ten times as much aneurine as the erythrocytes [520, 922], but since only one per cent. of the two former is found in blood their contribution to the total blood aneurine is only ten to twenty-five per cent. [520].

The aneurine level in the blood of the umbilical cord is nearly twice that of the maternal blood (11.6 micrograms per 100 ml. in the former, 6.79 in the latter), showing that the foetus and newborn obtain their aneurine at the expense of the mother's reserves [346, 506]. In the foetal tissues aneurine is present mainly as cocarboxylase [507].

Excretion. The kidney concentrates aneurine from plasma, perhaps twenty times or more [705], although there is no direct relationship between urinary and blood levels. The fact that diuresis can affect aneurine excretion profoundly suggests it is a non-threshold substance and renal clearance studies indicate that extensive tubular resorption of aneurine does not occur [903]. About five to eight per cent. is quickly eliminated unchanged in the urine and a greater part of the remainder is metabolized in the tissues [155]. The bulk of excreted aneurine is free, but a small amount is cocarboxylase. On a high intake the excretion rises to nine to thirteen per cent. [68, 71, 158]. If aneurine is injected parenterally the bulk is excreted in the next three hours [399], the excretion being delayed by 0.06 to one per cent. of zinc [784]. Excretion continues for many days [58]. The urinary excretion is linearly related to the intake but is not a simple threshold phenomenon. It varies from person to person and within very wide limits and is not determined

solely by the nutritional status of the individual [60]. On normal diets the daily urinary excretion varies from 36 to 625 micrograms. It normally exceeds 100 micrograms daily and is usually around 200 micrograms or more [71]. The fasting rate of excretion during two hours before breakfast is about one-half the average daily rate [177]. On an intake of 600 micrograms per 1,000 calories the excretion ranges from 100 to 224 micrograms daily [71]. Melnick and Field [72] place the minimal normal level of excretion after four hours at 15 micrograms; this corresponds to a minimal excretion of 4 micrograms per hour given by other workers [73]. Allibone and Finch [74] give the daily range of excretion of aneurine in children on a daily intake of 1 mg. as 10 to 400 micrograms in twenty-four hours. In the newborn excretion, varying from 4 to 22 micrograms daily, exceeds the intake for the first few days of life [75-77]. In spite of considerable variation in the urinary excretion from person to person, this is highly characteristic of the individual. The amount excreted is independent of the urinary volume [61]. Even when equilibrium has been established at a highly constant aneurine intake there are considerable day to day variations. One normal person may excrete twice or three times as much aneurine as another normal person on exactly the same diet [68]. In any one day these differences may be considerably larger than the mean differences. When the aneurine intake is increased from 1 to 2 mg. a day it requires a period of about six weeks for the excretion to reach equilibrium with the new intake level; half of this change occurs in the first ten days [68]. The excretion of pyramin, the pyrimidine-like component of the aneurine molecule, is far more constant than the aneurine and varies from 130 to 250 micrograms daily on intakes of from 0.6 to 2 mg. of aneurine daily [68]. The relationship between pyramin excretion and aneurine intake is linear on normal intakes of 1 to 2 mg. daily.

Since the major portion of aneurine administered in daily doses of more than 10 mg. is rapidly excreted in the urine, since doses of more than 35 mg. do not result in greater storage of aneurine in the tissues, there seems to be no justification for the use of larger doses of aneurine parenterally [80]. After complete saturation of the tissues with aneurine the body discharges excess of the vitamin at a rate which can be represented by a linear equation [68, 80].

The intake and urinary output of aneurine as reported by various investigators are given on p. 204 :

There is no correlation between the excretion of aneurine and the number of non-fat calories in the diet [580].

On a constant intake with physical activity constant the rate of aneurine excretion is not related to body weight, basal metabolic rate or surface area. It is generally lower at full activity (3,400 calories) than at limited activity or at rest [177].

The urinary excretion of aneurine is diminished in diabetes [80], during infections [786], in the aged [781], during exercise [911], and as a result of injury and hæmorrhage [88]. Diminished excretion has also been recorded in patients suffering from alcoholism, disseminated sclerosis and sprue [82]. It is increased in thyrotoxicosis [296] and during the administration of mercurial diuretics [914], sulphonamides [108] and salicylates [893], although there is diminished excretion if salicylates are given over a prolonged period. The excretion in pregnancy is stated to be within the same range as that of normal women [103, 171], that is approximately 100 to 200 micrograms daily.

According to some workers aneurine is excreted in the sweat in a concentration of 9 to 15 micrograms per 100 ml. [787, 788]. The loss is negligible under normal circumstances, but may become significant in those working in hot and humid environments and doing heavy work. Mickelsen and Keys [789] state that aneurine is present in negligible amounts in the sweat, e.g. 0.2 micrograms per 100 ml.

Aneurine is present in the fæces, which contain on an average 78 micro-

Author	Intake of Aneurine		Urinary Excretion in micrograms per twenty-four hours
	Micrograms	Micrograms per 1,000 calories	
Daum, <i>et al.</i> [98]	140	56	17
	200	80	16
	625	250	31
	1,000	400	51
Elsom, <i>et al.</i> [331, 713]	574	280	42
	651	350	56
Giff and Hauck [71]	1,500	600	100-224
Hathaway and Strom [96]	1,000	370-450	113
	1,000	370-450	116
	1,000	370-450	147
	580	180	5.8
Keys, <i>et al.</i> [92]	630	190	17.6
	700	210	2.1
	1,000	330	106
Keys, <i>et al.</i> [93]	1,000	330	92
	800	400	119
Mason and Williams [794]	860	—	175
Melnick and Field [165]	640	360	65
Oldham, <i>et al.</i> [95]	974	540	107
	“normal diet”	—	309
Roderuck, <i>et al.</i> [97]	1,180	400	331
	1,200	400	326
Sastri, <i>et al.</i> [580]	800-1,390	—	66-1,200 Av. 339

grams per 100 grams, although it varies considerably from person to person. Friedemann and co-workers [177] found that it varied from 0.29 to 1.22 mg. daily on a daily intake of 2.77 mg. Most of it is derived from the organisms of the gut as its concentration in the stools is independent of the intake [104, 107, 951]. On a diet restricted in aneurine the faecal excretion always exceeds the intake. The bulk of this aneurine is not available to the host as it is fixed in the bodies of the bacteria and cannot be washed out. The faecal aneurine of rats is not available to another animal on a low aneurine intake [56]. Even when the urinary excretion is almost zero aneurine is still present in the faeces. In man it is doubtful if the aneurine in the bacteria of the faeces is available because if bacterial growth is inhibited with phthalylsulphathiazole there is no evidence of a decreased excretion of aneurine and the B vitamins in the urine [127].

PHARMACOLOGY AND TOXICOLOGY

Aneurine is diuretic [185], possibly through a central rather than a renal effect [187]. Large doses given intravenously produce vasodilatation with a fall of blood pressure, bradycardia and respiratory arrhythmia and depression [110, 186]. Smaller doses increase the tonus of the isolated heart [721] and delay the onset of fatigue in the isolated perfused muscle [574]. In a concentration of 1 in 100,000, aneurine augments the effect of histamine on the isolated intestine [109].

In the concentrations in which it is found in the body aneurine may play a part in the transmission of nerve impulses (p. 199). But in very high concentrations, e.g. 150 mg./kg., it has a curare-like action, that is, it prevents the contraction of muscle when the nerve to the latter is stimulated without decreasing the contraction on direct stimulation [111, 131, 648, 920]. In concentrations that do not curarize, i.e. 15 to 30 mg./kg., aneurine blocks sympathetic ganglia [125]. In large doses aneurine is said to increase oxygen consumption [185], although it is claimed that it only does this in aneurine deficient animals after food [186].

Relatively enormous doses of aneurine, e.g. 50 mg./kg., are tolerated by most animals without toxic effects. Large doses intravenously produce a shock-like state in pigeons [932]. In man side effects have been reported after the parenteral administration of doses of 10 to 100 mg. Doses much in excess of 10 mg. daily are unwarranted, as they are not metabolized and are excreted unchanged in the urine [58]. Among the side effects that have been reported are vomiting, epigastric fullness, severe cramps, collapse and respiratory distress. Symptoms resembling anaphylactic shock with eosinophilia and controlled by adrenaline have been reported [772-777]. Many of the reactions reported are probably the result of sensitization, as they occurred after several injections had been given [663, 925]. An intradermal test is not conclusive evidence of sensitivity to aneurine, as the latter may give a weal and flare in normal and non-sensitive subjects [776]. Cases of intolerance are comparatively rare. Sudden death, however, has been reported following the intravenous injection of aneurine [134]. The intrathecal injection of aneurine is dangerous. It may cause severe reactions and signs of meningeal irritation [572, 863]. Applied in a high concentration to the cerebral cortex of dogs it causes epileptiform convulsions [138].

HUMAN REQUIREMENTS OF ANEURINE

The human requirements of aneurine have been calculated from: (a) animal data, (b) aneurine content of human diets, (c) production and relief of symptoms of aneurine deficiency in man by diets containing known quantities of aneurine, (d) excretion studies of aneurine.

Human Requirements based on Animal Data. From animal studies Cowgill [198] in 1934 arrived at the following formula:

$$\begin{aligned} \text{Daily aneurine requirement} &= \text{constant} \times \text{weight} \times \text{calorie requirements} \\ \text{in milligrams} &= 0.00142 \times \text{weight in kilograms} \times \text{calorie intake} \times 0.003 \\ &= \text{weight} \times \text{calorie intake} \times 4.26 \times 10^{-6}. \end{aligned}$$

According to this formula a man weighing 70 kg. and leading a moderately active life (needing, say, 3,000 calories) requires approximately 0.9 mg. of aneurine daily. This formula of Cowgill's is for the minimum requirement of the normal adult. It has been criticized on the ground that the fundamental relationship is not between aneurine and calories but between aneurine and carbohydrate intake [142]. Since fat and protein are aneurine sparing (p. 197) the requirements of aneurine are decreased on a high intake of fat and protein and a low intake of carbohydrate. Signs of aneurine deficiency (p. 238) have also been reported in volunteers subsisting on diets containing the quantity of aneurine calculated from the Cowgill formula [786].

Aneurine Requirements calculated from Dietary Studies. Calculations have been made of the aneurine content of normal diets from food tables [199, 200, 210, 213]. They vary from about 0.6 to 1.5 mg. It is, however, difficult to arrive at an exact figure owing to the wastage on the plate and during cooking, the considerable variability of the vitamin content of the same food, and the variations in the time and manner of cooking. With all these variables, considerable differences may be found in the aneurine content of the diets of two people living in the same house and doing their own cooking. On an average some thirty per cent. of the aneurine in the average diet is destroyed by cooking; in restaurant cooking it may be as much as seventy-five per cent. or even more. Stiebeling and Phipard [210] made an extensive survey of the diets of American families in 1939 and concluded that most people consumed more than 0.72 mg. of aneurine daily and that half the subjects examined received 1.5 mg. or more daily. Some in the low income groups in America are said to have a daily intake of as little as 0.5 mg. daily [799]. Lane, Johnson and Williams [792] carefully analysed representa-

tive samples of food for the aneurine content, and concluded that sixty-six to seventy-five per cent. of the American population consumed 0.8 mg. of aneurine per 2,500 calories. This was before the "enrichment" of flour with aneurine (p. 187), which would bring this figure up to at least 1.3 mg. An Australian survey reveals a daily intake of 0.84 to 0.88 mg. of aneurine [212].

Elsom and Machella [727] determined the aneurine intake of a number of normal subjects who ate as much food of varied type as they liked. No restriction was placed on quality, quantity or price and the food was not spoilt by over-cooking. The average consumption was 1.125 mg. of aneurine daily, with a range of from 1 to 2.15 mg. This serves to show that there are considerable variations in intake and probably requirements from person to person. A survey of Eastern diets reveals that the daily intake of aneurine may be as low as 0.3 to 0.6 mg. without beriberi supervening [207, 728].

Holt [140] states that on a uniform diet, carefully selected to include a given amount of aneurine, the requirements are lower than on a diet chosen naturally to satisfy appetite and taste. On selected "artificial" diets the minimal aneurine requirement lies between 0.13 and 0.17 mg./1,000 calories, whereas on a natural diet it is between 0.17 and 0.23 mg./1,000 calories. The same author considers that a range of 0.24 to 0.44 mg./1,000 calories protects against deficiency symptoms. He considers these values are valid for all age groups, manual workers and pregnant women. Young [143] from a dietary survey in Canada on 385 individuals concluded that the daily aneurine intake averaged only 0.2 mg./1,000 calories for adults and 0.22 mg. for children. On these very low intakes there were no deficiency symptoms.

Requirements based on Aneurine Deficiency Studies. The aneurine requirements of man have been determined by noting the appearance or removal of the manifestations of aneurine deficiency in subjects on graded intakes of the vitamin. These manifestations are described on pp. 238-240.

The aneurine requirements of man, based on these considerations, are given in the following table :—

Author	Daily Aneurine Intake on which Deficiency Symptoms were Observed	Minimum Daily Intake Considered Desirable for Physical Fitness
Daum, <i>et al.</i> , 1949 [144]	No disturbance of sensory and psychomotor functions on 0.25-0.3 mg. per 1,000 calories.	0.25-0.3 mg. per 1,000 calories.
Elsom, 1942 [786]	0.28 mg. per 1,000 calories.	0.35 mg. per 1,000 calories.
Foltz, <i>et al.</i> , 1944 [931].	0.33-0.38 mg. per 1,000 calories.	0.33-0.45 mg. per 1,000 calories.
Friedemann, <i>et al.</i> , 1949 [177].	0.25 mg. per 1,000 calories.	—
Hathaway and Strom, 1946 [159].	No deficiency symptoms on 0.37-0.45 mg. per 1,000 calories.	0.5-0.55 mg. per 1,000 calories.
Keys, <i>et al.</i> , 1942-44 [796, 797, 930].	0.23 mg. per 1,000 calories. (No symptoms at this level.)	0.23 mg. per 1,000 calories.
Melnick, 1942 [798]; 1944 [145].	0.26 mg. per 1,000 calories.	0.35 mg. per 1,000 calories (0.5 mg. per 1,000 calories for safe margin).
Williams, <i>et al.</i> , 1939-43 [149, 327, 715, 793, 794].	0.2-0.95 mg.	0.45 mg. per 1,000 calories (0.6 mg. per 1,000 calories for safe margin).

These estimates vary from 0.5 mg. to less than 0.23 mg. aneurine per 1,000 calories in relatively short-term experiments, and from 0.26 to 0.35 mg. per 1,000 calories for longer-term assessments. This wide range is due to the fact that periods of observation varied from a few weeks up to nine months, and evidence of aneurine deficiency varied from clinical signs of frank deficiency to the least detectable changes. Many months' deprivation of aneurine may be necessary for the appearance of deficiency symptoms; hence the necessity for long-term nutrition experiments. Keys and his co-workers [796] included simple strength tests, responses during brief exhausting work, prolonged severe work, and psychomotor tests of speed and co-ordination. They also estimated the glucose tolerance and blood pyruvate, lactate, glucose and haemoglobin at rest, during work and after recovery. Tuttle and his co-workers [160] studied the reaction time of volunteers on various intakes of aneurine; when this was low the reaction time was increased. It was found that subjects whose food requirement was approximately 2,500 calories daily maintained their normal reaction time when the diet contained 0.625 mg. of aneurine. Daum and her co-workers [144] noted changes in reaction time, aneurine excretion, maximum work output and the oxygen uptake for a specified amount of work when subjects were maintained on graded aneurine intakes. The aneurine intake giving the optimum physiological responses was considered to represent the desirable intake.

Requirements based on Excretion Studies. Aneurine is not stored to any extent in the body. Since it is excreted in the urine, measurement of urinary excretion on varying intakes has been used as a method for calculating the requirements of the vitamin. Although, broadly speaking, aneurine excretion is correlated with the intake in a well-nourished person there are considerable individual differences and considerable day-to-day variations on the same intake. One normal subject may excrete several times as much aneurine as another normal subject on the same diet. Normal aneurine excretions have been observed in beriberi [218]. The response to a test dose of aneurine has also been used. If a test dose of, say, 1 to 5 mg. of aneurine is administered the amount excreted will depend upon tissue reserves; if these are low much of the dose will be retained and the excretion will be low. The difficulty is in the interpretation of the results; a range of normal values has yet to be recognized. Some workers have correlated the excretion of aneurine and its excretion in response to a test dose with the presence or absence of deficiency symptoms. Unfortunately, much of the data obtained from excretion studies has not been submitted to statistical examination and has been obtained from short-term experiments [68]. Mickelsen and his co-workers [68] have shown that when the aneurine intake is increased from 1 to 2 mg. it requires a period of six weeks for aneurine excretion values to come to equilibrium with the new intake level. These workers claim that more reliable information is obtained from a study of the excretion of pyramin rather than aneurine (p. 244).

Holt [140] and Najjar and Holt [780] state that the "point of minimum aneurine excretion" is closely related to the beginning of sub-clinical aneurine deficiency, and they have calculated the minimum aneurine requirements by gradually reducing the aneurine intake and noting when the excretion falls to a minimum value.

Alexander and Landwehr [58, 161] have calculated the human requirements of aneurine by finding the difference between intake and excretion. The difference is the amount of aneurine utilized in the body. The daily aneurine intake of a thirty-five-year-old man weighing 180 lb. and consuming a 2,400-calorie diet was 1.3 mg., and the excretion calculated as aneurine and pyrimidine was 0.24 mg. The balance, 1.06 mg., or 0.44 mg. aneurine per 1,000 calories, was assumed to represent the daily aneurine requirement. When large supplements of aneurine were administered to the subject

Author	Type of Observation	Estimated Daily Aneurine Requirement
Alexander and Landwehr, 1946 [58, 161].	Difference between intake and excretion.	0.44 mg. per 1,000 calories.
Daum, <i>et al.</i> , 1948, 1949 [99, 144].	Changes in urinary excretion on graded intakes.	0.25-0.3 mg. per 1,000 calories.
Holt, 1942 [140].	Minimal aneurine intake for fasting level to fall to zero value.	0.26-0.31 mg. per 1,000 calories.
Mason and Williams, 1942 [715, 794, 795].	Excretion of 100 ± 10 micrograms in twenty-four hours and recovery of $20 \pm$ two per cent. of test dose of 1 mg. i.m.	0.4-0.45 mg. per 1,000 calories.
Melnick, 1942 [782, 798]	Intake noted at which aneurine excretion fell precipitously; intake noted at which prompt response in urinary excretion occurs after test dose of 5 mg.	0.35 mg. per 1,000 calories (0.5 mg. recommended).
Oldham, <i>et al.</i> , 1946 [95]	Urinary excretion and response to test dose on graded intakes from 0.14-0.51 mg. per 1,000 calories.	1 mg. or 20 micrograms per kg. of body weight.
Widenbauer and Wieland, 1939 [208].	Graded dose of aneurine given to a subject not excreting the vitamin. Daily intake noted when aneurine excreted in urine and blood level rose to 2-11 mg. per 100 ml.	0.37-0.55 mg.
Williams, Mason and Wilder, 1943 [149].	Critical level associated with urinary excretion and biochemical deficits in carbohydrate metabolism and presence of deficiency symptoms.	0.45 mg. per 1,000 calories (0.6 recommended).

approximately the same figure, 1.09 mg., was obtained. Alexander and Landwehr concluded that the aneurine present in the faeces is in the bodies of the intestinal organisms and not available to the host; faecal aneurine is therefore of no significance in aneurine metabolism studies (p. 204).

	Calories	Daily Aneurine Intake in mg. N.R.C.	Daily Aneurine Intake in mg. B.M.A.
Man (156 lb. or 70 kg.) :			
Sedentary	2,400	1.2	1.0
Moderately active	3,000	1.5	1.2
Very active	4,500	1.8	1.7
Women (125 lb. or 50 kg.)			
Sedentary	2,000	1.0	0.8
Moderately active	2,400	1.2	1.0
Very active	3,000	1.5	1.2
Last half of pregnancy	2,400	1.5	1.1
During lactation	3,000	1.5	1.4

Human Requirements of the Adult. Summarizing the results obtained by the various methods described it would appear that the minimum human requirements of aneurine are in the region of 1 mg. daily. This should be increased to allow for losses in cooking and wastage on the plate. The actual requirement depends upon the number of non-fat calories consumed and will therefore depend on occupation, metabolic rate, sex and other factors. Even in the normal subject there are wide fluctuations in requirements. The League of Nations Committee on Nutrition (1939) advised a minimum intake of 1 mg. of aneurine daily [211]. The Food and Nutrition Board of the National Research Council, U.S.A., 1948 [800] and the Nutrition Committee of the British Medical Association (1950) suggest the daily aneurine intake given in the table at the bottom of p. 208.

The Nutrition Committee of the British Medical Association (1950) adopted a basic estimate for all population groups, except nursing mothers, of 0.4 mg. daily per 1,000 total calories and 0.6 mg. daily per 1,000 non-fat calories. The intake suggested for the nursing mother is 1.4 mg. daily.

Requirements of Infants and Children. The Committee on Food and Nutrition of the National Research Council, U.S.A. (1948), suggest the following daily allowances of aneurine from infancy to adolescence [800] :

	Calories	Daily Aneurine Intake in mg.
Under 1 year	110/kg.	0.4
1- 3 years	1,200	0.6
4- 6 "	1,600	0.8
7- 9 "	2,000	1.0
10-12 "	2,500	1.2
Girls : 13-15 years	2,000	1.0
16-20 "	2,500	1.2
Boys : 13-15 years	3,200	1.5
16-20 "	3,800	1.7

The Nutrition Committee of the British Medical Association (1950) suggest a daily intake of 0.4 mg. aneurine up to 1 year of age ; 0.6 mg. between 2 and 6 years ; and 0.8 mg. between 7 and 10 years.

The requirements of infants have been calculated from the aneurine content of mothers' milk [219, 759, 901], which varies from 0.6 micrograms per 100 ml. in colostrum to 36 micrograms in milk, with an average of about 20 micrograms. Calculations from these data vary from 0.1 to 0.6 mg. of aneurine as the daily requirement with an average of 0.3 mg. daily.

If the infant were fed on cow's milk (average 0.035 mg. aneurine per 100 ml.) the average daily aneurine intake would be about 0.3 mg. daily. Thirty per cent. or more may be destroyed on pasteurization. As the metabolic rate of the infant and child per unit of body surface is greater than that of the adult, the aneurine requirements are relatively greater.

The requirements of infants have also been calculated from excretion studies [162, 220, 711]. Knott [711] increased the daily aneurine intakes of infants from 60 micrograms to 0.9 mg. daily and noted a marked rise in the excretion when the intake reached 0.24 mg. Holt and his co-workers [162] calculated the requirements by means of a urinary excretion procedure which involved determining the intake that would maintain urinary excretion at the upper limit of the zone of minimum excretion, which in the adult approximates to a condition of subclinical aneurine deficiency (p. 238). On this basis the requirement of infants varied from 0.14 to 0.2 mg. daily.

Another approach has been made correlating blood levels with intake. In the adult blood levels of 1 microgram or less per 100 ml. are associated with deficiency symptoms (p. 238). The optimum level is considered by Knott to be 5 micrograms. She found that a daily intake of 0.2 mg. of aneurine daily could not maintain a blood level of 5 micrograms in the infant. Schlutz and Knott [803] noted an improvement in appetite if the aneurine intake was increased to 54 to 60 micrograms per kg. body weight. This corresponds to 0.2 to 0.25 mg. for the newborn and 0.35 to 0.45 mg. up to six months.

The requirements of older children have been deduced from dietary surveys. Stiebelling and Phipard calculated that the average aneurine intake of children of 1 to 5 years in America is 0.8 to 1 mg. daily (60 to 75 micrograms per 100 calories). Similar figures were obtained by Knott [221] from aneurine retention studies for children of 4 to 7 years and by Benson and his co-workers [804] using excretion tests. Widdowson [164] calculated that the average intake of aneurine of over 1,000 children in Britain in 1937 was 0.6 mg. daily at 1 year to 1.1 mg. for boys and 0.9 mg. for girls at age 14 to 15 years. The intake per 1,000 calories fell from 0.5 mg. at 1 year of age to 0.35 mg. at 10 years. The relative requirements increase in the early years of life owing to greater metabolism and again in adolescence.

Requirements in Pregnancy and Lactation. Because a rat suckling her litter requires five times as much aneurine as normally [214] it has been assumed by some workers that the nursing mother requires three to five times as much aneurine as a normal woman [192, 199]. The League of Nations Committee on Nutrition, 1938 [216], suggested a daily intake of 2 to 3 mg. of aneurine in pregnancy and lactation. Other estimates based on dietary surveys or excretion tests vary from 1 to 5 mg. daily [210, 563-565, 808, 809]. It has been stated that from 4 to 5 mg. of aneurine daily is required by pregnant and nursing mothers to obtain an excretion corresponding to that of normal subjects [806, 809]. Oldham and her colleagues, however, found that the aneurine excretion was within normal limits on daily intakes of 1 mg. The requirements during lactation have been calculated by adding the aneurine content of a day's supply of mothers' milk—about 0.4 mg. according to Knott [801]—to the requirement of the moderately active normal woman, which is 1.2 mg. daily. The total is 1.6 mg., which approximates to the requirements recommended by the National Research Council, U.S.A. (1948) which is 1.5 mg. daily [800]. The Nutrition Committee of the British Medical Association (1950) suggest that in the first half of pregnancy the daily requirement is 1.0 mg. daily; in the second half 1.1 mg. daily; and during lactation 1.4 mg. daily.

There is no reason why the pregnant woman should require very much more aneurine than an active woman consuming the same number of calories. During lactation, when aneurine is excreted in the milk, the requirements may be slightly increased. According to Roderuck, Williams and Macy [166] only eight per cent. of the aneurine intake is excreted in the milk. If this is so the aneurine requirements of the nursing woman are only slightly in excess of those of the normal woman.

Aneurine Requirements under Special Conditions. Any levels set up as dietary standards can only be approximate. The individual's requirements are influenced by numerous factors, such as augmented metabolism, faulty metabolism, the quantities and ratios of carbohydrate, fat and protein in the diet, and the activity of the individual. Aneurine requirements are increased in conditions associated with increased metabolism, e.g. in hyperthyroidism [120, 121, 296] and during exercise [197]. The increase in the former condition may not be entirely due to a raised metabolism and may be complicated by diuresis and hepatic dysfunction. Dinitrophenol, which raises the B.M.R. and produces pyrexia, does not, however, increase aneurine requirements [168]. The necessity for increased aneurine requirements in fever is inconclusive according to Keys and Mickelsen [173]. Mills and his co-workers [725, 892]

claim that the aneurine requirements are increased at high environmental temperatures, being doubled, for example, on rising from 65°–91° F. This is denied by other workers [170, 172].

Aneurine may be lost through the excretory channels, e.g. in diuresis and diarrhoea, so that an increased intake is necessary to compensate for excessive excretion. Dann and Cowgill [223] showed that fifty to seventy per cent. of the aneurine intake may be lost in diarrhoea owing to the hastening of food through the gut, and Cowgill [224] was able to produce aneurine deficiency in dogs by vigorous diuresis. The increased need for aneurine said to occur in diabetics [414] may be due to diuresis as well as to perverted carbohydrate metabolism.

According to Mills and his co-workers [174] the aneurine requirement per gram of food rises sharply with advancing age in rats. In this animal the requirement seems to be largely conditioned by the mass of metabolizing tissue and relatively independent of calorie intake under conditions of voluntary *ad libitum* feeding.

Harris, Ivy and Friedemann [176] found that the aneurine requirements are not increased by physical work under conditions simulating high altitudes (15,000 feet).

In the rat alcohol, taken in addition to the normal diet, does not increase the need for aneurine [823].

HUMAN DISEASE ASSOCIATED WITH ANEURINE DEFICIENCY BERIBERI

Incidence. The incidence of beriberi is greatest in those regions where polished rice and refined cereals form the bulk of the diet. Rice is the staple foodstuff of half the human race, and in India eighty to ninety per cent. of the total calories are supplied by rice. To provide sufficient vitamins, the rice must be supplemented with maize, legumes or fish. When the supply of these fails, the incidence of beriberi increases. It is endemic in Japan, Southern China, the Philippines, East Indies, the Malay Peninsula, and Southern India.

In a post-war survey carried out in the Philippines beriberi was found in nearly thirteen per cent. of the population; the mortality was 132 per 100,000 of population [218]. The mortality is greatest in infants.

A fifth of all disease in Malaya is attributed to beriberi, and in the Philippines it ranks second to tuberculosis as a cause of death. Between 1920–29 there were on the average 17,000 deaths from beriberi annually in Japan. According to a report by Fehily [680] eighteen per cent. of the admissions to the Infant Welfare Centre in Hong Kong show clinical signs of beriberi. The disease also occurs sporadically in small outbreaks on board ship, among beleaguered troops—e.g. at the siege of Kut in 1916—and in prisons and mental asylums. It has been reported prevalent in Labrador and Newfoundland, where white bread, molasses and salt meat form the major part of the diet of many of the inhabitants. The condition of frank beriberi as met with in the East is practically unknown in England, although a few cases which develop among seamen may find their way to English ports. Yudkin [228], who made a search of the literature in 1938, failed to find a single reference to true beriberi reported in England. A fatal case in an English child of three is reported by Allibone and Baar [184]. Palmer [245] gives details of an English girl suffering from anorexia nervosa, who had been on a diet deficient in aneurine for some years and who was originally diagnosed as a mental case. Careful examination showed that she was a mild case of beriberi. An English case of “Rand scurvy”—a combined aneurine and ascorbic acid deficiency—is described by Young [673]. Beriberi associated with alcoholism (p. 234) has been reported in the United States but rarely in this country.

Ætiology. It has been generally supposed that beriberi is a deficiency disease due to lack of aneurine. Deficiency diseases, however, are never limited to lack of a single factor and, although there is an aneurine deficiency in beriberi, the disease is probably a multiple deficiency syndrome. However, a deficiency of aneurine is almost certainly a major cause, as Burgess [196] has shown that in prisoners of war beriberi was seldom seen when the aneurine/non-fat calorie ratio was over 0.3 mg. per 1,000 calories; when it fell below that figure the incidence of beriberi varied inversely with it. It is not certain whether the neuropathy in beriberi is due directly to aneurine deficiency or to an intoxication with pyruvic acid and allied intermediate products of carbohydrate metabolism. It is now recognized that the treatment of beriberi is more satisfactory with aneurine and foods or concentrates rich in the B complex than with pure aneurine alone. Recent observations on induced aneurine deficiency (p. 238) have shown that it is impossible to produce beriberi experimentally by diets poor in aneurine only. Polished rice, which is the staple food where beriberi is endemic, is not only deficient in aneurine but also in vitamins A, D, E and B₆, riboflavine, nicotinic acid, pantothenic acid, choline, calcium and iron. A deficiency of vitamin A and riboflavine in animals has been shown to produce degenerative changes in the spinal cord and peripheral nerves (pp. 42, 295). Clinically few patients present all the classical signs attributed to any single avitaminosis; actually any one case if carefully examined shows those of several. Certain manifestations are common to beriberi and pellagra, e.g. weakness, nervous irritability, vague malaise, lassitude, mental confusion, depression and inability to concentrate.

Cardiac and neurological lesions can be produced in monkeys on diets deficient in aneurine [178, 181]. The condition, however, is not exactly analogous to that of human beriberi.

Certain Indian and Japanese writers have postulated a toxin as a cause of acute beriberi [805]. Japanese writers in particular believe infantile beriberi to be caused by a toxin in the mother's milk, because the infant recovers when taken from the breast. Methylglyoxal, which has been found in the blood of patients with beriberi, is thought by some to be this toxin. Stannus [762], who accepts the toxin theory, suggests that the methylglyoxal is formed during the breakdown of carbohydrate in skeletal or heart muscle, and that in the absence of glutathione as a co-enzyme it cannot be broken down by glyoxalase. He postulates the possibility of a primary deficiency of glutathione in beriberi. Haynes and Weiss [699], however, were unable to produce the cardiac manifestations of aneurine deficiency by the injection of methylglyoxal, pyruvic acid or lactic acid.

It is not known for how long the diet must be depleted of aneurine for the onset of beriberi, although it is stated that the symptoms appear after about three months dietary deprivation [195, 702]. Certain predisposing factors play a part in the development of the disease. These include poverty, increased physical exercise, infection, fever, hyperthyroidism, pregnancy, lactation, fatigue, dietary fads and idiosyncrasies, alcoholism, digestive disturbances and diseases interfering with the absorption of food. Nixon [734] points out that many Chinese women appear normal in early pregnancy, but are often *in extremis* towards the end from beriberi. The frequent association of malaria and beriberi, commented upon by Cowgill [198], is due to the heightened metabolism of malaria increasing the vitamin requirements.

Cases of beriberi resulting from inadequate vitamin absorption as a consequence of prolonged vomiting in such conditions as pyloric obstruction, prolonged diarrhoea, fistulæ of the gastro-intestinal tract or short circuiting of the bowel have been reported. The disease can also result from unbalanced diets, persistent vomiting, and alcoholism [230-235].

Clinical Signs and Symptoms. Various clinical forms of beriberi have been described. Each case, however, presents individual variations, and mixed forms are seen.

Infantile Beriberi. This is common in the East among infants in the first few months of life and is characterized by a very rapid onset and acuteness, so that an apparently healthy child may die rapidly from the disease. Cardiovascular symptoms predominate. This has given rise to the assumption, referred to on p. 212, that it is due to toxic metabolites [810]. Bray [811] gives a good clinical description of the condition. The infant suffers from anorexia and is disinclined to feed from the breast, milk is regurgitated, but water is not; it is restless and tender over the abdomen, particularly over the liver; abdominal distension is present and is accompanied by colicky pain, vomiting and paroxysmal screaming; constipation, diminished excretion of urine and water retention occur. The latter leads to œdema (Fig. 72) and an increase in weight, so that the infant looks plump, although wasting occurs later. There follows tachycardia (200 per minute), tachypnoea, dyspnoea and aphonia due to œdema of the larynx. The latter is responsible for the peculiar grunt or "beriberi" cry said to be characteristic of infantile beriberi. Later cyanosis, signs of right-sided cardiac enlargement and failure, congestion of the lungs and engorgement of the liver occur. The serous cavities and tissues become filled with fluid to produce a generalized œdema. Finally come signs of increased intracranial pressure with meningism, rigidity, twitchings, drowsiness, coma and death. Each phase may only last a matter of hours and the whole condition a day or two. The sudden paroxysms of pain cause the body to be held tense and rigid, although true convulsions do not occur. Infantile beriberi is rare in England, although it has been described on the Continent and showed an increase there during the last war. The mortality in the East before the introduction of an extract of rice polishings (tikitiki) was very high (seventy-four per cent.), but this has been reduced considerably since the introduction of specific treatment. Post-mortem there is enlargement of the right ventricle and effusions into the pleural, pericardial and abdominal cavities. In addition there may be œdema or congestion of the liver, spleen and kidneys and œdema of the brain and lungs. Intercurrent disease often obscures the picture.

Women with manifest signs of beriberi may nurse infants having no apparent signs of infantile beriberi, and conversely seemingly healthy women may be nursing infants with manifest signs of the disease [810]. Such women and infants with no manifest signs are presumably in the latent stage. Congenital beriberi has been described, chiefly in the Orient, although it has been reported in America [944]. The child is born with almost fatal cyanotic manifestations, aphonia, tachycardia and cardiac enlargement. Treatment of infantile beriberi consists of giving 10 to 20 mg. of aneurine daily, and improvement of the diet of the mother. Fehily [810] states that the manifestations of aneurine deficiency are so common among the Chinese in Hong Kong that they are regarded as the physiological effects of child-bearing.

In the differential diagnosis of infantile beriberi the following must be considered: overfeeding, the results of which may resemble the vomiting of infantile beriberi; bronchitis, or bronchopneumonia, which are often complications of infantile beriberi; dyspepsia; meningitis; nephritis; peritonitis, which the gastro-intestinal syndrome of infantile beriberi may mimic; diphtheritic paralysis, which may be suggested by the dysphagia, aphonia and symptoms of circulatory failure in infantile beriberi; laryngismus stridulus, which may be suggested by the cyanosis and dyspnoea of infantile beriberi, although there is no "crowing" and Chvostek's sign is negative; congenital syphilis, which may be considered in view of the enlarged liver, œdema and loss of weight. Unless the diagnosis is entertained infantile beriberi may be easily overlooked in regions where the disease is not endemic.

The *adult or chronic type of beriberi*, in contrast to the infantile, is usually insidious in onset, except in cases of acute cardiac beriberi. Clinically the disease is characterized by a triad of symptoms—cardiovascular disturbances, neuritis and œdema—and various forms are termed "dry" (neuritic, para-

FATAL BERIBERI IN A CHILD

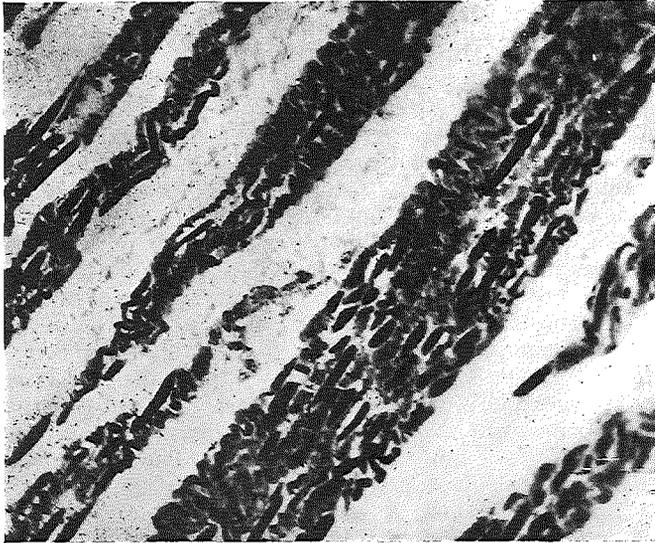


FIG. 64.

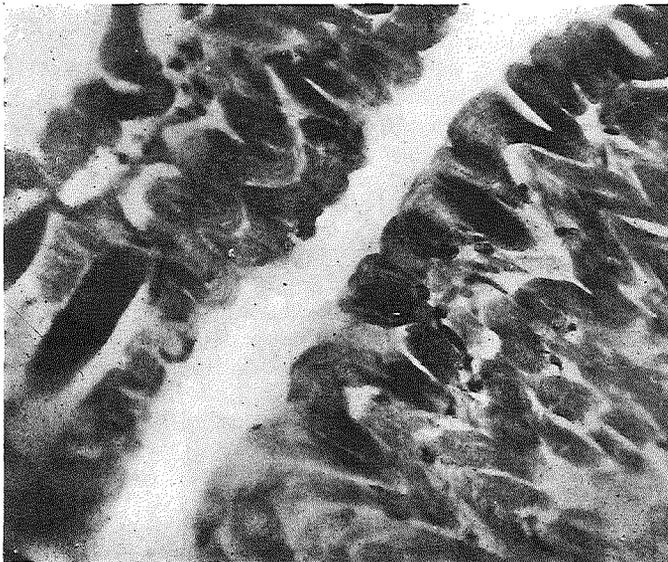


FIG. 65.

FIGS. 64 and 65. Quadriceps Femoris Muscle. Hæmatoxylin and Eosin $\times 85$ and 380. Muscle fibres are atrophic. Some have an indistinct transverse striation and a wave-like spiral appearance similar to that seen in amyotonia congenita.

A FATAL CASE OF BERIBERI IN A CHILD

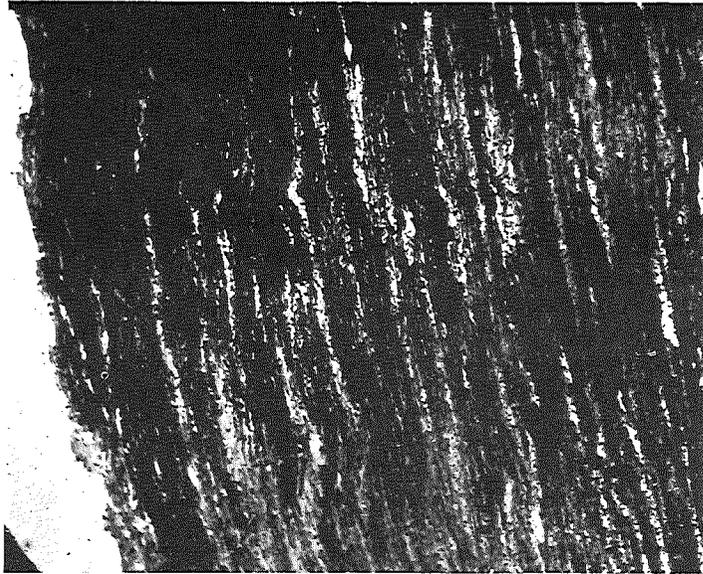


FIG. 66. Spinal Cord. Weigert-Pal $\times 85$. Extensive areas of patchy demyelination. Swellings of the myelin sheaths are visible under higher power.

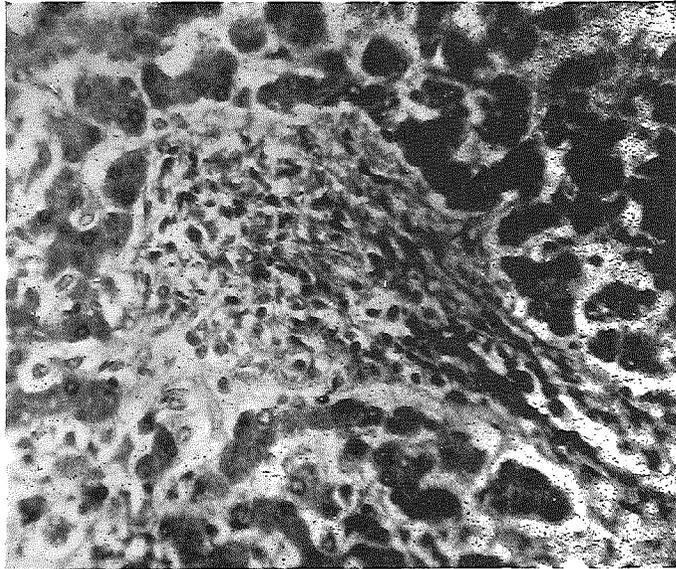


FIG. 67. Liver showing Periportal Space with Cellular Infiltration. $\times 380$. Under low power there are patches and strands of irregularly arranged liver cells separated by a network of broad strands of connective tissue. The liver cells show some atrophy and vacuoles in the cytoplasm. The periportal tissue is increased, consisting mainly of fibroblasts and collagen fibres infiltrated with small round cells.

plegic), "wet" or "cardiac," according to the prevailing symptoms. Mixed cases are also quite common. Neuritis is perhaps the most constant finding, although cardiac symptoms may be present. Early complaints are a feeling of fatigue, cramps in the legs, heaviness and stiffness of the legs with areas of paræsthesia and tenderness along the nerve trunks. Soon after the patient may notice headache, insomnia, anorexia, dyspnoea, tachycardia, nervousness, irritability, depression, lack of interest and initiative, and tenderness of the calf muscles on squeezing. Memory and concentration may be poor and there is a shift in the personality type toward the psychoneurotic. Circulatory symptoms include palpitation, shortness of breath, dizziness, an unexplained tachycardia, variable cardiac murmurs and a slight rise of blood pressure. These symptoms are vague, non-specific and ill defined and seldom give rise to a diagnosis of beriberi.

If deficiency is prolonged the major manifestations of beriberi slowly appear. In *dry beriberi* (see Fig. 69) the nervous system is primarily affected.



FIG. 68. A Fatal Case of Beriberi in a Child. Thickened endocardium of pulmonary conus. $\times 150$. The subendocardial connective tissue is increased and contains many elastic fibres.

The clinical picture is one of ascending, symmetrical bilateral peripheral neuritis. Initially weakness, stiffness and cramps in the legs are complained of, walking for short distances is unimpaired, but weakness is apparent after prolonged exertion (e.g. a mile walk), when the patient's legs will suddenly collapse under him. Later distances of a hundred feet may be sufficient to cause collapse. There may be a burning sensation in the feet,* and a numbness round the dorsum of the foot and ankle with a weakness in dorsiflexion of the ankle joint. Vibration sense may be diminished. The achilles and patellar reflexes are increased at first, then diminished and finally disappear. The ankle jerks are lost early, a sign of diagnostic importance. Weakness spreads upwards, first involving the extensor muscles of the foot, then the muscles of the calf, and finally the extensors and flexors of the thigh, which waste. Toe and foot drop can now be demonstrated. The affected muscles become tender, numb and hyperæsthetic, so that they are tender on palpation.

* The "burning feet" syndrome is discussed elsewhere (p. 320). It is probably not associated with aneurine deficiency as it may occur while the latter is being administered [195, 203, 273].

Deep sensation, elicited by compression of the tendo achilles (Abadie's sign) is increased. The hyperæsthesia extends in the form of a band round the limb (stocking and glove distribution) with anæsthesia following in its wake ; loss of sensation over the tibia occurs early and is diagnostic. Atrophy of the muscles and skin follows, so that the limb has a wasted and shiny appearance. When tested electrically the muscles show the reaction of degeneration. Laxity of the ligaments of the knee joint may occur and give rise to hyperextension (Fig. 70). When the signs and symptoms are pronounced in the legs the upper extremities become involved, the hands and arms being affected first. Thomson [813] claims that neuritic symptoms may appear in the hands and arms before the legs. Burning, numbness and loss of power in the hands

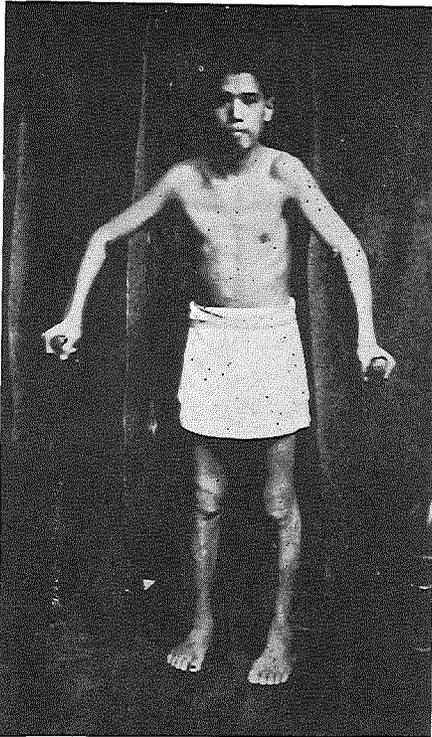


FIG. 69. Dry or Paraplegic Beriberi. The arms and legs are chiefly affected. The leg muscles have atrophied and the patient exhibits a characteristic posture.



FIG. 70. Beriberi. Recovery from dry beriberi, showing hyperextension of the legs with posterior displacement of the tibia, due to laxity of the ligaments.

are experienced, followed by loss of tendon reflexes, wrist drop (see Fig. 71), hyperæsthesia and anæsthesia. Palmar and plantar erythema have been reported [203]. The grip becomes so poor that the sufferer cannot button his clothes or pick up small objects, and may find difficulty in feeding himself, although there is rarely paresis of the muscles of the face, tongue or pharynx. There is loss of deep sensation. The gait becomes ataxic since the patient loses the power to raise the toes, and to avoid scraping them, he walks by lifting the hips, swings the legs, which are held wide apart (Fig. 69), and assumes a characteristic steppage gait. That has been likened to walking in wet clothes or stiff clay. The gait is also protective because of the tenderness of the feet. The ataxia is due to muscular weakness and not inco-ordination.

As the disease progresses the patient becomes bedridden and suffers great pain from the pressure of the bed and clothes on tender muscles. Severe

pains in the extremities, sufficient to prevent sleep, were experienced by prisoners of the 1939-45 war suffering from beriberi. Epicritic sensation is first affected, then temperature, pain and vibration sense. The muscles of the upper extremity, trunk and diaphragm may be involved, and muscular contractures and lack of muscular co-ordination may occur. Loss of sphincteric control does not occur until very late. The spinal cord may be involved with symptoms of spastic paraplegia, a positive Romberg test and a positive Babinski reflex [203]. The initial mild mental symptoms may be followed by mental confusion similar to that seen in toxic infectious states; insomnia, nervousness and emotional instability may also be present. Months may elapse before severe symptoms occur, which may be precipitated by an infection or severe privation. The eighth nerve may be affected, although rarely, with resulting tinnitus and deafness. Lesions of the optic nerve have



FIG. 71. Dry or Paraplegic Beriberi. Wasting of the extensors of the wrist and wrist drop.

been described [146, 193, 249, 751, 753] and were classed as prominent symptoms by Japanese writers earlier in the century. They include loss of visual acuity, bilateral central scotomata, concentric contraction of the field of vision, temporal pallor of the disc, some papilloedema, retrobulbar neuritis and optic nerve degeneration. These, however, may be due to a multiple vitamin B deficiency.

Cardiovascular and respiratory symptoms predominate in acute *cardiac beriberi*, which is described as "wet" if oedema is present. The first detailed and accurate description of the beriberi heart was made by Aalsmeer and Wenckebach [921]. The clinical picture is primarily that of cardiac over-activity and congestive heart failure and is similar to the clinical picture in thyrotoxicosis and arteriovenous aneurysm. The principal manifestations are dyspnoea and orthopnoea, palpitation on exertion, precordial pain, tachycardia and oedema [241, 244]. This fulminating acute type, known as *shōshin*, or "acute pernicious" beriberi heart [241], is a serious threat to life, many patients dying suddenly of heart failure if treatment is not instituted

at once. The "beriberi heart" has long been known to clinicians, working in the East. The heart is enlarged both to the right and left, although mainly to the right, the liver swollen, tender and pulsating, the veins of the neck engorged, and the pulse small, rapid and thready. The carotids pulsate violently and pulsation is visible or palpable in the epigastrium and jugulars. Breathing may be so laboured as to suggest respiratory obstruction. Numerous functional murmurs and signs of pulmonary congestion are common. There may be a transient elevation of the systolic and diastolic blood pressures, the pulse pressure and venous pressure are increased, and the electrocardiogram may show distinct changes (p. 236). It is of low voltage and there is an indefinite inverted or flattened T wave in leads I, II and III, deviation of the RST segment, shortening of the P-R interval and prolongation of the Q-T interval. These changes are non-specific and quickly disappear on treatment. On palpation a bounding quality is noted in the larger arteries and "pistol shot" sounds may be heard on auscultation. Systolic murmurs and a loud sharp second sound over the pulmonary area may be heard. The heart sounds have also been likened to the beats of a pendulum clock, i.e., they are evenly spaced. The pulse quickens rapidly on exertion, and the pulse pressure is high because of a lowering of the diastolic pressure. The skin is usually warm, moist and of normal colour, and cyanosis is rare. Circulatory failure may be right or left sided. Weiss and Wilkins [242] and Blankenhorn [215] examined a number of "beriberi hearts" in America and, in contrast to Eastern beriberi, the heart was not always enlarged and rapid circulation was not constantly present.

Œdema is conspicuous in cases of "wet" beriberi (Fig. 72), beginning in the feet and legs and extending up the body to the face, eventually leading to ascites, hydrothorax and hydropericardium. This generalized œdema may mask the muscle wasting, and oliguria occurs while the œdema is developing. There are apparently no renal changes accompanying beriberi œdema, which is firmer than that of nephritis and of a hydrostatic nature. The rapid blood flow, warm extremities, flushed colour and increased pulse pressure indicate a generalized arteriolar dilatation. The rapid blood flow returns the blood to the right side of the heart at an increased rate. The heart being weaker than normal fails to deliver the blood to the lungs as fast as it is received, and congestion of the viscera commences. If pulmonary œdema develops the right heart fails and its chambers dilate. There is precordial distress or pain, which is aggravated by food, so that the patient eats little. Gastro-intestinal symptoms such as anorexia, diarrhoea, and vomiting may be present. Aphonia is common, especially in infantile beriberi and in chronic adult cases.

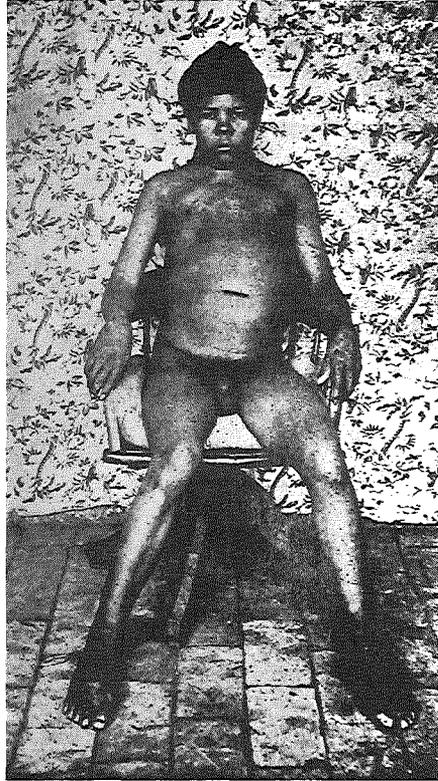


FIG. 72. Wet or Œdematous Beriberi. Note the ascites, with obliteration of the umbilicus, and œdema of the legs, scrotum and face.

A mild secondary anæmia and sometimes hyperglycæmia and glycosuria appear in the picture, and amenorrhœa often occurs. A mild microcytosis occurs in rats deprived of aneurine [591].

The circulatory dynamics of a case of cardiac beriberi was investigated by Burwell and Dexter [222] using cardiac catheterization. Before treatment the venous pressure was 300 mm. of water (normal 40 to 100 mm.), the pressure in the pulmonary artery 64/36 mm. mercury, the pressure in the right ventricle 65/17 mm. mercury (normal 30/18 mm.) and the cardiac output 11.8 litres per minute (normal 3 to 4.6 litres). The patient was given 10 mg. aneurine every six hours for thirty days and then re-catheterized, with the following findings : venous pressure 90 mm. water, pulmonary artery pressure 32/14 mm. mercury, right ventricular pressure 32/0 mm. mercury, and cardiac output 5.3 litres per minute.

Blankenhorn [215] has laid down certain criteria for the diagnosis of cardiac beriberi. They include : (a) enlarged heart with normal rhythm, (b) dependent œdema, (c) elevated venous pressure, (d) signs of peripheral neuritis, (e) non-specific changes in the E.C.G., (f) no other evident cause, (g) poor dietetic history, (h) improvement and reduction of heart size after treatment with aneurine. Thompson [813] has seen deficiencies associated with lack of other vitamins in beriberi patients in the Straits Settlements. Thus he describes eczema of the scrotum, curable by yeast and marmite ; cheilosis and corneal vascularization (pp. 306, 316), a red burning tongue and mouth responding to nicotinic acid ; hemeralopia ; and an anæmia curable by iron and marmite.

"Alcoholic beriberi" may be produced by chronic alcoholism, which leads to anorexia, chronic gastritis and so to deficient food intake. A vicious circle is set up. Alcoholic liquors supply a high calorie value with practically no aneurine—an ideal combination for the development of beriberi. Weiss and Wilkins [242] described 120 cases of "beriberi heart," many of which were attributed to alcoholism. Generally speaking, the cardiovascular symptoms predominate.

Diagnosis. The diagnosis of beriberi is made on a reliable dietary history, a careful physical examination, and the therapeutic test of administering aneurine. The dietary history reveals that the patient subsists on a diet abundant in over-milled rice, wheat or corn, or indulges in dietary idiosyncrasies, e.g. white bread and tea. The history may also reveal that the patient is a victim of anorexia nervosa, alcoholism or of some gastro-intestinal disease interfering with adequate absorption of water-soluble vitamins (p. 225). Persons on a low vitamin B intake, needing increased requirements on account of pregnancy, lactation, hard physical exertion or increased metabolism, are also likely to suffer from beriberi. In the case of infantile beriberi the mother's dietary history, especially during the latter months of pregnancy, may be of diagnostic importance and she may herself be suffering from latent beriberi.

In the more advanced disease the outstanding neurological manifestations are a symmetrical multiple neuritis, mainly of the lower limbs ; tenderness and cramps of the calf muscles ; pain on squatting ; dysæsthesiæ of the feet ; the characteristic steppage gait ; paralysis of the muscles of phonation, mastication, expression and respiration ; and anæsthesia of glove and stocking distribution. The cardiovascular picture is one of myocardial failure with a rapid circulation. Wenckebach [241] laid stress on the enlargement of the heart, cardiac murmurs, visible and palpable cardiac pulsation, arteriolar pulsation, venous engorgement, an enlarged liver, and œdema. These were the outstanding features of beriberi as seen by him in the Orient. The occidental beriberi heart does not show such a dramatic symptomatology, and Blankenhorn [215] has laid down less rigid diagnostic criteria for this (p. 220). The difference in the symptomatology of oriental and occidental beriberi is stressed by Aalsmeer [928]. The electrocardiographic changes in

beriberi are non-specific, the most frequent change being a low voltage and minor abnormalities in the T waves. The reversion of the E.C.G. to normal may lag behind clinical recovery.

Differential Diagnosis. The neuropathy must be distinguished from that due to other causes. In lead neuritis only the motor nerves and anterior horn cells are affected; pain and sensory nerve involvement are rare, the blood picture is characteristic, lead can be detected in increased quantities in the urine, and the blue line of the gums helps to establish the diagnosis. Wrist drop is one of the early signs. There is also a history of colic and exposure to lead.

Arsenical neuropathy and encephalopathy can simulate that resulting from aneurine deficiency and may indeed be due to a similar cause—an enzyme block at the pyruvic acid level in carbohydrate metabolism [226]. Sensory changes such as burning pain and paræsthesiæ occur with motor symptoms; the dermatitis of arsenical poisoning is, however, typical in appearance and distribution. Poisoning by other heavy metals may also cause a “biochemical lesion” similar to that produced by aneurine deficiency [227].

Peripheral neuritis due to external toxins and infections is abrupt in onset. Diphtheria produces an ascending paralysis beginning at the site of infection, e.g. faucial diphtheria leads at first to palatal paralysis.

The cardiovascular symptoms may resemble those of heart failure due to other causes, e.g. rheumatic, hypertensive, senile and syphilitic heart disease, and secondary to pulmonary disease, all of which can be distinguished by the history, clinical examination of heart and lungs, serological tests for syphilis, and blood-pressure readings. Thyrotoxicosis, arteriovenous aneurysm, cirrhosis of the liver and renal disease must also be considered. The œdema of renal disease is accompanied by albuminuria, low plasma proteins and low renal efficiency. “Hunger œdema” is probably due to a low protein intake, although Simonart [229] claims that it responds to treatment with aneurine.

Beriberi has also been diagnosed as tabes dorsalis, “rheumatism,” malaria, progressive muscular atrophy and lathyrism. Tabes can be excluded by serological tests for syphilis, examination of the C.S.F., the Argyll-Robertson pupil, lightning pains and the characteristic sensory pains. In both tabes and beriberi Abadie’s sign (pain on squeezing the calf muscles) may be positive. Malaria, the most protean of tropical diseases, is distinguished by a stained blood film, and in lathyrism there is no muscle tenderness or anæsthesia and the knee jerks are exaggerated (p. 74).

Pathology and Morbid Anatomy of Beriberi. Acute cases die from heart failure and the principal post-mortem changes are in the heart, which is large and dilated, particularly on the right. Wenckebach [241] also reported hydropic degeneration of the heart muscle, fibrosis of the myocardium and dilatation of the conus arteriosus, the latter being diagnostic according to him. In cases showing right-sided failure back pressure causes congestion of the liver, kidneys, spleen and intestines. Internal organs may also show fatty degeneration and cloudy swelling. Œdema of the lungs is often found as well as serous effusion in the pericardium, pleura and peritoneum. Legs, arms and thighs may be œdematous in “wet” beriberi. Weiss and Wilkins [242] describe hydropic degeneration of the myocardial fibres and the conduction bundle, “intercellular œdema” and an increase in collagen. Similar changes are seen in aneurine deficient rats [910].

Chronic cases usually die from intercurrent infections such as dysentery, typhoid or tuberculosis. The body is usually emaciated. The neurological lesions are noted chiefly in the sciatic nerve, but some degeneration may be seen in any peripheral nerve, the cranial nerves, the vagus and phrenic nerves and the sympathetic chain. Histologically there is degeneration of the myelin sheath with pigmentation and vacuolar degeneration of the cells of Schwann. The axis cylinders may show fragmentation or atrophy and Wallerian degeneration may be demonstrated. In the central nervous system

some degeneration of the sheaths of scattered fibres in the anterior and posterior nerve roots and posterior columns may occur. Changes in the ganglion cells of the medulla and pons have been described [247].

The muscles supplied by affected nerves are considerably atrophied and histologically there is evidence of cloudy swelling, fatty degeneration, loss of cross striation and shrinkage of sarcoplasm.

The post-mortem diagnosis of beriberi rests on (a) dilatation and hypertrophy of the heart, usually on the right, without evidence of organic cause; (b) visceral congestion; (c) oedema; (d) degenerative lesions in the peripheral nerves and spinal cord; and (e) absence of any other cause of death.

Prognosis and Treatment of Beriberi. The course is progressive unless treated and sudden death may occur from heart failure or secondary infection. The mortality varies from five to fifty per cent. according to the severity of the condition and the treatment used. Evidence of severe cardiac involvement, diaphragmatic paralysis, and serous effusions are of serious import and demand instant treatment. Once remission has occurred the prognosis is good if the patient is placed on a diet rich in the B vitamins. After treatment and recovery there is remyelination and regeneration; many of the nerve fibres are slowly restored to normal, although this is far slower than the clinical improvement.

For prophylaxis foods rich in aneurine and the vitamin B complex—cereals, peas, beans, peanuts and yeast—should be incorporated in the diet. It has been suggested that rice be enriched with aneurine, nicotinic acid and iron in areas where beriberi is endemic [209]; the provision of food yeast (*Torula utilis*, p. 193) has also been suggested. For many years an extract of rice polishings (*tikitiki*) has been used in the East as a preventive. The diets of patients in endemic areas suffering from debilitating diseases, chronic alcoholism, pellagra, sprue, cirrhosis, tuberculosis and other infections should receive special consideration.

The treatment of beriberi will depend on the severity of the condition. The acute state is a medical emergency, treated with bed rest and parenteral injections of 10 to 20 mg. aneurine daily; doses in excess of this are wasteful and unnecessary. As recovery sets in the dose can be reduced to 5 to 10 mg., given by mouth, unless impaired absorption is suspected. Venesection may be necessary to relieve the right side of the heart, and serous effusions may need paracentesis. In the chronic case 5 to 10 mg. of aneurine daily is adequate. At the same time the diet, which should be a high protein, high calorie one (4,500 calories), should be supplemented by foods rich in aneurine and by yeast (heat treated) or yeast extracts, or liver or rice polishings.

Specific treatment for the limbs may be needed. The patient should be rested in bed and the bedclothes should be kept off the limbs with a cradle, gentle passive movements of all joints and paralysed limbs should be performed several times a day and active movements encouraged as soon as possible. Massage and faradism can be employed after tenderness has gone. Analgesia may be required for pain and night cramps treated with quinine 3 to 6 grains night and morning.

In the cases of infantile beriberi 5 to 10 mg. of aneurine is given parenterally daily, and the mother treated as well if the infant is breast fed.

The response of acute beriberi to treatment is dramatic; improvement begins in a few hours. In severe neuritic cases pain is relieved in a few days, but complete clinical recovery may take many weeks or months. Sometimes residual neurological changes remain in cases of complete degeneration of the ganglion cells and axis cylinders.

OTHER MANIFESTATIONS OF ANEURINE DEFICIENCY

Factors conditioning Vitamin Deficiency. A state of vitamin deficiency, either mild or gross, may develop from a deficient intake due to a faulty diet,

in which case the deficiency may be said to be primary, or it may result from factors other than an inadequate diet, that is from conditioned deficiency. A conditioned deficiency is caused by factors interfering with the ingestion, absorption or utilization of essential vitamins, or by factors that increase their requirement, destruction, or excretion [236, 429, 430].

CONDITIONED VITAMIN DEFICIENCY

I. FACTORS INTERFERING WITH INGESTION

1. Personal :
 - Economic, ignorance, poor food habits, food faddism, eccentricity, alcoholism, anxiety.
2. Gastro-intestinal disease :
 - Anorexia : due to alcohol, anæsthesia, post-operative conditions, infectious disease and visceral pain.
 - Dysphagia.
 - Dyspepsia.
 - Nutritionally inadequate therapeutic diets as in :
 - (a) Gastro-enteritis.
 - (b) Cholecystitis and cholelithiasis.
 - (c) Ulcerative colitis.
 - (d) Peptic ulcer.
 - (e) Obesity treatment.
 - (f) Chronic renal, hepatic and cardiac disease.
 - (g) Carcinoma of stomach and œsophagus, cardiospasm.
 - (h) Intestinal obstruction.
3. Food allergy.
4. Mental disorders such as :
 - Neurasthenia.
 - Neurosis.
 - Psychoneurosis.
 - Psychosis.
 - Anorexia nervosa.
 - Migraine.
5. Operations and anæsthesia.
6. Loss of teeth.
7. Heart failure (anorexia, nausea and vomiting due to visceral congestion).
8. Parenteral administration of nutrients, e.g. saline, glucose, amino-acids.
9. Pulmonary disease (anorexia, vomiting due to cough).
10. Toxæmia of pregnancy (nausea and vomiting).
11. Neurological diseases interfering with feeding, chewing, and swallowing.

II. FACTORS INTERFERING WITH ABSORPTION

1. Diarrhœal diseases :
 - Ulcerative and mucous colitis.
 - Dysentery and intestinal parasites.
 - Intestinal tuberculosis.
 - Sprue.
2. Gastro-intestinal diseases associated with hypermotility or reduction of absorbing surfaces, e.g. carcinoma, sprue, colitis.
3. Gastro-intestinal and external fistulæ, strictures.
4. Short-circuiting operations on the bowel.
5. Vomiting.
6. Achlorhydria.
7. Biliary disease, especially obstructive jaundice.
8. Therapy—liquid paraffin, colloidal adsorbents and cathartics.

III. FACTORS INTERFERING WITH UTILIZATION

1. Hepatic dysfunction.
2. Diabetes.
3. Alcoholism.
4. Hypothyroidism.
5. Malignancy.
6. Therapy—sulphonamide and other drugs, radiation therapy.

IV. FACTORS INCREASING REQUIREMENT

1. Abnormal activity?—e.g. prolonged strenuous physical exertion.
Delirium.
Mania.
2. Fever.
3. Hyperthyroidism.
4. Pregnancy and lactation.
5. Abnormal environmental factors :
Excessive temperature, as in tropics and certain industries.
6. Therapy increasing metabolic rate, such as thyroid, insulin, fever therapy, parenteral dextrose, high carbohydrate diets.
7. Anoxia.

V. FACTORS CAUSING DESTRUCTION OF VITAMINS

1. Achlorhydria ?
2. Lead poisoning ? Trinitrotoluene poisoning ?
3. Therapy with :
Alkalis.
Sulphonamides, sulphones, antibiotics.
Arsenicals.
4. Antivitamins, e.g. thiaminase.

VI. FACTORS INCREASING EXCRETION

1. Polyuria as in :
Diabetes mellitus.
Diabetes insipidus.
Nephritis.
Diuresis induced by drugs.
2. Lactation.
3. Excessive perspiration ?
4. Therapy : excessive fluid intake—for urinary and other infections.

VII. FACTORS CAUSING DIMINISHED INTESTINAL SYNTHESIS

1. Sulphonamides.
2. Antibiotics, e.g. penicillin, terramycin, aureomycin, chloramphenicol.

Not all these factors operate in the case of all vitamins. Thus the requirements of vitamin A are independent of carbohydrate intake, basal metabolism and temperature. These factors will be discussed in so far as they concern aneurine.

I. Factors Interfering with Ingestion. An inadequate intake of aneurine may result from poverty, ignorance of what constitutes a balanced diet, poor food habits, food fads and eccentricity. We have seen patients with poor appetites, living on a high carbohydrate diet, who have neither the money nor the inclination to eat the right type of food, suffering from the manifestations of vitamin B deficiency—neurasthenic symptoms, fatigue, neuritis, sore tongue, mouth and lips, depression and insomnia [816]. Palmer [245] also describes a girl with eccentric food habits who lived largely on chocolate. Gastro-intestinal disease, especially if associated with anorexia, dysphagia,

dyspepsia, pain or vomiting, such as occurs in nervous dyspepsia, peptic ulcer, gastro-enteritis, gall bladder disease or ulcerative colitis, are noted for their interference with food intake. The patient, either because of pain or nausea, limits the quality and quantity of his diet. The nervous tense woman with "nervous dyspepsia" or an "irritable colon" associated with abdominal pain who gradually reduces her diet to tea and toast, and who subsequently develops a sore tongue and signs of peripheral neuritis is also well known. Deficiency diseases of a mild degree occur not only among patients who have followed self-imposed diets for both real and imaginary complaints, but also among patients who have been dieted for long periods by their physician for such conditions as allergy, peptic ulcer, biliary disease, nephritis, hypertension, colitis, diabetes and obesity. Wilbur [817] describes patients with marked loss of weight, stomatitis, peripheral neuritis and even pellagrous lesions following strict dietary treatment for non-organic digestive symptoms and for the relief of hay fever and asthma.

It is clear that many therapeutic diets even if well planned need to be supplemented by vitamins and minerals in which they are deficient. Particularly harmful are many of the slimming diets that are published in non-medical papers. Dried brewers' yeast and yeast extract are useful sources of the B vitamins for incorporation in therapeutic diets. Obstructive lesions such as carcinoma of the stomach and œsophagus and intestinal obstruction also limit food and hence vitamin intake.

In neuropsychiatric disorders, such as neurasthenia, the neuroses, psychoses and anorexia nervosa the patient may have no desire for food. In migraine and hyperemesis gravidarum the mere sight of food may induce nausea. Anorexia is also associated with alcoholism, anæsthesia, post-operative convalescence, infectious disease and visceral pain, and if prolonged may lead to severe vitamin deprivation. Alcohol, which contains no vitamins, produces deficiency disease by replacing other food and by causing nausea and vomiting by its irritant action on the stomach, in which it sets up a chronic gastritis. Alcoholism therefore produces aneurine deficiency by causing anorexia and by replacing aneurine-containing foods. Civilized man usually satisfies anorexia not by a limited food intake all round, but by snacks of carbohydrate foods, such as bread, toast and sugared beverages, which increase the relative aneurine requirement. Anorexia may also occur in old people, particularly if edentulous and leading a solitary existence. Lack of teeth results in the consumption of pappy carbohydrate foods, which if refined are poor in aneurine.

In heart failure and pulmonary disease the anorexia, nausea and vomiting due to visceral congestion and cough limit the intake of food. When saline, glucose and amino-acids are given parenterally because the patient cannot take food by mouth there is no aneurine, except the small amount stored in the body, to metabolize the glucose. Aneurine, nicotinic acid, riboflavine and ascorbic acid should be added to solutions for infusion if nothing is taken by mouth. Certain neurological diseases characterized by paralysis of the muscles of deglutition and cardiospasm may also interfere with the neuromuscular mechanism of swallowing. Patients with such conditions, as well as those with œsophageal stricture or carcinoma, or obstructive lesions in the gastro-intestinal tract, e.g. pyloric stenosis, cardiospasm and carcinoma of the stomach, may fail to receive sufficient food by the oral route and suffer from deficiency disease.

II. Factors Interfering with Absorption. Diseases interfering with the absorption of food play an important part in producing conditioned deficiency disease, even if the intake of food is adequate in all respects. In diarrhœal diseases, such as colitis (ulcerative and mucous), dysentery, intestinal parasitism [622], intestinal tuberculosis, sprue and pellagra, absorption of food and hence vitamins is impaired because it is rushed through the intestinal tract leaving little time for digestion, solution and absorption [430, 682]. The

absorbing surface of the gut may also be impaired, as in chronic ulcerative colitis and sprue, and the internal secretions may be so altered that absorption is imperfect. Absorption may also be modified by alkalis, adsorbents and lubricants (liquid paraffin) used in the treatment of these and other gastro-intestinal diseases. The water-soluble vitamins are adsorbed by such substances as aluminium hydroxide (e.g. Aludrox), fuller's earth and magnesium trisilicate [243], and liquid paraffin dissolves out the fat-soluble vitamins, A, D, E and K, which are thus lost to the body. Medicinal charcoal taken for therapeutic reasons removes considerable amounts of aneurine and riboflavine [592]. It is claimed that achlorhydria may impair the absorption of aneurine [44, 237], although this has been denied [55]. Vomiting, which is a common symptom of gastro-intestinal disease, interferes with the absorption of food and if unrelieved may precipitate aneurine deficiency. Achlorhydria may limit the absorption of some of the B vitamins [583, 586].

Obstructive gastro-intestinal lesions at or below the level of the stomach may interfere with the absorption of food. Thus a malfunctioning gastro-enteric anastomosis may obstruct the stomach and duodenum. Obstructive lesions of the small intestine have long been known to be associated with an anæmia and a clinical picture simulating that of pernicious anæmia and sprue.

Fistulæ, e.g. gastro-colic, which short-circuit the small intestine, wholly or in part, are causes, albeit uncommon, of deficiency diseases. In spite of an adequate food intake so little may reach the small intestine that severe loss of weight and malnutrition may result. Syndromes resembling those of sprue and beriberi have been reported in patients with such fistulæ [238]. Short-circuiting operations of the gastro-intestinal tract, e.g. gastrectomy, have long been known to produce macrocytic anæmia and there is some evidence that other deficiency conditions may be produced. Vitamin B deficiency may occur in ten per cent. of patients submitted to gastrectomy according to Welbourne, Hughes and Wells [701]. External intestinal fistulæ are uncommon, but they have been observed associated with deficiency disease.

Liver disease may lead to interference with the absorption of fat-soluble vitamins (A, D, E and K), and possibly with the intestinal absorption of aneurine [726].

From studies on sprue patients Frazer [583] has advanced a new mechanism of vitamin deprivation. He has found from intubation of a number of sprue cases that in achlorhydric phases large numbers of viable organisms reach the upper part of the small intestine, where they utilize the vitamins of the host. Frazer suggests that in the exacerbations of sprue, and possibly in other conditions such as pellagra, pernicious anæmia and nutritional macrocytic anæmia, there is a competition for essential nutrients between the host and bacteria in the absorption area of the intestine, which does not harbour bacteria in the healthy subject. This view is supported by the observation that in sprue parenteral administration of the appropriate vitamin often produces immediate relief of vitamin deficiency syndromes without affecting fat absorption.

III. Factors Interfering with Utilization. Evidence for the existence of factors interfering with the utilization of vitamins is largely circumstantial. It is known that certain vitamins cannot be utilized by the body as such. Thus carotene must be converted to vitamin A, aneurine to cocarboxylase, nicotinic acid to codehydrogenases I and II, and riboflavine to flavoprotein. The liver is considered to be the principal organ in which some of these conversions occur.

Cirrhosis and other diseases of the liver are believed to inhibit the utilization of aneurine, the excretion of which is lowered in hepatic disease. The high frequency of symptoms of aneurine deficiency in alcoholics is well known, and it is possible that hepatic dysfunction plays a part. It is believed by some

that diabetes interferes with the utilization of aneurine, although the evidence for this is conflicting. In malignant disease there appears to be a general vitamin deficiency.

Therapy with some sulphonamides and other drugs may interfere with the utilization of the B vitamins. It is known that deficiency symptoms can be produced by administering sulphonamide drugs to animals, although this may be due to the inhibition of the intestinal synthesis of some of the B factors (pp. 127, 136). Sulphadiazine may actually have an aneurine sparing action (p. 198). Sulphapyridine inhibits the activity of nicotinic acid in the experimental animal [664], but there is no evidence that it does in the pellagrin [429]. Deficiency symptoms have been produced in man following the administration of sulphonamides, sulphones and antibiotics such as penicillin, streptomycin, chloramphenicol and aureomycin [790]. Radiation sickness has been attributed to failure of co-enzyme formation from aneurine and nicotinic acid.

IV. Factors Increasing Requirement. It has already been stated that the aneurine requirement is proportional to the metabolic rate (p. 210). Fever increases the basal metabolism by 7.2 per cent. for each degree Fahrenheit, while strenuous physical exertion may increase it as much as fifteen times. It would be expected that the pyrexial patient requires more aneurine than normal. The occurrence of aneurine deficiency conditioned by fever, hyperthyroidism (p. 210), pregnancy and lactation (p. 210) is well recognized. Johnson and his co-workers [818] state there is an increased requirement of the B vitamins produced by moderately strenuous physical activity in farmers, soldiers and other workers. On a consumption of 4,000 to 5,000 calories daily deterioration in physical fitness occurred on diets deficient in the vitamin B complex. This was remedied by giving the entire B complex. Keys and his co-workers [797], however, obtained diametrically opposed results (p. 206). It is therefore debatable whether an increased requirement is to be expected in occupations associated with prolonged strenuous physical work and in mental patients with delirium or mania, showing an increase in psychomotor activity. Outbreaks of deficiency diseases such as pellagra were once common in mental hospitals. Excessively high environmental temperatures, such as might be experienced in the tropics and in certain industries, may increase the aneurine requirements (p. 211).

By increasing total metabolism various forms of therapy may produce a conditioned deficiency of aneurine. Drugs such as thyroid, thyroxine, and fever therapy increase metabolism, and hence the need for aneurine. Parenteral administration of glucose, insulin therapy and high carbohydrate diets do not increase total metabolism but increase the number of non-fat calories consumed and therefore the requirement of aneurine. The long-continued administration of glucose to patients on a poor diet may precipitate deficiency disease [665, 667]. When glucose is administered in this way over a prolonged period aneurine, nicotinic acid and riboflavine should be given as well. Excessive carbohydrate in the diet can also induce deficiency disease; beriberi due to this cause has, in fact, been reported [819].

V. Factors causing Destruction of Vitamins. Melnick, Robinson and Field [44] have shown that aneurine is stable in gastric juice between a range of pH 1.5 to 8. The presence of antacids, bile or pancreatic juice destroys aneurine. The administration of alkalis, as for peptic ulcer therapy, diminishes its excretion. Recent work suggests that some of the vitamins are concerned with the detoxication of drugs such as sulphonamides and arsenical drugs (pp. 421, 423). Certain foodstuffs contain a factor that may destroy or inactivate aneurine, particularly raw fish and meat. This factor, thiaminase (p. 228), is unlikely to be present in any quantity in most human diets, as meat and fish are usually cooked. It has been reported, however, that fifty per cent. of the dietary aneurine in man may be destroyed by diets containing raw clams [246]. An anti-aneurine factor is also stated to be present in

certain cereals, legumes and oil seeds [250], and the administration of live yeast withdraws aneurine from the gut.

VI. Factors Increasing Excretion. The possibility of the washing out of the body of the water-soluble vitamins must be considered in conditions associated with polyuria, such as diabetes mellitus and diabetes insipidus. As Cowgill and his collaborators [224] have shown, excessive diuresis produced by forcing fluids can bring on the symptoms of aneurine deficiency. The forcing of fluids over a long period of time as a therapeutic measure in general infections and infections of the urinary tract, and the loss of fluid by diuresis in oedema may precipitate a deficiency. The excretion of aneurine is considerably increased by the administration of mercurial diuretics such as mercuriophylline [54]. Lactation also results in a loss of aneurine from the body (p. 210), and is notorious in the East as a factor in the production of beriberi. Excessive perspiration has been said to result in appreciable losses of aneurine from the body, but this has not been confirmed (p. 203). In any case, loss of aneurine by this route would only be of significance under tropical conditions or in subjects doing heavy manual work in a hot environment.

Biochemically Induced Aneurine Deficiency. It has been recognized that a compound may so structurally resemble a vitamin or growth factor that it will be adsorbed in an enzyme system containing the vitamin in place of the latter. The difference, however, is sufficient to prevent the cycle of chemical reactions occurring. The enzyme system is thus "blocked," e.g. sulphanilamide blocks the system containing *p*-aminobenzoic acid (p. 135) and pantooyltaurine blocks that containing pantothenic acid (p. 120). If the enzyme system of which the vitamin is a component is blocked, the latter is not available for growth and development of the organism. Pantothenic acid and *p*-aminobenzoic deficiencies can be produced in animals by feeding the appropriate inactivating agent. Woolley and White [905] have produced aneurine deficiency in mice by the addition of a thiamin analogue, known as pyrithiamin, in which the thiazole nucleus is replaced by pyridine. If sufficiently large doses of aneurine are given to the animals recovery occurs. A competition between aneurine and pyrithiamin occurs. Animals fed large amounts of aneurine and a small amount of pyrithiamin fail to develop deficiency symptoms, but do so if the amount of pyrithiamin is increased sufficiently. Other aneurine analogues that produce deficiency symptoms by a similar competitive action are neopyrithiamine [612], oxythiamin [259] and butyl aneurine. Experiments involving the feeding of a biochemical inhibitor may considerably simplify the study of vitamin deficiency disease, as they circumvent the use of synthetic or purified diets, which are laborious to prepare and unappetizing to eat.

Another mechanism is involved in the destruction of aneurine by enzymes present in certain animals and plants. The paralysis that occurs in foxes on fox ranches as a result of feeding them raw fish (Chastek paralysis) is due to aneurine deficiency caused by an aneurine destroying enzyme, thiaminase [829]. This disease is fatal if untreated; it responds to treatment with aneurine or withdrawal of raw fish from the diet. Thiaminase splits aneurine into its pyrimidine and thiazole components through exchange of its thiazole moiety with an amine [820]; its action is inhibited by aminobenzylthiazolium salts [252]. Thiaminase can cause aneurine deficiency symptoms in chicks, cats and pigeons as well as foxes, and is in fact a useful tool for the production of aneurine deficiency. The importance of thiaminase in human nutrition is not known. In countries where raw, partly cooked or smoked fish constitutes a major constituent of the diet it may be a factor contributing to aneurine deficiency. Clams and salted herring have a very powerful thiaminase that may destroy fifty to sixty per cent. of the dietary aneurine; oysters and smoked salmon are innocent in this respect [246].

A plant factor from bracken fern, enzymic in nature, has been isolated which causes signs of aneurine deficiency and is responsible for disease in

horses and cattle feeding on fern pasture [256, 263, 651]. Other aneurine inhibitors have been described in grain [250], raw mutton [258] and the plant *equisetum* (horsetail) [770].

Nervous Lesions in Aneurine Deficiency. In animals suffering from acute aneurine deficiency Wallerian degeneration of peripheral nerve fibres occurs [289], and degenerative changes have also been described in the anterior and posterior nerve roots, tracts of the spinal cord, the medulla, pons, midbrain and internal capsule. In the deficient pigeon the first neuronal histological change is degeneration of the distal part of the axon, which proceeds towards the cell body, which becomes sclerosed; the large nerve fibres degenerate first [732]. If the deficiency is chronic, degeneration of the vestibular nerves results, which is often associated with cerebellar ataxia, and sometime degeneration of the cell bodies and peripheral fibres of the third and fourth cranial nerves. There is a progressive increase in the amplitude of the potentials, up to about three times normal, in the encephalogram of pigeons with aneurine deficiency. In the final stages there is a marked slowing down and depression of the brain potentials. The electroencephalogram returns to normal in a few hours if aneurine is given [821]. The neurological manifestations of aneurine deficiency in the pigeon are accompanied first by impaired function and then by degeneration of primary neurons of the proprioceptive nervous system and the central terminations of the optic nerves; after prolonged deficiency the efferent nervous system becomes affected. A number of degenerated fibres regenerate when aneurine is given, but severely injured ones continue to degenerate [732]. Increased amounts of alkaline phosphatase are present in the nerve cells, and a diminution of acid phosphatase in the axis cylinders [738]. If aneurine deficiency is continued long enough in animals the neurological lesions are irreversible [822]. The vascular changes in the brains of aneurine deficient animals have been studied by Prados and Swank [732], who found that hæmorrhages occurred in the brain accompanied by perivascular sclerosis and interstitial cell proliferation. The hæmorrhages were preceded by vasodilatation and were first perivascular and later infiltrating. The lesions were accompanied or preceded by degenerative changes in the neighbouring neurons and swelling or hypertrophy of the oligodendrocytes and astrocytes.

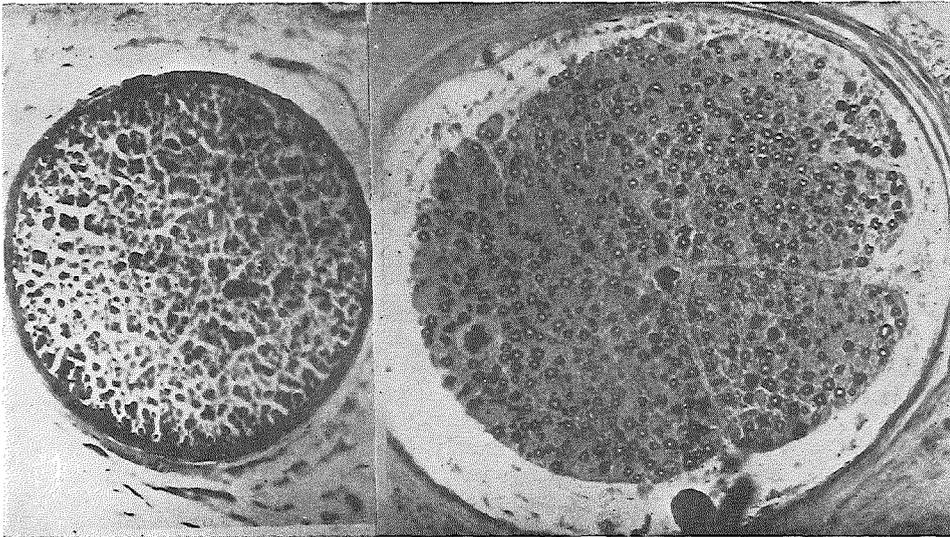
The dura of aneurine deficient rats shows hæmorrhagic lesions, including dilatation of the vessels and hyperæmia [608]. Everett [926] has studied the results of aneurine deficiency on the nervous system of the cat. After an initial period of anorexia the animal suffers from tonic convulsive seizures and disturbances of postural mechanisms, such as impairment of labyrinthine righting reactions, the vestibulo-ocular reflex and the pupillary light reflex. Dysfunction of the cerebellum is suggested by asynergia, ataxia and dysmetria. The peripheral nerves in the cat are not affected [269].

In the rhesus monkey changes in the peripheral nerves are absent, even with severe aneurine deficiency [180], but changes in the brain similar to those seen in Wernicke's encephalopathy in man have been reported by Rinehart [180]. Bilateral areas of degeneration occur in the corpus striatum, globus pallidus, substantia nigra, mamillary bodies, corpora quadrigemina, cerebellar cortex and nuclei of the third, sixth, eighth and tenth cranial nerves. Associated with these lesions are profound weakness, ataxia and occasional focal signs of cranial nerve weakness or hyperirritability.

In the human subject central degeneration is rare, although it may occur in beriberi. The nerves supplying the lower extremities are usually affected, the outstanding symptoms being intermittent tenderness of the calf muscles, burning of the soles of the feet, skin hyperæsthesia in a sock distribution, anæsthesia, muscular weakness and cramps. Tenderness of the calf muscles and hyperæsthesia of the soles are the earliest symptoms. Such symptoms, which are bilateral, can be observed before the onset of typical neuritic beriberi, in which diminished reflexes and muscular paralysis and atrophy

make their appearance. Vibration and position sense are impaired and ankle jerks may disappear. The terminal portions of the nerves are first affected and therefore the symptoms are more pronounced in the distal regions of the extremities, chiefly the lower, in which the sciatic nerve and its branches suffer. Histological changes (Fig. 73) are of late onset and may be still seen after symptomatic improvement. The first stages of recovery from the neuritis may be rapid, but many weeks may elapse before the complete use of the legs is regained. The slow improvement in severe neuritic cases under treatment is undoubtedly due to the long time taken for the remyelination of the nerve fibres.

In moderately advanced cases pain along trunks and along intercostal nerves may be complained of. Dropped foot and wrist, and muscular atrophy, especially of the thigh, may result, and then the upper extremities may be



FIGS. 73 and 74. Fig. 73 shows a transverse section of a branch of the anterior tibial nerve of a twenty-seven-year-old American woman suffering from aneurine deficiency. There is swelling and degeneration of the myelin sheaths. The myelin sheath count is 380 per sq. mm. (Osmic acid stain, $\times 150$). Fig. 74 is a section across the same nerve of a normal person as a control. The myelin sheath count is 6,840 per sq. mm. (Osmic acid stain, $\times 150$).

affected. As the sensory changes advance, pain and numbness increase. Finally the muscular and sensory changes may involve the trunk, contractures may occur, and the patient becomes bedridden. Involvement of the vagus may produce paresis of the vocal cords. The oculomotor nerves, particularly the abducent, may be affected, as in Wernicke's encephalopathy (p. 231). A neuritis of the tenth nerve, or its laryngeal branches, leading to changes in the voice have been described. Lesions of the optic nerve—diminished visual fields, temporal pallor of the disc, and primary optic atrophy—have been described in patients suffering from severe vitamin B deficiency, although several factors of the vitamin B complex may probably be involved [751]. No significant degeneration of the optic nerve is seen, however, in animals on diets deficient in aneurine or the vitamin B complex [550]

The neurological symptoms that may occur in such diverse conditions as nutritional, gastrogenous [260, 261], gestational [205, 262, 264] and alcoholic neuritis [266-268] and in Korsakoff's syndrome [257] have been attributed to aneurine deficiency. It is unlikely that there is only a deficiency of aneurine

in these conditions ; if a vitamin deficiency exists it is likely to be a multiple one. Gastro-intestinal conditions such as diarrhoea, colitis, sprue and pyloric stenosis associated with vomiting are associated with multiple avitaminosis (p. 225). Lewy [418] noted that the degree of peripheral nerve change in pregnant women on defective diets, as indicated by chronaximetric examination, coincided with the severity of the condition. Improvement followed the administration of vitamin B. If the diet contains adequate aneurine, pregnancy neuritis does not occur [270].

It was originally suggested by Wechsler [277] that diabetic neuritis might be due to a vitamin deficiency, and it was later claimed that the administration of aneurine relieved it [271, 280]. Needles [281], however, examined the diets of a number of diabetics and concluded that they contained an adequate amount of aneurine.

It has been suggested that alcoholic neuritis results from vitamin deficiency (p. 225). The alcoholic buys alcohol rather than food and suffers from anorexia and gastritis. These limit his food and vitamin intake and absorption. This is the most likely explanation of a deficiency rather than increased needs of aneurine due to the alcohol.

Some cases of nutritional neuropathy are not due to aneurine deficiency, as shown by the work of Grande and Jiménez [824], who investigated cases of neuropathy during the Spanish Civil War. They found that lactic acid was metabolized normally, and therefore concluded that the neuropathies they observed were not aetiologically related to aneurine. The diet in Spain consisted largely of bread, lentils, rice and soup and was not alarmingly low in aneurine. Pellagra was common, but not beriberi. These neuropathies were not cured by aneurine, nicotinic acid or vitamin A, but responded to treatment with yeast.

Wernicke's Encephalopathy. The disease known as superior hæmorrhagic polioencephalitis, or Wernicke's encephalopathy, was originally described in 1881 by Wernicke and is characterized by paralysis of the eye muscles, a reeling gait and disturbances of consciousness which usually terminate in fatal coma. The pathological changes are remarkable for their strict localization : pin-point hæmorrhages and glial proliferation of the corpora mamillaria, the hypothalamus, the thalamus, the peri-aqueductal grey matter, and the colliculi and the floor of the fourth ventricle (Figs. 75-79). Common clinical findings are nystagmus, diplopia, ophthalmoplegia, vomiting, muscular weakness, giddiness, anorexia, a dull mental apathy, with mental retardation, insomnia, confabulation, hallucinations, disorientation in space and time, and loss of memory for recent events. Less common are loss of visual acuity, fundal hæmorrhages, cranial nerve palsies, incontinence and ataxia. Wernicke's encephalopathy is closely related clinically to Korsakoff's syndrome (p. 252) and is often associated with alcoholism, although in twelve cases reported by Campbell and Biggart [284] only one was alcoholic.

The condition has been more recently described by Campbell and Biggart [284], Campbell and Russell [710], Ecker and Woltman [545], Wortis *et al.* [743], Spillane [146], De Wardener and Lennox [272], Boles [834], and Cruickshank [275]. It may occur in a variety of conditions, but is mostly associated with conditioned nutritional failure, e.g. in alcoholism, liver dystrophy, hyperemesis gravidarum [735], anorexia, chronic gastro-intestinal disorders and malnutrition. Many cases were seen in white prisoners of war in Eastern prison camps [272] ; it has also been described in infants [276]. The disease is generally considered to be due to acute aneurine deficiency, because it is always associated with a low intake or diminished absorption or utilization of the latter, and it responds to treatment with the vitamin. Experimental support for this view is afforded by the observations of Prickett [285], who observed foci of congestion, hæmorrhage and degeneration in the pons, medulla and cerebellum of aneurine deficient rats, and by Alexander and his co-workers [286], who produced similar lesions in aneurine

WERNICKE'S ENCEPHALOPATHY

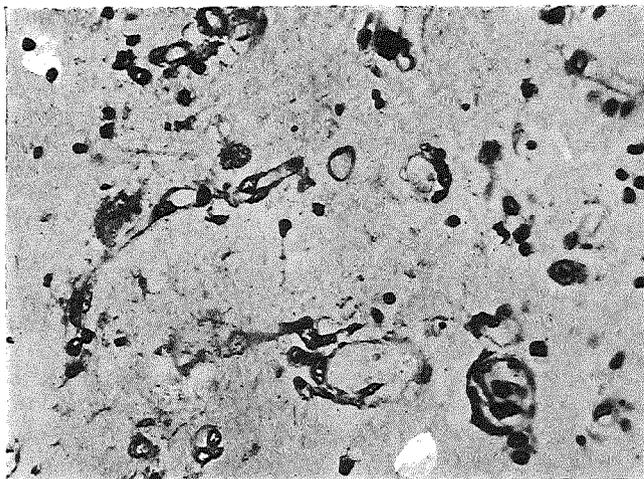


FIG. 75. Field from lesion in corpus mamillare, showing vascular dilatation and endothelial hyperplasia. Nissl stain. $\times 300$.

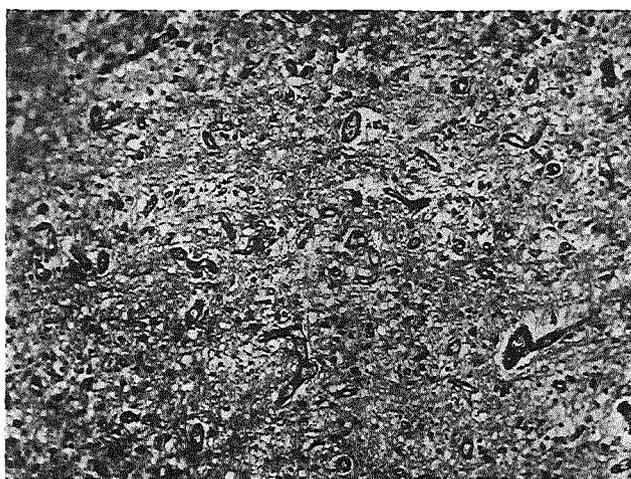


FIG. 76. Subacute case. Field from lesion in midbrain, showing vascular dilatation, thickening of capillary walls and cellular proliferation. $\times 65$.

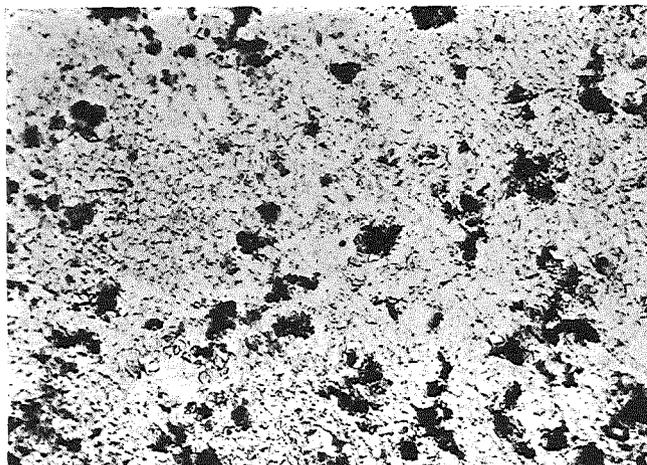


FIG. 77. Field from lesion in corpus mamillare, showing fat (dark granules) in proliferated microglia cells. Scharlach R and hæmatoxylin. $\times 250$.

WERNICKE'S ENCEPHALOPATHY

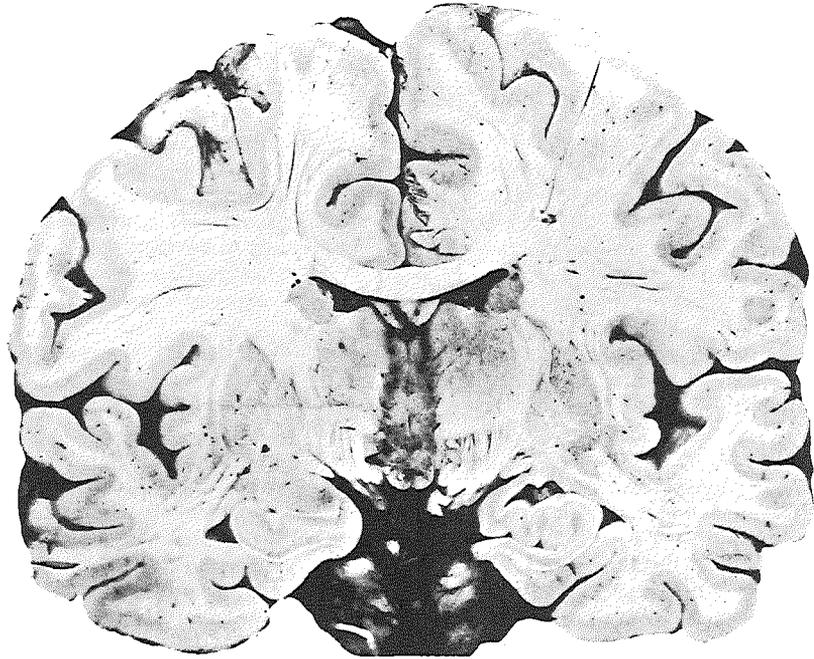


FIG. 78. Brain, coronal section, showing zone of congestion and petechiae around third ventricle and in corpora mamillaria.



FIG. 79. Brain, coronal section, showing similar zonal lesion around posterior end of third ventricle and upper end of aqueduct.

deficient pigeons. Rinehart [180] states that the lesions in the central nervous system of the rhesus monkey kept on an aneurine deficient diet correspond in a general way to Wernicke's encephalopathy, although there are minor differences, such as the relative absence of vascular changes and the severe changes in the corpus striatum (p. 229). The pathology of Chastek paralysis in foxes, produced by feeding them raw fish (p. 228), is similar to that seen in Wernicke's encephalopathy.

The condition responds to treatment with aneurine, although residual manifestations such as nystagmus, psychotic symptoms and mental deterioration may persist. Severe cases may be fatal in spite of treatment. Jolliffe and his co-workers [743] and Campbell and Biggart [284] consider that the condition is not due solely to aneurine deficiency and that nicotinic acid deficiency may play a part (see p. 369). The dramatic improvement often occurring after injecting aneurine suggests that Wernicke's encephalopathy is largely due to deficiency of this vitamin (p. 253).

Psychological Manifestations of Aneurine Deficiency. By maintaining volunteers on diets deficient in aneurine, deficiency symptoms have been produced. Mental symptoms resembling those of neurasthenia are common—intolerance of noise, peculiar sensations in the head, inability to concentrate, inattention to details, memory defects, irritability, "nervousness," anxiety, depression and insomnia [327, 331, 697, 713, 715, 826]. The individual becomes depressed, unco-operative, apprehensive, irritable and quarrelsome and suffers from lack of interest and ambition. In tests made by Brozek, Guetzkow and Keys [282] and by Henderson *et al.* [311] the Minnesota Multiphasic Personality Inventory showed significant changes in the scores on the three psychoneurotic scales—depression, hysteria and hypochondriasis. The Rorschach findings showed loss of spontaneity and an increase in tension. These workers consider that the neurasthenic symptoms of early pellagra (p. 360) are manifestations of aneurine deficiency. Horwitt and his colleagues [283] kept patients on diets containing restricted amounts of aneurine (0.2 to 0.4 mg. daily) for a period of three years. They noted an increased psychotic tendency, decrease in vibration sense and a rise in blood lactate and pyruvate.

Wernicke's encephalopathy is discussed on p. 231.

Cardiac Lesions in Aneurine Deficiency. In the experimental animal aneurine deficiency produces cardiac lesions. In the rat there is marked bradycardia, ectopic beats, widening of the PR interval, notched P waves, changes in the T waves and ST segments, dilatation of the right auricle and post-mortem necrosis of the cardiac muscle fibres with cellular infiltration [292, 294, 307, 896]. In the pig aneurine deficiency causes cardiac dilatation, myocardial necrosis, bradycardia, disturbances of AV conduction, heart block and necrosis of the Purkinje cells [292, 302, 830]. Swank, Porter and Yeomans [303] noted dilatation of the auricle of the aneurine deficient dog.

Cardiac lesions occur in man as a result of aneurine deficiency in the absence of symptoms of frank beriberi. They are often referred to as the "beriberi heart" and, although relatively common in the United States as a result of alcoholism and gross dietary deficiency, the condition is sufficiently rare in Britain to justify the publication of single cases [689-695]. Of 120 cases studied by Weiss and Wilkins [242] in America the commonest causes were chronic alcoholism, poor dietary history, drug addiction and the vomiting of pregnancy. The following signs and symptoms are commonly seen: tachycardia, arrhythmia, dyspnoea on exertion, reduced vital capacity, palpitations, cardiac murmurs, a bounding peripheral pulse, distended veins and sometimes oedema. The skin may be flushed and warm due to peripheral vasodilatation and the peripheral blood flow may be increased. These manifestations have been disputed by Roth, Williams and Sheard [927], whose observations, however, were made on subjects suffering from an induced aneurine deficiency. There is often cardiac enlargement, an increase in

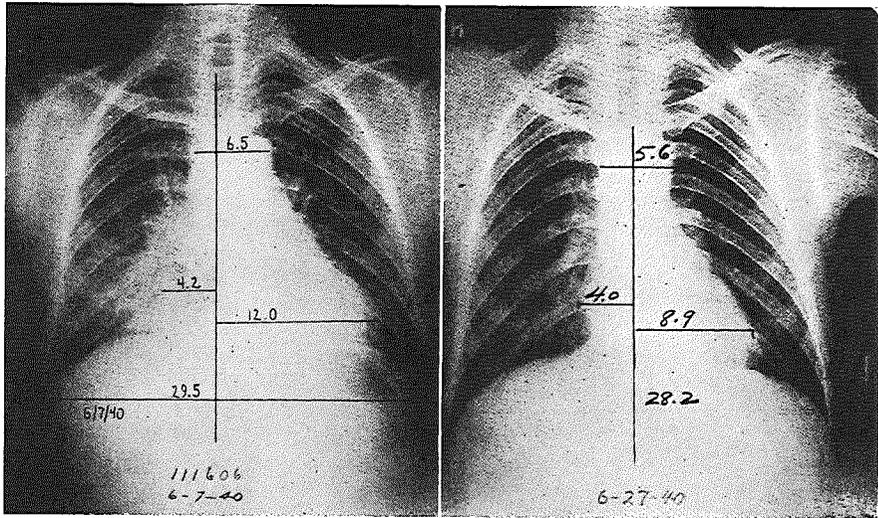


FIG. 80. X-ray of Patient suffering from Aneurine Deficiency, before and twenty days after Treatment. The measurements are in centimetres. The patient was an alcoholic who rarely ate more than one meal daily, and suffered from shortness of breath, tenderness of calves and legs and soles, pitting oedema of legs and an enlarged liver. The heart was enlarged on percussion and a systolic murmur was audible at the apex. The first X-ray shows enlargement of the heart, chiefly to the left. The second X-ray taken after twenty days' treatment with 10 mg. aneurine daily shows return of heart shadow to normal size. The oedema and systolic murmur disappeared. (Drs. Porter and Downs case.)

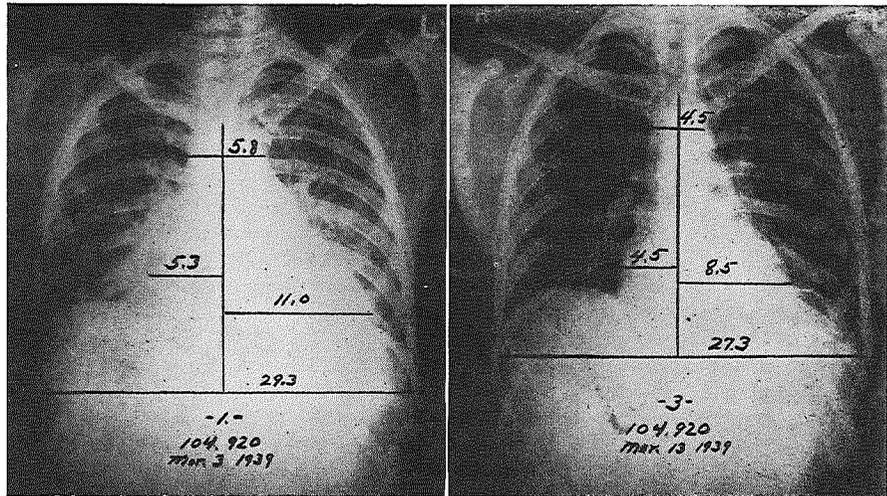


FIG. 81. X-ray of Patient suffering from Aneurine Deficiency, before and after ten days' Treatment. Measurements in centimetres. The patient was an alcoholic taxi-driver living largely on snacks, who had swollen and painful legs, and tingling of the fingers. The condition became worse and the patient had an attack of delirium tremens with tremors, over-activity, hallucinations and disorientation. On examination the heart was enlarged, knee and ankle jerks were absent, muscle weakness and tenderness were present in the arms and legs, which showed pitting oedema. There was a high-pitched blowing systolic murmur, gallop rhythm and enlarged liver. The first X-ray shows enlargement of the heart to right and left. The second X-ray, showing return of the heart to normal size, was taken ten days later, after daily injections of 12 mg. aneurine and yeast orally.

THE VITAMINS IN MEDICINE

E.C.G. IN ANEURINE DEFICIENCY

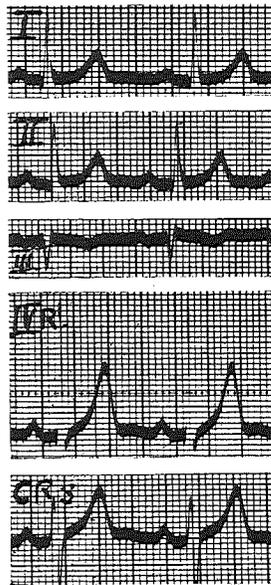
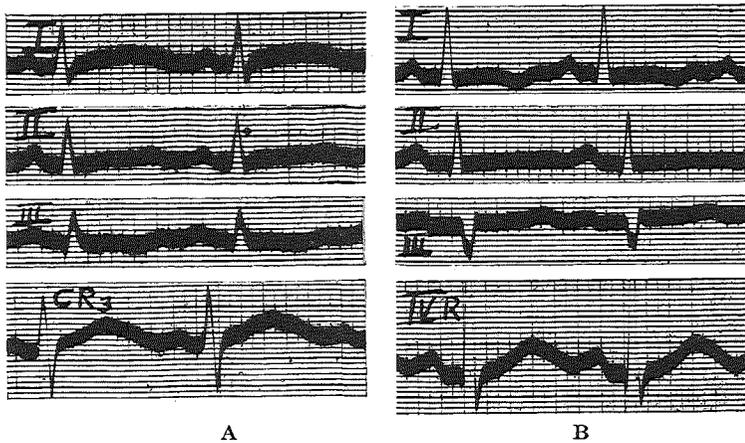


FIG. 82. Electrocardiogram from a Case of Circulatory Failure due to Aneurine Deficiency. The patient was an alcoholic with a typical history—shortness of breath, œdema of extremities, systolic murmur over the apex, enlarged liver and heart. The first E.C.G. shows sinus tachycardia, main deflections upwards in all limb leads, small S_1 and Q_3 , T_1 wide, shallow and upright, T_2 almost absent and T_3 inverted, and QT interval greatly increased (0.49 sec.). The second E.C.G. was taken twenty days after treatment with aneurine (20 mg. daily by mouth and 7 injections of 25 mg.). R_1 has decreased in height, the main deflections are downwards, T_1 is inverted, T_3 is upright, and RT_1 is curved with the convexity upwards. The QT interval is still increased to 0.5 sec. The tracing is similar to those seen in coronary disease. The third E.C.G. taken ten months after the first, shows normal tracings for all leads, and normal duration of electrical systole (QT = 0.42 sec.). Time markings in A and B are 1/25 sec. and 1/25 and 1/5 sec. in C.

E.C.G. IN INDUCED ANEURINE DEFICIENCY

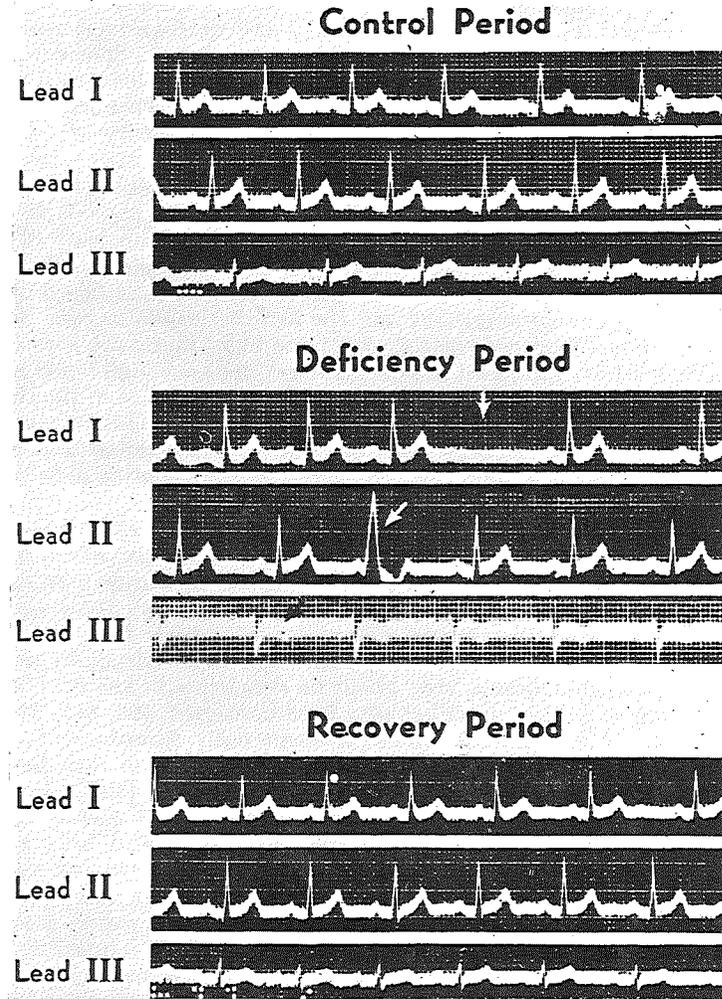


FIG. 83. Electrocardiogram in a Case of induced Aneurine Deficiency. The subject was placed on a diet of 0.47 mg. aneurine daily and an aneurine calorie ratio of 1. Changes in the E.C.G. occurred on the eleventh day after commencing the diet. They include sinus arrhythmia, sinus arrest, change in deviation of electrical axis and inversion of T_3 . After administering adequate aneurine the E.C.G. was restored to normal on the fifth day of treatment.

venous pressure and changes in the electrocardiogram, although these are non-specific. Auriculo-ventricular and intraventricular block have been reported [290]. Repeated attacks of aneurine deficiency may leave a residuum of permanent damage in the heart. The principal changes seen in the electrocardiogram are tachycardia, flattening and inversion of the T waves, changes in the PR interval and increased QT interval, increase in the length of systole and low voltage complexes.

Cardiac lesions due to aneurine deficiency are comparatively rare outside the tropics. They may occur as a result of restricted diets, deficient assimilation or any of the factors operating to produce aneurine deficiency described on p. 223. The recognition of cardiac dysfunction due to aneurine deficiency is based on the following diagnostic criteria: a history of nutritional deficiency, due to deficient intake or "conditioned" (p. 223); tachycardia not due to known causes, such as hyperthyroidism; palpitations; venous congestion; right-sided enlargement of the heart; oedema not in keeping with the cardiac and renal picture and the plasma protein level (the latter may be lowered in general malnutrition); and changes in the electrocardiogram. According to Porter and Downs [831] the cardiac output and oxygen consumption are increased and the arterio-venous oxygen difference is decreased. The blood pyruvate (p. 242) and blood sugar are increased and there is a higher pyruvate level in arterial blood [293]. A high blood pyruvate is of no diagnostic significance [253]. Aneurine is of no value in the treatment of cardiac conditions not due to aneurine deficiency. This has been convincingly shown by Weiss [629] and by Sutton, Friedemann and Simpson [253]. Heart disease of nutritional origin, not due to aneurine deficiency, has been described [686].

Aneurine Deficiency and Gastro-intestinal Symptoms. One of the earliest manifestations of aneurine deficiency in animals is anorexia, which is rapidly relieved by administering the vitamin. Other gastro-intestinal manifestations that have been observed in aneurine deficient animals are vomiting, loss of intestinal tonus [539] and impaired absorption of glucose from the intestine [20]. Radiological studies have shown that gastro-intestinal hypomotility, dilatation and stasis follow a pure aneurine deficiency in the rat [736, 737]. The production of peptic ulcers has also been described [306, 312, 313].

In man, as in the animal, anorexia is an early symptom of aneurine deficiency. Experiments on induced aneurine deficiency in man have often had to be terminated because of this. Constipation [327], hypo- and a-chlorhydria [308, 377], impaired gastric secretion, slow gastric emptying time and sluggish intestinal motility [304, 327] have also been reported, although Maxwell and his co-workers [879] failed to observe any significant radiological abnormalities in patients with low blood aneurine levels. Abdominal discomfort, heartburn and constipation occurred in a volunteer on a diet deficient in the vitamin B complex [331, 713]. In infantile beriberi gastro-intestinal symptoms are common; they include anorexia, regurgitation, abdominal distension, tenderness, vomiting and constipation.

Aneurine is often prescribed for the treatment of gastro-intestinal symptoms such as anorexia and constipation, and is a common constituent of proprietary "tonics." There is no therapeutic justification for this unless the patient is known to be suffering from aneurine deficiency. In the rhesus monkey aneurine has no laxative effect [833].

Leithauser [589] describes nine cases of abdominal distension severe enough to involve laparotomy in four owing to a mistaken diagnosis of appendicitis or carcinoma. The patients were alcoholics on restricted diets. Four were treated with aneurine with apparent relief of distension.

Experimental Aneurine Deficiency in Man. Many studies have been made, mainly in America, on the effects of a partial deficiency of aneurine in human subjects [92, 93, 282, 283, 311, 315, 327, 328, 331, 696, 697, 713, 715, 760, 793, 826, 890, 945]. The studies were carried on for varying periods, ranging from

a few weeks to three years (Horwitt *et al.* [283]); the daily aneurine intake varied from 0.15 to 0.5 mg. The deficiency symptoms varied widely, depending on the level of aneurine intake and the duration of the test. The outstanding symptoms are anorexia, fatigue and polyneuropathy. The following manifestations have been recorded.

General Symptoms. Fatigue, anorexia, loss of weight, dyspnoea on slight exertion, photophobia, backache, deterioration in physical fitness, thinning and loss of elasticity of skin, frequency of micturition, a decreased sense of well-being and anæmia. The anæmia is characterized by an increase in the mean corpuscular volume and hæmoglobin, macrocytosis, polychromasia and poikilocytosis [715]. The early stage of deficiency simulates neurasthenia, the later one anorexia nervosa.

Gastro-intestinal. Anorexia, nausea, vomiting, constipation, delayed emptying time of the stomach and bowel, gastric discomfort, hypo- and a-chlorhydria and heartburn. Radiologically the calibre of the jejunal loops is increased.

Cardiovascular and Vasomotor. Hypotension, palpitation, tachycardia, precordial pain precipitated by effort, sinus arrhythmia on exertion, pallor and a feeling of coldness. The heart is not enlarged and according to Williams and his co-workers [327] there is no œdema. This is recorded by Elsom and his collaborators [331, 713], although their subjects were suffering from a vitamin B complex and not a pure aneurine deficiency. Electrocardiographic changes that have been recorded include increase in length of systole, low voltage, shortening of the PR interval, increase in the QT interval, flattening of the T waves, depression of the ST segments, sinus arrhythmia and tachycardia [327, 328, 696]. The electrocardiographic changes are reversed by administering 10 mg. aneurine daily for a few weeks. There is no vasomotor disturbance as evidenced by skin temperature measurements and determination of rates of cooling and warming of body tissue [514]; this is in contrast to the moist, warm skin of beriberi (p. 219).

Respiratory. Dyspnoea on effort, reduction in respiratory efficiency.

Mental Changes. Listlessness, apathy, dulling of interest and ambition, inability to concentrate, confusion of thought and memory, loss of memory, depression, paranoid tendencies, irritability, quarrelsomeness, inattention to details, headache, tenseness, insomnia, impairment of foresight and judgment, hysteria and hypochondriasis. The results of applying personality tests are described on p. 234.

Nervous System. Depressed tendon reflexes, increased sensitivity to painful stimuli, vague paresthesiæ, polyneuropathy, muscle cramps, tenderness of calf muscles, diminished vibration sense and diminution of electrical irritability of peripheral nerves.

These experiments on human volunteers do show that aneurine can directly or indirectly lead to disturbances of function of the peripheral nerves of the lower limbs. If the deficiency is sufficiently prolonged it leads to a lower motor neurone type of lesion, which judged by the slow response to treatment is morphological and not readily reversible.

Psychomotor and Performance Tests. In subjects kept on a diet deficient in aneurine there is diminished work efficiency, as judged by bicycle ergometer tests [317, 320] and increased oxygen consumption [321]; diminished endurance; and deterioration in co-ordination tests involving strength, vision, hearing or speed [315, 318, 697, 836, 890, 930]. In some of the tests made the subjects were placed on diets deficient in the other B vitamins as well, so that in these cases it is difficult to ascribe any of the results obtained to the deficiency of one vitamin. Reaction time is diminished on low aneurine intakes, e.g. 0.20 mg. daily [319].

Laboratory Tests. There is diminished excretion of aneurine, a rise in blood sugar and glycosuria and diminished response to insulin [327]. According to the earlier work of Williams and his colleagues [327] the blood bisulphite binding substances (B.B.S.) are unchanged. Later work by the

same authors [793] and by others [283, 315] showed an increase in blood lactate and pyruvate at rest, during work and after the administration of glucose.

Diagnosis, Treatment and Prevention of Aneurine Deficiency. The symptomatology of early aneurine deficiency resembles that of neurasthenia. Diagnosis depends on a careful dietary history and a complete examination of the nervous and cardiovascular symptoms. The following manifestations are of significance. Tenderness of calf muscles, hyperæsthesia, muscle cramps and weakness, all of which appear before the more serious neurological complications; lost or diminished ankle and knee jerks and vibration sense may be observed. Anorexia, fatigue, dyspnoea on exertion, loss of weight and irritability are early symptoms. Factors predisposing to nutritional deficiency should be looked for (p. 223). A therapeutic test should be performed in suspected cases and, if there is no improvement after a few weeks' treatment, another diagnosis considered, although advanced cases may require many weeks' treatment before any improvement occurs. The value of laboratory tests in the diagnosis of aneurine deficiency is discussed on p. 242.

For the treatment of aneurine deficiency the measures laid down for that of beriberi should be followed (p. 222). The daily administration of 10 mg. of aneurine is adequate, the dose being reduced to 5 mg. as recovery occurs. Since there is likely to be a deficiency of other B vitamins, the diet should be supplemented with sources of the vitamin B complex such as yeast (heat treated), liver by mouth or injection, and the whole grain of cereals. The necessity for giving the whole of the vitamin B complex rather than massive doses of aneurine is stressed by Sydenstricker [767], who has observed that large doses of one vitamin may precipitate symptoms of a deficiency of another, e.g. ariboflavinosis. It has been shown that large doses of aneurine affect the excretion of riboflavine [768]. The rate of regeneration of nerve is about 1 mm. daily, so that in severe cases of aneurine deficiency many months or even a year may be required for complete recovery of some nerves, e.g. the sciatic. In paralysed cases irreversible changes in the nervous system may occur with permanent neurological damage.

For the prevention of aneurine deficiency in areas where it is likely to be endemic the following measures are suggested: whole wheat or other cereal bread should be substituted for white bread and refined cereals. If this is not possible because of the dietary habits and prejudices of the people the "fortification" of bread as practised in America (p. 187) should be considered. It is the height of human folly to extract essential nutrients from natural food, make them synthetically in the laboratory and then add them to the denatured foodstuff. Until the dietary habits of civilized man are reformed such measures may be necessary. The objection to whole-grain bread is one of custom. Infants can be weaned on to it and like it, and they will continue to eat it unless their parents do not; even peptic ulcer patients can tolerate it if it is gradually introduced into their diet.

METHODS USED FOR THE ASSAY OF ANEURINE

Biological Assay [427]. The most widely used biological assay method depends upon the cure of bradycardia in the rat suffering from aneurine deficiency [348-351, 379-381]. The assay is time consuming—it takes about fifty days to deplete the animals and three days to perform the test—and involves the use of a large number of animals. The catatorulin test of Peters [353, 354] depends on the fact that the oxygen consumption by brain tissue of avitaminotic pigeons is low and is stimulated by minute amounts of aneurine, e.g. 0.2 microgram.

Microbiological Assay. An assay method based on the use of the mould *Phycomyces blakesleeanus* and devised by Schöpfer [382] has been used by a number of workers for estimating aneurine in body fluids [356-359, 383, 638, 839, 840, 909, 948]. The method is based on the observation that the extent

of the growth of this mould is proportional within certain limits to the concentration of aneurine in the medium. After inoculating flasks, containing a known amount of aneurine and the test substance respectively, with spores of the mould, the flasks are set aside in the dark at 20° C. for four to ten days, when the mycelia of the mould are removed, washed and dried. The weights of the mycelia are approximately proportional to the amount of aneurine present.

A yeast fermentation method, based on the observation that aneurine increases the rate of production of carbon dioxide by yeast, is widely used [332, 362-365, 526, 852, 903]. By the use of a Warburg apparatus aneurine can be assayed on a micro-scale of 0.005 to 0.025 micrograms. Next to the thiochrome method, this is probably the most widely used and is comparable in accuracy.

Assay methods involving the growth of bacteria, such as *Staphylococcus aureus* [361], *Streptococcus salivarius* [853] and *Lactobacillus fermentum* [604, 605, 946] have also been used. These organisms are very sensitive to the presence of minute amounts of aneurine.

Thiochrome Method. Aneurine is oxidized quantitatively in alkaline solution to thiochrome (p. 185), which fluoresces under ultra-violet light and can be estimated fluorimetrically by comparison with a standard solution. This method of estimating aneurine was devised by Jansen [385] and adapted by a number of workers [386-402, 455-459, 599, 703, 841-850, 893, 949, 952]. It can be used for estimating free and combined aneurine in blood, urine and other body fluids and in foodstuffs. A micromethod capable of detecting 0.05 microgram of aneurine with an accuracy of \pm three per cent. has been devised [849]. Aspirin, salicylates, quinine and related drugs interfere with the thiochrome fluorescence [457, 843, 893].

The following steps are carried out in estimating aneurine by the thiochrome method: (a) the aneurine and cocarboxylase in the material under examination are extracted with dilute acid; (b) any cocarboxylase present is digested by clarase or other preparation rich in phosphatase; (c) selective adsorption of the aneurine from (a) and (b) by "decalso" or similar adsorbing agent; (d) elution of the thiamine; (e) oxidation of the thiamine to thiochrome by alkaline potassium ferricyanide; (f) extraction of the thiochrome with isobutyl alcohol; (g) measurement of the fluorescence produced by irradiating the thiochrome solution with ultra-violet light and comparison with a standard thiochrome solution.

Colorimetric Methods. When aneurine reacts in alkaline solution with a diazotized aromatic amine a coloured pigment is formed. Prebluda and McCollum [366, 367] have used this as a method of assaying aneurine. The method has been developed by Melnick and Field [368, 369] and others [413, 848, 851, 884]. The method is quite satisfactory if there is a relatively high concentration of aneurine in the substance examined, e.g. urine.

Kinnersley and Peters [371] have developed a test depending upon the formation of an azo dye between aneurine, formaldehyde and diazotized sulphanilic acid.

Estimation of Cocarboxylase. As much of the aneurine present in biological media is combined as the pyrophosphate or cocarboxylase, the latter must be hydrolysed before estimation. This can be done with yeast extract [407], diastase [458] or kidney extract [408], although a phosphatase preparation known as clarase is usually used. The normal blood cocarboxylase in an adult varies from 4.5 to 12 micrograms per 100 ml., with an average of 7 micrograms [377, 417]; it is lower in children [86].

LABORATORY METHODS PROPOSED FOR THE DETECTION OF ANEURINE DEFICIENCY

These depend upon the estimation of aneurine or bisulphite binding substances in the blood, urine or muscle, or upon so-called "loading" or "saturation" tests.

Estimation of Pyruvic Acid and Bisulphite Binding Substances. According to some workers there is an increase in the blood and urine pyruvate and bisulphite binding substances in subjects suffering from beriberi and aneurine deficiency (p. 240). It has therefore been used as an index of aneurine nutrition [84, 87, 154, 314, 322, 708]. A micro-method for its estimation has been devised [333]. The normal range of blood pyruvate is 0.3 to 1.28 mg. per 100 ml. [719, 322, 342, 344, 580]. The upper limit of normal is given as 1.3 mg. by Wortis and his co-workers [719].

An accumulation of pyruvic acid in the blood may be caused by retardation in its breakdown to carbon dioxide and water or by failure of resynthesis to glucose. The factors influencing the level of blood pyruvate are so numerous that a rise in the latter, without any other evidence, cannot be considered diagnostic of aneurine deficiency. Allibone [13], for example, has shown that there is a raised blood pyruvate in toxæmic and hæmolytic states, and others have observed it in patients with pyrexia [86, 322, 324], heart disease [343], hepatic cirrhosis [628] and a raised basal metabolic rate [719]. Allibone [13] and Joiner *et al.* [323] have suggested that in any case in which the resting blood pyruvic acid is raised, the response of the level to the administration of aneurine might serve as a therapeutic test for aneurine deficiency. Normal blood pyruvate values have been found in chronic beriberi [84], and subclinical symptoms of aneurine deficiency have been produced in volunteers without any change in the blood pyruvate [899]. Laing and his co-workers [577] found no correlation between the blood pyruvate and aneurine intake of a hundred unselected hospital patients; no correlation has been observed between blood aneurine and blood pyruvate levels [342].

While a high blood pyruvate *per se* cannot be considered diagnostic of aneurine deficiency, Williams and his co-workers [793] claim that a high blood pyruvate after the intravenous administration of glucose (0.4 gm. per kilogram of body weight) is of some diagnostic value. The highest level is obtained thirty minutes after injection, and this is considered the most significant reading. If the glucose is given orally (50 gm. in 250 ml., repeated in 30 minutes) specimens of blood are taken 30, 60 and 90 minutes later; maximal values are obtained after 60 to 90 minutes. It is claimed that in subjects receiving only 0.1 mg. of aneurine per 1,000 calories the blood pyruvate rises from 1.2 to 1.5 mg. per cent. to 2.3 to 2.6 mg. A return to normal after administering aneurine is confirmatory. It is also stated that in aneurine deficiency there is a delay in the return of blood pyruvate to normal after exercise. Taylor and McHenry [662] estimate the level of blood pyruvate three hours after ingestion of 100 grams of glucose.

Keys and his co-workers [93] reported a change in the lactate-pyruvate ratio in the blood of subjects on a restricted intake of the B vitamins. Goldsmith [343] studied this ratio in a number of normal subjects; under basal conditions it is 9 to 9.3 [580] and is unchanged following the administration of glucose, but increased after exercise. The ratio is lower than normal in patients with aneurine, riboflavine and nicotinic acid deficiency and in heart disease. It returns to normal on administering aneurine. According to Goldsmith a lactate-pyruvate ration of less than 7 is suggestive of aneurine deficiency. The test, however, is clearly not specific.

Horwitt and Kreisler [352] have elaborated a method for detecting subclinical aneurine deficiency, based on estimations of blood glucose, lactate and pyruvate following the administration of glucose and mild exercise. The subject under test is given by mouth 9 ml. of twenty per cent. glucose per kilogram of body weight after withdrawing a fasting basal blood sample. Sixty minutes later a mild exercise test is performed—walking up and down a flight of twenty-one steps 19 cm. high twice in sixty seconds—and five minutes later a further blood sample taken. From the data obtained an *index of carbohydrate metabolism* (CMI) is calculated from the following formula :

$$\begin{aligned} \text{CMI} &= \frac{L - \frac{G}{10} + 15P - \frac{G}{10}}{2} \\ &= \frac{1}{2} \left(L + 15P - \frac{G}{5} \right) \end{aligned}$$

G, L and P are milligrams of glucose, lactic and pyruvic acid respectively in 100 ml. blood. Normal values for the CMI are below 15; figures above this indicate a deficiency of aneurine.

This seems to be the most satisfactory laboratory test for detecting aneurine deficiency so far devised. It was based on observations over a period of four years on normal subjects, patients with aneurine deficiency, and subjects in whom aneurine deficiency had been artificially induced over a long period. It must be borne in mind that aneurine deficiency is not the only cause of elevated blood pyruvate and lactate levels. Cardiovascular and liver disease, infections, thyrotoxicosis, excitement, arsenic and phosphorus poisoning, pernicious vomiting of pregnancy [668] and eclampsia may all cause an increase.

Estimation of Blood Aneurine. Estimation of the blood aneurine has been used as a means of detecting aneurine deficiency. Levels of from 2 to 16 micrograms per 100 ml. of blood have been reported [356-359, 375, 377, 393, 403, 417, 839, 855-859], and various workers have assumed that levels of less than 3 to 7 micrograms of aneurine per 100 ml. of blood are indicative of aneurine deficiency [357, 375, 377, 417, 859]. Sinclair [355] considers values below 4.5 micrograms abnormal, and below 2 micrograms as abnormally low. Blood levels, however, reflect only the immediate past intake, and the wide range of values reported in the literature shows how little they can be relied on in assessing the nutritional status of the individual. Thus Benson and his co-workers [783] observed a range of 4.8 to 12.3 micrograms in 121 children, and twenty-two with aneurine deficiency had blood values within what was considered to be the normal range.

Estimation of the Urinary Excretion of Aneurine. The estimation of aneurine in the urine has also been used as an index of aneurine intake. The excretion figures, however, vary considerably in apparently adequately nourished subjects. Values from 20 to 1,200 micrograms a day have been reported [155-158, 165, 167, 328, 329, 369, 370, 386-389, 414, 580, 861, 862]. Excretions considered to be indicative of aneurine deficiency have been variously given from 30 to 100 micrograms daily [155-158, 165, 167, 328, 369, 386, 861]. Papageorge and Lewis [73] have utilized the "fasting hour" excretion of aneurine as an index of adequate intake. The "fasting hour" specimen is taken in the hour following completion of a twenty-four-hour period and after an over-night fast. They consider that the critical level is 4 micrograms, values below this suggesting an inadequate aneurine intake. Coryell and her associates [630] found that the mean "fasting hour" excretion was 8.4 micrograms in boys and 7 micrograms in girls. Salcedo and his co-workers [218] in the Philippines, using this excretion test in patients with beriberi, were unable to correlate the severity of the disease with the laboratory data, and Sastri and his co-workers [580] obtained values ranging from 3.4 to 90.4 micrograms on daily intakes of from 0.8 to 1.39 mg.

Subjects suffering from beriberi have been known to have an aneurine excretion within what is considered to be normal limits [218]. Giffit and Hauck [71] found striking variations in the aneurine excretion of individuals on the same intake and with the same level of metabolism. Others have found no evidence of diminished excretion of aneurine in patients suffering from vitamin B deficiency [891].

The use of a test dose has also been advocated in which the excretion is measured after a dose of aneurine is given by mouth or intramuscularly [155, 157, 165, 388, 414, 415, 526, 569, 630, 705, 782, 794, 862, 891]. We know little

of the factors involved in these tests, such as the metabolism of aneurine and its excretion (it is not a threshold substance), and in view of the wide individual variations obtained (from three to fifty-five per cent. of the dose according to one group of workers [580]) the results of such tests must be accepted with caution. Moreover, practically all the workers in this field have based their conclusions on observations that have not been submitted to statistical examination. Casual comparison of group averages has led to unwarrantable conclusions.

These excretion tests have been severely criticized by Mickelsen, Caster and Keys [60], who, in a four-year study, point out that most investigators have carried out their observations over a short period of time only, before, in fact, equilibrium was established between intake and excretion, *which may take as long as six weeks* if the intake is changed. They also state that in many cases the accuracy of the methods used for the determination of aneurine is open to question. Mickelsen and his co-workers placed their subjects on varying aneurine intakes and the same basic diet for periods of twenty-four to thirty-four weeks. The aneurine in the diet was estimated chemically and the total intake adjusted to a known value by supplements of pure aneurine. After a month's rest cross-over tests were done on the different subjects. The daily excretion of aneurine and pyramin (the "pyrimidine" of Pollock, Ellenberg and Dolger [724]) was determined and the results submitted to the analysis of variance. The variations in aneurine excretion appeared in a general way to be linearly related to intake, but the variations from person to person and even in the same person were so great that it was impossible to apply strict analysis of variance to the data. One subject might excrete two to three times as much aneurine as another on the same intake. The fact that a change in aneurine intake from 1 to 2 mg. required six weeks for aneurine excretion to come to equilibrium with the new intake shows the fallacy of employing the test dose, "load" or "saturation" tests planned over a twenty-four- or even forty-eight-hour period for estimating the nutritional status of the individual. If these variations observed by Mickelsen and his co-workers occurred in "normal" subjects under controlled conditions, one would expect an even greater variation among patients examined because of suspected deficiency disease, with their possible nutritional, metabolic and digestive peculiarities.

Pyramin (Pyrimidine) Estimation. Pollock, Ellenberg and Dolger [724] estimated the urinary excretion of pyramin (called by them pyrimidine) which they considered mirrored the body stores of aneurine. Mickelsen, Caster and Keys [60] have shown that measurement of the combined excretion of pyramin and aneurine is a reliable indication of the aneurine intake. The relationship between pyramin excretion and aneurine intake is exponential, with a curve approaching a plateau of about 400 micrograms of pyramin at high aneurine intakes. In the region of normal intakes (1 to 2 mg. daily) the relationship is very close to linear. Mickelsen, Caster and Keys state that pyramin excretion values are not subject to large individual differences such as are seen in urinary aneurine estimations. At low levels of aneurine intake the pyramin excretion is still measurable while that of aneurine becomes zero.

Muscle Biopsy. The aneurine reserves of the body have also been calculated from the aneurine content of human muscle. A small amount of muscle is obtained (5 to 15 mg.) with a sterile needle from the glutæal region, previously anæsthetized with procaine, and the aneurine content estimated [860, 902, 903].

THERAPEUTIC USES OF ANEURINE

Aneurine is often administered in large doses, e.g. 100 mg. Not only is this wasteful, since the maximum amount of aneurine that the body can metabolize is 10 mg. daily [58], but large doses of one B vitamin can precipitate symptoms of deficiency of another [767]. The maximum amount that can be

absorbed by mouth is 5 mg. daily [42]. There is no necessity to give aneurine parenterally unless impaired absorption is known or suspected. It has been given intrathecally [191, 396], but such severe reactions, including meningeal irritation, can occur from this unnecessary procedure that it is not recommended [572, 863].

Neurology. Neuritis. Although a deficiency of aneurine can cause neuritis, it is by no means certain that the relationship between aneurine and neuritis is a direct one. According to Walshe [360] aneurine is only "anti-neuritic" when the carbohydrate intake is high, although why neuritis should result from defective carbohydrate metabolism is unknown. In aneurine deficiency in animals and in beriberi in man the peripheral nerves undergo definite pathological changes (p. 229).

Unfortunately, many clinicians with an imperfect knowledge of the subject treated all forms of neuritis with aneurine, and many of them claimed to obtain beneficial results. The work was largely uncontrolled and often based on observations on small numbers of patients, most of whom were also treated by rest in bed, analgesics and often physiotherapy. Criteria of improvement were not laid down. Success of treatment was often measured by the subjective reports of the patient, by relief from pain and tenderness and by ability to walk or use the limbs. From 1936 onwards a spate of enthusiastic reports appeared, but further investigations during the last ten years have failed to substantiate these claims. Walshe [372], writing in 1945, stated: "Though I have sought it for over twenty years I have yet to see the case of polyneuritis, acute or chronic, that gave a clear and striking response to the administration of the vitamin B complex or thiamin, in whatever dosage and by whatever channel." Spillane [146] treated over 200 cases of polyneuritis with aneurine for four years during the 1939-45 war, the patients being malnourished natives, prisoners of war or internees. Those treated with aneurine were in bed and hospitalized just as long as those who received only rest and good food, and return of muscular power and reflex activity were always delayed. In spite of the bulk of literature testifying to the value of aneurine in the treatment of peripheral neuritis, a large amount of unpublished evidence has accumulated in the last ten years suggesting that it is of little value. As Spillane [140] points out, the acceptance of liver and insulin therapy was not long delayed, and if aneurine were of value in the treatment of polyneuritis, irrespective of its origin, we would not be ten to fifteen years debating the problem.

Shortly after aneurine became available commercially in 1935 Vorhaus, Willams and Waterman [271] studied the effect of administering 10 mg. of aneurine daily to 100 patients with neuritis of varying aetiology—41 with "metabolic neuritis," 20 with neuritis of infectious origin, 22 with localized neuritis, 11 associated with pernicious anæmia and 3 with pregnancy. The patients were observed for periods varying from three to fourteen months; 48 improved, 44 were symptom free and 8 failed to benefit. Russell [434] and Sciclounoff and Broccard [435] treated a number of mixed cases of neuritis with apparent benefit. Russell used doses of 0.8 to 1.6 mg., which would be present in a normal diet. Vorhaus [436] treated 520 mixed cases of polyneuritis with 3 to 10 mg. aneurine daily for an average period of nine weeks and concluded that treatment produced remission of symptoms in sixty-one per cent. and partial improvement in thirty-six per cent. Fifty per cent., however, had recurrences at the end of a year, and ninety per cent. at the end of three years. Re-administration of aneurine was effective in recurrent cases. The weak link in these reports is the absence of controlled observations and failure to consider the natural history of the disease, which often responds to rest and home treatment.

Kalaja [755] and Göth [864] treated a number of cases of neuritis of varying aetiology with aneurine and concluded that relief followed only in those cases associated with a deficiency of aneurine.

Infective Neuritis. Herpes Zoster. Unsupported claims have been made that aneurine relieves the pain of herpes zoster [338, 440, 633, 746]. One observer [633] naively observed that aneurine was only effective when used with other remedies, and another [439] only obtained relief in two cases out of sixteen.

Poliomyelitis. It has been claimed that aneurine deficiency is a predisposing cause of poliomyelitis [744]; however, the excretion of aneurine in children suffering from the disease is within normal limits [867]. In the rat a deficiency of aneurine *increases* the resistance of the animal to the Lansing strain of poliomyelitis virus [904]. Several workers have claimed that the administration of aneurine has a beneficial effect on the clinical course of poliomyelitis [444, 445, 745, 865]. Only small groups of patients were treated and no account was taken of the variability of the course of this disease. It is well known that some cases recover with virtually no paralysis, others remain badly paralysed, and in bulbar cases a fatal outcome is common. The assessment of any remedy in the treatment of this disease is not easy.

Diphtheritic Paralysis and Polyneuritis. It has been claimed that the duration of paralysis occurring in diphtheria patients can be shortened by the administration of aneurine [447-451]. Dieckhoff [453], Donovan and Bannister [707], Bøe [219] and Wassman [373], in controlled observations, found no evidence that aneurine had a prophylactic or therapeutic effect on diphtheritic paralysis or on the cardiovascular complications of diphtheria. Reinhard and Schwartz [454] recorded low excretions of aneurine in diphtheria patients (which may have been the result of the pyrexia), but they failed to observe any appreciable improvement in patients receiving the vitamin. Experiments on guinea-pigs injected with diphtheria toxin have shown that aneurine has no prophylactic or curative effect on post-diphtheritic paralysis [373, 453]. Remarkable improvement from day to day may be observed in patients with post-diphtheritic neuritis in the absence of any treatment whatever, and rapid improvement may occur both early and late in the course of the disease.

Neuritis in Leprosy and Tuberculosis. Leprosy is frequently complicated by neurological lesions such as localized anæsthetic patches, neuritic tenderness and swelling of peripheral nerves. It is stated that pain, tenderness and swelling of affected nerves are diminished by administration of aneurine [508-511]. According to Aring and Spies [433] neuritis in tuberculous patients is relieved by injections of 50 mg. of aneurine.

Nutritional or Metabolic Neuritis. A number of cases of neuritis appear to be directly related to defective nutrition and may be termed nutritional or metabolic neuritis. The better-known conditions of this type in which neuritis is encountered are pernicious anæmia, alcoholism, pregnancy, diabetes, pellagra, sprue, nutritional neuropathy, malignant disease and gastrogenous neuritis.

Pernicious Anæmia. Russell [434] and Aring and Spies [433] state that the neuritic symptoms that occur in pernicious anæmia are relieved by aneurine. The latter used doses of 50 mg. twice daily. Defective absorption of aneurine may occur in pernicious anæmia patients owing to achlorhydria. There is no evidence that aneurine has any effect on the cord symptoms of pernicious anæmia [868], which are both prevented and relieved by vitamin B₁₂ (p. 159).

Pellagra and Nutritional Neuropathy. Spies and his associates [374, 376, 461] reported relief of the symptoms of polyneuritis in pellagrins with aneurine, although they frequently observed deterioration of the mental state in the absence of other therapy. Lewy and his colleagues [378] observed that aneurine relieves the paræsthesiæ, numbness, pain in the calves, weakness and ataxia in pellagrins, while nicotinic acid had no such effect. They also made the interesting observation that large doses of aneurine precipitate the signs of riboflavine deficiency in pellagrins. It is generally considered that

aneurine alone affords little relief in nutritional neuropathy, such as seen during the last war in malnourished prisoners of war, natives and internees [146, 384, 404, 419, 420]. This condition is undoubtedly a multiple deficiency disease.

Alcoholic Neuritis. Until comparatively recently alcoholic neuritis was considered to result from the direct toxic effect of alcohol. In 1933 Wechsler [403] treated three cases of alcoholic neuritis by dietetic means and considered that the neuritis was due to a deficiency of vitamin B₁ or B₂. At the same time Blankenhorn and Spies [405] observed that treatment of fifty alcoholics with neuritis by means of high-calorie, high-vitamin diets supplemented with yeast or liver extract led to the disappearance of the neuritis. They also noted that there was no recurrence of the neuritis if alcoholics were allowed as much alcohol as they could consume, provided they received an adequate diet with yeast or liver injections. Similar observations were made by Strauss [462]. The chronic alcoholic drinks liquor instead of eating, and the gastritis and anorexia produced restrict still further the intake of food, while there is diminished absorption of what food is consumed. Jolliffe, Colbert and Joffe [266] calculated the aneurine requirements of forty-two alcoholics and claimed that there was a correlation between the degree of aneurine deficiency and the presence of polyneuritis, and that improvement varied with the intake of aneurine administered for treatment. In 1938 Goodhart and Jolliffe [268] compared a group of alcoholics given treatment with intravenous injections of 10 mg. of aneurine daily with another control group receiving what was considered to be an adequate diet. The aneurine group recovered more rapidly and completely than the control group. Other workers at this time recorded similar successes treating alcoholics with aneurine [463-468]. The conclusions of these workers were criticized by Meiklejohn [698], who pointed out that even if the diets of alcoholics are lacking in aneurine it does not prove a causal relationship between aneurine deficiency and polyneuritis. A diet lacking in aneurine is also likely to be lacking in other factors, and the administration of aneurine may result in an increased food intake, and hence these other factors.

Subsequent experience has not confirmed these earlier enthusiastic reports, which were generally based on uncontrolled observations on small groups. Brown [562] studied 236 alcoholics over a period of eighteen years at the Boston City Hospital. The average time spent by 118 of the patients on orthodox diet and treatment was compared with the same number given aneurine, liver and the vitamin B complex. The vitamin treated group were not discharged from hospital any sooner than the control group, ability to walk and relief from neuritis being used as the criterion of cure. Aring [868] also regards the treatment of alcoholic neuritis with aneurine as disappointing. He states that the aching, boring, stabbing pain is relieved but progress does not go beyond this. Walshe [372] also states: "I have never seen the severity or duration of a case of alcoholic polyneuritis mitigated by them (i.e. *aneurine or the vitamin B complex*)."

According to Jolliffe [406] aneurine is necessary for the metabolism of alcohol, and an alcoholic therefore has an increased need for aneurine. It has been postulated that the acetaldehyde formed by the metabolic oxidation of alcohol condenses with pyruvic acid with cocarboxylase as an enzyme, a reaction that can occur *in vivo* [410, 411]. This suggests the necessity of aneurine for the metabolism of alcohol. Lowry and his colleagues [412], however, found that the polyneuropathy in aneurine deficient rats is delayed by alcohol, and Westerfeld and Doisy [416] showed that the isocaloric substitution of alcohol for dietary fat or carbohydrate delays the onset of acute aneurine deficiency in pigeons. If these observations are applicable to man—there is of course no evidence that they are—they suggest that the consumption of alcohol may actually reduce the requirements of aneurine.

Pregnancy Neuritis. Neuritis may occur in pregnancy. According to

Vilter, Morgan and Spies [270] this is a manifestation of beriberi in pregnancy and does not develop if the diet is adequate in aneurine. The condition, which frequently follows hyperemesis gravidarum, is characterized by generalized weakness and malaise, followed by tingling, burning of the feet and other paræsthesiæ. The neuritis affects mainly the legs, is of a stocking distribution and is mainly symmetrical. There is tenderness of the calf muscles, hyperæsthesia on stroking the skin, hyperactive reflexes early in the condition and diminished or absent reflexes later. Foot drop may develop later and atrophy of the muscles and skin and vasomotor disturbances, such as sweating and cyanotic mottling, may occur. Degeneration of the myelin sheath, swelling of the cells, loss of Nissl substance and eccentricity of the cell nuclei have been described in the peripheral nerves [270, 869]. Undoubtedly pregnancy and lactation may predispose to nutritional deficiency in poorly nourished women. Such cases are rarely seen in this country but are more common in the East and in certain parts of America [565]. Low blood and urine levels of aneurine have been recorded in pregnancy [614, 566]; this may not be a sign of aneurine deficiency, but of more efficient utilization. The cause of the neuritis in pregnancy is obscure—it frequently clears up spontaneously and after parturition—and, in spite of the claims of several observers that it is relieved by the administration of aneurine [264, 265, 501, 558, 568, 614, 870] it is not certain that lack of this vitamin is the cause. Neuweiler [421] obtained variable results. Other diagnoses have been suggested, such as brachial neuritis, lumbodorsal root pains, acroparæsthesiæ and first dorsal rib pressure syndromes [265, 501]. True generalized severe polyneuritis is rare in pregnancy [869]. It occurs between the twelfth and twenty-fourth week of pregnancy and is preceded by very severe intractable vomiting and by emaciation. This may result from a condition of nutritional deficiency, but the factors responsible are unknown.

Gastrogenous Neuritis. Gastrogenous neuritis, associated with gastro-intestinal disease, has been described (pp. 225, 230). Ungley [260], Scott [261], Laurent and Sinclair [422] and others record cases of neuritis following gastric ulcer, pyloric stenosis, cardiospasm, dysphagia and neoplasms of the gastro-intestinal tract. Undoubtedly chronic lesions of the gastro-intestinal tract interfere with the digestion and absorption of food. Polyneuritis associated with dysentery and typhoid is described in text-books of medicine, but whether this is due to defective absorption of food or to a direct action of a toxin on the nerves is not known. The apparent relief of the symptoms of gastrogenous neuritis with aneurine is not conclusive, although it is rational to administer vitamins, parenterally in bad cases, to patients with chronic gastro-intestinal conditions and to patients on restricted diets. Spillane [146] points out that bedridden patients, whether suffering from nutritional deficiency or not, may develop neuritis and palsies of the limbs from pressure.

Pressure Neuritis. It has been claimed that pressure neuritis due to neoplastic growths has been relieved by the administration of aneurine [191, 479]. The evidence, however, is not very convincing.

Diabetic Neuritis. That neuritis may occur in diabetics is well known. Jolliffe and his colleagues [703] have estimated that it occurs in two per cent. of diabetics and claim that the administration of 10 mg. of aneurine a day relieves the condition in eighty per cent. of cases. Others have reported that aneurine has a beneficial effect [435, 436, 446]. Needles [871] and Rundles [423], however, were unable to observe any benefit, either from administering aneurine alone or with other B vitamins. There is no evidence that diabetic neuritis is associated with aneurine deficiency.

Toxic Neuritis. Neuritis can be caused by chemical toxins, but there is no conclusive evidence that it is the result of aneurine deficiency. Sexton and Gowdey [424] have pointed out the close similarity between arsenical encephalopathy and aneurine deficiency :

Arsenical Encephalopathy

1. Muscular weakness, particularly extremities; loss of muscular control, pain.
2. Paræsthesiæ in feet and hands.
3. Numbness, sensory disturbances.
4. Absence of patellar and Achilles reflexes.
5. Constipation.
6. Staggering gait.
7. Regional anæsthesia and areas of hyperalgesia.
8. Upward spread of paralysis.
9. Mental depression, psychosis, confusion, disorientation.
10. Dilatation of capillaries, hæmorrhages into the cord and cerebral cortex, cerebral œdema and petechial hæmorrhages.
11. Destruction of ganglion cells and anterior horn lymphocytes.
12. Focal vascular lesions which permit escape of blood into perivascular tissues.
13. Zones of predilection: corpus callosum, optic thalami, caudate nuclei, external capsule and brain stem.

Aneurine Deficiency

1. Muscular weakness, vague pains, lack of energy.
2. Paræsthesiæ in feet and hands.
3. Numbness in lower extremities, loss of vibration sense, muscle tenderness.
4. Absence of patellar and Achilles reflexes.
5. Constipation.
6. Staggering gait.
7. Areas of cutaneous anæsthesia and areas of hyperalgesia.
8. Gradual paralysis, spastic ataxia.
9. Psychosis, disorientation.
10. Hæmorrhagic areas in the brain, capillary dilatation.
11. Disintegration of ganglion cells.
12. Vascular degeneration of the cells of Schwann.
13. Lesions occur in periventricular grey matter around third ventricle, mamillary bodies, periaqueductal region, corpora quadrigemina.

Sexton and Gowdey found that oxophenarsine, an arsenical used in the treatment of syphilis, causes a significant derangement in carbohydrate metabolism, as evidenced by a fall in the lactate-pyruvate ratio (p. 242) and a rise in the blood pyruvic acid. The raised pyruvic acid suggests that carbohydrate metabolism is arrested at the pyruvic acid level, at which aneurine acts. They suggest that BAL (dimercaprol) and aneurine should be used in the treatment of arsenical encephalopathy. Hughes [425] also stresses the resemblance to chronic arsenical poisoning, and he also suggests that lead and thallium act as enzyme poisons and produce their toxic effects through a "biochemical lesion" in the metabolism of carbohydrate. It is known that some arsenicals, e.g. Lewisite, interfere with carbohydrate metabolism by attacking the sulphur groupings of the protein to which aneurine is linked as part of an enzyme system [426].

Claims have been made that aneurine relieves the neuritis that may occur in poisoning with such agents as tobacco [492, 493], arsenic [491, 492], lead [483, 484], mercury [494], thallium [485, 486], carbon disulphide [487], and sulphonamides such as sulphacetamide [490]. Most of the authors quoted mention one or two cases only without controlled observations. Aneurine has been found to be ineffective in the treatment of polyneuritis due to orthotricresyl phosphate poisoning ("Jamaica ginger paralysis") [433, 428].

Localized Neuritis. It has been stated that localized neuritis—such as sciatica, sacro-iliac, intercostal, ulnar, crural and shoulder girdle neuritis—is relieved by the administration of aneurine [440, 496–498, 502–505]. These claims have never been confirmed and were not controlled in any way. It is well known that such conditions often improve without any specific treatment.

Affections of the Cranial Nerves. Relief, in some cases described as

dramatic [524], has been stated to occur when patients suffering from trigeminal neuralgia are treated with aneurine [523, 524, 528]. Borsook, Kremers and Wiggins [528], who treated fifty-eight patients, state that it may be necessary to inject 10 mg. of aneurine daily for as long as six months to obtain maximum benefit. They claimed "marked improvement" in sixty-four per cent. of the cases treated. Rose and Jacobson [706], however, concluded that aneurine had no beneficial effect. As in many nervous conditions, the pain of trigeminal neuralgia shows so many spontaneous remissions that very carefully controlled observations are necessary in evaluating a new treatment.

According to Selfridge [530] deafness resulting from lesions of the eighth nerve is improved by injections of aneurine and nicotinic acid. Seven cases were treated by Veasey [531], who found that the results varied but was inclined to think that aneurine was of some value. Aneurine has also been used in the treatment of neuritis of the eighth cranial nerve [573] and tinnitus [658]. Childrey [681] used aneurine for the treatment of non-specific deafness, but found that very few cases received any benefit, which he confirmed by audiometer tests. He also treated several cases of tinnitus with aneurine and obtained relief in only one case. Using audiometer tests, Shambaugh and Jennes [875] were also unable to confirm the value of aneurine in the treatment of nerve deafness and tinnitus aurium. They state that many of the claims are based on the subjective evidence of patients and are insufficiently controlled. The evaluation of therapy for the improvement of hearing must be based on repeated audiometric tests, and to be significant there must be a sustained improvement of at least 10 decibels above the pre-treatment level.

Harris and Moore [544] state that daily doses of 20 mg. of aneurine and 250 mg. nicotinic acid produced remarkable improvement in patients with Ménière's syndrome. Of twenty cases, seventeen became entirely free from vertigo and the remaining three showed considerable improvement. Treatment was carried out for two to three months before maximum benefit was obtained. Atkinson [329] also described the treatment of nineteen cases of Ménière's syndrome by similar means.

Claims have also been made that injections of aneurine are of benefit in the treatment of facial paralysis [529] and optic neuritis [532, 533]. They have not been confirmed.

Diseases of the Spinal Cord. *Disseminated Sclerosis.* Moore [535] claimed that in five advanced cases of this disease treated with aneurine and nicotinic acid there was considerable subjective and objective improvement, which was not maintained when treatment was stopped. Stern [513] also reported benefit after the intraspinal injection of large doses of aneurine. Roch and Sciclounoff [440] treated seven patients with aneurine, four of whom were stated to show definite improvement. These investigators, however, were careful to state that "this may perhaps have been due to a natural remission in the disease and to hospitalization rather than to our interventions." Other workers have failed to report any benefit [503, 524, 633]. Masek [536] treated a number of cases of disease of the central nervous system, including disseminated sclerosis, with aneurine but did not observe that it had any beneficial effects.

Tabes. Wintrobe and his co-workers [881] produced spinal-cord lesions in pigs similar to the lesions of tabes dorsalis in man by feeding them diets deficient in the vitamin B complex. This prompted the trial of aneurine in the treatment of tabes. Metildi [537] and Reese and Hodgson [538] claim that the administration of aneurine, liver extract and yeast produces relief of pain, diminished ataxia and an increase in visual fields and sensory power. Aring and Spies [433] also report relief after administering large doses of aneurine (100 mg.) during tabetic crises. Stone [639] treated sixty-three tabetic patients with intraspinal injections of aneurine and also vitamin B

complex preparations, wheat germ oil, arsenic and bismuth. Some patients received fever therapy as well. In seventeen out of twenty-three patients with advanced tabes improvement occurred in gait, bladder symptoms, visual disturbances and lightning pains. The greatest improvement was shown in those receiving fever therapy. Kesert and Grossman [480] also report relief of lightning pains and gastric crises after injecting 50 to 100 mg. of aneurine intraspinally. Lack of controls, reliance on subjective symptoms and the multiplicity of treatments make such work difficult to evaluate. The anti-syphilitic therapy alone may have produced some benefit. Cochems and Kemp [882] treated a large group of tabetics with injections of aneurine for eight months; only nineteen per cent. obtained relief and definite proof of the value of aneurine in the treatment of tabes was lacking. These observations were made before the treatment of neurosyphilis with penicillin.

Pernicious Anæmia (Cord Symptoms). It has been claimed that the neurological complications of pernicious anæmia are relieved by injections of aneurine [716], although Aring [868] was unable to verify this. It is now known that the cord lesions of pernicious anæmia can be prevented by and cured with vitamin B₁₂, which appears to be the true extrinsic factor.

Other Diseases of the Nervous System. Several investigators have reported that aneurine produces some improvement in conditions such as Parkinsonism, progressive muscular atrophy, pseudohypertrophic muscular dystrophy, Friedrich's ataxia, amyotrophic lateral sclerosis and Sydenham's chorea [540, 685]. These reports have not been confirmed [572, 607].

Relief of Pain. Some investigators have reported that aneurine has an analgesic action. Stern [513] states that the intraspinal injection of 10 to 100 mg. of aneurine produces relief of the intractable pain occurring in such conditions as incurable cancer, disseminated sclerosis, thromboangiitis obliterans, tabes, Paget's disease, and osteoporosis of the spine [513]. Aring and Spies [433], however, were unable to confirm this reputed analgesic action in patients suffering from severe pain of nervous origin (carcinoma, cord tumour, trigeminal neuralgia) even when it was given in large doses. Krieg [521] and Ochsner and Smith [522] claim that parenteral or oral administration of aneurine is effective in relieving the pain of varicose ulcers and Sliosberg [515] has reported relief of pain in cases of painful amputation stumps. There is some evidence that aneurine relieves pain in nutritional neuropathy [146], although why it should do so in the above cases is obscure. Pharmacologically it is a vasodilator, but it is not known whether it is so in the doses in which it is used clinically. The vasodilator action of aneurine may explain its action in relieving the pain of migraine, which is reported by Bändler [627] and Palmer [872]. Palmer states that an injection of 60 to 120 mg. of aneurine intramuscularly can terminate an attack of severe migraine unaffected by ergotamine tartrate.

Aneurine has been used in dentistry to relieve the pain of "dry socket" after dental extractions [873, 874].

Summary. In spite of the early enthusiastic reports on the use of aneurine in the treatment of conditions of the nervous system these have not been confirmed. There is very little evidence of its value in the treatment of any neurological condition unless this is definitely associated with a deficiency of aneurine, either of intake or "conditioned" (p. 223).

Neuropsychiatric Disorders. It has been claimed that the simultaneous injection of 2 to 10 mg. of aneurine minimizes the occurrence of convulsions and prolonged coma in insulin shock therapy [548, 556], although Ziskind [740] was unable to confirm this. The observations of this worker are more likely to be correct as aneurine has no effect on the blood sugar level in man (p. 196).

It has been assumed, without any evidence, that since aneurine is essential for the metabolism of carbohydrate and since only the latter is used as a source of energy by the nerve cell, that aneurine administered therapeutically can effect the higher cerebral processes. This has led to its use to

stimulate mental functioning in normal persons and in the mentally deficient. Harrell [431] investigated the effect of 2 mg. of aneurine daily on the ability of children and young adults to perform certain psychological tests (underlining, code learning, reading, division, addition, multiplication, subtraction, arithmetical problems, number span, dart throwing, completion of geometrical designs, remembering word pairs, memorizing, intelligence tests, reaction time). It was claimed that the performance of the aneurine treated group was better than that of a control group, although it is doubtful if the differences were statistically significant. Visual acuity, weight increase and height increase were also stated to be greater in the aneurine treated group.

Using thirty-six pairs of identical twins as test subjects over a period of nine months Robertson and his co-workers [437] failed to find any statistically significant improvement in memory, intelligence tests, reasoning, code substitution and arithmetic. Unlike Herrell they observed no greater increase in weight and height in the aneurine treated group than in the control group.

Lewald and Alexander [578] administered aneurine to eight mental defectives for three months, but observed no improvement. Such a small group is not large enough for statistical evaluation. Rudolf [441] treated ninety certified mental defectives of both sexes, all ages and all grades with 3 mg. of aneurine daily for six months. They were specially selected as cases showing no improvement for at least a year before the commencement of treatment. Of the ninety cases, forty-four or forty-nine per cent. showed improvement as judged by intelligence quotient tests, quality of performance at work or school and general behaviour. No controls were used so that it is quite impossible to predict what would have happened to a similar group without treatment with aneurine.

From the available evidence it cannot be claimed that aneurine has any marked effect on the mental processes of either normal or mentally defective subjects.

Psychotic States Associated with Alcoholism. A number of workers have reported that patients suffering from Korsakoff's psychosis show considerable improvement after treatment with injections of aneurine [440, 471, 472, 579, 934]. Bowman, Goodhart and Jolliffe [579], from studies on fifty-one patients with the condition, claimed that a group treated with injections of 10 to 50 mg. aneurine daily showed a much better recovery rate than a control group treated by diet alone. They failed, however, to confirm this result statistically and were unable to state whether the increased incidence of recovery in the aneurine treated group was due to the form of therapy employed or to chance (i.e. spontaneous remission).

There is still some doubt about the part played by aneurine in the causation of Korsakoff's psychosis, which may be encountered after head injury, subarachnoid hæmorrhage, and other conditions unrelated to dietary deficiency. When associated with alcoholism recovery may occur without specific treatment. According to Jolliffe and Wortis [933] some patients with Wernicke's encephalopathy are left with a residual Korsakoff's psychosis after treatment with aneurine.

Delirium Tremens. The treatment of patients suffering from delirium tremens with aneurine parenterally in doses of 10 to 100 mg. daily has been advocated [474-476]. Caldwell and Hardwick [934] state that large doses of aneurine have lowered the mortality rate in this condition. According to Kiene and his colleagues [476] the condition is not due to the withdrawal of alcohol but to aneurine deficiency in the brain cells. Sciclounoff and Flagge [491] and Mainzer and Krause [593] state that aneurine is effective in cases of threatened delirium but is ineffective in an actual attack. Rosenbaum [631] and his co-workers are of the opinion that vitamin therapy does not influence the course of delirium tremens and Romano [582], surveying the literature since the last century, states that prognosis was as good then as it is now with vitamin therapy.

Wernicke's Encephalopathy. The aetiology of this condition has been discussed previously (p. 231). Most writers consider it to be a result of acute aneurine deficiency and to respond to treatment with large doses of the vitamin parenterally. Although 50 to 100 mg. is recommended the results are not likely to be any better with such high dosage than with doses of 10 mg. daily. Aring [868] considers that it is unusual for the associated mental deterioration to improve after administering aneurine. The effective relief of ophthalmoplegia with aneurine in some reports [743] suggests that a deficiency of the latter is responsible for the ocular palsies, although the disturbances of consciousness and ataxia may be due to other causes. Spillane [146] describes a patient who developed amnesia, confusion, bilateral ptosis and facial paralysis, ophthalmoplegia, and stupor. On recovering from the acute attack he was left with bilateral retrobulbar neuritis, nerve deafness, ataxia and symmetrical peripheral neuritis, none of which responded to treatment with aneurine.

Aneurine in Ophthalmology. There is no evidence that the administration of aneurine has any beneficial effect in any ophthalmological condition unless this is associated with a deficiency of aneurine or the vitamin B complex, e.g. nutritional retrobulbar neuritis. Toxic amblyopia is said by some investigators to improve after treatment with aneurine, but it is more likely that improvement was due to the removal of the offending agent [335, 634-637, 753]. Johnson [635] describes the treatment of five cases of amblyopia, due to the toxic effects of alcohol and tobacco, with 12 mg. of aneurine daily. All the patients were inveterate alcoholics and were possibly suffering from a vitamin B complex deficiency. Carroll [634, 938] allowed his patients to continue with their accustomed indulgence in alcohol and tobacco, and administered yeast, liver and aneurine. Vision was stated to have improved in twenty-one out of twenty-five patients. The results were claimed to be as good as those obtained with twenty-five controls. Some of the patients consumed a litre of spirits and ten to twenty cigars a day. Leinfelder and Stump [752] failed to observe any beneficial effects from giving aneurine to patients suffering from amblyopia due to tryparsamide.

Claims have been made that aneurine is effective in the treatment of optic neuritis, keratitis, dendritica and corneal herpes [532, 533, 637].

Aneurine in Vascular Disease. In spite of statements to the contrary there is no evidence that aneurine is of any value in the treatment of cardiovascular conditions unassociated with aneurine deficiency. Mild forms of aneurine deficiency may go undetected and cardiac signs and symptoms have appeared in volunteers on diets containing 0.36 mg. of aneurine daily (p. 239). In doubtful cases the therapeutic test may be applied. Aneurine is administered in doses of 10 mg. If the condition is due to aneurine deficiency improvement should occur within a few weeks; if it does not another diagnosis should be considered.

Moir and Battle [585] state that aneurine has a beneficial effect in patients with functional left mammary pain and hypertension. Improvement in such cases is most likely due to suggestion. Relief of pain is also claimed in patients with peripheral vascular diseases, such as thromboangiitis obliterans and intermittent claudication [587], although it has no effect on the course of the disease.

Claims have been made that ionization with an ointment containing acetylcholine and aneurine produces relief in frostbite, erythrocyanosis, varicose ulcers, eczema and neurodermatitis [908]. Any improvement in such cases may well be due to improvement in the peripheral blood supply by ionization with acetylcholine alone.

Aneurine in Gastro-intestinal Conditions. Many gastro-intestinal disturbances have been ascribed on clinical and experimental grounds to aneurine deficiency. On the other hand, it is more likely that these disturbances prevent effective absorption and so condition aneurine deficiency. In most

cases the deficiency states have been multiple and not pure aneurine deficiency, and "vitamin B" therapy has often meant the administration of concentrates containing not only aneurine but also other vitamins of the B complex. It is therefore difficult to assign an exact rôle to the effects of aneurine in these studies. Where aneurine deficiency can be incriminated as the cause of anorexia, or is associated with ulcerative colitis, vomiting, diarrhoea, gastro-intestinal diets, hepatic cirrhosis or impaired absorption then the vitamin should be given in adequate amounts, preferably by parenteral injection if absorption by mouth is debatable. There is no evidence that aneurine has any effect on the condition itself [739], although Cheney [598] describes the dramatic response of thirty-two cases of chronic diarrhoea and mucous colitis to treatment with aneurine.

Field, Robinson and Melnick [570] have found that patients receiving intensive alkali therapy for peptic ulcer and those with achlorhydria have subnormal excretions of aneurine. *In vitro* experiments showed that as much as fifty-six per cent. of aneurine is destroyed when incubated with bile or pancreatic juice in the absence of acid gastric juice. Patients suffering from peptic ulcer or achlorhydria may therefore develop an aneurine deficiency unless they take in more of the vitamin than will protect a normal individual.

Many claims have been made that aneurine is of value in the treatment of gastro-intestinal hypotonia. It has certainly been observed that the administration of foodstuffs rich in aneurine is helpful in overcoming atonic constipation, but the effect of the bran and fibre in these foodstuffs cannot be overlooked. The laxative action of yeast is also well known. There are many reports of success in the treatment of constipation by means of pure aneurine or a concentrate, but the difficulty of evaluating these reports is considerable, especially when defæcation with some individuals is almost a conditioned reflex [182].

Careful X-ray studies have been made by Wood, Splatt and Maxwell [642] on the effect of aneurine on gastric secretion and motility in man. They state that in doses of 3 to 10 mg. intramuscularly it has no effect on gastric secretion, although it hastens the emptying time in those persons whose gastric emptying time is habitually much longer than normal. It does not influence the rate of evacuation of the stomach of those whose emptying time is normal or rapid. Controlled studies on the supposed laxative action of aneurine have also been made by Loewe and Knox [643] in the rhesus monkey, the only animal suitable for measuring the effectiveness of cathartic drugs. They found that in doses of 1 to 100 mg. per kilo orally for periods of two to seventeen days it did not increase significantly the laxative effect of phenolphthalein. While a deficiency of aneurine may lead to an atonic condition of the bowels there is little evidence that the vitamin has a laxative effect in adequately nourished persons. It is possible that the clinical material for the observations recorded above was selected from poorly nourished hospital patients.

So-called gastro-intestinal diets are traditionally over-supplied with starches and sugars, and with foods containing insufficient vitamins. Functional disorders of the gastro-intestinal tract are frequently related to insufficient supplies of the vitamin B complex [602]. In such circumstances restriction to certain of the therapeutic diets will have an additive effect and may precipitate deficiency disease. Where anorexia is associated with aneurine deficiency the administration of the vitamin restores appetite [605].

It is believed that an insufficient intake of aneurine may be responsible for some gastro-intestinal symptoms in children and babies. Wilkins [324] records striking improvement in some cases among children after giving small doses of aneurine. Partial aneurine deficiency was also described by Clements [645] in at least eight per cent. of 150 infants breast-fed up to six months. The symptoms attributed to aneurine deficiency were failure to gain weight at the normal rate, constipation and vomiting. The adminis-

tration of aneurine to the child, or to the mother if still nursing, cured these symptoms. It should be noted that in these cases a claim is made for treatment with aneurine only in the presence of a definite deficiency of the vitamin.

In the treatment of gastro-intestinal conditions, e.g. peptic ulcer, by means of special diets, and after operations on the gastro-intestinal tract, supplements of aneurine, the vitamin B complex and ascorbic acid should always be given; from 2 to 5 mg. of aneurine and not less than 50 mg. of ascorbic acid per day should be administered.

Aneurine in Pregnancy. Reference has already been made to the increased need of aneurine in pregnancy. Some writers believe aneurine deficiency to be an ætiological factor in such disturbances of pregnancy as hyperemesis gravidarum, pregnancy neuritis, cardiovascular disorders and the toxæmias of pregnancy. While aneurine deficiency may cause neuritis and cardiovascular disturbances, which are amenable to treatment with the vitamin, it is more likely that hyperemesis gravidarum and pregnancy toxæmia cause aneurine deficiency; the former by repeated vomiting and loss of gastric hydrochloric acid needed for the absorption of aneurine (p. 226), the latter by hepatic dysfunction interfering with efficient utilization of aneurine (p. 226). Wernicke's encephalopathy (p. 231), which has been attributed to aneurine deficiency, is a terminal phase in hyperemesis gravidarum [734, 735] and Korsakoff's psychosis has also been reported [611].

Nixon [734] refers to the triad—œdema, toxæmia and aneurine deficiency—frequently seen in pregnant women in Hong Kong. The report of the University Clinic there shows an alarming increase in the number of cases of eclampsia and beriberi; the more severe the eclampsia the higher the incidence of aneurine deficiency, with concomitant increased mortality. Forty-five per cent. of the cases of eclampsia were complicated by aneurine deficiency. From a study of eight cases in this country Nixon showed that the excretion of aneurine was below that of normal controls, and the aneurine content of the placenta of eclamptic patients was also significantly low. Nixon's cases were seen in Hong Kong, where there is undoubtedly widespread beriberi amongst pregnant women which ranks high there as a cause of maternal death, being responsible for thirty-five per cent. of the cases. There is conceivably a relation between pregnancy toxæmia and frank beriberi. In the latter, congestion of the liver, with subsequent diminution in liver function, may be a contributory factor in the precipitation of pregnancy toxæmia. These observations of Nixon have been confirmed by King and Ride [470]. Neuweiler and Nyffenegger [596] state that there is a rise of blood bisulphite binding substances (p. 242) in hyperemesis gravidarum.

Most of the writers associating aneurine deficiency with the toxæmias of pregnancy diagnose the former from a low urinary excretion of aneurine. The fallacy of this has been pointed out before (p. 243). Rose and his co-workers [560] failed to note any decrease in the toxæmia during the latter part of pregnancy in a controlled group of patients receiving 3 mg. of aneurine and other members of the B complex daily. Horwitz and Farley [566] concluded from blood tests that thirteen of a group of 100 pregnant women were suffering from aneurine deficiency, and that ten of these developed neuritis and severe hyperemesis and six anorexia. The two latter conditions are likely to have caused a conditioned vitamin deficiency. Williams and Fralin [733] in a nutrition survey of over 500 pregnant women noted that eighty-four per cent. of a group showing excessive nausea and vomiting in early pregnancy had an aneurine intake of less than 2 mg. daily. It is difficult to say whether the low aneurine intake was the cause of the nausea and vomiting. The intake of other essential nutrients was also probably low. We would like to re-emphasize the view that vitamin deficiencies are never limited to lack of a single vitamin.

The use of aneurine in the treatment of toxæmia of pregnancy has been disappointing. Yasunami [559], working in Japan, states that he found

it effective in the treatment of the condition, particularly in preventing convulsions in pre-eclamptic patients. This has not been confirmed by work in America and Europe. Siddall [325], although he claims that the toxæmias of pregnancy are associated with a deficiency of aneurine, failed to observe any improvement in patients with pre-eclampsia after giving them daily injections of 1 to 7 mg. of aneurine for ten days. Strauss [657], Browne [886], and Kapeller-Adler and Cartwright [887] failed to observe any significant improvement in blood pressure, œdema or albuminuria in patients with toxæmia of pregnancy treated with aneurine in doses from 9 to 25 mg. daily. Kapeller-Adler and Cartwright [887] state that in severe cases aneurine actually intensified the signs and symptoms of pre-eclamptic toxæmia in the patients studied. They consider its use contra-indicated in view of the fact that it inhibits histaminase, an enzyme that normally hydrolyses histamine in the body. Kapeller-Adler believes that in normal pregnancy most of the histamine formed in the body is destroyed by histaminase, but that in the toxæmias of pregnancy it escapes destruction.

Widenbauer [609], Spitzer [610], Bernstein [571], Lund [568] and Willis [888] claim to have effectively treated hyperemesis gravidarum with aneurine. In some cases other preparations such as ascorbic acid, suprarenal cortex hormone, and pyridoxine were used. Spitzer used doses of 10 to 20 mg. of aneurine. Willis and others [888] gave doses of 25 to 50 mg. daily parenterally up to a total dose of 800 mg. The results were stated to be satisfactory, but not as good as with pyridoxine. Most of these observations were not controlled and their evaluation is, therefore, difficult. Hyperemesis causes aneurine deficiency (p. 223), but why the administration of large doses of aneurine should cure the former is difficult to understand. It may, of course, restore appetite and improve liver function by improved carbohydrate metabolism. Success has been claimed for many forms of treatment of hyperemesis, but it is probable that the success is due to the psychological effect of the treatment, particularly if this involves the use of the hypodermic needle.

According to Bickel [442] cardiovascular disturbances, due in certain cases at least, to aneurine deficiency, may develop during pregnancy in women who were previously apparently normal. He states that they are likely to appear in connection with pregnancy toxæmias. Daily injections of 50 mg. of aneurine were stated to produce a cure. Stähler [614] describes similar cases treated with injections of 10 mg. daily.

Large doses of aneurine and the B vitamins are said to relieve heartburn in pregnancy [889]. As aneurine has no spasmolytic action and has no effect on gastric secretion and motility its use for this purpose does not seem to have any rational basis.

Aneurine in Metabolic Diseases. Diabetes. Certain workers claim to have improved the carbohydrate tolerance of diabetics by administering aneurine. Thus Vorhaus and his co-workers [271] state that they obtained improvement in diabetes treated with aneurine, particularly in patients suffering from obesity, lack of appetite and a diminished metabolic rate. Sciclounoff [291] states that twelve of thirty-five diabetics showed improvement for periods of several days up to some months after supplements of aneurine; the glucose tolerance test curve was also lower after taking aneurine. Dienst [616] stabilized a group of diabetics on insulin and administered a preparation containing aneurine and claimed that carbohydrate tolerance was so improved that the patients required 10 to 20 units of insulin a day less. Hypoglycæmic reactions were said to be fewer. A fall in blood sugar and diminished glycosuria after administering aneurine to diabetics has also been reported [617].

Others have not been able to reproduce these results. Lawrence and Oakley [443] treated a large series of diabetics with aneurine, but could not record any effect, for better or worse, on the carbohydrate tolerance or insulin

requirement. Kaufman [670] could not observe that aneurine had any effect on the blood sugar of diabetics. Smith and Mason [555] kept two patients with severe diabetes on aneurine deficient diets and a third was given injections of glucose with and without the addition of aneurine; the vitamin had no effect on the severity of the diabetes or on the insulin requirements of the patients. Trasoff and Bordin [741], Robson [452], and Owens [684] could not observe any discernable effect on the severity of the disease after administering aneurine and the vitamin B complex to diabetics.

The relatively mild degree of depression of carbohydrate tolerance seen in animals and human beings after long periods of aneurine deprivation appears to represent a disturbance of metabolism unrelated to that involved in diabetes. Such "false diabetes" can be corrected by administering aneurine; true diabetes cannot. Glucose tolerance is impaired in the late stages of aneurine deficiency, but it must be both prolonged and severe [334].

No reduction in the insulin requirements of diabetics receiving large amounts of aneurine has been observed [684]; it is believed that aneurine deficiency may play a part in the causation of diabetic coma, in which the most severe damage falls on the brain, heart and kidneys, the cells of which are very susceptible to lack of aneurine. That disordered carbohydrate metabolism may occur in a diabetic coma is suggested by the finding by Markees and Meyer [460] of a raised blood pyruvate in diabetic acidosis. These investigators reported that recovery from diabetic coma induced in rabbits experimentally with alloxan is hastened by treatment with cocarboxylase (aneurine pyrophosphate) and riboflavine. They further state that a number of diabetics (unspecified) in coma have been treated with cocarboxylase with beneficial results, the patients recovering more rapidly, the alkali reserve rapidly increasing and the blood pyruvate falling [469]. Boulton and his co-workers [470] have made similar claims.

Gilliland and Martin [473] have confirmed the raised blood pyruvate in diabetic acidosis in alloxan-diabetic rabbits, and in diabetic patients with acidosis, but supplementing standard treatment for diabetic acidosis with cocarboxylase and riboflavine, as recommended by Markees and Meyer [469], did not accelerate recovery as measured by clinical improvement, a fall in the raised blood pyruvate and blood sugar and a rise in the alkali reserve.

It has been assumed that the aneurine requirements may be increased in diabetics. In theory the diminished oxidation of carbohydrate would be expected to decrease the requirement of aneurine. This has been shown experimentally by Lowry and Hegsted [477], who showed that the aneurine requirement of the animal suffering from alloxan-diabetes is less than that of normal controls. The animals showed no increased tendency to develop signs of aneurine deficiency. Caution must of course be exercised in the interpretation of such experimental findings, as alloxan-diabetes differs from human diabetes, particularly with regard to the capacity to survive without the help of insulin.

Hyperthyroidism. There is a superficial resemblance between the symptomatology of hyperthyroidism and aneurine deficiency—anorexia, diarrhoea, cardiac enlargement, tachycardia, fatigue, palpitations, impaired muscular strength, disturbed carbohydrate metabolism and "neurasthenic" symptoms. For this reason the administration of aneurine and the vitamin B complex has been recommended in the treatment of hyperthyroidism. The basic pathology of the two conditions is, however, quite different. In one the raised metabolism throws a strain on the cardiovascular system; in the other a deficiency of aneurine produces pathological changes in the heart muscle. Williams [296] and Davis and Bauer [936] observed a low blood cocarboxylase and a raised blood pyruvate in thyrotoxic patients. Williams found no correlation between these figures and the B.M.R.

Cowgill [198] administered 60 micrograms of aneurine per 100 calories of

the estimated total daily metabolism, which is increased in hyperthyroidism. Means and his associates [620] state that the clinical course of hyperthyroidism is favourably influenced by administering aneurine, although Jacobi and Pomp [621] could not confirm this, controls treated with rest and diet doing just as well as those receiving additional aneurine and vitamin A. Frazer and Ravdin [121] supplemented the routine pre-operative preparation of twenty-eight patients for thyroidectomy with 10 mg. of aneurine hypodermically every other day and 10 grams of yeast daily, and compared the results obtained with a control group of hyperthyroid patients not receiving aneurine. The aneurine had no antithyrototoxic action, nor had it any effect on the B.M.R. or on the severity of the post-operative crisis. It was considered, however, to bring down the pulse rate, increase weight and appetite and to diminish the time needed for pre-operative preparation. Williams [296] prescribed brewer's yeast and 10 to 20 mg. aneurine daily to all thyrototoxic patients under his care for four years; a distinct subjective improvement was recorded. Williams considers that the aneurine requirements are increased in hyperthyroidism, as aneurine is lost to the body in the sweat, fæces and urine as a result of hyperhidrosis, diarrhoea and diuresis.

Gout. Vorhaus and Kramer [624] have reported relief of pain in acute gout by administering aneurine. Kühnau [625] states that the blood nucleotides are raised in gout (5 to 10 mg. per millilitre; normal, 2 to 4 mg.); Birch and Mapson [626] have observed a similar rise in beriberi. Kühnau records that in patients with gout the intravenous injection of 10 to 20 mg. of aneurine is followed by disappearance of pain, swelling and redness and a fall in the blood nucleotides. He concludes that aneurine, as cocarboxylase, removes uric acid from the body by phosphorylating it and that in gouty subjects the formation of purines is so increased that the normal amount of aneurine in circulation is insufficient for its removal. Callahan and Ingham [575] treated nine cases of gout with 15 to 30 mg. of aneurine daily. They state that the period of disability was considerably reduced, although at the same time they diminished the purine intake of the patients, administered cinchophen and treated them with medicated baths.

Dermatology. Several workers have published uncontrolled observations on the treatment of skin conditions with aneurine. There is no rationale for its use and no evidence that it is effective in the treatment of any disease of the skin.

Shock. Govier and Greer [749] state that the average survival time of anaesthetized dogs, in which hæmorrhagic shock has been induced, is significantly greater in those animals treated with aneurine than in untreated animals. The dose given was 1 to 2 mg. per kilo followed by 0.5 mg. every two hours. The average survival time in the treated group was eight hours, in the untreated three-and-a-half. The administration of aneurine lowered the level of keto-acids, lactic acid and sugar in the blood of the bled dogs. This work was repeated with rabbits by Maycock [534], who was unable to confirm it. Govier [641] has studied the relationship between hæmorrhagic shock and the plasma aneurine level. He states that the resistance to shock is greater in animals with a raised plasma aneurine; they withstand more bleeding—forty-five per cent. more than controls with a low plasma aneurine—before developing severe hypotension, and they show a more rapid return to their normal blood pressure when hæmorrhage stops. Govier and Grieg [652] have shown that in dogs subjected to shock from hæmorrhage and in animals suffering from anoxic anoxia dephosphorylation of cocarboxylase occurs. If the bled dogs are given aneurine a resynthesis of cocarboxylase results. Govier believes that there is greater need for aneurine in hæmorrhagic shock because of the dephosphorylation of cocarboxylase; aneurine exerts its beneficial effect, according to him, by causing increased synthesis of cocarboxylase. Alexander [915] has shown that the concentration of total and phosphorylated aneurine in the liver rises in prolonged hæmorrhagic

shock. The non-phosphorylated aneurine of muscle also shows an increase, occurring at the expense of cocarboxylase.

It has been shown that in patients with severe injuries, hæmorrhage, infection and in those suffering from burns, there are considerable alterations in aneurine metabolism, as shown by a low urinary excretion [488, 489]. The metabolism of nicotinic acid, riboflavine and ascorbic acid is also affected, and it is suggested that 10 to 20 mg. of aneurine be given to such patients for the prevention of shock. Bergman and others [495], however, were unable to show that the administration of aneurine or ascorbic acid had any beneficial effect in the treatment of shock due to scalding burns in mice.

According to Grieg [499] destruction of three co-enzymes may occur in shock : cocarboxylase, codehydrogenase and alloxazine adenine dinucleotide. It would therefore appear rational to administer aneurine, nicotinic acid and riboflavine, which are components of these co-enzymes, to patients suffering from shock or conditions likely to result in shock, such as extensive burns, trauma and surgical procedures. Lund [889] recommends 10 to 20 mg. each of aneurine and riboflavine, 150 to 250 mg. of nicotinic acid and 1 to 2 grams of ascorbic acid. These vitamins alone do not of course satisfy the nutritional needs of the patient, which include a high protein, high carbohydrate diet supplemented by yeast, liver or liver extract.

Irradiation Sickness. It is claimed that the administration of aneurine affords some relief from the symptoms of irradiation sickness, which is characterized by nausea, vomiting, diarrhœa, nervous symptoms and headache, and which may occur after exposure to therapeutic doses of X-rays or the rays from radium. Martin and Moursund [654] state that these symptoms are prevented by giving 6 mg. of aneurine orally and a high carbohydrate diet for at least two days before exposure commences. If vomiting occurs it is frequently relieved by an intramuscular injection of 6 mg. Imler and Wammock [656] and Sponheimer [655] report that injections of 10 mg. of aneurine daily give rapid and complete relief from the more severe symptoms of irradiation sickness in the majority of cases. In severe cases the dose is increased from 15 to 30 mg. daily by injection. Results following parenteral therapy are said to be more effective than those following the oral route. The headache, nervous and digestive symptoms are reported to be relieved by this treatment, although according to Wallace [712], who employs 10 mg. of aneurine and 50 mg. of ascorbic acid parenterally, vitamin therapy does not control the diarrhœa. Johnston [653] claims that the best results are obtained with a combination of aneurine and nembutal. Whitmore [336], from observations on 122 cases, found that 6 to 9 mg. of aneurine daily prevented symptoms of irradiation sickness in eighty per cent. of the cases. The dose was increased if sickness developed. Another report from Bean, Spies and Vilter [940] favours the administration of 50 mg. of aneurine and 300 to 500 mg. of nicotinic acid daily. These workers state that the incidence of irradiation sickness is greater in patients consuming diets poor in the vitamin B complex. Once irradiation sickness was established, the administration of aneurine and nicotinic acid had little effect in relieving it, although it was found that if given in the doses stated before exposure, the onset of the sickness was largely prevented. Bean, Spies and Vilter suggest that the basic disorder in irradiation sickness is a disturbance in the respiratory enzyme systems, of which aneurine and nicotinic acid are components.

Aneurine and the Sulphonamides. Fleisch and De Preux [894] state that albuminuria and hæmaturia due to sulphonamides such as sulphapyridine, sulphathiazole and Irgamid (N-dimethylacroylsulphanilamide), can be diminished by administering large doses of aneurine ; aneurine has no effect, however, on the acute toxicity or lethal dose of the drugs in animals. Higgins [900] noted that the toxic effects of the sulphones—hyperæsthesiæ, loss of weight, anorexia, anæmia and lassitude—can be largely prevented in rats if they are given six times the normal allowance of aneurine, riboflavine and

pyridoxine. Yeast, however, is ineffective presumably because it cannot supply such large quantities of the B vitamins.

According to Kinnunen [500] aneurine plays a fundamental rôle in the acetylation of the sulphonamides. It increases the acetylation of sulphonamides in the rabbit, possibly by increasing the formation of the acetylating component, believed to be either ketene or acetyl phosphate, from the products of carbohydrate breakdown (pyruvic acid). Kinnunen has shown that sulphapyridine interferes with the formation of citrate in the carbohydrate cycle (p. 195). He believes that the neurotoxic effects of the sulphonamides are due to their acetylation, which interferes with the formation of acetylcholine. The reputed protective effect of aneurine (p. 259) is due to its catalysing the oxidation of pyruvate, which makes more of the acetylating component available.

The effects of the sulphonamides producing a partial aneurine deficiency by interfering with its intestinal synthesis (p. 204) has probably been greatly exaggerated. The administration of phthalylsulphathiazole to normal subjects makes very little difference to the excretion of aneurine in the urine or in the faeces [127]. In the rat also the administration of sulphonamides does not influence the urinary excretion of aneurine, suggesting that normally little of the aneurine synthesized in the gut by bacteria is absorbed.

According to Slater [619, 623] sulphadiazine and sulphamerazine have an aneurine sparing effect in man and the rat; sulphanilamide, sulphathiazole, succinylsulphathiazole and sulphapyridine have no such effect. The two former sulphonamides may act on the metabolic rate as they are the only sulphonamides which produce thyroid enlargement.

Uterine Cancer. Deficiency of vitamin B has been postulated as a factor in the aetiology of uterine carcinoma; this has led to the suggestion that the B vitamins might be used prophylactically against the development of cancer. Ayre and Bauld [541] state that women with carcinoma of the cervix uteri show signs of aneurine deficiency and hyperœstrinism, as evidenced by vaginal smears. They then postulate that in the presence of chronic vitamin B deficiency the liver does not inactivate endogenous œstrogen, excess of which is localized in the cervix, which is often the seat of chronic cervicitis. Œstrogen then produces metaplastic and eventually carcinomatous changes in the epithelial cells of the cervix. These views are based on the work of Biskind [512], Segaloff [542, 543] and Singher and his co-workers [546], who found that in the aneurine and riboflavine deficient rat the liver loses its ability to inactivate œstrogen. They are also supported by the observation that patients with severe liver damage excrete greater amounts of endogenous œstrogen and excrete a higher percentage of administered œstrogen than normal [547]. More recent investigations, however, have shown that there is no significant difference between the level of aneurine nutrition in women suffering from carcinoma of the cervix and normal controls [549], and that the failure of the liver to inactivate œstrogen in vitamin B deficient rats is due not to vitamin B deficiency *per se*, but to inanition. Greene and Peckham [550] have critically reviewed the literature on the subject.

Resistance to Fatigue. There are several reports on the beneficial effects of giving aneurine either alone or with other vitamin mixtures to increase the capacity and resistance to fatigue of athletes, soldiers and men engaged in heavy work [337, 660, 700]. These observations, which were largely uncontrolled, have not been confirmed. Many critically controlled studies on the subject have since been made and the conclusion drawn by four separate groups of investigators is that the administration of aneurine or members of the vitamin B complex to adequately nourished persons has no influence on work output, endurance in dynamic work, recovery of working capacity, or recovery from fatigue [338-340, 796, 797]. In one group investigated there was no change in the heart rate, oxygen consumption, respiratory

quotient, urinary excretion of nitrogen and ketone bodies, and blood lactate, nitrogen, glucose and B.B.S. [797]. The Council on Foods and Nutrition in America [341] has pointed out the waste of material and money in the indiscriminate administration of vitamin mixtures to workers in industry with a view to increasing output and diminishing fatigue. As the Council points out, if the workers are adequately nourished additional vitamins serve no useful purpose; if they are not adequately nourished they need more or better food and not vitamins out of a bottle. On the other hand, it has been reported that on diets deficient in aneurine and the vitamin B complex there is a decreased work output in trained subjects, loss of ambition and efficiency, and poor recuperation [584, 697, 836, 890]. This subject is dealt with in further detail on p. 239.

These observations are in keeping with those on rats. Aneurine in large doses has no effect on the work performance of rats receiving adequate supplies in their diet [912]. There is some evidence, however, that the vitamin may exert a pharmacodynamic effect on isolated perfused muscle. The total work output of the gastrocnemius muscle of the frog is significantly increased by perfusion with fluids containing 0.01 milli-equivalents of aneurine and calcium pantothenate per litre [943]. This concentration of aneurine is actually many times greater than that occurring in skeletal muscle and the effects may well be due to vasomotor changes. Lissák and his co-workers [525] were unable to confirm this effect of aneurine on isolated muscle.

Aneurine deficiency is not a significant factor in producing fatigue and other symptoms (effort intolerance, breathlessness, palpitation, precordial pain and subjective feelings of fatigue) in patients with the effort syndrome [937].

Morphine Addiction. Fitzhugh [666] observed that the irritability of rats addicted to increasing doses of morphine was reduced by aneurine. The vitamin also prevented the increase in irritability that follows morphine withdrawal. Himmelsbach [137] has been unable to confirm this clinically in the case of morphine addicts.

Other Clinical Uses of Aneurine. Aneurine has been used in the treatment of pink disease, although the earlier enthusiastic reports have not been confirmed [516-519]. It is a disease with remissions and exacerbations and eventual recovery or death from intercurrent infection, such as bronchopneumonia. Evaluation of a remedy in small numbers of cases is therefore difficult.

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CHAPTER IV

RIBOFLAVINE

HISTORY

IN 1932 Warburg and Christian [1] described a new "yellow enzyme" which they obtained from aqueous extracts of bottom yeasts. They claimed that it played an important rôle in respiration by forming part of an oxidation-reduction system, acting as a carrier of molecular oxygen to an oxidizable substrate. Warburg and Christian [2] later separated this "yellow enzyme" into a protein component and a pigment component, and noted that neither alone was catalytically active. In 1933 reports appeared from three independent research groups suggesting a relationship if not identity between vitamin B₂ or G, a heat labile factor of the vitamin B complex, and the water-soluble yellow-green fluorescent pigments found in many animal and plant products such as yeast, liver and kidney. Kühn [4, 5] and his associates in an attempt to isolate "vitamin B₂" from eggs obtained a water-soluble yellow-green fluorescent pigment, which like "vitamin B₂" had growth-promoting properties. They observed the similarity in distribution of the two substances and called attention to their probable relationship to the yellow enzyme of Warburg. Since pigments with apparent different vitamin activities were obtained from various sources they were looked upon as different members of a chemical group to which the term "flavin" was applied. The various flavins were called lactoflavin, hepatoflavin, ovoflavin, etc., according to their origin. Subsequent research has shown that they are all identical. At the same time Booher [6] in America showed that the growth-promoting activity of whey, which is a function of its "vitamin B₂" content, runs parallel to the amount of yellow pigment present.

Subsequent studies confirmed the identity of vitamin B₂ with the yellow pigment. After the elucidation of its structure and synthesis, it was decided in 1937 by the Council on Pharmacy and Chemistry of the American Medical Association to call it riboflavin and to abandon the terms vitamin G and B₂.

CHEMISTRY OF RIBOFLAVINE

Riboflavin was isolated from many natural sources by Kühn [5] and his collaborators and their work has been confirmed by a number of other investigators. Among the sources were egg yolk and white, milk, liver, kidney, urine, grasses, fish retinae, barley malt and yeast.

Riboflavin crystallizes in yellowish brown needles (Fig. 84). The needles have no sharp melting point, but darken at 240° C. and melt at 275°–282° C. with decomposition. Although stated to be water soluble its solubility is very slight, being only 12 mg. per 100 ml. at 27.5° C. It is quite insoluble in the fat solvents. It is very soluble in alkali solutions. The solubility in water is increased by adding urea or by the formation of a complex with boron, by means of which an 0.3 per cent. solution may be obtained. The solubility is also increased by the presence of tryptophane or nicotinamide [317]. Riboflavin is stable in strongly acid solution, but is unstable in the presence of alkali, or when exposed to light or irradiation with ultra-violet light, which cause irreversible decomposition. The vitamin is therefore stored in tubes covered with black paper or in amber-coloured ampoules. When exposed to daylight in neutral solution the ribose chain is split off to form lumichrome, which shows a greenish yellow fluorescence, but is devoid

of vitamin activity. In alkaline solution light splits off the four terminal carbon atoms of the ribose to form lumiflavin, which shows a bright blue fluorescence and like lumichrome has no vitamin activity. Riboflavin is relatively highly thermostable (e.g. only slight destruction at 120° C. for six hours) and uninfluenced by atmospheric oxygen in the dry state. Solutions of riboflavin exhibit a strong yellow-green fluorescence which reaches a maximum between pH 6.0 to 7.0. In milk at least ninety per cent. of the riboflavin appears to be in the free form. In most other sources, such as yeast, liver, and plants, it occurs conjugated with other compounds of high molecular weight.

Following the isolation of riboflavin in crystalline form, studies by Warburg and Christian [2] and by Kühn and Rudy [9, 10] in 1934, led to the

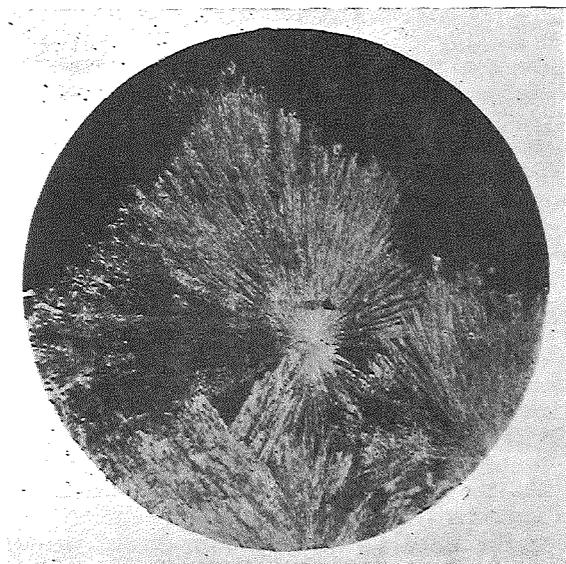
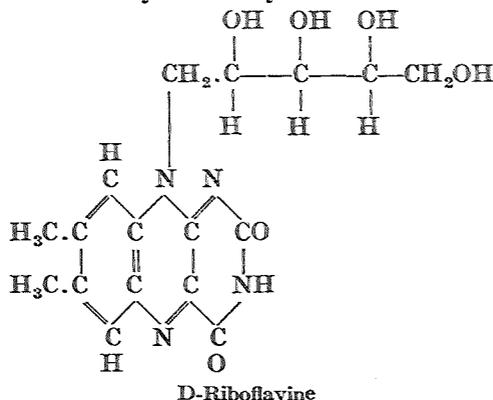


FIG. 84. Crystals of Riboflavin.

elucidation of its constitution. The synthesis of riboflavin, which was essential for the final proof of its chemical constitution, was effected in 1935 by Kühn [11] and his co-workers and by Karrer [12] and his collaborators. D-Riboflavin is 6 : 7-dimethyl-9-*d*-ribityl-isoalloxazine.



The fact that riboflavin exhibits a blue-green fluorescence in ultra-violet light serves as a basis for determining it by fluorometric methods [17-22,

145-148, 320, 321]. The vitamin can be separated from biological fluids or foodstuffs by adsorption on a cation exchange resin and eluted with pyridine [319]. It can be detected in biological material by fluorescence microspectroscopy [7]. It is also estimated by converting it into lumiflavin by exposure to light in alkaline solution and then determining the lumiflavin colorimetrically. Biological methods have been employed [14, 15]. A microbiological method has been developed which depends on the observation that the growth of a specific strain of *Lactobacillus casei* or *Lactobacillus helveticus* and the resulting production of acid is proportional to the amount of riboflavin in the medium [132, 133, 149-152, 323]. The physico-chemical methods for estimating riboflavin are reviewed by Hoffman [13] and Skiller [321], and microchemical methods by Bessey [322].

UNITS OF RIBOFLAVINE

The Bourquin-Sherman [16] method of estimation, originally described as a method of assay for vitamin B₂ or G in the old nomenclature, has been shown to be only an approximate measure of riboflavin. The method is not specific since it is based on growth responses, and many other factors besides riboflavin are essential for growth. The Bourquin-Sherman unit was defined as "the amount of vitamin B₂ which when fed daily to a standard rat during eight weeks will cause an average gain of 3 grams per week in addition to the average gain of the control test animals fed on a vitamin B₂ free diet." One Bourquin-Sherman unit has been variously reported as being equivalent to from 2 to 7 micrograms of pure riboflavin. The most recent figure is 2.19 micrograms [29]. There is no international unit.

Now that physico-chemical methods are available for the estimation of the vitamin, its concentration in a foodstuff or preparation can be expressed in milligrams or micrograms per given volume or weight of material.

DISTRIBUTION OF RIBOFLAVINE IN FOODS

Riboflavin is widely distributed in plants, which synthesize it, and in animal tissues. Among the best sources are yeast, milk, white of egg, fish roe, kidney, liver, heart and growing leafy vegetables; other fairly good sources are fish, meat and poultry muscle. Grains and legumes, although they contain it, are not particularly rich sources. There is an increase in riboflavin during germination.

In green vegetables the leafy portions and growing parts contain most riboflavin; as the leaves get older and dry the riboflavin content diminishes. It has been shown that the milk from cows fed on fresh young grass contains more riboflavin than those fed on dried or root crops, which accounts for the fact that the riboflavin content of milk is highest in summer. Milk, eggs and leafy green vegetables are the chief sources of riboflavin in the average dietary. The riboflavin content of human milk fluctuates during the day, varying from 21.8 to 25.8 micrograms per 100 ml. In early lactation it is 18 micrograms, and by the tenth day 22 micrograms per 100 ml. It can be increased to 67 micrograms by increasing the intake to 6 mg. daily [327]. Liver and yeast are excellent sources, but since they are only consumed in relatively small quantities they do not contribute much riboflavin to the diet. A variety of yeast known as "food yeast" (*Torulopsis utilis*) contains as much as 9 mg. of riboflavin per 100 grams [8]. Meat and poultry muscle are fairly good sources, but fish muscle is not. Fresh raw peas and beans are fair sources. White bread is a poor source. In natural materials where there is little respiration riboflavin is found mainly in the free state; in tissues which are respiring it is present mainly as Warburg's enzyme (p. 292).

Effect of Cooking, Curing, Freezing and Canning. Since riboflavin is fairly heat stable, it is not appreciably destroyed in the ordinary processes of

cooking, unless the medium is alkaline, although it is stated that the presence of cooking soda in a concentration of 0.12 per cent. causes no destruction [177]. The solubility in water will result in some loss in the cooking water if this is not consumed. The loss of riboflavine from food cooked by boiling is from fifteen to twenty per cent. [153]; slight losses also occur in pressure cooking [23]. With careful cooking up to practically one hundred per cent. of the riboflavine present in the raw material may be retained. Losses from roasting vary from nil to twenty-six per cent. [24, 126, 153]. The losses are greater if the food is cooked while exposed to light, e.g. up to forty-eight per cent. in the case of eggs, milk and chops [117]. In the cafeteria cooking of food the loss of riboflavine is greater, from twenty-two to forty-five per cent. [155].

Considerable quantities of riboflavine in milk may be destroyed if milk bottles are allowed to stand for any length of time exposed to the sun or bright daylight [309]. There is practically no loss of riboflavine in the pasteurization of milk [282]. In the curing of meat the losses of riboflavine are very small, e.g. about eight per cent. The loss in frying is about the same as in roasting (none to twenty-three per cent.) [24, 126]. Losses by drying or dehydration vary from nil up to fifteen per cent. [156, 281]. Quick freezing has very little destructive effect on the riboflavine in foodstuffs [25, 157]. Meat kept in cold storage for fifteen days loses fifteen per cent. of its riboflavine [24]. Canning and smoking have little effect on the riboflavine of foods, although in canning up to thirty per cent. or more may be lost in the liquid used to can the food [26, 157, 281]. The average retention in the canning of vegetables, including the riboflavine in the water, is ninety per cent. Appreciable amounts of riboflavine are retained in the preservation of food by brining and salting [280]. Dehydration of vegetables results in a loss of twenty to forty per cent. by the time the food reaches the table. Radio-frequency or high-frequency heating, which is used in the food industry, particularly in America, results in little loss of riboflavine [116]. Not all the riboflavine in foods is available. Everson [120] has shown that there is a significant difference in the availability of the riboflavine found in various food sources. The riboflavine in live yeast is not available to any extent; the yeast must first be destroyed by boiling before the riboflavine in the cells can be utilized [154]. It appears that live yeast cells in their growth and reproduction enter into active competition in the gut for the riboflavine ingested in the food. If yeast is given as a dietary supplement it must be boiled first.

Riboflavine Content of Foodstuffs

Food	Description	Micrograms of Riboflavine per 100 grams = 3½ ozs.
<i>Cereals</i>		
Barley	—	120-250
Buckwheat	—	80-160
Maize	Sweet, white or yellow	60-140
Oats	—	100-150
Rice	Unpolished	60-80
	Unmilled	120
	Milled and polished	47
Rye	—	140
Rye bread	—	40
Wheat	Whole grain	160-250
	Manitoba wheat	170
	Germ	480-1,500 (Av. 800)
	Bran	500-600
	Whole grain bread	180
	White bread (70%)	70 ; 60
	White bread (73%)	50

Food	Description	Micrograms of Riboflavine per 100 grams = 3½ ozs.
<i>Cereals—continued.</i>		
<i>Wheat—continued.</i>		
	White flour (73%)	85
	White flour (82½%)	100
	National flour (85%)	140-200 (Av. 150)
	National bread (85%)	100-200 ; 120-130
	Flour (U.S.A. enriched)	260-330
Spaghetti	—	80
<i>Proprietary Cereal Foods [294]</i>		
All-Bran	Kellogg's	360-480
Bran Flakes	Post's (with added vitamins)	210-290
Cerevim	Lederle Labs. (with added vitamins)	3,300
Corn Flakes	Kellogg's (with added vitamins)	80
	Post's (with added vitamins)	100
Force	—	160
Grape Nuts	With added vitamins	170-200
Quick Quaker Oats	—	140-180
Cream of Rice	—	80
	With added vitamins	620
Puffed Rice	Quaker Oats Co.	60
Rice Krispies	Kellogg's (with added vitamins)	70
Shredded Wheat	—	140-190
Soya Wheat	—	270
<i>Fruits</i>		
Apple	—	10-50
Apricot	Fresh	40-75
	Dried	100
	Tinned	24
Avocado	—	90-150
Banana	—	56-75
Blackberry	—	60
Blackcurrant	—	140
Cherry	—	40
Date	—	45
Fig	Fresh	5
	Dried	45
Grape	Fresh	15-40
	Dried	80
Grape-fruit	—	20-40
	Tinned	19
Guava	—	10-90
Melon	Cantaloupe	40-75
Orange	—	30-90
	Tinned	22
Peach	Yellow	60
	Tinned	22
	Dried	200
Pear	—	20-75
	Tinned	20
Pepper, green	—	50
Pineapple	—	5-55
Plum	—	30-45
Prune	Dried	160
Pomegranate	Juice	100
Pumpkin	—	45
Raisin	—	80
Raspberry	—	70
Strawberry	—	70
Squash	—	50
Tangerine	—	20-30
Water-melon	—	15-60
<i>Nuts</i>		
Almond	—	300-670
Brazil	—	70
Cashew	—	190

Food	Description	Micrograms of Riboflavine per 100 grams = $3\frac{1}{2}$ ozs.
<i>Nuts—continued.</i>		
Coconut	—	100-110
Pecan	—	106-300
Peanut	Raw	500 ; 170-750
	Roasted	160-500
Walnut	Butter	160-320
	—	130
<i>Vegetables</i>		
Alfalfa	Dried	1,500
Arrowroot	—	60
Artichoke	—	30
Asparagus	—	120
Bean	Fresh	110-175
	Dried	Up to 750
	Baked	50
Beet	Tops	170-300
	Root	50
Broccoli	Entire plant	250
	Flower	240
	Leaves	450
Cabbage	—	50-57
Carrot	Dehydrated	380-470
	—	50-60
Cauliflower	Dehydrated	280
	—	105-130
Celery	—	40-100
Chard	—	130
Cucumber	—	25-90
Date	—	4'-100
Eggplant	—	30-60
Endive	—	120-200
Grass	—	190
Kale	—	350-500
Lentil	Dried	315-400
Lettuce	—	45-150
Mango	—	50-100
Mushrooms	—	5-500
Onion	—	24-50
Papaya	—	150-180
Parsley	—	300
Parsnip	—	90
Pea	Fresh	150-200
	Dried	150-300
	Tinned	20-50
Peppers	Green	40-100
	—	29-40
	Peeled and raw	50
Potato	Chips	210
	Dehydrated	100
	—	80
Pumpkin	—	30
Radish	—	30
Rhubarb	—	30
Spinach	—	230-400
Sprouts	—	60-75
Sweet potato	—	60-75
Soya bean	—	280-750
Tomato	—	40-60
Turnip	Root	30-65
	Greens	350-560
Water-cress	—	250
<i>Dairy Products</i>		
Butter	—	10-37
Cheese	Camembert	830
	Cheddar	500-550
	Cottage	290
	Cream	140

Food	Description	Micrograms of Riboflavine per 100 gram = 3½ozs.
<i>Dairy Products—continued.</i>		
<i>Cheese—continued.</i>		
	Processed	430 ; 570
	Roquefort	450
	Swiss	370
	Velveeta	550
Eggs	Whole	250-440
	White	250
	Yolk	520
	Dried	1,230
Margarine	—	0-6
Milk	Cow's, average (new)	95 ; 150
	„ range (new)	135-210
	„ pasteurized	119-206
	„ cream	140
	„ dried	1,500
	„ „ (skimmed)	160-200
	„ evaporated	360-390
	Buttermilk	180
	Human	16-67
	Goat	40-80
<i>Meat Products</i>		
Calf	Muscle	140-220
	Kidney	2,100
	Liver	3,300
Chicken	Muscle	70-260
Duck	—	230
Lamb	Heart	270-350
	Kidney	2,000
	Liver	3,300
	Muscle	250
Luncheon meat	Canned	210
Ox	Heart	900
	Kidney	2,000
	Corned beef	100
	Liver	3,000
	Muscle	180-350
	Brain	140
	Tongue	270
Pig	Bacon	130-300
	Muscle	180-240
	Ham	190
	Heart	900
	Kidney	2,100
	Liver	2,700
Rabbit	Liver	2,700-3,500
	Heart	550-1,330
	Kidney	1,310
	Muscle	60
Meat extracts	—	1,540-2,580 ; 3,000-3,500
Sausage	Liver	1,120
Tripe	—	120
Turkey	—	190-240
Veal	—	260-280
<i>Fish</i>		
Carp	Muscle	40
Clam	—	120
Cod	Muscle	50
	Roe	900-1,130
Conger eel	Muscle	55
Crab	Flesh	350
Dogfish	Liver	370-540
	Roe	880
Haddock	Muscle	165
	Roe	1,420
Halibut	Muscle	185

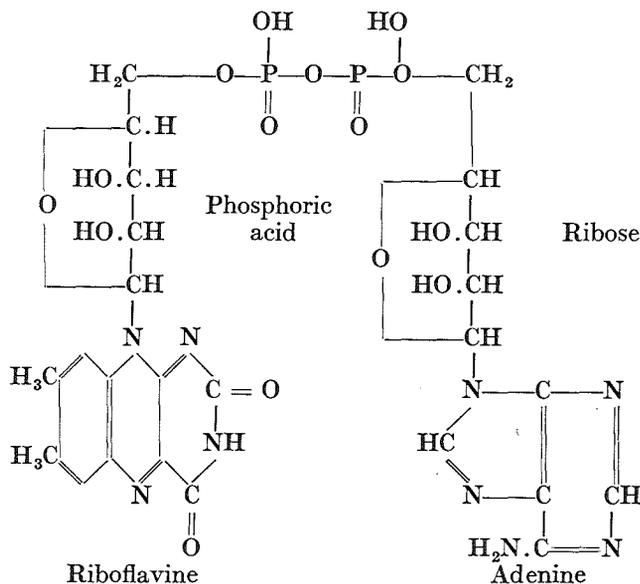
Food	Description	Micrograms of Riboflavine per 100 grams = 3½ ozs.
<i>Fish—continued.</i>		
Herring	Muscle	104
	Roe	385
Kippers	—	300
Lobster	Muscle	130
Mackerel	Muscle	200-330
	Roe	1,140
Oyster	—	130-460
Prawn	—	110
Salmon	Whole	160-220
	Tinned	160-180
Sardines	Whole	190-330
	Tinned	110-180
Shrimp	—	160
Trout	Roe	680
Tuna	—	130
Turbot	Muscle	137
<i>Miscellaneous</i>		
Ale	—	390
Beer	—	50-170
“Bemax”	—	1,050
Chocolate	—	290
Cocoa	—	390
Coffee	—	70
Honey	—	0-40
Ice cream	—	150-190
Jam	—	20
Macaroni	—	80
Malt	—	560
“Marmite”	—	5,300-6,500 (Av. 6,000)
Molasses	—	0-160
Royal jelly	—	820
Syrup	—	10
Tea	—	350
Yeast	Baker's	2,500-4,000 ; 3,500-8,000 ; 7,400
	Brewer's	1,800-3,000 ; 4,200
	“dried”	5,450
	“D.C.L.”	5,000
	<i>Torulopsis utilis</i> (food yeast)	5,000-9,000
	“Aluzyme”	4,500

PHYSIOLOGY OF RIBOFLAVINE

Riboflavine and Flavoprotein Enzyme Systems. In 1932 Warburg and Christian [1] isolated from yeast a yellow oxidation enzyme, which was subsequently shown by the researches of Kühn [31] and Theorell [32] to consist of an enzyme-like protein component closely associated with a flavin phosphoric acid, capable of undergoing reversible oxidation and reduction. Warburg [33] showed that the flavin was identical with riboflavine. Riboflavine phosphoric acid forms with a specific protein the “yellow oxidation enzyme” of Warburg, which was formerly thought to play an important part in tissue respiration. Although there may be some doubt about the function of this enzyme in living tissues, there is no question about the importance of other flavo-protein enzymes containing riboflavine such as D-amino acid oxidase [35], xanthine oxidase, succinic acid dehydrogenase [160, 161], and diaphorase [162], which act in isolated enzyme systems.

The flavoprotein enzymes containing riboflavine are co-enzymes linked to

an apoenzyme, which is a specific protein. There are two different types of riboflavine co-enzymes, namely, mononucleotides and dinucleotides. The mononucleotide is a riboflavine phosphate and the dinucleotide a riboflavine-adenine-dinucleotide. Many riboflavine co-enzymes have been discovered, the properties of which are dependent on the protein apoenzyme with which the riboflavine containing prosthetic group is conjugated. Riboflavine-adenine-dinucleotide is :



The riboflavine-containing enzymes form part of a cycle for the transference of hydrogen. The various reactions catalysed by these enzymes are given in the following table :

Enzyme	Hydrogen Donor	Hydrogen Acceptor
Warburg's yellow enzyme [31-33] .	Reduced codehydrogenases I and II.	Oxygen. Cytochrome <i>c</i> .
Cytochrome <i>c</i> reductase	Reduced codehydrogenase II.	Cytochrome <i>c</i> . Oxygen.
Diaphorase I	Reduced codehydrogenase I.	Cytochrome <i>a</i> and <i>b</i> .
Diaphorase II	Reduced codehydrogenase II.	?
Diaphorase [162]	Reduced codehydrogenases I and II.	?
D-Amino acid oxidase [35]	D-Amino acids	Oxygen.
Aldehyde oxidase [70]	Aldehydes	Oxygen-methylene blue.
Xanthine oxidase [163]	Xanthine	Oxygen.
Glucose oxidase	Glucose	?
Succinic dehydrogenase [160, 161] .	Succinic acid	?
Fumaric acid oxidase [165]	Reduced dyes	Fumaric acid.

Warburg's yellow enzyme probably plays little part in cellular respiration because of its turnover number. This is the number of times per minute that the enzyme can accept hydrogen from the substrate and transport it to the next acceptor in the series. In one cycle the turnover number of Warburg's yellow enzyme is 50, compared with the figure of 8,000 for some other riboflavine enzymes.

Riboflavine enzymes or flavoproteins form part of the system for the metabolism of carbohydrate. This also involves codehydrogenases I and II,

also known as co-enzymes I and II, which are complexes of nicotinic acid amide, adenine, ribose and phosphoric acid (p. 339). The carbohydrate substrate, e.g. lactic acid, is oxidized by dehydrogenation through the nicotinic acid enzymes, codehydrogenases I and II, which are reduced to the dihydro compound. These in turn are oxidized by the riboflavine enzymes, which are at the same time reduced to the dihydro compound. The reduced riboflavine enzymes are then re-oxidized by specific reactions involving loss of hydrogen. Thus the hydrogen may react directly with oxygen or it may react indirectly through the cytochromes *a*, *b* or *c*. The following scheme has been suggested for the oxidation of lactic acid to pyruvic acid, before the oxidation of the latter to carbon dioxide and water [164].

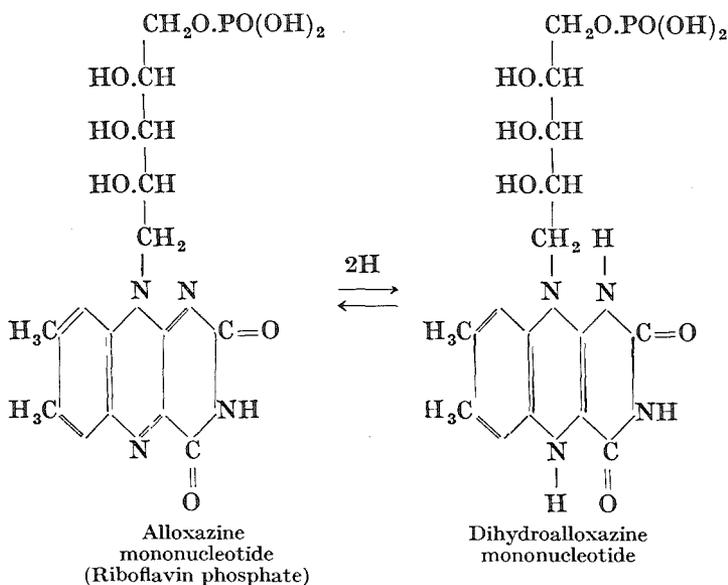
- I Lactic acid + lactic dehydrogenase + codehydrogenase I
→ pyruvic acid + reduced codehydrogenase I.
- II Reduced codehydrogenase I + flavoprotein
→ codehydrogenase I + reduced flavoprotein.
- III Reduced flavoprotein + cytochrome *b*
→ flavoprotein + reduced cytochrome *b*.

In the chain of the oxidative change of lactic acid *via* pyruvic acid to carbon dioxide and water it is probable that all the three vitamins, riboflavine, nicotinic acid and aneurine are essential, and that the absence of any one of them may interfere with the process.

The general scheme for the oxidation of a metabolite through the agency of one of the nicotinic acid co-enzymes and flavoprotein is as follows :

1. Substrate + enzyme + co-enzyme → Oxidized substrate + enzyme
+ reduced co-enzyme
2. Reduced co-enzyme + flavoprotein → co-enzyme + reduced flavo-
protein.
3. Reduced flavoprotein + X → flavoprotein + reduced X.

X is a hydrogen acceptor, e.g. cytochrome. The reduction and oxidation of the flavoprotein is supposed to occur by the addition and removal of hydrogen from the iso-alloxazine ring at positions 1 and 10 :



The fact that metabolically active tissues such as liver, kidney and heart muscle are rich in bound riboflavine, i.e. flavoprotein, suggests that riboflavine

plays the rôle of a respiratory catalyst [3]. It probably plays a part in the metabolism of muscle as even minute quantities (e.g. 10^{-5} mM per litre) can increase the work done by a contracting isolated muscle [285]. According to Leeman and Pichler [170] the riboflavin content of various parts of the brain is directly proportional to the rate of respiration. A fall in the alloxazine adenine dinucleotide content of the muscle, liver and brain occurs when an animal is subjected to hæmorrhagic shock [305]. The brain is the first tissue to suffer. A fall in the cocarboxylase content also occurs (p. 258).

Riboflavin may be involved in the formation of glucose and glycogen in the body. If rats deficient in riboflavin are exposed to a low oxygen tension they do not increase their liver glycogen to the same extent as normal animals; a normal response occurs if they are injected with riboflavin before the test. The blood glucose is also lower than normal in anoxic rats [244]. According to Drillhon [249] a rise of blood sugar occurs in riboflavin deficient rats; a fall is said to occur when riboflavin is injected into a normal rat [254].

Phosphorylation. Riboflavin must be phosphorylated before it can possess vitamin activity. This phosphorylation may occur as soon as it is absorbed from the intestinal wall, since preparations of intestinal mucosa can bring about the phosphorylation of riboflavin in presence of phosphates [36]. The phosphorylation of riboflavin probably occurs in the liver as well [3, 98], and human blood cells can synthesize both the phosphate and dinucleotide from riboflavin *in vitro* and *in vivo* [178].

According to De Preux [3] the liver not only stores riboflavin but also phosphorylates it, through the agency of the reticulo-endothelial system, since blockage of the latter reduces the amount of riboflavin phosphorylated, while stimulating it raises the degree of phosphorylation. There is evidence that other tissues, including muscle, can synthesize riboflavin dinucleotide [179].

Biosynthesis of Riboflavin. It has been clearly established that most micro-organisms including those in the rumen of herbivorous animals can synthesize riboflavin [106, 172]. Synthesis also occurs in the cæcum of the rat, but only under certain dietary conditions. Although riboflavin deficiency does undoubtedly occur in man, it has not always been reported in subjects receiving diets low in riboflavin. It has for example been reported in subjects on diets providing 1 to 1.28 mg. of riboflavin daily, while others have failed to observe it in subjects receiving less than 1 mg. daily (pp. 302, 303). The possibility that under certain conditions intestinal micro-organisms can synthesize riboflavin in man has been considered to account for this discrepancy. Najjar, Holt and their colleagues [297] have recently shown that in man the urinary and faecal excretion of riboflavin may exceed the intake. The faecal excretion in twelve subjects kept under observation for twelve weeks varied from 200 to 600 micrograms daily, although the intake was only 60 to 90 micrograms a day. The most likely explanation of this is that biosynthesis of riboflavin by the intestinal flora occurs.

Symptoms of riboflavin deficiency (angular stomatitis, red painful tongue and seborrhœic dermatitis) have been stated to occur in tuberculous patients undergoing treatment with streptomycin [102]. The symptoms disappeared after treatment with riboflavin. It is possible that streptomycin is excreted into the gut and destroys the organisms that synthesize riboflavin. If the intake of the latter is low symptoms of deficiency might then occur.

Riboflavin Deficiency in Animals. In the dog riboflavin deficiency causes bradycardia, cardiac arrhythmia, yellow mottling of the liver, degenerative changes in the central nervous system, collapse, and finally coma [49, 50]. These symptoms, which occur very rapidly, can be prevented by the administration of riboflavin. Pathologically, the most striking lesions occur in the liver and central nervous system, both of which are profoundly affected by changes in carbohydrate metabolism. Blood glucose and chloride fall and there is an eosinopenia [328]. The condition resembles that following adrenalectomy.

In the rat deprived of riboflavine the weight remains stationary ; the animal develops alopecia and an eczematous condition of the skin affecting specially the nostrils and the eyes, the rims of which become denuded of hair [123] ; and there is dullness of the cornea, blepharitis and conjunctivitis, the eyelids being stuck together with a serous exudate [51]. Water retention also occurs in the riboflavine deficient rat [46]. There is some evidence that the resistance of the rat to infection (e.g. typhus and leprosy) is lowered [52, 135] and that fertility is seriously impaired [136]. Increasing the fat level in a ration low in riboflavine has a deleterious effect on the growth of young rats ; the administration of adequate amounts of riboflavine completely corrects this deficiency [113]. These and other observations suggest some relationship between riboflavine and fat metabolism [124]. Rancid fat also accentuates the signs of riboflavine deficiency [333]. It has been shown by Warkany [61] that riboflavine deficiency in the rat may result in gross malformation of the offspring, e.g. syndactyly, microphthalmos, brachygnathia and cleft palate. Riboflavine is also essential for the nutrition of the pig, calf and monkey. Acute deficiency in the latter produces anæmia and a freckled dermatitis on the face, extremities and groin [238].

Riboflavine and the Eye. Conjunctivitis and keratitis occur in animals on riboflavine-free diets within seven to eight weeks, followed by a dullness of the eyeball and finally, according to some observers, opacity of the lens [38]. These eye lesions are no doubt due to defective metabolism in the lens and cornea following lack of the respiratory enzyme flavoprotein. The normal epithelium of the cornea of the rat has a high oxygen uptake, which falls if the animal is put on a diet deficient in riboflavine [296]. This fall in the oxygen consumption is probably due to necrosis of the epithelial cells of the cornea. Evidence for the rôle of riboflavine deficiency in the production of cataract is very conflicting. Day and other observers [38, 39] have described its occurrence in several species deprived of riboflavine, and its arrest in eighty-nine per cent. of the animals treated by the administration of riboflavine in doses of 120 micrograms twice weekly. Bourne and Pyke [65], however, could only induce cataract in twenty to thirty per cent. of their animals by depriving them of riboflavine, and György [66] observed no cataract in five hundred rats treated in this way. The problem was reinvestigated by Eckhardt and Johnson [67], who produced cataract in only two out of twenty-three rats kept on a diet poor in riboflavine and rich in galactose. The subsequent administration of riboflavine did not prevent the cataract from forming in the second eye. Baum and his co-workers [122] state that rats on diets completely devoid of riboflavine do not suffer from cataract ; they only do so if minute amounts of riboflavine are present, although a normal intake is non-cataractogenic. Riboflavine does not arrest the progress of lens opacities in the human eye [68].

The existence of a flavoprotein and a cytochrome-cytochrome oxidase enzyme system in the lens has been demonstrated [332]. These respiratory enzymes are more concentrated in the epithelium than the cortex or nucleus. The ratio of lactate and pyruvate concentrations in the various parts of the lens suggests that the metabolism of the epithelium is strictly aerobic while that of the cortex and nucleus is anaerobic.

Wolbach and Bessey [40, 41] state that corneal vascularization is an early, specific and most reliable criterion of ariboflavinosis, or riboflavine deficiency, in rats. They considered that this vascularization is a response to the respiratory needs of the corneal epithelium, in which oxidation occurs through the mediation of flavoprotein.

Riboflavine has been found in the retina of many species. Adler and Euler [181] consider that it plays some part in a light sensitive reaction because of the fluorescence of free riboflavine. They state that free riboflavine occurs in the fish retina and that it is therefore possible that in fish riboflavine acts as a photosensitizer by absorbing short-wave light and transmitting it

as light of longer wave length. In the mammalian retina, however, Pirie [180] was unable to demonstrate much free riboflavine; it is nearly all bound as riboflavine-adenine-dinucleotide, which is light stable. There is therefore no evidence that riboflavine acts as a photosensitizer in the mammalian retina. Heiman [176] believes that riboflavine is essential for the visual act and may be a factor in cone vision by functioning in the flavoprotein oxidation reduction system and by its power to intensify weak light. He accepts the unproven assumption that riboflavine acts as a photosensitizer in the retina. This, incidentally, is accepted by many writers as an established fact, although the only evidence for this is the work of Adler and Euler [181] on the frozen fish retina. According to Philpot and Pirie [250], practically all of the riboflavine in ocular tissues is present as the adenine dinucleotide, and is therefore not affected by light. They find that there is very little riboflavine in the cornea (0.2 microgram per gram), but much more in the lacrymal gland (6.5 microgram per gram). They therefore suggest that the riboflavine of the cornea is derived from the lacrymal secretions by diffusion.

Dimness of vision, impairment of visual acuity, photophobia, lacrimation, inability to see in a dim light, visual fatigue and corneal vascularization have been described as clinical manifestations of riboflavine deficiency (p. 313). Kimble and Gordon [44] have observed that individuals showing poor dark adaptation and a low vitamin A blood level, did not improve with vitamin A alone, but responded to the administration of both vitamin A and riboflavine. This observation may mean that riboflavine plays some part in the visual act; on the other hand it may play a part in the absorption and utilization of vitamin A. Pock-Steen [45, 78] observed that in 109 patients with incipient sprue and frank sprue many suffered from eye symptoms, the principal one being reduced visual acuity in dim light. This "twilight blindness," or aknephascopia, was considerably relieved by riboflavine, but not by vitamin A. The same observer also states that patients suffering from riboflavine deficiency show poor dark adaptation [158].

Riboflavine and Hæmatopoiesis. Miller and Rhoads [43] were able to produce in dogs a syndrome similar to sprue, a disease associated with a macrocytic anæmia, by feeding diets deficient in riboflavine. György and his co-workers [48] also noted that riboflavine causes a definite increase in hæmoglobin production above the basal level when fed to dogs in which anæmia was produced by a deficient diet. Rats kept on a riboflavine deficient diet develop leukopenia, granulocytopenia and sometimes anæmia, which is corrected by administering riboflavine [74]. Spector and his colleagues [182] have shown that dogs on a synthetic diet normal in all respects, but containing no riboflavine, develop a severe anæmia on slight bleeding. The dogs do not recover unless given riboflavine in a dosage of 30 micrograms per kilo daily, which appears to be sufficient for adequate hæmoglobin production. The anæmia produced by a deficiency of riboflavine was of the microcytic hypochromic type; when small amounts of riboflavine were given to the dogs after slight bleeding the anæmia was normocytic and hypochromic. Riboflavine also appears to play a rôle in determining the size of new blood cells according to Spector. Since *d*-amino-acids are deaminated by an enzyme system containing riboflavine-adenine-dinucleotide it is possible that riboflavine may be related to amino-acid metabolism and therefore possibly for the formation of blood proteins. Although Waisman [238] has proved that anæmia occurs in monkeys fed on diets deficient in riboflavine, in man there is no substantial evidence that riboflavine is essential for hæmatopoiesis; the anæmia in monkeys is not completely corrected by riboflavine, and only responds completely when pteroylglutamic acid is given [334]. Keys [28] failed to get any signs of anæmia in human volunteers kept for eighty-four days on a low intake of riboflavine. There is often an associated anæmia in human ariboflavinosis, but this responds to iron [289]. A deficiency of riboflavine reduces phagocytic activity [274] and causes a fall in the white count [28].

Riboflavine and Tumour Formation. Rats fed butter yellow (*p*-dimethyl-amino-azobenzene) or treated with it externally develop cancer of the liver. This is prevented by giving the rats riboflavine, which appears in such cases to have an anti-carcinogenetic effect [183]. This is of some interest, as the susceptibility of natives in the Far East and South Africa to primary carcinoma of the liver may be due to a dietary deficiency. The native diet in areas where these primary liver carcinomata are found is deficient in the vitamin B complex and riboflavine. The concentration of riboflavine in tumour tissue is low in comparison with that of normal tissues [184]. This is in keeping with the view that cancerous tissues have a deficient aerobic oxidation system. A diet rich in riboflavine increases the resistance to tumour formation in rats [258].

Riboflavine and Nitrogen Metabolism. In animals and in man there is a close correlation between nitrogen and riboflavine metabolism. An increase in the amount of protein ingested increases the riboflavine content in the liver of the rat [221]. In man the excretion varies inversely as the protein intake [234]. It would appear that riboflavine is released and excreted when reserve protein is depleted and stored when it is replenished. Thus normal persons will retain more than fifty per cent. of ingested riboflavine when in nitrogen equilibrium and less than fifty per cent. when in negative nitrogen balance [330]. If the latter is excessive, as after trauma or surgical procedures, and in untreated diabetes, more riboflavine is excreted than is ingested, but post-operatively, when the balance is positive, riboflavine is retained [144, 330].

Riboflavine Metabolism and Other Vitamins. Other vitamins appear to play some part in riboflavine metabolism. Thus the storage of riboflavine in the liver, which occurs in a normal animal after injecting the vitamin, is considerably influenced by the tissue stores of aneurine [173]. The riboflavine content of the tissues also falls considerably, mainly because of rapid excretion and poor absorption, in animals suffering from aneurine deficiency [174]. It has been argued that these changes in riboflavine metabolism are observed only in the terminal stages of aneurine deficiency and appear to be unspecific [175]. According to Delachaux [30] the metabolism of riboflavine is linked with that of aneurine, since administration of large quantities of the latter results in an increased excretion of riboflavine and *vice versa*.

Pantothenic acid appears to have a direct and specific function as part of the mechanism whereby riboflavine is stored in the liver after the ingestion of food [173].

Chemically Induced Riboflavine Deficiency. Vitamin deficiency can be induced in animals experimentally by feeding compounds structurally related to the vitamin. The symptoms of riboflavine deficiency in animals have been reported following the administration of 2 : 4 dinitro-7 : 8-dimethyl-10-ribityl-5 : 10 dihydrophenazine [284] and galactoflavine, in which the ribityl side chain of riboflavine is substituted by a dulcetyl [53]. Inversion of the hydroxyl groups in the side chain forms *d*-araboflavine, which is also a riboflavine antagonist in rats [58]. It is supposed that there is an active competition between the vitamin and its antagonist in an essential enzyme reaction and that the antagonist displaces the vitamin. 1 : 2-Dichloro-4 : 5-diaminobenzene can inhibit the formation of riboflavine by growing cultures of *B. megatherium* [326]. It is not an analogue, but 1 : 2-dimethyl-4 : 5-diaminobenzene may be a precursor of riboflavine and the dichloro-analogue may exert its selective action by inhibiting the biosynthetic process in which riboflavine is formed.

Pharmacology of Riboflavine. The pharmacology of riboflavine has been studied by Unna and Greslin [185], who state that in rats 10 grams per kilo and in dogs 2 grams per kilo orally fail to produce any toxic effects. The low solubility of the vitamin prevents its absorption from the gastrointestinal tract in amounts sufficient to produce toxic effects. Likewise the subcutaneous injection of doses of 5 grams per kilo produces no toxic

effects [81]. The sodium salt is more soluble and 300 mg. of this intraperitoneally is lethal in the rat. Death occurs within two to five days with signs of anuria due to renal blockage with crystalline concretions. The daily administration of 10 mg. of riboflavine to rats and 25 mg. per kilo to dogs for a period of four months produces no sign of toxic manifestations. The metabolism, circulatory and respiratory systems, and isolated smooth muscle organs of the animals are unaffected.

Absorption, Storage and Excretion of Riboflavine. Riboflavine is absorbed from the intestine and gastric hydrochloric acid is probably needed for its absorption. Phosphorylation is thought to occur in the intestine, although it can take place in the liver, blood and tissues. Riboflavine is not readily phosphorylated or absorbed in patients with gastro-intestinal disease; it is only utilized in such patients if it is injected [134]. The bulk of the riboflavine in the body is stored in the liver, heart and kidneys. Rats maintained on a low protein diet are unable to store the normal amount of riboflavine in the liver [287]. On the other hand, deficiency of aneurine causes increased storage of riboflavine in the liver [304]. If there is a considerable increase in intake there is only a slight increase in the amount stored in the liver; and even if the animal dies for want of riboflavine the quantity present in the liver, kidney and heart is only about a third of normal [54]. The body therefore appears to cling tenaciously to its stores of riboflavine. Human blood contains about 0.5 microgram per gram [111]. Much of it is in combination with the euglobulin of the plasma [300]. The free riboflavine in plasma is 0.84 ± 0.71 micrograms per 100 ml. [335]. Destruction of riboflavine in the body occurs, but to what extent is unknown. If riboflavine is injected there is an immediate concentration in the liver [57, 173]. The riboflavine content of the latter also increases during digestion and assimilation, being mobilized from other tissues [173].

When given intravenously the bulk is excreted into the small intestine, particularly the duodenum, from which it is reabsorbed [186]. It is largely destroyed in the large intestine, and some destruction also occurs in its passage through the kidney.

Certain factors influence the excretion of riboflavine. Thus in man the administration of large doses of aneurine, e.g. 1 to 10 mg., over a period, increases the excretion of riboflavine in the urine, although such dosage does not produce clinical riboflavine deficiency [187]. Chronic aneurine deficiency increases urinary excretion considerably in rats [288]. The protein intake also influences riboflavine excretion [188, 316]. An increased protein intake produces a diminished excretion and increased retention of riboflavine. If the carbohydrate fat ratio of the diet is low less riboflavine is excreted [316]. The excretion of riboflavine in rats is increased in experimental hyperthyroidism [189], and in man the excretion falls after exercise [286]. Cayer and Cody [110] found no difference in the riboflavine excretion of patients suffering from acute infections and various chronic diseases, except in patients with peptic ulcer who consumed large quantities of milk. Increased retention and decreased excretion occur as a result of hæmorrhage, infection and trauma (fractures, burns) [144, 171, 294] and in pregnancy [172]. The diminished excretion after severe burns parallels the severity of the injury and the upset of nitrogen metabolism [171].

Riboflavine is also excreted in the sweat. The estimates of the amount lost in this way are variously given as from 5 to 120 micrograms per litre and 10 micrograms per hour [193, 195]. Even accepting the highest figure of 120 micrograms per litre, this only corresponds to three per cent. of a good intake and is, therefore, a negligible loss. According to Sargent, Robinson and Johnson [278] there is no free riboflavine in sweat.

Riboflavine is excreted into the milk; on a daily intake of 3 mg. about 0.3 mg., or ten per cent., is excreted in the milk [112]. After delivery it is low (68 micrograms in twenty-four hours) but increases later [172].

If the diet is adequate, riboflavin is excreted in the urine in the form of uroflavin, a pigment almost identical with it in composition, properties and vitamin activity. There is a fairly general dependence of uroflavin excretion upon riboflavin intake [109].

Emmerie [55], who has studied the riboflavin excretion in man, estimates that the urinary output of flavin is from 819 to 1,250 micrograms daily. Strong [111] puts it at from 500 to 800 micrograms a day on a normal diet, and 50 to 150 micrograms on an intake of 1 to 2 mg. a day. This agrees fairly closely with the figures of Feder, Lewis and Alden [302], who found that the daily excretion was 500 to 1,000 micrograms, with an average of 800 micrograms, on a daily intake of 2 to 3 mg. According to Connors, Eckhardt and Johnson [192] the average riboflavin excretion of a group of normal subjects was 1,032 micrograms. Keys and co-workers [273] state that on an intake of 0.31 mg. per 1,000 calories daily, the average daily excretion is twelve per cent. of the daily intake. On an intake of 1.6 mg. daily the excretion is twenty-five per cent. of this; it drops to ten per cent. on a daily intake of 1.1 mg. [331]. An increased intake is reflected in an increased excretion; when the intake is considerably decreased, there is an increased retention and decreased excretion. In pregnancy on a low intake, e.g., 1.75 mg. daily, the urinary excretion is about seventeen per cent. of the intake; this rises with an increased intake and reaches seventy to eighty per cent. on an intake as high as 7 mg. daily [167]. For the first four days after birth excretion exceeds intake [312].

Riboflavin deficiency cannot be detected by a single determination of a twenty-four-hour specimen of urine. It fluctuates with the food intake and with the type of food eaten, e.g. liver. According to Sastri and his co-workers [316] the percentage of riboflavin excreted varies from forty to two hundred and fifty per cent. of the intake and bears no relation to the latter. Actually it is possible by placing human subjects on a diet low in riboflavin to depress the excretion to zero without evidence of clinical riboflavin deficiency [126]. Coryell and her co-workers [325] state that the fasting hour excretion in boys is 45 micrograms, and 38 micrograms in girls.

Axelrod, Spies and Elvehjem [109] have carried out "saturation tests" on human beings with riboflavin employing intravenous injections of 200 to 400 micrograms of riboflavin per kilo of body weight, but they were unable to detect any correlation between the amount of the test dose of riboflavin retained and the daily urinary riboflavin excretion. Sebrell and co-workers [119] state that in a condition of riboflavin depletion there is a close relationship between riboflavin intake and excretion. Axelrod, Spies and Elvehjem [109] observed that injected riboflavin was rapidly excreted in the urine, from thirty to forty per cent. being excreted after a test dose of 200 micrograms per kilo of body weight. They were also able to produce an uncomplicated riboflavin deficiency in the dog, in which the degree of retention of a test dose of riboflavin was found to be a measure of the riboflavin deficiency [125].

Another saturation test to detect human riboflavin deficiency has been devised by Najjar and Holt [126]. They inject 1 mg. of riboflavin intravenously after an overnight fast and follow the urinary excretion for half-hourly and hourly periods over four hours following the injection. Normally a marked excretion occurs in the first half hour, and during the second and subsequent hours the excretion falls off rapidly, approaching that of the initial control period. From thirty-two to seventy-two per cent. of the dose is retained. In riboflavin deficiency the excretion is much less, from eighty-one to ninety-three per cent. being retained in the four-hour test. As the weight of the individual also influences the excretion Najjar and Holt suggest a dose of 0.016 per kilo rather than a flat dose of 1 mg. for all subjects. Coryell and her co-workers [325] found the mean four-hour urinary excretion after a test dose of 1 mg. to be 351 micrograms for boys and 344 micrograms

Urinary Excretion of Riboflavine before and after Test Doses

Author	Subjects	Daily Dietary Intake of Riboflavine mg.	24 Hours Urinary Excretion mg.	Test Dose	Excretion After Test Dose
Emmerie [55, 56].	Normal.	?	0.952 0.885	5.71 mg. 4.24 mg.	3.28 mg. 1.20 mg.
Sebrell, <i>et al.</i> [119]	Normal. Ariboflavinosis.	2.54-3.68 0.5	0.793-1.265 0.024-0.119	5 mg. 2 mg.	50-80% Excretion rose from 34 micrograms to 1.48 mg. on 20th day.
Axelrod, <i>et al.</i> [109]	Ariboflavinosis.	0.3 per 2,000 cal.	0.058-0.091	0.2-0.4 mg. per kg. i.v.	30-40% excreted after 1 hour.
Najjar and Holt [126].	Normal.	?	0.236-0.270	1 mg. i.v.	?
	Normal. Ariboflavinosis.	?	0.3-0.68 in 4 hours 0.074-0.194 in 4 hours.	1 mg. i.v.	32-72% retained. 81-93% retained.
Swaminathan [190]	Normal.	Very low. 1.2-1.5	0.32-0.36	1-10 mg. orally.	80-85% excreted.
Swaminathan and Verma [191].	Ariboflavinosis.	0.4-0.5	0.05-0.2	5 mg.	10-20% excreted.
Connors, <i>et al.</i> [192]	Normal.	?	1.03	1 mg. i.v.	38.6% retained.
	Patients with rosacea keratitis.	?	0.62	5 mg. i.m. 1 mg. i.v. 5 mg. i.m.	21.5% retained. 21.3% retained. 47.5% retained.
Williams, <i>et al.</i> [200].	Ariboflavinosis.	0.76	0.06-0.15	2 mg. s.c.	0.103-0.348 mg. after 4 hours.
Harris and Scoular [159].	Normal.	1.3 1.25-2.5	0.46-1.8 36-51% of intake.	5 mg.	0.311-1.155 mg. 24-44% excreted in urine.
Goth [243].	Normal.	?	—	3-5 mg.	30-50% of test dose in 2 hours.
	Deficient.	?	—	3-5 mg.	Less than 30% of test dose in 2 hours.
Sastri, <i>et al.</i> [316].	Normal.	0.76-1.91	0.4-1.977	1 mg.	4.4-85.3% excreted.
Ruffin, <i>et al.</i> [62].	Normal.	?	0.8	5 mg.	3.3 mg. in 24 hours.
	Deficient.	?	0.4	5 mg.	Less than 2.2 mg. in 24 hours.

for girls. The excretion of riboflavine in a group of institution children in America was 0.032 to 0.055 mg. hourly; after a test dose of 1 mg. it rose to 0.273 to 0.387 mg. hourly [166].

Feder, Lewis and Alden [302] state that saturation tests give no more information on the level of riboflavine nutrition than do single riboflavine estimations on a fasting morning specimen of urine.

The results of test doses in the urinary excretion of riboflavine are given above.

The amount of riboflavine excreted in the faeces is determined largely by the degree of intestinal synthesis, which is affected by the nature of the diet but is largely independent of its riboflavine content. In man the faecal excretion is greater than the urinary, and on a low intake (e.g. 0.36 mg. a day) three times the intake [63]. The nature of the diet is important. When human volunteers were kept on a diet containing 1.33 mg. of riboflavine a day, the riboflavine being alternatively provided by a natural diet and a

synthetic one, the faecal excretion on the former was eighty-four per cent. of the intake, while on the latter only twenty-eight per cent. [64]. Carbohydrates such as dextrin, corn starch and lactose favour the development of the intestinal bacteria that synthesize riboflavine [69]. The work of Czaczkcs and Guggenheim [71] suggests that on a low protein diet the ability to store riboflavine in the tissues is reduced and urinary excretion is increased. The faecal excretion of riboflavine appears to be proportional to the number of viable bacteria in the faeces and presumably to the amount of riboflavine they synthesize. According to Oldham and co-workers [72] the urinary excretion of riboflavine in man varies inversely with the nitrogen balance. When the balance is positive forty to sixty per cent. is excreted; when it is negative it is only seven per cent. The faecal excretion is unaffected.

To what extent the riboflavine in the faeces is absorbed is unknown. According to Najjar and Barrett [108] most of the riboflavine synthesized by intestinal bacteria is retained by the bacteria and excreted in the faeces; the riboflavine is only liberated after the bodies of the bacteria disintegrate. Faecal excretion varies considerably from person to person, although the amount excreted by any one individual is fairly constant despite dietary variations [283]. According to Sastri and his co-workers [316] considerable quantities of riboflavine may be derived from the faeces, the urinary excretion in some cases being as much as 250 per cent. of the intake in the food.

HUMAN REQUIREMENTS OF RIBOFLAVINE

The requirements of riboflavine have been calculated from (a) an analysis of normal diets, (b) from the intake of riboflavine by subjects suffering from ariboflavinosis (riboflavine deficiency), (c) from studies with experimental diets, (d) from excretion studies.

Dietary surveys have been made by a number of observers [59, 104, 197, 275]. According to these surveys the riboflavine intake of the average adult varies from 1.1 to 2 mg., although a good many persons in low income groups probably receive no more than 0.7 to 0.8 mg. A survey of the diets of English munition workers made in 1943 revealed that the average daily intake of riboflavine was 1.1 to 1.3 mg. [207]. If a diet deficient in riboflavine is consumed over a period, symptoms of ariboflavinosis develop (p. 305). Many observers have attempted to note the dietary intake of riboflavine that just produces such symptoms, and they assume that a figure slightly above this represents the minimum riboflavine requirement [119, 277, 290]. Some workers state that deficiency symptoms do not occur on intakes of 1.5 mg. or more daily, but appear on an intake of 0.3 to 0.5 mg. [119, 198], although this has been disputed [199].

Experimental diets have been administered to volunteers to see if symptoms of ariboflavinosis appear [60, 119, 199, 200, 227, 273]. Deficiency symptoms have been reported on diets containing from 0.5 to 0.55 mg. daily [60, 119, 260, 331], although some workers have not confirmed this [168]. Williams and his colleagues [200] and Keys and his co-workers [273] failed to observe deficiency symptoms on a daily intake of 0.31 to 0.35 mg. of riboflavine per 1,000 calories, or 0.9 mg. for the sedentary man. In Keys' study tests included work on a treadmill, an anaerobic work test, a glucose tolerance test, tests of muscle power and psychomotor tests. Blood studies and slit-lamp examination of the eye showed no abnormality. The average urinary excretion was twelve per cent. of the intake. Friedemann and his co-workers [227] also kept volunteers on a daily intake of 0.95 mg. riboflavine for five to seven months without observing any signs of deficiency. All the subjects remained in good health. According to Hagedorn [168] there is no correlation between the riboflavine intake and the appearance of lesions characteristic of ariboflavinosis. In all these studies it is possible that the period of deprivation was too short. Horwitt and co-workers [260] produced the typical

facial and scrotal lesions of ariboflavinosis in subjects kept on a daily riboflavine intake of 0.5 mg. for periods ranging from four to ten months. It would appear that an intake of 0.5 mg. of riboflavine daily is a critical level at which deficiency symptoms may occur after several months.

Excretion studies have been employed to calculate the riboflavine requirements of man [55, 56, 111, 119, 169, 200, 203, 260, 283, 291]. The daily intake of riboflavine that causes neither a progressive decrease nor increase in excretion when graded amounts are administered to subjects on a basal diet is taken to be the normal requirement. Some workers accept an arbitrary figure for the excretion of a test dose of riboflavine as evidence of a satisfactory intake. Oldham and her co-workers [291] accept an excretion of twenty per cent. of a test dose in fours as adequate.

Author	Daily Requirement Calculated from Excretion
Snyderman, <i>et al.</i> [169]	0.4 mg. (infants weighing 6-9 kg.).
Oldham, <i>et al.</i> [291]	0.5 mg. per 1,000 cal. (children 2-5 years).
Sebrell [119]	0.04-0.05 mg. per kg. (adults).
Williams, <i>et al.</i> [200]	0.5 mg. per 1,000 cal. (adults).
Strong [111]	1-2 mg. minimum (adults).
Brewer, <i>et al.</i> [203]	1.3-1.5 mg. per 2,100-2,300 cal. (adults).
Horwitt, <i>et al.</i> [260, 331]	1.1-1.6 mg. (adults).

These figures range from 1 to 3 mg. for an adult, the most recent figures being between 1 and 1.5 mg. This is approximately the range found by other methods.

The following are the recommended daily allowances of riboflavine suggested by the Food and Nutrition Board of the National Research Council, U.S.A. [1948]:

	Daily ^{est} Riboflavine Requirements in mg.	Calories
Man (70 kg.):		
Moderately active	1.8	3,000
Very active	1.8	4,500
Sedentary	1.8	2,400
Woman (50 kg.):		
Moderately active	1.5	2,400
Very active	1.5	3,000
Sedentary	1.5	2,000
Pregnancy (latter half)	2.5	2,400
Lactation	3.0	3,000
Children up to 12 years:		
Under 1 year	0.6	110/2.2 lb.
1-3 years	0.9	1,200
4-6 "	1.2	1,600
7-9 "	1.5	2,000
10-12 "	1.8	2,500
Children over 12 years:		
Girls, 13-15 years	2.0	2,600
16-20 "	1.8	2,400
Boys, 13-15 "	2.0	3,200
16-20 "	2.5	3,800

These figures for the riboflavine requirements of man may need revision in the light of work on the human biosynthesis of riboflavine (p. 295). To what extent this normally occurs in man is not known, nor is it known how much is absorbed.

The Nutrition Committee of the British Medical Association (1950) has suggested the following daily allowances of riboflavine :

	Daily Allowance of Riboflavine in mg.
Children up to 1 year	0·6
„ 2- 6 years	0·9
„ 7-10 „	1·2
Adult male doing very light work (2,250 cal.)	1·4
„ „ „ light work (3,000 cal.)	1·8
„ „ „ medium work (3,500 cal.)	2·1
„ „ „ heavy work (4,250 cal.)	2·6
„ „ „ extra heavy work (5,000 cal.)	3·0
Female 11-14 years	1·6
„ 15-19 „	1·5
„ 20+ „	0·9
„ adult (2,000 cal.)	1·2
„ doing light work (2,500 cal.)	1·5
„ „ heavy work (3,000 cal.)	1·8
„ „ very heavy work (3,750 cal.)	2·2
Pregnancy, first half	1·5
„ second half	1·6
Lactation	2·1

It is assumed from animal studies that the daily requirement of riboflavine in man depends upon sex, age, degree of physical activity and calorie intake, and is increased in pregnancy, lactation and fever. Requirements are increased as a result of physical exercise [47] and a high fat diet [57]. Ingestion of large amounts of nicotinic acid by patients on a deficient diet is said to increase riboflavine requirement [115]. Mills [202] has shown that the requirements of riboflavine are independent of environmental temperature (cf. aneurine). In traumatic conditions (fractures) and after severe burns the riboflavine requirement may be increased as there is marked retention and diminished excretion of the vitamin [144, 171].

THE RIBOFLAVINE DEFICIENCY SYNDROME. ARIBOFLAVINOSIS. DISEASES ASSOCIATED WITH RIBOFLAVINE DEFICIENCY

Historical. The essential features of riboflavine deficiency, or ariboflavinosis, were described as far back as 1911 by Stannus [84], who clearly described a group of symptoms including soreness of the tongue and lips, with a sodden excoriated condition at the angles of the mouth (which he termed angular stomatitis) and palpebral fissures, and a characteristic lesion at the free border of the prepuce, the vulva and anus, together with a dermatosis of the scrotum, often spreading to the skin of the adjacent thighs. Stannus described the smooth tongue devoid of papillæ and denuded of epithelium—the so-called magenta tongue of ariboflavinosis—some twenty-five years before riboflavine was discovered. He knew that these lesions were the result of dietary deficiency but beyond that he could not go as the vitamins had not then been differentiated. Shortly afterwards, in 1918, Scott [139] described a condition among Jamaican coolies that showed some of the symptoms described by Stannus and in addition a central neuritis, photophobia, indistinctness of vision, ulceration and discharge of the eyelids and a burning sensation. A few years later Goldberger and Tanner [245] also gave an account of these symptoms, which they produced experimentally in American prisons. They noted that the condition was cured by autoclaved yeast. In 1930 Fitzgerald Moore [246] confirmed the observations of previous workers and added retrobulbar neuritis as part of the syndrome (p. 315),

which he stated was common in West Africa, West Indies and Malay, and was responsible for the blindness of thousands of natives. The dietary origin of the syndrome was confirmed by curing it with marmite, a source of the vitamin B complex. Moore [247] later showed that neither vitamin A nor nicotinic acid deficiency played any rôle in the ætiology of the syndrome. Landor and Pallister [248] completed the clinical picture by describing the neurological symptoms—pains, tingling and weakness of the legs, rombergism, exaggerated knee and ankle jerks, diminished sensation in the feet, and defects in the touch, pain and deep sense pathways. These were also curable by marmite or liver. In 1938 Sebrell and Butler [82] published their observations on induced riboflavine deficiency in man.

Incidence of Ariboflavinosis. Judging from the literature riboflavine deficiency is relatively rare in the British Isles. It has been described here by Duckworth [138], Deeny [204] and Scarborough [205], but the number of cases recorded is few. In certain parts of America, particularly the Southern States, it is stated to be common. Thus Goldsmith [206] states that forty to sixty-seven per cent. of hospital patients in Louisiana show some evidence of riboflavine and nicotinic acid deficiency. A medical survey of Newfoundland in 1944 revealed that riboflavine deficiency was comparatively common in that country [34].

According to Farber and Miller [223] riboflavine and nicotinic acid deficiency is common in tuberculous patients. Lesions stated to be characteristic of riboflavine deficiency were observed in twenty-five per cent. of 400 patients in an American sanatorium. Riboflavine deficiency is very common among the Chinese according to Hou [105], and from the literature it would seem to be common in India [85, 198, 235, 236] and among African natives [84, 143, 201]. Braun, Bromberg and Brzezinski [129] have reported the presence of riboflavine deficiency among pregnant women in Israel; the condition improved after delivery and after administration of riboflavine.

Jones and his co-workers [277] noted that in a camp of over 10,000 men of mixed races in North Africa, some 1,746, or seventeen per cent., showed

Clinical Manifestations attributed to Riboflavin Deficiency (Ariboflavinosis)

Lips	Tongue	Skin		Eyes	Neurological
		Face	Elsewhere		
Cheilosis Angular stomatitis. Vertical fissuring. Crusting of lips. Stomatitis. Burning of lips. Redness and desquamation of lips.	Glossitis. Magenta tongue. Flattened papillæ and epithelium. Burning tongue. Fissured tongue. Dysphagia. Patchy oval desquamation.	Seborrhœic der- matitis on alæ nasi, nasolabial folds, eyelids and ears. Shark skin eruption. Comedones. Capillary dilatation. Flushing of face.	Vaginitis. Dry itching dermatitis of hands, scrotum, vulva and anus.	Roughness of the eyelids. Lacrimation. Photophobia. Blurred vision. Poor vision. Visual fatigue. Phlyctenular conjunctivitis. Impairment of visual acuity. Burning of the eyes. Twilight blindness. Circumcorneal injection. Corneal vascu- larization. Retrobulbar neuritis. Blepharospasm. Opaque corneal infiltrates. Nebulæ. Corneal epithelial dystrophy. "Rosy eyes," Rubeosis. Mydriasis. Iritis. Pigmentation of the iris.	Paræsthesia, particularly in legs. "Burning feet." Exaggerated knee and ankle jerks. Rombergism. Partial optic atrophy. "Cerebellar syndrome." Ataxia. Muscular weakness. Nystagmus. Vertigo. Tremor. Clonic contractions. Dysdiado- kokinesis. Dysmetria. Mental apathy

signs of ariboflavinosis. The outstanding features were stomatitis and cheilosis; the lesions responded to riboflavine therapy in a selected group of cases.

Lesions of Lips. In 1938 Sebrell and Butler [82] induced a deficiency syndrome in eighteen women, who were given a diet complete in all respects, except that it was deficient in the vitamin B complex. Daily supplements



FIG. 85. Lips and Tongue in Ariboflavinosis. The case shows considerable erosion at the corners of the mouth (angular stomatitis). The lips are denuded, red and cracked and covered with crusts of blood. The tongue is fiery red and cracked.

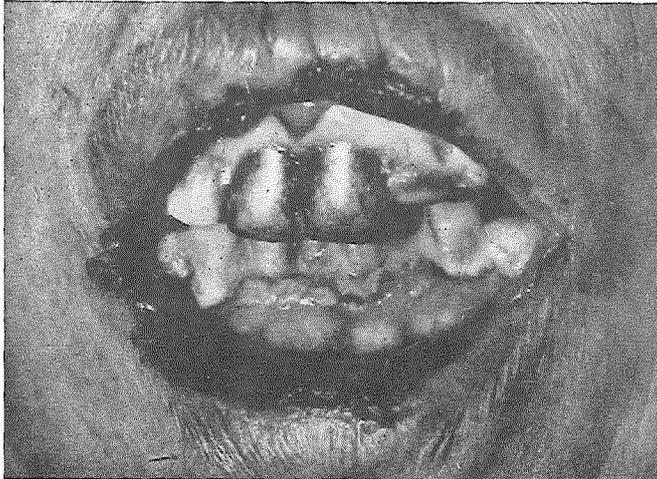


FIG. 86. Ariboflavinosis, showing Cheilosis. A case showing cheilosis with denuded lips and blood crusts. There is also considerable hyperplasia of the gums suggesting scurvy.

of aneurine were given to eliminate any deficiency symptoms of this vitamin. Within ninety-four to 130 days ten of the women developed cheilosis,* which began as a pallor of the mucosa of the lips in the angles of the mouth, and was followed by maceration and bilateral transverse fissures (Fig. 85). The lesions, which remained moist and became covered with a honey-coloured crust

* There has been some confusion over the term cheilosis and angular stomatitis, which some writers use interchangeably. Cheilosis is a lesion of the vermillion of the lip, angular stomatitis the lesion at the muco-cutaneous junction at the corner of the mouth.

(Fig. 86), were identical with those described by Stannus [84] under the name angular stomatitis and by earlier workers. The fissures extend 1 to 3 mm. on to the mucous membrane of the mouth and up to 10 mm. on the skin. They are usually shallow, but may be 0.5 mm. deep. The lips became abnormally red along the line of closure and showed a marked increase in vertical fissuring, due to superficial denudation of the mucosa (Figs. 85 and 86). Sebrell and Butler also described a fine, scaly greasy desquamation on a mildly erythematous base in the nasolabial folds, on the *alæ nasæ*, in the vestibule of the nose and on the ears. This syndrome was termed ariboflavinosis by Sebrell and Butler and hypo-riboflavinosis by Stannus [240]. It is identical with the *pellagra sine pellagra* and the *formes frustes* of pellagra described by Stannus and others between 1911 and 1935. Further cases resembling those of Sebrell and Butler were described by Jolliffe and his co-workers [89].

Sebrell and Butler treated four of the ten volunteers with 1 to 2 mg. of synthetic riboflavine daily for three to ten days and then with doses corresponding to 0.025 mg. per kilo of body weight. This was later increased to 0.05 to 0.075 mg. per kilo [87]. All the lesions disappeared in from five to forty-seven days, but in controls treated with 100 mg. of nicotinic acid daily the cheilosis was definitely worse. It cleared up in the controls, however, when they were given 0.25 mg. of riboflavine per kilo of body weight. One woman had the typical skin lesions of pellagra and cheilosis. After thirty days' treatment with nicotinic acid the pellagrous lesions healed, but not the cheilosis, which became worse, although it rapidly yielded to riboflavine in a few days. Jolliffe's cases cleared up with daily doses of 5 mg. [89].

Sydenstricker [86] and his colleagues have also described five patients who showed evidence of pellagra or were pellagrins and presented lesions corresponding to those described by Sebrell and Butler. The cheilosis and fissures in the corners of the mouth disappeared when riboflavine was given in rather large doses of 20 to 75 mg. a day orally, or 10 to 50 mg. parenterally; the riboflavine seemed more effective parenterally than orally. In every instance response to riboflavine was relatively slow, and in the presence of an inadequate diet nicotinic acid given concurrently seemed to have no adjuvant effect. Particular interest was aroused in two cases in which dermatitis, cheilosis and conjunctivitis appeared to be cured by riboflavine.

Although Kruse and Horwitt and co-workers [91, 260] were able to duplicate Sebrell and Butler's results, some doubt has been cast upon the experimental production of pure riboflavine deficiency. Thus Boehrer, Stanford and Ryan [199] were unable to observe manifestations of riboflavine deficiency in volunteers on a daily riboflavine intake of 0.47 mg. The experiment only lasted for five weeks; possibly a longer period than this is necessary before symptoms appear. Williams and his collaborators [200] also failed to reproduce the oral and facial lesions described by Sebrell and Butler. They kept volunteers on diets providing 0.875 mg. of riboflavine daily for a period of ten months without observing any signs ascribed to riboflavine deficiency. Clinical examination of the volunteers showed no abnormal findings, and the following laboratory tests were normal: serum calcium, phosphorus and protein; blood lipids; blood counts; blood glucose, pyruvic acid; gastrointestinal motility as shown radiologically; urinalysis; and slit lamp examination of the eyes. Machella and McDonald [208] doubt the existence of the ariboflavinosis syndrome. They have treated twenty patients with lesions attributed to the syndrome with riboflavine without success. It is possible that not only riboflavine deficiency but deficiency of other factors of the vitamin B complex plays a part in producing the syndrome known as ariboflavinosis [240].

Validity of Angular Stomatitis * as a Manifestation of Riboflavine Deficiency. Angular stomatitis is not a specific sign of ariboflavinosis and may occur

* This is referred to as cheilosis in much of the literature cited. It is preferable to retain cheilosis for the lesions of the vermillion of the lips.

independently of the latter. Spies [107, 210] has shown that it commonly occurs in children and that *Staphylococcus aureus* and *Streptococcus hæmolyticus*



FIG. 87. Ariboflavinosis. The lids, particularly the lower, of both eyes are macerated and stuck together. There are also long wide fissures at the angles of the mouth (angular stomatitis).



FIG. 88. Ariboflavinosis. The case shows dermatitis of both eyelids, extending from the margin of the lids to 3 to 6 mm. outwards with a dark red discoloration and papular lesions and crusts of exudates scattered over the lesion. The lesions responded to treatment with riboflavine and healed in six days.

can frequently be cultured from the fissures. The condition often develops in children who dribble or constantly lick because of the abnormal amount of moisture at the corner of the mouth, whence the name *perlèche*, from *lecher*,

to lick. One of the authors has also observed fissuring at the corners of the mouth in two cases of Parkinsonism, in which there was no question of nutritional deficiency, and in which the condition was undoubtedly due to drooling of the saliva. Ellenberg and Pollack [211] have observed deep granulomatous fissures at the corners of the mouth with no involvement of the lips and glossodynia in thirty-four patients with no history of any nutritional defect and in whom laboratory studies showed no sign of avitaminosis. There was no response to intensive riboflavine therapy, either by mouth or parenterally. The cause of the lesion was eventually traced to badly fitting dentures causing mal-occlusion in thirty-two of the patients and mechanical defects in closure of the jaws in the remaining two patients. The skin at the corners of the mouth is constantly moist and becomes macerated and infected. Other writers have also observed angular stomatitis due to badly-fitting dentures and local trauma which did not respond to riboflavine, but disappeared when the dentures were changed. There is also evidence that angular stomatitis may be associated with such diverse conditions as hypochromic anæmia [213, 214], sensitivity to the constituents of chewing gum [215], denture plastics [209] and the dye in lipstick [218]. Other causes that have been incriminated are plastic cigarette holders, some toothpastes, antiseptic lozenges, moustache wax, cosmetics and the chewing of tobacco. Angular stomatitis may also occur in chronic illnesses, especially arthritis, and as a result of treatment with antibiotics such as penicillin, aureomycin and chloramphenicol [324]. It is probable that angular stomatitis due to iron deficiency is more common than that due to riboflavine [213].

Numerous other reports show that the relationship of angular stomatitis to ariboflavinosis is not as clear cut as originally suggested and that as a symptom of the latter it is non-specific. Machella and McDonald [127, 208] failed to improve thirteen cases of angular stomatitis by treatment with riboflavine. Some responded, however, to nicotinic acid, vitamin B₆ and the entire vitamin B complex given as brewers' yeast. Smith and Martin [216] also noted that angular stomatitis may disappear after the administration of vitamin B₆. The observation of Machella [127] that hæmorrhagic lip lesions may respond to ascorbic acid suggests that some cases are scorbutic. Youmans [222] also found that some cases of angular stomatitis were refractory to treatment with riboflavine.

Dermal Lesions of Ariboflavinosis. In the experimental production of ariboflavinosis Sebrell and Butler [82, 87] noted in addition to the labial changes a seborrhœic dermatitis of the face present on the alæ nasi, nasolabial folds, eyelids and ears. They were described in greater detail by Jolliffe [89], Spies [90], Sydenstricker [86] and others. One of the first descriptions of this seborrhœic dermatitis in pellagrins was given by Stannus [84]. The facial lesions consist of filiform excrescences of a seborrhœic nature, apparently derived from sebaceous glands, varying in length up to 1 mm., closely and sparsely scattered over the face. Although the characteristic location is in the nasolabial folds the excrescences occur on the alæ nasi, the bridge of the nose, above the eyebrows, about the ears and other parts of the body (Fig. 89). The lesion has the appearance of a seborrhœic dermatitis on an erythematous base; the skin over the excrescences is fine, scaly, greasy and desquamating. The skin over the nose has a "shark skin" appearance. The extruded sebum becomes inspissated in the pores with the development of fine hair-like protruding comedones resembling the urea frost of uræmia, only it cannot be rubbed off. It is prominent over the nose, malar eminences and forehead. Seborrhœic changes are stated to be present in the more severe cases of deficiency. In many cases there is also a crusty superficially eroded lesion just inside the nares, and there may be a vertical fissure at the mucocutaneous junction (Fig. 89). The eyelids often show a dermatitis and may be macerated and stuck together [105], as shown in Figs. 87 and 88.



FIG. 89. Ariboflavinosis before Treatment. A pellagrin showing filiform seborrheic excrescences on the forehead, nose, cheeks, lips and chin, and around the nasolabial folds. There are also moist pale patches in both corners of the mouth with vertical fissures on the lips.

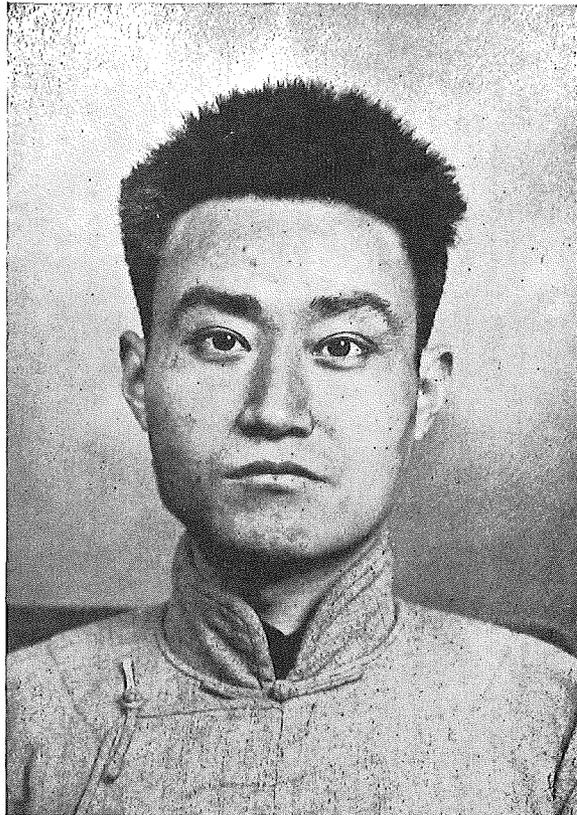


FIG. 90. Ariboflavinosis after Treatment with Riboflavine. The same patient as in Fig. 89 after treatment with riboflavine (15 mg. for the first two days, 10 mg. for the next seven to ten days, and then 5 mg. daily for another week). The lesions have disappeared.



FIG. 91. Lesions of Ariboflavinosis in a Pellagrin. Before treatment. There is a pellagrous dermatitis on the hands. At the corners of the mouth are fissures around which serous crusts have accumulated. The lips are dry and chapped and there are seborrhœic excrescences in the naso-labial folds, both sides of the nose and the bridge and in between the eyebrows. Photophobia is also present. These lesions are characteristic of ariboflavinosis.



FIG. 92. Ariboflavinosis in a Pellagrin. After treatment. The same patient as in Fig. 91 after treatment with riboflavine. The lesions have practically disappeared.

Sydenstricker [217] also mentions a dry brown itching dermatitis of the hands and scrotum or vulva associated with cheilosis and glossitis and responding to the riboflavine therapy. Purcell [143] describes a syndrome, which includes scrotal dermatitis and superficial glossitis and which disappears on treatment with riboflavine. Mitra [219] gives an account of an oro-genital syndrome among Indians, characterized by angular stomatitis, glossitis and scrotal dermatitis. The lesions did not respond to nicotinic acid, but did to yeast or 5 mg. of riboflavine daily. Some cases, however, appeared to do better on a mixture of riboflavine and nicotinic acid. As far back as 1911 Stannus [84] described a dermatosis of the free border of the prepuce, the vulva, anus and scrotum, spreading to the thighs, and he now thinks that this is part of the riboflavine deficiency syndrome [240]. Scrotal dermatitis is one of the commonest skin lesions.

Tongue Lesions of Ariboflavinosis. Jolliffe [89], Kruse [91] and their co-workers have described a specific type of glossitis associated with riboflavine deficiency and quite distinct from that attributed to nicotinic acid deficiency. The tongue in the latter may be sore before any changes are visible. Then desquamation of the epithelium of the lingual papillæ occurs, beginning at the tip and sides of the tongue and finally spreading over the entire dorsum. The tongue is scarlet, dry, atrophic and very painful. The tongue in ariboflavinosis on the other hand is described as magenta or purplish red in colour. It is clean and the epithelium does not desquamate over the papillæ, but is flattened and swollen. The papillæ are flattened or mushroom-shaped rather than atrophic, giving the tongue a pebbly or granular appearance. The tongue may be painful and burn when food is taken. Later irregular patchy denudation may occur giving rise to a condition known as "geographical tongue." Sydenstricker and his co-workers [115] have examined the papillæ microscopically and liken them to "a dead jelly fish that has been washed up on the beach . . . a round translucent hemisphere with the capillaries lying deeply in a loose coil." The scarlet tongue of pellagra or nicotinic acid deficiency owes its colour to desquamation of the epithelial cells of the papillæ; the capillary loops then become more readily visible. In the magenta tongue of ariboflavinosis the capillaries dilate and proliferate and possibly the circulation is slowed. This may account for the magenta colour of the tongue in contrast to the pink colour of the normal tongue.

Weisberger [220] describes a type of glossitis in riboflavine deficiency which does not correspond to the generally accepted picture. He states that the glossitis is characterized by a primary lingual coating followed by a patchy oval desquamation with an atrophic centre and raised edges. Weisberger states that this type of glossitis responds to treatment with riboflavine. Sydenstricker [115] noted a magenta-coloured glossitis in pellagrins on diets poor in riboflavine treated with nicotinic acid. The tongue returned to normal on exhibiting riboflavine. The tongue changes are not reversed by riboflavine in long-standing cases of deficiency.

Rosenblum and Jolliffe [221] record that some patients suffering from vitamin deficiencies still had glossitis even when treated with both nicotinic acid and riboflavine, and that it did not return to normal until the whole vitamin B complex and vitamin B₆ were given. Machella and McDonald [208] failed to improve six patients with the so-called magenta tongue of ariboflavinosis by giving them riboflavine. Some did improve when yeast and vitamin B₆ were given.

Painful tongue (glossodynia) is not always due to riboflavine or other vitamin deficiency. It is often due to iron deficiency, excessive smoking, irritating foods and drink, electrogalvanic currents produced by dissimilar metallic dentures, and neurogenic and psychogenic causes [226]. It may be the only symptom of thrombosis of a small intracranial vessel (Alvarez). Smith [37] describes a number of cases of fissured tongue with changes in the epithelium and papillæ that did not respond to treatment with riboflavine

and other B vitamins. Glossitis may also occur in pernicious anæmia, sprue and pellagra and as a complication of oral treatment with antibiotics such as chloramphenicol and aureomycin [324].

Ocular Manifestations of Ariboflavinosis. In 1939 Spies and his co-workers [90, 228] observed in patients suffering from malnutrition an ocular lesion, characterized by bulbar conjunctivitis, lacrimation, burning of the eyes and failing vision, that was cured by administering riboflavine. At the same time Sydenstricker and his colleagues [86] in a study on riboflavine deficiency noted that conjunctivitis and photophobia were prominent symptoms. In the same year Pock-Steen [45] described "twilight blindness" in patients with sprue or incipient sprue that was relieved by riboflavine and not by vitamin A. Many of these patients also suffered from ocular conditions such as reduced visual acuity, conjunctivitis, keratitis, and mydriasis, which were attributed to riboflavine deficiency. The work of Bessey and Wolbach [41] and Eckhardt and Johnson [67] in 1939 showed that the earliest sign of riboflavine deficiency in the rat is corneal vascularization. This was followed



FIG. 93. Ariboflavinosis. A case showing injected vessels in the conjunctivæ, corneal opacities and phlyctenules at the margin of the cornea.

in 1940 by the papers of Kruse, Sydenstricker and their colleagues [91, 92, 115] on the ocular changes of riboflavine deficiency in man. They stated that corneal vascularization was a constant finding. It is now known that this is not pathognomonic of riboflavine deficiency, although it may occur in cases of ariboflavinosis (p. 317).

Conjunctivitis. Gross injection of the vessels of the bulbar and fornix conjunctivæ have been described in subjects with riboflavine deficiency [91-93]. This has been referred to as "conjunctivitis," although no infection was present (Fig. 93). Hou [105], who has seen many cases in China, adds phlyctenular conjunctivitis as a symptom of ariboflavinosis. He states that in China the ocular lesions are more commonly seen than the facial and oral ones.

It is stated that in India many patients with angular stomatitis and other signs of ariboflavinosis also suffer from angular conjunctivitis of the Morax-Axenfeld type [293]. This conjunctivitis, as well as the other signs of ariboflavinosis, is reported to have disappeared after treatment with 3 to 5 mg. riboflavine daily, although sometimes doses of 40 mg. daily were needed.

Photophobia. Photophobia (Figs. 87, 88, 91) was observed in forty-three out of forty-seven patients with ariboflavinosis examined by Sydenstricker [92].

Johnson and Eckhardt [93] and Hou [105] also found that it was a prominent symptom in their patients. Itching, burning, blepharospasm, a sensation of roughness of the eyelids, lacrimation, mydriasis, blurred vision, inability to see in a dim light, and visual fatigue are also described as common in patients with ariboflavinosis [91, 92, 115]. These lesions clear up in twenty-four to forty-eight hours after giving riboflavine.

Diminished Visual Acuity and Eye Strain. Diminished visual acuity was observed in twenty-nine of forty-seven patients studied by Sydenstricker [92]. It was also noted in subjects with ariboflavinosis by Johnson and Eckhardt [93] and by Hou [105]. The symptoms occurred in the absence of errors of refraction or opacity of the lens. Impaired visual acuity may result from such symptoms as burning of the eyes, lacrimation, blepharospasm, corneal opacities and iritis, which are all stated to occur in ariboflavinosis. Vitamin A deficiency may cause some of these symptoms, and it is quite likely that a diet deficient in riboflavine—that is, lacking an adequate quantity of milk, eggs, green vegetables and whole grain—is also deficient in vitamin A. The work of Kimble and Gordon [44] suggests that riboflavine may be necessary for the proper utilization of vitamin A. They found that some subjects with poor dark adaptation did not respond to treatment with vitamin A unless riboflavine was given as well. It is possible that the diminished visual acuity, photophobia and “twilight blindness” considered to be associated with riboflavine deficiency may be in part due to interference with the regeneration of visual purple in the rods and cones. Sydenstricker and others [92] report considerable improvement in visual acuity in their cases treated with riboflavine.

“Eye strain” has been stated to fall into this group. Pett [229] states that of a group of 232 persons doing close work and with many complaining of eye strain, thirty-seven per cent. showed signs of riboflavine deficiency. Twenty-eight were given 3 mg. of riboflavine daily for three months and sixteen showed much improvement. However, a similar number in a control group showed improvement on a placebo. This illustrates the importance of suitable controls in studies of this kind.

Corneal Opacities. Bessey and Wolbach [41] in their studies on riboflavine deficiency in rats noted opaque infiltrates into the cornea from the edge of the limbus, and Sydenstricker and his co-workers [92] observed corneal opacities in eighteen of his forty-seven cases of ariboflavinosis. Superficial nebulæ, seen by the naked eye as a slight “steaminess” and on slit-lamp examination as a fine superficial diffuse opacity, were noted in all these cases and superficial punctate opacities in two of them. Interstitial nebulæ and posterior punctate opacities were sometimes seen, although rarely. Johnson [130] noted corneal ulcers appear over these opaque infiltrates in severe cases of ariboflavinosis. These corneal opacities are probably caused by infiltration of the corneal epithelium and substantia propria with leucocytes. With riboflavine therapy these corneal nebulæ and ulcers heal, often completely, but if untreated the opacities become permanent and scar tissue may form from corneal ulcers. According to Sydenstricker [92] the interstitial nebulæ clear more rapidly than the superficial ones, and the posterior nebulæ disappear last of all. It must not be forgotten that a deficiency of other vitamins may result in corneal lesions. Thus vitamin A deficiency may cause corneal opacity, scarring of the cornea and even perforation in severe cases (p. 73), and corneal lesions are also seen in animals on diets deficient in pantothenic acid, tryptophane, pyridoxine and the amino-acids isoleucine and valine [224, 255].

A deficiency of vitamin A and riboflavine may in fact occur in the same individual. Verma [232] describes a syndrome which appears to combine the manifestations of a deficiency of both these vitamins and which is characterized by partial degeneration of the optic nerve, phrynoderma, sore mouth, night blindness, xerosis, conjunctivitis, photophobia and impairment

of vision. The condition responds to treatment with shark-liver oil, a potent source of vitamin A, and yeast, a source of the vitamin B complex including riboflavine. The oral lesions cleared up with riboflavine alone.

Spies and his co-workers [42] observed corneal ulceration and infected exudates in riboflavine deficient subjects and state that ocular symptoms may occur independently of cheilosis and other symptoms.

Cataract. Conjunctivitis and keratitis occur in animals on diets free from riboflavine, followed by dullness of the eyeball and finally, according to some observers, opacity of the lens, although the latter observation has been doubted (p. 296). In Sydenstricker's series [92] cataract was observed in six, but they were all elderly patients and it is difficult to say whether the cataract was the result of riboflavine deficiency. According to Wagner, Richner and Karbacher [68] riboflavine does not arrest the progress of cataract in the human eye. A deficiency of other factors may cause cataract in the experimental animal, e.g. tryptophane [230]. In man it cannot be considered as yet that cataract is a result of ariboflavinosis.

Iritis. Sydenstricker and his co-workers [91, 92] observed severe iritis in four out of forty-seven of their cases, and mild iritis, characterized by moderate congestion of the iris with accumulation of pigment on its anterior surface, in somewhat under half of the cases. In light-coloured irises the pigment appeared as dark clumps of "hazel spots"; in brown irises the pigment caused "veiling of the normal architecture." As these changes disappeared with riboflavine therapy they were considered to be part of the ariboflavinosis syndrome.

Rubeosis Iridis. This is a peculiar non-inflammatory vascular proliferation affecting the iris and mostly seen in diabetics, although diabetes is not an essential factor in its causation. Festoons of newly formed blood vessels are seen on the surface of the iris, and in some cases the condition appears more particularly in the sphincter region, where the vessels anastomose to form a network encircling the pupil. In other cases patches of anastomosing vessels are seen in the periphery. Stannus [233] suggests that rubeosis is a manifestation of riboflavine deficiency, as he has cured the condition with riboflavine in doses of 5 mg. daily. After forty-eight hours the vascular network on the iris is difficult to see, and after a week completely disappears.

Nutritional Amblyopia. Métivier [128] has given an account of ocular manifestations observed in Trinidad which he considers are due to riboflavine deficiency. One he calls "tropical nutritional amblyopia," which is the nutritional "retrobulbar neuritis" of Moore [246, 247]. Stannus [84]. Landor and Pallister [248] and Scott [139], later termed "malnutritional amblyopia" by Stannus [292], who described it first in 1911. Many of the earlier writers on pellagra, e.g. Stambio of Milan (1794), were aware of some of these eye symptoms. There is failure of central visual acuity, diminution in the size of the visual fields, dimness of vision, flickering of images, disappearance of images, difficulty in recognizing objects and persons, rings and haloes about lights at night, disturbances of colour vision, scotomata, pallor of the temporal halves of the discs, and partial optic atrophy. Some patients describe how objects come into vision and then disappear; others say they can only see parts of printed words. These symptoms are attributed to failure of the nutrition of the optic nerve or the retinal elements and, according to more recent writers, are the result of ariboflavinosis, since recovery from some of the symptoms occurs on administration of 4 to 5 mg. of riboflavine daily [128]. Foods rich in the vitamin B complex, such as wheat germ, yeast and marmite, are also effective in causing improvement. Wilkinson and King [256, 301] have described a deficiency syndrome seen in Hong Kong in 1940 with amblyopia as a predominant symptom accompanied by soreness of the tongue, angular stomatitis, giddiness, weakness of the limbs, temporal pallor of the discs, acroparæsthesiæ, scrotal eczema and swelling of the ankles. Visual acuity was reduced in some cases to finger counting at 3 feet within a

few weeks of onset. The condition cleared up with yeast and dietetic measures and also with nicotinic acid and riboflavine, although the authors regarded the syndrome as a result of nicotinic acid deficiency. The amblyopia, however, cleared most rapidly when the patients were given riboflavine. Thus 3 mg. of riboflavine daily brought vision from 6/60 to 6/9 or 6/6 in a week or ten days. It was also found that a full well-balanced diet helped to restore visual acuity. The complete syndrome is more likely to be due to a deficiency of riboflavine and protein rather than of nicotinic acid.

Métivier [128] claims that he has observed 192 cases of a condition hitherto unrecorded which he calls essential corneal epithelial dystrophy. It is characterized by a faint greyish-white disturbance in the corneal epithelium made up of fine points like dots and commas, and it runs typically in a double line transversely across the cornea at the level of the lower part of the pupillary areas. It stains with fluorescein or Bengal red. At times the double line is incomplete, but minute prolongations usually extend above and below it; in some cases almost the whole of the corneal surface is covered with faint greyish-white points. Other symptoms associated with this eye condition are photophobia, lacrimation, pallor or atrophy of the temporal half of the optic disc, burning and numbness of the feet, cheilosis, dry parched skin, sore tongue and "rosy eyes." The last-mentioned is an apple-pink injection of the ocular conjunctiva exposed in the interpalpebral area with dilated vessels that run into the limbus. Recovery occurs from the epithelial dystrophy and rosy eyes after two weeks' treatment with 5 mg. of riboflavine daily, and only after five to twelve weeks when foods rich in the vitamin B complex are given. The burning and numbness of the feet were only relieved by aneurine.

A number of papers were written in the immediate post-war period on nutritional amblyopia seen in prisoners of war, mainly those in Japanese hands. The observations were carried out by physicians many of whom had never left their homeland before, and, deprived as they were of access to the literature, most of them believed they were describing a new syndrome. They added little to the clinical descriptions of the earlier workers on the subject; being prisoners of war themselves and living under terrible conditions, it is remarkable that they kept such good clinical records. Among the authors are Spillane and Scott [78], Talbot [79], Smith [88], Reed [212], Smitskamp [252], Wilcockson [239] and De Raadt [259]. De Raadt describes Pick's visions, or partial visual vertigo, in which some part of the visual field moves in relation to the surrounding objects, e.g. letters may appear to be detached from a word or a person may appear to move into an adjoining room through a wall.

Snow-blindness. According to Tisdall [237] snow-blindness may be due to riboflavine deficiency. He examined 400 Indians in Hudson Bay and noted that five per cent. were blind. The blindness he attributed to riboflavine deficiency because the diet is poor in riboflavine and because the light reflected from the snow, being very strong, causes local destruction of riboflavine in the eye. Pure riboflavine is destroyed by ultra-violet light, but in the eye it is present as riboflavine-adenine-dinucleotide, which is not inactivated by light [250].

Corneal Vascularization. In 1940 Sydenstricker, Sebrell, Cleckley and Kruse [91, 92] examined the eyes of forty-seven patients with riboflavine deficiency and noted vascular changes in forty-five of them. They stated that the earliest and most common sign of ariboflavinosis is circumcorneal injection (Fig. 95), that is, proliferation and engorgement of the bulbar conjunctival capillaries of the limbic plexus. The lesion, if not grossly visible, is seen on slit-lamp examination. They describe the earliest change as marked proliferation and engorgement of the limbic plexus with the production of great numbers of narrow capillary loops, which outline the extreme margins of the scleral digitations and obliterate the narrow avascular zone

between the plexus and the sclerocorneal junction. They state that if untreated the lesion progresses to corneal vascularization within a short time, but rapidly regresses if treated with riboflavine. In untreated cases the cornea is described as being invaded first by very small capillaries arising from apices of loops surrounding the scleral digitations and lying just beneath the epithelium. They soon anastomose to form a tier of loops from which more capillaries develop and extend centripetally to form secondary arcade capillary loops. The process of anastomosis and loop formation proceeds until extensive vascularization of the cornea results (Fig. 95). The capillaries are empty at first but fill with red blood cells in a few days. Many of the vessels are so small that they are invisible to the naked eye or with a loupe but are visible under the corneal microscope or slit-lamp. The Sydenstricker school believe that these eye lesions are due to riboflavine deficiency because they clear up when riboflavine is administered; they are present in persons living on diets poor in riboflavine; the administration of other vitamins has no effect; the lesions are associated with cheilosis and glossitis; and similar lesions are produced in animals kept on diets deficient in riboflavine.

Bessey and Wolbach [41], from animal observations, state that corneal vascularization is an early and specific sign of riboflavine deficiency and occurs as a compensatory mechanism to bring the blood into closer contact with the corneal epithelium. This being normally avascular contains no haemin compounds, and Wolbach and Bessey assume that probably the oxidation of metabolites within it occurs through the agency of the riboflavine enzyme system, the riboflavine diffusing in from the limbic plexus. They believe that in ariboflavinosis blood vessels grow into the cornea in an attempt to overcome the local anoxia by bringing available riboflavine into closer proximity with the corneal cells. This view has been widely accepted. The work of Philpot and Pirie [250], however, makes this explanation unlikely. They have shown that whereas the cornea contains less riboflavine than any other ocular tissue (0.2 microgram per gram), the lacrymal gland contains as much as 6.5 micrograms per gram. They therefore suggest that the cornea receives its riboflavine from the lacrymal secretions rather than from the blood of the limbic plexus.

Validity of Circumcorneal Injection and Corneal Vascularization as Manifestations of Riboflavine Deficiency. After the observations of Sydenstricker and his colleagues in 1940 on the eye symptoms of ariboflavinosis, circumcorneal injection and corneal vascularization were accepted as certain diagnostic signs of the condition and were used in nutrition surveys as an index of riboflavine nutrition. Since 1942, however, many investigations have shown that neither of these signs is diagnostic of riboflavine deficiency, although they may occur in riboflavine deficient subjects.

Stannus [240], who has examined between four thousand and five thousand eyes by slit-lamp microscopy for signs of ariboflavinosis, states that there is not a narrow avascular zone between the limbic plexus and the corneo-scleral junction, and that he has seen both circumcorneal injection and corneal vascularization without any evidence of riboflavine deficiency. He states that the area between the limbic plexus and the corneo-scleral junction may appear avascular because the blood vessels may be constricted and empty. They become visible, however, on using drugs such as dionine, which dilate the vessels. The limbic plexus, which is a capillary bed and liable to great variation within physiological limits, becomes congested and engorged on the slightest provocation. Engorgement occurs in all varieties of conjunctivitis, in those whose eyes are exposed to heat and dust, cold wind, bright light, mild infection, chemical irritants and even by rubbing the eye [241, 242]. The "proliferation" of the limbic vessels is thus only apparent because vessels which were once empty and invisible become filled with blood and visible. Hence circumcorneal injection is not pathognomonic of riboflavine deficiency.

The work of Scott [257], Ferguson [242], Gregory [241], Stannus [292, 240] and others has conclusively shown that corneal vascularization can occur in the absence of riboflavine deficiency. Anderson and Milam [77] in a nutritional survey of over one thousand individuals, both black and white, found



FIG. 94. Normal Eye. There is no proliferation of the limbic vessels and no penetration of cornea by blood vessels.

no correlation between corneal invasion and the level of riboflavine in the diet. Corneal vascularization may occur in riboflavine deficient subjects and is then associated with photophobia, impaired vision, itching and a feeling of grittiness in the eyes. The limbic plexus is congested and there is a bilateral symmetrical superficial vascularization of the cornea, in which small vessels grow inwards from the marginal loops of the limbic plexus under the corneal

epithelium in a regular arcade all round the cornea and extend towards the centre. According to Stern [311] riboflavine deficiency always causes corneal vascularization if it is continued long enough. He believes that the condition may be precipitated by conditioning factors such as chemical or mechanical



FIG. 95. An eye showing circumcorneal injection. This was claimed by Sydenstricker to be an early sign of ariboflavinosis. It is not pathognomonic.

trauma to the cornea in the presence of a subliminal riboflavine deficiency.

Some other conditions in which corneal vascularization may occur are vitamin A deficiency [41], tryptophane deficiency [230, 255], injury to the corneal epithelium by chemical irritants, diseases causing pannus, such as trachoma, phlyctenular keratitis and any superficial keratitis [241]. The instillation of a simple irritant, such as five per cent. soap solution, into the

conjunctival sac may cause collapsed afunctional blood vessels in the cornea to become engorged [279]. Another observation that requires some explanation is that of Boehrer, Stanford and Ryan [199], who failed to observe corneal vascularization in volunteers on diets low in riboflavine (approximately 0.5 mg. daily), yet it appeared in a control subject receiving 3.5 mg. of riboflavine daily.

Neurological Manifestations of Riboflavine Deficiency. Workers in the English colonies who wrote before the recognition of riboflavine as a factor in human nutrition described certain neurological symptoms which did not fit into the clinical picture of beriberi or pellagra. Stannus [240] is inclined to believe that they are the result of riboflavine deficiency, with the possibility that lack of another factor of the B complex may be involved. Scott [139], during an investigation of natives in Jamaica, included a central neuritis and a burning sensation of the feet in his symptomatology. This burning sensation of the feet is also mentioned by other writers, such as Landor and Pallister [248], who also observed pains and weakness of the legs, rombergism, exaggerated knee jerks, "stocking" anaesthesia and retrobulbar neuritis followed by partial optic atrophy. According to Spillane and Scott [78] optic atrophy occurred in forty per cent. of prisoners of war on diets deficient in riboflavine. Reference is also made to the "burning feet" syndrome (also called "happy feet," "electric feet," and nutritional melalgia) by a number of workers who observed the condition in prisoners of war between 1941 and 1945 [252-259]. The appreciation of touch, temperature and deep sensation were also affected, and in late cases the authors remarked that the symptoms resembled those of subacute combined degeneration of the cord. Grande Covián and Jiménez Garcia [251], writing in 1940 after the Spanish Civil War, noted the association of glossitis and cheilosis with loss of visual and auditory acuity, paræsthesiæ, a burning pain in the soles of the feet and ataxia. Peraita [318] calls the burning extremities seen in deficiency states "the paræsthetic-causalgia syndrome," which was also seen during the Spanish Civil War. Moore [246, 247] observed that in his early cases complaining of sore lips and tongue there were no changes in the fundi, but later pallor of the temporal halves of the discs was noted, in some optic atrophy, in others signs of optic neuritis. Central scotomata and some retraction of the visual fields were also present. This was also noted by Wilkinson and King [256, 301]. The nervous symptoms that Moore described were mental dullness, muscular weakness, ataxia and paræsthesiæ.

Smitskamp [252] and De Raadt [259] state that otoneurological symptoms may occur, such as tinnitus, vertigo, nerve deafness, hyper-excitability of the vestibular apparatus for cold-water stimulation, and loss of tonal differentiation.

Stannus [240] accepts these symptoms as manifestations of riboflavine deficiency, and to them he has added others which he has observed. These fit into a condition resembling the "cerebellar syndrome"—muscular asthenia and hypotonia, ataxia, jerky clonic contractions, dysmetria, dysdiadokokinesis, nystagmus, vertigo, tremor, typical gait and pendulum knee jerks. The patient shows mental apathy, hyperemotionalism and a Parkinson-like facies. Cerebellar manifestations, including ataxia, dysmetria and decomposition of movement, have been noted by Ransome [303] in Singapore coolies who may have been suffering from ariboflavinosis.

Kwashiorkor. Hughes [299] believes that the underlying deficiency in kwashiorkor (infantile pellagra, malignant malnutrition (p. 363)) is ariboflavinosis. He points out that the epithelial lesions of kwashiorkor (glossitis, blepharitis, angular stomatitis, scrotal and vulvar dermatitis, and seborrhœic lesions of the face) resemble those of ariboflavinosis in the adult; histological appearances are also similar. The fatty liver and pre-terminal coma of kwashiorkor resemble experimental riboflavine deficiency in dogs. Hughes claims that the epithelial lesions improve within a few days of giving ribo-

flavine. It is unlikely that kwashiorkor results from a pure riboflavine deficiency; inanition, protein deficiency and dehydration probably play a part.

Pathogenesis of Ariboflavinosis. Stannus [240] believes that the varied lesions of riboflavine deficiency are a manifestation of an acute functional derangement of the capillary circulation of the affected parts. The first tissue to suffer from riboflavine deficiency is the endothelium of the capillary system. The capillaries undergo a reversible functional disturbance—a capillary dysergia—resulting in loss of tone and dilatation. Normal cellular metabolism is upset and tissue functions are disturbed. The first tissues to suffer are those with the largest number of capillaries, those whose metabolism is greatest, and those with a very specialized function. The interference with tissue cell metabolism is probably of the nature of an anoxia, using this term in its widest sense. If the anoxia is not too prolonged, recovery of function takes place in the capillaries when supplied with adequate riboflavine, otherwise irreversible processes leading to pathological changes occur. According to Stannus the skin lesions of ariboflavinosis, which occur where the skin is thin or highly specialized, and at the mucocutaneous junctions about body orifices (lips, palpebral fissures, nares, prepuce, vulva, anus, scrotum), are due to capillary congestion, with resulting impaired nutrition of the skin. The colour of the magenta tongue (p. 312) is the result of capillary dilatation and a sluggish blood flow. While Stannus grants that the changes in the cornea are due to anoxia following lack of riboflavine, he believes that the visual disturbances have no immediate connection with the eye, but are part of a lesion in the central nervous system. The neurological manifestations are the expression of a metabolic disturbance in nerve tissue produced by capillary dysergia, the more vascular tissues being the first to suffer, e.g. the grey matter and the cerebellar neurophyl. Stannus [253, 292] points out that the neurological lesions of ariboflavinosis are essentially affections of the sensory nerves.

Diagnosis of Ariboflavinosis. The diagnosis of ariboflavinosis is made on the history, clinical examination and response to treatment with riboflavine. If the condition does not rapidly respond to treatment with riboflavine, it is not ariboflavinosis. Laboratory tests have proved disappointing. Blood and urine studies have been made and saturation tests have been devised for the laboratory diagnosis of riboflavine deficiency, but they are of doubtful value and have not received general acceptance as normal standards have not been laid down (p. 300). Suvarnakich, Mann and Sure [335], however, believe that the riboflavine content of serum should be of value in the diagnosis of human riboflavine deficiency. They state that the serum level of free riboflavine and flavine adenine dinucleotide in normal subjects is 0.84 ± 0.71 micrograms and 2.32 ± 0.42 micrograms respectively per 100 ml. Nitrogen balance and protein intake do not affect these levels. It is generally accepted that ariboflavinosis cannot be determined by single estimations of the twenty-four-hour excretion of riboflavine [126]. It is also possible to depress the excretion of riboflavine to zero without producing evidence of deficiency symptoms [126]. Feder, Lewis and Alden [302] state that an excretion of 0.53 to 0.8 microgram of riboflavine per ml. of urine denotes an adequate intake, while values below 0.3 microgram per ml. denote a deficient intake of riboflavine. According to Johnson and his co-workers [314] excretion while fasting of less than 20 micrograms per hour is the lower limit of normal, and 200 micrograms the lower limit four hours after an oral test dose of 5 mg. Sinclair [315] gives an excretion of 30 micrograms per hour while fasting as abnormal, and 10 micrograms as denoting deficiency; he states that a blood level of less than 12 micrograms per 100 ml. also denotes deficiency. Lossy, Goldsmith and Sarett [329], however, have shown that riboflavine excretion is not diminished in patients suffering from clinical vitamin B complex deficiency.

In the clinical examination the characteristic seborrhœic facial lesions, the

glossitis, and the lesions in the region of the anus, vulva and prepuce are of diagnostic importance, but circumcorneal injection and corneal vascularization, although they may be present, are non-specific (p. 317). Angular stomatitis may be present, but it may be due to causes other than ariboflavinosis (p. 309). The neurological lesions of diagnostic value are burning feet, retrobulbar neuritis followed by partial optic atrophy, loss of visual acuity, sensory changes, and the cerebellar syndrome of Stannus (p. 320). A poor dietary history—one lacking meat, cheese, eggs, green vegetables, milk—is suggestive. Diagnosis may be assisted by the therapeutic test. If the lesions do not show some signs of resolution in a few days after giving 10 mg. of riboflavine daily, the condition is not due to riboflavine deficiency.

In the differential diagnosis of ariboflavinosis, pellagra, sprue, idiopathic hypochromic anæmia, subacute combined degeneration, disseminated sclerosis, and cerebellar lesions must be considered. Glossitis, lesions of the lips, anus, scrotum and vulva, burning feet, muscular weakness and retrobulbar neuritis may occur in pellagra. Angular stomatitis and skin lesions similar to those seen in ariboflavinosis have been reported in sprue and idiopathic hypochromic anæmia [103]. As Landor and Pallister [248] pointed out, the neurological symptoms in the late stages of what is now known as ariboflavinosis resemble those of subacute combined degeneration (paræsthesiæ of legs, ataxia, impaired appreciation of touch, pain and temperature, rombergism, exaggerated knee and ankle jerks, nystagmus, achlorhydria, glossitis). Disseminated sclerosis may also be confused with some of the neurological symptoms reported in ariboflavinosis (retrobulbar neuritis, misty vision, paræsthesiæ, muscle weakness, nystagmus, vertigo, emotional changes, exaggerated reflexes). The neurological manifestations listed by Stannus (p. 320) are almost identical with those of the cerebellar syndrome.

Treatment of Ariboflavinosis. Most cases respond to treatment with riboflavine in doses of 5 to 10 mg. by mouth daily. The average case responds to 5 mg. by mouth daily [115], although Jolliffe [89] prefers to give 50 mg. intramuscularly at the beginning of treatment for a few days, followed by 10 mg. daily by mouth. If the patient suffers from achlorhydria, vomiting, diarrhœa, hepatic disease or other disorder preventing absorption or utilization, the riboflavine is given parenterally in doses of 10 mg. of the sodium compound [92, 115]. Larger doses, although wasteful, are non-toxic [81]. Yeast in doses of 60 to 90 grams daily is also curative. Sydenstricker [86] warns against giving large doses of a single vitamin for long periods in the treatment of avitaminosis, as although it may cure the major manifestations of the condition under treatment, it may precipitate a deficiency of another member of the vitamin B complex (p. 240). He therefore gives other members of the B complex such as yeast, crude liver extract, wheat or rice bran extract with the riboflavine. Food yeast (*Torulopsis utilis*) is a rich source of riboflavine, and 10 grams a day is sufficient to eliminate riboflavine deficiency at a cost of one farthing a day [8]. In addition to vitamin therapy the diet must include generous amounts of food rich in the vitamin B complex, e.g. whole grain cereals, meat, milk, liver, eggs and cheese, otherwise the patient will relapse as soon as riboflavine is withdrawn.

Following the administration of riboflavine, photophobia, burning, itching and blepharospasm are relieved in twenty-four to forty-eight hours and visual acuity slowly improves. Oral lesions begin to improve in three days or so, but complete resolution may take weeks [91, 92].

The improvement in the glossitis can be followed by tongue prints. The tongue is wiped dry and covered with ink by means of an inking pad. Then stiff white glossy paper is placed on the tongue with a rolling motion and quickly removed. Serial tongue prints so obtained are valuable in following the type and progress of the glossitis (Figs. 111 to 120).

There is no information on the speed with which the neurological manifestations attributed to ariboflavinosis resolve.

Plummer-Vinson's Syndrome. This syndrome is characterized by glossitis, anæmia, dysphagia and achlorhydria and is practically confined to women. It has been ascribed to ariboflavinosis [142, 225] on account of some resemblance in the symptomatology of the two conditions, although there are conflicting reports on the value of riboflavine in the treatment of the condition [73, 306]. Pellagra has many symptoms in common with Plummer-Vinson's syndrome as well. Sjögren's syndrome (filiform keratitis and dryness of the mucous membranes) is believed by Franceschetti [101] to be due to riboflavine deficiency. The increased sedimentation rate and the frequent involvement of joints suggests, however, an infective ætiology.

Clinical Uses of Riboflavine

Apart from its use in the treatment of deficiency states the value of riboflavine in therapeutics has yet to be shown.

Johnson and Eckhardt [93] noted the resemblance between acne rosacea and the flushing of the face and prominence of capillary dilatation on the cheeks and nose of some of their patients with ariboflavinosis. They therefore suggested that acne rosacea is due to riboflavine deficiency, the persistent congestion and redness, telangiectases, seborrhœic hyperactivity with dilated follicles, and thickening and hyperactivity of the subcutaneous tissue being part of the physiological response of the blood vessels to deficient oxidative reactions (cf. Stannus' theory of capillary dysergia, p. 321). Johnson and Eckhardt claim that the condition responds to treatment with 1 to 2 mg. of riboflavine daily, although Sulzberger and Cope [313] were unable to confirm this. Johnson and Eckhardt further state that they isolated *Demodex follicularis* from the skin of rosacea patients and that they were able to infect with it the skin of riboflavine deficient rats, but not the skin of normal rats. They believe that the *Demodex* is a secondary invader of skin containing dilated capillaries.

Johnson and Eckhardt [93, 130, 192] state that rosacea keratitis is of nutritional origin. They noted that the diet of patients with this condition is low in milk, liver and eggs—good sources of riboflavine—and that many of them show some degree of riboflavine deficiency as judged by retention tests. Patients with rosacea retained 47.5 per cent. of a dose of 5 mg. given intramuscularly, compared with normal controls who retained 21.5 per cent. The validity of these tests is open to doubt. Conners, Eckhardt and Johnson [192] postulate that corneal disease may result from a dietary deficiency of riboflavine or from a disturbance of riboflavine metabolism. They claim to have successfully treated rosacea keratitis, marginal corneal ulcers and catarrhal corneal infiltrates with 1 mg. riboflavine intravenously daily, supplemented by a vitamin B complex preparation containing 830 micrograms of riboflavine per ounce, 1 ounce being taken orally three times a day.

There is a suggestion that psoriasis improves if patients are treated with riboflavine intramuscularly (5 to 10 mgm.) [336].

As Wise [270] points out, many factors, including riboflavine deficiency, which have been suggested as ætiological in rosacea keratitis, are probably not fundamental, although they may be contributory. He administered large doses of riboflavine to twenty-one patients with the condition, but failed to cure the eye lesions. Spontaneous remissions often occurred without any change in diet. Fish [271, 298] states that rosacea keratitis may occur without riboflavine deficiency and is not cured by treatment with it. A series of forty-five cases all of whom had cutaneous lesions as well failed to benefit from riboflavine therapy. Many of them became worse owing to the withdrawal of atropine during the test. Fish considers that neither acne rosacea nor rosacea keratitis are manifestations of ariboflavinosis and that most symptoms are due to secondary infection, which can be controlled by the sulpha drugs. She points out that the type of corneal vascularization seen in ariboflavinosis is not the same as that present in rosacea keratitis.

Grimsdale [95] has used riboflavine with apparently beneficial results in the treatment of corneal ulcers, photophobia and non-infective conjunctivitis, without any typical signs of ariboflavinosis. Rones and McKay [131] gave riboflavine to twelve patients with diverse chronic corneal lesions and without signs of riboflavine deficiency. The cases, which included phlyctenular keratoconjunctivitis, corneal ulceration, superficial punctate keratitis and sclerosing keratitis were all stated to benefit from the oral administration of 5 to 10 mg. of riboflavine daily, including cases which had not responded to previous local therapy. Stern and Landau [121] claim to have effectively treated several cases of eczematous keratitis with riboflavine; they consider that the condition results from a number of factors (infectious agents, mechanical irritation) associated with ariboflavinosis superimposed on a tuberculous diathesis.

Sydenstricker and his colleagues [91, 115] have observed several cases of syphilitic keratitis in which treatment with riboflavine was followed by rapid improvement during periods in which antisyphilitic treatment was not given. This has been confirmed by Cosgrove and Day [137] and Clark [140], although Wagener [100] failed to obtain any improvement. Kruse and Sydenstricker [91, 92] discuss the relationship of syphilis to ariboflavinosis and keratitis, and raise the question whether syphilis produces keratitis only when the nutrition of the cornea is impaired, as in ariboflavinosis.

Castellanos [276], accepting the erroneous assumption that strong light inactivates the riboflavine of the eye (p. 297), states that vernal conjunctivitis is caused by ariboflavinosis. Of one hundred and five patients treated with local anaesthetics, adrenaline and riboflavine, 1 to 3 mg. daily, he claims that ninety-two per cent. showed immediate improvement. Stern [106] confirmed this in the palpebral form with papillary hypertrophy, which improved under treatment with 5 mg. of riboflavine twice daily. As in neither case were the observations controlled and vernal conjunctivitis is a persistently recurrent condition, further proof is required of any association between it and ariboflavinosis.

Verma [293] observed that riboflavine is effective in the treatment of angular conjunctivitis of the Morax-Axenfeld type without giving any local treatment. Most of his patients were, however, suffering from symptoms of ariboflavinosis and the clearing of the conjunctivitis may well have been due to improvement in their general condition. Landau and Stern [118] noted improvement in patients with trachomatous pannus treated with riboflavine; these were also patients with symptoms of ariboflavinosis. Schwartzman, Dragutsky and Rook [97] reported the successful treatment with riboflavine of a case of Ritter's disease (dermatitis exfoliative infantum), the essential lesion of which is a generalized inflammation of the skin followed by suppuration and necrosis resembling pemphigus. Riboflavine in doses of 2 mg. three times a day was given and the skin dressed with fifty per cent. cod-liver oil in paraffin. Rapid improvement and cure in eighteen days were reported. Topping and Knoefel [99], on a purely experimental basis, claim to have treated a case of pemphigus with riboflavine after all other treatment failed, but Wolf and Lewis [76] failed to observe any response in a case treated with 100 mg. daily. Vorhaus and his co-workers [272] report complete healing in five cases out of six of decubital ulceration treated with 5 mg. of riboflavine daily. Complete healing occurred in from seven to thirty-four days without any other treatment.

Atkinson [231] believes that the causative factor in Ménière's syndrome is a deficiency of nicotinic acid and riboflavine, deficiency of the latter being the cause of damage to the organ of Corti. He claims that the administration of nicotinic acid and riboflavine results in considerable improvement in patients suffering from the condition.

It has been claimed that riboflavine and the vitamin B complex minimize the oral side-effects sometimes seen after the administration of the antibiotics.

chloramphenicol and aureomycin (atrophic glossitis, redness of the oral mucous membranes and angular stomatitis) [324]. These antibiotics alter the balance among the organisms in the mouth, favouring the development of fungi but not bacteria. It is difficult to see how treatment with riboflavine or other vitamins can influence this as the symptoms, although they superficially resemble those of ariboflavinosis, have a totally different pathology.

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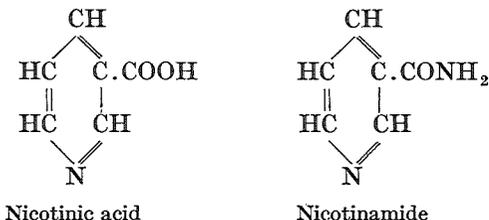
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CHAPTER V
NICOTINIC ACID

NIACIN

HISTORY

NICOTINIC acid, a member of the B complex, is β -pyridine carboxylic acid. The amide, nicotinamide, is a component of a complex enzyme system.



For several years the significance of nicotinic acid in nutrition remained undiscovered. Although it was discovered in 1867 and found in yeast concentrates nearly forty years ago, little interest was taken in the physiological rôle of pyridine derivatives until 1938, when Warburg [1] and his colleagues showed that nicotinamide is the active group of the co-enzyme now known as codehydrogenase II (p. 339). At the same time Kühn and Vetter [2] isolated nicotinamide from heart muscle, and others obtained it from cozymase. As a result of this work considerable interest centred around nicotinic acid as a factor in nutrition. Thus Frost and Elvehjem [3] observed that it had growth stimulating properties, and others showed that it was an essential growth factor for a number of micro-organisms.

A disease of dogs known as black tongue—characterized by glossitis stomatitis, diarrhoea, and typical skin lesions—bears a close resemblance to human pellagra. During their studies on chick pellagra, Elvehjem [4] and his associates isolated nicotinic acid from liver concentrates that were active in curing the condition. They then tested the effect of nicotinic acid on dogs suffering from black tongue; it was curative. This suggested its therapeutic use in the treatment of human pellagra. The first report of its successful use in this connection was made by Elvehjem, Madden, Strong and Woolley [4] in September, 1937. A number of papers by other investigators confirming this appeared in rapid succession. Nicotinic acid was at first hailed as the PP or pellagra preventing factor, but it is now known that pellagra is a multiple deficiency disease and that lack of nicotinic acid is only one of the factors in its causation.

In 1942 the Food and Nutrition Board of the U.S.A. National Research Council suggested the terms niacin and niacin amide for nicotinic acid and nicotinic acid amide respectively [156]. The Food and Nutrition Board suggest that the original names be retained in scientific literature.

CHEMISTRY OF NICOTINIC ACID

Nicotinic acid is a white crystalline solid, melting at 228°-229° C., soluble in water (1 in 60 at 25° C.) and alcohol (1 in 80 at 25° C.). It is not oxidized or destroyed in the ordinary processes of cooking, or by exposure to air, light or alkalis. It can be made bacteriologically sterile by autoclaving

without loss of potency. Being an acid it readily forms salts and an amide, which are also physiologically active. The *pH* of a 1 per cent. solution is 3.

Nicotinic acid has been estimated in body fluids and foodstuffs by a variety of methods [335]. One chemical method depends on the colour produced with cyanogen bromide, which can be estimated photometrically either directly or after condensation with various amines, such as aniline [8], metol [9], *p*-aminoacetophenone [11], aminopropiophenone [334], *m*-phenylenediamine [262] and orthoform [16]. The condensation product with cyanogen bromide can be estimated photometrically [326], fluorometrically [5], or by spectrophotometry [14]. Another method depends on the formation of a coloration when the dry material under test is fused with 2 : 4-dinitrochlorobenzene [13]. None of these methods is specific, as the same colours are given by other pyridine derivatives. Microbiological methods have been devised

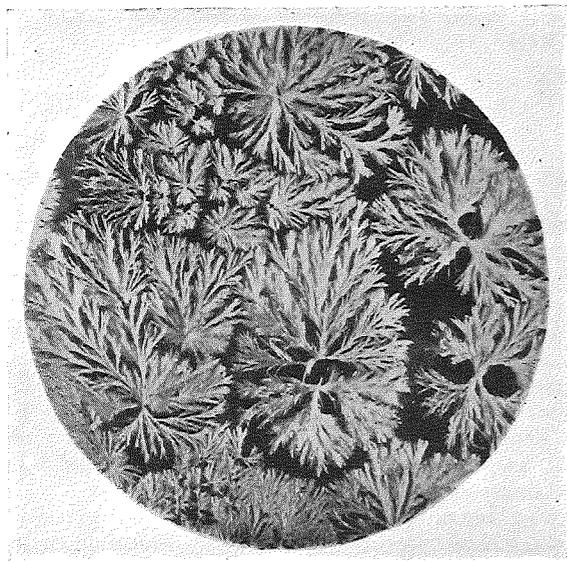


FIG. 96. Nicotinic Acid Crystals.

[335], such as the *Lactobacillus* test [12], in which the amount of lactic acid produced by *Lactobacillus arabinosus* is proportional to the nicotinic acid content of the medium. Other methods depend on the use of bacterial enzymes present in bacteria which have been adapted to grow on nicotinic acid [155], and the use of *Proteus* HX19 [6] and yeast [7]. A micromanometric method for estimating nicotinic acid combined as diphosphopyridine nucleotide (codehydrogenase I, p. 339) has been devised. It depends on the liberation and measurement of carbon dioxide during a

reaction catalysed by diphosphopyridine nucleotide. The pyridine nucleotides can also be estimated by adsorption on active charcoal, elution with ten per cent. pyridine and spectrophotometric analysis of the eluate [318].

As a considerable proportion of the nicotinic acid in biological material is combined, it is freed by vigorous hydrolysis with acid or alkali. This produces dark pigments that interfere with colorimetric estimation. Either the pigment or the nicotinic acid is adsorbed or the pigment removed by extraction with non-aqueous solvents such as ethyl acetate or ethyl laurate.

DISTRIBUTION IN FOODSTUFFS

Nicotinic acid occurs in all living cells. Liver, the adrenals, kidney, yeast, whole grain products, flesh foods ("meat"), mushrooms, and peanuts are among the best sources. Denatured cereals, such as white flour or polished rice, fruit and vegetables in general and milk are poor sources. Meat extracts contain appreciable quantities, e.g. a teaspoonful, such as is used to prepare a drink, may contain 10 mg. [164]. Free nicotinic acid is not found in living cells, but as the amide or as part of complex enzyme systems in which it is chemically bound (p. 339).

Nicotinic acid is concentrated mainly in the aleurone layer of cereals and

the bran and not, like aneurine, in the scutellum. In America bread is "fortified" by the addition of nicotinic acid so that the content is about 16 mg. per pound. It is recommended that in Britain bread should contain 1.6 mg. of nicotinic acid per 100 grams of bread as a minimum [34]. An increase in the nicotinic acid content of oats and rice occurs on germination, but not of wheat, barley or maize [35].

Since nicotinic acid is heat stable and resistant to oxidation and the action of light, very little is lost in food during cooking and processing. Any losses that do occur result from the vitamin diffusing into the cooking water, which is usually thrown away. In the cooking of meat eighty to eighty-five per cent. is retained after roasting, seventy-seven per cent. after frying, and sixty-five per cent. after braising; the total retention in the meat and drippings in domestic cooking averages seventy per cent. and may be as high as 100 per cent. in stewing [19, 165, 166]. In the curing of meat some eighty-four per cent. is retained [166]. Food cooked in cafeterias and restaurants usually contains less nicotinic acid than food cooked at home. Losses up to sixty per cent. have been recorded [167]. In the cooking of vegetables the loss of nicotinic acid is from eight to twenty-two per cent.; the cooking water contains twelve per cent. on an average (range two to forty per cent.) [170]. In the dehydration and canning of meat products eighty-eight to ninety-four per cent. of the nicotinic acid is retained [263]; in the case of vegetables it is seventy-seven to a hundred per cent. [39]. If the cooking water is thrown away it will be less.

Nicotinic Acid Content of Foods

Food	Description	Nicotinic Acid in micrograms per gram
<i>Cereal Foods</i>		
Barley	Red	47
	Pearl	27.5-30
Bread, wheat	White	6.6-10.0
	Wholemeal	37-42
	National bread (1943)	10.6
	Enriched (U.S.A.)	33
Biscuits (dry)	—	20-30
Buckwheat	—	44
Corn	—	15.6-26.0
	Meal	17.6
Macaroni	Flour	3
	—	21
Millet	—	8
Oatmeal	—	6-11
Oats	—	11.3-16.0
Rice	Polished	14
	Unpolished	44-66
	Milled	16
	Bran	300
	Polishings	284-1,400
	Flour, whole	9 ; 12.2 ; 17
Rye	„ bleached	7.3
	—	20
Semolina	—	21
Spaghetti	—	3
Tapioca	Boiled	3
Wheat	Whole	28-41 ; 54-80 ; 55
	Flour, white	6-9
	National flour—	
	85% extraction (1943)	13.3-24.3 ; 17
	82½% „ (1944)	18
80% „	16.3	
70% „	8.4-12.0	

Food	Description	Nicotinic Acid in micrograms per gram
<i>Cereal Foods—continued.</i>		
<i>Wheat—continued.</i>		
	Germ	34-70
	Bran	250-460
	Middlings	92-177
	Screenings	192
	U.S.A., whole	54
	„ white	8
	„ “enriched”	31
<i>Proprietary Cereal Products</i>		
[264]		
All Bran	Kellogg's	160-185
Bemax	—	60
Cerevim	Kederle (Vitamin concentrate added)	203
Corn Flakes	Kellogg's (Vitamin concen- trate added)	16
	Post's (Vitamin concentrate added)	13
Cream of Rice	—	17
Cream of Wheat Force	Vitamin concentrate added . —	16-20 41
Grape Nuts	Post's (Vitamin concentrate added)	39-49
Oats	Quaker	10
Rice Krispies	Kellogg's (Vitamin concen- trate added)	80
Shredded Wheat Soya Wheat	Kellogg's —	42-45 34
<i>Vegetables and Vegetable Products</i>		
Asparagus	—	11
Beans	Green	3.5-7.6
	Lima	4
Beets	Root	4-6.4
	Greens	3.0
Broccoli	Leaves	9 ; 14.4
Brussels sprouts Cabbage	— —	4 1.2-4
Carrot	Dehydrated	28-30
Cauliflower	—	4 ; 14.7
Celery	—	4.8-6.6
Cucumber	—	1.8-2.6
Egg plant	—	1.09-3.2
Endive	—	6
Kale	—	7.2
Kohlrabi	—	8
Lettuce	—	2.7
Mushrooms	—	2-5
Onion	—	69 ; 31
Parsnips	—	1.0-5
Peas	—	2
Peppers	Green	7-21
Potatoes	Green	2.0 ; 4
	White	11.8
	Sweet	12.9
	Peeled	6.7
	Dehydrated	48
Pumpkin	—	7
Radish	—	1.5-24

Food	Description	Nicotinic Acid in micrograms per gram
<i>Vegetables and Vegetable Products—continued.</i>		
Soya bean	Flour	7.2-24
	Bread	13.5
Spinach	—	5-7.2
Squash	—	9.6
Tomatoes	Whole	5.8
	Juice	1.0
	Ketchup	20
Turnip	Root	6.9
	Greens	5
Watercress	—	10
<i>Beans and Nuts</i>		
Almond	—	11.7 ; 50
Bean	Kidney, dried	17.1-28
	Lima, dried	12.7-18.3
	Broad, dried	21
Chestnuts	—	10
Coconut	—	18.2
Cowpeas	—	22
Lentils	Dried	31
Peas	Dried	18-28
Peanuts	Raw	86
Peanut butter	—	162 ; 186
Pecan	—	9
Soya	Bean	22-29
Walnut	—	12
<i>Fruits</i>		
Apples	—	0.9-5
Apricots	—	30
Banana	—	3-6.1
Cherries	—	1.4
Cranberries	—	12.9
Dates	—	8 ; 21.8
Figs	Fresh	10
	Dried	20
Grapes	—	4 ; 8.4
Grape-fruit	Juice	1.5-2.1
Guava	—	10
Lemons	—	1.5-1.9
Limes	—	1.0-2.7
Melon	—	8
Orange	—	2.2
Peaches	—	3.9-5
Pears	—	0.9-2.0
Plums	—	1.18-5.6
Pineapples	—	1.36
Prunes	Dried	17
Raisins	—	2.9-6.3
Raspberries	—	3
Strawberries	—	2.3-2.6
Jam	—	2
<i>Dairy Products</i>		
Cheese	Cheddar	2
	Others	1-16
Eggs	Whole	1.0
	Dried	2

Food	Description	Nicotinic Acid in micrograms per gram
<i>Dairy Products—continued.</i>		
Milk	Cow's, fresh	0.8-1.0
	" condensed	1.8
	" skimmed, powdered	6-8.9
	Human	2.6 ; 1.76-2.45 ; 1.83-3.3
Cream	—	10
Ice-cream	—	1
<i>Meat and Meat Products</i>		
Beef, fresh	Brain	35-49
	Heart	68-84
	Kidney	73.4 ; 100
	Liver	120-179
	Muscle	46-63.9
	Tongue	71
	Pancreas	58.4
Beef	Extract	375-1,025
	" Corned "	24-95
Chicken	Breast	86 ; 151
	Muscle, leg	72
	Liver	80-152
Duck	Muscle	30
Frankfurter	—	24
Goose	—	30
Meat extract	—	2,000 ; 1,000-1,200
	Mutton and Lamb	Muscle
Pork	Brain	32
	Heart	60 ; 80
	Kidney	60
	Liver	176
	Brain	64
	Heart	40 ; 73
	Kidney	98
	Liver	140-228
	Muscle	40-61
	Bacon	20-44
Rabbit	Ham	41 ; 88
	Loin	88
	Smoked ham	82
	Brain	12
	Muscle	65-126
	Liver	143-220
	Turkey	—
Veal	Heart	106
	Liver	176
	Muscle	65-170
<i>Fish</i>		
Average	Lean	42
	Flesh	23
	Liver	16
	Roe	15.2
Clams	—	10
Crab	—	28
Haddock	—	9
Halibut	—	30-60
	Milt	23.6
	Roe	23.6
Herring	Flesh	29-40

Food	Description	Nicotinic Acid in micrograms per gram
<i>Fish—continued.</i>		
Mackerel	—	55-72
Oyster	—	13
Salmon	Fresh	74 ; 84
	Tinned	60
Sardine	—	48-74
Shrimp	—	10-19
Trout	—	35
Turbot	Muscle	23
<i>Miscellaneous</i>		
" Aluzyme "	—	34
" Bemax "	—	60
Bouillon cubes	—	up to 270
Chocolate	—	11
Honey	—	0
Coffee	—	100
Malt extract	—	75-134
" Marmite "	—	600 ;
		527-672
Molasses	—	28 ; 39
Royal jelly	—	59
Tea	—	70
Yeast	Brewer's	300-1,000
	Baker's	400-500
	D.C.L.	250-350
	<i>Torulopsis utilis</i> (food yeast).	400-450

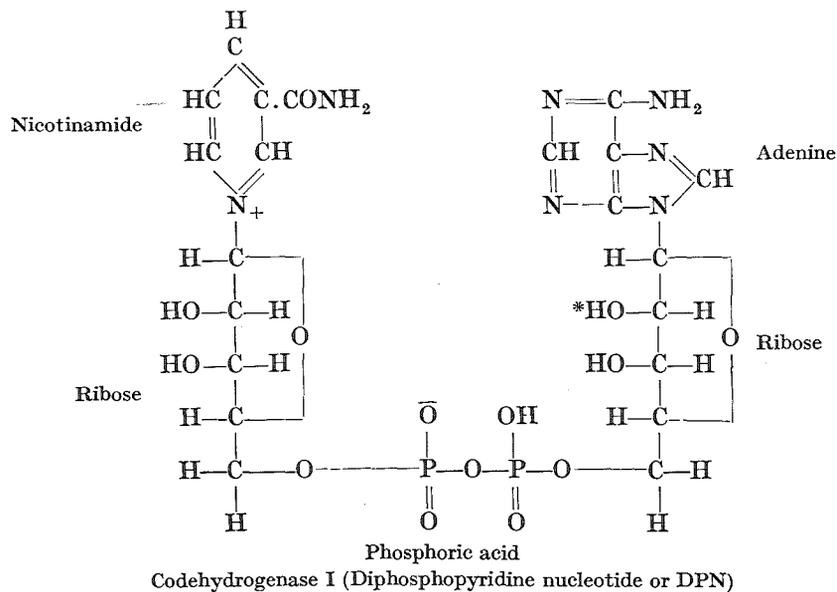
THE PHYSIOLOGY OF NICOTINIC ACID

Nicotinic Acid and Enzyme Systems. Nicotinic acid, like riboflavine, forms part of complex enzyme systems concerned with hydrogen transport in the living cell. The enzymes consist of an apoenzyme and a co-enzyme. The apoenzyme is a specific protein, believed to have no enzyme action itself, which is linked to the co-enzyme, the prosthetic group of the enzyme system. There are two co-enzymes associated with hydrogen transporting enzymes (dehydrogenases) known as codehydrogenases I and II, formerly known as co-enzymes I and II.

Codehydrogenase I (p. 340), or diphosphopyridine nucleotide (DPN), is a complex of one molecule of nicotinic acid amide (nicotinamide), one of adenine, two of ribose and two of phosphoric acid [17, 18].

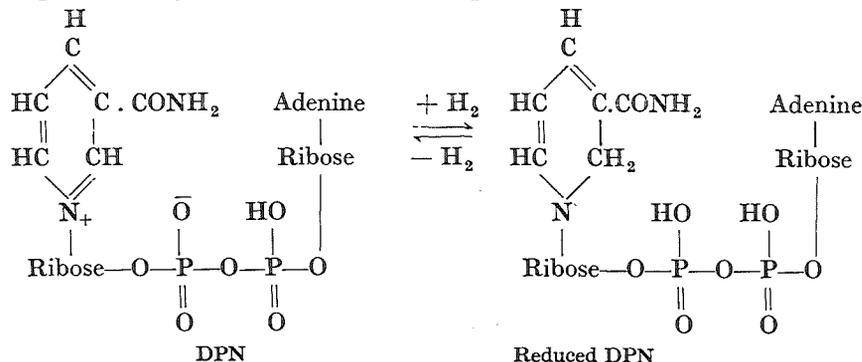
Codehydrogenase II contains three molecules of phosphoric acid (triphosphopyridine nucleotide, TPN). It is structurally similar to DPN with an additional molecule of phosphoric acid, which is attached to the second hydroxyl group of the ribose * [319]. Both occur in animal and plant cells, although DPN appears to be present to a greater extent. All living cells can synthesize the codehydrogenases from nicotinic acid. Yeast and red blood cells [20] contain relatively large amounts of both. Fresh yeast contains about 500 micrograms per gram, and human muscle 100 to 400 micrograms per gram. Synthesis of DPN from nicotinic acid probably occurs in both the nucleated blood cells [157] and the erythrocytes [48] of the blood. In uncomplicated nicotinic acid deficiency there is a decrease in the DPN content of liver and muscle [190].

The function of the codehydrogenases is to catalyse the dehydrogenation of various substrates. The following table gives some of the dehydrogenation reactions in which they are involved [158]:



Substrate and Dehydrogenation Product	Source of Apoenzyme	Codehydrogenase
Lactic acid \rightleftharpoons pyruvic acid	Heart muscle	DPN
Alcohol \rightleftharpoons acetaldehyde	Yeast	"
Malic acid \rightleftharpoons oxalacetic acid	"
Triosephosphate \rightleftharpoons phosphoglyceric acid .	Skeletal and cardiac muscle	DPN
$2R.CHO + H_2O \rightarrow R.CO_2H +$ R. CH_2OH (aldehyde mutation).	Liver	"
Formic acid $\rightleftharpoons CO_2 + H_2O$	Seeds and <i>E. coli</i>	"
β -hydroxybutyric acid \rightleftharpoons acetoacetic acid	Heart muscle	"
Glucose-6-monophosphate \rightarrow phospho- gluconic acid.	Yeast, erythrocytes	TPN
Decarboxylation and dehydrogenation . .	Yeast	"
Glucose \rightleftharpoons gluconic acid	Liver, yeast	DPN or TPN
Citric acid \rightleftharpoons α -ketoglutaric acid . . .	Liver, yeast	TPN

During the dehydrogenation the codehydrogenase absorbs two atoms of hydrogen from the substrate to form a dihydro-compound. This in turn gives up its two atoms of hydrogen to molecular oxygen, i.e. it is oxidized, and codehydrogenase is reformed. The codehydrogenases thus undergo a reversible reduction-oxidation process, the nicotinamide part of the molecule being involved in the change. It is believed that upon reduction the nitrogen of the pyridine ring is reduced from the quinquevalent to the tervalent condition.



DPN and TPN are also co-enzymes involved in phosphorylation and pyruvic acid oxidation. They form part of an oxidation-reduction system with the flavoproteins (p. 294). Since they function in the metabolism of carbohydrate, they are essential for growth and activity.

DPN cannot transfer hydrogen directly to molecular oxygen, but requires cytochrome reductase to accept its hydrogen. The two flavin enzymes cytochrome reductase and diaphorase (p. 293) are catalysts for the transfer of hydrogen from DPN and TPN through the cytochrome system to oxygen. DPN and TPN serve as part of the intracellular respiratory mechanism of all cells. When coupled with a number of specific proteins they serve for the transport of hydrogen from a number of substances to other respiratory catalysts and make possible the ultimate combustion of metabolites to carbon dioxide and water with the release of energy. They function in anaerobic as well as in aerobic metabolism. The anaerobic degradation of glucose is catalysed by DPN and adenosine di- and tri-phosphate (p. 194).

According to Wald and Hubbard [43] the conversion of retinene to vitamin A₁ is a reduction in which two atoms of hydrogen are transferred to retinene from reduced DPN. This is catalysed by an enzyme in the outer part of the rods of the retina. A second enzyme system reduces DPN, using hexosediphosphate or one of its derivatives as a hydrogen donor.

The content of DPN and TPN in the blood can be increased, e.g. by eighty-five per cent., by the administration of excessive quantities of nicotinic acid; the increase varies with the amount of nicotinic acid administered [20, 21]. It has been suggested that the determination of DPN in blood might be used as a test for human nicotinic acid deficiency. It is, however, of no diagnostic value since the blood level in pellagrins may fall within the normal range, although in many cases it is lowered [20]. After nicotinic acid therapy the level of DPN and TPN in the blood of pellagrins is raised [22]. Axelrod [23] and his associates determined the DPN content of striated muscle in normal subjects and in pellagrins and they found that it decreases as the pellagra becomes more severe. A fall in DPN occurs in heart muscle rendered ischaemic by ligation of the coronary artery [346]. Low values of DPN and reduced DPN have also been observed in the blood of patients with congestive heart failure; these are restored to normal by digitalis therapy [345].

It has been shown that a number of pyridine derivatives related chemically to nicotinic acid have a nicotinic-like action and can replace it in the codehydrogenases. These derivatives include the salts of nicotinic acid, nicotinamide, the N-diethylamide of nicotinic acid (nikethamide), ethyl nicotinate and other nicotinic acid esters [287], nicotinuric acid, nicotinamide-glucosido-iodide, quinolinic acid, pyrazine 2 : 3-dicarboxylic acid and pyrazine monocarboxylic acid. Nicotinamide, nikethamide, quinolinic acid, 2 : 6-dimethyl pyridine-3 : 5 dicarboxylic acid and pyrazine mono- and 2 : 3 dicarboxylic acids have been found to be effective in the treatment of pellagra in place of nicotinic acid [57-60]. Not only are these compounds active in doses comparable with that of nicotinic acid (up to 1,000 mg.), but they also cause an increase in the concentration of DPN and TPN in the blood. It is assumed that these compounds are converted in the body into nicotinamide. The nitrogen atom in the pyridine ring must apparently be unsubstituted for the compound to show nicotinic acid activity. Thus trigonelline (p. 348), in which the nitrogen is methylated, is inactive. This is understandable as in the codehydrogenases the nitrogen atom of the pyridine ring links up with the ribose moiety of the molecule; in trigonelline it is blocked.

It is known that sulphonamides such as sulphapyridine and sulphathiazole prevent bacterial growth by interfering with the functioning of chemically related enzyme systems (p. 228). West and Coburn [173] noted the chemical similarity between sulphapyridine and nicotinamide—both have a pyridine ring—and reported *in vitro* experiments with *Staphylococcus aureus* on the

basis of which they suggested that sulphapyridine exerts its bacteriostatic effect by interfering with the formation of co-enzymes from nicotinamide. The co-enzyme systems are essential for cell respiration in micro-organisms. This has been confirmed [174]. Apparently sulphapyridine inhibits the action of nicotinic acid by preventing the formation of the co-enzyme systems in which nicotinic acid participates [184]. It does not affect the activity of preformed co-enzymes [175]. Sulphapyridine not only blocks nicotinic acid in the nutrition of micro-organisms, but it inhibits its curative effect in canine black tongue [36]. Other sulphonamides such as sulphadiazine, sulphanilamide, sulphapyrazine and sulphaguanidine, are unable to block the nicotinic acid co-enzyme systems, presumably because they differ structurally from nicotinic acid in having no pyridine ring [179].

Nicotinic Acid and Porphyrin Metabolism. It has been suggested that nicotinic acid is associated with porphyrin metabolism, since many of the manifestations seen in pellagra, such as abdominal distress, diarrhoea, pigmentation of the skin, and photo-sensitivity are often found in patients exhibiting acute toxic porphyrinuria [24]. It has been stated that the porphyrin output in pellagra is approximately related to the severity of the skin and mucous membrane lesions and that the excretion returns to normal on a diet rich in yeast and liver and with the regression of the disease [25]. It is generally agreed now that the excretion of coproporphyrin in pellagrins lies within normal limits [26, 27, 31] and is of no aid in the diagnosis. Rosenblum and Jolliffe [26] have found that the porphyrinuria in pellagra may decrease without the administration of nicotinic acid, or increase with the administration of nicotinic acid, and while the manifestations of pellagra are regressing. Liver dysfunction, which is nearly always present in pellagrins, may explain the porphyrinuria of pellagra, either in the form of a disturbance in hæmoglobin breakdown and the production of excessive coproporphyrin, or of the inability of the liver to excrete porphyrin in the bile. Porphyrinuria in old persons has been observed to disappear after the administration of nicotinic acid [176]. This may have been due to improvement of impaired liver function.

Watson [29] has shown that a colour reaction in the urine of pellagrins, frequently mistaken for that of porphyrin, is in reality due to the pigment uroscopin. This is present in the urine of many individuals and is not related to nicotinic acid deficiency or pellagra [30, 177].

Nicotinic Acid and Carbohydrate Metabolism. It is now certain that DPN and TPN function in respiratory oxidation systems as carriers of hydrogen and are essential for the metabolism of carbohydrate. Fat has a nicotinic acid sparing action, probably because the energy metabolism is shifted from carbohydrate to fat (cf. aneurine, p. 197) [32]. It is possible that more nicotinic acid is required for the metabolism of fructose than of glucose [325]. It is known that the energy of nerve tissue is derived solely from the combustion of carbohydrate, and that it is liberated stepwise by means of several enzyme systems, one of which contains nicotinic acid. A break in the chain of carbohydrate oxidation may explain some of the mental symptoms of pellagra and nicotinic acid deficiency. Pellagrins are stated to show a hypersensitivity to insulin, becoming hypoglycæmic more readily than normal subjects after an injection of insulin [180]. This is probably due to impaired liver function and adrenal damage. Glucose storage is delayed in pellagrins, who show a "lag" type glucose tolerance test curve, with a blood sugar 15 to 30 mg. above resting level even after three hours [273]. Large doses of nicotinic acid are said to increase storage of glycogen in the liver of rats [337]. The view that nicotinic acid has a hypoglycæmic action in normal subjects and diabetics rests on slender evidence [178 268,]. More recent re-investigation shows that neither nicotinic acid nor nicotinamide have any effect on the blood sugar and acetone bodies of normal and diabetic subjects [33, 41, 44]. Banerjee and his colleagues [49] state that nicotinic acid has no effect on the blood sugar of normal rabbits, and that

it has no effect on glucose tolerance or on glycosuria. Janes and Myers [76] observed ketosis in alloxan treated diabetic rats receiving nicotinic acid. Lazarow, Liambies and Tausch [317] protected rats against diabetes produced by alloxan with nicotinamide; Banerjee and his co-workers [49] were unable to show a consistent protection.

Whatever the effect of nicotinic acid in physiological quantities is on enzyme systems controlling the metabolism of carbohydrate, the administration of quantities in excess of normal requirements has virtually no effect on the blood sugar in health or in the diabetic.

Nicotinic Acid and Hæmopoiesis. The acidosis and dehydration of dogs suffering from black tongue, which is due to nicotinic acid deficiency, is accompanied by hæmoconcentration. If the animals are kept alive with saline injections they suffer from severe anæmia, with red cell counts as low as 0.75 million per cubic millimetre; the total white count is about 2,500 per cubic millimetre, and the bone marrow hypoplastic. Erythropoiesis stops at the erythroblast level. Administration of nicotinic acid, or its amide leads to a rapid restoration of the red and white cells to normal [267]. Since immature nucleated erythrocytes respire they probably utilize the codehydrogenases. Pigs fed a nicotinic acid deficient diet develop a normocytic anæmia [79]. The association of anæmia with pellagra has long been known, but since it is a multiple deficiency disease the anæmia cannot be specifically ascribed to nicotinic acid deficiency. It is variable in type (p. 363) and sometimes responds to iron therapy alone. The administration of nicotinic acid to malnourished anæmic subjects raises the pyridine nucleotide content of the blood cells, but has no effect on that of normal subjects [320].

Nicotinic Acid Requirements of Micro-organisms. Nicotinic acid or one of its derivatives is a growth factor for a number of organisms, some of which are used for the microbiological assay of the vitamin (p. 334). Some organisms, e.g. those of the coliform group, can synthesize nicotinic acid. Organisms requiring nicotinic acid are *S. aureus*, *C. diphtheriæ*, *P. vulgaris*, *S. paradysenteriæ*, *L. arabinosus*, *L. casei*, *S. lactis*, *S. faecalis*, *L. mesenteroides*, *K. pneumoniae*, *B. mellitensis*, many yeasts and *Mycobacteria* [140] and, of particular interest, an X-ray induced mutant strain of *Neurospora crassa* (p. 344). *B. influenzae* can only utilize nicotinic acid in the form of codehydrogenases. Using different organisms it is possible to estimate nicotinic acid, nicotinamide, nicotinuric acid and DPN and TPN in the presence of one another. Nicotinamide has marked tuberculostatic activity [330], and a derivative of one of its analogues, isonicotinyl hydrazine (isoniazid), has been employed in the treatment of tuberculosis [347].

Biosynthesis of Nicotinic Acid. It is now established that nicotinic acid is synthesized by organisms in the human gut [266, 269, 302, 303], mainly by *B. coli* [265]. The action is accelerated *in vitro* by ornithine, but whether this amino-acid is essential *in vivo* is not certain. According to Ellinger [302] about sixty per cent. of the daily nicotinamide requirements of man can be synthesized by the flora of the human intestinal tract. It is not known how much of this nicotinic acid is absorbed by the gut, although some of it is released by the bacteria. Najjar [269] has observed on daily intakes of as little as 1.5 to 2 mg. of nicotinic acid an excretion of N'-methylnicotinamide equivalent to that found after the ingestion of food furnishing 20 to 30 mg. of nicotinamide. According to Denko and his co-workers [181] little or no absorption occurs from the large intestine, since on a restricted intake of nicotinic acid the fæces may contain large amounts, and the urine very little. Benesch [303] has shown that under aerobic conditions bacterial synthesis of nicotinic acid occurs in gut, but under anaerobic conditions the organisms in the cæcum destroy two-thirds of the nicotinic acid. He therefore suggests that in the normal cæcum an equilibrium is struck between organisms which produce and those that destroy nicotinic acid. Symptoms of nicotinic acid deficiency can be produced in man by the administration of sulphonamides

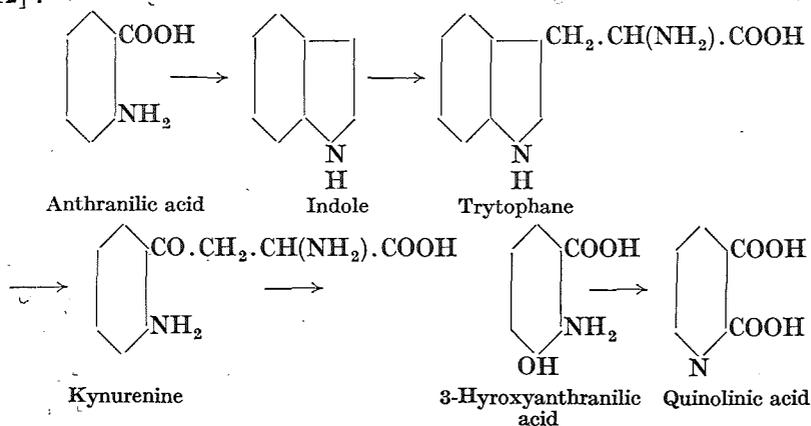
such as succinylsulphathiazole or sulphaguanidine [38] and by penicillin [301], all of which inhibit the growth of intestinal organisms.

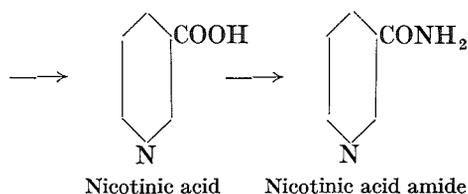
Some animals, such as the rat and sheep, do not need endogenous sources of nicotinic acid. Intestinal synthesis occurs in the intestine of the rat and some of it is formed independently of bacteria, possibly from tryptophane [42].

Nicotinic Acid and Tryptophane. It has been known for over 200 years that pellagra is endemic in districts in which maize is eaten as a staple article of food (Casal, 1735). Although maize is a poor source of nicotinic acid, it is not for this reason that a maize diet predisposes to pellagra but because this cereal is deficient in tryptophane [77]. This explains why foods rich in protein, such as meat and milk, which also contain tryptophane, are effective in the treatment of pellagra. The importance of tryptophane in this connection is that it is the precursor of nicotinic acid. If it is administered in daily doses of the order of 6 gm. it will induce a remission in pellagra in the absence of other treatment [82] and will cause an increased excretion of the nicotinic acid metabolite, *N'*-methylnicotinamide. Human adults and infants not suffering from pellagra given 1 to 5 gm. of tryptophane daily excrete a much larger amount of *N'*-methylnicotinamide in the urine than is found under basal conditions [94, 97]. At first it seemed that tryptophane exerts its effect through stimulating the bacterial synthesis of nicotinic acid in the intestine [70, 71]. The present view is that the conversion of tryptophane to nicotinic acid occurs in the tissues and not as a result of the action of intestinal micro-organisms. This is supported by the observation that rats deprived of their intestinal bacteria show no impairment in their ability to convert tryptophane to *N'*-methylnicotinamide [73]. In man the intravenous administration of tryptophane on a constant diet produces a prompt and considerable rise in the urinary excretion of *N'*-methylnicotinamide equal in magnitude to that provoked by the oral administration of tryptophane [74]. It also causes a rise in the pyridine nucleotides in the red blood cells [355]. D-Tryptophane is more effective than the L-compound [311]. Free tryptophane serves as an excellent source of nicotinic acid, but ingested as protein it is less active [107]. In the rat the excretion of *N'*-methylnicotinamide is proportional to the casein content of the diet [321], and in the growing animal dietary tryptophane plays a more important part than the nicotinic acid of the diet in the synthesis of pyridine nucleotides [336].

Tryptophane is more effective than nicotinamide and as effective as nicotinic acid in increasing the liver pyridine nucleotides of the rat [343, 349]; given in excess it is converted into pyridine nucleotides in the liver and not degraded or excreted [344].

Studies with mutant strains of the mould *Neurospora crassa* have thrown some light on the intermediate stages in the conversion of tryptophane to nicotinic acid. A possible biosynthetic route is from anthranilic acid [66, 342]:



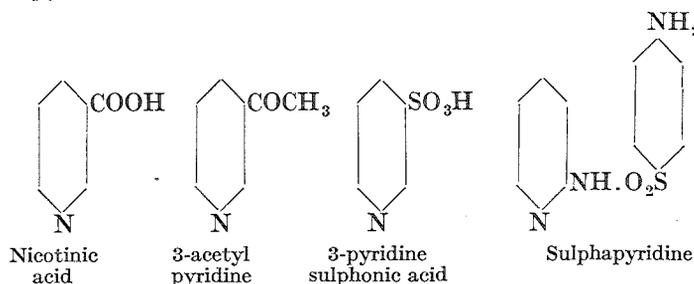


The conversion of 3-hydroxyanthranilic acid to nicotinic acid has been confirmed using the N^{15} isotope of nitrogen [328]; the amino-group of 3-hydroxyanthranilic acid is the precursor of the pyridine nitrogen atom of nicotinamide [342]. 3-Hydroxyanthranilic acid, tryptophane and kynurenine [327] can be used as a substitute for nicotinic acid by the rat [80]. Studies with isotopic tracer elements, such as C^{14} , confirmed the conversion of tryptophane to kynurenine in the intact animal [81] and its conversion to nicotinic acid independently of the intestinal bacteria [325], although the exact stages of the reaction are still conjectural [81]. Quinolinic acid may possibly be an intermediate metabolite [168, 342]. 3-Hydroxyanthranilic acid has been detected in human urine, particularly in that of tuberculous subjects [359].

In animals pyridoxine may be essential for the conversion of tryptophane to nicotinic acid, and there is evidence that it plays a part in tryptophane metabolism in man [310, 311]. Animals on a pyridoxine deficient diet excrete considerable quantities of xanthurenic acid after the administration of tryptophane, but little N' -methylnicotinamide or other nicotinic acid derivatives [103]. Pyridoxine appears to be necessary for the conversion of tryptophane to tissue pyridine nucleotides [358]. It is presumed that pyridoxine functions, as pyridoxal phosphate, in a manner similar to its role in other transaminase or decarboxylase systems [321].

Riboflavin also appears to be essential for the conversion of tryptophane into nicotinic acid; riboflavin and pyridoxine probably function in the conversion of kynurenine to 3-hydroxyanthranilic acid [345].

Nicotinic Acid Antagonists. For many years the pellagra-producing properties of corn have been attributed to a toxic factor present in the grain. This conception has been supported by the recent discovery of antivitamin. In 1945 Woolley [112] demonstrated that the administration to mice of 3-acetylpyridine, which resembles nicotinic acid structurally, produced a disease with a symptomatology similar to that seen in nicotinic acid deficiency. Its effect can be reversed by giving tryptophane or nicotinic acid. A search was made for a pellagra producing compound in corn. Woolley [113] obtained a toxamin from corn active in doses of 1 mg. 3-Pyridine sulphonic acid and sulphapyridine also act as nicotinic acid antagonists in the dog [115, 116].



In 1946 Kodicek, Carpenter and Harris [130] reported that indole-3-acetic acid, which is present in a relatively high concentration in maize, produces signs of nicotinic acid deficiency in rats. But they could not repeat their observations subsequently, nor could Rosen and Perlzweig [136]. The amino-acids, *dl*-threonine and *dl*-phenylalanine, can aggravate nicotinic acid deficiency [139].

Pharmacology. The toxicity of nicotinic acid has been studied by Unna [56]. Four to five grams of nicotinic acid per kilo of body weight are necessary to produce acute toxic effects in mice and rats; the amide is twice as toxic in these animals, although in human beings the amide is better tolerated than the acid itself. Nicotinic acid is not toxic to rats, chickens and dogs if taken over a prolonged period (two months) in doses of 2 grams per kilo of body weight. Toxic doses result in ataxia and cyanosis. Symptoms resembling anaphylactic shock have been reported in man after doses of 50 mg. intravenously, but the occurrence of such symptoms must be very rare [149]. Temporary leucopenia after administering nicotinic acid has been reported, and this too must be extremely rare [353].

Nicotinic acid possesses a pronounced vasodilator action, which was observed by the earlier workers when they used it in the treatment of pellagra [61-63]. The effects noted both in pellagrins and in normal persons include flushing of the face and neck, a sensation of heat, tingling and itching, which come on within seven to ten minutes and last about thirty minutes. There is light dizziness, thumping in the head, headache, and sometimes nausea, vomiting and transient abdominal pain; the blood pressure is not appreciably affected. An urticarial rash, palpitations, cyanosis of the nails and mental depression have also been described. Reddening and flushing of the skin may occur with either an increase or a decrease in the skin temperature. The symptoms are transitory and harmless, although disturbing. Sebrell and Butler [61] include gastro-intestinal symptoms, substernal oppression, and pruritis among the symptoms. Oral doses of 100 to 300 mg. or 20 to 25 mg. intravenously will cause an increase of skin temperature. The dose that causes flushing may be as little as 2 mg. in susceptible persons, or as much as 300 mg. Bean and Spies [64] have shown that this response is also given by the sodium, ammonium, and monoethanolamine salts of nicotinic acid and by its ethyl ester, and by pyrazine monocarboxylic acid, all of which are effective in the treatment of pellagra. β -Pyridylcarbinol, the carbinol of nicotinic acid, and the tetrahydrofurfuryl ester are also vasodilators [149], but nicotinamide is not [65]. This vasodilator action of nicotinic acid is checked by glycine in doses of 30 to 60 gm. and by adrenaline [182].

Loman and his colleagues [137] have shown that nicotinic acid is a peripheral vasodilator, and they infer that its vasodilator action is arteriolar. There is no significant change in body temperature or metabolic rate so that the vasodilatation is presumably not compensatory to increased heat production, but probably to a local effect on the arterioles in the skin [182]. The flushing, itching and heat of the skin, increased motility of the stomach and the secretion of gastric hydrochloric acid that occur after administering nicotinic acid are similar to those produced by histamine. Nicotinic acid, however, has an anti-histamine action on the bronchi and gut [159].

According to Moore [126] nicotinic acid is a vasodilator of cerebral and spinal vessels, although this is disputed by Scheinberg [170], who observed no change in the blood flow in the vessels. In the rabbit hyperæmia of the kidney follows the injection of 10 to 20 mg. of nicotinic acid per kilo of body weight [285]. Aring and his co-workers [141] have shown that nicotinic acid and quinine nicotinate administered intravenously increase the rate of intracranial blood flow in human beings for twenty to sixty minutes, without any significant change in blood pressure. Since Moore [126] noted an increase in the width of the pial vessels in the cat after the injection of nicotinic acid, presumably these vessels, at least, are involved in the process, which occurs within several minutes of the injection. The dilator effect of nicotinic acid roughly parallels the reaction of the skin. Nicotinic acid derivatives which do not cause flushing of the skin (e.g. nicotinamide) do not increase the rate of intracranial blood flow.

It was concluded by Popkin [67] that this vasodilator effect of nicotinic acid was too inconstant and evanescent to be of therapeutic value, but

Abramson [68] found a significant increase in the blood flow to the hand and forearm, although not to the leg, after administering 100 to 300 mg. of nicotinic acid by mouth or 20 to 25 mg. intravenously. The effect was due to local changes at the periphery, rather than to an increase in cardiac output, since neither the pulse nor the blood pressure were affected. Loman [137] has shown that nicotinic acid can cut short a Raynaud attack artificially produced by the injection of adrenaline into the brachial artery.

The vasodilator effect of nicotinic acid in the lower extremity is inferior to that of the drug priscoline (priscol) [169].

Nicotinic acid even at concentrations of 1 in 10^{-6} has a stimulating effect on the isolated frog and mammalian heart [160]. In the case of failure of the myocardium, it increases the cardiac excursion, reverses abnormal rhythms and augments coronary flow.

The intravenous injection of nicotinic acid in normal subjects is followed by a rise in indirect reaction serum bilirubin, which reaches a maximum in one and a half hours and returns to the initial value in six to eight hours. There is also an immediate stimulation and excretion of bile and increased elimination of urobilin, which reaches a maximum in two to three hours and returns to normal in twenty-four [40]. In pathological conditions of the liver the bilirubin level fails to return to normal values in eight hours and the bilirubin in circulation is of the direct reacting type. Both sodium nicotinate and nicotinamide exert a powerful choleric and chologogue effect [331].

Nicotinamide, but not nicotinic acid, appears to enhance the bacteriostatic effect of penicillin on *Staphylococcus aureus* [172]. The effect is not observed with other streptococci or *B. coli*. On the other hand, it exerts a marked inhibitory effect on the growth of *Mycoderma tuberculosis* [330].

Frankau [183] has shown in a series of carefully controlled experiments, the results of which were submitted to statistical analysis, that the administration of nicotinamide in doses of 50 to 200 mg. to active young men results in increased efficiency in carrying out fairly severe tests involving both physical effort and co-ordination. There was a well-marked diminution in the time taken to complete the test and less fatigue in the subjects receiving nicotinamide compared with controls.

Shock and Sebrell [163] could not observe any change in the work output of isolated muscle perfused with a dilute solution of nicotinic acid.

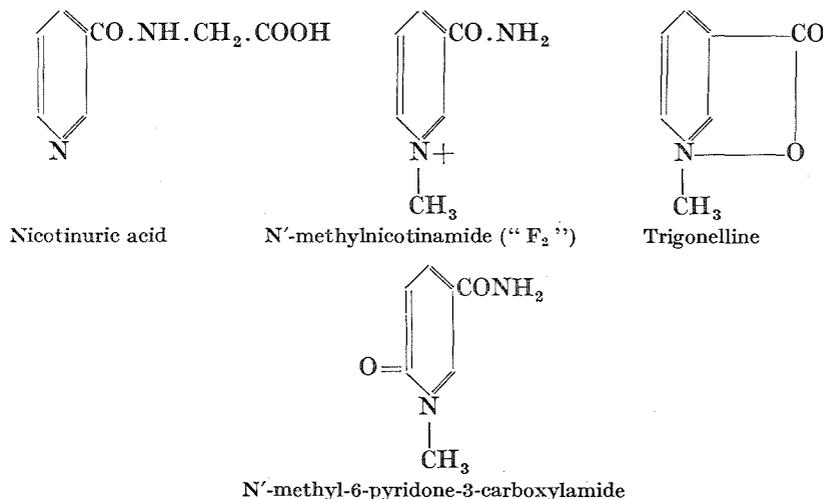
Absorption, Storage and Excretion of Nicotinic Acid. Nicotinic acid is present in foodstuffs mainly as co-enzymes from which it is readily released. It is not known whether these are absorbed directly as such or hydrolysed to nicotinic acid or nicotinamide first. If nicotinic acid or the amide are given by mouth they are absorbed unchanged. Nicotinic acid is converted into the amide after absorption into the blood stream. In normal persons the blood nicotinic acid ranges from 260 to 830 micrograms per 100 ml. of blood with a mean of about 438 micrograms [8, 11, 45-48, 84]. On a daily intake of 12 to 16 mg. it is about 600 micrograms per 100 ml. [185]. A rise occurs after severe exercise [197]. The greater part, about ninety per cent., is in the blood corpuscles [84] and is present as the co-enzymes [186]. Ingestion of nicotinic acid causes a rise in the co-enzymes in the blood of both pellagrins and normal persons [22]. The co-enzyme content of the erythrocytes is raised to about three times the normal value on an intake of 200 mg. of nicotinic acid daily. Ingestion of the amide, however, produces little or no rise in the co-enzyme content of blood. Large doses of riboflavine do not affect the absorption or storage of nicotinic acid [312].

Nicotinic acid is present in practically all tissues, mainly as co-enzymes; the liver contains more than any other organ. There is a direct correlation between the tissue nicotinic acid and the DPN content of muscle and liver and the nicotinic acid intake in the diet [190]. In pellagra it is stated that the DPN content of striated muscle is lower than normal, but the amount in erythrocytes shows only a slight decrease. A period of months is required

to deplete the body of sufficient of its stores of nicotinic acid to produce pellagra. Chronic alcoholics and others with liver damage may have difficulty in storing nicotinic acid [198]

Like other vitamins nicotinic acid is secreted in the milk, which contains about 128 to 336 micrograms per 100 ml. [196]. It is excreted in the sweat, which contains from 20 to 100 micrograms per 100 ml. [187, 188].

After the ingestion of nicotinic acid or nicotinamide, these compounds and their derivatives appear in the urine. The derivatives are the two co-enzymes (p. 339), nicotinuric acid, N'-methylnicotinamide (nicotinamide methochloride) [229], and N'-methyl-6-pyridone-3-carboxylamide [300]. The so-called "total nicotinic acid" of some workers includes nicotinic acid, nicotinamide, nicotinuric acid and trigonelline. According to Holman and De Lange [329] trigonelline plays no part in nicotinic acid metabolism. Most of the metabolite described as trigonelline is in fact N'-methylnicotinamide [283]. It forms a fluorescent compound with acetone or methylethyl ketone, which serves as a method for its estimation [313]. It exerts an anti-pellagra action in man [49]. It is excreted by glomerular filtration and tubular secretion [304]. The substance formerly designated as "F₂," a fluorescent metabolite of nicotinic acid, has been identified as N'-methylnicotinamide. It is probably formed from L-methionine, or homocystine, which function as methyl donors, and nicotinamide in presence of magnesium ions and a source of phosphate such as adenosinetriphosphate [388]. Vitamin B₁₂ in the presence of methyl donors increases the excretion of N'-methylnicotinamide [348].



If radio-active nicotinic acid is administered to mice only sixty per cent., as measured by radio-active methods, can be detected in the urine [274]. According to Ellinger and Abdel Kader [214] under normal conditions man eliminates nicotinamide, nicotinic acid and mainly N'-methylnicotinamide. They state that extra-dietary nicotinic acid or nicotinamide is eliminated almost exclusively as N'-methylnicotinamide, and to a small extent as nicotinamide [214]. According to Ellinger and Coulson [283] the mean excretion of N'-methylnicotinamide is 7.5 mg. daily, with a range of 2 to 8 mg.; this represents approximately 15 per cent. of a daily intake of 40 mg. of nicotinic acid. Fitzpatrick and Tompsett [333] give a range of 2 to 12.5 mg. with a mean of 5.8 mg.

Methods have recently been elaborated for the estimation of N'-methyl-6-pyridone-3-carboxylamide in urine [292, 295], and it is now possible to account for the major part of a dose of ingested nicotinic acid or nicotinamide

in the urine. Holman and De Lange [292] have been able to account for seventy-three to eighty per cent. of an orally administered dose of 500 mg. of nicotinamide in the urine as *N'*-methyl-6-pyridone-3-carboxylamide, *N'*-methylnicotinamide and the total acid-hydrolysable derivatives of nicotinic acid. According to Perlzweig, Rosen and Pearson [309] 3.6 to 12 mg. (mean 8.6 mg.) of the 6-pyridone compound is excreted in twenty-four hours by subjects on a normal diet. They claim that eighty-two to eighty-nine per cent. of an oral dose of nicotinamide can be recovered in the urine as nicotinic acid, *N'*-methylnicotinamide and the 6-pyridone compound.

Many workers have attempted to diagnose nicotinic acid deficiency by estimating various nicotinic acid derivatives in the urine. As formerly most workers did not include *N'*-methyl-6-pyridone-3-carboxylamide in the excretion products the method has not been of much value in the past. Ellinger, Benesch and Hardwick [291] claim that the excretion of methylnicotinamide after a dose of 100 mg. of nicotinamide yields useful information on the nicotinic acid stores of the body. The exact extent to which nicotinic acid is synthesized in and absorbed from the gut is not known (p. 343). It may be an important source. Many factors influence the excretion of nicotinic acid. The amount excreted depends upon the intake of protein (i.e. tryptophane) [94] and on changes in the diet and on drugs [25]. Thus it is low on a diet of maize and is diminished by barbiturates, poorly absorbed sulphonamides such as phthalylsulphathiazole and succinylsulphathiazole, and by drugs exerting a toxic action on the liver. According to Ellinger and Coulson [283] the excretion of nicotinic acid is influenced by exercise, the presence of methyl donors and the efficiency of the body methylating mechanism. It is stated that the excretion in the newborn is greater than the amount present in the milk and that formed by biosynthesis in the intestine [52, 314]. This must represent nicotinic acid synthesized by the infant or derived from the mother. On the second day after birth the excretion is 3.8 mg. and drops to 60 micrograms by the seventh day [142]. Smoking is followed by an apparent increase in the excretion of nicotinic acid [53]. There is an increase in the excretion of nicotinic acid derivatives in pregnancy [289] and a decrease in typhoid [55], probably associated with increased metabolism, and in sprue even when additional nicotinic acid is administered [179]. Cayer and Cody [202] observed that the urinary excretion in hospitalized patients with acute and chronic illnesses differed little from those of a control group without organic disease. Levenson, Lund and their co-workers [127, 128], on the other hand, state that abnormally small amounts of nicotinic acid derivatives are found in the urine of patients suffering from severe injury, hæmorrhage and infection.

HUMAN REQUIREMENT OF NICOTINIC ACID

Nicotinic acid is essential for the nutrition of most animals ; it is stated to have a specific growth promoting property [357]. It is impossible to give an absolute figure for the nicotinic acid requirement of man. This is conditioned by numerous factors, including the protein intake, the quality of the protein (particularly its tryptophane content), the presence of nicotinic acid precursors in the diet, the possible presence of "anti-pellagra" compounds, the relative amounts of other members of the vitamin B complex and the calorific value of the diet. Diets poor in protein or containing much corn, which is deficient in tryptophane, contain little nicotinic acid. The studies of Woolley [112, 113] indicate that the pellagragenic nature of corn may be due to the presence of an anti-vitamin (p. 345). It is also known that the pyridoxine in the diet plays a part in the conversion of tryptophane to nicotinic acid. Bacterial synthesis of nicotinic acid in the intestine is undoubtedly important as an extra-dietary source of the vitamin. It is possible, as Benesch [303] pointed out, that some intestinal bacteria are con-

cerned with nicotinic acid synthesis, while others are breaking it down. Normally a balance is struck between the two processes, but intestinal infections and infestations, diarrhoea, sprue and other gastro-intestinal diseases may upset the balance and increase the need for dietary nicotinic acid.

Dietary studies have shown that diets providing only 3 to 5 mg. of nicotinic acid need not precipitate pellagrous symptoms [281, 282]. It has been estimated that the nicotinic acid intake of Goldberger's volunteers, who developed pellagra in 1913-15 on a diet containing much corn, was 12 mg. daily, whereas the control subjects did not develop the disease on an intake of 7.2 mg. daily (p. 351). More recently Goldsmith and her co-workers [360] have found that 7 mg. of nicotinic acid daily can prevent pellagra even if a diet containing much corn, and hence poor in tryptophane, is consumed. In India the typical rice diet provides from 7 to 9 mg. of nicotinic acid daily, yet pellagra is rare, probably because the protein of rice is of high quality [177]. In contrast, pellagra is rife in Moldavia, where corn is the staple food and the daily nicotinic acid intake is of the order of 15 mg. [286]. This is greater than the 11 mg. daily calculated to be present in the average American diet [204], providing 2,500 calories daily, or the English diet, which contained 9 mg. when the all-white loaf of seventy per cent. extraction was consumed [143]. The present-day diet in Britain probably provides from 11 to 16 mg. of nicotinic acid daily [207]. Very poor diets among some American families are stated to contain only 4 to 6 mg. of nicotinic acid daily [205, 206].

The Food and Nutrition Board of the National Research Council of the U.S.A. in 1948 advised these daily allowance of protein and nicotinic acid :

Subjects	Calories	Protein	Niacin
		grams	mg.
<i>Man</i>			
Sedentary	2,400	70	12
Moderately active	3,000	70	15
Very active	4,500	70	18
<i>Woman</i>			
Sedentary	2,000	60	10
Moderately active	2,400	60	12
Very active	3,000	60	15
Last half pregnancy	2,400	85	15
During lactation	3,000	100	15
<i>Children under 12 years</i>			
Under 1 year	110/2.2 lb.	3.5/2.2 lb.	4
1-3 years	1,200	40	6
4-6 years	1,600	50	8
7-9 years	2,000	60	10
10-12 years	2,500	70	12
<i>Boys and girls over 12 years</i>			
<i>Boys</i>			
13-15 years	3,200	85	15
16-20 years	3,800	100	17
<i>Girls</i>			
13-15 years	2,600	80	13
16-20 years	2,400	75	12

The figures recommended by the National Research Council of America (1948) are given in the accompanying table. These appear to be unnecessarily high if one considers the pellagra-preventive properties of milk, eggs and green vegetables, although in a corn-eating area they may be only just adequate. It is of considerable interest that the Italian pellagrologists of two centuries also recommended a diet containing these foodstuffs for the prevention and cure of pellagra.

Nutritionists in Britain suggest that a daily intake of 14 mg. of nicotinic acid is adequate [220]; they recommend that the minimal nicotinic acid content of flour should be 1.6 mg. per 100 grams, which is approximately the content of eighty per cent. extraction flour.

It has been estimated that the infant at six months obtains 1 to 2 mg. of nicotinic acid daily from its milk and relatively little from other sources. This low intake is probably adequate because milk is an excellent source of tryptophane.

The Nutrition Committee of the British Medical Association (1950) has suggested the following daily allowances of nicotinic acid :

	Daily Allowance of Nicotinic Acid
Children up to 1 year	4 mg.
„ 2 to 6 years	6 „
„ 7 to 10 years	8 „
Adult male doing light work	12 „
„ „ „ medium work	14 „
„ „ „ heavy work	17 „
Adult females (acc. to calorie intake)	8-15 „
Pregnancy	10 „
Lactation	14 „

DISEASES ASSOCIATED WITH DEFICIENCY OF NICOTINIC ACID PELLAGRA

History. The word pellagra, which is a corruption of the Italian *pelle agra*, meaning rough skin, was first used in 1771 by Frapolli, who insisted that the skin lesions of the disease were precipitated by exposure to sunlight. The first comprehensive study of pellagra was made in 1730 by Gaspar Casal, a physician of Oviedo, whose name is commemorated in the lesion of the neck known as Casal's necklace (p. 358). The disease was first recognized as a clinical entity in Spain and Italy but it was soon observed in many other European countries. In 1864 it was first described in North America, although it was undoubtedly widespread before that time. In both Italy and America pellagra became a national menace. In 1881 there were 100,000 cases in Italy with a population of 16,500,000; in 1830 five per cent. of the population were said to be afflicted. The modern history of pellagra starts in 1914 when Funk postulated that pellagra was a food deficiency disease. This was confirmed by the classical investigations of Goldberger and his associates in the years 1913-15. Attempts to transmit pellagra by inoculation of healthy persons with the blood, secretions and preparations made from pellagrous lesions failed, thus showing that the disease was not infectious. After the discovery in 1937 that nicotinic acid cured black tongue in dogs—a disease showing some resemblance to human pellagra—it was used in the treatment of the latter, with good results. It soon became apparent, however, that nicotinic acid, although it relieved some symptoms of the disease, was not curative. Liver and a diet rich in the vitamin B complex were found to be more effective.

Distribution of Pellagra. Pellagra, which is endemic in maize-eating areas, occurs among practically all races, chiefly in rural districts where there is a limited choice of food. The disease was once common in Italy, where it was a major problem, but with a rise in the level of nutrition of the population it is now disappearing; less than 100 cases are reported annually, in comparison with numbers running into six figures recorded in the last century.

By far the largest number of cases occur in America chiefly in the Southern States among the negroes. It has been estimated that in 1938 nearly half a million persons in the United States suffered from pellagra and that 3,500 died from it every year [208]. This was an improvement on conditions a

decade earlier, when 7,000 died annually in the Southern States, with a death rate of 22.4 per 100,000 of population. The latter figure was reduced to 5.1 in 1940. A considerable improvement occurred after 1929 owing to the free distribution of yeast to sufferers and the growing of crops on small holdings by the rural population. In the Northern States pellagra occurring among the white population is of alcoholic origin. According to Spies [209] one to two per cent. of the admissions to the medical wards of Cincinnati General Hospital, Ohio, U.S.A., suffer from pellagra.

Pellagra is also met with in Spain, Portugal, the Balkans, Greece and Turkey, South America, India, China, Japan and the Straits Settlements. During and after the Civil War of 1937-39 in Spain there was an alarming increase in all deficiency diseases, particularly pellagra, 30,000 cases of which were observed in Madrid [145]. In Chile there are 3,000 cases annually with a mortality of twenty-six per cent. [210]. Pellagra is rare in Africa, except in Egypt and an area on the east coast. The distribution and incidence of pellagra in warm climates is admirably reviewed by Stannus [75] in a series of papers in the *Tropical Diseases Bulletin*.

Pellagra is rare in Great Britain, although it is certain that a proportion of the mild cases go unrecognized. Before the nutritional nature of the disease was known it was not uncommon to observe cases in mental hospitals and institutions. It is still met with in psychiatric practice [211] and sporadic cases are occasionally reported [72]. The disease has been reported as a complication of morphine addiction [217]. In 1942 Deeny [138] reported on sixteen cases of pellagra in Northern Ireland, where he says mild forms are relatively common and often pass unrecognized, the patient being diagnosed as suffering from neurasthenia, dyspepsia or eczema. A patient of one of the authors suffering from mild pellagra was treated for six months for eczema without the true nature of the condition being diagnosed [212].

Ætiology. Pellagra is a multiple deficiency disease. In 1937, when nicotinic acid was found to be of value in the treatment of the condition, it was at first concluded to be due to nicotinic acid deficiency. But nicotinic acid does not cure pellagra. Indeed some symptoms of pellagra do not respond to nicotinic acid at all. Moreover, there is no correlation between the incidence of pellagra and the nicotinic acid content of the diet. There were, for example, some inhabitants in Madrid during the Spanish Civil War with a low intake of nicotinic acid, yet they did not get pellagra [203]. In any case there is never an uncomplicated nicotinic acid deficiency; if the diet is lacking in nicotinic acid, it is lacking in other factors. Pellagra, as it occurs endemically, is a disease with disturbed metabolic relationships involving nicotinic acid, pyridoxine, tryptophane, possibly adenine, amino-acids, proteins, aneurine, riboflavine and folic acid. It is interesting to note that for many years the belief was widely held that, since pellagra was endemic in many maize-eating areas, it was caused by a toxin in the grain. This was definitely disproved in 1910, when Stannus [75] described an outbreak of pellagra among African natives on a diet of rice and beans; many cases of pellagra were seen in Japan among white prisoners of war, whose diet was low in calories, protein and the vitamin B complex.

The pellagra syndrome has not been produced experimentally in man by diets deficient in nicotinic acid only. Briggs and his colleagues [281] noted a sunburn-like erythema in subjects kept for forty weeks on diets containing only 4 mg. of nicotinic acid daily; there were no other symptoms of pellagra.

There are many external factors that play a part in precipitating an attack of pellagra. They include the following:

Infestations and Infections. These include malaria, schistosomiasis, amœbic and bacillary dysentery, ankylostomiasis and intestinal tuberculosis. They operate by interfering with the absorption of food (diarrhœa), increasing the general metabolism owing to pyrexia, or by the parasite absorbing foodstuffs from the host.

Increased Metabolism. It is well known that pellagra occurs in women during pregnancy and lactation and among men in prison camps doing forced labour. In females the greatest incidence is in the child-bearing period.

Lesions of the Gastro-intestinal Tract. So-called "secondary" or conditioned pellagra has been recorded following some lesion in or an operation on the gastro-intestinal tract, such as carcinoma of the alimentary tract, œsophageal stricture, gastric and duodenal ulcer, chronic gastritis, enteritis, ulcerative colitis, pyloric stenosis, rectal stricture, short-circuiting operations on the intestine [215], cardiospasm [216, Fig. 97], and gastro-intestinal fistulæ. These operate by interfering with the absorption of essential nutrients. There is an excellent review by Bean, Spies and Blankenhorn [261] on secondary pellagra.

Alcoholism. Pellagra due to alcoholism is not uncommon in the northern

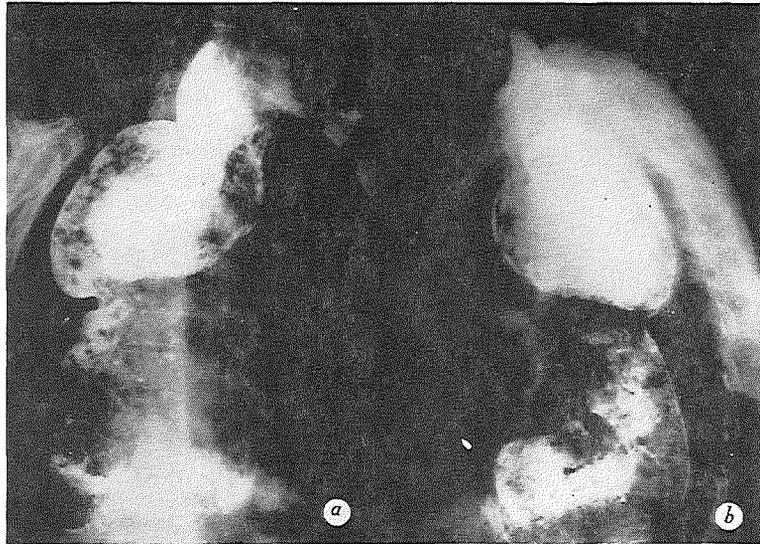


FIG. 97. "Secondary" Pellagra conditioned by Cardiospasm. Anterior and lateral views of X-ray after swallowing barium meal, showing enormously dilated and tortuous œsophagus with a constriction in the middle third. Same case as Fig. 99.

United States. Strong alcohol irritates the gastro-intestinal tract, and causes gastritis and secondary infection, which result in faulty absorption of food (cf. p. 225). The alcoholic consumes calories but not food.

Restricted Food Intake. The quality and quantity of food consumed may suffer as a result of eating unbalanced diets. Food faddists, eccentrics, asylum inmates, and "slimming patients" have been known to develop pellagra. Pellagrins may become insane (p. 360), and the insane may develop pellagra because of nutritional failure. Gastric disturbances and gastritis are also common in psychotics.

Sunlight and Physical Trauma. Exposure to sunlight or any physical trauma may be a precipitating cause of pellagra (Fig. 98). From a study of 465 cases Ruffin and Smith [85] noted that not only sunlight, but radiation from an electric heater will precipitate not only the dermatitis of pellagra, but also oral and gastro-intestinal symptoms in convalescent patients. This was prevented by liver extract. Sunlight acts as an irritant; exposure to any other form of irritation such as tight clothing, repeated friction, irritating sweat or friction between body surfaces (thighs, nates, scrotum) may cause

skin lesions in pellagrins. There is a distinct seasonal incidence, which is higher in the late spring and early summer. Analysis of diets shows that this seasonal wave cannot be explained solely in terms of dietary deficiency [78].

Diets containing much maize, or tryptophane-deficient protein, or unbalanced amino-acid mixtures are pellagrigenic (p. 344). Pellagra does not develop if such diets are supplemented with milk, eggs, meat and green vegetables. Woolley [112, 113] has extracted a toxamine from corn, which is

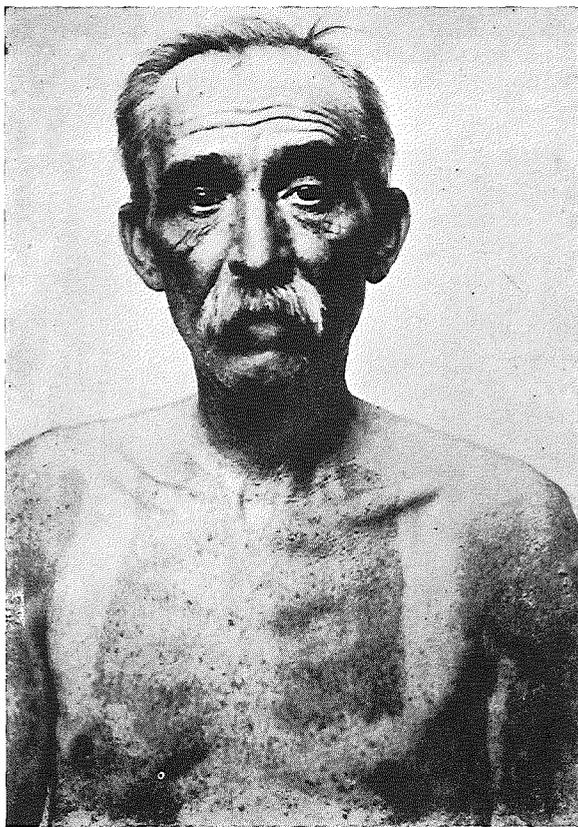


FIG. 98. Pellagra. The patient is a labourer wearing trousers but no shirt whilst working in the daytime. A pellagrous dermatitis is present on the part of the arms and body exposed to light. It is absent over the area protected by the patient's braces. Exposure to light may be a precipitating cause of the skin lesions of pellagra.

known to contain a nicotinic acid inhibiting factor [77, 230]. If the corn is treated with lime or alkali the pellagrigenic property is lost [351, 352]. This is of interest as pellagra is rare in Mexico where much corn is eaten in the form of *tortilla*, which is made with lime water. A deficiency of adenine may possibly be a factor in the aetiology of pellagra [146].

Fatty infiltration of the liver and liver damage is a common finding among pellagrins. These liver changes have been extensively studied by Gillman and Gillman [270, 276].

Clinical Signs and Symptoms of Pellagra. It is apparent from the study of a number of pellagrins, that there is a long prodromal period of ill-health with insidiously advancing symptoms, which at first appear trivial, but gradually increase in intensity. It takes four to eight months for the full-blown manifestation of the disease to appear [322]. Loss of weight,

strength and appetite, insomnia, vertigo, headache, "dyspepsia," anorexia, sore tongue and mouth, and constipation or diarrhoea are common prodromal symptoms, and appear without obvious cause. In the preclinical stage constipation is common. Irritability, loss of memory, and depression may be common complaints. Other early symptoms include abdominal pain, nervousness, palpitation, flight of ideas, inability to concentrate and mental confusion, dizziness on rising, pains in the limbs and vague alimentary symptoms. It is clear that the early syndrome presents no uniform clinical picture, and in the early stages a diagnosis of neurasthenia may easily be made. The diagnosis of preclinical pellagra can be made if these signs and symptoms are

SECONDARY PELLAGRA

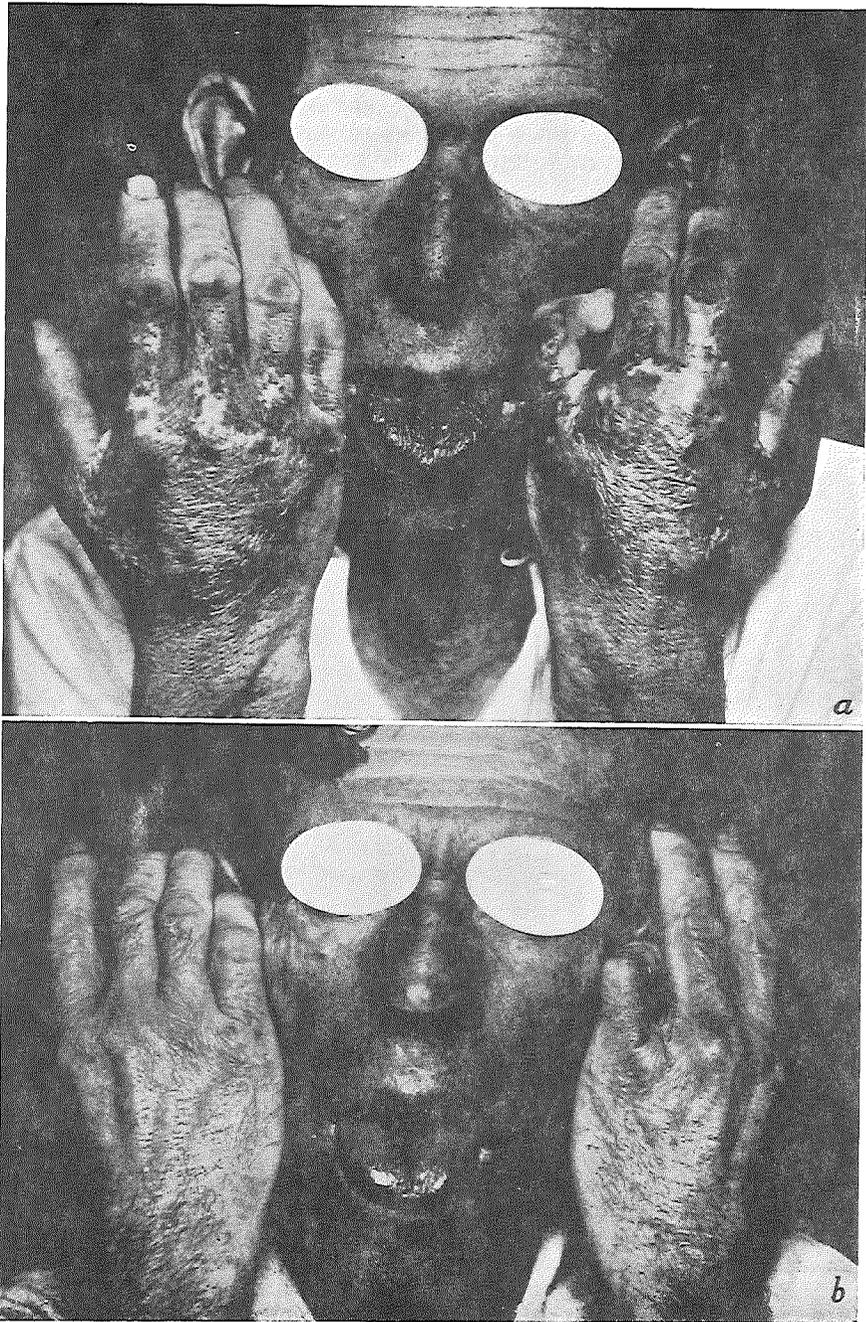


FIG. 99. "Secondary" Pellagra. The pellagra was due to cardiospasm (see Fig. 97). The upper figure is before treatment. Note the dermatitis on the back of the hands, neck and nose. The lips are cracked and the tongue red, swollen and beefy looking. The lower figure is after treatment with nicotinamide and high vitamin diet.

associated with grossly inadequate nutrition, with persons suffering from gastro-intestinal disease or who have been submitted to surgery of the gastro-intestinal tract, or with persons whose vitamin requirements have been increased by pregnancy, lactation, infection, diseases of the thyroid, or increased physical exercise (as in prison camps). At this stage the disease is readily arrested by the provision of nicotinic acid and other vitamins in high doses, supplemented by liver, yeast, and an adequate diet.

The facies of pellagra often exists before typical manifestations appear. According to American workers, the pupils are usually dilated (Fig. 106), the

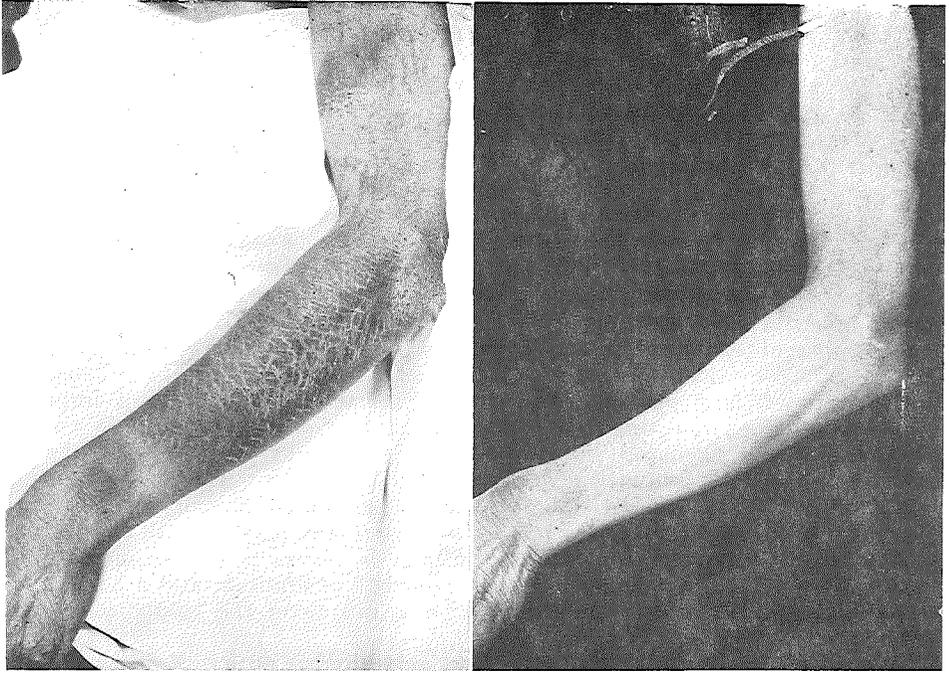


FIG. 100. Pellagrous Dermatitis. The heavily pigmented and cracked condition of the skin is characteristic. A case treated at the London Hospital.

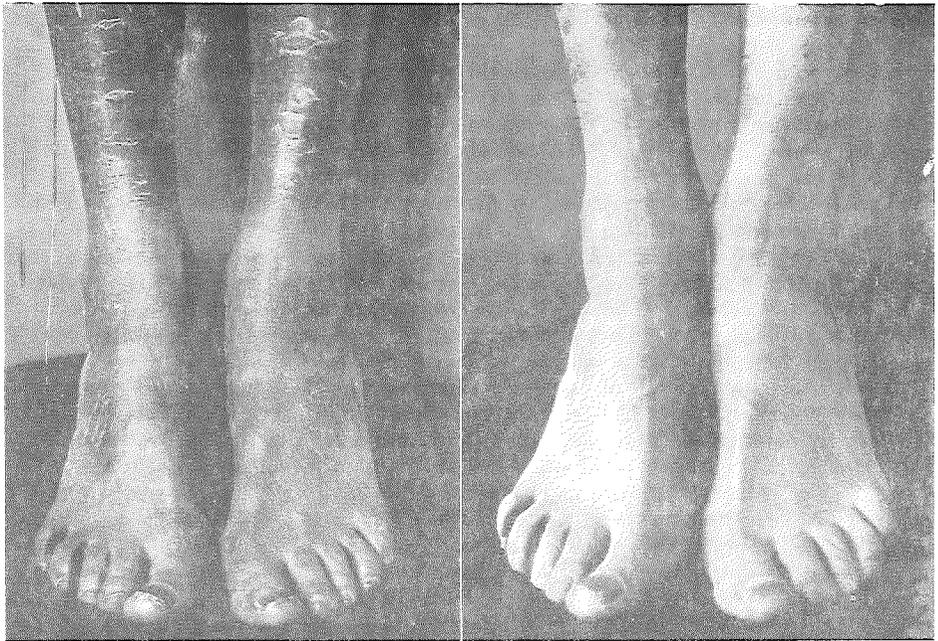
sclera is bluish and leaden coloured, the eyes and eyelids move slowly, and there is a characteristic dull lifeless stare (Fig. 106). There is an anxious or querulous expression around the eyes, which is so marked that it may sometimes be of diagnostic aid. The typical pellagrin is profoundly miserable. The ambulant pellagrin has frequently a muddy complexion and a slightly pigmented or macular eruption over the face, particularly the alæ of the nose and exposed surfaces of the neck, long before a typical dermatitis appears.

General Symptoms. These are summarized in the full-blown case in the mnemonic "Diarrhœa, Dermatitis and Dementia." The general symptoms include insomnia, loss of weight and strength, vague burning sensations over almost any part of the body, vertigo, staggering and sometimes tinnitus. The head may feel dull, there may be difficulty in concentrating, and cephalgias

PELLAGRA



FIGS. 101 and 102. Pellagra. A case admitted to the London Hospital under Dr. S. L. Simpson. The arm on the left shows the condition before treatment; brown pigmentation and thickening of the skin, which is cracked and glossy. The same arm, after a fortnight's treatment with a preparation containing the vitamin B complex, but not aneurine, is shown on the right. The skin is now approximately normal.



FIGS. 103 and 104. Pellagra. The feet of the same patient shown in Figs. 101 and 102 before and after treatment with a vitamin B complex preparation. The skin in the photograph on the left is highly pigmented and shiny. The skin is nearly normal on the right.

varying from a sensation of fullness or pressure, localized or general, to boring or stabbing pains are complained of. Any of the prodromal manifestations previously described may be met with. So-called "neurasthenic" symptoms occur in the prodromal stage. More than half of all severe cases have a macrocytic hyperchromic anæmia, and a number show achlorhydria. Pellagra may present no clear-cut clinical picture. The following manifestations are often seen.

Gastro-intestinal Symptoms. These precede the other symptoms and lesions and are usually the presenting ones. Loss of appetite, nausea, indigestion, vomiting, abdominal pain and constipation or diarrhœa are early complaints. Glossitis (Figs. 99, 111-113) and stomatitis are among the first symptoms. The tongue is indented and fiery red, first at the tip and edges and later the entire tongue is smooth, glazed and denuded of superficial epithelium and papillæ (Figs 112, 113) and is painful and inflamed as in sprue (p. 151). Often there are areas of superficial ulceration over the mucous membranes of the tongue and mouth, and smears from these show large numbers of Vincent's organisms. Dysphagia, a scalding sensation in the mouth, increased by highly seasoned foods or hot drinks, may be so painful that the patient refuses food; vomiting may be serious. There may be a salty, bitter or bad taste in the mouth and the pain on swallowing may cause increased salivation. Œsophagitis is common and food may burn all the way down. Œsophagoscopy under local anæsthesia shows a hyperæmic and œdematous mucosa with multiple small punctate ulcerations, and a barium swallow shows up many small constricted areas along the course of the œsophagus [272].

About fifty per cent. of pellagrins have achlorhydria even after histamine stimulation. In some cases hydrochloric acid reappears in the stomach after treatment with nicotinic acid. Radiological examination of the stomach reveals an atrophic mucosa associated with hypotonia, hypomotility and retarded evacuation [219].

In the early stages of pellagra the patient may be constipated; it is only later that diarrhœa becomes a prominent feature, although it is by no means constant, even in severe pellagra. Chronic diarrhœa when it does occur is distressing, the patient passing anything from three to thirty stools a day. These are liquid, profuse, foul and gaseous; sometimes they resemble those of sprue or those of dysentery, if they contain blood and mucus. Frequent defæcation produces a burning feeling in the rectum and proctoscopy shows general inflammation of the mucous membranes. The restricted food intake and the diarrhœa lead to emaciation. Many pellagrins have an irregular temperature with an evening rise up to 101° F. Albuminuria is said to be present in twenty per cent. of the cases.

Skin Lesions. Pellagrous dermatitis has a characteristic appearance and is distributed in those parts of the body subject to exposure and mild trauma due to tight clothing. The lesions are precipitated by exposure to sunlight, fires and radiant heat. They are distributed on the face, neck (Casal's necklace), dorsal surfaces of the hands and lower forearms (Figs. 99-103, 105-108), elbows, and the dorsum of the feet and lower legs in bare-footed persons. There may also be patches over the sternum, scrotum, labia and anus and other regions subjected to mechanical irritation or the action of the body secretions. These are typical sites, but the dermal lesions may occur on any part of the body. They are usually bilaterally symmetrical and are sharply demarcated from the adjacent healthy skin (Fig. 105), although Bean, Spies and Vilter [271] have described a number of cases of unilateral or asymmetrical pellagrous dermatitis. At first the skin lesions are erythematous and somewhat resemble sunburn, but later they change to a reddish brown colour, a fine branny or sometimes coarse desquamation occurs about a fortnight later, and the underlying skin is thickened. Permanent pigmentation may develop in pellagrins who have been subject to repeated occurrences of dermatitis.

The skin becomes scaly and over the legs and hands may sometimes present a typical appearance, which has been likened to cracked enamel or crazy paving (Figs. 83, 84). Sometimes the skin is uniformly smooth and shiny (Fig. 103). In severe cases the skin over a large area of the body may resemble that of a well-roasted turkey.

Hyperkeratosis (Figs. 109, 110) with callus formation is characteristic in chronic pellagrous dermatitis, and commonly appears over skeletal pressure points (knee, elbow, instep, front and back of the ankle), and may precede the exfoliating dermatitis and other manifestations of pellagra, particularly in those not exposed to sunlight. Indeed this may be noted by the potential pellagrin long before the prodromal appearances of the disease. This hyperkeratosis varies in appearance. Over the knees and instep the lesions may be wrinkled, or there may be fissures, or the hyperkeratosis may be nodular. The hyperkeratotic skin commonly shows pigmentation ranging from a light yellow, through brown to black.

These hyperkeratoses are an exaggerated response to irritation. They may also occur on the soles of the feet, although they do not necessarily occur over pressure points as they occur on bedridden patients. A fairly common type of hyperkeratosis is a diffuse thickening of the skin over the fingers, especially over the knuckles; it may be smooth and white or fissured, rough and pigmented.

Another skin manifestation is an ichthyosis-like change, which may be overlooked or attributed to chapping. It is worse in winter than in summer, but it may also be conditioned by exposure to heat. The usual site of these ichthyosis-like lesions is the antero-lateral aspect of the calves and less frequently the forearms. In a few cases the large thick plaques simulate alligator skin. A fine bran-like desquamation may also be seen.

The skin lesions sometimes become crusted and secondarily infected, particularly in natives, whose local remedies, e.g. dung, usually make the condition worse.

The genital and anal regions are often affected and the lesions appear at the same time as those of the tongue and mouth. An irritating secretion is poured out by the vagina which may macerate the perianal region. The lesions are red, macerated and often infected, particularly with Vincent's organisms. These lesions occur in some fifty per cent. of females with severe pellagra [222].

In pellagra there is an over-activity of the sebaceous glands (dyssebacia), with the formation of inspissated sebum blocking the mouths of the sweat glands. This lesion, which according to Smith [222] occurs in twelve per cent. of pellagrins, may be due to riboflavine deficiency (p. 309).

Lesions of Mouth and Lips. Pellagrous glossitis (Figs. 99, 111-113), which is common, is characterized by swelling and redness of the margins and tip of the tongue with indentations made by the teeth. Hyperæsthesia of the tongue frequently precedes objective signs. At times large red fungiform papillæ appear against a background devoid of filiform papillæ. As the disease progresses desquamation of the superficial epithelium leaves a scarlet, smooth, dry and beefy-looking tongue (Fig. 99). The desquamation may be irregular giving the appearance of the "geographical tongue." During desquamation secondary infection with Vincent's organisms and monilia frequently occurs, producing a thick white coating, which is ultimately shed. As the tongue becomes red and swollen, fissures and aphthous ulcers develop on its surface. The inflammatory process extends to the buccal mucosa, gums, lips and pharynx, producing reddening and superficial ulcerations. In advanced pellagra biopsy of the tongue shows extensive fibrosis of the submucosa and the adjacent muscular tissue.

The lips are often red and scaly (cheilosis) and fissures appear at the corners of the mouth (angular stomatitis). These lesions are due to riboflavine deficiency and do not respond to nicotinic acid (see p. 306).

There is a low incidence of dental decay and caries in chronic pellagrins [340]. Spies and his co-workers [341] have shown that there is a high content of nicotinic acid in the saliva of patients with severe dental caries, and a low content in the saliva of those with sound teeth.

Mental Symptoms. In pellagrins mental symptoms develop in one-third to a quarter of the cases if untreated. It has been estimated that in Italy, when pellagra was rife, four to ten per cent. of pellagrins became permanently insane. Symptoms are exceedingly varied. A feeling of tenseness, irritability, mental depression and emotional instability are fairly common. Patients



FIG. 105. Pellagra. A fatal case photographed at Napsburg Mental Hospital, Herfordshire. The dermatitis on the dorsal aspect of the hands is bilateral and sharply demarcated from the adjacent skin. There are pellagrous lesions on the forehead, cheeks, chin, lips, and in angles of the mouth. The skin on the dorsum of the forearm is thickened and pigmented. The patient was insane and died in a mental hospital.

weep without cause and insomnia is frequent. Melancholia, lethargy, and stupor are common, but confused states with hallucinations are also seen, as well as excitement, mania and delirium. The mental symptoms, which are often the first to appear, are particularly amenable to nicotinic acid therapy.

The mental symptoms of pellagra have been specially studied by Frostig and Spies [93], who describe the symptoms of the initial nervous syndrome. They are: hyperæsthesia to all forms of sensation; increased psycho-motor drive; increased emotional drive with a definite trend toward depression and apprehension; weariness and increased fatigue; headaches and sleeplessness; loss of memory; and confusion. In general the patients appear to have anxiety states with depressive features. There are also types in which excitement, mania, hallucination and deli-

rium may occur. A toxic confusional psychosis is very common and a clinical picture resembling Korsakow's syndrome has been described. The earlier pellagrologists recorded acute confusional insanity, stupor, hallucinations, acute delirium, catatonia, manic depressive states and dementia.

Psychosensory disturbances occur in all the special senses. Patients dislike bright light and colours, noises cannot be tolerated, music upsets them, odours and tastes may be so disagreeable as to cause nausea and vomiting. The patients can be described as being "on edge," irritable and tense. Many abnormal skin sensations are observed. Prominent complaints are dizziness, difficulty in maintaining balance, flickering stars and dark spots in front of the eyes.

The psychomotor drive is increased—the patient is fidgety, moves about a great deal, and is quarrelsome. He complains that a sudden noise or flash of light makes him jump and twitch. Emotional reactions are increased. The patient is more excitable and sensitive than usual; he is often depressed, sad and gloomy, and he is in a constant state of apprehension. Many patients express various fears, frights and phobias, although they may try to suppress them. The emotional outlook is gloomy and pessimistic and imminent danger is constantly expected.

In spite of the increased motor drive and restlessness the patients complain of weakness and fatigue. They tire readily at their work. There is a conflict between restlessness and fatigue, with the former often prevailing. Sleeplessness is also a common symptom, the patient falling asleep between 12 p.m. and 2 a.m. and waking again at 5 a.m. Sick headaches are common, resembling those of migraine, and occurring suddenly. The pain is localized in the forehead and temples and is accompanied by scintillating scotomata. As in migraine nausea and vomiting are frequent. Developing pellagra often causes a breakdown in personality. Individuals previously strong, courageous and enduring become shaky, weary and apprehensive before clinical pellagra can be diagnosed. Severe pellagrous psychoses occur in ten per cent. of untreated pellagrins. The patient may have periods of depression and apprehension followed by confusion, hallucinations, delirium, disorientation, and confabulation. A paranoid condition is often observed. Tremor, jerky movements and rigidity of the body may accompany these symptoms. In cases with severe depression the patient may have a mask-like expression and sit in one position staring into space for hours without moving.

The mental symptoms may precede the other symptoms of pellagra, so that a potential pellagrin may easily be diagnosed as "neurasthenic" or a paranoid. This is important because the mental condition clears rapidly in a few days with nicotinic acid therapy, whereas a case of true neurasthenia or paranoia remains unaffected. Early mental changes are due to "biochemical lesions" in the brain.

Neurological Lesions. These may precede, accompany or follow the other lesions of pellagra and were well recognized by the nineteenth-century



FIG. 106. Pellagra. A pellagrin showing mental symptoms. Note the characteristic dull, lifeless vacant stare and the dilated pupils. There are pellagrous lesions on the face and forearm. Melancholia, lethargy and stupor are common.

pellagrologists. Pain and cramps in the calves, numbness, burning or itching of the extremities, dizziness, vertigo, tinnitus, formication and other paræsthesiæ, headache, ataxia, tremors, spastic paralysis, Rombergism, sensory loss, diplopia, nystagmus and absent knee jerks have been reported. Peraita [89] observed the following neurological lesions in pellagrins during the Spanish Civil War : vertigo, diminished visual acuity, acroparæsthesiæ, causalgia and retrobulbar neuritis. The latter manifested itself in the form of central scotomata and missing letters and interrupted words on attempting to read. Hypohydrosis was also observed [284]. Burning hands and feet are



FIG. 107. Pellagra in a Child. Note the butterfly-like distribution of the dermatitis on the face. Pigmentation is present on the exposed parts and is absent from those covered by clothing.

almost pathognomonic [221]. This symptom was observed by earlier workers and was re-described by medical officers among prisoners of war in Japanese prison camps. The condition is characterized by severe burning and aching of the feet, associated with hyperæsthesia, raised skin temperature and vasomotor changes in the feet. The pains are more severe at night, interfering with sleep, the patient seeking relief by pacing the room or putting the feet in cold water. The condition became known as "painful feet," "burning feet," "hot feet" and "happy feet," the last named suggesting the dancing movements of the sufferer. Stannus [221] attributed the "burning feet" syndrome to ariboflavinosis, although Gopalan [226] claimed to have relieved the syndrome with pantothenic acid, other members of the vitamin B complex being ineffective.

De Raadt [275] has described a group of oto-neurological symptoms associated with the early stages of pellagra seen in Dutch prisoners of war in Batavia. These include vestibular hyper-irritability, vertigo, tinnitus, subjective deafness, headache, nystagmus, weakness of convergence and lateral gaze. The underlying pathology is a degenerative brain-stem encephalopathy. Visual defects were noted by the older pellagrologists, who recorded asthenopia, photophobia, reduction in the visual fields, central scotomata and optic atrophy. These symptoms have been more recently described by Fitzgerald Moore [223], Landor and Pallister [224] and Wilkinson and King [225].

Genito-urinary. Burning on urination, albuminuria, casts and indicanuria may be present. Libido is decreased, sterility common, and menstrual disorders are often seen [228]. Porphyrinuria occurs, but is an inconstant finding and of no diagnostic importance (p. 342).

Cardiovascular. The blood pressure is normal or slightly subnormal, and in severe cases the pulse rate is increased. Death in severe cases frequently occurs after vasomotor collapse and syncope.

Mainzer and Krause [87] studied the electrocardiographic records of a number of pellagrins, and in many the electrocardiogram was abnormal. That these abnormalities had a causal relationship to pellagra is demonstrated by the fact that their development ran parallel to the clinical course of the disease, and by the rapid disappearance in some cases after treatment with nicotinic acid and aneurine. Tachycardia was observed mostly when the disease was at its height, and bradycardia during convalescence. The most frequent electrocardiographic changes were a low voltage and notching of the ventricular complex, inversion of the T wave and shortening of the PR-interval. These changes are, however, not characteristic of pellagra.

Rachmilewitz and Braun [218] also noted marked changes in the electrocardiogram, particularly in the T waves, which were flat in T₁ and inverted in T₂, T₃, T₄, and were reversed by administering nicotinic acid.

Other Manifestations. Anæmia. Anæmia is common among pellagrins, although its occurrence is inconstant and it bears no direct relationship to the achlorhydria. The type varies; hypo- and hyper-chromic varieties and various colour indices have been recorded. There is an increased output of urea, even on a low protein intake. The polyuria and diarrhœa leads to loss of sodium, potassium and chloride ions from the body [324].

Pellagra sine Pellagra. Forms of pellagra have been described in which skin lesions are absent—*pellagra sine pellagra*. The outstanding lesions are stomatitis, glossitis, cracked lips and sores in the corners of the mouth, and although it is claimed that some cases have responded to treatment with nicotinic acid, it seems more likely from recent work that these symptoms are due to riboflavine deficiency (p. 307).

Infantile Pellagra. The condition formerly known as infantile pellagra, or kwashiorkor, in Africa is probably not pellagra at all, i.e. not pellagra in infancy. The syndrome is characterized by a failure of growth, œdema, steatorrhœa, lowered plasma albumin, macrocytic anæmia, "crazy pavement" dermatosis, depigmentation of hair, bowel symptoms such as diarrhœa, a deficiency bowel pattern on radiological examination [277] and a fatty liver. The condition has been named malignant malnutrition by Trowell [95], who attributes it to general malnutrition and debility accentuated by tropical parasitic and helminth infections. The crazy pavement dermatosis suggests pellagra, but the resemblance is superficial. It has been suggested by Altmann and Murray [90] that the condition is primarily due to a protein deficiency, although the administration of protein hydrolysates aggravates the condition [91]. Gillman and Gillman [270, 276] reported an excellent response to dried stomach. The fatty changes in the liver, which have been described by Gillman and Gillman, may be due to deficiency of lipotropic factors such as choline and methionine. The late and final results

are hepatic cirrhosis, pancreatic fibrosis and a form of nephritis. Davies [144] suggests that malignant malnutrition is primarily a pancreatic disorder due to malnutrition and that in consequence the liver becomes infiltrated with fat.

Diagnosis of Pellagra. The diagnosis of full-blown pellagra is made on the dermatitis, stomatitis, glossitis, mental and gastro-intestinal symptoms, the dietary history and response to the therapeutic test with nicotinic acid and foods rich in the vitamin B complex. The diagnosis is not difficult in cases with a characteristic pellagrous dermatitis, especially if this is symmetrical and shows a seasonal exacerbation, but in the absence of the latter,



FIG. 108. Pellagra in an English Schoolgirl. The dermatitis is present on the forehead, nose, cheeks, chin, neck and hands. There are lesions at the corners of the mouth as in ariboflavinosis (p. 306). Such cases are seldom seen outside mental hospitals.

predominating gastro-intestinal symptoms, glossitis and stomatitis may simulate sprue (p. 151). The neuropathy, glossitis and anæmia of pellagra without dermatitis may cause confusion with pernicious anæmia in the tropics and with subacute combined degeneration. The anæmia in pellagra is often of the normocytic hypochromic type and the hæmoglobin commonly fifty to seventy per cent. Achlorhydria is common. A history of repeated attacks of the disease, particularly in the spring, and of dietary deficiency helps in the diagnosis. The skin lesions may be mistaken for those of erythema multiforme, erythema solare, occupational dermatitis, syphilis, lupus erythematosus and toxic dermatitis. The nervous manifestations have to be differentiated from those of hysteria, ergotism, lathyrism and general paralysis of the insane. In old people with arteriosclerotic changes and accompanying mental symptoms there

may be lesions of the hands and face, which may cause confusion in diagnosis. Other conditions in which some of the signs and symptoms of pellagra may appear are tuberculous enteritis, chronic pancreatitis, stomatitis of varying aetiology, neurasthenia and Vincent's angina.

The Plummer-Vinson syndrome has some symptoms in common with pellagra—glossitis, dysphagia and anæmia. The possibility should always be borne in mind that pellagra may be associated with other diseases such as syphilis, tuberculosis, tropical diseases and conditions mentioned on p. 352, which may act as predisposing causes, and to which pellagra may be secondary.

Laboratory Tests for Diagnosis of Nicotinic Acid Deficiency. So little is known about the fate of nicotinic acid in the body and so many factors influence the excretion of the vitamin that tests for nicotinic acid deficiency

THE SKIN IN PELLAGRA

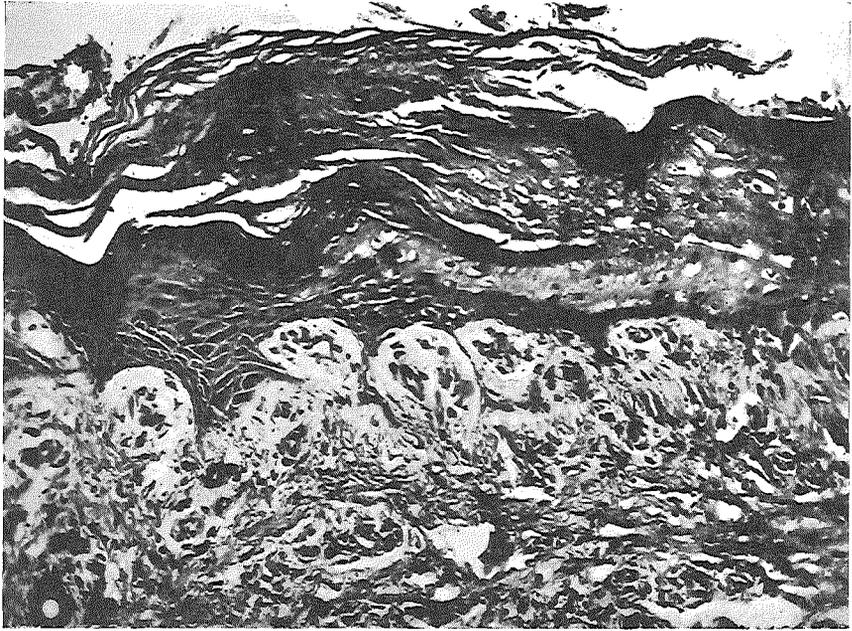


FIG. 109. The Skin in Pellagra. Low-power photomicrograph showing hyperkeratosis, oedema and distortion of the rete pegs of the epidermis.

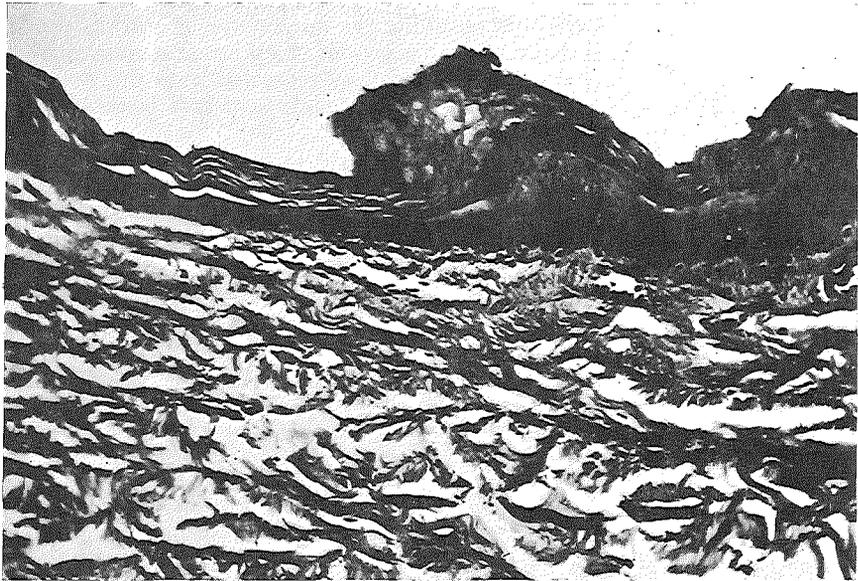


FIG. 110. The Skin in Pellagra. Low-power photomicrograph showing hyperkeratosis, atrophy of the epidermis, and oedema of the cutis.

based on excretion tests are of little value, although Ellinger, Benesch and Hardwick [291] claim that the excretion of methylnicotinamide after a dose of 100 mg. of nicotinamide reflects fairly accurately the stores of nicotinic acid in the body. The method was used as an index of nicotinic acid nutrition in R.A.F. personnel [288]. Apparently deficient subjects may excrete only half as much methylnicotinamide after a test dose of nicotinic acid as normal subjects, but so do subjects from a mixed public ward in a general hospital [286]. It is claimed that measurement of the excretion of N'-methyl-nicotinamide for one hour, with the patient in the fasting state, or for longer periods after a test dose yields helpful information [294, 315]. Johnson and his co-workers [316] claim that an excretion of 0.03 mg. or less of this compound in one hour while fasting indicates a subnormal storage in the tissues, and that less than 0.5 mg. in four hours after the oral administration of 50 mg. of nicotinamide indicates deficiency. A urinary excretion of less than 95 micrograms per 100 ml. per hour fasting is said to be below normal, and 45 micrograms or less to denote deficiency [323]. Goldsmith and her co-workers [360] noted pellagrous lesions in subjects excreting 0.5 to 0.6 mg. daily.

The excretion of porphyrin in pellagrins is within normal limits and is of no help in diagnosis (p. 342).

Blood levels of less than 400 micrograms of nicotinic acid per 100 ml. are stated to be below normal, and less than 200 micrograms extremely low [323].

Pathology of Pellagra. Post-mortem the only external appearances that are of diagnostic value are the skin and mouth lesions; emaciation occurs late. The pathological lesions are frequently obscured by complicating diseases, such as bacillary dysentery and tuberculosis.

The most striking histological skin changes are hyperkeratosis (Figs. 109, 110), parakeratosis, acanthosis, hyperplasia of the sweat glands, dilatation of the papillary blood vessels, moderate lymphatic infiltration and plugging of the hair follicles with dry sebaceous material [232, 233]. Slight oedema of the deeper portions of the epidermis occurs and cells of the basal layer undergo multiplication. The skin lesions are sharply limited to the zone between the corium and epidermis. Generally speaking the microscopic picture is similar to that found in chronic inflammatory diseases of the skin. The skin changes are to a considerable extent reversible and may represent a specific response on the part of the skin to a deficiency of nicotinic acid and possibly other members of the vitamin B complex.

Degeneration of the nerve fibres of the skin in early pellagra has been described. Vesicular formations in the epidermis may occur, and if they become infected the epidermis sloughs off, leaving an atrophic or horny lamellar corium. The pigmentation is due either to the formation of melanin in the malpighian and basal layers of the epidermis, or to the formation of granules of an iron pigment in the epidermis. In old lesions atrophy and reduction in the cells of the malpighian layer occurs.

As a rule little can be seen macroscopically in the gastro-intestinal tract. Greenfield and Holmes [71] describe the *état mamelonné* of chronic gastritis; enteritis has been described. The walls of the colon may be thickened and red, and covered with pseudo-membranous patches and minute grey cysts, formed by distension of the crypts of Lieberkühn.

Vedder [231] states that the gastro-intestinal tract is considerably inflamed and frequently ulcerated, particularly in the small intestine, colon and rectum. To what extent these changes are terminal ones following emaciation is difficult to decide. Fatty degeneration of the viscera and atrophy of the thyroid and adrenal glands have also been described.

The nervous lesions are late in appearance. The most common lesions in the spinal cord consist of areas of scattered symmetrical myelin degeneration in various tracts, particularly in the posterior and lateral columns. The peripheral portions of the fibres are frequently spared. In most cases the

afferent tracts are more affected than the efferent, although in occasional cases no lesions in the posterior columns have been observed. Chromatolysis of the posterior root ganglia with loss of Nissl granules is most frequent in the dorsal, lumbar and lower cervical regions. In the grey matter there is pigmentation of the cells of the anterior and posterior horns, the latter appearing to be degenerated from the cervical region downwards. The cells in Clark's column are particularly affected. In the anterior horns in the lumbar region the cell bodies are swollen and the nucleus is displaced to the periphery as a result of chromatolysis [231]. In the brain the frontal lobe is most frequently involved. The large pyramidal cells, in scattered foci, show chromatolysis with nuclear displacement and accumulations of fat. In late cases gliosis occurs. There is some wasting of the brain with excess fluid in the ventricles. Pathologically the lesions bear some resemblance to those of subacute combined degeneration.

Leigh [356] states that there is a retrograde cell degeneration affecting certain groups of nerve cells throughout the nervous system, the Betz cells being invariably affected. He considers that involvement of the Betz cells is pathognomonic.

Prognosis and Treatment. Most cases develop in late winter or spring, become more and more severe for two or three months and then slowly improve. The patient may recover completely or vague symptoms may remain. Recurrences may occur every spring, and with each attack the patient becomes weaker and more emaciated until death occurs, in the average untreated case, in about five years. Acute cases have been described in which death may occur in the first attack owing to severe gastric and nervous involvement. Recovery readily occurs following effective treatment, but relapse is common.

Nicotinic acid, sodium nicotinate, or nicotinamide relieve the acute mental symptoms in a dramatic fashion, and also improve the alimentary tract and skin lesions, but have little effect on the neuropathy, or on the lesions of the lips and face. Nicotinamide has an advantage over nicotinic acid in that it does not produce vasodilatation of the skin, flushing, itching and other reactions (p. 346); the dosage of nicotinamide is the same as that of nicotinic acid. Reactions from the latter are less likely if it is given after food.

Treatment of Mild Cases. Patients with mild or subclinical pellagra recover rapidly on a diet containing adequate quantities of nicotinic acid and other members of the vitamin B complex. Plenty of red meat, meat extracts, liver, eggs, fresh vegetables, milk and yeast extract or brewers' yeast should be incorporated in the diet. Some commercial yeasts may not supply sufficient nicotinic acid to prevent pellagra [278]. Small doses of nicotinic acid or nicotinamide, 25 to 50 mg., two or three times daily after meals are helpful. Exposure to direct sunlight, rough clothing, and skin trauma should be avoided.

Treatment of Moderately Severe Cases. A case of moderately severe pellagra should be confined to bed until the skin lesions have disappeared. The same general and dietary treatment as described in the mild cases should be followed. Nicotinic acid or, better, its amide is given in doses of 50 to 200 mg. daily after food. The lesser-known members of the vitamin B complex are best administered in the form of boiled yeast (1 to 6 oz. daily), or yeast or liver concentrates. Crude liver concentrates are given in doses of a tablespoonful three times daily. Supplements of vitamins A and D (fish liver oils), C and iron (ferrous sulphate gr. 3 to 5 t.d.s.) should be included and a high calorie diet (2,500 to 4,000 calories) provided. Riboflavine, 3 to 10 mg. daily, and aneurine 5 to 10 mg. twice daily, after meals, help to control associated riboflavine and aneurine deficiencies. After recovery the dosage of nicotinic acid is reduced to a maintenance dose of 50 mg. once or twice daily. Pure vitamin preparations are not so effective as an adequate diet.

Treatment of Severe Cases. Severely ill patients should be hospitalized and treated as emergencies, as they may collapse and die within a day or so. Diarrhoea and dementia are often present so that normal feeding has to be abandoned at first. The patients are usually dehydrated from the diarrhoea and the associated glossitis, dysphagia, anorexia and vomiting prevent the ingestion of food. The administration of vitamin concentrates by mouth is therefore useless in the early treatment of the disease. The patient is given intravenous infusions of five per cent. glucose in normal saline in doses of 500 to 1,000 ml. two or three times daily for the first day or so. This may be continued if the patient cannot take fluids by mouth or has severe diarrhoea. It is not advisable to give yeast at this stage as it cannot be retained and makes the diarrhoea worse. Nicotinic acid or the amide is given in the saline drip in doses of 10 to 20 mg. as a single dose, this amount being repeated at hourly intervals. Too large a quantity at once may cause a reaction. Some workers have found six intravenous injections of 50 mg. a day (total 300 mg. daily) satisfactory. If nicotinic acid is tolerated by mouth and there is no severe diarrhoea, 300 to 500 mg. daily for a week is given orally in divided doses. To the saline glucose drip may be added 100 mg. of aneurine 50 mg. of riboflavine and 200 mg. of ascorbic acid. Intravenous therapy is continued until the diarrhoea and vomiting have subsided. The patient is then given a liquid diet supplemented by nicotinic acid (300 to 500 mg. daily), yeast, meat extracts, liver extract and aneurine and riboflavine in the doses mentioned. Occasionally patients do not respond readily to nicotinic acid; in such cases favourable results are often obtained by crude liver extracts given parenterally in doses of 5 c.c. daily. Liver extracts and stomach extract are sometimes life-saving in severe cases [270]. When improvement has occurred a maintenance dose of nicotinic acid once or twice daily usually suffices. Within one to three days of beginning treatment the fiery tongue and soreness of the anus and vagina subside; the dermal erythema blanches; the acute mental manifestations often vanish overnight; and the papillae of the tongue regenerate after seven to fourteen days. The diarrhoea, however, may persist for five to ten days. The mental changes unless of long standing are usually reversible.

Symptomatic Treatment. A mild alkaline mouth wash may be used for the stomatitis, but the teeth should not be brushed as the gums are tender. For the dermatitis dressings of calamine may be used; if there is secondary infection this may require local treatment with penicillin cream or other antibiotics if available, but not with sulphonamides, which may produce skin sensitization. Sedatives may be required at first for the uncontrollable mental patients, who are, however, usually amenable after the first few days on nicotinic acid therapy. Tinct. opii 30 minims every four hours and succinylsulphathiazole by mouth help to control the diarrhoea in the early stages. If the hæmoglobin is below fifty per cent., blood transfusions may be given, and iron as ferrous sulphate 3 to 5 mg. t.d.s. and yeast administered to control the anæmia. It is important to correct achlorhydria if present, as this interferes with the absorption of iron and vitamins.

Relapses are common once the patient passes out of the care of the hospital or physician. This is not surprising. How can the pellagrin, whose disease is usually due to poverty, afford a diet containing adequate protective foodstuffs? The problem of pellagra is not medical but economic. In America pellagrins are encouraged to keep chickens or a cow or to cultivate kitchen gardens or small holdings. Spies [88] states that a mixture of twenty-five per cent. brewers' dried yeast, sixty-seven per cent. peanut butter and eight per cent. peanut oil in daily doses of 2 ounces is an inexpensive and palatable dietary supplement and tends to prevent pellagra, beriberi and riboflavine deficiency.

Nicotinic Acid Psychoses. In some of the older accounts of "typhoid pellagra" psychotic symptoms were commonly described. It is believed by

Jolliffe and others [99, 100] that nicotinic acid deficiency is responsible for an encephalopathic syndrome, which has a mortality of eighty-nine to one hundred per cent. unless treated. A hundred and fifty cases were studied by Jolliffe, who observed that the condition was associated with deficiency diseases, particularly with deficiency of the vitamin B complex. The clinical picture is characterized by clouding of consciousness, changing cogwheel rigidity, and uncontrollable sucking and grasping reflexes; there may also be oculomotor disturbances varying from bilateral nystagmus to complete ophthalmoplegia. Sydenstricker [101] adds hebetude grading into profound stupor, delirium, and agitated depression. According to Jolliffe this syndrome may occur independently or in association with pellagra or with polyneuritis or with both. Sydenstricker, however, observed it in nineteen cases in the absence of a complete syndrome of pellagra or a history of pellagra.

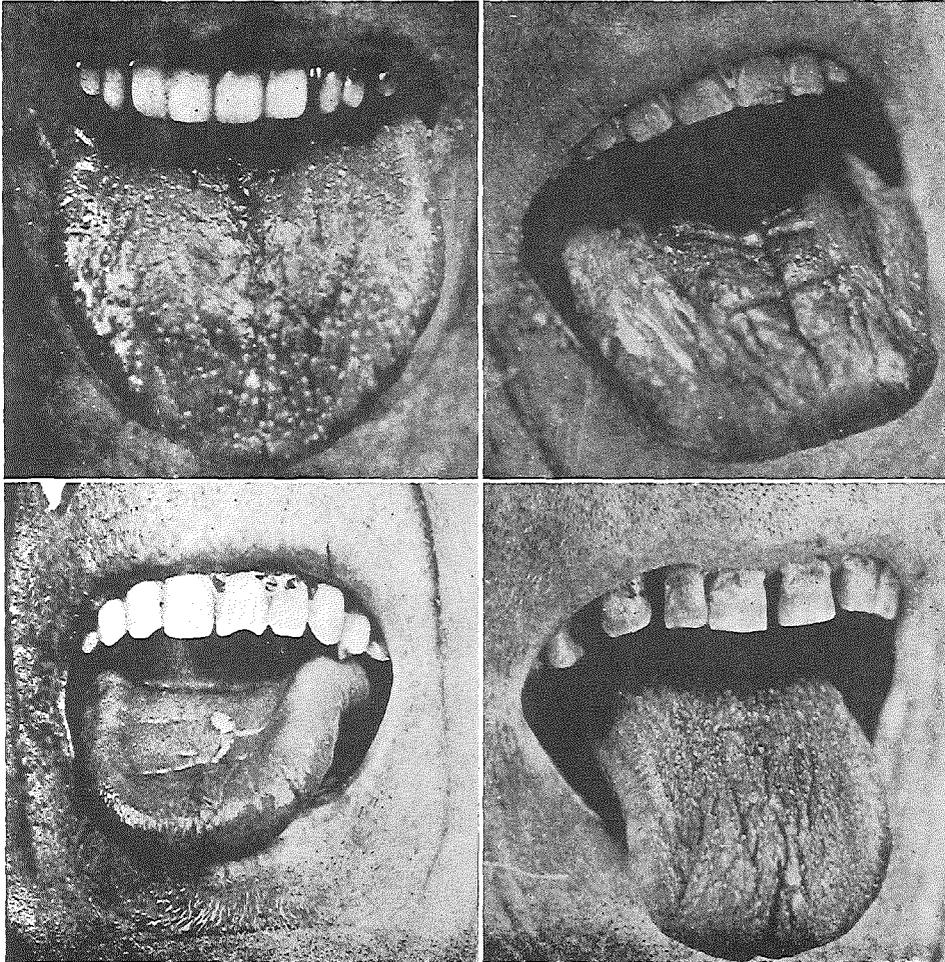
This syndrome treated by hydration and aneurine had a mortality of one hundred per cent. in one group, and a mortality of sixty-two per cent. when treated with hydration and the entire B complex. In the hands of Jolliffe this was reduced to 31.8 per cent. with nicotinic acid, which was injected in 100 mg. doses up to a total of 500 mg. a day; later, this was increased to 1,000 mg. a day in 100 mg. doses. Sydenstricker used 100 to 300 mg. of sodium nicotinate in normal saline containing five per cent. glucose, which was given intravenously, and 100 mg. of sodium nicotinate intramuscularly. The cure was described as dramatic. Recovery occurred within a few days when the patients were given a high calorie diet supplemented by nicotinic acid and the vitamin B complex, aneurine and riboflavine. A control group presenting stupor of demonstrable origin was employed by Sydenstricker; they did not respond. The workers on this subject are convinced that a therapeutic test with nicotinic acid is justifiable in cases of unexplained hebetude or unconsciousness with a bad dietary history.

Jolliffe believes that this encephalopathic syndrome results from a complete and acute nicotinic acid deficiency, while the pellagra syndrome represents a partial and more prolonged deficiency of nicotinic acid. Patients showing the encephalopathic syndrome but no signs of pellagra represent a complete nicotinic acid deficiency which develops so rapidly that the changes of pellagra do not have time to occur.

Sydenstricker [234] also draws attention to the psychoses formerly classified as "toxic," "exhaustion delirium" and "psychosis, cause undetermined" not infrequently seen in general hospitals and seen sometimes after surgical operations or after delivery. In most cases there is no history of frank dietary deficiency, although some patients are alcoholic, some have been dieted for medical or surgical reasons; others have their vitamin requirements increased by fever or infection. Intravenous saline and glucose infusions without food by mouth sometimes precipitate an attack. The onset of delirium, hallucinations or mania is abrupt, or after a short period of confusion. An important diagnostic sign is the fluctuation of the condition, the patient improving or relapsing for no obvious reason. The tongue is frequently dry, clean and red--the so-called "toxic tongue." Rarely are there any classical signs of nicotinic acid deficiency present. Patients, particularly middle-aged or elderly, showing mental confusion, delusions, hallucinations, stupor, manic excitement and confabulation are often admitted to hospital with a provisional diagnosis of uræmia, arteriosclerotic or senile dementia, neurosyphilis, drug intoxication, or a cerebral vascular accident. Some are even admitted to mental wards. Gottlieb [235] has described several patients admitted to a London hospital under such circumstances.

Spillane [147] has observed Arabs picked up in the street unconscious responding dramatically to nicotinic acid. The symptomatology included stupor, tremors, rigidity and grasping and groping movements. Stupor, delirium and acute psychotic symptoms were also seen by Spillane in under-

THE TONGUE IN NICOTINIC ACID AND RIBOFLAVINE DEFICIENCY



FIGS. 111 to 114. The Tongue in Nicotinic Acid and Riboflavine Deficiency.

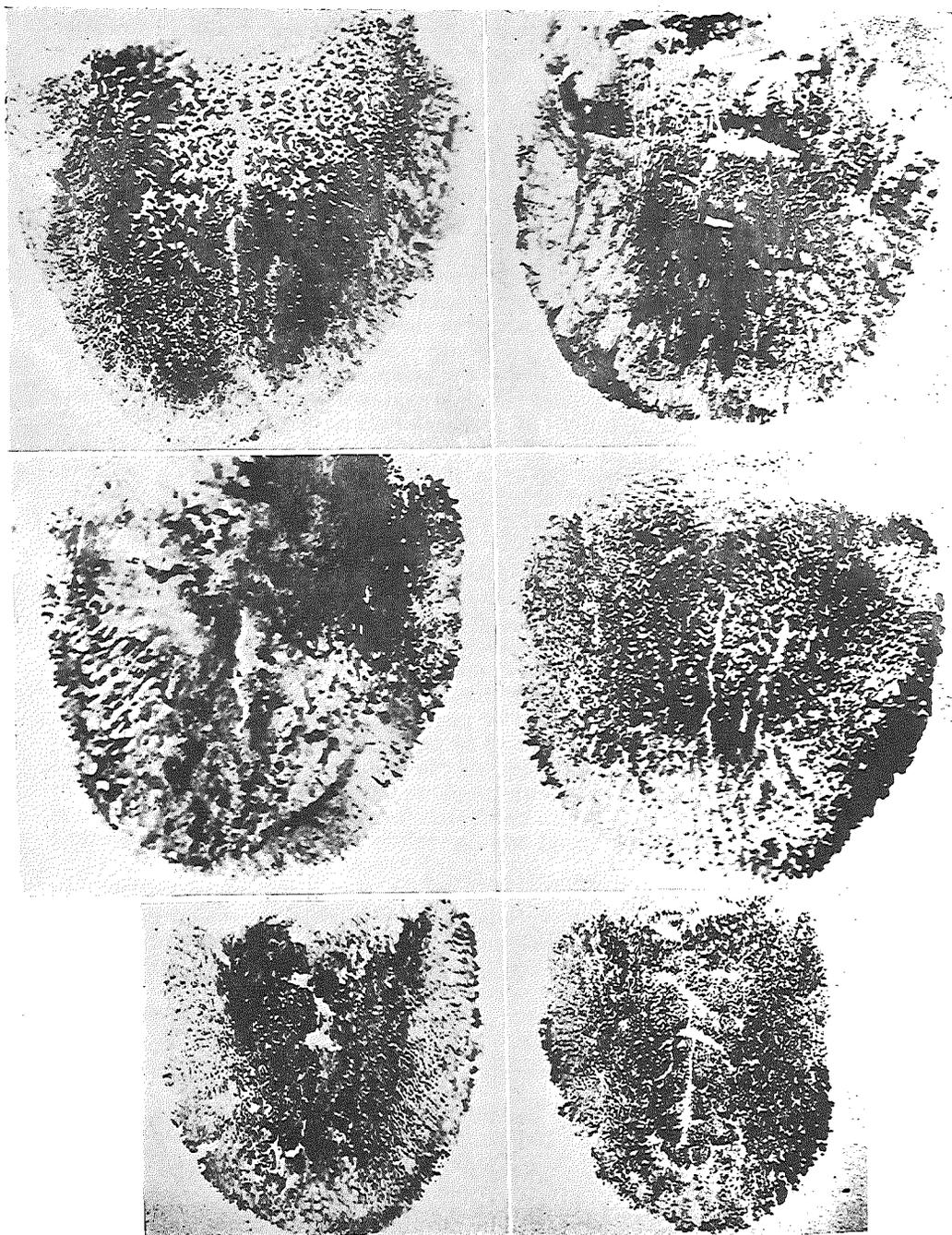
FIG. 111 (*upper left*). Hypertrophy of the papillæ in a patient with nicotinic acid deficiency.

FIG. 112 (*upper right*). Tongue, which was fiery red, showing atrophy of the papillæ. From a case of nicotinic acid deficiency.

FIG. 113 (*lower left*). Bald atrophic tongue due to nicotinic acid deficiency.

FIG. 114 (*lower right*). Tongue showing fissuring and hypertrophy of some of the papillæ from a case of riboflavine deficiency. The tongue was magenta coloured.

NICOTINIC ACID DEFICIENCY. TONGUE PRINTS



FIGS. 115 TO 120. Tongue Prints from Cases of Nicotinic Acid Deficiency.

FIGS. 115 and 116 (*upper left and right*). Tongue print showing progressive atrophy of the papillæ.

FIGS. 117 and 118 (*middle left and right*). Tongue print showing atrophy of the papillæ on left, with improvement on the right two weeks after treatment with nicotinic acid.

FIGS. 119 and 120 (*lower left and right*). Tongue print of same case showing return of more papillæ with vitamin therapy.

nourished German prisoners of war. Wexberg [98] points out that some cases of senile dementia have nutritional deficiency as a background. These respond to treatment with nicotinic acid, which if given in sufficiently large doses produces a dramatic improvement in the mental condition in twenty-four to forty-eight hours. For immediate treatment Sydenstricker [234] suggests 100 mg. of nicotinic acid or 30 mg. of nicotinic acid amide every hour for ten hours during the first two days, continuing this dosage longer if necessary. This is given by mouth, stomach tube, or parenterally if the patient is stuporous or unco-operative. Once improvement sets in the daily dosage is reduced to 100 mg. of nicotinic acid five times a day, or 150 mg. of nicotinamide daily. Later, 25 mg. of nicotinic acid three times daily should be sufficient. Sydenstricker also gives aneurine in doses one-tenth of that of the nicotinic acid, i.e. 50 mg. daily at first. Yeast in quantities of 15 to 30 gm. daily or other sources of the vitamin B complex such as yeast extract or wheat germ preparations are added to the diet.

Mainzer and Krause [121] gave large doses of aneurine in a case of delirium tremens associated with severe gastro-intestinal symptoms without effect, but the administration of nicotinic acid in doses of 500 mg. daily made all pathological manifestations disappear within twelve hours. They believe that the prompt response to nicotinic acid favours the assumption that lack of vitamin is an important factor in the development of delirium tremens. May [122] also observed that nicotinic acid in daily doses of 600 mg. brought about improvement in four cases of severe psychosis with pellagrous dermatitis accompanying chronic alcoholism.

Calvo Melendro [236] and Washbourne [306] treated cases of depressive psychoses without an obvious nutritional deficiency with nicotinic acid, 300 to 400 mg. intravenously. Medlicott [299] claimed striking benefit in cases of confusional and schizophrenic psychoses and exhaustion following delirious mania by treatment with nicotinic acid. Lehmann [298] also records a case of confusional state passing into a Korsakoff's psychosis after cerebral injury treated successfully with 25 mg. of nicotinic acid by mouth three times a day and a daily intravenous dose of 50 mg. Gregory [354] treated fifty-four cases of senile psychosis with doses of 300 mg. three times a day orally and 100 mg. daily intravenously with dramatic or considerable improvement in twelve. It is possible that any beneficial effects seen in these cases were due to an increase in cerebral blood flow due to the vasodilator action of nicotinic acid.

Sydenstricker and Cleckley [153] report that thirty-eight patients in stuporous states or in active psychoses without evident cause showed prompt and very often impressive improvement after treatment with nicotinic acid, in total dosage varying from 100 to 4,500 mg. orally. Pellagra and other deficiency states were absent. Sydenstricker and Cleckley believe that the symptoms of many cases of toxic psychosis, exhaustion delirium and unexplained clouding of consciousness, may be relieved by nicotinic acid. In some cases very large amounts of nicotinic acid, e.g. 4,500 mg., were necessary to obtain satisfactory results.

Lingual Manifestations of Nicotinic Acid Deficiency. Kruse [96] claims that the examination of the tongue affords a method of detecting nicotinic acid deficiency. He states that atrophy of the tongue, fissures and denudation and reduction of the filiform papillæ occur in nicotinic acid deficiency, and that these lesions are reversed by nicotinic acid in doses of 200 mg. daily. Sevringhaus and Kyhos [104] noted that thirty men out of 102 in a prison camp showed the tongue changes described by Kruse, which yielded to treatment with 50 mg. of nicotinic acid daily. While tongue changes may occur as a result of nicotinic acid deficiency, these are not diagnostic of the latter. Upper dentures and iron deficiency anæmia are the commonest cause of denudation of the filiform papillæ; fissures of the tongue can also be congenital and symptomless and are often seen on routine examination of

patients. Glossitis may be due to badly fitting dentures and the chewing or smoking of tobacco ; it may also occur in sprue and pernicious anæmia. In all probability the vitamins are so closely inter-related that it is difficult to attribute specific lesions, e.g. of the tongue, to a deficiency of any one vitamin. Glossitis does not necessarily result from pure nicotinic acid deficiency.

Bakwin and his co-workers [105] concluded that the tongue lesions commonly seen in children are not due to nicotinic acid deficiency.

Induced Nicotinic Acid Deficiency. Attempts have been made to induce nicotinic acid deficiency in man by sterilizing the gut with sulphonamides or penicillin and thus preventing the biosynthesis of nicotinic acid. Hardwick [110] described the development of stomatitis and acute dermatitis after the administration of sulphaguanidine ; the lesions disappeared after the administration of nicotinic acid. Ellinger and Shattock [148] observed symptoms suggestive of nicotinic acid deficiency in a patient receiving penicillin by mouth ; withdrawal of penicillin and administration of nicotinic acid by mouth resulted in cure of the condition. There is no proof that these lesions were due to nicotinic acid deficiency, as sulphonamides and penicillin may inhibit the biosynthesis of other vitamins as well as that of nicotinic acid.

So far no one has produced a pure nicotinic acid deficiency by omitting nicotinic acid alone from the diet. Several workers have described the results of a vitamin B complex deficiency, but which members of the complex were responsible for the symptoms is not known.

NICOTINIC ACID THERAPY

Nicotinic acid has been used in the treatment of a number of clinical conditions, some associated with deficiency disease, while others are not. In the latter group it presumably acts pharmacologically and not as a vitamin. Nicotinamide can be used instead of nicotinic acid, and in the same dosage, in the treatment of conditions associated with defective nutrition. It is in fact preferable to nicotinic acid if the latter is to be injected or given in large doses, since nicotinic acid is liable to produce flushing and vasodilatation, which although not harmful, may prove alarming to the patient (p. 346). Nicotinamide is free from these side effects. If nicotinic acid itself is given, it is best to keep the single dose within 50 to 100 mg. after meals to avoid the vasodilator effect. When nicotinic acid is given for its vasodilator action it cannot be replaced by nicotinamide, which is devoid of any such action.

Oral Conditions. "Trench Mouth." Vincent's organisms are commonly found in the buccal lesions of pellagra and disappear with nicotinic acid therapy. King [111, 151, 239] has attempted to demonstrate an association between nicotinic acid deficiency and the syndrome variously described as "trench mouth," Vincent's disease, ulcerative gingivo-stomatitis and fusospirochætal stomatitis. The condition is characterized by the formation of a pale yellowish-grey epithelium progressing to ulceration on the gingivo-dental margins. It is accompanied by bleeding from the diseased capillaries, soreness and fœtor oris. King believes that nicotinic acid deficiency, reduced tissue resistance (colds, infections), chronic gingivitis, and oral trauma are contributory factors in the ætiology of trench mouth. He distinguishes three types : (a) *fulminating*, with rapid onset, occurring sporadically in spring and summer ; (b) *subacute non-ulcerative* type, with a comparatively high incidence throughout the year ; (c) "*flaring*" *subacute*, resembling (a) in symptomatology, but differing in that there is a history of subacute disease. Nicotinic acid in doses of 200 mg. daily, with a maintenance dose of 100 mg. for seven to fourteen days was stated to be effective in the fulminating type, but less satisfactory in the subacute and "flaring" types. King states that he found the best treatment to be local application of hydrogen peroxide and

chromic acid and nicotinic acid by mouth. Ascorbic acid therapy was disappointing. He states that most cases clear up given nicotinic acid, riboflavin and ascorbic acid. Good results with nicotinic acid therapy in the treatment of trench mouth are also claimed by Miller, Greenhut and Roth [243], Schwartzman and Grossman [244] and Smith [245].

These views have been criticized by other workers. Ungley and Horton [241] noted that eighty-five per cent. of a group of British naval ratings suffered from trench mouth, but they were unable to relate the incidence of this to nicotinic acid or ascorbic acid deficiency. Nicotinic acid, 500 mg. daily,

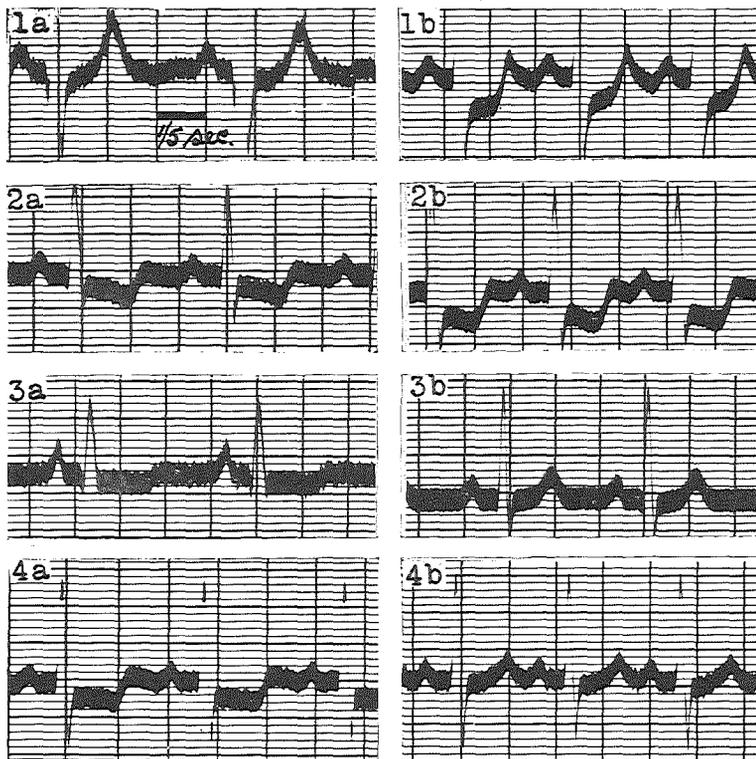


FIG. 121. Electrocardiograms in a Patient with Cardiac Ischæmia, after Administration of Nicotinic Acid and Glyceryl Trinitrate.

- (1a) At rest. (1b) After exercise inducing pain.
 (2a) At rest. (2b) 25 minutes after 200 mg. of nicotinic acid which failed to cause flushing.
 (3a) At rest. (3b) 24 minutes after 300 mg. of nicotinic acid, which caused flushing.
 (4a) At rest. (4b) 4 minutes after chewing glyceryl trinitrate gr. 1/100.

caused no improvement. Cuthbert and Williams [242] and Stammers [259] also found no evidence of nicotinic acid deficiency in trench mouth nor did they find that nicotinic acid without local treatment had any effect on the course of the disease. Stammers' conclusions were based on a study of over 1,000 cases.

Coulson, Ellinger and Smart [288] examined the nicotinamide methochloride excretion after a test dose of nicotinamide, which is considered to be an index of nicotinic acid nutrition (p. 366), in a number of R.A.F. personnel with normal gums and found it to be higher than in those with various types of gingivitis. The difference was statistically significant, but was not considered to be of ætiological importance, since other factors have a marked

influence on the results. Among subjects from the same economic or social class with similar feeding habits and similar diet the excretion of nicotinamide methochloride after a test dose of nicotinamide was the same whether gingivitis was present or not. Cocker and Bigger [108] conclude that gingivitis can be adequately treated by local treatment without vitamins.

Cardiovascular Diseases. It has been suggested that the vasodilator action of nicotinic acid might be utilized in the treatment of peripheral vascular disease, especially in extremities with a diminished blood supply [68, 112, 113, 137]. The effective oral dose is in the region of 100 to 300 mg. or 20 to 25 mg. intravenously. Whatever favourable effects it might have, they are of short duration. Loman [137] and his colleagues have shown that nicotinic acid cuts short a Raynaud attack produced artificially by adrenaline injected into the brachial artery. Green and Salber [154] state that a considerable improvement occurred in a case of hemiplegia treated with 150 mg. of nicotinic acid three times daily for fifteen days, and Furtado [246] claims that a dose of 50 to 200 mg. daily gave considerable relief in a case of cerebral thrombosis. As only single cases were reported and both conditions often result in natural recovery, it is difficult to comment on this form of treatment.

Moncrieff [162] has used nicotinic acid with good results in angina pectoris. Neuwahl [247] found that the administration of nicotinic acid by mouth decreased the severity and number of attacks of angina pectoris in a number of cases, but in some the effect was only transient. He, therefore, gave it in the form of an intravenous drip of a 0.05 per cent. solution in isotonic saline. One infusion of 100 to 300 mg. produced beneficial results, which were maximal in twelve to twenty-four hours. In most cases a course of six infusions spread over three weeks was given. Six cases showed complete or almost complete regression of symptoms over a period of three to seven months after treatment. The nicotinic acid was stated to produce a fall in blood pressure and slowing of the heart. According to Stokes [260] the changes in the electrocardiogram of cardiac ischaemia in man following the administration of nicotinic acid suggest that it can improve the coronary blood flow (Fig. 121). This only occurs after giving doses large enough to produce peripheral flushing. In a controlled clinical trial Stokes was unable to confirm the beneficial effect reported by others of nicotinic acid in the treatment of angina. In ten cases of angina that were all relieved and prevented by glyceryl trinitrate only three received slight benefit from the administration of nicotinic acid in doses of 200 mg. daily (50 mg. q.d.s.).

Soupanki [240] concluded that nicotinic acid is inferior to theophylline in the treatment of coronary disease.

It is claimed that the carbinol of nicotinic acid, β -pyridylcarbinol, is an effective vasodilator given by mouth. It has been used in the treatment of peripheral vascular diseases such as arteritis, acrocyanosis, arteriosclerosis and intermittent claudication [332, 361]. The furfuryl ester of nicotinic acid (trafuril) is also an effective vasodilator and has been used locally in the form of a cream for similar conditions.

Skin Diseases. Greenberg [248] showed that the urinary excretion of nicotinic acid was within normal limits in a number of patients with various dermatoses. Tisdall, Drake and Brown [114] treated some cases of acrodynia with nicotinic acid on account of the superficial resemblance between pellagra and acrodynia, but they observed no demonstrable improvement on nicotinic acid alone. Birkhäuser [249] treated ten patients suffering from chilblains with 50 mg. of nicotinamide twice daily for several weeks and reported promising results. It is difficult to understand what effect the nicotinamide had, as it is devoid of any vasodilator action. Nicotinic acid has been used for the treatment of chilblains, in doses of 50 mg. three times a day [145], with apparently good results.

Ferreira-Marques [117] states that nicotinic acid and its amide have an antipruritic action. He claims to have effectively treated 150 patients with

EFFECT OF NICOTINIC ACID ON SKIN LESIONS IN A DIABETIC

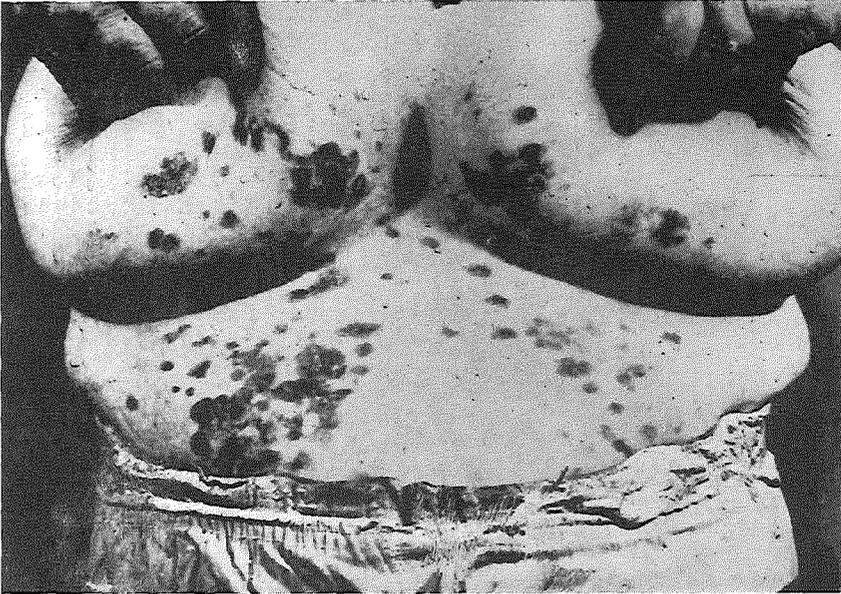


FIG. 122. Diabetic with scaly, red, indurated and dry skin lesions before treatment.

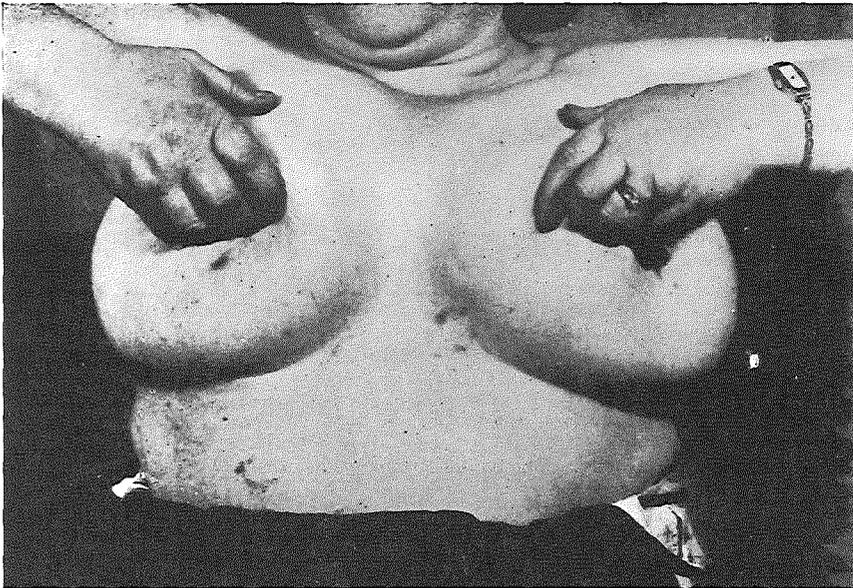


FIG. 123. Same patient as in Fig. 122 after treatment with nicotinamide 250 to 450 mg. daily. No local treatment was given and the diabetes was uncontrolled.

EFFECT OF NICOTINIC ACID ON SKIN LESIONS IN A DIABETIC

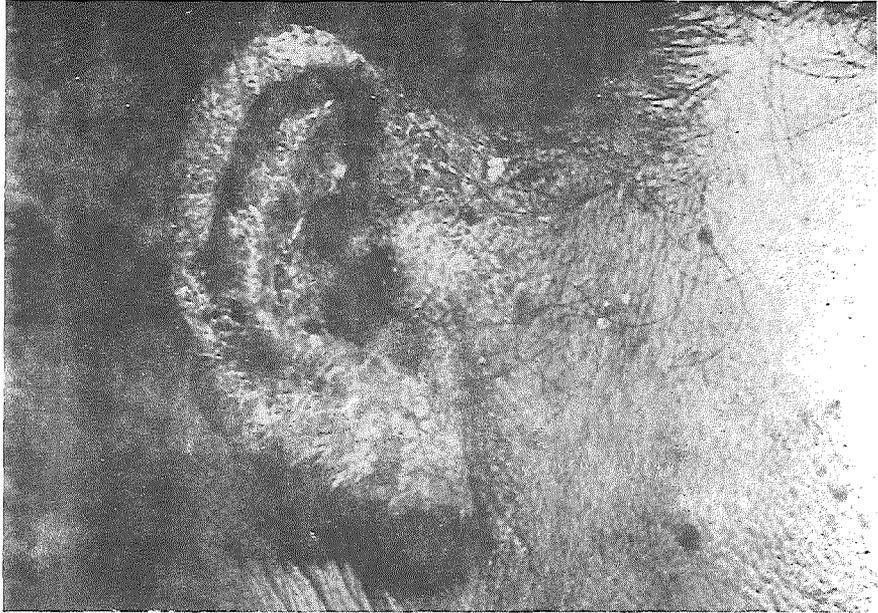


FIG. 124. Diabetic with red, indurated, dry and scaly skin eruption of the ears of three years' duration. There were deep fissures behind the ears and lesions on the breasts as in Fig. 122, and in the pubic, intergluteal, sacral and olecranon regions.



FIG. 125. Same patient as in Fig. 124 after treatment with nicotinic acid 200 mg. daily. The diet and dosage of insulin remained unchanged.

nicotinamide, sixty-eight of them carefully controlled, suffering from simple pruritus, pruritus vulvæ, pregnancy pruritus, senile pruritus, Hebra's prurigo, Besnier's prurigo, Fox-Fordyce's disease, and pruritus and prurigos caused by irradiation. The dose was 200 mg. of nicotinamide five times a day. The same author has treated lichen planus with penicillin and nicotinic acid [307], and tuberculous skin lesions with nicotinic acid, riboflavine and iron [308].

Nicotinic acid in doses of 50 to 200 mg. four times daily is stated to relieve the pruritus and improve the cutaneous manifestations of dermatitis herpetiformis [213]. This is ascribed to the pyridine ring in nicotinic acid, since improvement also occurs with sulphapyridine but not with other sulphadiazines not containing the pyridine ring. β -Pyridyl carbinol, the carbinol of nicotinic acid, is also effective [350].

Harris and Derian [133] have pointed out that chronic bromide intoxication shows marked parallelism with the symptomatology of pellagra. They claim that treatment of bromism with large doses of nicotinic acid causes rapid disappearance of symptoms.

Diabetes. Neuwahl [178] claimed to have observed a well-marked temporary improvement in the carbohydrate tolerance of diabetics being treated with nicotinic acid for vascular disease. He states that further investigation on a group of twelve diabetics showed that the administration of nicotinic acid or nicotinamide diminished the requirements of insulin needed to keep the blood sugar of the diabetics within normal limits. The dosage was of the order of 500 mg. three to five times daily to begin with, the dose being subsequently reduced as the blood sugar came down. The nicotinic acid and nicotinamide were given in enteric coated tablets.

Skin disturbances such as pruritus, dermatitis and intertrigo are common in diabetics, and owing to dietary restrictions some degree of avitaminosis may result. Rudy and Hofmann [250] state that these skin disturbances are most frequently due to vitamin deficiencies, particularly of nicotinic acid, rather than to disturbed carbohydrate metabolism as was formerly thought. Pellagrous dermatitis in diabetes is often seen and is sometimes diagnosed as psoriasis. Rudy and Hofmann have treated the skin lesions in a number of diabetics by the administration of nicotinic acid or nicotinamide (Figs. 122-125). They state that complete cure may take from a few days to a few months, and that more stubborn cases require large doses parenterally as well as orally. The dosage given was from 150 to 300 mg. daily in divided doses. The vitamin B complex was also given in the form of yeast.

Asthma. Maisel and Somkin [251] first published a preliminary report on the treatment of asthmatic attacks with nicotinic acid. Severe attacks were controlled by the slow intravenous injection of 100 mg. of nicotinic acid, which was stated to produce improvement lasting from three to fifteen hours. Some chronic patients were improved by oral medication, 200 mg. three times daily and on retiring. The patients noted flushing after administration of the nicotinic acid followed by the expulsion of tenacious mucous plugs. The beneficial effect was attributed to a vasodilator effect on the blood vessels or relief of bronchospasm. Neuwahl [252] noted improvement in four cases, but three were made worse. Melton [253] gave nicotinic acid in doses of 50 to 100 mg., usually intravenously, to nineteen cases of asthma during acute paroxysms and obtained definite improvement in sixteen cases; two had marked exacerbations. The tests were controlled by injections of sterile water. Nicotinic acid was also given over a long period in doses of 50 mg. two or three times daily and 100 mg. at night to thirty cases and the frequency of attacks was stated to have been reduced in sixteen. Relapse occurred after discontinuing the nicotinic acid. The fact that it makes some cases worse should be borne in mind.

Neurology. Selfridge [123, 124] used nicotinic acid, nicotinamide and sodium nicotinate in the treatment of some thirty cases of high tone deafness, and in many the results were said to be striking. He believes that an under-

lying nutritional deficiency explains the nerve changes, and all cases observed by him gave a history of faulty diets. He states that both aneurine and nicotinic acid gave a response in the hearing curve, but that the greatest improvement came from the use of nicotinic acid.

Harris and Moore [125] have described the treatment of twenty cases of Ménières syndrome with 250 mg. of nicotinic acid and 20 mg. of aneurine a day; seventeen became entirely free from vertigo. They point out, however, that treatment may have to be continued for several months to obtain complete relief. Atkinson [119, 161, 280] has also used nicotinic acid in the treatment of certain types of Ménière's syndrome. He states that patients suffering from this condition may be divided into two groups by an intradermal histamine test. There is a small group sensitive to histamine that can be treated by desensitization with the latter, and a large group, insensitive to histamine, the attacks being the result of primary vasospasm. In this group Atkinson found that relief could be obtained by vasodilator drugs, the most satisfactory of which was nicotinic acid. He gives an initial dose of 30 mg. intramuscularly, and if this is tolerated a dose of 25 to 30 mg. intravenously. This is repeated daily or every second day for six to eight doses, increasing the dose by 5 mg. each time to the maximum tolerated, which is usually 50 mg., but may be as high as 75 mg. After a few days oral treatment is started as well, usually 50 mg. two or three times daily. Following intravenous therapy, intramuscular therapy is started and given daily for one to three months, and then successively decreased to five, four, three, two and one administrations a week. At the same time 100 to 150 mg. are given daily by mouth. After several months' treatment oral therapy alone is tried. Atkinson believes that nicotinic acid deficiency may result in nerve deafness and tinnitus, and ariboflavinosis in middle-ear deafness.

Atkinson [296] has also treated migraine with nicotinic acid on the assumption that some types are due to a primary vasospasm. Treatment begins with the intravenous injection of 25 to 35 mg. of nicotinic acid intramuscularly, the dose being increased 5 mg. daily until a dose of 50 mg. is reached. Seventeen patients out of twenty-one obtained complete relief or improved. These results were confirmed by Grenfell [118] but not by Friedman and Brenner [305]. Atkinson [297] has treated over 200 cases of tinnitus aurium with nicotinic acid. From fifteen to fifty-five per cent. were relieved, and from fifty-two to eighty-five per cent. improved.

Goldzieher and Popkin [123] have treated 100 consecutive patients with severe headache due to various causes with intravenous injections of nicotinic acid. Seventy-five were completely relieved with doses of 100 mg., the relief appearing to be correlated with the degree of flushing produced. Good results were obtained in the treatment of migrainous headache and in that following spinal puncture. Liedholm and Radner [134] observed improvement in patients with headaches following encephalography treated with intravenous sodium nicotinate. The well-known "malarial headache," which is exceptionally severe, is relieved by nicotinic acid in oral doses of 50 to 100 mg. [129].

The surgical treatment of trigeminal neuralgia is not completely devoid of risk and any medical method of treatment is worth investigation. Adams and Robinson [120], of Leeds, have reported that the paroxysms of the disease can be relieved by nicotinic acid in doses of from 50 mg. twice daily to 75 mg. four times daily. Furtado and Chicorro [254] also used nicotinic acid because they had observed that the pain of trigeminal neuralgia is often accompanied by vasomotor changes in the trigeminal area. Their method is to give a daily intravenous injection of 100 to 200 mg. In four cases out of eight a few injections gave relief lasting for months, and when an injection was given during a paroxysm the relief of pain was immediate.

Moore [126] has treated cases of disseminated sclerosis with injections of 60 to 160 mg. of nicotinic acid and 33 mg. aneurine two or three times

weekly. Considerable subjective and objective improvement occurred in all patients, and he attributes it in part to the vasodilator action of nicotinic acid improving the blood supply to the brain and spinal cord, thereby intensifying the action of aneurine. Loman [137] and his colleagues, however, state that nicotinic acid is a relatively ineffective cerebral vasodilator, since it fails to influence the cerebrospinal fluid pressure and does not dilate the retinal vessels, which are comparable to the pial vessels.

Other Uses. Graham [135] has used nicotinic acid in the treatment of seventy patients undergoing X-ray therapy. Only those suffering from severe nausea and vomiting were given nicotinic acid in doses of 30 to 200 mg. three times a day. The results obtained were stated to be better than with other forms of treatment. Similar claims are made by Kepp [131], who treated his cases with 500 mg. of nicotinamide daily. Bean, Spies and Vilter [279] state that patients on diets poor in the vitamin B complex readily develop irradiation sickness, which can be prevented or relieved by administering nicotinic acid or aneurine a few days before exposure. Well-fed patients had little reaction to the same dose of X-rays that made patients deficient in the vitamin B complex sick. The same authors record a case of classical beriberi and pellagra developing after irradiation therapy. They suggest that the basic disorder in irradiation sickness is a disturbance in the respiratory enzyme systems, of which nicotinic acid, aneurine and other members of the vitamin B complex are components. The excretion of urinary pigments and DPN and TPN after irradiation therapy is similar to that observed in pellagrins. As the diet is often already deficient in patients needing radiotherapy, it would appear rational to supplement it with the vitamin B complex before commencing treatment.

Owing to the low excretion of nicotinic acid and its derivatives in surgical patients suffering from hæmorrhage, burns, infection and trauma, it has been suggested that supplements of nicotinic acid of the order of 500 mg. a day be given at first, reducing this dose to 75 mg. daily [127, 128].

Since sulphonamides and antibiotics, such as penicillin, administered orally destroy the bacteria that synthesize nicotinic acid in the gut, increased amounts of nicotinic acid are often administered when these drugs are given for any length of time.

Experimentally it has been shown that the oral administration of 0.5 to 0.75 per cent. of nicotinamide in the diet will markedly suppress the spread of tuberculosis in experimentally infected mice [255]. This amount is roughly equivalent in anti-tubercular activity to 1 mg. of streptomycin four times daily over the same test period. It would be impossible to give doses of nicotinamide of this order to human patients. Farber and Miller [256] have shown that patients with tuberculosis often show evidence of nicotinic acid deficiency, which responds rapidly to diet and specific treatment with nicotinic acid. Isonicotinyl hydrazine, a derivative of an analogue of nicotinic acid, is a powerful tuberculostatic drug that has recently been introduced for the treatment of tuberculosis [347]. Unfortunately it readily produces drug resistance.

Rachmilewitz and Braun [257] made serial electrocardiographic examinations in fifty cases of typhoid fever, and in thirty-five there were changes in the T waves, which became flat, iso-electric or diphasic. On the supposition that the cause of the E.C.G. abnormalities were due to a metabolic disturbance in the heart muscle due to vitamin deficiency the patients with T wave changes were given nicotinic acid, and in half the cases treated the E.C.G. spontaneously returned to normal.

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