

CHAPTER VI

ASCORBIC ACID

(VITAMIN C)

HISTORY

SCURVY has been a menace to seafarers, explorers, and armies since classical times, although the first recorded accounts of the disease are to be found among the writings of the physicians who accompanied the crusaders in the thirteenth century. The decline of scurvy in Europe coincides with the introduction of the potato in the early seventeenth century and the increased consumption of vegetables and fruit. Vasco da Gama, who sailed round the Cape of Good Hope in 1498, describes how a hundred of his crew of a hundred and sixty perished from scurvy at sea. Sir Richard Hawkins' "Observations in his Voyage to the South Sea" (1593) contains an account of the prevention and treatment of scurvy with lemon juice. Jacques Cartier (1535) during his exploration of Canada found that the native Indians prevented the disease by drinking a decoction of pine needles, which we now know contains ascorbic acid.

Lind in his famous book, "A Treatise on the Scurvy" (1757), mentions the value of fresh citrus fruits and green vegetables in the treatment of scurvy. He gave patients suffering from the disease various forms of treatment, including two oranges and a lemon a day. Only those receiving the oranges and lemons showed any improvement. During his voyage round the world between 1772 and 1775 Captain Cook kept his men free from scurvy by including in their dietary as much fresh food as possible, including fruit and vegetables. In 1804 regulations were introduced into the British Navy providing all ratings with daily rations of lemon juice, and similar provisions were made by the Board of Trade in 1865.

To-day frank scurvy is almost a disease of the past. It is still met with, however, in isolated parts of the world and during wartime. During the war of 1914-1918 outbreaks occurred among British troops at the siege of Kut, and several cases occurred among civilians in Glasgow, Newcastle and Manchester owing to a shortage of potatoes.

Modern nutritional work on scurvy dates from 1907, when Holst and Frölich of Christiania tried to produce beriberi in guinea-pigs by means of restricted diets, but scurvy resulted instead. Henceforth the guinea-pig was used in all experimental work on scurvy. After Funk's postulation in 1912 of a scurvy-preventing or antiscorbutic vitamin, vitamin C, attempts were made to isolate it from orange and lemon juice. Between 1924 and 1929 Zilva [1] and his co-workers succeeded in obtaining a concentrate from lemon juice. In 1928 Szent-Györgyi isolated vitamin C, which he called hexuronic acid, from cabbage and adrenals without knowing it during the course of studies on cellular oxidation. In 1932 Waugh and King [2] isolated it from lemons and identified it with the hexuronic acid of Szent-Györgyi. In the same year Waugh and King [4] showed that the antiscorbutic activity of hexuronic acid was identical with that of the vitamin C obtained from orange juice.

The structural formula of vitamin C was established in 1933 as a result of the work of Haworth, Hirst and their co-workers [6], Karrer [7], and a number of other investigators. In the same year Reichstein [8] and his colleagues in Switzerland synthesized the D- and later the L-form of the

vitamin. Almost simultaneously Haworth and Hirst [9] synthesized it in Britain. In 1933 the name ascorbic acid was suggested for vitamin C, and this is now the pharmacopoeial one. It is the L- form.

CHEMISTRY OF ASCORBIC ACID

Ascorbic acid forms colourless crystals melting at 190° – 192° C., with a specific rotation of $+23^{\circ}$ in water and $+48^{\circ}$ in alcohol. It is freely soluble in water, and slightly soluble in acetone and the lower alcohols, but insoluble in benzene, ether, chloroform and fats. It condenses with aldehydes, acetone and other ketones in the presence of mild dehydrating agents to form stable crystalline derivatives, and forms salts through an enolic hydrogen atom.

If kept dry and not exposed to light ascorbic acid is stable for a considerable time. Visible light and ultra-violet light have a markedly destructive effect; hence it is stored in yellow-coloured bottles. In solution stability

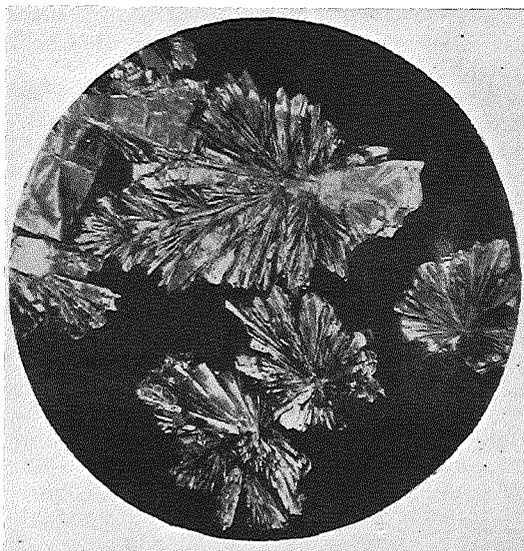
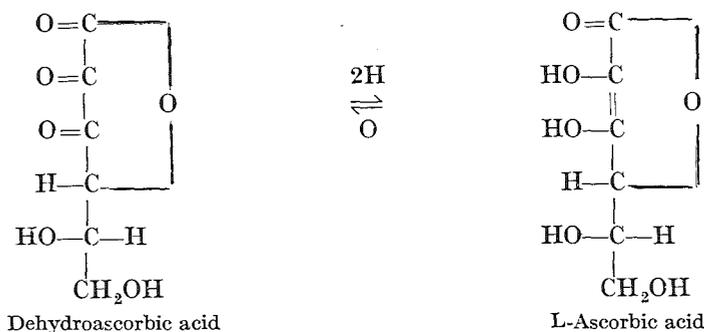


FIG. 126. Crystals of Ascorbic Acid.

depends upon many factors. The presence of traces of iron and copper ions (e.g. 20 micrograms per litre) rapidly catalyses its oxidation. Ascorbic acid is stable in the presence of aluminium and stainless steel. In aqueous solutions below pH 7.6 it is not oxidized on exposure to air unless traces of copper or other such catalyst are present. The rate of oxidation is directly proportional to the square root of the concentration of copper ions, and inversely proportional to the square root of the pH [38]. But in the presence of air or oxygen destruction is rapid and complete when the pH is above 7.6 and heat is used. Autoclaving at 120° C. for twenty minutes in oxygen at pH 8 results in a loss of forty-nine per cent.; in carbon dioxide and nitrogen only twelve per cent. at pH 8, and four per cent. at pH 3.2 [13]. It has been found that solutions of ascorbic acid can be stabilized by the addition of small amounts of fruit acids such as tartaric and citric acids. In foods factors are present inhibiting oxidation [3]. Numerous substances such as tissue extracts, glutathione, cysteine, thiourea, carbon monoxide, cyanides and halide ions inhibit oxidation. Solutions of ascorbic acid and its sodium salt are quite stable if kept in air-free vessels or in an inert atmosphere of nitrogen or carbon dioxide. The first oxidation product is dehydroascorbic acid, which

can be reduced to ascorbic acid by hydrogen sulphide, cysteine and glutathione.



Dehydroascorbic acid is as potent an antiscorbutic as ascorbic acid itself. It is utilized by man in the same way as ascorbic acid, which it can replace weight for weight.

Ascorbic acid is reversibly oxidized by a number of organic compounds such as methylene blue, quinones and indophenol dyes, which are used in its estimation. Aqueous iodine also oxidizes it, two atoms of iodine reacting with one of the vitamin. In the presence of alkali iodine quantitatively oxidizes it to oxalic and trihydroxybutyric acids.

Estimation of Ascorbic Acid [5]. The reactions of reduced ascorbic acid with 2 : 6 dichlorophenolindophenol, the blue colour of which is discharged, and of dehydroascorbic acid with 2 : 4-dinitrophenylhydrazine are most widely used for the estimation of ascorbic acid in foodstuffs and body fluids. Before assay the ascorbic acid must be extracted with one to two per cent. oxalic acid, or metaphosphoric acid, five to ten per cent., with one per cent. thiourea or ten per cent. stannous chloride, all of which inhibit oxidation.

The reaction with dichlorophenolindophenol is not specific, as sulphhydryl groups (cysteine, glutathione), thiosulphates, pyridinium compounds (used as urinary antiseptics), nicotinic acid and its derivatives, riboflavine, aspirin, atropine, iron and reductones interfere. Reductones are produced from hexoses when foods are heated in alkaline solution and reduce the blue dye in a manner indistinguishable from that of ascorbic acid. Dehydroascorbic acid, which functions in the body as ascorbic acid, is not estimated by indophenol and must first be converted into ascorbic acid by hydrogen sulphide. The interference from reductones is largely eliminated by titration in the presence of formaldehyde or by the method of Harris and Mapson [10], which depends on the principle of the continuous flow, by which the complete time course of a reaction can be followed. At pH 3.5 and 20° C. after 0.28, 0.55, 0.75 and 1.1 seconds respectively, sixty-four, eighty, eighty-four and eighty-nine per cent. of the reaction with ascorbic acid is complete, whereas other values are obtained with other reducing substances. By plotting curves of reaction times both ascorbic acid and interfering substances can be estimated when present together. Hydrojuglone, or 1, 4, 5-trihydroxynaphthalene, is another interfering substance in nuts, buds and catkins [11]. Coloured fluids interfere with the titration end-point, but the colour can be eliminated by using a photo-electric colorimeter or by electrometric titration. Another possible method of separating ascorbic acid from interfering substances is by paper partition chromatography using phenol-acetic acid as a solvent [12]. The method of Roe and Kuether [360], depending on the reaction between 2 : 4-dinitrophenylhydrazine and dehydroascorbic acid to form a red compound which can be estimated by the photo-electric colorimeter, avoids the interfering substances previously mentioned. It has been modified by Roe and his colleagues [15] to permit the determination of ascorbic acid, dehydro

ascorbic acid and diketogulonic acid, which was formerly estimated as dehydroascorbic acid, in the presence of one another. Diketogulonic acid has no antiscorbutic activity and gives falsely high values if not allowed for.

Ascorbic acid has also been estimated by its ability to decolorize methylene blue [583, 584]. It can be estimated spectrographically in blood and urine using the reagent *perinaphthindanetrione* or 2-nitro*perinaphthindanetrione* to produce a colour reaction [129]. The method is reasonably specific, the only interfering substances being reductones, reductic acid and cysteine.

Bioassay is rarely used, but is still the final criterion for judging whether a preparation has an antiscorbutic action. Preparations are assayed by prophylaxis or cure of scurvy in guinea-pigs, from the rate of growth of the lower incisors of the guinea-pig, from the length of the odontoblasts, and from growth response [20]. The increase in serum alkaline phosphatase in scorbutic guinea-pigs observed after a critical dose of ascorbic acid has also been used [891].

UNITS OF ASCORBIC ACID

Since the availability of pure ascorbic acid the unit based on a biological assay has been unnecessary. The International Unit, which was in use before its isolation and synthesis, is equivalent to 0.05 mg. of ascorbic acid.

DISTRIBUTION OF ASCORBIC ACID IN FOODS

Ascorbic acid appears to be present in all living tissues, but fresh fruits and plants are the best sources. Among the richest sources are rose hips and haws, black and red currants, strawberries, "greens" and the citrus fruits (mainly in the peel). Plums, apples (see, however, Bramley's seedling), pears and melons contain negligible quantities, while potatoes, spinach, cabbage, sprouts, kale, broccoli, watercress and turnips (root and tops) contain relatively large amounts. Half a pound of potatoes supplies about 30 mg. of ascorbic acid a day, and an ounce of black currants provides an average of 50 mg. Black-currant purée is a very good source [811]. Generally speaking, the ascorbic acid content of fruit and vegetables increases to a maximum just before ripening and then decreases steadily. The content is influenced by climatic factors, method of growing, the soil, season, time of picking, variety and the time taken to reach the table [16, 17]. It is highest in late summer and autumn, e.g. spring potatoes only contain 5 to 10 mg. of ascorbic acid and autumn tubers 20 to 33 mg. Variations may be found in different fruits and leaves on the same plant. In fruits the outer portion contains more than the centre, except in potatoes, in which the skin contains less than the rest [901]. Dried legumes and cereals are poor sources of ascorbic acid.

The richest source of ascorbic acid is rose hips, which should be picked before they are over-ripe, otherwise they may lose half their content. Rose hips are converted into syrup, which since 1942 has been issued by the Ministry of Food, with a declared potency of 175 to 200 mg. per 100 ml. It has been shown, however, that such syrup loses its ascorbic acid fairly quickly. In three to five months there may be a loss of fifty per cent. in the ascorbic acid content; in one case it was ninety per cent. [894]. A high sugar content and the presence of sulphur dioxide minimizes the destruction of the vitamin in fruit syrups on storage [914]. Half a pound of potatoes, a helping of cabbage or brussels sprouts, and an ounce of watercress daily supply sufficient ascorbic acid provided the foods are not spoilt in cooking and provided the cooking water is consumed as well. Small but appreciable quantities are present in animal products. Indeed it is possible to remain free from scurvy on a diet consisting solely of underdone meat (p. 439), and skimos, who live largely on animal flesh, do not suffer from scurvy. The chief animal sources of ascorbic acid are glandular tissues, particularly the adrenals, and actively functioning tissues.

The ascorbic acid content of cows' milk is very variable. The value drops as soon as it leaves the cow and cools down. Even in twenty-four hours raw milk may lose twenty to thirty per cent. Traces of copper in the vessels, sunlight and pasteurization reduce the quantity still further [34]. Unless special care is taken pasteurization may result in the loss of thirty to sixty per cent. of the ascorbic acid. Even after the delivery of domestic milk the small residual amount of ascorbic acid is further decreased by keeping on the doorstep (p. 396) and by reheating [832]. Exposure to ultra-violet light on the doorstep causes loss of ascorbic acid. A pint of commercial milk contains from 4 to 13 mg.; the same amount of mother's milk contains 14 to 30 mg. in this country.

Effect of Cooking. When ascorbic acid is heated in solution with alkalis it is rapidly inactivated. In natural foodstuffs there are stabilizing factors such as organic acids (tartaric, citric) and sugars (fructose, sucrose, glucose) preventing its destruction. During cooking a proportion of the ascorbic acid is extracted and passes into the cooking water; from twenty-five to sixty per cent. may be lost in this way if the cooking water is not consumed. As soon as vegetables or fruits are gathered an enzyme known as ascorbic acid oxidase is liberated, and this slowly oxidizes ascorbic acid. It is formed when fruits or vegetables are bruised, macerated or steeped in water. Ascorbic acid oxidase is a specific copper protein, blue-green in colour, with a molecular weight of 150,000 and containing six copper atoms per molecule [18]. Its activity does not depend upon its copper content, the copper being non-ionizable [19, 23]. Fruit and vegetables should therefore not be kept too long before cooking. Cooking in iron, copper or badly tinned vessels causes destruction of ascorbic acid; vessels of alkali-free glass (pyrex), stainless steel, aluminium or enamel have no deleterious effect. Boiling fruits and vegetables with the lid on the vessel for the minimum period of time in the smallest quantity of water conserves the maximum quantity of the vitamin [883]. The material should be plunged straight into boiling water to destroy the ascorbic acid oxidase as soon as possible and cooked with the vessel lid on. The rate of boiling is important on account of the ratio of water to vegetables at the end of cooking. The loss occurring on cooking fruit and vegetables varies enormously. The amount of ascorbic acid retained may vary from twenty-seven to seventy per cent. [14]. Considerable losses occur on rejecting the cooking water. Keeping dishes hot causes further destruction of ascorbic acid, particularly if soda is added for cooking purposes. Soda, however, may diminish the cooking time of vegetables such as peas and thereby conserve ascorbic acid.

A considerable amount of ascorbic acid is retained in jam making, about thirty to forty per cent. of that present in the original fruit escaping destruction; the sugar and the sulphur dioxide used for preserving fruit pulp protect against oxidation [914]. The content slowly declines on keeping, e.g. twenty to thirty per cent. after three months [902], although the figure is very variable and the loss may be as little as ten per cent. after six months' keeping. Black-currant syrup keeps well; even after a year's storage. The content is affected by exposure to light, air and elevated temperatures [899].

Boiling conserves more ascorbic acid than steaming, but in the latter process there is practically no waste in the cooking water, which usually goes down the kitchen sink and may extract as much as sixty per cent. of the ascorbic acid present in the food. As served, steamed green vegetables actually contain twenty-five per cent. more ascorbic acid than when boiled. Baked potatoes contain approximately as much ascorbic acid as boiled, and weight for weight chips contain more, as much water is driven off in frying [896]. Mashing or whipping up potatoes and keeping them hot destroys their ascorbic acid rapidly. Most of the vitamin is preserved if the potato is cooked and served in its skin [882]. Chopping vegetables before or after

cooking destroys a considerable amount of ascorbic acid, particularly if a steel or metal knife or chopper is used [21]; a shredded lettuce loses eighty per cent. in one minute [22].

Stewing and baking are slow methods of cooking and hence more ascorbic acid is lost than by boiling or steaming; a stew pot kept going for hours is certainly unlikely to contain much ascorbic acid. Frying, being a quick method, does not cause very much destruction of ascorbic acid provided the temperature is not too high [896].

Pressure cooking, if timed correctly, does not result in any more destruction of ascorbic acid than other methods of cooking [1023]; the average is twenty-two to twenty-eight per cent. [30]. It may result in less destruction because practically no water is used, and therefore little is leached out [27, 28]. If pressure cookers are misused considerable destruction of vitamins may occur. Waterless cooking results in less loss of ascorbic acid than any other method; about seventy-three per cent. is retained [30].

Radio-frequency or high-frequency heating, which has attained an important position in the food industry in America, causes no more destruction of ascorbic acid in foods than ordinary methods of cooking [74].

Restaurant, cafeteria and Army meals usually contain little ascorbic acid because of the long time elapsing from the peeling and preparation of the vegetables to their appearance on the plate [898, 900]. The hot plate and steam table cause rapid inactivation. Olliver [896] has shown that keeping cooked cabbage hot for fifteen minutes results in a twenty-five per cent. loss of ascorbic acid; this is increased to seventy-five per cent. after ninety minutes.

Effect of Storing, Cold Storage, Canning, Dehydration, Preserving, etc. Loss of ascorbic acid may occur if fresh foods are stored for any length of time between purchase and consumption [25]. According to Olliver [29] there is little loss in cabbage stored for a week under normal conditions, although other authorities state that there is a drop of fifty to eighty per cent. after two to four days' storage [25]. Temperature, freshness, care in handling, exposure to the sun and strong wind are important factors. A loss of ten per cent. for every day's storage has been reported for green vegetables and bruised soft fruits [896]. Root crops keep better because there is less surface and they are protected by a tougher epidermis, although losses may be considerable if they are stored for months. Fruit and vegetables should not be cut up and left for hours before consumption.

There is no appreciable loss of ascorbic acid in fruits and vegetables kept in cold storage or treated by the "quick-freeze" process [26, 885]. Generally speaking, frozen foods contain more than canned foods [51]. Some losses do occur as a result of processing, such as washing and blanching, particularly if this is prolonged, so that the overall loss may be as much as forty per cent. [50]. There is hardly any loss in the ascorbic acid of vegetables and fruits kept in domestic refrigerators for a few days. Little loss occurs during defrosting after freezing, and after cooking the defrosted food much ascorbic acid is retained, e.g. up to eighty per cent. [904]; peas lose forty to fifty per cent. [903]. Even if kept cold or refrigerated after cooking, cooked food rapidly loses its ascorbic acid.

Pickling, curing and salting result in complete destruction of ascorbic acid. The vitamin is largely retained in pulped fruit if this is treated with sulphur dioxide, e.g. sulphited black currants retain sixty-three per cent. of their ascorbic acid for sixteen months [36].

Canned foods are excellent sources of ascorbic acid, about sixty-seven to ninety-three per cent. being retained in the process [33, 35, 886]. If the food is blanched first a further fifteen to thirty per cent. may be lost [35], although the loss may be as high as seventy per cent. in the case of beans [37]. The more rapid the processing the more ascorbic acid is retained. A good vacuum is required for the retention of the vitamin during storage. A considerable

amount (about fifty per cent.) is present in the canning liquid. Cold canned foods usually contain more ascorbic acid than foods cooked in the household. Storage in the can results in a slow loss of ascorbic acid ; this can be minimized by storage in a refrigerator [886]. Contamination with copper of the can destroys ascorbic acid rapidly ; this has been overcome by suitably lining the can. Once the can is opened the vitamin is fairly stable for a few days if the food is kept in a refrigerator.

Dehydrated vegetables have recently been popularized. The degree of ascorbic acid retention depends on the method of preparation. Sun-dried foods contain little or none. The material should be cooked or scalded rapidly before dehydration to inactivate ascorbic acid oxidase ; sulphite, or dehydration in the absence of air, retards the oxidation of ascorbic acid. According to Olliver [29] about sixty per cent. is lost in the factory dehydration of cabbage. On reconstitution dehydrated cabbage only recovers about fifty per cent. of the original moisture content, and during domestic cooking on an average about thirty per cent. is retained in the cooked food. About fifty per cent. is extracted, and the remaining twenty per cent. is destroyed [29]. This compares favourably with the cooking of fresh cabbage. From eleven to fifty-six per cent. of the ascorbic acid is lost in the dehydration of potatoes [906]. Further loss occurs on storing, e.g. fifty per cent. in three months. Dehydrated vegetables are best cooked by placing directly into boiling water and cooking for twenty minutes, and not by preliminary soaking in cold water, which leaches out some fifty per cent. of the ascorbic acid [897, 910].

Considerable amounts of ascorbic acid are retained in fruit preserved by domestic processes [36]. Black currants and gooseberries preserved as whole fruit by heat processing methods in glass or metal containers retain sixty-one and sixty-eight per cent. after eighteen months' storage ; raspberries retain forty-seven per cent., and strawberries twenty-five per cent. The use of plastic seals for covering heat-processed jars of fruit leads to considerable loss of ascorbic acid, e.g. eighty per cent. [36].

Spray drying of liquids such as milk results in a loss of about twenty per cent. of the ascorbic acid ; roller drying causes a loss of thirty per cent., and evaporation about sixty per cent.

Ultra-violet light inactivates ascorbic acid [832]. This is important if jams, preserves, etc., are kept for weeks or even months in shop windows. Such preparations should be marketed in tins or in amber-coloured glass. The conditions are not the same as in fresh foodstuffs exposed to solar ripening, when enzymes, pigments and organic acids protect the ascorbic acid from inactivation. Fifty per cent. of the ascorbic acid in milk may be destroyed in one hour if it is left in a glass bottle on the doorstep in bright sunlight [832].

Ascorbic Acid Content of Foods. The ascorbic acid content of various foods before and after cooking and canning is given in the following table :

Ascorbic Acid Content of Foods

Foodstuff	Description	Ascorbic Acid in mg. per 100 grams or 100 ml. (3½ oz.)	Remarks
<i>Fruits</i> Apple . . .	—	8-22	Av. 8
	Cooked	4	
	Skin	6.1-15.5	
	Jam and jelly	2	
	Bramley seedling	16 ; 22	
	„ „ peel	83	
	„ „ dried	11	
Cox's orange pippin	14.4		

ASCORBIC ACID

Foodstuff	Description	Ascorbic Acid in mg. per 100 grams or 100 ml. (3½ oz.)	Remarks
<i>Fruits—continued</i>			
Apricot . . .	Fresh	4-11	80-100% retained
	Canned	5	
	Dried	1-2	
Avocado . . .	Jam	2-12	10-16
Banana . . .	Ripe	10	
Barberry . . .	Raw	81	
Bilberry . . .	Cooked	7	
Blackberries . . .	Raw	10	20 when raw
	Cooked	8	
	Jam	5	
	Jelly	2	
Blueberry . . .	Raw	4-75	Av. 10
Cherry	Raw	2-8-10	3
	Cooked	3	
Cranberry . . .	Juice	15	
Currant, black . . .	Raw	108-419	Av. 150
	Juice	90-360	
	Cooked or canned	90	
	Jam	50	
	Juice	140	
	Purée	70	
	Raw	30-45	
" red	Cooked	23	6
	Jam	6	
" white		23	
Custard apple . . .	Raw	1-3-15	
Damson	Cooked	3	
Elderberry . . .	Raw	8-10	
Fig	Fresh	2-2-8-72	0
	Dried	0	
Gooseberry . . .	Fresh	25-40	40 mg. when raw 40 mg. when raw
	Cooked	20	
	Jam	11	
Grape	Raw	4	37-50
	Juice	37-50	
Grapefruit . . .	Oil gland layer	314	90-100% retained
	Mesocarp	219	
	Endocarp	76.6	
	Canned	41	
	Canned, sweetened	45-50	
	Ditto after 6 months	39-46	
" " 12 "	34-41		
Greengage . . .	Marmalade	5-5	5-0-6-5 mg. when raw
	Raw	5-0-6-5	
	Cooked	2-7-5-7	
Guaya	Jam	2	75
" (S. African) . . .		250	
Haw	Raw	49-500	
Hip, rose	Fresh	67 up to 4,800	418 mg. when raw " " "
	Brought to boil	394	
	Boiled 5 min. with sugar	238	
	" 10 " " "	216	
	Jelly	100	
	Syrup	175-200	
			Ministry of Health

Foodstuff	Description	Ascorbic Acid in mg. per 100 grams or 100 ml. (3½ oz.)	Remarks
<i>Fruits—continued</i>			
Huckleberry	—	30	
Lemon	Juice	30-78	Av. 45
	Pulp	14-16	
	Peel	100-205	
	Marmalade	10	42 mg. when raw
Lime	Pulp	20-60	
	Juice	16.8-62.5	Av. 37
Litchi	—	2 ; 20	
Loganberry	Raw	35	
	Boiled	22-26.7	38.8-48.8 mg. when raw
	Canned	31-35	"
Mango	Raw	25	
Medlar	Raw	2.0	
Melon	Raw	3-21	
	Cantaloupe	30-42	
	Water melon	1.0-7	
Mulberry	Raw	6.6-21	
Nectarine	Raw	8-24	
Olive	Raw	15	
Orange	Pulp	16-47	
	California (juice)	52	
	Brazil (pulp)	34-62	
	Jaffa "	33-54	
	Navel "	52-98	
	Juice	22-89	Av. 58
	Peel	75.8-210	
	Canned	94-100% retained	
	Marmalade	7-14	50 mg. when raw
Papaya (pawpaw)	Raw	36-115	Av. 45
	Skin	116	
Peach	Raw	2-17	Av. 10
	Canned	60-90% retained	
Pear.	Raw	3-7	
	Canned	1.5	
	Cooked	3	
Peppers	Green	125-180	
	Red, ripe	150	
Persimmon	—	100	
Pineapple	Raw	3-13	
	Canned	8	
Plum	Fresh	3-7	
	Dried prune	1.0-2.0	
	Boiled	2.2-2.9	4.6 mg. when raw
	Canned	2.2-2.5	" "
Pomegranate	Raw	6-15.6	
Pumpkin	Raw	5	
	Cooked	2	
Quince	Raw	9-12	
Raspberry.	Raw	19-37	Av. 30
	Canned	3.9-8	
	Jam	8	
Rowan (moun- tain ash)	—	35-50	
Strawberry	Raw	46-234	Av. range 50-80
	Boiled	25-37	71.4 mg. when raw
	Canned	21-35.7	" "

Food stuff	Description	Ascorbic Acid in mg. per 100 grams or 100 ml. (3½ oz.)	Remarks
<i>Fruits—continued</i>			
Tangerine . . .	Pulp	10-36	
	Juice	10-78	
Whortleberry . . .	—	5	
<i>Nuts</i>			
Almond	—	<19.3	
Cashew	—	180	
Chestnut	—	6	
Cob	—	3-15	
Coconut	—	0.4-13.4	
Hazel	—	2.74 ; 15	
Peanut	—	10	
Pecan	—	2	
Walnut	Unripe	160	
	Chutney (home made) .	98% of original	
	Chutney (commercial) .	40% of original	
<i>Vegetables</i>			
Artichoke, globe	Raw	9	
	Cooked	6	
„ Jerusalem	—	7	
Asparagus	Whole	35	
	Canned	80-100% retained	
	Cooked	30	
Bean, broad	Raw	27.7-37	
	Boiled	10	
	Canned	14.7-17.6	
Bean, green, snap or string	Raw	19-25	
	Canned	40-75% retained	
	Cooked	7.2-20.2	
	Dried	0	
Bean, soya	Black, dried	30-40	
	Green, dried	17.75	
Beetroot	Root	15	
	Cooked	5	
	Canned	2	
	Tops	34-50	
Broccoli	Entire plant	70-110	
	Boiled leaves	22	32 mg. when raw
	Dehydrated	6.0	
Cabbage	Raw	60-118	Av. 70
	Cooked	20-43% retained	
	Fresh, boiled 10 mins. .	11-57 ; 70	
	„ „ 15 „	49	
	„ „ 30 „	29	
	„ „ 60 „	22	
	„ „ 90 „	15	
	„ „ 90 „	5	
	Dehydrated (uncooked)	220-376	30-80% retained
	„ (cooked)	42-62 ; 190	Av. 60-70%
Carrot	Raw	5-10	
	Cooked in skin	90% retained	
	Shredded	80% retained	
	Canned	2	
Cauliflower	Raw	69-75	
	Cooked	37-40	

Foodstuff	Description	Ascorbic Acid in mgr. per 100 grams or 100 ml. (3½ oz.)	Remarks
<i>Vegetables—contd.</i>			
Cauliflower— <i>cont.</i>	Dehydrated (uncooked)	290	
	„ (cooked)	17-60	
Celery . . .	Stalks . . .	5-7	
Chard . . .	—	35-42	
Chives . . .	—	70-119	
Corn (sweet) . . .	—	10	Av. 6-12
Cucumber . . .	—	8	
Dandelion . . .	Leaf . . .	100	
Endive . . .	Unblanched . . .	15-24	
	Blanched . . .	4	
Garlic . . .	—	14	
Grass . . .	Fresh . . .	68 ; 75.3	
Horseradish . . .	—	90	
Kale . . .	Raw . . .	100-150	
	Boiled . . .	23-40	
	Dehydrated . . .	170-295	
Kohlrabi . . .	Raw . . .	60-117	
Leek . . .	Raw . . .	15-20	
	Cooked . . .	10-15	
Lettuce . . .	—	8-18	
Lucerne (alfalfa) . . .	Fresh . . .	73-380	
Mango . . .	—	25-60	
Marrow . . .	Raw . . .	11	
	Cooked . . .	2	
Mint . . .	—	39	
Mushroom . . .	—	3	
Mustard and cress . . .	—	37	
	Seeds . . .	44	
Nasturtium . . .	Leaves . . .	200-465	
Nettle . . .	Leaves . . .	50 ; 72	
Onion . . .	Bulb, raw . . .	15-30	
	Spring . . .	25	
	Cooked . . .	6	
	Dehydrated . . .	37	
Parsley . . .	Leaves . . .	154-209	
Parsnip . . .	Raw . . .	18-30	
	Cooked . . .	4-10	
Pea . . .	Fresh green . . .	25	
	Dried . . .	0	
	Boiled . . .	14-16	
	Quick freeze . . .	12-23	
	Canned . . .	10	45-90% retained
	Split . . .	2	
Pepper . . .	Green . . .	120-180	
	Red . . .	150	
Potato . . .	Tubers, raw . . .	11-36	Av. 18
	New . . .	20-33	
	Old . . .	5-10	
	Stored 180 days . . .	10	18 mg. raw
	Baked . . .	7	„ „
	Peeled and raw . . .	19	
	„ „ boiled . . .	52% retained	
	„ „ sliced and boiled . . .	47% retained	
	Boiled whole . . .	66% retained	
	„ „ cold . . .	2-6	18 mg. raw
	„ „ unpared and mashed . . .	11-31	87% retained
	„ „ and creamed . . .	2	18 mg. raw
	„ „ fried . . .	2-1	„ „
	Chips . . .	60% retained	

Foodstuff	Description	Ascorbic Acid in mg. per 100 grams or 100 ml. (3½ oz.)	Remarks
<i>Vegetables—contd.</i>			
Potato— <i>contd.</i>	Dehydrated	16-25	60% retained
	„ reconstituted	6	25% retained
Potato, sweet	—	22-33	
	Boiled whole	100% retained	
Pumpkin	Cooked	2	
Radish	Root	29-36	
Rhubarb	Fresh	20-25	
	Cooked	4	
	Jam	2	
Shallot	Bulb	7-6	
Spinach	Leaves	50-80	
	Boiled	15 ; 71	
	Quick freeze	32	
Sprouts	Fresh	65-150	
	Boiled	43-85	
	Quick freeze	51	
Squash	Raw	29	
	Cooked	7-17	
Swede	Root, raw	44	
	„ cooked	22	
Tomato	Raw	10-38	
	Canned	14-21	17.5 mg. (U.S.A.)
	Juice	16-33	
	Late	5-8	
	Canned after 6 months	7-18	
Turnip	Root	30	
	Cooked	13-15	50-60% retained
	Tops	100-140	
	Boiled	18	35 mg. when fresh
	„	17% destroyed 28% diffused	
Watercress	—	61-89	
<i>Dairy Products</i>			
Milk	Cow's, raw	0.5-2.96	Av. 2.2 mg.
	„ colostrum	1.77	
	„ boiled	0.4	0.7 mg. raw
	„ pasteurized	0.3-5.8	Av. 1.3 mg.
	„ evaporated	0.4-2.76	
	„ skimmed, dried	1.58-6.27	
	„ whole dried, by roller process	6.9-9.7	12.3 per reconstituted litre
	Goat's, raw	0.5-2.0	
	„ boiled	0.4	0.9 mg. fresh
	Human	1.2-10.8	Av. 5.1 (U.S.A.) 3.2-3.8 (U.K.)
	„	8-10	well-fed mothers
	„	1.2-4.0	poorly fed mothers
	„	7.0	colostrum, 10 days post partum
	„ milk depots	1.31 ± 0.2	
Cheese	—	nil	
Eggs, hen's	—	nil	
„ duck's	—	0.3-1.3	
<i>Fish</i>			
Carp	Liver	4.5-11.3	
	Muscle	0.5-1.88	
	Roe	20-24	

Foodstuff	Description	Ascorbic Acid in mg. per 100 grams or 100 ml. (3½ oz.)	Remarks
<i>Fish—continued.</i>			
Clam . . .	—	30	
Cod . . .	Liver	26.7	
	Roe	120-160	
Crab . . .	Liver	27	
	Muscle	13	
Eel	Liver	9.8-11	
	Muscle	1.4	
Haddock . . .	Roe	10	
Herring . . .	Roe	20	
Lobster . . .	Liver	24	
	Muscle	5	
Mackerel . . .	Roe	40	
Mussel . . .	Liver	30	
	Muscle	3	
Oyster . . .	Liver	12	
	Muscle	3	
Salmon . . .	Whole fish	89-215	
	Chilled	9	
	Canned	0	
	Roe	14	
Scallop . . .	Liver	11.5	
	Muscle	3	
<i>Meat and Poultry</i>			
Calf	Liver	30-50	
	Muscle	7.8	
Duck	Liver	13 ; 68	
	Muscle	7.8	
	Heart	24.2	
Fowl	Muscle	1.5-6.0	
	Liver	28-43	
	Brain	11.4-26	
	Heart	3.8 ; 4.6	
	Kidney	10.8	
	Liver	35	
Ox	Muscle	1.6 ; 2.2	
	Liver	27-40	
Pig	Muscle	1.7	
	Brain	25	
	Kidney	14	
	Liver	11-41	
Rabbit . . .	Muscle	1.9	
	Muscle	0.42-3.4	
Sheep	Brain	15.4	
	Heart	6.2	
	Liver	25-50	
	Kidney	10.9	
	Muscle	2.5	
	Muscle	2.5	
<i>Miscellaneous</i>			
Beer	—	nil	
Corn	—	2.1	
Cider	—	trace	
Coffee . . .	Freshly ground	56-61	
Honey	—	0-20	
Rice	—	2.4	
Tea	Fresh leaves	120	
Wheat	—	2.6	
Yeast	Baker's	1.6	

What is perhaps more important than the ascorbic acid content of a foodstuff when raw or cooked by a special process is the content of the food as served at the table. Fincke and her colleagues [53] calculated the ascorbic acid content of an average helping of food as served on the plate in American college dining halls. Their figures are given below :

Ascorbic Acid Content of Food as Served [53]

Food	Mean of all Values Obtained in Four College Dining Halls (mg. per portion served)
Apple, raw	8
" baked	4
" rings	1.0
" sauce	0.7
Apricots	7.1
Asparagus	8.4
Beans, baked	2.1
" green	1.1
Beef, roast	2.2
Beets	4.9
Broccoli	27.9
Brussels sprouts	24.3
Cabbage, cooked	11.1
Carrots, raw	2.1
" cooked	1.6
" and celery, cooked	2.2
" and peas, cooked	0.7
Cauliflower	28.4
Celery, raw	1.6
Cherries	4.5
Chicken	2.2
Chili	10.1
Corn	4.2
Cucumber	1.3
Custard	1.2
" banana	3.1
Egg, cooked	1.8
Farina	1.0
Figs	1.1
Fruit, mixed	9.2
" juice, mixed	4.6
Goulash	7.4
Grapefruit	48.1
" juice	45.2
Ham	1.4
" loaf	3.9
Hash	1.2
Jam, jelly, honey and marmalade	1.0
Ketchup	3.6
Lemon	4.3
Lemonade	8.7
Liver	7.1
" sausage	1.2
Meat loaf	2.5
" pie	2.5
" sandwich	0.6

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Food	Mean of all Values Obtained in Four College Dining Halls (mg. per portion served)
Milk	1.6
Olives	1.2
Onions, raw	2.7
" boiled	3.3
Orange	59.8
Parsnips	2.8
Peaches, canned or dried	3.4
Pears, canned	0.5
Peas	4.5
" and turnips	2.6
Pickles	0.7
Pie, apple	0.4
" pumpkin	—
Pineapple juice	23.3
Plums, canned	1.3
Pork chop	2.1
" roast	1.1
" sausage	1.3
Potato chips	4.0
Potatoes, baked	10.5
" boiled	6.1
" creamed	4.1
" fried	1.9
" mashed	2.5
Prunes	6.6
Radishes	3.2
Rhubarb	2.7
Salad, apple	4.7
" " and carrot	5.1
" " " nut	2.0
" " " orange	20.0
" " " raisin	1.7
" banana	1.7
" " and pineapple	6.4
" cabbage	26.3
" " and celery	9.8
" " " raisin	25.4
" " " tomato	19.3
" carrot	3.7
" " and celery	3.7
" " " pineapple	1.1
" " " radish	5.1
" " " raisin	3.3
" cottage cheese	1.1
" dressing, French	0.8
" fruit	9.3
" " in gelatin	1.0
" grapefruit	14.1
" " and orange	26.6
" green pepper	4.8
" lettuce	1.6
" macaroni	1.2
" mixed	1.9
" potato	1.0

Food	Mean of all Values Obtained in Four College Dining Halls (mg. per portion served)
Salad, radish	1.0
„ tomato	4.0
„ „ aspic	0.4
„ tuna fish	1.1
„ vegetable	11.6
Sauerkraut	4.0
Soup	3.2
„ bean and pea	12.2
„ cream of tomato	4.6
„ pea (split)	0.8
„ potato	1.8
„ tomato	5.0
„ vegetable	6.1
„ „ chowder	7.7
Spaghetti and tomato	3.8
Spinach	9.4
Squash	6.1
Stew	3.9
Strawberries	61.6
Stuffing	2.0
Sweet potato	12.0
Tomato juice	5.5
„ sauce	18.9
Tomatoes, sliced	24.0
„ stewed	2.9
Turnips	10.8
Veal	4.1
Vegetables, mixed	2.9
Watercress	10.0

PHYSIOLOGY AND FUNCTIONS OF ASCORBIC ACID

Ascorbic acid is synthesized by all the higher plants, probably by simple organisms such as moulds and bacteria, and by many animals except the guinea-pig and the primates. In the rat, which synthesizes its ascorbic acid, radio-active tracer studies have shown that glucose is the source of ascorbic acid [209]. It is formed during the germination of seeds.

The rôle of ascorbic acid as an activator for the growth of tissues is well recognized. It stimulates the growth of fowl monocytes [464] and neoplastic tissue, e.g. sarcoma cells [465]. It has a beneficial effect on the formation of fibres in fibroblasts in tissue culture [657]. Epithelial sheets of tissue will not grow in media devoid of ascorbic acid [955] and young actively growing cells show a high concentration of the vitamin [340].

Collagen Formation. Ascorbic acid is essential for the formation of intercellular substance in the animal organisms [39, 40]. Wolbach and his associates [41, 44] in a series of papers from 1926 to 1937 showed that reticulum and collagen are not formed in the scorbutic animal. Normally in the intercellular substance fibroblasts lie in an amorphous ground substance within which fibrils of a reticulum are found as wavy bands of collagen. The fibrils are cemented together by a translucent matrix. In the scorbutic animal the ground substance and fibroblasts are present but no collagen is formed. After the administration of ascorbic acid bundles of collagenous material are

formed within twenty-four hours. The rôle of ascorbic acid as a prerequisite for the formation of intercellular material in the human subject has been established by the work of Hunt [77] and Crandon [68] (pp. 413, 485).

Once collagen is formed ascorbic acid is not essential for its maintenance [46, 1033]: it is only needed for the replacement of that destroyed by wear and tear [1033]. Hyaluronic acid a component of connective tissue ground substance, is not formed in the scorbutic state [54]. Ascorbic acid does not serve as a precursor or component of collagen; this has been shown in metabolism experiments using ascorbic acid containing radio-active carbon, C^{14} [55].

Although the exact mode of action in collagen formation is not known, recent work with cortisone has thrown some light on the subject. It has been shown that adrenocorticotrophic hormone (A.C.T.H.) and cortisone can prevent or cure lesions of experimental and clinical collagenous diseases. Ascorbic acid is known to be directly implicated in the activity of the adrenal cortex (p. 428), so that it seems possible that some of the mesenchymal changes in scurvy are not due directly to lack of ascorbic acid but to hyposecretion of the adrenal cortex steroids. Cortisone inhibits many of the manifestations of scurvy [56], and it is an attractive hypothesis to suppose that deficiency of ascorbic acid leads to insufficiency of the glyco-corticosteroids of the adrenal, lack of which may result in defective collagen formation.

Structure of Teeth. In the guinea-pig deficiency of ascorbic acid produces lesions in the tooth pulp and dentine [47], changes occur in the odontoblasts, which become shorter and spindle shaped and finally produce a substance resembling bone, which fills the pulp canal. The dentine becomes porotic and resorbed and, instead of new dentine, a spongy, porous bonelike material is laid down. The pulp tissue and newly formed bone become atrophied and resorbed with the formation of small cysts. Alveolar resorption in the jaw occurs and extends to the outer edges of the mandible [60]. If the deficiency of ascorbic acid is absolute the pulp is shrunken and completely detached from the dentine [41, 42], and the enamel is hypoplastic [49]. Fish and Harris [48] noted changes in the enamel and cement of scorbutic guinea-pigs. Boyle, Bessey and Wolbach [43, 44] observed the effect of ascorbic acid deficiency on the parodontal structures of guinea-pigs. The teeth became loosened, alveolar bone underwent rarefaction and periodontal membrane widened. Resorption of alveolar bone and cementum with loss of collagenous fibres of the periodontal membrane and depletion of the hæmopoietic cells of the bone marrow were observed microscopically. In severe scurvy the teeth could be easily removed. Scorbutic monkeys showed necrosis of the gingivæ and of the periodontal membrane, intercancelous tissue and bone [64]. According to Wilton and Thorell [65] ascorbic acid deficiency prevents maturation of young dentine cells and causes reversion of mature cells to a state resembling immature cells.

There is no evidence that in man dental decay is associated with deficiency of ascorbic acid [916].

According to Roff and Glazebrook [57] parodontal disease in man may be due to a deficiency of ascorbic acid (p. 463).

Bone Formation and Repair. Ascorbic acid is also essential for the formation of bone and cartilage (see Fig. 127). The gross and histological changes seen in the bones of scorbutic animals are similar to those seen in other structures. The lesions are commonest at the costochondral junction, the distal end of the femur, the proximal end of the tibia, femur and wrist. In the affected bones bone formation ceases and the existing osseous shell becomes rarefied, with an increased tendency to fracture on slight trauma, particularly at the epidiaphysial junctions in growing bones, and false motion at the costochondral junctions. These are the results of failure to form intercellular material. In young bone the osteoblasts lose their characteristic

shape and migrate from the trabeculae to the diaphysis. Formation of cartilage and bone matrices ceases. The osteoblasts become surrounded by liquid and give rise to an apparent region of oedematous connective tissue at the ends of the diaphysis. Microscopically rarefaction of the cortex is observed, bone ceases to grow, and the normal junction is replaced by a zone of connective tissue poor in collagen and in which are embedded fragments of calcified cartilaginous matrix, devoid of osteoid tissue. Controlled experiments show that the connective tissue cells of the marrow are osteoblasts that have migrated and reverted to fibroblasts. Ascorbic acid appears to be necessary for the proper functioning of the osteoblasts, which in its absence revert to their prototype and attempt to form a fibrous union between epiphysis and diaphysis.

Owing to the continued proliferation of the osteoblasts in the periosteum of the bones of scorbutic animals, stripping of the periosteum from the bone cortex occurs. Kodicek and Murray [100] have observed overgrowth of subperiosteal bone and porosis in animals suffering from prolonged ascorbic acid deficiency; the overgrowth is trabecular, not compact bone, and is converted into the latter on administering ascorbic acid. Lack of intercellular material in the neighbouring blood vessels leads to subperiosteal hæmorrhages, which strip the periosteum off the bone and are characteristic of the scorbutic state (Fig. 170). The response to treatment with ascorbic acid is dramatic. Within twenty-four hours new intercellular substance is formed, the fibroblasts are surrounded by a thin shell of osteoid material, trabeculae are formed, proliferation of osteoblasts in the periosteum eases, and hæmorrhage from the fragile capillaries stops. Capillary formation, which is essential in growing tissue, is resumed.

Mouriquand [61-63] has described the bony defects resulting from acute and chronic ascorbic acid deficiency. In acute deficiency softening and rarefaction of the bone occurs, particularly in the region of the femoral or tibial epiphyses. In chronic deficiency he describes a syndrome resembling chronic osteo-arthritis, with osteophytic outgrowths, pseudoankylosis, decalcification of the epiphyses and diaphysis, and periosteal thickening. Complete decalcification and disappearance of the neck and head of the femur have also been observed. Partial avitaminosis in the female guinea-pig results in the birth of dystrophic young [1024]. Murray and Kodicek [52] describe stiffening of the joints in scorbutic guinea-pigs.

The deposition of bone salt in both normal and regenerating bone is retarded in scorbutic animals, but not in the bone of animals receiving adequate ascorbic acid. A deficiency of ascorbic acid lowers the alkaline phosphatase of both bone and blood [880], and Bourne [87] has demonstrated that there is less phosphatase activity in regenerating bone from scorbutic animals than that from normal guinea-pigs. Follis [78] has shown that in the epiphyseal cartilage of scorbutic guinea-pigs there is no cytochrome oxidase and the cells contain no glycogen, no mucopolysaccharide and a reduction in ribose nucleic acid. This is in contrast to the findings in normal bone. Follis [917] believes that some of the changes in cartilage seen in scurvy result from a combination of non-specific inanition and mechanical disturbances, resulting from the absence of osteoblastic activity in the shaft beneath the cartilage.

Ascorbic acid is essential for the formation of callus in the union of fractured bones. Fractures heal badly in a subject, human or animal, deficient in ascorbic acid. Wolbach and Howe [41, 42] have demonstrated the complete failure of natural callus formation in experimental scurvy and the resorption of old callus and the refracture of united fragments of bone has been described in scurvy since early times. Callus is normally consolidated into compact bone by thickening of the trabeculae; in the scorbutic state this does not occur [52]. It has also been shown that the excretion of ascorbic acid falls rapidly in an animal with multiple experimental fractures [58] and

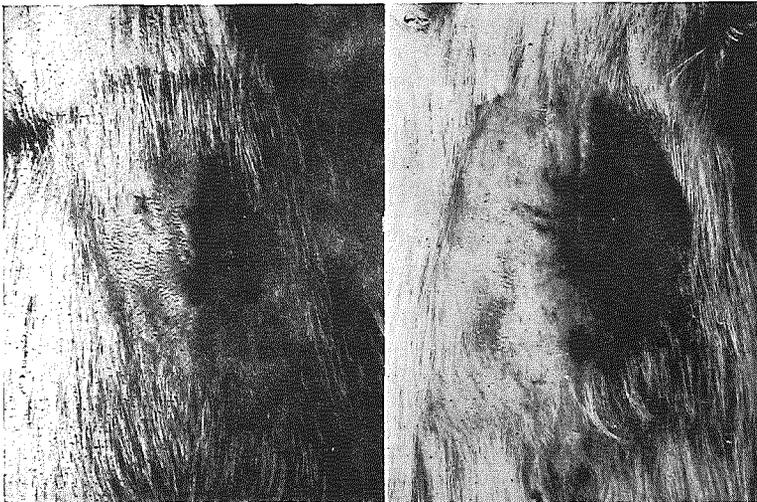
ASCORBIC ACID AND WOUND HEALING



FIG. 127. Growing point of cartilage in a chick embryo, showing cartilage cells laden with ascorbic acid granules. Silver stain ($\times 300$).



FIG. 128. Ascorbic acid and Wound Healing. Section of wound in skin of cat after seven days. In the scar tissue are numerous histiocyte-like cells containing granules of ascorbic acid. Silver stain ($\times 300$).



Control.

Subscorbutic.

FIGS. 129 and 130. Ascorbic Acid and Wound Healing. Abdominal incisions of guinea-pigs twenty-one days after operation (life size). Both healed by first intention. *Left*, control animal receiving adequate ascorbic acid; *right*, subscorbutic animal. In the control the scar is practically invisible. In the subscorbutic animal it is puckered, stretched, sunken and shows a mauve discoloration.

ASCORBIC ACID AND WOUND HEALING

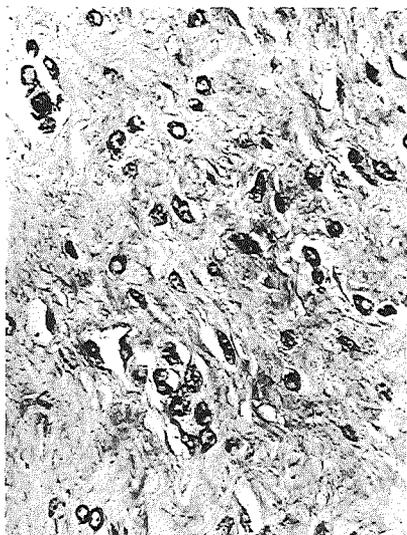


FIG. 131. Control.

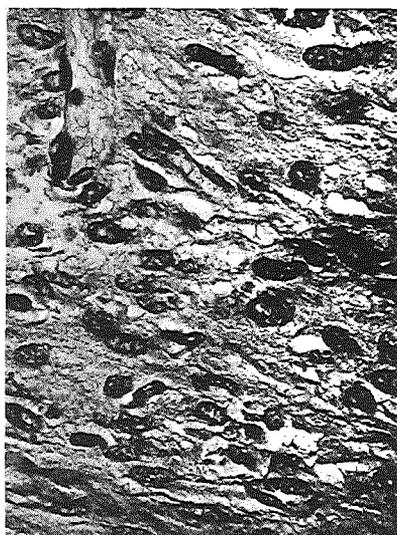


FIG. 132. Subcorbutic.

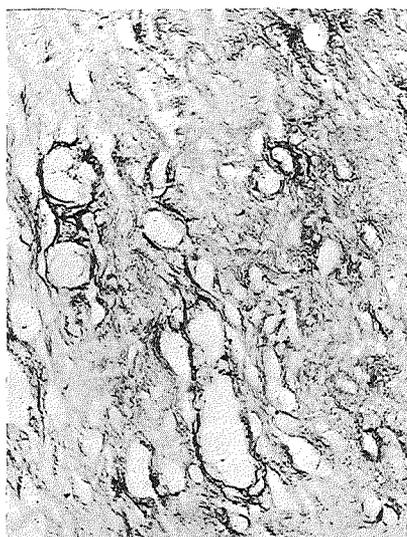


FIG. 133. Control.

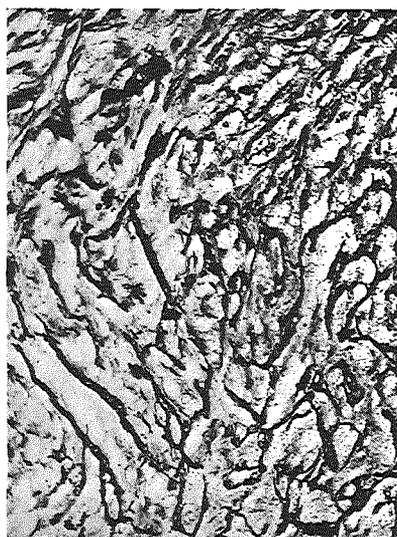


FIG. 134. Subcorbutic.

FIGS. 131 to 134. Ascorbic Acid and Wound Healing. Sections from the abdominal scars of guinea-pigs killed twenty-one days after operation ($\times 300$). Figs. 131 and 133 are sections from a control animal; Figs. 132 and 134 from a subcorbutic animal. Fig. 131 shows fibrocytes and Fig. 132 fibroblasts, stained with hæmatoxylin and van Gieson stain. There is no van Gieson staining intercellular substance in the scar of the subcorbutic animal. Fig. 133, stained with silver, shows black silver-stained reticulum fibres only around the blood vessels. The rest of the ground or intercellular substance is mature collagen and appears translucent yellow in section. In contrast in Fig. 134, from the subcorbutic animal, the whole of the intercellular substance consists of coarse irregular bands of precollagen, which stains densely black with silver.

that the degree of healing in bone is proportional to the ascorbic acid intake. Bourne [920] has shown that the optimum formation of bony trabeculae in the injured femora of guinea-pigs is brought about by the administration of 2 mg. of ascorbic acid daily and that anything less than 1 mg. seriously retards the formation of bony trabeculae. Provided the level of ascorbic acid in the tissues is optimal, additional vitamin does not accelerate bone regeneration. This was shown by Bourne [921] in the case of rats, which synthesize their own ascorbic acid.

Fractures are sometimes associated with damaged muscle fibres. In the scorbutic animal more of the damaged fibres degenerate than normally, and the muscle tissue so lost is replaced by large masses of hyperplastic connective tissue, which is avascular or nearly so [52]. In the scorbutic animal defective capillary formation in the region of a fracture can be demonstrated; after the administration of ascorbic acid capillary proliferation and active hyperaemia occur [59].

It has long been known that degeneration of striated muscle occurs in

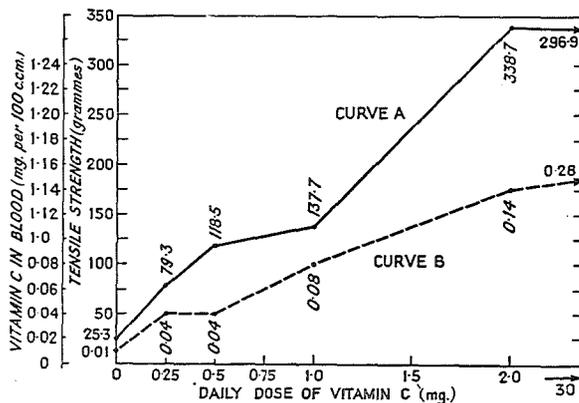


FIG. 135. Tensile Strength of Wounds and Ascorbic Acid Intake. Curve A, tensile strength of wounds in fifty-two guinea-pigs on varying intakes of ascorbic acid; the tensile strength of wounds increases with increasing ascorbic acid intake, up to an optimum of 2 mg. Curve B is the total blood ascorbic acid in fourteen guinea-pigs on varying doses of vitamin C. The blood ascorbic acid rises with increased intake of ascorbic acid. A maximum is reached when the intake is 2 mg. daily.

scurvy; hyaline degeneration and fragmentation of the fibres are described. Boyle and Irving [1028] noted hyaline changes only in chronic scurvy. In acute scurvy myofibrils became detached from the sarcolemma.

Wound Repair. Clinical and laboratory observations have shown conclusively that the rate and efficiency of primary wound healing depend on the ascorbic acid and protein concentration in the tissues. By special histochemical staining techniques with silver nitrate, due to Bourne [70], Gough [387] and Giroud and Leblond [71], it has been shown that appreciable quantities of ascorbic acid are mobilized from the tissues and concentrated in healing wounds and young granulation tissue [69]. At the same time there is a diminished excretion of the vitamin in the urine [923]. The lowest excretion occurs six to nine days after wounding and precedes the period of most active collagen formation. The tensile strength of scorbutic guinea-pig wounds is considerably less than normal [72, 73, 925]. The tensile strength of the wound is in fact proportional to the ascorbic acid content of the wound and of the blood [924-926]. After surgical operations the tensile strength of wounds in the human is decreased if the plasma ascorbic acid falls below 0.2 mg. per ml. [852]. In the guinea-pig a daily intake of 2 mg. is adequate for optimal

ASCORBIC ACID AND WOUND HEALING

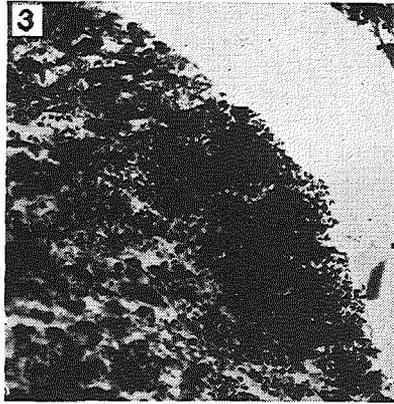


FIG. 136. Unorganized blood clot in wound of a guinea-pig receiving no ascorbic acid. Masson trichrome stain ($\times 100$). Section made after a week's healing. Tensile strength of wound 46 grams.

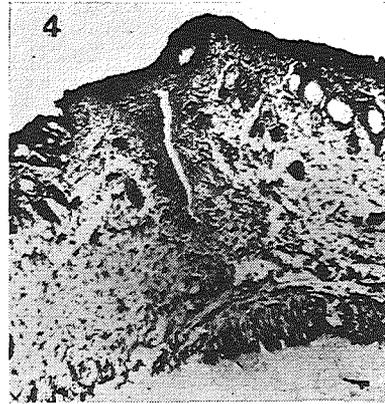


FIG. 137. Section of wound of guinea-pig receiving 0.25 mg. ascorbic acid daily. Hæmatoxylin and van Gieson stain ($\times 50$). Little scar tissue is present. Although the surface of the wound is covered by epithelium, there is a large empty space beneath the epithelial layer. Tensile strength of scar 34 grams.

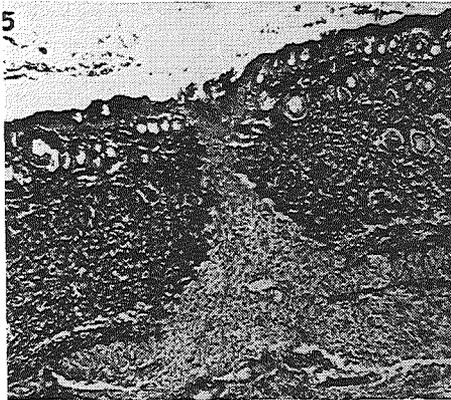


FIG. 138. Section of wound of guinea-pig receiving 0.5 mg. ascorbic acid daily. Hæmatoxylin and van Gieson stain ($\times 50$). There is a well-marked difference between the normal connective tissue and scar tissue. This is due to the small amount of fibres and large numbers of cells in the scar. The epithelium has covered the scar completely. Tensile strength of scar 62 grams.

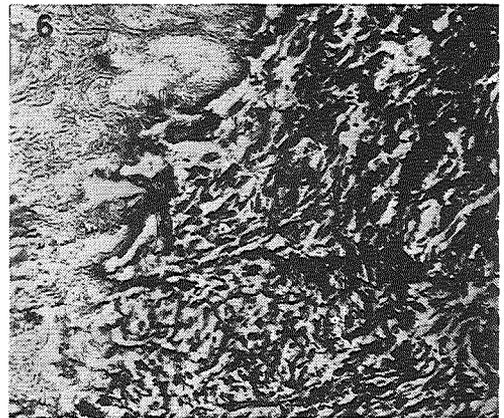


FIG. 139. Reticular preparation made from wound shown in Fig. 138 ($\times 120$). On the left is normal connective tissue, on the right are numerous reticular fibres and cells in the scar.

ASCORBIC ACID AND WOUND HEALING



FIG. 140. Section of wound of guinea-pig receiving 1 mg. of ascorbic acid daily. Hæmatoxylin and van Gieson stain ($\times 50$). The scar contained about the same amount of fibrous tissue as the scar in the guinea-pigs receiving 2 mg. of ascorbic acid (Figs. 142, 143), but more of the fibres were reticular. Tensile strength of scar 158 grams.

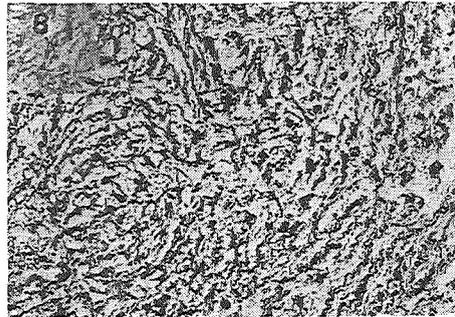


FIG. 141. Reticulum preparation of wound, section of which is shown in Fig. 140. Numerous fine reticular fibres, but few cells, are present.

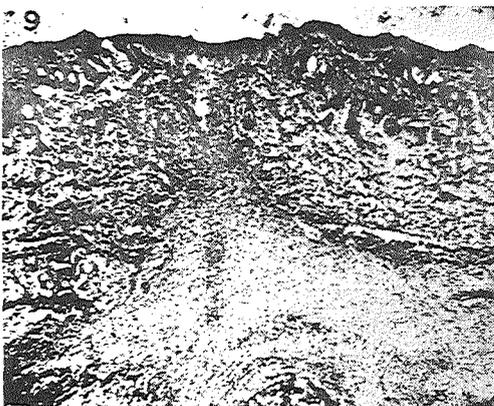


FIG. 142. Section of wound of guinea-pig receiving 2 mg. of ascorbic acid daily. Hæmatoxylin and van Gieson stain ($\times 50$). There is abundant collagen in the scar. Tensile strength of scar 295 grams.

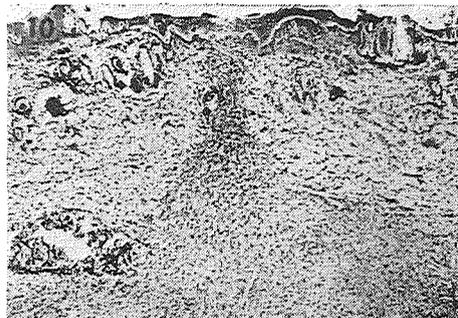


FIG. 143. Reticulum preparation of wound section of which is shown in Fig. 142 ($\times 50$). Few reticular fibres are present and the inter-fiber staining of scar tissue is similar to that of normal connective tissue.

healing ; less than this delays the process, although more than this has no significant effect on the rate of healing or on the tensile strength of the wound (Figs. 136-143). In the scorbutic guinea-pig the wound scar is puckered, stretched, sunken, loose, irregular and almost avascular, and the removal of catgut ligatures, either by phagocytosis or by extrusion, is delayed [77]. Some fibroblastic proliferation occurs in the wound, but the fibroblasts do not form syncytia and there is very little extra-cellular material. If ascorbic acid is administered normal fibroblasts appear and fine reticular fibres and much extra-cellular material are formed. The latter consists partly of acid mucopolysaccharides of the hyaluronic acid or chondroitin sulphate type [54]. It is possible that epithelial regeneration can occur in the absence of ascorbic acid ; it does so in the cornea and the gum periosteum [79]. When, however, healing of a wound demands new formation of collagenous tissue a deficiency of ascorbic acid delays epithelialization because of lack of a satisfactory collagenous base for the regenerating epithelium. There is diminished phosphatase activity in the wound of a scorbutic animal [80].

In 1940 Crandon [68] proved conclusively that ascorbic acid is necessary for the healing of wounds in man. This investigator placed himself on a scorbutic diet for six months and after three months a wound was made in his back and a biopsy specimen removed. This showed good wound healing, with ample intercellular substance and capillary formation (Fig. 144). Another wound was made after six months, when the subject was scorbutic and with a zero blood ascorbic acid. Ten days after the second wound was made a biopsy specimen showed that beneath the skin, which appeared to be well healed, there was only blood clot and no evidence of healing ; it was in fact necessary to insert a rubber drain. There was lack of intercellular substance and capillary formation (Fig. 145). Then 1,000 mg. of ascorbic acid was injected intramuscularly and ten days later a further biopsy made of the wound. There was now good healing, with the formation of ample intercellular substance (Fig. 146). As a result of inadequate collagen formation in the subject deficient in ascorbic acid, fibroblastic proliferation occurs but there is no intercellular material (Fig. 147) and blood vessels do not penetrate the poorly developed granulation tissue. Leakage of blood from the fragile capillaries forms hæmatomata, which are not organized or absorbed, and the superficial skin scar is split by them or by extravasations of blood. Phagocytosis is delayed and the surrounding structures are not incorporated in the scar tissues of the wound. Infection occurs more readily than normally in scorbutic wounds, as noted by Farmer [864], who repeated Crandon's observations on human volunteers. Hunt [77], from a study of twenty-eight surgical cases that came to post mortem, considered that the poorest collagen formation occurred in those patients most deficient in ascorbic acid. Carney [97], however, failed to observe any relationship between plasma ascorbic acid levels and satisfactory wound healing in military hospitals. Wounds most commonly disrupt on or about the tenth day and it is possible that ascorbic acid deficiency is an important cause of wound disruption. In spite of a low level of ascorbic acid nutrition at the time of operation normal wound healing results if ascorbic acid is given orally or parenterally post-operatively [852]. Sprinkling ascorbic acid on the wound of a rat, which synthesizes its own ascorbic acid, has no beneficial effect [85].

Wolfer and his co-workers [75] made observations similar to those of Crandon on nine human volunteers. They concluded that a diet deficient in ascorbic acid diminishes the tensile strength of the wound by fifty per cent. from three to five days up to the fourteenth post-operative day. They consider that when circumstances for healing are not good, as in the presence of undue wound tension or diminished blood supply, failure of primary wound healing is far more likely to occur in subjects deficient in ascorbic acid.

Abscesses do not heal well in scorbutic animals. The appearance and increase of macrophages is delayed, bacteria readily escape from the abscess

WOUND HEALING IN EXPERIMENTAL HUMAN SCURVY



FIG. 144. Section from wound of a human subject on a scorbutic diet for three months. An experimental wound was made and sutured and a biopsy specimen obtained eleven days later. Normal healing has occurred (see text, p. 413).



FIG. 145. Section from wound of a human subject on a scorbutic diet for six months, showing absence of healing. The wound appeared to heal by first intention. Ten days later a biopsy specimen was taken. As soon as the skin was divided it was found that the tissues under the skin had not healed at all and that the wound contained firm dry blood clot. This is shown in Fig. 145 by the large empty space beneath the epidermis (see text, p. 413).

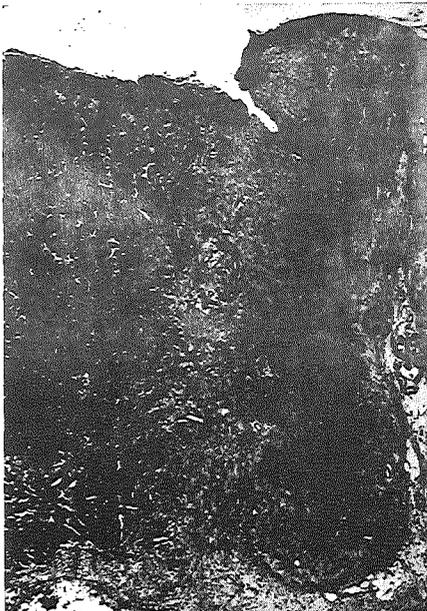


FIG. 146. Same case as Fig. 145 after ten days' treatment with 1 gram of ascorbic acid daily. Another incision was then made across the same wound that had formerly failed to heal. A section of this shown above, shows that normal wound healing has occurred.

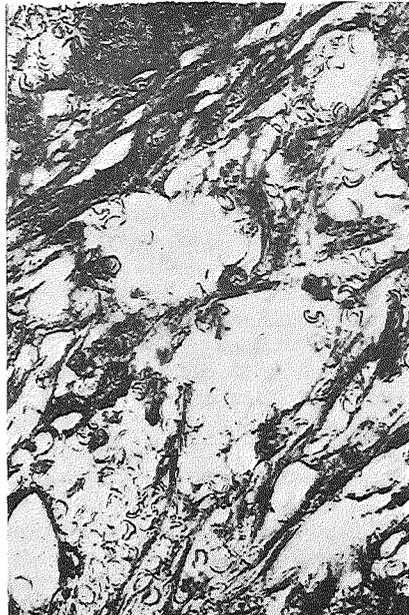


FIG. 147. Section from wound of human subject on a scorbutic diet for six months. Note formation of granulation tissue without intercellular substance.

into the surrounding tissues, and the necrotic centres in the abscess are not walled off [928].

Hines and his co-workers [890] have shown that ascorbic acid is essential for the regeneration of damaged nerve. The tibial nerve of guinea-pigs was crushed and the rate of regeneration studied on diets containing graded amounts of ascorbic acid. Regeneration was impaired when the ascorbic acid intake fell below an optimal level of 2.5 mg. daily. It does not follow that ascorbic acid has any specific action on nerve tissue, as any deficiency in collagenous intercellular material in the tissues supporting a nerve would retard its regeneration.

Capillary Resistance. The hæmorrhagic manifestations of scurvy have been stated to be due to abnormal capillary fragility. Morphological changes have not been detected in the capillaries and, although it has been suggested, it has not been proved that this increased fragility is due to a defect in the cement substance binding the capillary endothelium or in the pericapillary connective tissue and collagen sheath. Actually the minute structure of the capillary wall is not well known.

There are a number of clinical methods employed for the estimation of capillary permeability. These are described on p. 464. They can be classed as positive or "negative" pressure tests. In positive pressure tests the venous return from a limb is dammed back in order to increase the pressure within the capillaries; in negative pressure methods, suction is applied to the skin and this "negative" pressure is transmitted to the underlying capillaries. Capillaries which cannot stand the test pressure are ruptured and petechial hæmorrhages result, and the ease with which such petechiæ can be produced is considered as a measure of the strength of the capillary. An area is marked off on the skin of a limb, usually the arm, the pressure applied under controlled circumstances for a given time and the petechiæ counted under standard illumination with or without the aid of a lens. There is no correlation between the positive and negative tests [66] and many authors do not give adequate directions for carrying out their tests. There are too many variables for the method to be of any value in the detection of ascorbic acid deficiency, even if it could be shown that if all these variables are controlled there is a true correlation between capillary fragility and ascorbic acid deficiency. The petechial count depends on the part of the body tested, the temperature, texture and degree of vascularization of the skin, age, time of the day, and the season of the year [930]. False positive results are liable to be obtained during menstruation, after hot baths, in septic and acute infections (e.g. diphtheria), in certain blood diseases (anæmia, purpura, hæmophilia), as a result of drug therapy (arsphenamine and related drugs), and in acute nephritis, malignant disease, allergic states and hypertension. Other factors influencing capillary fragility are skin structure, barometric pressure, external temperature, exposure to light [1026], and activity of the adrenocortical system [1027]. Munro, Lazarus and Bell [66] have also noted an apparent improvement in capillary strength to occur spontaneously. The fact that capillary fragility shows an increase in the spring suggests it may be related to a fall in the ascorbic acid intake that occurs about this time, although the effect of sunlight, wind, humidity and perhaps other meteorological influences cannot be excluded. Crandon [68] noted that even five months on a scorbutic diet did not significantly change his capillary fragility and Lazarus, Munro and Bell [66] state that one-third of scorbutic subjects do not show any signs of decreased capillary fragility, whilst one-third of undernourished subjects without clinical scurvy do show capillary weakness. They also note that there is frequently no marked impairment of capillary strength when human subjects are experimentally deprived of fruit and vegetables for several weeks or months. On the other hand, they state that some subjects with weak capillaries may show an improvement in capillary strength after receiving fruit juices. Göthlin in 1933 [82, 83] sought to establish a correlation

between capillary fragility and the level of ascorbic acid nutrition, but the majority of workers since that date have failed to correlate them [66-68, 81, 347, 553, 877, 929]. According to Lazarus, Munro and Bell [67] petechial counts may occur in patients with scurvy, but clinical improvement after treatment with ascorbic acid is not correlated with a lowering of the petechial count. These workers consider that determination of the capillary fragility is of no diagnostic value in scurvy because of the wide variations in the results obtained.

The problem has also been approached from the histological side. No important changes in blood vessels have been described which can be attributed to ascorbic acid deficiency. Capillary permeability in the scorbutic guinea-pig has been studied by Elster and Schack [89] by observing the rate of transport of the dye T-1824 (Evans' blue) across the capillaries. After administering T-1824 intravenously to scorbutic and normal guinea-pigs, they found no qualitative or quantitative differences in the tissue distribution of the dye, indicating that capillary permeability was unaltered in the scorbutic state.

Observations by Lee and Lee [88] suggest that ascorbic acid might be one of the factors essential for the functioning of certain peripheral vasomotor mechanisms. Others have noted a greatly diminished vascular response in the perfused hind limbs of scorbutic guinea-pigs. These findings could be explained by an adrenaline-protecting action exerted by ascorbic acid.

They have shown that in scurvy there is failure of the contractile mechanism of the small vessels, resulting in their dilatation and a sluggish blood flow (Figs. 162-165).

Ascorbic Acid and the Hæmopoietic System. It is generally considered that ascorbic acid plays a part in erythropoiesis, which is usually depressed in scurvy, particularly in the first two years of life. Anæmia is of regular occurrence in scorbutic guinea-pigs, blood films from which show slight poikilocytosis, anisocytosis, polychromasia and stippling [90]. If the disease is fatal an increased number of reticulocytes appear in the peripheral circulation just before death. This has been observed in human scurvy [94]. The bone marrow is hyperplastic, with an increasing number of normoblasts, suggesting arrest of maturation. The administration of orange juice to scorbutic guinea-pigs produces a reticulocytosis within three days, followed by an increase in red cells and hæmoglobin concentration. The anæmia is not associated in the experimental animal with iron deficiency because it does not occur if ascorbic acid is administered with a scorbutic diet, nor does iron prevent its development or effect its cure [99].

Although anæmia is frequent in human scurvy its occurrence is inconstant. Of forty-three patients with severe scurvy seen by Brown [84] nine showed little or no anæmia. Mettier, Minot and Townsend [86] found that about a third of a large group of scurvy patients suffered from severe anæmia, a third were mildly anæmic, and the remaining third had slight or no anæmia. Anæmia is not a constant finding in infantile scurvy [92], and it does not occur in sub-scorbutic ascorbic acid deficiency [870]. Crandon [68], who existed on a scorbutic diet for six months, showed a slight fall in blood hæmoglobin, which was not surprising as 6 litres of blood were withdrawn for laboratory tests, but it responded to iron therapy while other symptoms of scurvy persisted. In another study of this kind conducted on volunteers on a scorbutic diet calculated to contain 1 mg. of ascorbic acid daily there was no sign of anæmia, although other symptoms of scurvy were present [108]. However, these experimentally induced single vitamin deficiencies cannot be compared with a malnutrition syndrome such as scurvy. Lozner [110] reported cases of ascorbic acid deficiency that responded to iron and not ascorbic acid. There was no evidence that the cases were scorbutic and the diagnosis was made on low plasma ascorbic acid measurements. Croft and Snorf [96] have been cited as presenting evidence that the anæmia of scurvy

may be due to lack of factors other than ascorbic acid. Actually there was no evidence that their patients suffered from scurvy; they suffered from a number of other diseases and had a low plasma ascorbic acid. There are no characteristic hæmatological findings in scurvy. The anæmia when it does occur conforms to no particular type; it may be macrocytic, normocytic or microcytic. Mild hypochromia is common, but not constant. McMillan and Inglis [93], in a study of forty-three scorbutic subjects, found macrocytic anæmia in two, normocytic anæmia in eighteen, microcytic anæmia in fourteen, and microcytic hypochromic anæmia in six. There are no characteristic changes in the bone marrow, which is normoblastic [84] and normally cellular or hypercellular [103]. Differential counts of nucleated marrow cells show a relative increase in normoblasts [103]. The bone marrow has also been described as hyperplastic [86], hypoplastic [95] and megaloblastic [98]. Vilter and his co-workers [103] noted signs suggesting accelerated blood destruction in scorbutic patients, namely, reticulocytosis, slight jaundice and increased urobilinogen output in urine and fæces. These signs and the anæmia disappeared when ascorbic acid was given. *In vitro* ascorbic acid can take part in a reaction involving the conversion of hæmoglobin into bile pigments [933]. It has often been observed that the anæmia of scurvy improves when the patient is put to bed and given a diet restricted in ascorbic acid [84, 103].

The conflicting reports on scorbutic anæmia that have appeared may be explained. Scurvy, like other diseases of malnutrition, is probably a multiple deficiency state, and lack of other factors as well as ascorbic acid may influence blood formation. Infection and hæmorrhage may also play a part.

The possibility that ascorbic acid may influence the absorption and utilization of iron cannot be overlooked [109, 116]. Lin [684] has shown that anæmia in subjects with ascorbic acid deficiency did not respond to ascorbic acid alone, but did to ascorbic acid and iron. The administration of ascorbic acid increases the iron level in the blood [934].

It has been supposed that ascorbic acid aids the absorption of iron from the gut, from which it is absorbed in the ferrous state. Bergheim and Kirch [118] state that ascorbic acid reduces iron to the ferrous state in the stomach. Tötterman [113] found no correlation between iron and ascorbic acid metabolism in normal subjects. Administration of ascorbic acid produced no change in the serum iron, hæmoglobin, erythrocyte or reticulocyte values, nor had prolonged oral or parenteral administration any effect on the serum iron in patients with infections, in which the latter is lowered. Tötterman also found anæmia resulting from infection to be refractory to treatment with iron and ascorbic acid. These results are at variance with those of Albers [106], who states that ascorbic acid given intravenously to pregnant women causes a rise in the serum iron within three hours. Others have reported a rise in the serum iron following the administration of ascorbic acid [934]. In patients with anæmia due to iron deficiency injection of ascorbic acid caused a fall in the serum iron; after treatment with iron and ascorbic acid the serum iron rose. The serum iron in patients with infection showed considerable fluctuation after treatment with ascorbic acid. According to Sinclair and Duthie [115] the utilization of iron by patients with rheumatoid arthritis is not materially improved by the administration of ascorbic acid.

Ascorbic acid may be essential for the conversion of folic acid to folinic acid [117] and might therefore affect hæmatopoiesis indirectly. According to May and his co-workers [131] lack of ascorbic acid plays a part in the ætiology of the megaloblastic anæmia of infancy, probably by interfering with the utilization of folic acid. Barron [101] suggests that ascorbic acid may play a rôle in maintaining hæmoglobin at a normal physiological level because it reduces the red cell count to normal in polycythæmia produced experimentally by cobalt salts. The treatment of human polycythæmia with ascorbic acid is described on p. 480. It has no effect on polycythæmia due to continued exposure to low air pressures [725].

The administration of doses of 100 to 300 mg. of ascorbic acid produces changes in the blood chemistry—a decrease in blood sodium and chloride and an increase in blood potassium [316].

ASCORBIC ACID, INFECTION AND IMMUNITY

Blood Levels and Excretion in Infection. In infections there is a lowering of the blood ascorbic acid and diminished excretion. This has been demonstrated in animals and man in a number of infections, such as tuberculosis, pneumonia, diphtheria and rheumatism [119–127]. Thus Faulkner and Taylor [123] found that the average serum ascorbic acid of a group of healthy subjects was 1.31 mg. per 100 ml., whereas in patients with infectious diseases it was 0.65 mg. per 100 ml. The organs of animals suffering from infections contain considerably less ascorbic acid than those of healthy control animals [138]. The lowered blood ascorbic acid and lowered excretion are not due to pyrexia *per se* but due to the infectious process [152].

This fall in the plasma ascorbic acid and diminished excretion in infections can be partly accounted for by the migration of the vitamin to the white blood cells (p. 420) and the adrenals (p. 430).

Ascorbic Acid and Immunity. There is some evidence to show that ascorbic acid may be concerned with acquired immunity. It is probably concerned with antibody formation. Juszat [149] found that the addition of 100 mg. of ascorbic acid to an immunizing dose of horse protein caused a five- to seven-fold increase in the specific precipitin production in rabbits. This was confirmed by Madison and Manwaring [153] and others [141, 142, 148, 153]. Cameron [140] found that the blood of guinea-pigs treated with diphtheria toxoid and receiving 3.6 mg. of ascorbic acid daily contained more antitoxin than controls similarly treated but receiving only 0.9 mg. of the vitamin daily. Birkhaug [150] claimed that supplements of 10 mg. ascorbic acid produced a significant inhibition of the tuberculin reaction in guinea-pigs. He noted that the inhibition of the tuberculin reaction was correlated with the urinary excretion of ascorbic acid and the amount of the latter stored in the adrenal gland. Heise and Steenken [151] were unable to confirm these observations. Steinbach and others [818, 819] found that the administration of ascorbic acid to tuberculous guinea-pigs increases the tolerance to repeated large doses of tuberculin, and Büsing [154] observed that the administration of ascorbic acid to rats or rabbits infected with pneumococci or staphylococci increased the survival time.

Much of the confusion on the subject arises from the failure to recognize that conclusions drawn from studies on one species (e.g. the guinea-pig) that needs an exogenous supply of ascorbic acid cannot be applied to others (e.g. the rat) that synthesize their own ascorbic acid and cannot develop the symptoms of deficiency. The latter species can be used to study the effects of an excess of ascorbic acid, but never a deficiency. Another source of confusion is failure to distinguish between the response following a single injection of an antigen (e.g. the primary response in the case of diphtheria toxoid), and the response after the animal has received one or more doses (secondary response). The blood antibody response to the first dose of antigen is small, slow in developing, rises and falls rapidly and is less affected by the amount of antigen than the secondary response. The blood antibody response to more than one dose of antigen is large, develops quickly, rises rapidly, is maintained for a relatively long time and its magnitude depends on the dose of the antigen. Long [130] has shown that the primary antitoxin response of guinea-pigs deficient in ascorbic acid to alum precipitated diphtheria toxoid is not significantly reduced, whereas the secondary response is.

The effect of ascorbic acid on the immune state is obscure. The small effect of a deficiency on the primary response suggests that ascorbic acid is

concerned more with the metabolic systems controlling the production of antibodies in cells already in a state of "secondary responsiveness" than with the response of those cells to primary conditioning by the antigen (Long).

Ascorbic Acid and Bactericidal and Antitoxic Action. Ascorbic acid has bacteriostatic and bactericidal activity. In suitable concentration it prevents the development of pneumococci, streptococci, staphylococci, *Haemophilus pertussis*, *Clostridium tetani* [154, 156]; at higher concentrations the organisms are destroyed. These concentrations, however, are many times greater than those present in human blood, in which it can have little bactericidal action. The bactericidal action of blood is, in fact, independent of its ascorbic acid content [815], and if the vitamin is given to human subjects with low ascorbic acid levels it does not result in an increase in the bactericidal action against staphylococci, *B. coli* and *S. typhosum* [160, 169]. If the ascorbic acid in blood is oxidized there is no decrease in its bactericidal action against the same organisms.

Ascorbic acid inactivates certain toxins, e.g. those of *Clostridium oedematiens*, *Cl. histolyticus*, *Cl. tetani*, *B. dysenteriae* and *H. pertussis* [115-160]. These toxins are not only neutralized *in vitro*, but if the ascorbic acid given is *in vivo* it can raise the resistance against toxins. Certain strains of poliomyelitis virus are inactivated by ascorbic acid, and according to Jungeblut [161] it has some curative effect *in vivo* in infected monkeys, although this is disputed by Sabin [162]. Ascorbic acid has a virucidal action on influenza A virus [136].

The toxin of *Corynebacterium diphtheriae* particularly affects the adrenal glands, in which the level of ascorbic acid falls (p. 430). It has been suggested that ascorbic acid and the suprarenal cortex may play an important part in the anti-infective processes of the body (p. 430). Many workers have stated that the degree of virulence of a standardized diphtheria toxin, as measured by guinea-pig assay, is dependent on the ascorbic acid intake of the animal, and that an increased intake of the vitamin gives increased protection [164-166]. According to Jungeblat [817] diphtheria toxin is inactivated *in vitro* by ascorbic acid, particularly in the presence of cupric ions; this was confirmed by Willison [936], who found that 2M.L.D. of diphtheria toxin is detoxicated by 1 mg. of ascorbic acid at 37° C.

Zilva [170], Torrance [171] and others [207] have been unable to confirm these observations. Zilva claims that guinea-pigs treated with ascorbic acid show no more resistance to diphtheria than do deficient controls. Torrance could not confirm the inactivation of diphtheria toxin by ascorbic acid, nor could he detect any significant fall in the ascorbic acid of the adrenals of animals treated with toxin. Willison [936] claims that failure to demonstrate the inhibitory effect of ascorbic acid on diphtheria toxin is due to too short a period of incubation and to the use of the wrong pH.

Ascorbic Acid and Complement. Between 1938 and 1940 several reports were published establishing a direct correlation between the ascorbic acid level of the blood and its complement activity. Complement, which is a non-specific anti-bacterial property of blood, resides in four major components of the blood, two of which are globulins, one a carbohydrate and one of unknown nature. Ecker and his colleagues [172, 175] stated that *in vivo* there is a correlation at concentrations of ascorbic acid below 1 mg. per 100 ml. of serum. They claim that in scurvy the administration of graded doses of ascorbic acid is paralleled by a corresponding increase in the complement titre. Simola and Brunius [516] and Marsh [173] also claimed to have observed a lowering of complement titre in animals with ascorbic acid deficiency. Chu and Chow [174] recorded similar observations on thirty-eight patients on basal diets receiving increasing quantities of ascorbic acid.

More recent work has failed to confirm these observations, which were based on average results without statistical evaluation. Zilva [176] and Chakraborty [469] could find no significant change in the complement titre

of scorbutic guinea-pigs. Spink, Agnew and Michelson [855] showed that a fall in plasma ascorbic acid in both guinea-pigs and adult humans is not accompanied by a reduction in the complement titre, and that neither the *in vitro* nor *in vivo* addition of ascorbic acid results in change of complement titre. They further state that ascorbic acid can be removed chemically from blood without changing the titre of complement. Similar observations were made by Rice and Boulanger [131]. Kodicek and Traub [937] were unable to find any significant change in complement in guinea-pigs partially or completely deficient in ascorbic acid. These observations are in agreement with those of Crandon and his colleagues [68] in human scurvy (p. 453), Feller and co-workers [856], and Natvig [214]. Deeny and his collaborators [938] carried out investigations on eighty patients suffering from acute infections in private practice in Ireland. The ascorbic acid and complement values of the blood were estimated, but they were unable to establish any linear relationship between the two in health or disease; when the two factors were plotted there was a random distribution of the points. Further indirect evidence of lack of any correlation between ascorbic acid and complement titre is the observation that although newborn infants have a higher plasma ascorbic acid concentration than their mothers (p. 444), they have a significantly lower complement activity [937].

Feller and his associates [856] have also studied the following immunological phenomena in relation to ascorbic acid and also vitamin A nutrition :

- (a) Capacity of nasal secretion to inactivate influenza virus.
- (b) Titre in blood serum of neutralizing antibodies for influenza virus.
- (c) Activity of lysozyme in the nasal secretion.
- (d) Phagocytic activity.
- (e) Complement titre of blood serum and of polymorphonuclear neutrophile leucocytes in whole blood for pneumococci.

The results of the various immunological tests were not significantly influenced by marked changes in the plasma levels of ascorbic acid or vitamin A, or by a period of severe ascorbic acid deficiency followed by a large excess of the vitamin. The authors of the work point out, however, that because of the multiplicity of factors involved in the mechanisms of virulence, susceptibility and resistance broad conclusions cannot be drawn from the study.

Ascorbic Acid and Leucocytosis. Ascorbic acid is taken up in large quantities by the leucocytes of the blood [182], and in concentrations of 0.25 to 1 p.p.m. stimulates their respiration [268]. It has been demonstrated by special cytological methods by Tonutti and Matzner [102] in the leucocytes of the lungs of guinea-pigs with pneumonia. They observed an increase in the alveolar phagocytes, which were laden with ascorbic acid granules, which sometimes contained as much as 0.3 per cent. The alveolar exudate also contained considerable ascorbic acid in its cells. According to Cuttle [104] considerable quantities of ascorbic acid are stored in the white blood cells, since in leucocytosis an excessive amount of the vitamin can be absorbed and retained and the amount in the blood cells is increased. These abnormalities bear a direct relationship to the number of circulating leucocytes. The increased utilization of ascorbic acid in infections (p. 418) may be due to the accompanying leucocytosis. When erythrocytes and leucocytes compete for ascorbic acid *in vitro* it is taken up preferentially by the leucocytes [843].

Cottingham and Mills [939] observed that ascorbic acid deficiency severe enough to retard growth produces a corresponding reduction in phagocytic activity. The leucocytes of adequately fed guinea-pigs took up an average of 18.3 micro-organisms per cell *in vitro*, with ninety-nine per cent. of the cells showing evidence of bacterial destruction by the end of one hour. On a diet deficient in ascorbic acid, phagocytosis was reduced to 7.3 bacteria per cell, with intracellular digestion reduced to seventy-four per cent. Nungester and Ames [132] observed that in guinea-pigs deficient in ascorbic acid the peritoneal exudate was tinged with blood and contained few white

cells. They found that the phagocytic activity of these cells was related to their ascorbic acid content. This correlates with the observation of Perla and Marmorston [143] that the cellular response to intraperitoneal irritation is poor in scorbutic guinea-pigs.

It has been stated that the injection of ascorbic acid provokes a leucocytosis in infective states or in leucopenia [105]. From histological studies on experimental fractures in rabbits it would appear that the injection of ascorbic acid causes an increased proliferation of the reticulo-endothelial elements [137]. This occurs even in animals on a diet containing an adequate supply of the vitamin. Meyer [181] determined the opsonic index of three subjects and found that the injection of ascorbic acid caused a marked rise in the phagocyte count, in one case as much as six times the normal. The injection of doses of from 50 to 750 mg. of ascorbic acid into normal individuals is stated to cause considerable leucocytosis, e.g. up to an increase of sixty-eight per cent. [854].

Crandon [68] found that whilst on a scorbutic diet his white cell count fell from 5,000 to 3,500. After an injection of 1,000 mg. of ascorbic acid, it rose to 5,000 and later to 9,000. Faulkner [184] observed that the administration of large doses of ascorbic acid in various infective conditions was accompanied by appreciable reticulocyte responses analogous to those following the administration of ascorbic acid to patients with the anæmia of scurvy (p. 453).

Bacchus and Toompas [107] observed that ascorbic acid did not produce a leucocytosis in the rat, which synthesizes its own ascorbic acid, although it did produce a considerable eosinophilia if administered with adrenaline.

This work on ascorbic acid and infection is difficult to interpret. The bulk of the evidence shows that it plays some part in immunity phenomena and what is vaguely termed "resistance to infection."

Ascorbic Acid as a Detoxicating Agent. *Arsphenamines.* It was observed by Sulzberger and Oser [185] in 1935 that large doses of ascorbic acid reduced and inhibited the susceptibility of the skin of the guinea-pig to experimental sensitization with neoarsphenamine. This was confirmed by Cormia [186], who states that possibly sensitization does occur, but that ascorbic acid inhibits the cutaneous reactions. McDonald and Johnson [270] state that ascorbic acid has some protective action against arsphenamine reactions in guinea-pigs, but they could find no relation between the amount administered and the degree of protection. These results were questioned by Cohen [187], who in carefully controlled studies showed that there was no difference in sensitivity to neoarsphenamine between guinea-pigs on a diet deficient in ascorbic acid and those receiving an adequate supply. Chapman and Morrell [192] obtained results exactly opposed to those previously described; their guinea-pigs on a diet low in ascorbic acid were actually less sensitive to arsphenamine than those on a normal diet.

These conflicting results are undoubtedly due to differences of technique. Thus Martin and Thompson [940] state that ascorbic acid is most effective in protecting mice against the toxic effects of neoarsphenamine if it is injected two hours before the latter, whilst McChesney, Barlow and Klinck [941, 942] claim that the maximum protective effect is obtained if neoarsphenamine and ascorbic acid are injected in the same solution intravenously, and that if the ascorbic acid is given two hours before the neoarsphenamine its protective action is lost. They state that a dose of neoarsphenamine which represents LD_{50} kills only ten per cent. of the animals if this is injected with an equal weight of sodium ascorbate. The amount of ascorbic acid needed to exert a protective effect is between a quarter and an eighth of the weight of the arsphenamine or half a mole of ascorbic acid for a mole of neoarsphenamine. These investigators state that at a level of three moles of ascorbic acid per one mole of neoarsphenamine, the dose of the latter may be increased to 700 mg. per kilo with no greater toxicity than that produced in controls

receiving 400 mg. per kilo. If the ascorbic acid is injected simultaneously at another site the detoxifying action is somewhat decreased but not entirely eliminated. Friend and Ivy [133] found that ascorbic acid had a protective effect against the organic arsenical chlorarsen if ascorbic acid were given for nine days before and for three days after the arsenical.

Evidence for the increased tolerance of the human organism to the arsphenamines after ascorbic acid therapy is conflicting. Dainow [188] claims that the tolerance for toxic drugs, including the arsphenamines, depends upon the body reserves of ascorbic acid. He treats all cases of erythrodermia and toxic reactions due to parenteral arsenic therapy with oral or intravenous doses of 100 to 300 mg. of ascorbic acid daily, and he states that the period of treatment is considerably reduced. Intravenous or intramuscular doses of 300 mg. a day controlled many of the symptoms of neoarsphenamine intolerance in twenty-two cases quoted by Welker [250]. Large intravenous doses of ascorbic acid (500 mg. a day) followed by high maintenance doses of 100 to 200 mg. by mouth were given by Cormia [193] to patients who had previously suffered from neoarsphenamine dermatitis. He found that they were able to tolerate more of the same arsenical without further reactions, although he admits that his results were not so spectacular as those of Dainow. Actual cases of neoarsphenamine dermatitis cleared up without fourteen to eighteen days after giving 100 to 200 mg. of ascorbic acid by mouth.

Falconer, Epstein, and Mills [194] studied a group of seven patients in whom attacks of thrombopenic purpura repeatedly occurred after the administration of neoarsphenamine and bismarsen. At no time and in none of the patients was any appreciable modification of sensitivity to the drugs observed during or after the administration of 100 mg. doses of ascorbic acid.

The blood ascorbic acid of a number of patients showing signs of sensitivity to neoarsphenamine was examined by Friend and Marquis [195]. The values were from 0.13 to 0.35 mg.—well below normal levels. It was concluded that a low blood ascorbic acid was the result of a toxic reaction to the neoarsphenamine rather than a predisposing factor to such reactions. This view is open to criticism because pre-reaction blood values were not determined, and four out of twelve patients receiving arsphenamine without reactions had low ascorbic acid blood levels. Farmer and his colleagues [196] have also demonstrated that patients hypersensitive to neoarsphenamine show very low ascorbic acid blood levels. In patients showing severe symptoms of intolerance a fall in the blood ascorbic acid occurred in spite of the oral administration of the vitamin during treatment. It was frequently observed that a marked lowering of the blood level followed the administration of neoarsphenamine in patients showing no intolerance to the drug. Delp and Weber [820, 821] also state that patients showing sensitivity to arsphenamines have low ascorbic acid levels. By giving 300 mg. of this vitamin a day orally and 300 to 500 mg. intravenously every other day they claim that the sensitivity of such patients is diminished.

Bundesen [822] and his colleagues have carried out extensive observations on the detoxifying action of ascorbic acid in arsenic therapy. They have found that ascorbic acid prevents the *in vitro* oxidation of neoarsphenamine and mapharsen. A number of patients were patch tested with neoarsphenamine, and positive reactors retested with the drug to which ascorbic acid had been added. Not a trace of reaction was found in thirty-two out of thirty-eight who formerly reacted to neoarsphenamine alone. Further studies by Bundesen and his co-workers suggest that the majority of hypersensitive patients whose local cutaneous reaction to neoarsphenamine is fully prevented by ascorbic acid should be able to tolerate intravenous neoarsphenamine if the blood ascorbic acid is maintained at a sufficiently high level to inhibit the formation of toxic products of oxidation of the neoarsphenamine. This work has been confirmed and extended by Abt [215].

Beerman and his co-workers [197] claim that clinically the incidence of

reactions to antisymphilitic arsenicals is reduced fifty-eight per cent. if the substances are dissolved in a one per cent. solution of methyl glucamine ascorbate before administration. White [216] has been unable to confirm this in animal tests.

Lead and Gold. The plasma ascorbic acid of the rat is markedly decreased by the intraperitoneal administration of gold chloride [134]. Sande [198] observed that the ascorbic acid content of the organs of guinea-pigs that had received injections of gold salts was much lower than that of control animals. Three patients after treatment with gold developed symptoms of intolerance and a lowered capillary resistance; one patient also had signs of liver injury. After treatment with 100 to 200 mg. of ascorbic acid a day given intravenously the symptoms of intolerance rapidly disappeared. Dainow [188] describes the treatment of erythrodermia due to gold therapy with intravenous injections of 0.2 gram of ascorbic acid. Cohen and his collaborators [135], however, failed to observe any beneficial effect from giving 300 mg. of ascorbic acid to sixty-five patients receiving 118 courses of gold injections.

Studies on lead poisoning were made by Holmes and his co-workers [199], who observed four hundred men exposed to lead over a period of a year. Thirty-four had symptoms of lead poisoning. Half were treated with additional doses of 100 to 200 mg. of ascorbic acid a day for several weeks, and in most the vitamin was said to be more effective in removing the symptoms of lead poisoning (irritability, insomnia, skin pigmentation, nervousness) and restoring the blood picture than in the controls who received the usual therapy with calcium. Marchmont-Robinson [245], from a study of over three hundred lead workers, concluded that 50 mg. of ascorbic acid a day protected them against the effects of chronic lead absorption. Pillemer [201] in a well-controlled experiment with guinea-pigs found that in two series of forty-four guinea-pigs on a subclinical scurvy intake of ascorbic acid, the degree of lead poisoning that developed after a month's ingestion of lead carbonate was more severe in comparison with that in two groups of twenty-four animals saturated with the vitamin.

Dannenberg [200], Evans [943] and their co-workers were unable to confirm these observations. Evans and his colleagues studied a group of four hundred workers in a tetraethyl lead factory. The level of ascorbic acid nutrition was low. The administration of 100 mg. daily failed to have any effect on the lead concentration in the blood or on its elimination in the faeces and urine. No difference was noted in the physical condition, number and severity of complaints, erythrocyte count, number of stippled erythrocytes or haemoglobin percentage.

Frommel and Loutfi [1061] state that the electrocardiographic disturbances produced in guinea-pigs by the injection of bismuth sodium thiodiglycollate are diminished by the simultaneous injection of 0.1 gram ascorbic acid per kilo.

Sulphonamides and Other Drugs. Claims have been made that the toxic effects of certain drugs can be prevented or minimized by the administration of ascorbic acid. Thus Dainow [202] and Bickel [203] state that some of the toxic effects of the sulphonamides (particularly sulphapyridine) can be prevented, or at any rate relieved, by the simultaneous administration of ascorbic acid in daily doses of 0.5 gram given intravenously. According to Dainow the urinary excretion of ascorbic acid is considerably decreased in patients receiving sulphonamide, and in animals there is a fall in the ascorbic acid content of the brain, liver and testicles. He argues that the normal ascorbic acid reserves are mobilized to detoxicate the sulphonamides. Dunlop [204] has been unable to confirm Dainow's statement that ascorbic acid increases the tolerance of patients to sulphapyridine, and others have found no difference in the survival rate of rats and guinea-pigs treated with toxic doses of sulphanilamide alone and with sulphanilamide and ascorbic acid [825, 1058]. Pelner [1014] gave full doses of sulphadiazine to fifty

patients together with 100 mg. of ascorbic acid daily, and stated that none of them suffered from any reactions. As no controls were observed it cannot be said that ascorbic acid definitely exerted any protective action. Contrary to Dainow's observations, Holmes [932], Kinnunen [168], Ekman [163] and Longenecker [167] observed an increased urinary excretion of ascorbic acid in patients given sulphathiazole, and recommended supplements of 100 mg. daily to allow for this. According to Ekman increased excretion is due to increased synthesis of the vitamin in the tissues. He states that ascorbic acid is concerned with the oxidation and detoxification of cyclic compounds in the body.

It has also been stated that ascorbic acid diminishes the toxicity of such substances as anaesthetics [213], benzene [177, 205, 206], trichlorethylene, T.N.T. [823], barium [178] and barbiturates [949].

There is some doubt about the detoxifying effect of ascorbic acid on T.N.T.

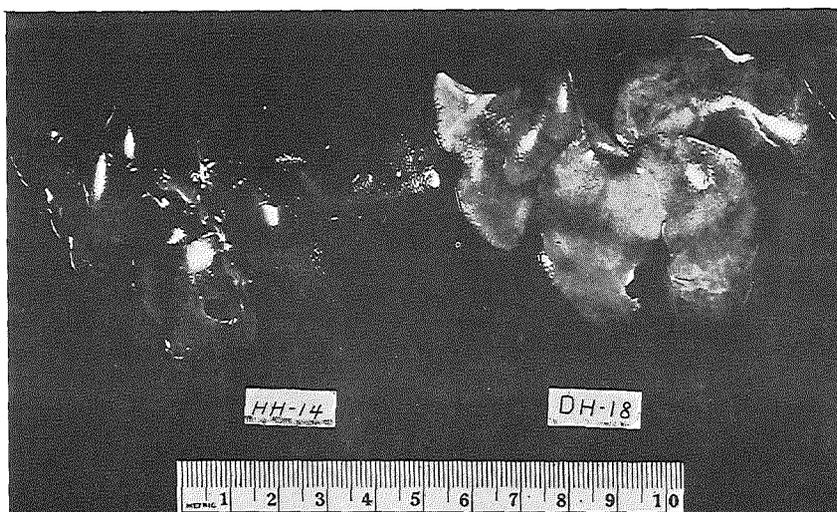


FIG. 148. The protective action of ascorbic acid against hepatotoxic agents. The illustration shows the livers of two guinea-pigs, both fed on a scorbutic diet for sixteen days before being given 25 mg. of hydrazine, a hepatic poison, for two days. Guinea-pig HH-14, whose liver is seen on the left, received daily injections of 30 mg. of ascorbic acid; guinea-pig DH-18, whose liver is shown on the right, did not. Macroscopically the liver of guinea-pig HH-14 was practically normal in appearance. The liver of guinea-pig DH-18, however, was pale, yellow, friable, and showed signs of fatty degeneration. It was sixty-eight per cent. heavier than the liver of guinea-pig HH-14.

Smith and his colleagues [950] were unable to observe any protective effect in cats, rats or guinea-pigs. In the United States a recommendation has been made that munition workers exposed to T.N.T. should receive at least 100 mg. of ascorbic acid daily.

Ascorbic acid appears to increase the rate of excretion of amphetamine (benzedrine) [252] and to increase the rate of metabolism.

Kimball [210] and Frankel [211] believe that the toxic effects of sodium diphenylhydantoin (soluble phenytoin), a drug used in the treatment of epilepsy, are more pronounced in patients deficient in ascorbic acid. According to Drake [599] in the experimental animal sodium diphenylhydantoin causes an increased excretion of ascorbic acid in the urine, and a lowering of the body reserves of the vitamin [599]. Gruhzit [212], however, found that the administration of this drug to animals had no effect whatsoever on the absorption and utilization of ascorbic acid, and others have reported that it has no effect on the ascorbic acid blood levels of patients [682]. An

PROTECTION OF ASCORBIC ACID AGAINST LIVER TOXINS

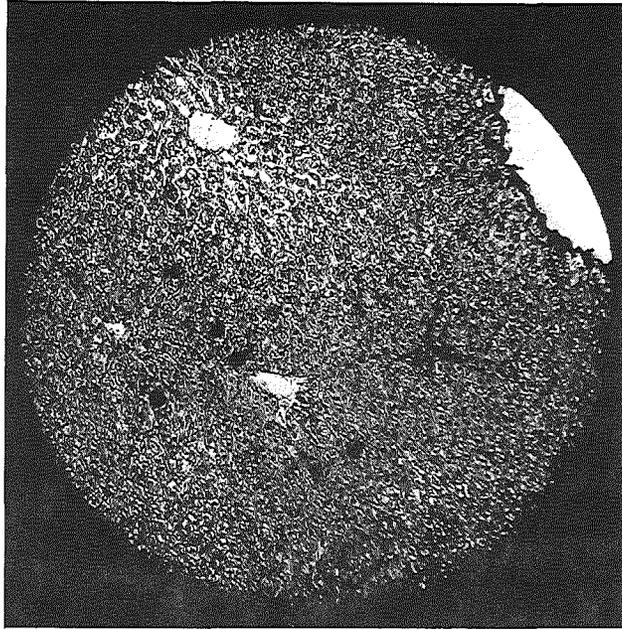


FIG. 149. The protective action of ascorbic acid against hepatotoxic agents. A section of the liver of guinea-pig HH-14 (see Fig. 148), given 30 mg. of ascorbic acid daily and then 25 mg. of hydrazine for two days. Hæmatoxylin and eosin stain ($\times 100$). The cellular appearance is almost normal. Under a magnification of $\times 400$ some hydropic changes in the cytoplasm are visible.

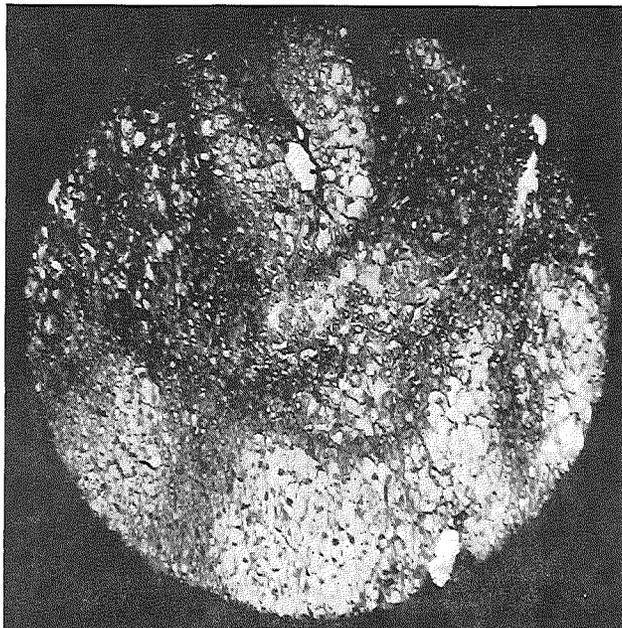
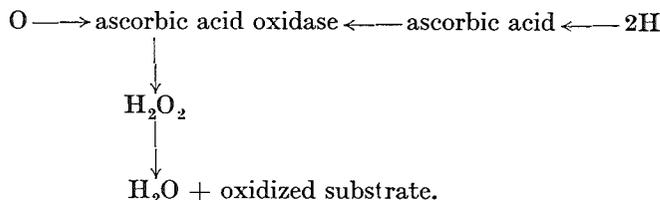


FIG. 150. The protective action of ascorbic acid against hepatotoxic agents. A section of the liver of scorbutic guinea-pig DH-18 (see Fig. 148) damaged by hydrazine. Hæmatoxylin and eosin stain ($\times 100$). Although the magnification is the same as in Fig. 149, the cells are increased in size and there is general loss of architecture and destruction of the cells and nuclei following fatty necrosis.



The cytochrome-indophenol oxidase system has been shown to act as a catalyst in the aerobic oxidation of ascorbic acid [219]. It is suggested that glutathione might reduce oxidized ascorbic acid [222]. Prunty and Vass [274] have shown that the concentration of glutathione in human red blood cells varies inversely with that of the plasma ascorbic acid.

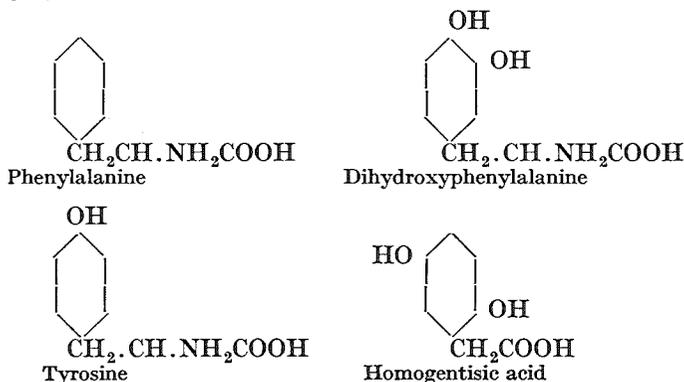
Harrison [254], von Euler and Klussman [255] state that slices of fresh tissues from scorbutic animals have a lower oxygen uptake than controls and that it is restored by adding ascorbic acid. This could not be confirmed by Stotz and his co-workers [220]. The oxidation of certain fatty acids in the liver proceeds at a higher rate in the presence of ascorbic acid [224].

Ascorbic Acid and Amino-Acid Metabolism

As far back as 1930 Szent-Györgyi [231] wrote: "Hexuronic acid (i.e. ascorbic acid) completely inhibits the formation of pigment in all systems in which a melanoid pigment is formed through the oxidation of a phenol." Abderhalden [232] showed that *in vitro* ascorbic acid inhibited pigment formation from adrenaline and from 3:4-dihydroxyphenylalanine ("dopa") both in the presence and absence of the enzyme tyrosinase. This has been confirmed by others. Many investigators have reported a decrease in the deposition of pigment in Addison's disease and a bleaching of existing pigmentation after administering large quantities of ascorbic acid (p. 430). This bleaching effect has been attributed to direct reduction of melanin by ascorbic acid [872].

These observations suggested ascorbic acid might play a rôle in amino-acid metabolism. Sealock [217] observed that the administration of tyrosine and dopa to guinea-pigs on a maintenance allowance of ascorbic acid produced signs of ascorbic acid deficiency. When albino animals, which are incapable of synthesizing melanin, were used no deficiency symptoms were produced. This suggests that ascorbic acid is used in the transformation of tyrosine and dopa into melanin.

Alkaptonuria (phenylketonuria, tyrosinosis) is normally encountered as an inborn error of metabolism. There is a defect in the enzyme systems involved in the oxidation of phenylalanine or tyrosine and their derivatives, such as homogentisic acid, which appears in the urine and causes it to darken considerably on standing. Alkaptonuria has been produced experimentally in the guinea-pig by



Sealock and his co-workers [217] by administering large amounts of phenylalanine, tyrosine or dopa in the absence of adequate amounts of ascorbic acid. It has also been produced in the white rat. Homogentisic acid is therefore a step in the normal pathway of phenylalanine and tyrosine metabolism. The amount present in the urine is inversely proportional to the ascorbic acid intake, and when this is adequate homogentisic acid disappears from the urine [225]. This occurs in man. Ascorbic acid is, however, ineffective in correcting the metabolic defect when given to subjects suffering from hereditary alkaptonuria [218].

Premature infants fed on cow's milk are unable to metabolize the tyrosine and phenylalanine in the milk proteins in the absence of ascorbic acid. They excrete *p*-hydroxy phenylpyruvic and *p*-hydroxyphenyl lactic acids [272].



Full term infants show this error of metabolism only if they are given additional tyrosine or phenylalanine. In both full term and premature infants it is corrected by ascorbic acid. Pteroylglutamic acid also prevents it [238]. An enzyme system in liver oxidizes tyrosine, but this is absent from the livers of scorbutic animals. The scorbutic liver, however, regains its power to oxidize tyrosine *in vitro* and *in vivo* after the addition of ascorbic acid [226]. The oxidation of "dopa" occurs chiefly in the kidney rather than the liver [221, 223]. According to Painter and Zilva [230] the normal metabolism of tyrosine is mediated by intestinal bacteria, the accumulation of hydroxyphenyl compounds being due to the absence of the modifying influence of ascorbic acid on the intestinal flora responsible for the breakdown of tyrosines. The oxidation of tyrosine to "dopa" *in vitro* by ascorbic acid and oxygen has been demonstrated [229].

Deranged tyrosine metabolism is also seen in human scurvy [238, 251]. Scorbutic infants and adults excrete in the urine *p*-hydroxyphenyl compounds (*p*-hydroxyphenyl lactic acid and *p*-hydroxyphenyl pyruvic acid) when given tyrosine; these disappear when ascorbic acid and pteroylglutamic acid are administered [236, 238]. Increased excretion of *p*-hydroxyphenyl compounds in scorbutic monkeys is diminished by ascorbic acid, but not by pteroylglutamic acid.

Benzoquinoneacetic acid has been identified by Fishberg [257] in the urine of subjects on diets deficient in ascorbic acid. It is an intermediate in the catabolism of tyrosine and phenylalanine.

If a high protein diet is fed to rats the ascorbic acid in the blood and in those tissues that metabolize large amounts of amino-acids (liver, kidney, muscle) is lower than when diets high in fat or carbohydrate are eaten [279]. Administration of ascorbic acid increases the concentration in the active organs but not in the blood. These observations suggest that tissues metabolizing large amounts of amino-acids have increased utilization of ascorbic acid.

Ascorbic Acid and Hormones. The Adrenals [1036]. The cytochemical work of Bourne [227], Gough [387], and Giroud and Leblond [228] has demonstrated that ascorbic acid is particularly abundant in the adrenal, the pituitary, corpus luteum [266] and glandular tissue (Figs. 151 to 157). No other organ in the body contains as much ascorbic acid as the adrenal (4.60 ± 0.34 mg. per gram of fresh tissue). Large quantities are present in the Golgi apparatus

Demonstration of Ascorbic Acid in Tissues by
Silver Staining Technique

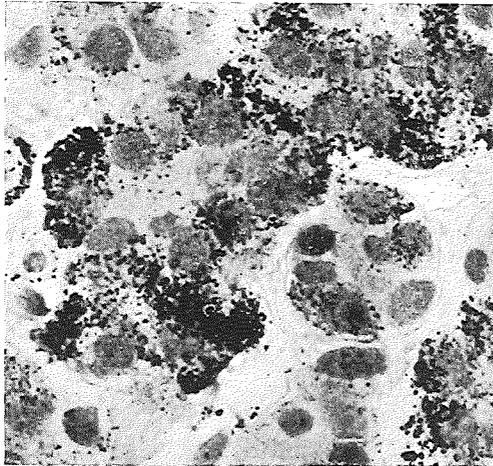


FIG. 151. Ascorbic Acid in the Pituitary Gland. Deposits of silver representing granules of ascorbic acid in chromophil cells.

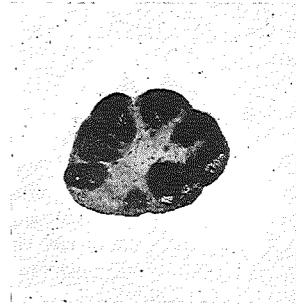


FIG. 152. Ascorbic Acid in Corpus Luteum of Dog. After staining for fifteen minutes with 0.4 per cent. silver nitrate solution.



FIG. 153. Ascorbic Acid in the Pituitary of a Dog. After staining for fifteen minutes with 0.4 per cent. silver nitrate solution.



FIG. 154. Ascorbic Acid in Interstitial Cells of Testis. After staining for fifteen minutes with 0.4 per cent. silver nitrate solution.

of the adrenal cortex [271]. The connection between ascorbic acid, adrenaline and pigment formation has already been mentioned (p. 429). The administration of ascorbic acid to guinea-pigs is said to increase the adrenaline content of the adrenals [244]. Vogt [282], however, has found no correlation between the amount of adrenaline secreted and the ascorbic acid content of the blood leaving the adrenal gland. Many workers claim that low blood and urinary ascorbic acid values occur in patients with Addison's disease [237, 239], and they have observed a striking diminution in the pigmentation of the skin that occurs in this disease after the administration of ascorbic acid (500 mg. daily) [235, 237, 239, 872]. The evidence on the storage and excretion of ascorbic acid in patients with Addison's disease is conflicting. Sendroy and Miller [240] state that the urinary excretion of ascorbic acid is low because of renal insufficiency. Jenovese and others [241] state that although the urinary excretion of ascorbic acid is low, blood plasma levels are within normal limits.

From cytochemical studies Deane and Morse [291] and Sayers [862, 863] have demonstrated a close connection between the adrenocorticotrophic hormone of the pituitary (A.C.T.H.) and ascorbic acid. A single dose of A.C.T.H. given to a rat or guinea-pig causes a fall in the cholesterol and ascorbic acid of the adrenals; it has been suggested that both of these are essential for the synthesis and release of adrenal cortical hormones [320]. It appears that there is an optimal ratio of adrenal cholesterol to adrenal ascorbic acid for normal steroid production in the adrenal cortex [1018]. The fall in the ascorbic acid content of the adrenals of the hypophysectomized rat has been used by Sayers [296] for the assay of A.C.T.H. activity. Various noxious stimuli, e.g. hæmorrhage, burns, cold, muscle trauma and nerve stimulation, and the administration of adrenaline [288] rapidly reduce the ascorbic acid in the adrenals [281]. The subjection of rats to heat stress causes a fall in the adrenal ascorbic acid [477]. This fall can be prevented by the previous administration of cortical hormone [283]. The administration of salicylates also causes a fall in the ascorbic acid of the adrenals [1021]; the effect is not specific as it is caused by other monohydroxybenzoic acids [1022]. Ascorbic acid deficiency in guinea-pigs causes a fall in the adrenal 11-corticosteroids [269] and adrenal cholesterol [275]. Reports on the effect of A.C.T.H. on the metabolism of ascorbic acid in man are contradictory, some observers reporting no significant changes in the urinary excretion and others an increased excretion.

Stefanini and Rosenthal [298] observed a fall in the plasma ascorbic acid and a low urinary excretion of the vitamin in two patients treated with A.C.T.H.; in both a hæmorrhagic diathesis occurred, suggestive of that seen in scurvy.

Hyman and his co-workers [299] studied the effect of A.C.T.H. and cortisone in guinea-pig scurvy. Although A.C.T.H. caused a fall in the adrenal ascorbic acid it prolonged life and delayed the onset of scorbutic symptoms. The work of Clayton and Prunty [329] suggests that there is an increase of adrenal cortical activity in scurvy as judged by adrenal hypertrophy and an increased excretion of cortical hormone from the increase of 17-ketosteroid excretion. Both of these changes are reversible by cortisone, although this has no effect on the disease. According to Clayton and Prunty, the 17-ketosteroid excretion is increased in the early stages of scurvy, and in the fully developed condition it is maximal, although this is denied by Banerjee and Deb [1025]. Adrenal cortical activity in scurvy is increased by A.C.T.H. even in the absence of ascorbic acid.

Schaffenburg, Masson and Corcoran [56] found that cortisone also inhibits many of the manifestations of scurvy, probably because of its action on mesenchymal tissues. Treager and his associates [303] administered A.C.T.H. to five patients with clinical scurvy. The blood eosinophil response (Thorn's test) was the same as in normal subjects, and did not change after adminis-

Demonstration of Ascorbic Acid in Tissues by
Silver Staining Technique

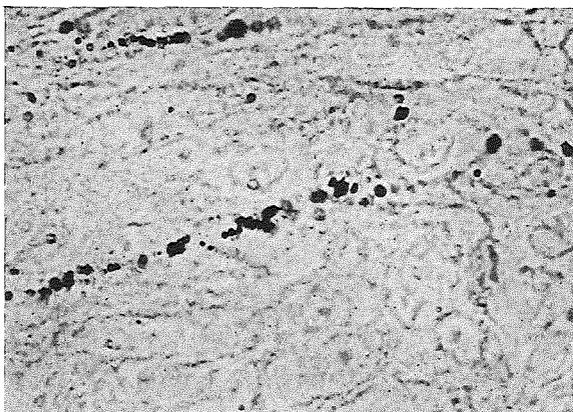


FIG. 155. Ascorbic acid in Embryonic Nerve Tissue. Ascorbic acid granules are seen scattered along a developing axone in a chicken embryo. Silver stain ($\times 800$).

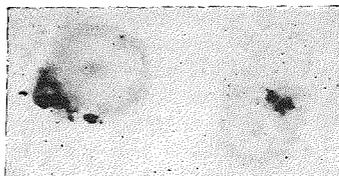


FIG. 156. Ascorbic acid in the Adrenal Cortex. Cells from the secretory zone of the adrenal cortex of the rat, showing ascorbic acid aggregated in the Golgi region of the cells. Silver stain ($\times 2,000$).

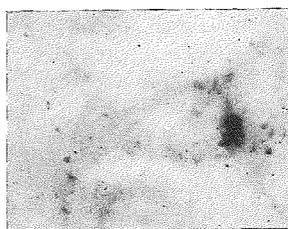


FIG. 157. Ascorbic acid in the Adrenal Medulla. Cell in the adrenal medulla of a cat, showing ascorbic acid aggregated in the nucleus near the Golgi region. Silver stain ($\times 1,200$).

tering ascorbic acid. Eisenstein and Shank [377], however, observed a fall in the circulating eosinophils in scorbutic animals after administering A.C.T.H. Other tests by Treager showed a normal adrenal cortical activity (normal serum sodium and potassium). These observations suggest that ascorbic acid in the adrenal is not essential for the formation of the corticosteroids, although theoretically the small amount of ascorbic acid still present might suffice. Patients dying of scurvy sometimes present a syndrome of hypotension and shock resembling an Addisonian crisis. Perhaps it is only terminally that the adrenal ascorbic acid becomes exhausted.

Ascorbic acid given to animals exposed to stress exerts certain protective actions which are related to adrenal cortical function. Thus the exposure of rats to cold normally causes an increase in weight of the adrenals, which is prevented by giving ascorbic acid [304]; the previous administration of ascorbic acid prevents signs of the "alarm" reaction in animals under the stress of adrenaline [107]; the injection of ascorbic acid alters the eosinopenia of stress in intact rats and delays the eosinophilia characteristic of stress in adrenalectomized rats [308]; under stress the excretion of ascorbic acid is greater than the intake, but if A.C.T.H. is injected the urinary excretion of ascorbic acid falls and the plasma and cell levels rise [310]. In normal dogs and rats the administration of A.C.T.H., cortisone or D.O.C.A. decreases the urinary excretion of ascorbic acid [316].

In the "alarm" reaction of the general Adaptation Syndrome (Selye) the ascorbic acid is aggregated peripherally in the cells of the adrenal cortex. The "resistance" phase is characterized by two types of cell in the cortex, one with the ascorbic acid arranged peripherally in the cell, the other with the ascorbic acid diffusely distributed in the cytoplasm. In the "exhaustion" phase the cells of the cortex are enlarged but their ascorbic acid content is diminished [311].

When ascorbic acid is given to normal subjects in doses of 100 to 300 mg. intravenously there is a fall in blood sodium and cholesterol and a rise in blood cholesterol and potassium (cf. electrolytes in Addison's disease); if A.C.T.H. is administered at the same time it can prevent this disturbance in the blood electrolytes and facilitates storage of ascorbic acid in the adrenal gland [316]. Sayers [320] has expressed the view that ascorbic acid and cholesterol take part in the formation of A.C.T.H., although this is difficult to understand as the administration of A.C.T.H. to the rat or guinea-pig causes a fall in the adrenal cholesterol.

The slow increase in the cell ascorbic acid compared with the plasma ascorbic acid suggests that something of a barrier exists between the cell and the plasma to the entry of ascorbic acid. In the presence of A.C.T.H. more ascorbic acid passes into the cell and at a faster rate [323]. It is of interest that ascorbic acid is conserved in the blood cell and not in the plasma in ascorbic acid deficiency (cf. Crandon, p. 454). The plasma level can be lowered to zero, but the cells still hold appreciable amounts. The reduction of cell ascorbic acid in ascorbic acid deficient animals becomes even more difficult when A.C.T.H. is administered [316].

Pituitary. A progressive fall in the plasma ascorbic acid, similar to that seen after adrenalectomy occurs in the hypophysectomized rat [293]. Stress reproduces the same effect. After the administration of adrenal cortex extract the level reverts to normal. According to Skelton and Fortier [293] the pituitary-adrenal system plays a rôle in the increased synthesis of ascorbic acid facilitated by stress.

Thyroid. Most workers agree that the administration of thyroid, thyroxine or the thyrotropic hormone results in a reduction in the ascorbic acid content of the liver, adrenal, thymus and kidney [258, 261, 265]. Paal and Brecht [262], however, observed a rise in the adrenal ascorbic acid. A fall in tissue and plasma ascorbic acid after administering thyroid has also been reported [244], and what is surprising, anti-thyroid drugs such as

thiourea and thiouracil have the same effect [244]. That ascorbic acid promotes the storage of glycogen is suggested by the work of Morelli and d'Ambrosio [289] and of Fischbach and Terbrüggen [295]. These workers could not observe any effect on the part of ascorbic acid on the glycogen of the livers of animals treated with thyroxine. According to Hirsch [326] and Steffen and Zais [331] large doses of ascorbic acid partially prevent the fall in liver glycogen produced in guinea-pigs or rats by thyroxine. This conflicting evidence has since been re-examined by Crabtree and Trikojus [333], who state that the effect of the administration of thyrotropic hormone or of thyroxine is uninfluenced by large doses of ascorbic acid, as judged by liver glycogen values, thyroid, adrenal and body weight and by histological examination of the thyroid. They found that the liver can concentrate ascorbic acid when large doses of this are given at the same time as thyroid or thyrotropic hormone.

Demole and Ippen [258] and Belasco and Murlin [243] stated that in the guinea-pig the loss in weight caused by thyroxine can be checked by ascorbic acid, provided enough is given. In thyrotoxic patients the ascorbic acid excretion is said to be low [264]; the administration of large doses of ascorbic acid is without effect on the basal metabolic rate [259].

The effect of thyroxine on the ascorbic acid level in the adrenal is variable [260]. First the level falls and then it rises. The administration of anti-thyroid drugs such as thiouracil results in an initial increase in the ascorbic acid of the adrenals, which atrophy [334]. Thyroidectomy on the other hand produces a final decrease in the ascorbic acid concentration in the gland.

Ascorbic Acid and Carbohydrate Metabolism. The metabolism of carbohydrate is disturbed in scorbutic guinea-pigs, as shown by diminished glucose tolerance, low liver glycogen, high blood sugar and a low insulin content of the pancreas [275, 335]. Although the adrenaline content of the adrenals is increased it has been shown by removal of the adrenal medulla that adrenaline is not responsible for the lowered glucose tolerance [242]. Banerjee and Deb [275] have suggested that the altered carbohydrate metabolism in scorbutic guinea-pigs may be due to the combined deficiency of adrenal cortical hormone and insulin. A diabetic type of glucose tolerance curve has been described in human subjects on low ascorbic acid intakes; this curve is said to return to normal on giving adequate ascorbic acid [665, 668].

Conflicting results have been reported on the effects of injecting ascorbic acid into normal human subjects. A fall in blood sugar has been described after the intravenous injection of the vitamin [248, 669], although this has been denied by others [670]. There is no evidence that the administration of ascorbic acid has any effect on the glucose tolerance of diabetics, even when given in large doses [257]. The state of ascorbic acid nutrition in diabetics is no worse than that of normal controls [256]. The injection of insulin into normal or diabetic subjects produces a fall in the plasma ascorbic acid and in the urinary excretion [249, 253]; this effect wears off and is followed by a rise in plasma concentration and increased excretion of ascorbic acid, and finally by a return to normal levels [247]. There is no loss of ascorbic acid, but a redistribution in the body. There is a transfer of ascorbic acid from the plasma to the white cell-platelet layer of the blood.

Dehydroascorbic acid is said to be diabetogenic [338, 826]. Diabetes in rats produced by dehydroascorbic acid is reversed by bilateral adrenalectomy [1029]. Alloxan is diabetogenic and when injected into rabbits causes a fall in the plasma ascorbic acid [339].

Lactic acid is stated to accumulate in the muscles of animals deficient in ascorbic acid [254]. Crandon [68], who induced experimental scurvy in himself, noted impairment in ability to perform aerobic work, e.g. running, jumping, but anaerobic work e.g. working an ergograph with the fingers or forearm, was unaffected.

Much of the literature on ascorbic acid and carbohydrate metabolism is

confusing and needs careful interpretation. Experimental work has sometimes been done on animals synthesizing ascorbic acid, e.g. the rat, sometimes on animals needing an exogenous supply, e.g. the guinea-pig. Observations on humans have been made on subjects whose state of ascorbic acid nutrition was unknown or on diabetics who recently came under control. Tolerance of diabetics often improves with hospitalization. Changes in blood sugar following the administration of ascorbic acid do not necessarily show that the latter affects carbohydrate metabolism. Any changes may be due to alteration of renal threshold or to a temporary effect on storage mechanism.

Interrelationship with Other Vitamins. *Vitamin A.* Sure and his co-workers [273] showed that depletion of vitamin A in the rat is followed by a fall in the concentration of ascorbic acid in the tissues. Pirie and Wood [341] also demonstrated a fall in the ascorbic acid content of the aqueous humour, and Mayer and Krehl [345] state that one of the first symptoms of vitamin A deficiency is depletion of the animals' ascorbic acid reserves as evidenced by scorbutic symptoms curable by ascorbic acid. Mapson and Walker [349], while they agree that scorbutic symptoms appear in animals on diets deficient in vitamin A, attribute them not to ascorbic acid deficiency, but to restriction in total food intake. It is also stated that ascorbic acid prevents the appearance of symptoms of vitamin A deficiency [352].

Aneurine. A diet low in aneurine is stated to cause a temporary lowering of plasma ascorbic acid [351]. Large doses of aneurine cause toxic effects in scorbutic guinea-pigs [353]. If rats are made deficient in aneurine, ascorbic acid synthesis almost ceases [361].

Riboflavine. Riboflavine increases the survival time of scorbutic guinea-pigs [354].

Folic Acid and Vitamin B₁₂. See p. 161.

Vitamin P. Rutin, a compound with a vitamin P like action (p. 732), increases the apparent biological action of ascorbic acid when this is supplied in sub-maximal amounts [355]. The mechanism of this action is uncertain, but it could be explained by the protective effect of rutin preventing the oxidation of ascorbic acid. Papageorge and Mitchell [358] state that rutin has a "sparing effect" on adrenal ascorbic acid. They attribute this to the antioxidant action of rutin on ascorbic acid and adrenaline, the latter when oxidized accelerating the oxidation of ascorbic acid.

Absorption of Ascorbic Acid. Ascorbic acid is absorbed from the small intestine by a simple diffusion mechanism, the rate of absorption varying directly with the concentration of the ascorbic acid ingested [284]. In the rat about sixty per cent. of the ingested ascorbic acid is absorbed. If large portions of this organ are resected absorption of the vitamin is inadequate [332]. The degree of absorption depends on the concentration in the intestinal contents, rather than on the concentration in the blood tissues [957].

Certain strains of bacteria, including *B. coli*, can decompose ascorbic acid *in vitro* [278], although whether they exert any destructive action in the gut is open to question. Glucose has a protective action and may inhibit their action *in vitro* [280]. A number of pathogenic enteric organisms, e.g. *Salmonella*, *E. typhosus*, *Proteus morgagni*, can decompose ascorbic acid, and it is conceivable that in pathological gastro-intestinal conditions they may prevent adequate absorption [959]. Gould and Shwachman [960] conclude that about twenty-seven per cent. of ingested ascorbic acid is destroyed somewhere in the gastro-intestinal tract. Melnick, Hochberg and Oser [363], however, state that nearly ninety-nine per cent. of an oral dose of 200 mg. of ascorbic acid is absorbed. Ascorbic acid oxidase in foods is probably rapidly destroyed in the gastro-intestinal tract.

Farmer and his co-workers [284, 285] believe that in man the absorption of ascorbic acid from the intestine is almost complete in health, because on normal intakes the faecal excretion is negligible, less than 5 mg. daily, and even on large doses of 1,000 mg. daily does not exceed 15 mg.

Under abnormal conditions intestinal absorption may be incomplete owing to the operation of certain factors interfering with it. Abnormal bowel motility, e.g. in diarrhoea and catharsis, may be sufficiently severe to interfere with absorption, even if large doses are given. Abt and his co-workers [287, 831] have shown that there is tenfold increase in the faecal excretion of ascorbic acid in infants receiving sufficient magnesium sulphate to produce semi-liquid stools. Up to a quarter of the intake of ascorbic acid was found in the faeces.

The absorption of ascorbic acid appears to be impaired in patients with achlorhydria [473]. At any rate patients with this condition have abnormally low plasma ascorbic acid levels. The administration of alkaline powders and alumina gel (aluminium hydroxide gel) does not interfere with the absorption of ascorbic acid [277, 332].

Utilization of Synthetic Ascorbic Acid. With the availability of vitamins in chemically pure form the question arose whether the synthetic form was utilized as well as the natural. It has long been postulated that fruit and vegetables contain an unknown factor required for the utilization of ascorbic acid [367, 881]. In a recent investigation by Crampton and Burton [370] the authors reported that orange, apple and tomato juice show a thirty-five to forty-five per cent. higher ascorbic acid potency than is indicated by chemical assay. The matter has been investigated by a number of workers [371, 372, 373, 379], who have all shown that synthetic ascorbic acid is utilized just as well as the natural vitamin in vegetables and fruit. According to Hollinger [380] some individuals utilize synthetic ascorbic acid better than ascorbic acid in green vegetables. The availability of ascorbic acid in the latter is about eighty-five per cent. [383].

Storage and Distribution in the Body. Ascorbic acid is found in all the tissues and fluids of the body. About four-fifths of the ascorbic acid intake is destroyed. This occurs in the caecum according to Reid [425]. Muscle contains about 2 mg. per 100 grams. Glandular tissue, particularly the adrenals, is rich in the vitamin. The adrenals contain more than any other organ or tissue (p. 428), and in response to various factors the ascorbic acid level varies (p. 429). There is also a high concentration in the intra-ocular fluid, ciliary body, iris and lens [961, 962]. In the adult animal ascorbic acid probably enters the aqueous humour by a process of secretion; the level is much higher (e.g. 50 mg. per 100 ml.) than in blood [364]. Ascorbic acid is stored in those organs and tissues with a high metabolic activity. Tumour tissue has a high ascorbic acid content [290]. On exposure to cold there is a considerable increase in the ascorbic acid stored in the liver and kidneys [405].

Ascorbic acid is present in the blood, C.S.F [297], saliva, gastric juice [292], and milk. The amount in C.S.F. varies from 0.7 to 2.1 mg. per 100 ml.; Strait and his co-workers [307] obtained values twice this. It may be increased from 1.8 to 4.1 mg. after a 1 gram dose of ascorbic acid. The amount in saliva is 0.07 to 0.25 mg. per 100 ml., and in gastric juice 1.05 mg. per 100 ml., when fasting and 0.9 mg. after stimulation [292]. There is no correlation between the amount in the saliva and gastric juice and in the blood and urine [292]. This suggests that the secretion of ascorbic acid by the gastric mucosa and salivary glands is a physiological property of their cells.

The blood ascorbic acid is subject to considerable variation, particularly in the plasma. The most recent figure for plasma ascorbic acid based on the examination of 110 normal subjects is 0.3 to 2.1 mg. per 100 ml. with an average of 0.98 mg. [292]. Other workers give a range of 0.6 to 2.5 mg. per 100 ml. [302]. In some working class families the lower level might fall as low as 0.1 mg. [300]. As the plasma level varies with the intake a wide range of values has been reported from zero to 20 mg., the latter being reached after the administration of large doses of the vitamin. However, the correlation is not absolute. Thus Putnam and his co-workers [294] noted that a daily intake of 32 mg. among Mexican Indians maintained a plasma

level of 1.12 mg. of ascorbic acid per 100 ml., while inhabitants of North Carolina had plasma levels below this on an intake of nearly 150 mg. daily.

The major part of the ascorbic acid is in the cells, not the plasma. Sargent [385] has determined the partition of the blood ascorbic acid between whole blood, plasma and the red blood cells. The ratio of ascorbic acid in the plasma and cells is given by the equation $A_p = A_c - 0.45$, A_p and A_c being the concentration of ascorbic acid in plasma and cells respectively expressed in mg. per 100 ml. According to Roe, Kuether and Zimler [388] and Daubenerkl [397] the distribution of ascorbic acid between the plasma and the whole blood is related to the level of ascorbic acid in the blood. They state that at whole blood levels of ascorbic acid below 0.6 mg. per 100 ml. the plasma content is lower than the whole blood content; at whole blood levels of 0.6 to 0.9 mg. the concentration in plasma is approximately the same as in whole blood; and at whole blood levels above 0.9 mg. the plasma content is higher than the whole blood content. Heinemann [301] states that the concentration of ascorbic acid in the cells is consistently greater than that in the plasma, but he did not consider the wide range of blood values of Roe and his co-workers. According to Butler and Cushman [590] the average amount of ascorbic acid in the platelets and white cells is 34 mg. per 100 ml., with a range of 29 to 43 mg. An M.R.C. report [407] gives the figure 16.6 mg. per 100 ml. on a daily intake of 20 mg. of ascorbic acid. The ascorbic acid is combined with the serum albumin in plasma [385, 396]. In patients suffering from serious illnesses the ascorbic acid in the plasma is lower than that in the whole blood [388]. Thus both dietary intake and underlying pathological processes operate to produce the resulting pattern of distribution of ascorbic acid in the blood.

Wilson and Lubschez [408] correlated the intake of ascorbic acid with the plasma and white cell layers. They give the following figures :

Daily Intake	Plasma Ascorbic Acid mg. per 100 ml.	Ascorbic Acid in White Cell Layer mg. per 100 ml.
0.5-1.9 mg. per kilo . . .	0.4 mg.	20 mg.
1.5-2.9 mg. per kilo . . .	0.7 mg.	25 mg.
9 mg. per kilo	1.4 mg.	25 mg.

Thus, beyond a certain intake there is no increase in the ascorbic acid of the white cells. It would appear that intakes of much more than 2 mg. per kilo daily are normally quite unnecessary. Wilson and Lubschez found that during convalescence from intercurrent febrile illnesses intakes of 3 to 9 mg. per kilo were necessary to maintain white cell levels of 25 mg. per 100 ml. or more.

Dodds and Macleod [312] correlated the plasma ascorbic acid with the daily intake in forty-one adults of mean weight 57 kilo. The wide range of values indicates that any estimation of the ascorbic acid intake based on plasma content would be quite fallacious.

Daily Intake	Plasma Ascorbic Acid mg. per 100 ml.	Range mg. per 100 ml.
32 mg.	0.48 ± 1.37	0.34-0.62
57 mg.	0.72 ± 0.21	0.51-0.93
82 mg.	0.93 ± 0.196	0.74-1.13
107 mg.	1.05 ± 0.170	0.88-1.22

There is a seasonal variation in the blood ascorbic acid, the highest levels coinciding with the highest intake, i.e. in August [113]. It is lowest in April.

It has been stated that in pregnancy and lactation the blood ascorbic acid is lower than normal [305, 402], but Fredrikson [276] found no evidence for this, or for the statement that the blood ascorbic acid varies according to the stage of pregnancy, independent of seasonal variations. The ascorbic acid content of foetal blood is considerably higher than that of the maternal blood [276, 406]. Hamil and his co-workers [406] found that the umbilical cord blood ascorbic acid ranged from 0.66 to 2.18 mg. per 100 ml.; the corresponding figures for the blood ascorbic acid of the mothers was 0.04 to 1.19 mg. There is a decline in the blood ascorbic acid in the first week post-partum. The whole blood ascorbic acid is higher in males than females [411].

Ascorbic acid is secreted in the milk, the content of which depends on the mother's intake. Surveys in Britain and Australia show that the average ascorbic acid content of fresh mother's milk is about 3.5 mg. per 100 ml. [414, 416], with a range of 3.2 to 3.8 mg. [415]. A survey made in Chicago gave the figure 5 mg. per 100 ml. [419]. Colostrum contains the most ascorbic acid (7.2 mg. per 100 ml.) and there is a progressive fall as lactation proceeds. The amount of ascorbic acid in milk varies according to the time of the year; in English mother's milk it varies from 2.5 mg. per 100 ml. in March to 5.1 mg. in September-October. Kon and Mawson [415] found a mean daily intake of 5.9 mg. in a group of women they studied in 1944. The output in the milk was 3.2 to 3.8 mg. per 100 ml.

Excretion of Ascorbic Acid. Ascorbic acid is excreted in the faeces, urine and sweat. The faecal excretion is normally of the order of 5 mg. daily but may be increased in diarrhoea and gastro-intestinal disorders (p. 223). The loss in the sweat is negligible, even under conditions of profuse sweating, when it varies from 0.8 to 2.7 mg. daily [964-966]. Much of this is in the form of dehydroascorbic acid.

The urinary excretion of ascorbic acid depends on the intake [313]. If large supplies are administered to persons whose stores are low the urinary excretion does not rise to normal levels at once. The excretion remains low until the blood and tissues have taken up much of the ascorbic acid. When this has occurred the blood and tissues are "saturated." * On an approximately constant water intake there is no correlation between the volume of urine and the excretion in the urine; there is only correlation between urinary volume and urinary excretion when the fluid intake alternates from day to day between widely different levels [964]. Excretion is not affected by diuresis [550]. Ascorbic acid like glucose is considered to be a "threshold" substance. The renal threshold, that is the level at which ascorbic acid leaves the blood and passes through the kidney into the urine, has been calculated by giving supplements of ascorbic acid and finding the blood level at which there is a sudden sharp rise in excretion. The figure given varies from 1.0 to 1.4 mg. per 100 ml. of plasma [314, 430, 967], although Crandon [68] states that it may be much lower, e.g. 0.85 mg. per 100 ml., in a state of severe ascorbic acid deficiency, and Friedman and his co-workers [319] consider that ascorbic acid is excreted in the urine at all plasma levels. The renal threshold is a relatively stable figure for any one individual and varies little [430].

Excretion does not follow degree and rate of absorption but depends on the rate of absorption by the tissues. The excretion never equals the amount ingested, even when the body is saturated, as some is destroyed in the tissues. Half to two-thirds of the ingested ascorbic acid is metabolized to dehydroascorbic acid, some of which undergoes irreversible destruction to diketogulonic acid and oxalic acid [865]. Dehydroascorbic acid and diketogulonic acid are found in all tissues; the ratio of the former to ascorbic acid is increased in scurvy [1032]. In man dehydroascorbic acid and di-

* This is a misleading and meaningless word, but it is used in the literature to denote a state of ascorbic acid storage in the body. The body is said to be "saturated" when the administration of a large dose of ascorbic acid results in a rapid increase in the excretion. By the same token a diabetic would be "saturated" with sugar.

ketogulonic acid are excreted in approximately equal amounts, and together form about twenty per cent. of the "total ascorbic acid" excreted [1031]. Using ascorbic acid containing radio-active C^{14} , it has been shown that in guinea-pigs sixty-six per cent. of an ingested dose is broken down and expired as carbon dioxide and twenty-two per cent. is eliminated in the urine [798]. According to Basu and Ray [322] there is no proportional relationship between average excretion and the state of "saturation" unless the excretion is about 30 to 40 mg. daily. The excretion varies enormously in health and disease and, unless exceptionally low, is of little significance (see p. 441). The daily excretion may be less than 5 mg. in infants, and in the adult may vary from almost zero to 50 mg. daily. The common range of excretion is between 10 and 50 mg. daily [240, 314, 375]. Small amounts of dehydro-ascorbic acid are excreted in the urine [905], the ratio of this to ascorbic acid varying from 0.05 to 0.4. The urinary excretion of ascorbic acid is lower in the winter months owing to the lower intake during this season. Excretion is not influenced by menstruation [438].

If large doses of ascorbic acid are administered, e.g. 10 mg. per kilo or 600 mg., after a response has occurred, then ninety-four to ninety-eight per cent. of the daily excretion is excreted in the first thirteen hours, and seventy-eight to eighty-eight per cent. in the first seven hours [800]. Not more than fifty to sixty per cent. of the ascorbic acid administered can be recovered from the urine when full saturation is reached on this dose [800].

The mechanism of the excretion of ascorbic acid by the kidney has been studied by Ralli [317] and her colleagues. Studies on the simultaneous ascorbic acid and creatinine urinary clearance in the dog, and ascorbic acid and inulin clearance in man showed that ascorbic acid is excreted in the urine by a process of filtration and active tubular reabsorption. This view is supported by the observation of Leblond [318] that the concentration of ascorbic acid in the capsular fluid of the frog is the same as that of the plasma. The reabsorptive mechanism for ascorbic acid appears to be limited to a maximal rate, so that when the vitamin is presented to the tubules by the glomerular filtrates at a rate exceeding this maximum, the excess is excreted in the urine. The excretion of ascorbic acid in a given individual, therefore, depends on (a) the blood plasma level, (b) the rate of glomerular filtration, and (c) the maximum rate of tubular reabsorption. In a later paper Ralli [319] has shown that the ascorbic acid excretion in the urine does not become zero, even with very low concentrations of ascorbic acid in the plasma, but ultimately falls to a minimum and constant value and is then independent of the plasma concentration. At lower excretion levels the excretion of ascorbic acid does not depend on the concentration in the blood nor on the filtration rate through the kidneys, but on the rate of tubular reabsorption, which is extremely variable [317, 319].

Any condition which reduces renal function impairs the excretion of ascorbic acid and may, therefore, falsify any conclusion regarding the level of ascorbic acid nutrition when this is based on excretion tests. Sendroy and Miller [240] compared the urinary excretion of patients suffering from renal diseases with that of normal individuals. They found that an abnormally slow excretion of a test dose of ascorbic acid does not necessarily indicate a low level of the vitamin in the body, because renal damage retards excretion even when the intake of ascorbic acid is adequate. The effect of decreased kidney function on the ascorbic acid clearance was found to run parallel to the effect on the urea clearance. Similar findings are reported by Wright and his co-workers [550, 576]. No appreciable destruction of ascorbic acid occurs in the urine stored in the bladder [321].

Many foods, drugs and external conditions influence the excretion of ascorbic acid. Diets high in protein, cysteine and methionine accelerate excretion in the rat [439, 504, 980]; glucose decreases tubular reabsorption in the dog and leads to an increased excretion [441]. Body rest and posture

have no effect. Conditions of stress, such as exposure to cold [405], burning, hæmorrhage, muscle trauma and severe exercise [443], lead to increased excretion (p. 448). Under stress the excretion may be greater than the intake [310], due to mobilization of ascorbic acid from the adrenals, which become depleted. There is a decreased excretion during the healing of wounds [923] and in patients recovering from severe injury, hæmorrhage and infection [446]. The excretion of ascorbic acid is markedly decreased by exposure of human subjects to the anoxic conditions of an altitude of 18,000 feet [974]; at an altitude of 35,000 feet and breathing one hundred per cent. oxygen there is increased excretion [799]. The observation that ascorbic acid increases the altitude tolerance of mice is of interest in this connection [975]. Pyrexia *per se* probably has no effect on ascorbic acid excretion (p. 448).

Various drugs affect the excretion of ascorbic acid. Insulin [247], ammonium chloride [324, 449], salicylates [450] and anti-histamine drugs [330] increase the urinary excretion of ascorbic acid. So do thiouracil [168], thyroid, 2 : 4-dinitrophenol [994], local anæsthetics [915], atropine, aspirin, cincophen, barbiturates, amidopyrine, adrenaline, chloroform, chloretone, paraldehyde, stilbœstrol, œstradiol and sulphonamides [168, 324, 325, 327, 328]. Sodium bicarbonate causes a lowered urinary excretion of ascorbic acid [449]. The urinary excretion of ascorbic acid is markedly increased under hot and moist environmental conditions, compared with normal or hot and dry conditions [964].

Increased and decreased absorption of ascorbic acid due to drugs or other factors is not necessarily due to changes in the utilization of the vitamin, nor does it indicate that requirements are increased. Drugs may change renal tubular absorption, e.g. the œstrogens [327], or they may alter the permeability of the kidney, or ascorbic acid might be mobilized from the adrenals or white blood cells. The salicylates increase the excretion of ascorbic acid by depleting the ascorbic acid in the adrenals [502].

HUMAN REQUIREMENTS OF ASCORBIC ACID

Requirements based on Dietary Studies. The human requirements of ascorbic acid have been determined by observing the minimum intake needed to prevent the appearance of scorbutic or deficiency symptoms and from dietary surveys. The minimum amount that prevents scurvy may not be sufficient for the maintenance of good health. Thus Stepp and Schröder [346] found that the dose of ascorbic acid preventing scurvy in guinea-pigs does not prevent dental lesions. Perhaps some idea of the ascorbic acid requirements can be obtained from a study of the infant, which receives some 40 to 50 mg. daily in its milk provided its mother is healthy and well nourished. On the other hand Nansen, the explorer, lived on a meat diet for nearly a year, and this could not have supplied much more than 10 mg. daily, yet he kept in good health. The synopsis overleaf gives some of the methods used for assessing human ascorbic acid requirements based on dietary studies and the daily intake suggested.

Certainly a very low intake of ascorbic acid appears to be compatible with good health. Meat- and fish-eating races such as the Eskimos probably consume no more than 20 mg. daily. Fox [347] kept a prisoner for ten months on a diet of cooked meat, which could not have supplied much more than 10 mg. daily. Although he had manual work to do, he showed no deficiency symptoms; the urinary excretion was about 1 mg. daily.

A very thorough investigation was carried out by the Accessory Food Factors Committee of the Medical Research Council between 1944-46 to determine the ascorbic acid requirements of the human adult [407]. Twenty subjects aged twenty-one to thirty-four were given a diet adequate in all respects except that it supplied only 1 mg. ascorbic acid daily. One group

Author	Method	Suggested Daily Intake of Ascorbic Acid
Rietschel and Mensching [342]	Smallest intake compatible with health and absence of scurvy.	10-15 mg.
Medical Research Council [407]	Prevention and cure of scurvy.	10 mg.
Kalk and Brühl [384]	Daily dose required to cure scurvy.	10 mg.
Pi Joan and Lozner [873]	Minimum daily intake compatible with good health.	15 mg.
Leeson, H. J., <i>et al.</i>	Minimum daily intake compatible with good health.	25 mg.
Fox, <i>et al.</i> [344]	Minimum daily intake compatible with good health.	15 mg.
Fox, <i>et al.</i> [347]	Diet of cooked meat.	? Probably 10 mg.
Crandon, <i>et al.</i> [68]	Daily consumption necessary for re-excretion of ascorbic acid in urine after treatment of scurvy.	30-45 mg.
Johnson, <i>et al.</i> [835]	"Saturation" after two months deprivation of ascorbic acid.	75 mg.
Najjar, <i>et al.</i> [343]	Daily intake that did not produce deficiency symptoms over period of eighteen months.	25 mg.
Kyhos, <i>et al.</i> [386]	Cure of gum lesions in prisoners with zero ascorbic acid in plasma.	50-75 mg.

received no supplementary ascorbic acid, another received 10 mg. daily, and another 70 mg. daily. The group receiving no ascorbic acid developed scorbutic symptoms after seventeen to twenty-one weeks, characterized by hyperkeratosis of hair follicles, perifollicular hæmorrhages, hæmorrhages of the gums, delayed wound healing and ecchymoses of the limbs. These symptoms were cured by 10 mg. of ascorbic acid daily; the results were no better with 20 mg. daily. No scorbutic or any other symptoms appeared in a group of volunteers receiving 10 mg. of ascorbic acid daily for four hundred and twenty-four days, and they remained in good health. Their plasma ascorbic acid was less than 0.05 mg. per 100 ml., and white cell ascorbic acid content was between 1.5 and 3 mg. per 100 ml. The scorbutic group had a plasma ascorbic acid of zero to 0.03 mg. per 100 ml. and a white cell level of 1 mg. or less per 100 ml. About one hundred days elapsed between the disappearance of ascorbic acid from the plasma and the first clinical signs of scurvy.

Although a daily intake of 10 mg. protects against scurvy, this is a marginal figure, and to provide a safety margin it is probably wise to double or even treble this figure. Tests on physical fatigue leave some doubt whether 10 mg. daily is optimal [407]. Thus Hagtvet [409] noted scorbutic symptoms in polar explorers doing heavy work on ascorbic acid intakes of 5 to 15 mg. daily. Fox [347], however, observed no deficiency symptoms in a native doing heavy work on an intake of 10 mg. daily.

Dietary surveys of the population have been made. To get satisfactory results the ascorbic acid must be assayed in the food as it is presented at the table and not before or just after cooking. The diet of an individual or a group must be examined for a week and representative samples analysed. Values calculated from food tables will give highly fallacious results.

Author	Class of Subjects Examined	Daily Intake of Ascorbic Acid in Food in mg.
Widdowson and Alington [833]	"Middle class."	{ 57 mg. in 1935 27 mg. in 1941
McNee and Reid [971]	Royal Navy and civilians.	15 mg.
Stuhl [972]	British soldiers	>25 mg.
Stamm, <i>et al.</i> [1042]	R.A.F. personnel.	17-26 mg.
Ungley [973]	Navy personnel.	16-30 mg.

No deficiency symptoms were observed on low intakes of 15 mg. of ascorbic acid daily.

Requirements based on a Study of Capillary Fragility. In 1931 Göthlin [348] stated that capillary fragility is a measure of the level of ascorbic acid nutrition, and he employed it to determine the human requirements of the vitamin. From simultaneous determinations of capillary fragility tests and blood ascorbic acid levels Göthlin stated capillary fragility becomes pathological at plasma levels of 0.1 to 0.14 mg. ascorbic acid per 100 ml. He put the minimum human daily requirements of ascorbic acid at 20 to 30 mg. for a 60-kg. subject. More recent work suggests that the determination of capillary fragility is of no value in detecting ascorbic acid deficiency (p. 415). In a Medical Research Council investigation on experimental scurvy deprivation of ascorbic acid showed no significant effect on capillary resistance [407].

Requirements based on Examination of Urinary Excretion of Ascorbic Acid. Many workers have attempted to find the human ascorbic acid requirement from studies on the urinary excretion of the vitamin [348, 356, 357]. An arbitrary excretion figure, e.g. 25 mg., has been adopted and the daily intake found that produces an excretion in excess of this. This is considered to be the daily requirement. The excretion of ascorbic acid is not a measure of the adequacy of the intake. An almost zero excretion is compatible with good health (p. 438). Even if an arbitrary excretion figure is accepted as a measure of adequate intake, excretion is affected by so many factors that it fails to be an accurate measure of intake. The renal threshold, for example, is exceedingly variable and may fall between 0.85 and 1.4 mg. per 100 ml. Deeny and his collaborators [970] have shown that marked hourly variations in excretion occur at all physiological levels of intake and that they are independent of the rate of flow of the urine.

“Saturation” or “loading” tests have been devised to assess ascorbic acid requirements in place of simple excretion studies. The technique of the test depends on the hypothesis that after the administration of a given dose of ascorbic acid the tissues and organs must first be “topped up” with an adequate supply before the blood level rises to the renal threshold so that increased quantities are excreted through the kidneys in the urine. Briefly the method consists of determining the intake that causes a sharp rise in the urinary excretion (p. 467). The authors see no reason why “saturation” with ascorbic acid should be essential for good health, although many workers believe it is. The values obtained by saturation tests vary from 30 to 100 mg. daily as shown in the following table :

Author	Daily Ascorbic Acid Requirement
van Eekelen, 1936 [365]	60 mg.
Schnetzer, 1937 [369]	40 mg.
Widenbauer, 1937 [376]	30-50 mg.
Heinemann, 1938 [368]	0.8 mg. per kilo (= 50 mg.)
Kellie and Zilva, 1939 [359]	30-50 mg.
Hauck <i>et al.</i> , 1939 [374]	1-1.6 mg. per kilo
	(= 65-100 mg.)
Todhunter and Robbins, 1940 [375]	1.6-1.7 mg. per kilo
	(= 100 mg.)
Lewis <i>et al.</i> , 1943 [976]	75 mg.
Kline and Eheart, 1944 [377]	1.4-1.8 mg. per kilo
	(= 75-100 mg.)
Haines <i>et al.</i> , 1947 [366]	70 mg.

Hauck and her associates [374] first saturate the individual with 200 mg. ascorbic acid daily for six days; saturation is confirmed by an increase of excretion following a test dose of 400 mg. The test is repeated two or three times. Finally a series of similar tests is conducted on graded doses of ascorbic acid and the smallest amount found that will induce a similar response to the test dose of 400 mg. as obtained in the preliminary tests. This is the maximum requirement to maintain tissue saturation. Todhunter and Robbins [375] gave the subject a basal diet furnishing 20 mg. of ascorbic acid daily and the response in urinary excretion to a 400 mg. test dose, following a four-day period when 200 mg. daily was taken, was determined for three successive periods. Widenbauer [376] gave the individual under test a standard diet containing little ascorbic acid for several days and calculated the average daily excretion. This served as a preliminary blank. Test doses of 200 to 500 mg. of ascorbic acid were then given until "saturation" was reached; this was arbitrarily assumed to be reached when at least fifty per cent. of the last test dose was excreted in twenty-four hours, or half this in twelve hours. The daily dose was then adjusted to give an excretion of ascorbic acid slightly higher than the preliminary blank. This dose was given for seven days, the urine being titrated daily to find the average excretion. The daily requirement was found by taking from the average daily intake during the final period the average daily excretion during the same period less the average daily excretion of the preliminary period. A similar method was employed by Lewis and her co-workers [976]. Figures for the human requirements of ascorbic acid based on excretion and saturation tests are based on purely arbitrary assumptions. Both the size of the test dose given and the criterion of adequate excretion vary from one worker to another. It has not been proven that an individual in a state of ascorbic acid saturation is any healthier than one who is not.

Requirements calculated from Blood Levels. Ascorbic acid requirements based on plasma blood levels must be accepted with considerable caution (p. 469). The range of so-called normal values is wide. Many observers believe that plasma ascorbic acid values below 0.5 to 0.7 mg. per 100 ml. indicate ascorbic acid depletion, and that values of 0.7 to 1 mg. per 100 ml. indicate mild deficiency. Purinton and Schuck [988] consider 0.8 mg. per 100 ml. indicates an adequate intake. Ralli and her associates [378] obtained the mean value 1.2 mg. for a group of students. Isolated blood plasma figures are of little value. Dodds and Macleod [997] and others [294] have shown that there is no consistent relationship between plasma ascorbic acid levels and intake. Dodds and Macleod brought their subjects into equilibrium or slight ascorbic acid deficiency as shown by plasma ascorbic acid averages which slowly decreased or were just maintained. This was accomplished by an initial intake of 32 to 35 mg. daily. Then the intake was gradually increased by large test doses over periods of eight to ten weeks and plasma levels found. An intake of 1 mg. per kilo. (60 mg. for a 60-kilo. subject) increased plasma ascorbic acid for all subjects studied, and this was considered to represent the daily requirement. On the other hand, Wright and his co-workers [404] found supplements of 25 mg. of ascorbic acid a day sufficient to maintain whole blood or plasma values when human subjects were on a diet containing no ascorbic acid. Bessey, White [858], Horwitt [859] and others [861] gave supplements of ascorbic acid until there was a rise in the plasma level; this corresponded to 50 mg. daily. Fincke and Landquist [977] arbitrarily fixed a plasma level of 0.8 mg. per 100 ml. as an index of adequate intake and found that 40 to 90 mg. of ascorbic acid daily was necessary to maintain this. Goldsmith and her associates [551] considered the optimum plasma level to be 1 mg. per 100 ml. and found that an intake of 70 mg. daily provided for this.

It is now established that the plasma ascorbic acid level, unless near zero, is of little value in assessing the ascorbic acid reserves of the body. Blood

plasma levels of 0.02 mg. per 100 ml. have been recorded in the absence of scurvy [367]. According to a Medical Research Council report [407] a plasma value below 0.1 mg. per 100 ml. indicates a daily intake of about 20 mg.

The ascorbic acid of whole blood or the white cell and platelet layer is of far greater significance than the plasma level (p. 469). This can now be done on 0.1 ml. blood [413]. Whole blood ascorbic acid values reflect the tissue reserves of the vitamin, plasma values represent an overflow. Owing to the technical difficulties involved in the estimations very few determinations have been made in conjunction with dietary studies or nutrition surveys.

Wilson and Lubschez [408] have shown that to maintain a level of 25 mg. per 100 ml. of ascorbic acid in the white cell layer, which is considered an optimal level since it will not rise much above this whatever the intake, an intake of 100 mg. ascorbic acid daily is necessary. They consider that daily intakes above 200 mg. unnecessary and inadvisable.

Correlation of Ascorbic Acid Levels in Blood and Urine. According to Faulkner and Taylor [314] plasma ascorbic acid values above 1.4 mg. per 100 ml., the renal threshold, correspond to a state of "saturation," and lower values correspond to an "unsaturated state." The renal threshold, however, has a very wide range, from 0.85 to 1.4 mg. per 100 ml. [68, 314, 430, 967]. This makes such a test valueless. Roberts and co-workers [981] determined blood concentrations and the urinary response to a test dose on intakes ranging from 32 to 82 mg. of ascorbic acid daily. A blood level of 0.7 mg. per 100 ml. and an excretion of fifty per cent. of a 300 mg. test dose in twenty-four hours were arbitrarily used as criteria indicating a satisfactory intake. On these criteria 62 to 72 mg. daily was considered adequate for girls of six to twelve years.

Ralli and her co-workers [378] argued that the amount of ascorbic acid required daily would be the smallest amount necessary to maintain a normal plasma level. At this level the amount excreted should be small and should remain fairly constant so that the maximum capacity of the kidney tubules would be exceeded. Then if more than the daily needs were given excretion should rise promptly. In the technique used by Ralli the subject is given a diet containing 5 mg. of ascorbic acid daily. The twenty-four-hour ascorbic acid excretion and the plasma level are determined and then graded intakes of ascorbic acid given. On an intake of 50 mg. daily for one hundred and twenty-seven days the plasma ascorbic acid averaged 0.4 mg. per 100 ml. Increasing the intake of ascorbic acid did not alter the amount excreted as long as the intake was below 100 mg. daily. At this intake there was a sharp rise in the urinary excretion and a plasma level of 1 mg. per 100 ml. It was therefore concluded that to maintain tissue saturation and a plasma level of 1 mg. per 100 ml. the daily intake of ascorbic acid must be at least 100 mg. daily. This was considered to be the optimum daily requirement.

Haines and her co-workers [366] placed groups of healthy adults for six weeks on diets providing 30, 53 and 70 mg. ascorbic acid daily after preliminary saturation with 400 mg. of ascorbic acid. At the end of six weeks the tissue reserves of ascorbic acid were judged by fasting plasma ascorbic acid values. Intake was considered adequate if the fasting plasma levels reached a plateau and if fifty per cent. of a test dose of 400 mg. of ascorbic acid was excreted in twenty-four hours. A daily intake of at least 70 mg. of ascorbic acid was considered necessary to satisfy these criteria.

Purinton and Schuck [988] consider that a plasma level of 0.8 mg. or more per 100 ml. indicates an adequate level of ascorbic acid nutrition. To determine the human requirements they measured the ascorbic acid plasma concentration and urinary elimination of sixty-three healthy individuals in response to a test dose of 500 mg. The subjects were given a diet containing 14 to 15 mg. of ascorbic acid and fasting urine and plasma levels determined. Then the test dose was given orally or intravenously and the plasma ascorbic acid measured at regular intervals until the absorption of the dose was indi-

cated by a return of the plasma value to the fasting level. The urine was also analysed at definite intervals for twenty-four hours following the test dose. By subtracting the amount of ascorbic acid excreted in the test period from the quantity administered in the diet and the test dose a retention figure was obtained. By subtracting the quantity excreted under fasting conditions from this retention figure a value was obtained which was considered to be the quantity of ascorbic acid metabolized by the individual. Using this method Purinton and Schuck consider that the requirements of the young adult are of the order of 100 mg. daily.

REQUIREMENTS UNDER VARIOUS CONDITIONS

Infancy. The ascorbic acid in the umbilical cord is one and a half to four times that of the maternal blood at the time of delivery [276, 389-391, 406], due to the placenta exerting a selective action in filtering ascorbic acid from the blood [978]. The foetus withdraws considerable amounts of ascorbic acid from the mother in pregnancy and stores it; the maternal blood ascorbic acid falls as pregnancy proceeds, and at delivery is less than a half of its value during the first twenty-eight weeks of pregnancy [391]. The blood ascorbic acid of the newborn falls to less than half its value ten days after birth [389]. Storage probably occurs in the liver as reserves in that organ fall considerably within the first month of life [393].

Soon after birth the infant needs a further supply of ascorbic acid, which it receives from its mother's milk with or without supplements of vegetable and fruit juices. Cow's milk and dried milks contain very little ascorbic acid by the time they reach the infant.

Two weeks after birth the plasma ascorbic acid of the infant is only 0.1 mg. per 100 ml. if it is not receiving supplements of ascorbic acid [394]. The unreliability of basing infants' requirements on plasma ascorbic acid levels is pointed out by Snelling [401], who found very low values in the absence of signs of scurvy.

Human milk has a very variable ascorbic acid content, which depends on the mother's intake. Although surveys have shown that in Britain it varies from 2.5 to 5.1 mg. per 100 ml. with a mean of 3.5 mg. [414], it can vary from 1 to 15 mg. per 100 ml. A day's milk may provide from 15 to 30 mg. of ascorbic acid; in some American mothers investigated by Munks and her co-workers [424] the daily output in the milk was 12 to 54 mg., the majority yielding 30 mg. or more. According to Ingalls and his co-workers [395] a mother receiving 20 mg. ascorbic acid daily can provide 20 to 50 mg. in a day's supply of milk. If these figures are correct the mother's stores of ascorbic acid must be severely drained or synthesis must occur in the body. A mother might provide as much as 5 grams of additional ascorbic acid during the lactation period.

Pasteurized milk contains very little ascorbic acid (range 0.3 to 5.8 mg. per 100 ml.), so that infants receiving this and no supplement of ascorbic acid may obtain as little as 1.5 mg. daily. Ingalls [399] describes three cases of scurvy in premature infants fed pasteurized milk only, all of them dying within fifty-seven days. A scorbutic dietary for this period will exhaust the ascorbic acid stores of an infant. Ascorbic acid should be given to these infants in amounts corresponding to those found in breast milk (15 to 30 mg. daily), beginning within the first few days of life.

Neuweiler [400] calculated the requirements of infants one to two weeks old by a test dose technique. He assumed that infants with a satisfactory ascorbic acid intake should excrete within twenty-four hours fifty per cent. of a test dose of 30 mg. ascorbic acid given subcutaneously. This corresponds to a dose of 300 mg. for an adult (p. 468). According to Neuweiler the young infant requires 6 mg. per kilo daily, or 20 to 25 mg. for an average-sized infant.

According to Dann [871] the requirements of premature infants are

related to protein intake. After a period of saturation with ascorbic acid breast-fed infants retain a larger part of a test dose of ascorbic acid than do infants fed on cow's milk. This could be explained by the difference in the protein content of the two milks (human, 1.5 grams per 100 ml.; cow's, 3.5 grams per 100 ml.), bearing in mind that ascorbic acid is related to amino-acid and protein metabolism in premature infants (p. 427).

It is reasonable to assume that the physiological requirements of an organism for a particular nutrient are satisfied by the diet provided in its normal environment. In the case of the infant requirements of ascorbic acid are satisfied by that present in the breast milk of an adequately nourished mother. In Britain freshly expressed mother's milk contains on an average 3.5 mg. ascorbic acid per 100 ml. with a range of 2.5 to 5.0 mg. [414]. This would prove 15 to 30 mg. of ascorbic acid daily to the average infant. There is no evidence that British infants are suffering from ascorbic acid deficiency. American figures are higher. According to Pratt and his co-workers [392] the ascorbic acid content of the milk of an unselected group of mothers in Detroit varied from 12 to 54 mg., with the majority around 30 mg. or more. Widenbauer [410] gives values ranging from 18 to 78 mg. daily; Neuweiler [400] writing in 1937, considered that an infant then obtained 25 mg. in the milk in the early days of life, reaching 50 mg. in six months. Whether the infant needs all this ascorbic acid is another matter. The various analyses of mother's milk show that the average infant may obtain anything from 15 to 50 mg. of ascorbic acid daily, omitting extreme figures. This corresponds to 2.5 to 8 mg. per kilo. This amount is supplied by the mother, whose diet should contain liberal amounts of fresh or freshly cooked fruit and vegetables. Certainly artificially fed infants run the risk of scurvy if they do not receive supplements of ascorbic acid in the form of vegetable or fruit juices or the synthetic vitamin. Pasteurized cow's milk may provide only 1.8 mg. of ascorbic acid daily, although the average ranges from 4 to 6 mg. daily. To make certain of an adequate ascorbic acid intake the artificially fed infant should receive 25 mg. of ascorbic acid daily. It is considered that premature infants should have 50 mg. ascorbic acid daily [430]. Twenty-five mg. is provided by any of the following: 1 tablespoon of black currant juice; 2 tablespoons of black currant purée; 1½ ounces of red currant juice, or orange juice, or grapefruit juice, or lemon juice; ½ ounce of rose hip syrup; 3½ ounces of cabbage or spinach water, or the same volume of turnip or tomato juice. As infants are given water in between feeds this can be given in the form of recently prepared vegetable water or diluted fruit juice. Breast-fed infants can also be given these in case the ascorbic acid in the milk is insufficient.

Requirements of Children and Adolescents. These have been estimated by means of saturation tests (p. 467); estimating the amount of ascorbic acid necessary to produce a rise in plasma level (or conversely reducing the intake until a fall in the plasma level occurs); correlation of blood levels and urinary excretion; and from dietary studies. The table on p. 446 summarizes some of the work on this subject.

There appears to be a considerable discrepancy between the actual intake of ascorbic acid and the ideal intake calculated from arbitrary laboratory tests. Clearly the majority of children are not suffering from ascorbic acid deficiency which these tests suggest. Trials have shown, however, that some groups of children in apparently normal health benefit from an additional intake of ascorbic acid, their physique and resistance to infection being slightly better than those of control children on normal diets [816].

Requirements of Adults. These have been calculated by the methods described on pp. 439 to 444. The minimum requirements fall between the values 10 to 50 mg. daily, and the optimum between 30 to 100 mg. The higher figures are based on saturation tests, blood levels and other laboratory procedures and the lower ones on clinical or dietary studies. Dietary surveys

THE VITAMINS IN MEDICINE

Author	Method	Age Group and Daily Requirement of Ascorbic Acid
Everson and Daniels, 1936 [421].	Graded supplements given until sudden rise in excretion occurred.	Under 5 years, 120 mg. or 7 mg. per kilo.
Widenbauer, 1937 [376] .	Oral test doses until "saturated" (50% of test dose excreted in 24 hours).	3 years, 22 mg.
Bessey and White, 1942 [858].	Dose of ascorbic acid needed to produce rise in plasma level.	5-13 years, 40-50 mg.
Roberts, <i>et al.</i> , 1943 [981].	Blood concentrations (over 0.7 mg. per 100 ml.) and urinary response to test dose (50% excreted in 24 hours).	6-12 years, 62-72 mg.
Meyer and Hathaway, 1944 [445].	Saturation test.	Under 5 years, 25 mg.
Storvick, <i>et al.</i> , 1949 [382]	Intake needed to maintain a plasma ascorbic acid of 0.8 mg. per 100 ml.	Girls 16-19 years, 70 mg ; boys 18 years, 90 mg.
James, 1943 [982] . . .	Analysis of ascorbic acid in diet.	Schoolchildren, 10-15 mg.
Booth, <i>et al.</i> , 1942 [984] .	Analysis of ascorbic acid in diet.	Schoolchildren, 15 mg.
Widdowson and McCance, 1942 [996].	Analysis of ascorbic acid in diet.	Public schoolboys, 15-20 mg. ; schoolchildren, 32 mg.
Harris and Olliver, 1943 [893].	Analysis of ascorbic acid in diet.	Inmates of children's home, 19-24 mg. spring and winter; 24-55 mg. summer and autumn.

Recommended Daily Allowance of Ascorbic Acid
National Research Council, U.S.A.

	Calories	Ascorbic Acid mg.
<i>Man</i> (154 lb., 70 kilo.)		
Sedentary	2,400	75
Physically active	3,000	75
With heavy work	4,500	75
<i>Woman</i> (123 lb., 56 kilo.)		
Sedentary	2,000	70
Moderately active	2,400	70
Very active	3,000	70
Pregnancy (latter half)	2,400	100
Lactation	3,000	150
<i>Children up to 12 years</i>		
Under 1 year	110 per kilo.	30
1-3 years (27 lb., 12 kilo.)	1,200	35
4-6 years (42 lb., 19 kilo.)	1,600	50
7-9 years (58 lb., 26 kilo.)	2,000	60
10-12 years (78 lb., 35 kilo.)	2,500	75
<i>Children over 12 years</i>		
<i>Girls</i>		
13-15 years (108 lb., 49 kilo.)	2,600	80
16-20 years (122 lb., 55 kilo.)	2,400	80
<i>Boys</i>		
13-15 years (108 lb., 49 kilo.)	3,200	90
16-20 years (141 lb., 64 kilo.)	3,800	100

(p. 440) show that not many individuals, in Britain, at any rate, receive the intake of ascorbic acid that is considered optimal according to laboratory tests.

National Research Council (U.S.A.) Recommendations. In 1941 the Committee on Food and Nutrition of the National Research Council drew up a table of the probable daily requirements of various vitamins. These were tentative figures for use in planning dietaries and allowed for a considerable wastage in the preparation of food. The values were revised in 1945 and 1948. Those for ascorbic acid are given on p. 446.

The Nutrition Committee of the British Medical Association (1950) recommended a much lower daily intake—30 mg. daily for adolescents of both sexes ; 20 mg. for an adult male irrespective of occupation ; 10 mg. for children up to one year old ; 15 mg. for children two to six years ; and 20 mg. for children over seven years.

Requirements and Age. The ascorbic acid requirements are probably related to basal metabolic rate [988], so that adolescents, particularly boys, require more than subjects in any other age group. It has been stated incorrectly that requirements are increased in old age because the subjects tested had a low excretion or showed increased retention on test dosing [452, 683]. The probability is that they were judged by standards of young adults and their food intake, including that of ascorbic acid, was much lower than normal. The subjects were hospital patients and not normal subjects.

Requirements in Pregnancy and Lactation. During pregnancy there is a lowered excretion of ascorbic acid [417], and blood plasma levels fall progressively right down to term [390, 442, 989]. This would be expected as ascorbic acid is stored by the fetus at the expense of the mother (p. 444). Neuweiler [417] found that it was necessary for pregnant and nursing women to ingest larger test doses of ascorbic acid than normal subjects to maintain the same level of excretion. Using saturation tests (p. 471) various workers have calculated the daily requirements of pregnant and nursing mothers [376, 410, 414, 420, 423]. The values fall between 33 mg. and 100 mg. It is not surprising that the plasma ascorbic acid and the urinary output should be low in the lactation period ; the ascorbic acid is secreted into the milk and not excreted in the urine. The work of Munks and her colleagues [419] demonstrated the wide variations in ascorbic acid metabolism among normal lactating women and the unreliability of accepting levels of the vitamin in the blood and urine as an index of adequate intake. The amount excreted in the urine bore no relation to the intake or that present in the milk. Intakes of 100 mg. ascorbic acid daily were often associated with a low urinary excretion and fasting blood levels were frequently as low as those considered by some authorities to indicate scurvy (e.g. 0.1 mg. per 100 ml.). A low blood and urine ascorbic acid did not seem to have any deleterious effect on mother or child. When supplements of ascorbic acid were fed the bulk appeared in the urine and not the milk [392]. There is an upper limit, or threshold value, about 7 to 8 mg. per 100 ml., which cannot be exceeded whatever the mother's intake of ascorbic acid [418].

To supply the infant with 15 to 30 mg. of ascorbic acid daily it would seem that during lactation the mother's intake should be at least 45 to 60 mg. daily. This can normally be obtained from a good diet. The requirements recommended by the National Research Council (1948) are 100 mg. daily in pregnancy and 150 mg. during lactation. These quantities are certainly very generous and there can be very few women in Britain who obtain such an intake. The Committee on Nutrition of the British Medical Association (1950) suggested 40 mg. daily in pregnancy and 50 mg. during lactation. It should be possible to obtain enough ascorbic acid from a good diet without having recourse to supplements of the synthetic vitamin.

Requirements and Exercise. Experimental evidence concerning the effect of ascorbic acid on exercise is conflicting. Several workers have re-

ported a decreased excretion after severe exercise, and diminished efficiency on low intakes of ascorbic acid [336, 443, 455, 462]. Other workers claim that the administration of ascorbic acid increases efficiency and wards off fatigue [846, 990]. Much of this work has been uncontrolled and not submitted to statistical examination. Subjective statements by volunteers undergoing fatigue tests are difficult to interpret. Johnson and others [835] have shown that two months' deprivation of ascorbic acid leads to no detectable deterioration in physical vigour, and that supplements of 75 mg. of the vitamin daily do not produce any benefit in manual workers with respect to well-being, physical vigour and efficiency. This work was controlled and evaluated statistically.

It has not been proven that additional ascorbic acid is needed in severe exercise. Low blood and urine figures could be explained by redistribution in the body, e.g. storage in the adrenals as part of a stress phenomenon (p. 432). If the requirements of ascorbic acid are related to metabolic activity [988] one would expect them to be increased by exercise, although the best experimental evidence is against this.

Faulty Absorption. Some gastro-intestinal conditions interfere with the absorption of vitamins (p. 225). In such cases an increased intake is necessary to compensate for this.

Effect of Drugs. Many drugs alter the excretion of ascorbic acid, but unless they affect general metabolism there is no evidence that they affect the normal requirements of the vitamin.

Requirements and Infections. A diminished excretion of ascorbic acid has been observed in a number of infections [119-127, 432-437]. The plasma ascorbic acid is also lower than usual [123]. The fall in plasma and urinary ascorbic acid is not due to the pyrexia *per se* but is associated with the infective process [992, 993]. This fall is due to mobilization of ascorbic acid from the plasma to the white cells which become laden with ascorbic granules (p. 420), and to the adrenals. There is no direct evidence that infection increases the requirements of ascorbic acid. The fall in plasma and urine levels are quite unreliable guides of adequacy of intake (p. 441). Most workers, however, assume that requirements are increased.

Requirements and Raised Metabolism. A decreased excretion of ascorbic acid has been observed in patients with diseases characterized by increased local or general metabolism, e.g. malignant disease [440], leukaemia [474], and hyperthyroidism [264]. In the experimental animal drugs increasing general metabolism such as thyroid, 2:4-dinitrophenol and insulin increase the excretion of ascorbic acid [994]. As requirements are related to metabolic rate, requirements are probably increased in the conditions mentioned.

Requirements and Climate. The ascorbic acid requirements are not raised by a high environmental temperature. In tests carried out by Henschel and his associates [869, 879] the work performance of subjects at high environmental temperatures (122° F.) was uninfluenced by high or low intakes of ascorbic acid. It is not certain whether climate has any effect on ascorbic acid requirements. Scurvy is a disease of temperate and not tropical climates. This may be because native races consume more raw food than the inhabitants of temperate lands.

DISEASES ASSOCIATED WITH ASCORBIC ACID DEFICIENCY SCURVY

Ætiology and Incidence. Scurvy is a deficiency disease considered to be due mainly to an insufficient intake or absorption of ascorbic acid. It has been maintained that lack of this vitamin is not the sole causative factor, since cases of scurvy have been described resistant to treatment with pure ascorbic acid. Some of these cases, however, responded to parenteral but not to oral treatment with the vitamin. Mawson [444] has described a case

of scurvy in which the ascorbic acid intake was adequate, although the excretion and the blood level were low. "Renal scurvy" was diagnosed, implying that the patient had an abnormally low renal threshold for ascorbic acid and was therefore unable to store sufficient in the tissues to prevent scurvy. With the discovery of other vitamins in fruit juices, particularly vitamin P, it has been suggested that scurvy, like beriberi and pellagra, is a multiple avitaminosis. According to Szent-Györgyi and Scarborough (pp. 731, 738) scurvy is a disease due to a combined deficiency of ascorbic acid and vitamin P. Cameron and Mills [1015] gave vitamin P but not ascorbic acid to a case of classic scurvy. The hæmorrhagic features promptly disappeared, but the other manifestations were unaltered. This is at variance with the observation of Mouriquand and Edel [456, 471] who found that the administration of vitamin P precipitated hæmorrhages in guinea-pigs on a scorbutic diet.

How long does it take for a healthy adult to become scorbutic when fed on a diet otherwise adequate but devoid of ascorbic acid? There have been various answers to this question. According to Lind it occurs between four and six months at sea on a scorbutic diet. Mensching [342] lived on a diet poor in ascorbic acid for a hundred days without getting scurvy. Crandon [68], who lived on a diet adequate in all respects, but lacking ascorbic acid, observed that scurvy developed after a hundred and thirty-two days of total deprivation. Symptoms of scurvy appeared in a group of Chinese refugees after three to eight months on a deficiency camp diet of cereals and salt turnip [834]. In infants scurvy develops between the second and ninth month [514]. Farmer [864] observed no clinical evidence of scurvy, except hyperkeratotic papules surrounding the hair follicles on the legs, in volunteers on a scorbutic diet (0 to 10 mg. ascorbic acid daily) for five months. In a trial carried out in Britain under the M.R.C. the first sign of scurvy—hyperkeratosis of hair follicles—appeared after seventeen to twenty-one weeks; after twenty-six to thirty-four weeks perifollicular hæmorrhages; and after thirty to thirty-eight weeks swelling and bleeding of the gums [407].

Factors responsible for the actual appearance of scurvy in addition to a scorbutic diet are climate, composition of the food in relation to other vitamins and minerals, constitution of the subject, condition of internal secretions, presence of infection, and the condition of the alimentary tract. According to Lund [460] patients with gastric lesions have low reserves of ascorbic acid and are often near the scorbutic level. The causes of scurvy met with in the population at large are "ignorance, apathy and poverty" [927]. According to McMillan and Inglis [927] there was an increase in scurvy during the last war. In 1941-42 scurvy formed over three per cent. of all admissions to medical wards in an Edinburgh municipal general hospital. According to the Ministry of Food the ascorbic acid intake of Edinburgh and Glasgow was much below the average for all towns in Britain. McMillan and Inglis attributed the relatively high incidence of the disease firstly to ignorance, particularly in males living on their own ("bachelor scurvy"), and to the need for potatoes and vegetables in the diet. Secondly to apathy because such foods require preparation and cooking, and thirdly to poverty, making it impossible to buy an adequate diet. Pure scurvy, although common in the days when ships went to sea carrying their own food for months, is now rare. It is often accompanied by signs of other vitamin deficiencies such as neuritis (aneurine), glossitis (nicotinic acid), and cheilosis (riboflavine).

Clinical Manifestations of Scurvy. The adult suffering from scurvy complains of tiredness and weakness and pain in the limbs. An early manifestation is the appearance of hyperkeratosis of the hair follicles, which is followed by perifollicular hæmorrhages, particularly on the legs, which are brawny and tender.

As navigators and explorers have observed, weakness is one of the early complaints of scurvy. This is associated with fatigue on exertion, palpitation,

anorexia and breathlessness. The patient sits, rather than stands or walks, and when he does stand he flexes his legs. Hæmorrhage from the gums and stomato-gingivitis, hypertrophy of the dental papillæ (see Fig. 158), followed by loosening of the teeth are common symptoms of scurvy; the loosening of the teeth is due to the resorption of the alveoli of the jaw bones. One of the most reliable early signs of scurvy is gingivitis. The characteristic swollen boggy and tender gums of scurvy occur only when teeth are present and are most marked about defective teeth (Figs. 158, 159). Not only may hyperæmia and hæmorrhage of the gums occur, but epithelial degeneration, ulceration and even gangrene. Owing to the infection around the base of the teeth the breath is foetid. Other hæmorrhagic manifestations include hæmaturia, melæna with diarrhœa, pin-point hæmorrhages in the gut with subsequent ulceration, menorrhagia, metrorrhagia, epistaxis and subperiosteal hæmatomata. The latter cause the limbs to be painful to the touch. Extravasations of blood from larger vessels into the muscles may occur (Fig. 161), as well as beneath mucous membranes, in the gums, conjunctivæ and lining of the serous cavities and joints. Peritoneal irritation may occur through extravasation of blood into the peritoneal cavity. Hines [448]

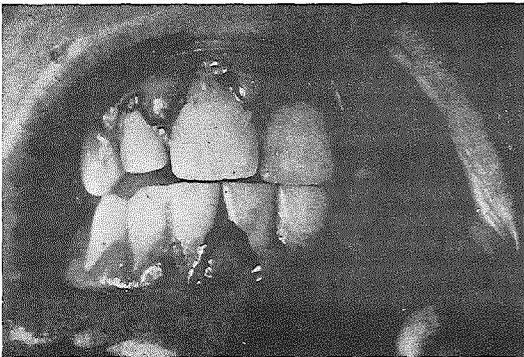


FIG. 158. Scorbatic Gingivitis. The patient is an orphan on a very poor diet. Hæmorrhage from the gums and hypertrophy of the dental papillæ are clearly seen. The teeth appear to be healthy, although irregular.

describes three cases of scurvy that presented as acute surgical disease of the abdomen. Two were subjected to surgery and bleeding was shown to be the cause of peritoneal irritation. Petechial hæmorrhages are also present (Fig. 160). Purplish discoloration may occur around the joints, abdomen, face and popliteal fossa. Farmer [864] observed petechiæ following slight trauma in volunteers kept on a scorbatic diet for five months. The scleræ are slightly icteric.

The hæmorrhagic manifestations have been stated to be due to abnormal capillary fragility, although more modern work negatives any connection between ascorbic acid deficiency and increased capillary fragility (p. 415). According to Lee and Lee [88] there may be a failure of the contractile mechanism of the small vessels, resulting in their dilatation and a sluggish blood flow (Figs. 162-165). Scarborough believes that vitamin P may play a part in the hæmorrhagic manifestations of scurvy (p. 738). Only a third of all cases of scurvy show an increased capillary fragility [860].

The complexion is sallow, dirty yellowish grey and cadaveric, and the extremities may be cold and cyanotic. Long splinter-like hæmorrhages are sometimes seen under the nails.

The anæmia of scurvy is discussed on p. 453. It is not a constant finding and conforms to no particular type. It may be macrocytic, normocytic or microcytic. A mild hypochromic and normocytic anæmia is common. In their cases Spies and his co-workers [103] observed a persistent reticulocytosis and moderate leucopenia and thrombopenia. The anæmia of scurvy is probably not due to ascorbic acid deficiency alone, as a simple uncomplicated deficiency does not produce scurvy [68, 407], and anæmia when it does occur sometimes responds to treatment with iron alone [110]. Usually the serum iron is normal [103]. The icteric index is 10 to 20, suggesting hæmolysis

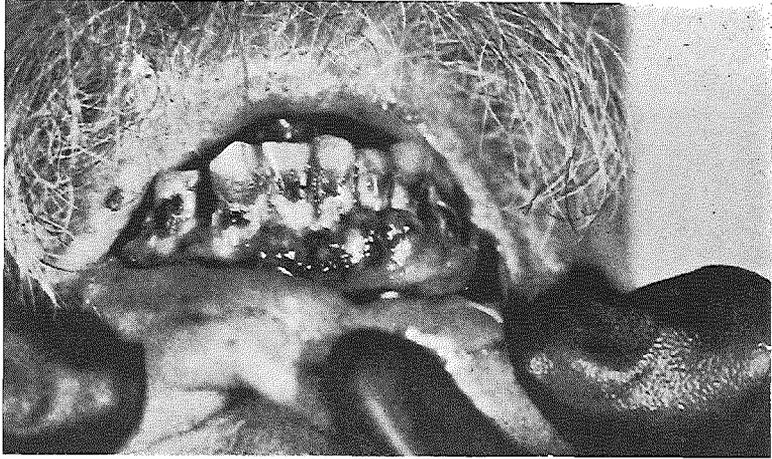


FIG. 159. Scurvy. A patient with scurvy showing considerable swelling, sponginess and discoloration of the gums.

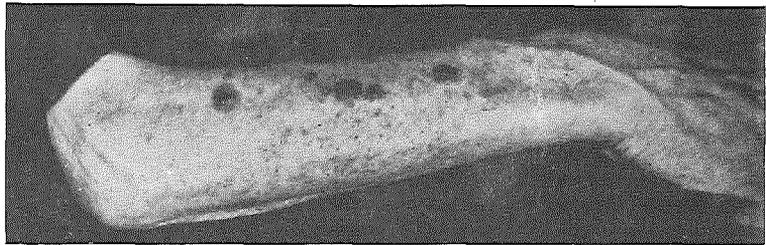


FIG. 160. Scurvy. Forearm of the same patient as in Fig. 159 showing bleb-like extravasations of blood resulting from slight traumata a year previously. Petechial hæmorrhages resulting from a recent tourniquet test are also seen.

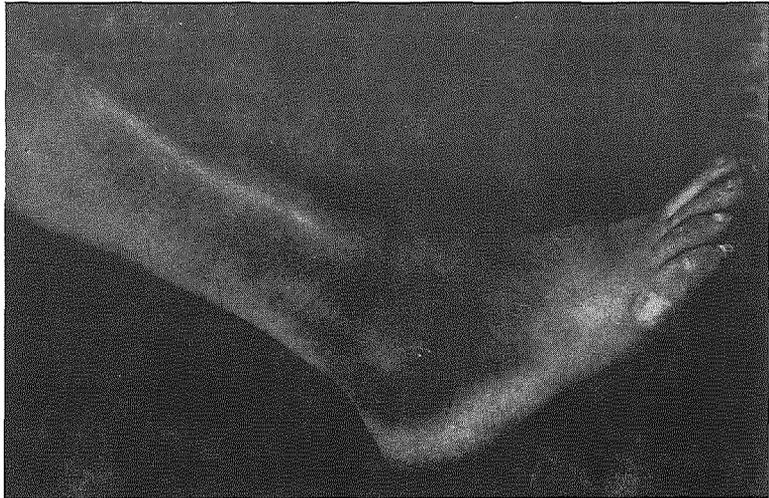
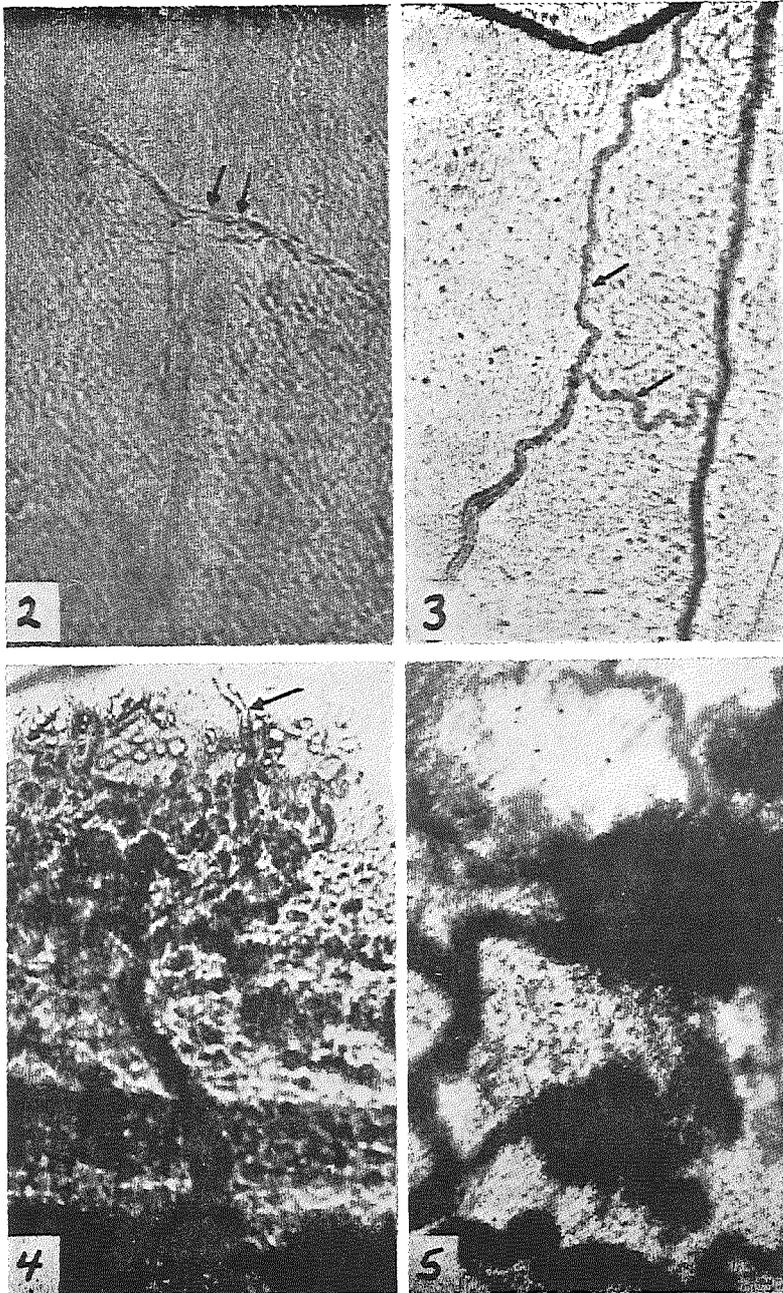


FIG. 161. Scurvy. The same patient as in Fig. 159, showing extravasation of blood around right ankle and on right shin. Some oedema of the feet is present.

THE PERIPHERAL VASCULAR SYSTEM IN SCURVY



FIGS. 162 to 165. The Peripheral Vascular System in Scurvy (approximately $\times 120$).

FIG. 162 (*upper left*). Normal animal : arteriole in an environment of adrenaline, 1 in 5 million. The lumen of the capillary (arrows) is empty, due to contraction under the influence of adrenaline.

FIG. 163 (*upper right*). Scorbutic animal : a terminal arteriole in an environment of adrenaline, 1 in 5 million. Both capillaries (arrows) have failed to contract and are filled with a sluggish stream of blood.

FIG. 164 (*lower left*). Scorbutic animal : small venules draining the capillaries are partly surrounded by small hæmorrhages, seen as dark masses in the centre of the figure. At the bottom of the figure two large masses of blood are visible outside the main venule.

FIG. 165 (*lower right*). Scorbutic animal, showing larger petechiæ along the tributary branches of the collecting venules.

as there are no signs of liver dysfunction [103]. Red cells may be found in the urine.

Infection with its resulting pyrexia is common. The depleted ascorbic acid reserves of the patient suffering from scurvy are said to render him more susceptible to infection. If death occurs it is due to intercurrent infection, e.g. bronchopneumonia, or it suddenly occurs with syncope. Crandon [68], however, contests these views. Although he suffered from frank clinical scurvy for a month and subclinical scurvy for several months, he remained free from infection, with the exception of "colds."

Crandon [68] placed himself on a diet free from ascorbic acid, but containing all the other essentials, including a complete range of all the other vitamins and minerals. During the first four months all the clinical findings were negative. There was a slight loss of weight, a fall in the metabolic rate and a feeling of weakness and fatigue on slight exertion. It was only after a hundred and thirty-four days had elapsed that clinical manifestations of scurvy appeared. The loss in weight, which eventually reached a maximum of 27 lb., was attributed to the monotonous diet rather than to the absence of ascorbic acid. The following description is taken from the paper by Crandon and his co-workers [68].

Skin Lesions. After a hundred and thirty-four days had elapsed small hyperkeratotic papules began to develop over the buttocks and posterior aspects of the calves; there was also noticeable fragmentation of hairs. These lesions resembled the follicular keratitis of vitamin A deficiency. Each papule contained an ingrown hair, which could be seen if the hyperkeratotic plug was picked or scraped off, leaving a small slightly bleeding crater. There was also marked dryness of the skin, particularly over much of the extensor surfaces and backs of the hands. The existence of vitamin A deficiency was ruled out because 30,000 units of vitamin A were being taken daily and biophotometer readings and plasma vitamin A values were within normal limits.

After a hundred and sixty-one days small perifollicular hæmorrhages or petechiæ appeared on the legs, particularly after standing for long periods. After six months they were abundant over the whole of the leg, and on the thighs took the place of the hyperkeratotic papules. The administration of ascorbic acid rapidly cleared these lesions.

Wound Healing. The experiments of Crandon and others on the healing of wounds have been previously described (p. 413).

Teeth and Gums. During the first five months no changes were observed in the teeth or gums. At the end of six months the gums were slightly boggy on pressure, but no other gross changes were observed. A biopsy of the gums showed normal tissue. It is interesting to note, however, that X-ray films of the teeth showed occasional interruptions of the lamina dura. No trace of bleeding occurred on brushing the teeth.

Farmer [864] kept several volunteers on a scorbutic diet (0 to 10 mg. ascorbic acid daily) for five months, but failed to observe any changes in the gums, teeth or jaws, either with the naked eye, radiologically or with the biomicroscope. This period may not have been long enough (see M.R.C. report [407]).

Hæmatological Picture. Although blood was lost by venesection on four occasions and as a result of the numerous blood estimations no anæmia developed. The percentage hæmoglobin showed a slight fall during the third month of the diet, but was due to iron deficiency since it was corrected by the administration of ferrous sulphate. These observations are in accord with those of other workers in this field [407].

The white cell count averaged 5,000 at the beginning of the experiment and no appreciable change was observed until after a hundred and thirty-two days, when the count fell to 3,500 and later to 3,200. Following the administration of ascorbic acid, the count shot up rapidly to 5,000 and then to 9,000.

In experimental scurvy the prothrombin time is not appreciably increased [1049].

Capillary Fragility. The capillary fragility test as determined by the technique of Göthlin (p. 464) remained negative. At the end of five months when frank scurvy was present, there were fewer petechiæ on the arm after applying a blood pressure cuff for ten minutes at 100 mm. mercury than there were in normal controls. The negative pressure method of Dalldorf (p. 465) also gave negative results. This is in keeping with the most recent work on the subject, which negatives any connection between ascorbic acid deficiency and increased capillary fragility (p. 415).

Blood Pressure. This remained constant at 120 systolic, 70 diastolic, except on one occasion when it dropped temporarily owing to a large loss of blood.

Resistance to Infection. Contrary to expectations based on the work on ascorbic acid and infection (p. 418 *et seq.*) there was almost complete freedom from respiratory infection, even though the experimental period included the winter months. Blood complement determinations were normal throughout the period of observation even when frank scurvy was apparent.

Fatigue. Fatigue appeared at the beginning of the third month and became progressively more marked. Careful tests after prolonged ascorbic acid deficiency showed impaired capacity for aerobic work, e.g. walking and running on the treadmill. No great change could be detected in the capacity for doing anaerobic work, e.g. muscular movements measured by an ergograph. Tests of harder grade work—a run to exhaustion at seven miles an hour—showed that running was only possible for sixteen seconds, whilst following the administration of ascorbic acid the time was increased to sixty-six seconds and to eighty-four seconds after normal diet had been resumed. The performance whilst in the scorbutic state was equivalent to that of a man in the eighth decade of life.

Crandon has drawn attention to the fact that scurvy might have appeared much sooner if he had been submitted to extreme muscular fatigue. After it had been decided to terminate the experiment, fatigue and languor were considerably diminished within twenty-four hours of administering ascorbic acid.

Farmer [864] has also studied fatigue phenomena in young volunteers kept for five months on a scorbutic diet (0 to 10 mg. ascorbic acid daily). A measurable decrease in work output occurred and the subjects complained of severe fatigue after three months on the diet. Errors were increased and there was loss of interest in work and motivation. Threshold of perception, co-ordination of motion on a pursuit meter and critical fusion frequency of visual flicker showed little or no change from the normal.

Blood Ascorbic Acid. The plasma ascorbic acid rapidly fell and reached zero after forty-one days on the diet, remaining at this level for thirteen weeks before the first evidence of clinical scurvy appeared. On the other hand, the ascorbic acid level in the white cell platelet layer of the centrifuged blood fell gradually from a relatively normal level of 28 mg. per 100 c.c. on the seventieth day to 4 mg. on the eighty-second day, but did not reach zero until shortly before the appearance of clinical scurvy. These observations and the fact that normal wound healing occurred after three months of ascorbic acid deficiency, when the plasma level had been zero for forty-four days, suggest that plasma values are a poor index of the ascorbic acid status of the individual. As pointed out later (p. 470) the white cell platelet level of ascorbic acid in the centrifuged blood is a more accurate measure of the degree of ascorbic acid deficiency than plasma determination.

Miscellaneous Observations. The basal metabolic rate fell at one time as low as minus twenty-two per cent. This was probably due to loss of weight, or inanition, or both, rather than to ascorbic acid deficiency. Insulin and glucose tolerance tests were normal. Gastric analysis revealed a large drop

in the free and total acid ; this was restored after administration of ascorbic acid. There was a lowering of the total phosphorus content of muscle, and an increase of the phosphagen phosphorus. All other tests on blood, urine, the stools, and X-ray films and electrocardiograms were within normal limits.

The opinion is expressed that the long interval that elapsed before clinical scurvy appeared was probably due to the absence of complicating factors, such as growth, infection or multiple avitaminosis. The study proves that clinical scurvy as ordinarily met with is undoubtedly a multiple deficiency disease, whereas Crandon was suffering from severe uncomplicated ascorbic acid deficiency. It has been observed elsewhere (pp. 212, 352) that simple vitamin deficiencies do not exist except experimentally.

Fox [344] and his colleagues studied nearly a thousand mine labourers in Africa on diets low in ascorbic acid—generally about 15 mg. a day. Some of their findings can be correlated with those of Crandon. For example, no changes in capillary fragility were detected ; there were no signs of anaemia ; the gums remained healthy ; resistance to infection was not diminished ; the healing of wounds was not impaired unless ascorbic acid was *totally* withheld for six months. Even when the blood ascorbic acid was nil there was no sign of scurvy. Fox and his co-workers concluded that deprivation of ascorbic acid, unless extremely severe and prolonged, does not of itself lead to the appearance of scurvy in the absence of precipitating factors, such as infection.

Infantile Scurvy. This is still seen in children in spite of general improvements in infant feeding. Under the names of *Barlow's disease* and *scurvy rickets* it was frequently observed a few years ago. Scurvy rickets is a misnomer as a separate entity ; scurvy and rickets may occur in the same child. For the reasons previously given (p. 444) infantile scurvy is more prevalent in the artificially fed infant than in the breast-fed, particularly if the infant is given overboiled, dried or condensed milk. The disease usually makes its appearance in children of two to twelve months and is rare after eighteen months. The greatest incidence is between the seventh and ninth month. A case of scurvy at nineteen days has been reported by Follis [428], who regarded it as congenital. Faulkner [835] states that the incidence of scurvy among children admitted to the Boston City Hospital is 0·14 per cent. Follis [428] observed an incidence of scurvy of 5·3 per cent. in 1,303 children autopsied at Johns Hopkins Hospital, Baltimore. In only nine per cent. of the cases was scurvy diagnosed on clinical grounds ; the remainder were diagnosed radiologically. Evans [426] gives a much lower incidence, ninety-three cases in 106,800 infants, or 0·09 per cent., diagnosed clinically and radiologically. Barlow's observation that scurvy is seldom seen in breast-fed infants has been confirmed [428].

The onset of the disease is gradual and the child is usually brought to the family doctor because he screams when his limbs are touched, because he does not use his limbs, or because of bruising of the orbit and limbs, and hæmorrhage from the gums, bowel or urinary tract. On examination the position of the legs is characteristic ; they are flexed, widely abducted and externally rotated (Fig. 169). This position is largely due to the extreme pain caused by subperiosteal hæmorrhages, which are most likely to occur around the bones of the legs. Careful interrogation of the mother on the question of diet is necessary.

On examination the infant is usually wasted, pale and fretful and is terrified of being touched. There may be bruising on the face or body (Fig. 169), and on deep palpation acutely tender swellings may be felt deep to the muscles, particularly of the leg. There is a failure to gain weight—which may be masked by œdema—weakness, rapid shallow breathing, a rapid pulse, diarrhœa and vomiting. If there is retrolbulbar hæmorrhage the eye may be proptosed. The temperature is usually slightly raised, about 100° F. ; the fever in both clinical and experimental scurvy has never been satisfactorily

INFANTILE SCURVY

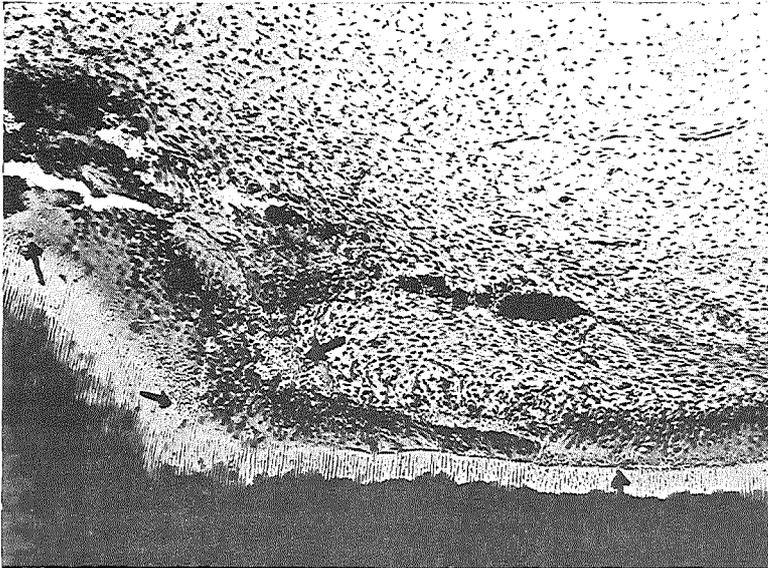


FIG. 166. Infantile Scurvy. Photomicrograph of a Premolar Tooth ($\times 90$). Hæmatoxylin and Eosin. There are numerous recent hæmorrhages (arrows), many between the predentine and odontoblast layer, and calcification in the pulp at the site of a former hæmorrhage.

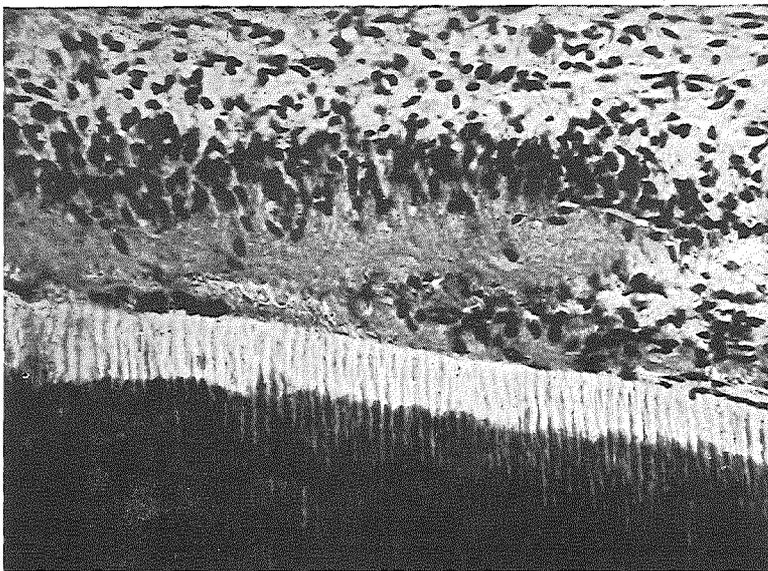


FIG. 167. Infantile Scurvy. Photomicrograph of a Premolar Tooth ($\times 320$). Hæmatoxylin and Eosin. The odontoblast layer has been raised from the predentine by a previous hæmorrhage. Calcification is beginning at the internal surface of the predentine. There is a layer of homogenous material, which is not collagen, on the outer side of the odontoblasts, which have lost their normal morphology.

INFANTILE SCURVY

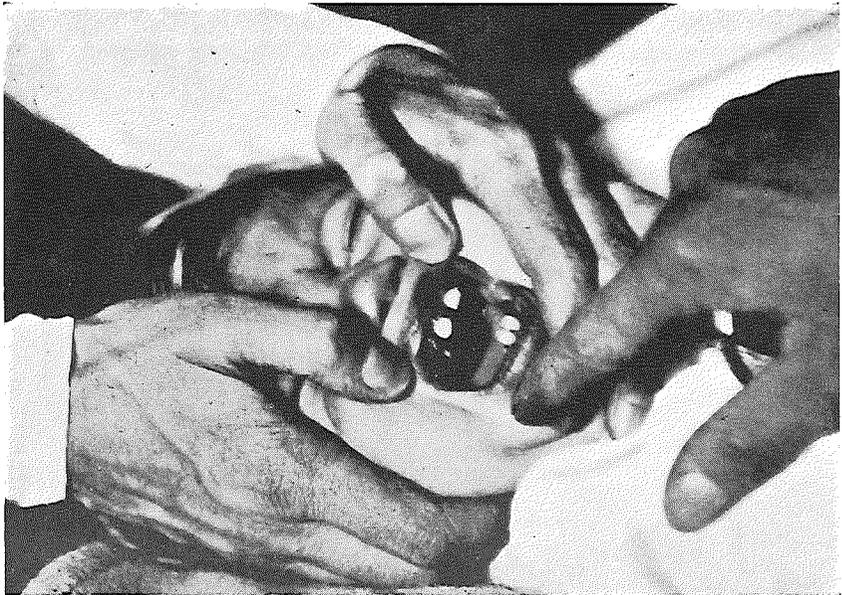


FIG. 168. Infantile Scurvy. Swollen, spongy and hæmorrhagic gums in a child of ten months suffering from scurvy.

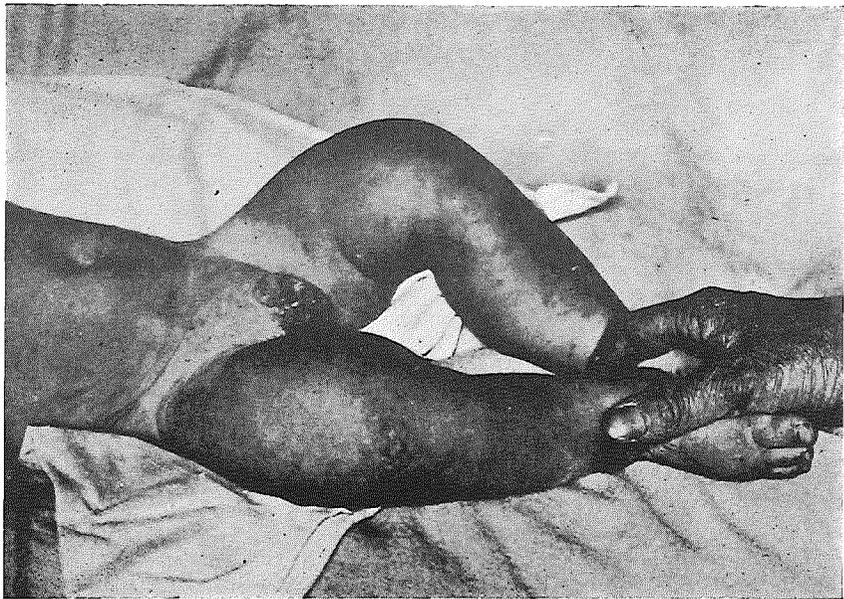


FIG. 169. Infantile Scurvy. The same child as in Fig. 168. There is gross hæmorrhage under the periosteum of the femora ; both legs, which are in the characteristic position of flexion, abduction and external rotation, are swollen and tender ; and there are hæmorrhages into the tissues of the legs and scrotum.

explained, but is probably due to the absorption of blood from the hæmorrhages. Hæmorrhage from the gums (Fig. 168), bowel, or urinary tract may be observed, but the mouth lesions do not occur if the infant is edentulous (cf. Adult Scurvy, p. 450). The gums are swollen, red and dusky if the teeth are due to erupt. Intracranial hæmorrhage has been reported. A most

INFANTILE SCURVY

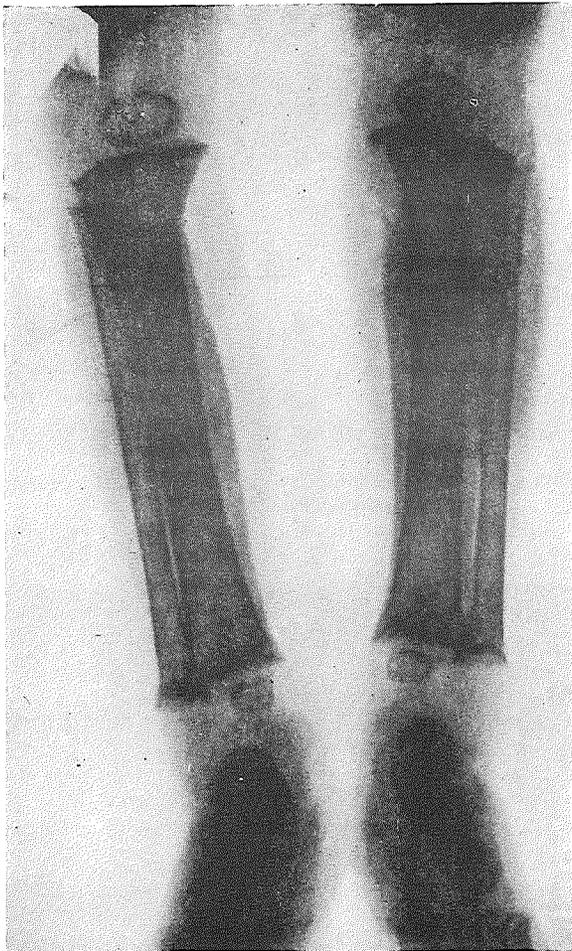


FIG. 170. Infantile Scurvy. Radiogram. Note decalcification of the epiphyses with a well-defined periphery ; calcified subperiosteal hæmorrhages, which show as a dark shadow on the medial side of the tibiæ and between the tibia and fibula of the left leg ; and the dense metaphyseal borders of the diaphyses.

helpful physical sign is the characteristic ridge enlargement of the costochondral junction [428].

Moderate secondary anæmia is often present with hæmoglobin levels in severe cases as low as forty per cent. of normal. Examination of the blood and urine shows the absence of ascorbic acid or markedly reduced levels. A few red blood cells in the urine are of diagnostic importance.

These signs and symptoms are those of frank scurvy. Mild scurvy or scurvy in its early stages can only be diagnosed radiologically (Fig. 170). As

a result of the observations of Pelkan [500], Fraenkel [509], Baetjer [499], Wimberger [502], Schwartz [505] and Bromer [500] ten X-ray signs have been recognized :

(1) A zone of rarefaction immediately posterior to the zone of preparatory calcification ; the "scurvy line," the framework marrow (Bromer), the *Gerüstmark* of the German writers.

(2) A broad, finely irregular edge of dense shadow around the centre of ossification, with rarefaction of the central portion (Wimberger's sign).

(3) A finely irregular broadened intensely calcified zone of preparatory calcification at the epiphyseal end of the long bones, the so-called "white line of Fraenkel."

(4) A small spur at the lateral edge of the epiphysis (Pelkan).

(5) Separation of the epiphyses, which have a signet-ring appearance.

(6) A ground glass transparency of the shaft, with clouding or obliteration of the trabecular structure visible in normal bones.

(7) Thinning of the cortical shadow, often represented by a narrow white line.

(8) Subperiosteal hæmorrhages, occurring only in the late stage (Fig. 170).

(9) Subperiosteal fractures in the ends of the diaphysis.

(10) Enlargement of angulation of the costochondral and vertebral junctions of the ribs.

Taken alone the majority of these signs are not pathognomonic. Signs (2), (3) and (4) are not absolutely characteristic of scurvy and may also be seen in rickets, lead and phosphorus poisoning and after administering vitamin D preparations. The most important sign is (1).

There are three signs diagnostic of latent scurvy, known as Pelkan's triad. They are : (a) a broadened epiphyseal line ; (b) a dense shadow around the centre of ossification of the epiphysis ; (c) absence of trabeculations in the shaft. Park [514], on the other hand, states that there are no early radiological signs, and that reliance must be placed on early clinical signs. This is disputed by Follis [428], who could only diagnose six out of sixty-nine cases of infantile scurvy clinically.

The healing of scorbutic bone can be demonstrated radiologically after two weeks' treatment [500]. At first the broadened epiphyseal line loses its finely irregular appearance and becomes sharply outlined. The scurvy line not only disappears but becomes more heavily calcified than the rest of the shaft. Years after clinical cure radiological examination still shows oval, definitely circumscribed areas of rarefaction in the interior of the epiphyseal centres of ossification [1000].

Morbid Anatomy and Pathology of Scurvy. The principal lesions are hæmorrhagic and skeletal. The former have already been described. The skeletal lesions resemble those of the guinea-pig, the commonest sites being at the costochondral junctions, the distal ends of the femora, and the proximal ends of the tibiæ and femora and the wrists. The lesion is characterized by rarefaction of the cortex and conical widening of the bone, inhibition of bone growth and replacement of the normal junction of bone and cartilage by a zone of connective tissue poor in collagen and containing fragments of densely calcified cartilage with no osteoid tissue. These changes result from the inability of the osteoblasts to form normal osteoid tissue in the absence of adequate ascorbic acid with consequent attempts at fibrous tissue union between the epiphysis and diaphysis. Epiphyses may be separated and fractures occur.

The dental lesions in human scurvy include hyperæmia and œdema of the pulp, degeneration of the odontoblast layer with cyst formation, destruction and calcification of vessels and necrosis and calcification of areas in the pulp. The dentin becomes porotic and resorption occurs along Tomes' canals, which widen into spindle-shaped and round spaces. Abnormal and irregularly canalized dentin is formed. The commonest lesions are in the apical third of

the tooth. Lesions may occur in the teeth before they do in bone. The loosening and falling out of the teeth in advanced scurvy is due to the rarefaction of the alveolar bone.

The gingival lesions consist of hyperplasia of the papillæ, development of granulation tissue and finally gangrene; they do not occur unless teeth are present.

In the older literature on scurvy œdema, particularly around the ankles (see Fig. 161), enlargement of the heart, hydropericardium and hydrothorax were described. Neuritic changes, such as degeneration of the peripheral nerves and large anterior horn cells have been described, as well as sensory disturbances and reduced or absent knee-jerks. Ulceration of the gastrointestinal tract, atrophy of various organs and endocrine glands are also mentioned. It is almost certain that all these changes do not result from ascorbic acid deficiency; inanition, repeated hæmorrhage, multiple vitamin deficiencies and infection all play a part.

The morphological changes of scurvy are greatly modified by growth, activity, stress, trauma and the presence of complicating factors increasing the ascorbic acid requirements. Generally speaking the extent and severity of the lesions diminish with age, particularly in the skeleton, the bones most affected being those in which growth happens to be most active at the time of the deficiency. Stress (motion, pressure) exerts an important effect on the site and extent of hæmorrhage. Infections may precipitate scurvy in a person bordering on this condition.

In the scorbutic guinea-pig pathological changes occur in the cells of the liver and kidney (Figs. 171-173) as evidenced by the deposition of trypan blue in these cells after subcutaneous injection of this dye [1001]. Fatty metamorphosis also occurs in the liver cells [472]. Aschoff and Koch [39] described advanced fatty degeneration in the liver cells in human cases of scurvy, although this may have been due to multiple deficiencies. Beyer [952] observed that fatty degeneration of the liver produced by toxins occurred more readily in scorbutic guinea-pigs than in those receiving adequate ascorbic acid (Figs. 148-150).

Deranged tyrosine metabolism occurs in human scurvy. This is discussed on p. 427.

A close relationship between ascorbic acid metabolism and adrenal cortical function is suggested by evidence from animal experimentation (p. 430), and diminished adrenal cortical activity might therefore be expected in human scurvy. This does not appear to be so, because the blood eosinophil response to A.C.T.H. (Thorn's test) is normal, and blood glutathione, potassium and sodium in scurvy are normal and unaltered by ascorbic acid therapy [303].

Diagnosis of Scurvy. Clinical diagnosis of mild scurvy is difficult and uncertain. In frank scurvy in the adult the diagnosis is made on the multiple petechiæ, hæmorrhagic manifestations, mouth lesions, extreme fatigue and bad dietary history. In isolated cases the disease may be confused with purpura. Mercurial stomatitis may resemble the oral lesions of scurvy, but it does not present the other features. Acute leukæmia should be considered in the differential diagnosis; here the blood count is diagnostic.

Diagnosis in the infant is not always easy, although the typical drawn up and immobile appearance of the legs (p. 169) and the acute pain on touching the limbs are characteristic of the severe case. The finding of a few blood cells in the urine is a most valuable sign. Osteomyelitis, with its painful limbs, and poliomyelitis, with its immobility, may cause some confusion, although high fever is against the diagnosis of infantile scurvy, and in the latter there is no true paralysis or neurological disturbance. Rheumatic fever, which is, however, rare below the age of two, syphilitic epiphysitis, and Parrot's disease (syphilitic pseudoparesis) may have to be considered. Radiological examination of the bones will help to establish the diagnosis.

PATHOLOGICAL CHANGES IN LIVER AND KIDNEYS IN SCURVY

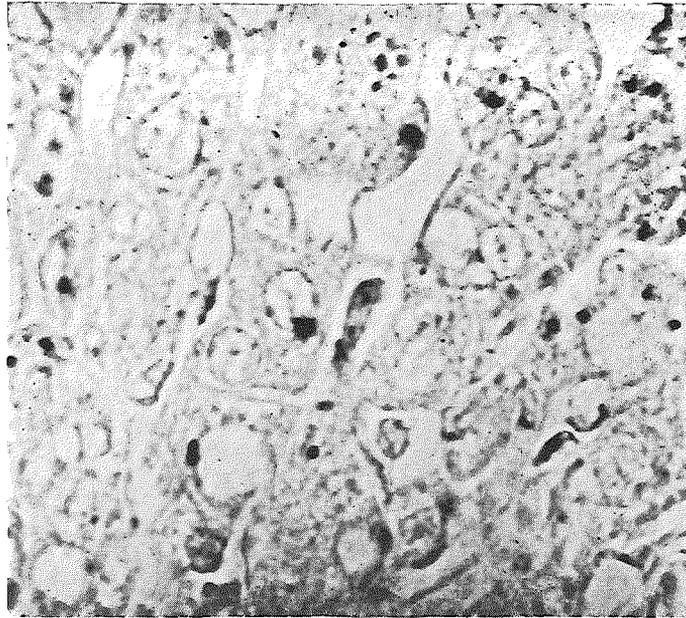
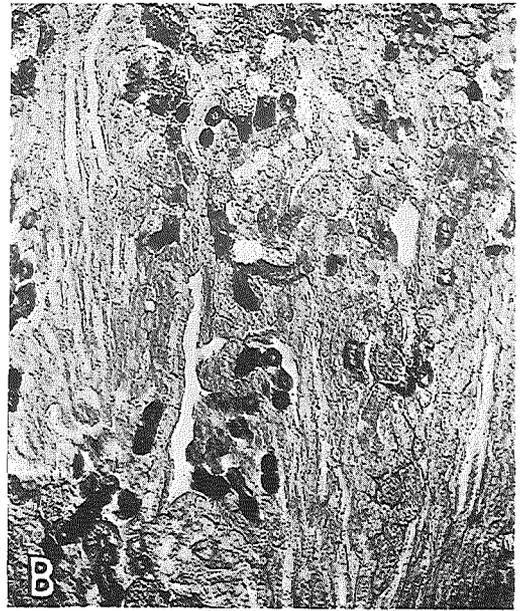
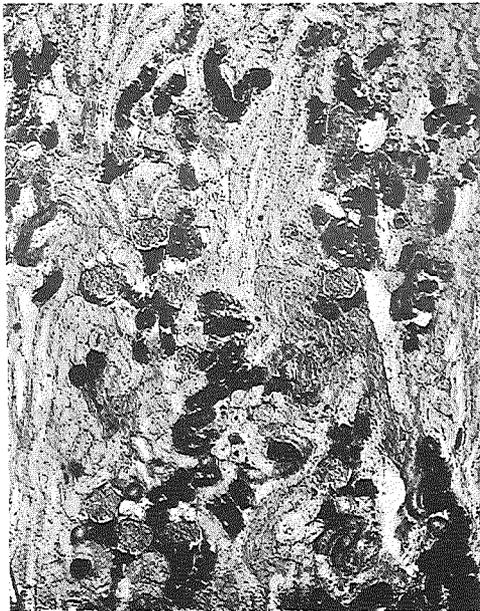


FIG. 171. Pathological Changes in the Liver in Scurvy. Section of liver of a scorbutic guinea-pig after intravenous injection of trypan blue dye, which is visible as dark dots in the cytoplasm of the liver cells. Large fat vacuoles, indicating fatty degeneration, are also present in the liver cells. Control animals showed neither concentration of dye in the liver cells nor fatty degeneration. As trypan blue has an affinity for pathologically altered tissues, these changes are evidence of hepatic damage from ascorbic acid deficiency.



FIGS. 172 and 173. Pathological Changes in Kidney in Scurvy. Left (A) is section of renal cortex of a scorbutic guinea-pig after intravenous injection of trypan blue, showing large granular masses of dye in the cells of the proximal convoluted tubules. Right (B) is a section of the renal cortex of a control animal showing only slight amounts of dye. As trypan blue has an affinity for pathologically altered tissues, the changes are evidence of renal damage in ascorbic acid deficiency.

Special Tests for Scurvy. A number of tests for the detection of ascorbic acid deficiency have been devised. These are described later (p. 464).

Treatment of Scurvy. Treatment in uncomplicated cases consists in the administration of fruit juices and large doses of ascorbic acid, e.g. 200 to 300 mg. two or three times a day by mouth, taken preferably before or with meals, since gastric acid probably assists in absorption. There is no need to give ascorbic acid parenterally unless there is nausea or vomiting or the patient has gastro-intestinal lesions likely to interfere with absorption. The parenteral dosage is somewhat smaller than the oral dose. Overdosage is wasteful, but does no harm [847]. In cases of mild or latent scurvy and infantile scurvy 100 mg. daily by mouth is probably adequate. Fruit juices are also recommended, as cases have been described that are resistant to pure ascorbic acid. Intensive ascorbic acid treatment is continued until the vitamin is freely excreted in the urine or until the lesions are healed, and then a maintenance dose of 50 to 100 mg. daily given orally.

Many cases of scurvy are complicated by other vitamin deficiencies, and these should be searched for and treated. An enriched nutritious diet should be given to the scorbutic patient as well as specific vitamin therapy. The local lesions are treated symptomatically, e.g. mouth washes and mild disinfectants for the mouth to prevent infection; light splints for the legs in infantile scurvy.

Prevention is better than cure, particularly in the case of infantile scurvy. This need never occur if infants, particularly the bottle fed, are given fruit and vegetable juices sufficient to supply 20 to 40 mg. of vitamin a day (p. 445).

SUBCLINICAL SCURVY, THE PRESCORBUTIC STATE, SUBVITAMINOSIS C, AVITAMINOSIS C (HYPOVITAMINOSIS C, PARAVITAMINOSIS C, ASYMPTOMATIC SCURVY)

A state of ascorbic acid deficiency without the clinical manifestations of scurvy has been described under the above names. Diagnosis is not made on definite clinical grounds, but usually from laboratory tests or from the dietary history. The conception of subclinical forms of scurvy is due to Hess [447], who as far back as 1917 pointed out that an asymptomatic stage of scurvy with well-marked skeletal lesions may precede clinical infantile scurvy. It has been observed that skeletal lesions characteristic of scurvy can be demonstrated in animals suffering from ascorbic acid deficiency without clinical signs of scurvy [449]. In the human being a group of symptoms characterized by susceptibility to infection, unreasonable loss of vitality, lessened endurance, gingivitis, vague pains, bodily fatigue, loss of appetite and mild digestive disturbances have been attributed, probably incorrectly, to ascorbic acid deficiency. Certainly a low degree of excretion has been detected in persons showing such a group of symptoms, but, on the other hand, it is known that an individual may excrete very little ascorbic acid and have low plasma values and yet be in excellent health. Dahlberg, Engel and Rydin [836] carried out carefully controlled observations on five thousand soldiers and concluded that half of them suffered from ascorbic acid deficiency as judged by urine and saturation tests, yet their health was as good as the other half, who received an extra 50 mg. of ascorbic acid daily. Many workers state that they have been unable to recognize "subvitaminosis C" as a clinical entity and deprecate the use of this expression applied to subjects unsaturated with ascorbic acid or who excrete only small amounts. Hjärne [911] has been unable to correlate the general clinical condition of children with their blood ascorbic acid level.

On the other hand, children in the poorer classes sometimes receive suboptimal amounts of ascorbic acid [837-839] and respond to improved diets [451]. The beneficial effects of the so-called Oslo breakfast, with its fresh vegetables or salad, has been observed by school medical officers. The

results may be due to improved nutrition generally and not to an increased intake of ascorbic acid only. It has been stated, with very little evidence, that the increased incidence of infection and general fatigue in the spring [451] are due to the gradual depletion of the body's stores of ascorbic acid over the winter months when fresh foodstuffs are scarce.

Many cases of inflammatory dental disease such as gingivitis have been attributed—probably incorrectly—to lack of ascorbic acid, and not so much to lack of dental hygiene [995]. It is unlikely that the average case of gingivitis is due to ascorbic acid deficiency (see p. 476).

According to some investigators anæmia is prevalent in states of ascorbic acid subnutrition. This is difficult to reconcile with the observations on scurvy and anæmia (pp. 453-454) and with the observation of Lozner [457] that normal regeneration of hæmoglobin can occur in patients whose plasma contains no ascorbic acid.

So-called ascorbic acid deficiency as judged by urine, saturation and blood tests has been detected in a number of patients suffering from various clinical conditions, and there has been a tendency to associate these ætiologically with ascorbic acid deficiency. This is an uncritical approach because apparent ascorbic acid deficiency, judged by laboratory tests, can be demonstrated in apparently healthy controls. It is far more likely that in the case of the diseased patient his disease produces a "conditioned" ascorbic acid deficiency. Thus infections and diseases with increased metabolism may increase the requirements of ascorbic acid; gastro-intestinal diseases will interfere with its absorption and the patient on a "gastric diet" receives a very low intake; in nervous and mental diseases there is often a restricted intake of food, and hence of ascorbic acid. The remarks on conditioned vitamin deficiency in the chapter on Aneurine (p. 223) apply equally well to ascorbic acid. Croft and Snorf [521] estimated the blood ascorbic acid of a hundred unselected patients, and of these thirty-eight were considered to be suffering from ascorbic acid deficiency as their blood levels were below 0.4 mg. per 100 c.c. Most of them suffered from gastro-intestinal conditions, which undoubtedly conditioned the deficiency. None, however, showed any definite scorbutic signs.

Gingival Manifestations. Kruse [995] states that macro- and microscopic examination of the anterior gums affords a simple, convenient and objective method of detecting ascorbic acid deficiency. In many cases he says that the gingival manifestations are readily seen naked eye; in some only by bio-microscopy. Both gums are not equally affected, the upper gum often, although not invariably, showing the more advanced process. The sites affected are involved in a definite order: interdental papillæ, the marginal gingivæ, and then the alveolar gingivæ. Kruse divides the changes into acute and chronic, with three stages in each.

In the *acute process* the subsurface vascular papillæ are engorged and dilated, and under the microscope enlarged and congested capillaries are visible. In mild cases this change is restricted to the interdental papillæ and then to the marginal gingivæ. In more severe cases the vascular reaction may be seen all over the gum. At this stage there is no swelling. In the second stage the gum is reddened, first at the points of the interdental papillæ, spreading to the bases and then the marginal gingivæ. In more marked cases the whole gum is intensely red due to the deeper vessels becoming larger and engorged. At this stage there is very little or slight swelling. The subsurface capillary papillæ become less discrete and distinct. In the third stage the reddened gums swell and, although the changes may be restricted to the interdental papillæ, more often the marginal gingivæ are affected and form a red swollen collar projecting around the necks of the teeth. Frequently the entire gum is red and swollen and the swelling may be so intense as to stretch the gum and give it a glossy appearance. The gum may recede slightly from its free edge, increasing the length of the crown, and if it is very swollen a

sulcus may form between the gum and teeth, becoming filled with calculus or infected *débris*. Infection of the gum and bleeding gums are common. These changes precede those of frank scurvy in which the gums are congested, bleeding, ulcerated and spongy and are covered with necrotic material. Later the teeth become loose and fall out and the alveolar process becomes necrosed.

In the chronic process the subsurface vascular papillæ show slight dilatation and engorgement, so that gums appear reddened and slightly swollen, the process commencing in the interdental papillæ, extending to the gingival margin and finally over the whole gum. In the second stage the redness and swelling are slowly masked by œdema and infiltration, so that the gum appears swollen and pale. The process may be confined to the papillæ or spread over the whole gum. Atrophy, in the form of pitting, occurs in the third stage. The pits or depressions occur first on the interdental papillæ, then on the gingival margins and finally over the whole gum. Although often visible macroscopically the pits are best seen microscopically. In the next stage the atrophy becomes more profound and the pits gradually disappear. The papillæ get smaller and recede from the gum, leaving the cement exposed. Finally the whole gum shows pronounced atrophy, becomes white in colour, and the teeth are loosened and extruded.

Kruse states that these changes are reversible by giving ascorbic acid. He remarks that in all cases a long period of time is needed for recovery, more than a year in some cases, even with intensive treatment.

Kruse's observations conflict with those of Crandon [68], Farmer [1078] and the Medical Research Council team [407]. After six months on a scorbutic diet Crandon failed to observe any pathological changes in his teeth and gums, although the level of ascorbic acid in the plasma and white cell layer was zero. This has been confirmed by Farmer and his colleagues [864], who kept volunteers on a scorbutic diet for five months, but failed to observe any pathological changes in the gums, teeth or bones of the jaw as observed with the naked eye, biomicroscope and X-rays. The Medical Research Council workers stated that gum changes only appear after the skin lesions. As in Crandon's case the level of ascorbic acid in the plasma and white cell layer was zero. Kruse's work has been severely criticized by King [536], who states: "*(His)* conclusions disclosed such unfamiliarity with gingival pathology and gingival capillaroscopy as to render them of dubious value."

Gums in apparently normal people often bleed on touching with a probe. Redness, swelling and bleeding of the gums can be caused by trauma and œstrogens, and can occur during menstruation and pregnancy and as a result of over-smoking.

LABORATORY METHODS USED FOR DETECTING ASCORBIC ACID DEFICIENCY

Capillary Fragility Tests. Tests involving the measurement of capillary fragility were the first to be used for the detection of ascorbic acid deficiency. In the *positive pressure method* devised by Göthlin [82, 83], also known as the Rumpel-Leede test, the exact technique is as follows: A circular area 60 mm. in diameter is marked off in the antecubital fossa and a blood pressure cuff placed at least 2.5 cm. above this and pumped up to 50 mm. mercury pressure, which is maintained for fifteen minutes. After the pressure is reduced the number of petechiæ is counted. If many are present the test is repeated not less than forty minutes later on the other arm at a lower pressure of 35 mm. of mercury. The results are graded according to the following scheme:—

- Grade I. No petechiæ within the examined skin area at 50 mm. pressure for fifteen minutes.
- Grade II. Petechiæ appearing at 50 mm. mercury, but less than six in number.

Grade III. More than six petechiæ at 50 mm. mercury, but two or less at 35 mm. mercury.

Grade IV. At least two petechiæ at a pressure of 35 mm. mercury.

The pressure of the cuff must be infradiastolic so that there is an uninterrupted flow of blood to the forearm.

The test is carried out at room temperature (16°–21° C.) and the patient should not have a hot bath on the day of the test or take any exercise within three hours of it. Göthlin considers that more than six petechiæ at 50 mm. pressure for fifteen minutes are abnormal and he believes that this capillary fragility test serves as a measure of the physiologically indispensable minimum requirement for ascorbic acid. From simultaneous ascorbic acid tests on blood a positive Göthlin test is stated to correspond to a blood level of between 0.1 and 0.14 mg. of ascorbic acid per 100 c.

Wright [522] modified the test by inflating the pressure cuff midway between the systolic and diastolic pressures for fifteen minutes and then counting the petechiæ in a 2.5 cm. circle on the flexor surface of the forearm. A petechiæ count of 0 to 10 is considered normal ; from 10 to 12 borderline ; and above 12 abnormal.

Dalldorf [523] employed a *negative pressure method* for determining capillary fragility. He applied a 1 cm. suction cup to the skin of the upper arm near the deltoid insertion and applied varying pressures for one minute. If petechiæ appeared at a negative pressure of 25 mm. of mercury or below, the capillary fragility was considered abnormal.

Scarborough [510] determined the capillary resistance in three standard areas on the arm, using negative pressure applied for half a minute.

The capillary fragility test has been widely used, but the results obtained are not consistent. Re-investigation of the test by a number of workers has shown that it is of no value in the diagnosis of scurvy or ascorbic acid deficiency [66–68, 81, 347, 350, 526–532, 553, 1010]. For a more detailed discussion see p. 415.

The Intradermal Test. In 1937 Rotter [533] described a test for the diagnosis of ascorbic acid deficiency depending upon the power of the ascorbic acid of the skin to decolorize the blue dye, 2 : 6-dichlorophenolindophenol. His test consisted of injecting 0.1 ml. of a 1 in 400 dilution of the dye into the skin of the volar surface of the forearm. According to Rotter, if the individual is saturated the ascorbic acid in the skin decolorizes the dye in less than five minutes ; normal cases decolorize it in five to ten minutes, and unsaturated individuals require longer than ten minutes. Shortly after its introduction several workers used the test and considered it adequate as a rough test to estimate ascorbic acid deficiency [534, 535, 1072] and Slobody and his co-workers [1002], who modified the test, considered that there was a correlation between Rotter's decolorization time and the plasma ascorbic acid level. They injected 0.05 ml. of N/300 dye solution and stated that decolorization took more than fourteen minutes if the plasma ascorbic acid was below 0.3 mg. per 100 ml.

More recently a number of other workers have submitted the test to extensive trial, but they have found no correlation between the decolorization time of Rotter and the clinical condition of the patient, or between it and ascorbic acid levels in blood or urine [537–541]. Technically it is difficult to inject small quantities of solution with precision, but even using an improved syringe capable of injecting exactly 0.01 c.c., Goldsmith [540] could obtain no correlation between the intradermal test and blood serum values. Changes in position and temperature considerably affected decolorization time. Goldsmith subjected the test to a statistical study and her results indicated no specificity for the method. MacLenathen [539] found that the variations in the decolorization time were too great for any normal standard to be set up, and there was frequently a wide variation in the response at

areas within a few centimetres of one another. Holland [488] found that variations in decolorization time occurred when the test was performed simultaneously on both arms. Other factors not considered in devising the test are the possibilities of other reducing substances in the skin, and local changes in the circulation, oxygen supply, and lymphatic drainage of the forearm. With all these possible variables the test does not appear to have any clinical value.

Urinary Excretion of Ascorbic Acid. Single determinations of ascorbic acid in the urine are of no value as an index of ascorbic acid nutrition. The twenty-four hour excretion has been used, but this is equally valueless as it only reflects the immediate intake and not the extent to which tissues are affected. Twenty-four hour collections of urine are preserved by adding enough twelve per cent. metaphosphoric acid to the receptacle to maintain a final concentration of two to three per cent, or two per cent. oxalic acid is added. An alternative but less satisfactory method is to add 10 ml. of glacial acetic acid to every 100 ml. of urine, which is kept in a refrigerator or dark bottle. 8-Hydroxyquinoline is also a good preservative. Using acetic acid there is a loss of ten per cent. of ascorbic acid for every hour's storage; with phosphoric acid the loss in the same time is only one per cent. [1003]. Wright [555] has shown that even with preservative a total loss of twelve per cent. of the ascorbic acid may result on storing the urine until the next day; in warm weather with partially filled bottles the loss may reach forty-six per cent. [556]. The principle of the estimation is to take a measured amount of recently standardized 2 : 6-dichlorophenolindophenol and determine the amount of urine which has to be added to it to discharge its colour, the titration being carried out rapidly. The dye is made up in aqueous solution at a strength of about 0.1 per cent., so that 0.05 ml. (i.e. 0.5 mg.) is equivalent to roughly 0.025 mg. of ascorbic acid. The solution should be freshly prepared and standardized against crystalline ascorbic acid, 0.025 gram of which are dissolved in 50 ml. of water for the purpose of standardization. The purity of the ascorbic acid is further checked against 0.01 N iodine solution. In titrating the urine a 2 ml. microburette reading to the nearest 0.01 ml. is used. The urine is run from the burette into the dye solution conveniently contained in a pointed centrifuge tube.

2 : 6-Dichlorophenolindophenol is also decolorized by other substances present in the urine, e.g. drugs [559] and some normal products of metabolism (p. 392). Richter and Croft [1003] estimate the "true" ascorbic acid in urine by eliminating thiosulphates and sulphur-containing substances in the urine with lead acetate before titration with indophenol dye. Another method of estimation using indophenol dye is to add excess of the latter and extract the excess with xylene, the difference giving the amount reacting with the ascorbic acid in the urine [459]. The use of hydrogen peroxide and formaldehyde eliminates the interference of iron, sulphites and reductones [459].

Ascorbic acid can be estimated in urine by an entirely specific method depending on its oxidation to dehydroascorbic acid, which is separated as the crystalline 2 : 4-dinitrophenylhydrazine derivative [360] (p. 392).

Excretion tests are of no value as a measure of ascorbic acid deficiency. The excretion in health and disease varies enormously, from almost zero to 50 mg. daily (p. 437). Kassan and Roe [367] examined a series of apparently normal medical students and found that fourteen excreted no ascorbic acid, yet they had no signs or symptoms of ill health. Roe and Hall [360] state that no less than fourteen out of fifty subjects on an apparently adequate diet showed no excretion of ascorbic acid. Pijoan [873] lived on a diet poor in ascorbic acid and excreted none in the urine for twenty months without any ill effect. Fox [344] has repeatedly observed low excretions for African natives in good health. Conversely the urinary excretion of ascorbic acid is not always as low as would be expected in cases of frank avitaminosis. Schultzer [369], for example, found mean daily excretions in scorbutic

patients ranging from 13 to 24 mg. According to some observers a daily excretion of 24 mg. of ascorbic acid would indicate an adequate intake of the vitamin. Kellie and Zilva [359] have shown that the amount excreted can be made to vary at will by adjustments of the diet.

There are several factors, including a fluctuating renal threshold, which may affect the urinary excretion of ascorbic acid besides a diminished intake (p. 437). Deeny [970] has shown that marked hourly variations occur in the excretion on a standard intake, and at all physiological levels of intake. The position is well commented on by Sinclair [453]: "There is no reason why a person in full health should be expected to excrete any ascorbic acid; he is not expected to excrete glucose in the urine."

Saturation Tests (Test Dose). The urinary excretion of ascorbic acid following the administration of a test dose of the vitamin has been used as a criterion of the body stores and as a means of detecting early states of ascorbic acid deficiency. The rationale of the test is based on the hypothesis that, following the administration of ascorbic acid, the requirements of the tissues for the vitamin are satisfied before its concentration in the blood rises to the threshold value and is eliminated in the urine. If a test dose of ascorbic acid is given to a normal subject receiving 25 to 50 mg. daily a large rise in the urinary excretion of ascorbic acid occurs on the first day of the test or certainly on the second; if the ascorbic acid intake is lower than normal the response is less or delayed by one or more days [560, 866].

Oral Test Dose. In the original method of Harris and Ray [385] twenty-four-hour specimens of urine were collected on consecutive days to determine the "resting level" of excretion and response after repeated daily test doses noted. Then Harris and Abbasy [356] introduced a simplified method. When first seen, the subject emptied the bladder; three hours later a specimen of urine was obtained, any passed during the interval added to it, and the whole titrated for ascorbic acid. The same programme was repeated on the second and third day. The next day the test dose of 700 mg. of ascorbic acid per 10 stones of body weight was given in water and the urine collected three to six hours later, i.e. the time of peak excretion. The test was repeated on subsequent days until a sharp rise in excretion occurred. According to Harris subjects on an intake of 25 to 50 mg. of ascorbic acid per 10 stones body weight showed a sharp rise in the urinary excretion of ascorbic acid on the first or certainly the second day of test dosing. Harris calculated what he considered to be the body deficiency of ascorbic acid by multiplying the test dose by the number of days' dosing needed to obtain a sharp rise in the urinary excretion of ascorbic acid. In a later paper Harris [1004] defines "saturation" as sufficient storage of ascorbic acid in the body that an excretion of 50 mg. or more occurs within four to five hours of a test dose of 700 mg. ascorbic acid per 10 stones body weight. With an intake of 45 to 75 mg. or more of ascorbic acid daily a response occurs on the first day of test dosing. On an intake of 37 mg. ascorbic acid daily the response occurred on the first or second day of dosing; on 23 mg. daily the response is on the second or third day. Some of Harris's test subjects required ten days' dosing for saturation.

Since the original work of Harris in 1935 many workers have devised methods for assessing the level of ascorbic acid nutrition by a test dose technique. The test doses vary from 200 to 600 mg. Beck and Schorlemmer [568] and Gander and Niederberger [569] collect the urine over a period of six hours after a 300 mg. test dose, and consider that a concentration of 5 mg. or more of ascorbic acid per 100 ml. urine is adequate. Pemberton [562] gives a test dose of 70 mg. per stone body weight and states that a sharp rise in the excretion should occur by the sixth hour. Goldsmith and Ellinger [567] found that it occurred between the first and sixth hour after a dose of 600 mg. Engelfried [907] states that six-hour excretion after a test dose of 200 mg. is satisfactory. These methods have been criticized by Richardson and

Mayfield [561], who question the validity of the six-hour period. They find that the volumes of urine and the amount excreted at each urination differ considerably even in the same person, and they could find no correlation with the excretion at six hours and twenty-four hours.

Others have given a test dose of 200 to 600 mg. ascorbic acid and consider an excretion of fifty per cent. or more of this within twenty-four hours or twenty-five per cent. in twelve hours a satisfactory index [376, 462, 563, 877]. Spellberg and Keeton [357] and Youmans [565] consider an excretion of thirty per cent. of a test dose in twenty-four hours an adequate index of satisfactory intake. Wright [566] regards an excretion of less than twenty per cent. in twenty-four hours a sign of suboptimal intake. Baumann [564] gives a daily dose of 50 to 100 mg. of ascorbic acid daily and states that normal subjects excrete sixty to eighty per cent. of the test dose on the third or fourth day.

Parenteral Test Dose. Some workers give the test dose parenterally. This route is unnecessary, because it has been shown that ninety-nine per cent. of a test dose given orally is absorbed (p. 434). It may also give erroneous results because, if given intravenously or even intramuscularly, ascorbic acid quickly reaches the blood stream and begins to be excreted before the tissues can absorb it. The size of the test dose and the interpretation of the results are even more variable than with the oral test dose. Berryman and others [905] have shown that there is a considerable variation in the amount excreted after the intravenous injection of 200 mg. ascorbic acid, even on a fixed oral intake of 100 mg. in the diet. Wright [570] gives the very large dose of 1,000 mg. of ascorbic acid intravenously and considers an excretion of 400 mg. after five hours or 500 mg. after twenty-four hours an index of adequate intake. Ralli and her co-workers [572] regard an excretion of 40 mg. within three hours after an intravenous test dose of 100 mg. as evidence of saturation. Kastlin and Schlesinger [573] found that normal subjects excreted forty per cent. of an intravenous dose of 500 mg. within four hours. Van Eekelen and Heinemann [574] warn against the erroneous conclusions that might be drawn from the intravenous injection of large doses and employ a dose of 4 mg. per kilo. subcutaneously. Practically the whole of this is excreted in six hours, peak excretion occurring at three hours. Remel and Schenk [611] consider an excretion of less than 18 to 20 mg. of ascorbic acid after an injection of 200 mg. indicates a deficient intake.

Several investigators [240, 550, 576] have pointed out that in saturation tests a delay in excretion may occur in persons with impaired renal function (see p. 438). Ludden and Wright [550] state that erroneous values may be observed in such persons when the ascorbic acid content of single urine specimens obtained three, five, six or eight hours after any test dose, of whatever size, are used as criteria of the state of ascorbic acid nutrition. They have introduced a modified technique, which they say is valid even in cases of impaired renal function, with the exception of uræmia. The test is conducted as follows :

The patient omits breakfast on the morning of the test. Immediately after he has micturated and discarded the preliminary specimen of urine, 1 gram of ascorbic acid in 10 ml. of normal saline is given intravenously. A specimen of urine is collected exactly one and a half hours after the injection and another exactly five hours after. The twenty-four-hour output can then be predicted from the formula, $\frac{ab}{1.26 a - 0.27 b}$, where a and b are the ascorbic acid excretions over one and a half and five hours respectively. Wright and Ludden term this expression the *Saturation index*. The formula is arrived at from a definite correlation between the percentage of the first five hours' excretion of ascorbic acid excreted during the first hour and a half, and the percentage of the twenty-four hours' excretion excreted during the first five hours.

Criticism of Test Dose Studies. In criticism of the test dose technique for detecting ascorbic acid deficiency it must be admitted that it has not yet been demonstrated that "saturation" with ascorbic acid is a normal state; in fact it is definitely abnormal because very few subjects are saturated with respect to ascorbic acid if they rely on natural sources of the vitamin. Further many people in the best of health have been shown to be unsaturated. As Crandon [68] has shown, it is possible to feel in good health and excrete no ascorbic acid. There is no reason why ascorbic acid should be excreted in the urine any more than glucose. Excretion and saturation tests do not measure the ascorbic acid reserves of the white cell layer and the tissues, which retain their ascorbic acid even when the urinary excretion is low.

The measurement of the level of ascorbic acid nutrition by the test dose technique is a purely arbitrary procedure. A massive and unphysiological dose of ascorbic acid is given and the excretion studied over a period of several hours, the dose varying from one worker to another. The interpretation of the results also depends upon the individual worker. Some give daily supplements until there is a sudden rise in the urinary excretion; others consider that the subject is saturated when fifty per cent. of a test dose is excreted. If these arbitrary figures are accepted, and there is no reason why they should be, there are still sources of error. The renal threshold is variable (p. 437); the excretion of ascorbic acid also depends upon the acid-base equilibrium and the composition of the diet; and Deeny [970] has shown that the excretion of ascorbic acid on a given intake varies markedly from hour to hour, thus throwing considerable doubt on the value of the test dose technique in which the excretion of ascorbic acid is found during a period of four to six hours after giving the test dose. Berryman and his co-workers [905] have shown that there is considerable variation in the ascorbic acid excretion after a test dose.

The futility of using excretion studies as an index of ascorbic acid nutrition has been demonstrated by Lowry, Bessey and Birch [1034], who found that an intake of 1 gram daily for three months did not affect the ultimate excretion of the vitamin; the unwanted excess was metabolised.

Estimation of Ascorbic Acid in the Blood. The 2:6-dichlorophenolindophenol titration test has been applied to the estimation of ascorbic acid in blood. A number of techniques using this test have been developed for the estimation of ascorbic acid in the plasma [360, 577-586]. Oxalated blood is first centrifuged and the plasma deproteinized with metaphosphoric acid. After recentrifuging, the clear liquid is titrated against the dye indicator or methylene blue as for urine. Using a methylene blue micro-method Butler and Cushman [1006] estimate the ascorbic acid in 0.2 ml. of capillary blood. A method that is commonly used is that of Roe and Kuether [360], which depends on the reaction between 2:4-dinitrophenylhydrazine and dehydroascorbic acid to form a red compound which can be estimated colorimetrically. Isolated analyses of blood plasma values have proved quite worthless in estimating degrees of ascorbic acid subnutrition. Estimation of the plasma ascorbic acid is only a measure of the immediate nutritive or metabolic level and is dependent on the recent dietary intake. It does not reflect the tissue stores.

High fasting values may indicate satisfactory nutrition [466], but low values do not provide a reliable index. Healthy persons have ascorbic acid plasma levels ranging from 0.1 mg. per 100 ml. [591] to 2.43 mg. per 100 ml. [578]. According to McMillan and Inglis [927] there is no relationship between the blood figures and the clinical picture in scurvy. Dodds and Macleod [312] correlated plasma ascorbic acid with the daily intake. The wide range of values indicated that conclusions on the level of ascorbic acid nutrition based on plasma levels could be entirely fallacious. Urbach, Hickman and Harris [1035] found that even after administering 100 to 180 mg. of the vitamin daily for six months the final plasma ascorbic acid was no different from that

of controls. The threshold value may vary in different individuals (p. 437), and plasma values are higher during menstruation [1007]. Some observers consider that plasma values below 0.5 to 0.7 mg. per cent. indicate a state of ascorbic acid depletion, and that values from 0.7 to 1 mg. indicate mild deficiency. These figures are purely arbitrary ones, based on "saturation" tests, and there is no clinical justification for them. Ralli [378] and her associates believe "saturation" to be the ideal, with blood plasma values of 1 mg. per 100 ml. or over. According to Bryan [466] and his co-workers saturation occurs with intakes of 1.7 to 1.9 mg. of vitamin per kilo. of body weight. It has never been satisfactorily demonstrated that the body is saturated with respect to ascorbic acid in normal healthy persons.

On a daily intake of 60 to 80 mg. of ascorbic acid, which covers the recommended intake of the National Research Council, U.S.A., the plasma ascorbic acid is about 0.7 mg. per 100 ml. The general opinion is that a fasting plasma level of 0.6 to 0.8 mg. or more is adequate, although this range is purely arbitrary and there is no justification for it.

Crandon [68], Fox [344], Rietschel [342] and others have shown that plasma ascorbic acid may be exceptionally low without clinical evidence of ascorbic acid deficiency. According to Rietschel plasma levels of 0.2 to 0.3 mg. are not abnormal. He kept a subject on a diet free of ascorbic acid for one hundred days without producing scurvy; the ascorbic acid plasma level was zero. Pijoan [873] also maintained a plasma level of 0.0 to 0.2 mg. per 100 ml. for twenty months without ill effects. Dagulf [463] reports that three hundred and twenty-six healthy Swedish people had an average plasma ascorbic acid of 0.22 mg. per 100 ml. In scurvy, too, the values are not always exceptionally low, though they all fall within the range of 0.0 to 0.5 mg. There is certainly complete lack of correlation between the clinical condition and plasma ascorbic acid values. Thus Croft and Snorf [521] noted blood values of only 0.12 mg., and Kassan and Roe [454] levels of 0.02 mg., without any signs of scurvy. Owens [257] has also observed levels below 0.4 mg. in fifty diabetics without clinical evidence of ascorbic acid deficiency. Holmes [637] examined the plasma ascorbic acid in sixty healthy children and low levels were observed, which persisted over a period of ten months. The ability to maintain a fixed level of ascorbic acid in the plasma, no matter how small, indicates a positive ascorbic acid balance [873].

There is evidence that accurate information on the level of ascorbic acid nutrition can be obtained from analyses of whole blood [301, 360, 408, 477, 714] or of the white cells and platelets [588-590]. Bessey, Lowry and Brock [413] have devised a method for estimating ascorbic acid in whole blood using 0.1 ml. of blood. Butler and Cushman [590] have shown that whole blood, and the white layer of the centrifuged blood of subjects whose ascorbic acid nutrition would be considered poor may contain measurable amounts of ascorbic acid (25 to 38 mg. per 100 ml.) even when the plasma level is zero. Crandon [68], whilst on a scurvy producing diet, noted that after forty-one days his plasma ascorbic acid was zero, but even after eighty-two days the white cell platelet value was 4 mg. per 100 ml. It would appear that the apparent ascorbic acid content of whole blood or of the white blood cells plus platelets of individuals not suffering from infection or leukæmia provides a far more reliable index of ascorbic acid deficiency than plasma values. Whole blood determinations afford the best index for "saturation." What is important is the tissue reserves of ascorbic acid (not the plasma level), which is best measured by the concentration in whole blood or the white cell layer. If this falls steadily the intake of ascorbic acid is inadequate. According to Butler and Cushman [590] the average normal ascorbic acid of the white cells and platelets is 34 mg. per 100 grams, with a range of 29 to 43 mg. Even massive doses of 1 gram daily do not raise it above normal levels [1034]. They have shown that ascorbic acid passes from the plasma to the red blood cells and that the distribution ratio of the plasma concentration to the red

cell concentration varies with the state of ascorbic acid nutrition. The distribution between plasma and whole blood is related to the level of ascorbic acid in the blood. At whole blood levels below 0.6 mg. per 100 ml. the plasma content is lower than the whole blood content; at whole blood levels above 0.9 mg. per 100 ml. the plasma content is higher than that of whole blood [388, 397]. In patients with leukæmia the concentration of reducing substance in the white cell layer is as high as 140 mg. per 100 ml. when the plasma ascorbic acid is as low as 0.2 mg. If the plasma level of ascorbic acid were taken as an index of ascorbic acid nutrition, these patients would have been considered to be suffering from considerable deficiency.

The plasma values do not reflect the amount of ascorbic acid stored in the body; they merely represent an overflow, and when this is high enough ascorbic acid is excreted. Whole blood values indicate the extent of saturation down to a state of marked depletion, and while values above 5 mg. per 100 ml. exclude scurvy, values below this are equivocal. Farmer [864] observed zero plasma ascorbic acid values in volunteers on scorbutic diets without clinical evidence of scurvy. It took seventy days on the diet for the plasma ascorbic acid to fall to zero. White blood cell concentrations of ascorbic acid are the best indication of ascorbic acid deficiency. Technical difficulties attached to estimating its concentration in white cells have prevented its routine use.

Blood tests for ascorbic acid deficiency have been used in which a test dose of vitamin is injected intravenously or intramuscularly and the blood levels studied at varying intervals afterwards. Kajdi [592] and her co-workers proposed a new saturation test, in which the plasma ascorbic acid is determined before and four hours after the injection of 200 mg. of the vitamin. From these figures is calculated the *ascorbic acid index*, which is defined as the initial plasma value multiplied by the increase in the plasma four hours after the injection of the vitamin multiplied by 100. Kajdi takes a four-hour interval because she finds that the ascorbic acid blood plasma level reaches a constant value by that time. The test dose of 200 mg. was chosen because smaller amounts did not sharply illustrate the difference between depleted and partially depleted stores of ascorbic acid, and larger doses were no more accurate. Kajdi states that an ascorbic acid index below 0.8 indicates clinical scurvy; indices between 0.8 and 6 show a low reserve; and an index of 10 or over corresponds to optimal ascorbic acid nutrition. It is claimed that in sixty-four out of seventy cases correct information on the ascorbic acid status of the patient was obtained, laboratory findings being checked against the condition found on clinical examination. Elmy and With [584] employ a saturation test using 500 mg. of ascorbic acid and determine the plasma level before the injection and eight times within the following four hours. This seems an unnecessary elaboration with much inconvenience to the patient.

Goldsmith and Ellinger [567] examine the plasma ascorbic acid in the fasting patient and again one and three hours after the administration of 600 mg. of ascorbic acid. They consider that the ascorbic acid status is adequate if the original level is over 0.7 mg. per 100 ml., rising to 2 mg. or more three hours after the test dose. Similar tests have been employed using smaller intravenous test doses (100 to 300 mg.) [593, 594, 842].

A method of estimating ascorbic acid deficiency based on a test dose of 500 mg. intravenously with observations on both the blood level and urinary excretion over a four-hour period is described by Kastlin and his collaborators [573]. They claim that the urinary excretion after four hours is proportional to the urinary excretion in a twenty-four-hour period. Ascorbic acid is first estimated in a blood sample and specimen of urine after fasting for twelve to fifteen hours, and then 500 mg. of the vitamin given intravenously. A blood specimen from the opposite arm is collected five minutes later. Subsequent blood and urine specimens are obtained one, two, three and four hours later.

Then the blood ascorbic acid in milligrams per cent. and the vitamin excreted in milligrams are plotted against time. The total urinary excretion in milligrams and the percentage of the test dose excreted are computed. Kastlin and his fellow-workers state that a typical curve of a "saturated" person shows the following characteristics: (a) The fasting blood level of ascorbic acid is 0.7 mg. per cent. or higher. (b) The five-minute period is relatively high—from 4.5 to 9 mg. per cent. (c) The rate of return of the blood concentration from the five-minute peak to the four-hour level is gradual, showing that the tissues have no great avidity for ascorbic acid. (d) The four-hour blood level is well above the fasting level. (e) Urinary excretion of the test dose is greatest in the first hour and the total urinary excretion in four hours is forty per cent. or more of the test dose.

The typical severe ascorbic acid deficiency curve is stated to show a marked deviation from the normal. (a) The fasting blood level of ascorbic acid is below 0.4 mg. per cent. (b) There is only a slight variable rise in the blood level after five minutes. (c) The blood concentration falls rapidly to near the fasting level. (d) The total urinary excretion ranges from a few milligrams to twenty per cent. of the test dose. The authors of this test state that it may show evidence of severe vitamin deficiency when there are no clinical signs of scurvy.

Rinehart and Greenberg [785] read the plasma ascorbic acid three and five hours after an oral dose of 15 mg. per kilo of body weight. For the sake of simplicity they classify the resulting blood plasma curve as flat (peak below 0.5 mg. per cent.), medium (peak 0.5 to 0.9 mg. per cent.) or high (peak above 0.9 mg. per cent.). According to Rinehart and Greenberg the flat curves reflect tissue depletion of ascorbic acid and the medium curves moderate depletion.

These plasma studies, like others described, are based on arbitrary standards and ignore the fact that blood plasma levels do not reflect tissue stores, but only the immediate intake, and can be very low in the absence of scurvy or ill health. The urinary excretion is an overflow from the plasma.

Ascorbic Acid in Cerebrospinal Fluid. Rohmer, Bezssonoff and Sacrez [1011] state that the level of ascorbic acid nutrition can be followed by estimating the vitamin in cerebrospinal fluid. There is no parallelism between the cerebrospinal fluid levels and the urinary excretion. Owing to the greater trouble of obtaining cerebrospinal fluid, the necessity for the patient to remain in bed after spinal puncture, and all the risks attending the removal of cerebrospinal fluid, the method has little to recommend it.

ASCORBIC ACID IN THERAPEUTICS

The only disease that specifically responds to ascorbic acid is scurvy, yet its use has been advocated in the treatment of a number of conditions.

With regard to dosage and administration there is no need to give the vitamin parenterally, unless there is any reason to suspect deficient absorption (as in persistent vomiting, colitis, diarrhoea), since absorption from the intestine is practically complete even when very large doses are given (p. 434). In severe cases of scurvy relief may be more rapid if it is given parenterally. Intravenous injection is not recommended as the renal threshold may be rapidly exceeded and a large part of the dose excreted in the urine. Intramuscular injection is therefore preferable. As a dietary supplement 100 mg. a day in divided doses is adequate. When given therapeutically there is no justification for repeated doses in excess of 100 to 200 mg., as once the plasma level begins to rise the excess spills over into the urine (p. 437). Farmer [864] has shown that when the plasma and white cell ascorbic acid are zero the administration of 100 mg. daily will eventually produce tissue saturation. A patient grossly deficient in ascorbic acid requiring it parenterally may need

500 mg. daily for several days until the tissues are satisfied. It should be taken several times a day, rather than in one large dose.

Ascorbic Acid in Infections. The results of experimental studies on immunity and infection are very conflicting (pp. 418-421). In human infections there is certainly a fall in plasma and urinary ascorbic acid, but this is almost certainly due to redistribution, the vitamin passing from the plasma into the white cells (p. 420) and possibly into the adrenals (p. 430). Ascorbic acid is bacteriostatic and bacteriocidal, but only in concentrations much higher than can ever be achieved in the tissues. On the basis of the lowered excretion during infective states many workers have given large doses of ascorbic acid to patients suffering from infections. There is no evidence from controlled observations that this procedure has any effect, beneficial or otherwise, on the course of the infection or the condition of the patient. Assuming that ascorbic acid does play a part in immunity phenomena and leucocytosis (p. 420), the normal intake is presumably sufficient for this purpose. As long as a patient is excreting ascorbic acid in the urine the needs of the body are being satisfied. Brown [509] and his co-workers state: "There is no clear-cut unchallenged demonstration that ascorbic acid can exert a direct effect against a clinical bacterial infection."

Diphtheria. The evidence that ascorbic acid has a protective action *in vivo* against diphtheria toxin is conflicting (p. 419). Whether the administration of ascorbic acid as a therapeutic measure has any effect on the progress of the disease clinically is very doubtful. It is claimed by some workers that the administration of ascorbic acid to patients suffering from the toxic form of diphtheria lowers the mortality rate [604-606]. This has been denied by others, who have observed no favourable effects [486, 607, 617]. Ascorbic acid has no effect on the Schick reaction [598, 608].

Pneumonia. Tonutti has shown that in pneumonia large amounts of ascorbic acid are taken up by the white cells (p. 420). Gander and Niederberger [432] have attempted to correlate the seasonal incidence of ascorbic acid deficiency with that of pneumonia; according to them the peak mortality months for pneumonia coincide with those for the lowest level of intake (about March). The figures of Anderson [931] do not bear this out. His mortality figures for pneumonia in Glasgow show that the highest mortality is in January and the quarter preceding it. He states that the number of deaths in January is twice the average of the other eleven months. According to Glazebrook and Thomson [816] the incidence of pneumonia was lower in a large group of adolescent boys receiving supplements of ascorbic acid than in a control group that did not.

Gander and Niederberger [432] treated fifteen patients with ascorbic acid and recorded that considerable improvement occurred if the patients were saturated with the vitamin on the first day of the illness. Then the temperature dropped by crisis by the third day. A "remarkable improvement in the general condition" both early in the course of the disease and in convalescence was described. The scheme of dosage employed was 500 mg. intramuscularly and 300 mg. by mouth. During the next three hours 900 mg. of ascorbic acid by mouth was given. Some cases received 1,800 mg. a day. No controls were used in this study. Hochwald [609, 610] states that if 500 mg. of ascorbic acid is given intramuscularly every two hours an attack of lobar pneumonia can be cut short on the first day. If given on the second day the course of the disease can be shortened, whilst later treatment, although it does not arrest the course of the disease, lessens its severity. According to Hochwald [610] the improvement in general condition (prostration, dyspnoea), the fall in temperature, and improvement in the blood picture is particularly striking. Hochwald [610], Kalk and Frobenius [613] and Walther [614] consider that pneumonia is an allergic-hyperergic condition; a nasopharyngeal infection precedes the lobar pneumonia by two or three weeks and sensitizes the body. Others have claimed that large doses of ascorbic acid produce clinical improvement [612, 615, 616].

Tuberculosis. A diminished excretion of ascorbic acid and low plasma levels have been reported in tuberculous patients [126, 127, 633, 634, 849, 850]. As in other infections, these low levels are due to redistribution of ascorbic acid in the body. As healing in tuberculosis is characterized largely by the formation of connective tissue, for which ascorbic acid is essential, it is possible that ascorbic acid deficiency may delay healing and have an unfavourable effect on the course of the disease. Getz and Koerner [849] state that an extreme degree of ascorbic acid deficiency appears to make the prognosis worse in tuberculous patients. In a follow-up paper based on a study of 1,100 men Getz and his co-workers [1030] concluded that marked vitamin A and ascorbic acid deficiency occurred in the 28 men of this group who developed pulmonary tuberculosis. Other factors, e.g. poor housing and living conditions, exposure to infection, protein intake and occupation, which were not taken into account, may have been contributory. Other workers have failed to observe any parallelism between the severity of the disease and plasma and urine levels [493, 633].

Animal experiments suggest that ascorbic acid deficiency predisposes to tuberculous infection. Thus Greene [619] and his co-workers found a shortened survival period and a decrease in the body weight of infected guinea-pigs on a scorbutic diet, and De Savitsch [620] found smaller lesions and a greater increase in weight in animals inoculated with tubercle bacilli and fed sources of ascorbic acid than in the inoculated and untreated controls. Russell, Read and Rouse [889] have shown that compared with controls there is a greater dissemination of tubercle bacilli in the viscera of infected scorbutic animals, and that the tuberculous lesions show more extensive caseation. They observed that the deposition of fibrous tissue around the tubercles was slightly greater in the organs of control animals receiving adequate ascorbic acid. These observations are in agreement with the early laboratory studies on scorbutic guinea-pigs, which often died of tuberculosis. McConkey and Smith [621] concluded that the administration of tuberculous sputum to guinea-pigs was not the sole cause of intestinal ulcers. Their control animals given adequate ascorbic acid developed ulcers in only two instances, compared with twenty-six in the group suffering from ascorbic acid deficiency. Steinbach and Klein [809] have claimed that the daily injection of ascorbic acid into tuberculous guinea-pigs increased their tolerance to repeated large doses of tuberculin. Similar observations were made by Birkhaug [150], who reported that ascorbic acid inhibits the tuberculin reaction in tuberculous guinea-pigs. He also records that large doses of the vitamin caused a significant increase in weight and reduction in the tuberculous lesions of guinea-pigs infected with tuberculosis. The histochemical studies of Tonutti [102] show that re-infection of experimental animals after a previous sensitization results in the accumulation of cells in the lung alveoli laden with ascorbic acid. In addition the bronchioles are often seen to be choked with desquamated cells saturated with ascorbic acid. Such studies explain why the plasma and urine concentration of ascorbic acid are lower in tuberculosis.

It is doubtful if the administration of ascorbic acid beyond the normal requirements is of any benefit to tuberculous patients, although its use has been advocated [625-632]. Most of the workers who claim that it has a beneficial effect speak of general improvement, but produce no evidence in the form of radiological changes, gain in weight or lowering of the sedimentation rate. Heise and Martin [624] claim that the administration of ascorbic acid lowers the sedimentation rate but produces no clinical improvement. Sweany and his colleagues [635] thought that ascorbic acid was of some benefit and prolonged life in advanced cases, but they were unable to observe any radiological improvement. McConkey [1012] thought that cod-liver oil and tomato juice were helpful in preventing laryngeal tuberculosis as a complication of pulmonary tuberculosis. To what extent vitamins A and D and the fatty acids in the oil might play a part is unknown. Erwin and others, at

Liverpool [636], and Kaplan and Zonnis [850] saturated tuberculous patients with ascorbic acid for six months, but failed to observe any significantly favourable results, as judged by the usual clinical criteria, when compared with control patients.

Pertussis. Ascorbic acid in a concentration of 80 mg. per 1,000 ml. inhibits the growth of *Hæmophilus pertussis* [649]. Although such a concentration cannot be reached in the plasma, it can in the white cells [102]. Animal experiments also suggest that the virulence of *H. pertussis* is reduced if injected with ascorbic acid. Following these observations several enthusiastic but uncontrolled reports appeared on the value of ascorbic acid in the treatment of whooping cough [650-652]. The administration of 100 to 300 mg. daily was said to reduce the number of whoops considerably and to shorten the duration of the disease from weeks to days [651]. A carefully controlled study by Gairdner [654] at Great Ormond Street Hospital for Children failed to confirm these observations. Stringent criteria of diagnosis were recognized: recovery of *H. pertussis* from a cough plate, a paroxysmal cough, lymphocytosis and a sublingual ulcer. The number of paroxysms was counted and the weight of the children carefully controlled. The duration of illness and gain in weight did not differ significantly in the treated and control group.

Respiratory Infections. Glazebrook and Thomson [816] state that the duration of an attack on tonsillitis is less in adolescents treated with ascorbic acid than in controls not receiving the vitamin.

Good results have been reported in the treatment of the common cold with large doses of ascorbic acid, e.g. 300 to 1,000 mg. daily [662, 663, 741]. These trials were uncontrolled and based on the subjective judgments of the patient. In a trial conducted among Dutch factory workers the prophylactic use of ascorbic acid and quinine was stated to reduce the incidence of the common cold [844]. However, the number of working days lost was the same in the treated and untreated group. Carefully controlled observations by Cowan, Diehl and Baker [742], Glazebrook and Thomson [816], Brown [506] and several Scandinavian workers [836, 841, 857] have shown that there is no evidence that ascorbic acid has any statistically significant effect in preventing the common cold or affecting its duration. Dahlberg, Engel and Rydin [836] carried out a mass experiment on 2,500 Army conscripts, one half receiving 200 mg. of ascorbic acid, the other half acting as controls. No difference was noted in the frequency or duration of colds, fever, endurance test or diseases of any description in the two groups.

Rheumatic Fever. The ascorbic acid excretion is lower than normal in patients with rheumatic fever as in other acute infections [435, 467, 468, 638, 639]. Rinehart [638] noted in 1934 that guinea-pigs infected with β -streptococci developed an arthropathy that could be prevented by ascorbic acid. As the ascorbic acid excretion in patients with rheumatic fever is low Rinehart argued that the disease might result from the combined effect of infection and ascorbic acid deficiency. The experimental work of Rinehart has been confirmed but his conclusions doubted [640, 641]. Schultz and others [640, 643-645] pointed out that the lesions in Rinehart's guinea-pigs only superficially resembled those of rheumatic fever. He argued correctly that the low ascorbic acid excretion in rheumatic fever is the result and not the cause of the infection, which he found to be uninfluenced by administering large doses of ascorbic acid [643]. Abt and his co-workers [152] found the clinical course of acute rheumatic fever uninfluenced by the administration of ascorbic acid. During treatment the patient with rheumatic fever may excrete more ascorbic acid than the average febrile patient because salicylates increase excretion (p. 439). This has been shown by Keith and Hickman [646]. Salicylates increase the excretion of ascorbic acid by depleting the supplies in the adrenals [622].

In 1950 Masse! and his co-workers [623] published a preliminary report

on seven patients, suggesting that large doses of ascorbic acid had anti-rheumatic activity. They certainly gave much larger doses—4 grams daily—than other workers. This dose was given for periods of eight to twenty-six days. The temperature was reported to have fallen rapidly and the joint manifestations improved, although the effect on the sedimentation rate was variable. If this is confirmed it is interesting to speculate on the mode of action. The relation of ascorbic acid to adrenal cortex activity and the value of A.C.T.H. and cortisone in the treatment of rheumatic diseases suggests that the effect might be mediated through the adrenal cortex. Rinehart [436] states that ascorbic acid has a favourable effect on the sedimentation rate, weight and arthritis in rheumatoid spondylitis. Glazebrook and Thomson [816] state that the incidence of rheumatic fever was lower in a group of adolescents receiving supplements of ascorbic acid than in a control group that did not.

Rheumatoid Arthritis. Rinehart also believes that a chronic ascorbic acid deficiency is an important aetiological factor in rheumatoid arthritis [436]. There is a diminished excretion and low plasma ascorbic acid in this condition [436]. Hall and his co-workers [507] saturated patients suffering from rheumatoid arthritis with ascorbic acid for eight months but observed no clinical improvement that could be attributed to the vitamin. Sherwood [647]. Hare and Williams [648] claimed to produce clinical improvement by giving arthritics ascorbic acid. Carefully controlled assessment, independent of subjective statements by patients, are necessary in this type of investigation. Conditions for assessing the value of drugs in rheumatism have been laid down by Quin, Mason and Knowelden [544].

Selye [515] reported the aggravation of arthritis in rats, induced by injecting formaldehyde into the joints, by the use of desoxycorticosterone acetate (DOCA) and its prevention or cure by A.C.T.H. Brownlee [517], using Selye's technique, reported beneficial effects on formaldehyde-induced arthritis using DOCA and ascorbic acid. However, this work could not be confirmed by others, including Selye himself [518-520]. Dugal [716] noted improvement in formaldehyde-induced arthritis using ascorbic acid alone.

Just before the observations of Brownlee, Lewin and Wassén [524] claimed that the injection of DOCA and ascorbic acid produced dramatic amelioration of symptoms in patients with rheumatoid arthritis comparable with the effects of cortisone or A.C.T.H. Their report was quickly followed by a number of others based on few cases and with little objective evidence [547-549]. Careful investigations by subsequent workers, based on objective data and with control injections, have failed to confirm that DOCA and ascorbic acid have any specific value in the treatment of rheumatoid arthritis [542-546].

Dental and Oral Conditions. The importance of ascorbic acid in the development of the teeth is discussed on p. 406.

Although there is general agreement that an adequate intake of ascorbic acid is necessary for normal tooth structure and growth there is no evidence that a deficiency plays any part in the aetiology of dental caries in human beings. Schiötz [674] observed that the teeth of children fed on liberal diets containing plenty of milk, orange juice, apple and carrot were less carious than those of children from poor districts whose food lacked these articles of diet. There are so many dietary factors involved here that the beneficial effects of the diet cannot be ascribed solely to any one of them. Controlled animal studies by Boyle [49] show that on low intakes of ascorbic acid the deposition of dentine in the incisor teeth is much retarded, but the enamel continues to be deposited at the normal rate. No correlation was found between ascorbic acid deficiency and the incidence of dental caries. According to Sandberg and Dagulf [525] no correlation exists between the values for blood ascorbic acid and either the absolute frequency of dental caries or the increase of dental caries studied over a period of two years.

While the existence of gingivitis in scorbutic or sub-scorbutic human beings and guinea-pigs is not doubted, there is considerable difference of opinion on the part played by minor degrees of ascorbic acid deficiency in the aetiology of gingivitis as met with in general medical and dental practice. A careful clinical examination should be made before attributing bleeding gums to ascorbic acid deficiency. A mistaken diagnosis of this was made in four cases; the true diagnosis was uræmic toxæmia [884].

Earlier workers recorded that supplements of fruit juices or synthetic ascorbic acid in doses of 100 to 400 mg. daily had a favourable effect on gingivitis and the healing of bleeding and sore gums [672-675]. Ascorbic acid deficiency, as evidenced by a diminished excretion or low blood levels, was also recorded by a number of observers in patients with these conditions [673, 675, 805, 812]. Much of this work was uncontrolled, the ascorbic acid assays were often doubtful and, since in some cases local treatment was also carried out at the same time, the conclusions are not acceptable. A number of workers have since failed to find any significant relationship between the intake of ascorbic acid and the incidence of gingivitis [68, 680, 748, 779, 1016]. Burrill [1017], from observations on nearly 1,400 patients, noted that plasma ascorbic acid levels in patients with gingivitis and periodontal disease tend to be lower than controls. The differences between the two groups were, however, small and probably not statistically significant. The seasonal variations in the plasma ascorbic acid and the incidence of gingivitis were such that no causal relationship between a low level of ascorbic acid nutrition and gingivitis could be established. Burrill states that a patient likely to neglect his mouth will also neglect his diet; ascorbic acid subnutrition and oral infection originate from the same cause rather than from one another.

Roff and Glazebrook [57, 678] examined six hundred boys on the training ship *Caledonia* and observed that stomato-gingivitis associated with ascorbic acid deficiency was common amongst these boys, whose normal intake averaged only 25 mg. a day. Three hundred of the boys were saturated with ascorbic acid (200 to 300 mg. a day), and then the dose reduced to a maintenance level of 50 mg. a day, so that the total intake was 75 mg. a day after saturation. The same number of boys in a control group received the same diet, but no supplements of ascorbic acid. Before treatment the incidence of gingivitis and gingivo-stomatitis in the group was 17.6 per cent.; after six weeks' treatment with ascorbic acid it fell to 4.9 per cent. The corresponding figures in the control group before and after receiving dental treatment but no supplements were 16.3 and 12.64 per cent. The boys in this group suffering from gingivo-stomatitis were then treated with ascorbic acid, and usually within fourteen days a characteristic colour change was observed in the gums, which lost their deep red colour, became firmer and assumed their normal pink appearance. Roff and Glazebrook are of the opinion that marginal gingivitis is due to bad dental hygiene, since it usually responds to dental treatment, whereas gingivo-stomatitis, which they regard as subscorbutic, does not.

These observations were supported by those of Campbell and Cook [679], who distinguished between simple gingivitis and ulcerative, ulcero-membranous or fuso-spirochætal gingivitis (Vincent's disease). According to Campbell and Cook, in simple gingivitis the gums are sore and bleed on pressure and the condition clears rapidly with doses of 300 mg. of ascorbic acid daily. With the ulcerative type pain and bleeding are more intense, there is a characteristic foetor and smears show pus, spirochætes and fusiform bacilli. Treatment is more difficult and does not yield to ascorbic acid alone, but requires in addition local treatment with chromium trioxide and hydrogen peroxide or magnesium sulphate paste.

Stuhl [892] and Buchanan [1009] record a low excretion of ascorbic acid in patients with ulcerative gingivitis in the British Army and Navy. This is

probably not directly related to gingivitis. Stuhl and Buchanan state that the effects of local treatment (gentian violet two per cent.) are enhanced by the administration of ascorbic acid. Buchanan states that ulcerative gingivitis does not clear up with normal dental treatment and is progressive unless local treatment is supplemented by treatment with 700 mg. of ascorbic acid daily until the patient is saturated.

The observations of Roff and Glazebrook, Campbell and Cook, Stuhl and Buchanan have been criticized by King [536], who states that the grading of lesions was not described in sufficient detail and that little indication was given of criteria of cure.

There is considerable difference of opinion on the aetiology of ulcerative gingivitis (ulcerative gingivo-stomatitis, Vincent's disease, "trench mouth," fuso-spirochætal gingivitis). Pincus [999] doubts whether it is associated with ascorbic acid deficiency, since the condition may clear up with local treatment alone and the use of ascorbic acid without the latter gives variable results. Pincus observed two hundred men with ulcerative gingivitis and states that many were getting enough ascorbic acid. Cuthbert and Williams [986] also failed to show any degree of ascorbic acid subnutrition in sailors suffering from ulcerative gingivitis. The level of ascorbic acid nutrition was no worse in these men than in non-infected healthy seamen. They found that the administration of the vitamin did not accelerate cure of the gingivitis, and they were inclined to think that the chief factor in the spread of the disease is contagion, e.g. due to "French kissing," inadequate washing of crockery and dirty oral habits. The view of Ministry of Health dentists is that "there is ample evidence that ascorbic acid therapy is ineffective" in the treatment of ulcerative gingivitis [985].

Kent [983] believes that much confusion over treatment has resulted because there are two clinical entities, latent scorbutic gingival ulceration and ulceromembranous gingivitis or Vincent's disease. In the former local therapy fails and the response to ascorbic acid is dramatic; in the latter local therapy is necessary. Linghorne and his co-workers [554] found no improvement in cases of gingivitis treated with large doses of ascorbic acid for five months. But they also state that gingivitis is developed more frequently in subjects with a low blood ascorbic acid than in those with high levels. When the gingivitis was cleared up the provision of 75 mg. ascorbic acid daily appeared to have a delaying effect on recurrence. Yet these workers did not consider gingivitis scorbutic because they remarked that "the histological appearances of the gingival tissues in no way resembled the changes seen in scurvy."

MacDonald [979] noted that there was a high incidence of bleeding gums and gingivitis in naval ratings. He was unable to show that it was related to ascorbic acid deficiency and the condition cleared up with local treatment. Ungley and Horton [1016] made similar observations on naval patients with sore and bleeding gums, in whom scurvy or "subscurvy" was absent. Local causes, such as infection and calculus, were sufficient to account for the gum condition, and both ascorbic acid and nicotinic acid were ineffective. Day and Shourie [958] found that ascorbic acid was ineffective in the treatment of gingivitis and associated conditions in Indian children. Lesson and his co-workers [490] treated one hundred and twenty-nine subjects with gingivitis with 25 to 125 mg. ascorbic acid daily, but observed no improvement after three months' treatment. Stammers [951] strongly advocates local treatment in ulcerative gingivitis. He finds ascorbic acid and nicotinic acid therapy disappointing, with a response rate of only eighteen per cent. Recently Stamm, Macrae and Yudkin [918] found that twenty per cent. of a group of nearly three thousand R.A.F. personnel suffered from bleeding gums, but no greater improvement was obtained by treatment with ascorbic acid than with inert control tablets. Those with "sponginess" as well as bleeding of the gums showed no more improvement after taking ascorbic acid than when

given control tablets. These workers concluded that the incidence of bleeding gums is not related to ascorbic acid deficiency.

These conflicting views on ascorbic acid and gingivitis are undoubtedly due to confusion over the ætiology and symptomatology of inflammatory conditions of the gums and the absence of established standards for the normal gum. Many investigators do not state the exact type of lesion treated and the criteria for assessing recovery, or for assessing vitamin deficiency.

The recent observations of Farmer and his colleagues [864] suggest that there is probably no connection at all between ascorbic acid deficiency and dental lesions, excluding of course frank scurvy. They kept volunteers on a scorbutic diet for five months but failed to observe any changes in the gums, teeth or bone of the jaw as observed with the naked eye, radiologically or with the biomicroscope. The plasma ascorbic acid and the ascorbic acid in the white cell layer were zero. These observations suggest that the oral conditions ascribed to ascorbic acid deficiency by some writers are in reality due to pre-existing caries and gingivitis or improper dental hygiene and are not related to ascorbic acid deficiency.

It is stated by Campbell and Cook [853] that the oral administration of ascorbic acid to patients before and after dental extractions plays an important rôle in the healing of the wounded gum tissue and the absorption of the alveolar bone margins. They also report that pain and bleeding, particularly persistent hæmorrhage after extractions, are considerably reduced. The patients were given 300 mg. of vitamin daily until "saturated," and then 100 mg. daily as a maintenance dose. Persistent hæmorrhage was treated with 500 mg. of the vitamin. There is no evidence that such large doses as this were necessary.

Hæmatology. In scurvy erythropoiesis is depressed in the bone marrow ; the anæmia usually responds to treatment with ascorbic acid (p. 416). Recent work in this country has drawn attention to the increased incidence of anæmia during the war, particularly among pregnant women. An inadequate intake of iron has been suggested as the cause, but it may be that ascorbic acid deficiency plays a part as well [557]. Israels [557] suggests that any patient whose anæmia does not respond to iron should be given ascorbic acid. Davidson and Donaldson [794] treated hypochromic anæmia in children with iron and observed that daily supplements of 25 mg. of ascorbic acid had no effect in raising the hæmoglobin level. Dyke and Della Vida [734] state that a number of pernicious anæmia patients, in spite of their maintenance dosage of liver, showed a progressive fall in the red blood count. This was checked by giving 100 mg. of ascorbic acid daily for a month, the red blood count increasing by 1,000,000 per c.mm. These observations suggest that in pernicious anæmia, even with ample dosage of liver extract, hæmopoiesis is subnormal if there is any degree of ascorbic acid deficiency.

The prompt response of the petechial hæmorrhages of scurvy to the administration of ascorbic acid suggested its use in hæmorrhagic conditions other than scurvy. There have been reports on its use in the treatment of purpura [685-694], but these early enthusiastic reports have not been substantiated by later workers [695-702]. Scarborough [969] treated fifteen patients with purpura with ascorbic acid without success, and Davis [558], from an analysis of 1,200 cases of Schonlein Henoch purpura, concluded that ascorbic acid was of no value. Ascorbic acid is also ineffective in hæmorrhagic states [717-719].

It has been claimed that hæmaturia is a manifestation of ascorbic acid deficiency and responds to treatment with the vitamin [693, 709-711]. There is no evidence that hæmaturia is associated with ascorbic acid deficiency except in cases of scurvy. The vitamin has no effect in controlling hæmaturia due, for example, to renal lesions [712]. Claims have been made for the effectiveness of ascorbic acid in the treatment of leukæmia [720, 722], but they have not been confirmed [575, 721, 724].

Vogt [721], Carrie [723] and Ellis [618] state that leucopenia from irradiation with X-rays can be corrected with ascorbic acid. Ellis states that a sufficiently large dose can prevent the fall in the white count that follows irradiation. Field and Reckers [587] have been unable to confirm this in dogs. There is apparently a fall in the ascorbic acid of the plasma and tissues of animals exposed to roentgen irradiation [622]. Kalk [724] claims that ascorbic acid is effective in the treatment of granulocytopenia in doses of 500 to 1,000 mg. daily.

Lian [1008] and Deeny and his co-workers [963] showed independently that familial idiopathic methæmoglobinæmia, one of the "inborn errors of metabolism," and characterized by a permanent slaty blue colour of the skin and methæmoglobin in the blood, responds to treatment with 300 to 400 mg. of ascorbic acid daily and sodium bicarbonate. The general health in this disease does not suffer, as there is sufficient functioning oxyhæmoglobin. Treatment with ascorbic acid or methylene blue results in disappearance of the bluish colour of the skin, which becomes normal and remains so as long as treatment is maintained. Ascorbic acid reduces methæmoglobin to hæmoglobin. Normally methæmoglobin reduction in erythrocytes takes place through the oxidation of triose-phosphate and lactate, and the system requires co-enzyme I [602]. The cause of idiopathic methæmoglobinæmia is congenital absence of a factor in the erythrocyte mechanism that in normal red cells rapidly reduces methæmoglobin [603]. The observations of Lian and Deeny have been confirmed by a number of other workers [595-597, 600, 603].

The studies of Barron [101] suggest that ascorbic acid might play a part in maintaining the hæmoglobin of the blood at a normal physiological level. He found that in polycythæmia induced by administering cobalt to animals the red cell count could be reduced by ascorbic acid. A fall in the hæmoglobin also occurs [642]. Deeny [726] observed that ascorbic acid and sodium bicarbonate caused a marked fall in the red cells count in two patients with polycythæmia vera. Kandel and LeRoy [727] had previously failed to find any beneficial effects from giving ascorbic acid in this condition.

Ascorbic acid protects erythrocytes *in vitro* from the hæmolytic action of hypotonic solutions of saline [807]. If this is confirmed *in vivo* ascorbic acid may be of therapeutic value in the treatment of patients having increased fragility of the red cells.

Ascorbic Acid in Dermatology. During his experiments on induced scurvy Crandon [68] noted that after a hundred and thirty-four days on a diet practically free from ascorbic acid small hyperkeratotic papules began to develop over the buttocks and posterior aspects of the calves. Noticeable fragmentation of the hairs and marked dryness of the skin were also noted. It was proved that these skin lesions were not the result of vitamin A deficiency, as 30,000 units of this vitamin were being taken daily. The administration of ascorbic acid rapidly cured these skin lesions.

It has been suggested that ascorbic acid is intimately connected with pigment formation and pigmentary disorders of the skin (p. 430), in which it is encountered for the most part in the basal layer of the epidermis and in hyperpigmented structures. The formation of pigment in the skin depends on the ability of the melanoblasts to produce it, probably through the oxidation of certain organic substances, including L-3 : 4-dihydroxyphenylalanine ("dopa"). All pigment producing cells stain black with *dopa* reagent. According to Szent-Györgyi [231] ascorbic acid inhibits the formation of pigments formed through the oxidation of phenolic substances, such as *dopa*, and by virtue of its location in the pigment areas of the skin might conceivably be able to prevent oxidation and subsequent pigment formation. This has been verified by *in vitro* experiments [232-234]. On the other hand the hyperpigmentation of the nipples and areolæ of castrated guinea-pigs receiving œstrogens was uninfluenced by giving ascorbic acid [729]. Cornbleet [730]

found that human skin contained less ascorbic acid after ultraviolet irradiation. He further observed that a section of skin from a negro injected with ascorbic acid reacted more to silver nitrate than a section of skin from a white person who had received a similar amount of ascorbic acid. He inferred from this that the pigment fixed the ascorbic acid in the skin.

If ascorbic acid has an inhibiting effect on pigment production it might explain the hyperpigmentation that occurs in scurvy and Addison's disease. It is stated that the pigmentation of this condition is diminished by the administration of the vitamin [235, 237, 239, 872].

Ascorbic acid has been recommended in the treatment of a number of skin conditions such as psoriasis [508, 731-733], urticaria [736], erythema multiforme [739], and infantile eczema [740]. The evidence is not impressive. A low excretion of ascorbic acid has been noted in lupus vulgaris and erythematosus, but administration of the vitamin had no effect on the course of the disease [737, 738]. Ascorbic acid is said to be of value in the prevention and treatment of poison oak dermatitis [475].

Lever and Talbott [744] examined the blood ascorbic acid in a hundred and eighty-one patients suffering from various skin lesions, including dermatitis, psoriasis, urticaria, lupus vulgaris, lupus erythematosus, eczema, exfoliative dermatitis, pemphigus and acne vulgaris. The results were compared with those of sixty-eight apparently healthy persons. Great variations were found, but there was no significant correlation between the blood ascorbic acid and the development of the skin lesions, and large amounts of the vitamin were given to eighteen patients showing low levels for ten weeks, but without any improvement in their clinical condition. Braestrup and Hansen [745] observed that ascorbic acid had no beneficial effect on the clinical condition of patients with skin lesions.

The follicular lesions commonly seen in this country are probably those of keratosis pilaris and have no direct association with a deficiency of ascorbic acid or vitamin A [476, 492].

Ophthalmology. According to Friedenwald, ascorbic acid plays an important part in the nutrition of the ocular tissues and is essential for the secretion of aqueous humour [962]. According to him, ascorbic acid is secreted into the aqueous humour by the ciliary body and this is the source of the ascorbic acid in the lens. In several animal species the aqueous humour contains twenty to fifty times as much ascorbic acid as the blood plasma [487]. This high concentration in a body fluid is exceptional for, in general, ascorbic acid is more concentrated in body cells than in fluids. Other parts of the eye, e.g. the retina, vitreous humour, the ciliary processes, the cornea and the lens, contain high concentrations of ascorbic acid [489, 494, 601, 747, 962]. The concentration of ascorbic acid is greatest in the superficial layers of the cornea [653] and falls after injury [494]. Lyle and McLean [755] treated patients suffering from the following inflammatory conditions of the cornea with ascorbic acid: dendritic corneal ulcer, disciform keratitis, sclerosing keratitis, superficial punctate keratitis, post-vaccinal keratitis and corneal ulceration, all of which occurred as complications of eye injuries in airmen. One thousand mg. ascorbic acid was given intravenously as well as local treatment. Improvement was stated to be dramatic and was attributed largely to the treatment with ascorbic acid. As local treatment was given as well, it is impossible to assess the part played by ascorbic acid. Similar observations were made by Summers [659], who used penicillin as well, so that the part played by each substance could not be evaluated.

Campbell, Ferguson and Garry [655] made superficial and deep heat injuries on the corneas of normal and ascorbic acid deficient guinea-pigs. They found that the healing of superficial lesions was not impaired by ascorbic acid deficiency, but the deeper lesions, involving the substantia propria of the cornea, healed more slowly in deficient guinea-pigs. Even after healing was complete structural weakness of the wound persisted for as long as three

weeks after injury in the scorbutic animals. Boyd and Campbell [658] extended these observations to injuries of the human cornea. They concluded that the administration of large doses of ascorbic acid (1.5 grams daily) to patients with corneal ulcers had no significant effect on the healing time of those that were superficial, but it significantly accelerated the healing of deep ulcers. There was no evidence that the patients treated suffered from ascorbic acid deficiency.

Campbell and Ferguson [660] observed that ascorbic acid deficiency in guinea-pigs significantly increased the incidence of vascularization of the cornea after heat injury.

Livingstone and Walker [756] state that mustard gas injuries of the rabbit cornea can be mitigated by large doses of ascorbic acid. The effect must be pharmacological as rabbits do not suffer from ascorbic acid deficiency. The dose was large—six daily injections of 500 mg. Mann and Pullinger [757] could not confirm these observations.

The healthy lens is particularly rich in ascorbic acid, the level of which is greatly reduced or absent in a lens with cataract [489, 746]. According to Bellows [489], galactose induced cataracts in rats can be delayed by giving large doses of ascorbic acid.

There is no good evidence that ascorbic acid deficiency has any bearing on the aetiology of cataract [747]. In large doses ascorbic acid may exert some pharmacological effect as, according to Josephson [750], cataracts in rats caused by dinitrophenol can be arrested by the administration of ascorbic acid.

Friedenwald [749] states that the visual acuity of diabetics, in whom cataract is common, is improved by administering ascorbic acid. Others have failed to confirm this [257].

Yudkin [753] claims that ascorbic acid is of value in ocular conditions associated with vascular disturbances, e.g. hæmorrhagic retinitis and choroiditis and hæmorrhage into the vitreous from local arteriosclerosis. Traube [754] reports a case of conjunctival bleeding treated with ascorbic acid. As there is no evidence that ascorbic acid is associated with capillary resistance it is difficult to understand why it should be effective in the control of hæmorrhage of this type.

Allergic Conditions. Evidence that anaphylactic shock in sensitized animals can be minimized by the injection of ascorbic acid is conflicting. It has been stated that animals receiving small amounts of ascorbic acid are more sensitive to a second dose of horse serum than those receiving an adequate intake of ascorbic acid [758]. Large doses of ascorbic acid are stated by some workers to prevent or minimize the anaphylactic shock that occurs in animals sensitized to horse serum or histamine [759-761]. The blood ascorbic acid rises in fatal histamine shock [778]. Hochwald [767] claimed that if given intraperitoneally fifteen to forty-five minutes before the shock dose 100 mg. of ascorbic acid protected two-thirds of a group of guinea-pigs against anaphylactic shock. Others have failed to observe any association between ascorbic acid and anaphylaxis [762-763]. Yoshikawa [764] states that large doses inhibit anaphylactic shock, while small doses intensify it. There is apparently a species difference as ascorbic acid intensifies protein shock in rats [773]. Dragstedt [671] reported that ascorbic acid failed to prevent peptone shock in dogs.

According to Friedmann [828] a deficiency of ascorbic acid in guinea-pigs causes a loss of smooth muscle responsiveness to non-specific stimulation and Raffel and Madison [829] state that it influences the antibody response to specific antigenic stimulation. If this be so then ascorbic acid influences two of the basic factors in anaphylaxis, i.e. smooth muscle contractivity and antibody production.

Schiødt and Søggaard [766] claim that the chance of getting serum sickness after anti-pneumococcal serum is diminished if ascorbic acid is given. The figures are not convincing and have not been confirmed [765].

Hochwald [767] stated that ascorbic acid taken regularly in doses of 500 to 1,500 mg. is useful in aborting asthmatic attacks, and Hagienco and others [769] claimed to have stopped attacks by intravenous injection of doses of 200 to 300 mg. The latter stated that attacks cease after one to five injections every fifteen minutes. Others [770, 848] have described the successful treatment and prevention of asthma with ascorbic acid. This received some support from the observation that it prevents acetylcholine bronchospasm in guinea-pigs [771]. According to Goldsmith and her associates [552] there is some relationship between the blood ascorbic acid and the frequency and severity of asthmatic attacks. Others have failed to confirm these observations [768, 772]. Hunt [772] administered 100 mg. of ascorbic acid a day to twenty-five asthmatics but was unable to observe any diminution in the severity or incidence of the attacks, nor had the ascorbic acid any effect in diminishing the amount of adrenaline required.

Holmes [681] treated twenty-five hay fever patients with 200 to 500 mg. daily and claimed that remarkable improvement occurred. The observations were not properly controlled. Brown and Ruskin [667] also treated hay fever patients during the ragweed pollen season with high doses of ascorbic acid, 250 mg. three to four times daily, and reported that half of their sixty patients showed "an improvement of fifty per cent. or more." Friedlander and Feinberg [661] state that hay fever patients have a normal ascorbic acid nutrition and that large doses have no effect in the treatment of hay fever and other allergic conditions. Others have treated hay fever with ascorbic acid without benefit [664, 666].

Long and his co-workers [814, 830] have shown that ascorbic acid partly but substantially desensitizes guinea-pigs made sensitive to tuberculin by injection of B.C.G. vaccine. This desensitization is inhibited by a factor in cabbage and so does not occur in guinea-pigs maintained on a diet containing this. Dehydroascorbic acid also desensitizes, but is not inhibited by the cabbage factor [827]. Long assumes that dehydroascorbic acid is the desensitizing agent and that ascorbic acid desensitizes only after oxidation to dehydroascorbic acid; the cabbage factor (probably a sulphhydryl compound—SH) may act by inhibiting the oxidation of ascorbic acid. The desensitizing action of A.C.T.H. and cortisone on tuberculin allergy in the guinea-pig may be due to their facilitating the oxidation of ascorbic acid by antagonizing the —SH compounds (e.g. glutathione) in the tissues. The fact that alloxan, which is known to combine with —SH compounds, has similar anti-allergic activity is consistent with this view.

Cornforth and Long [1020] suggest that cortisone desensitization to B.C.G. can be explained in the following way. Cortisone facilitates the oxidation of ascorbic acid to dehydroascorbic acid, which inhibits the enzyme phosphoglucomutase, as a result of which glucose-1-phosphate accumulates; this compound has been shown to be intimately concerned in desensitization.

Gastro-Intestinal Diseases. Patients suffering from gastric and duodenal ulcers on special diets consisting of milk, eggs, white bread and fish have a low intake of ascorbic acid and may show signs of deficiency disease if such diets are continued for a long time [478-485]. It has been estimated that strict peptic ulcer diets may provide not much more than 5 mg. ascorbic acid daily [484]. The intermittent gastric alkalinity resulting from the use of antacids does not appear to interfere to any extent with the absorption of ascorbic acid [491]. In some cases peptic ulcer patients have been rendered almost scorbutic by adherence to diets that contain very little ascorbic acid [332, 481, 775]. Platt [775] described four cases of adult scurvy resulting from strict adherence to special diets. The patients suffered from hæmaturia, purpuric rashes, epistaxis and bleeding gums, all of which cleared after administering ascorbic acid. The importance of ascorbic acid in wound healing makes it advisable that patients with peptic ulcers should have an adequate amount of ascorbic acid included in their diets. Hunt [77] described

post mortems on twenty-eight patients dying several days after perforation of a peptic ulcer, or after operations on the stomach. In those patients with a very low level of ascorbic acid nutrition wound infection was common, and collagen formation, which is essential for wound repair, was either poor or absent. Microscopically the wounds were like those of scorbutic guinea-pigs. There is no reason to believe that ascorbic acid is ætiologically related to peptic ulceration, as was suggested by Smith and McConkey [676] and Roe and others [806].

The production of experimental peptic ulcers in dogs by cinchophen can be prevented by the administration of ascorbic acid [677]. This may act by detoxifying cinchophen and it does not necessarily follow that ascorbic acid has any effect on the prevention or healing of human peptic ulcers. Some workers have claimed that its administration promotes their healing, but the evidence for this is very slender [776, 777].

According to Abt, Chinn and Farmer [473] the absorption of ascorbic acid is defective in most patients with achlorhydria. It is unlikely that hydrochloric acid is needed for the absorption of ascorbic acid as the administration of alkalis does not seem to interfere with absorption [277, 332, 491].

Obstetrics. The requirements of ascorbic acid during pregnancy and lactation have been mentioned previously (p. 447). Some workers have stated that the diet of most pregnant women is deficient in ascorbic acid, but this view is based on excretion or blood studies, which have probably led to false conclusions [874, 998]. For example, Williams and Fralin [874] make the sweeping statement that only two per cent. of women consume an adequate diet in pregnancy.

Javert and Stander [989] state that lack of vitamins C and K may be a factor in the pathogenesis of threatened and spontaneous abortion and ante-partum bleeding. Of seventy-nine patients with threatened, spontaneous or habitual abortion sixty-nine per cent. had a lower plasma ascorbic acid than normal controls. King [703] also noted low blood ascorbic acid levels in cases of inevitable and complete abortion. Skin ecchymosis, epistaxis, bleeding gums and vaginal bleeding were common, but these symptoms are not necessarily associated with a deficiency of ascorbic acid; only two of them are seen in scurvy. Javert and Stander treated thirty-three patients with threatened abortion with ascorbic acid, vitamins E and K, minerals, trace elements and progesterone and they claim that the incidence of abortion was reduced in comparison with controls. So many preparations were used that it is impossible to ascribe the fall in the abortion rate to any one of them. Other workers state that ascorbic acid given either prophylactically or for treatment prevents threatened abortion progressing to inevitable abortion [781-783]. The reports are unconvincing because of lack of adequate control observations. It is well known that women who have previously aborted often become pregnant again and proceed to term with the delivery of a healthy live infant without any specific form of treatment.

Käppeli [780] observed that ascorbic acid increases uterine tone in the isolated guinea-pig uterus, producing a slow steadily increasing contraction which either becomes rhythmic or tetanic. According to this observer it produces either relaxation or a tetanic contraction in the human uterus. He states that in doses of 100 to 250 mg. given orally or intramuscularly it prolongs the uterine contractions caused by pituitary extract. The effect begins in five to ten minutes after intramuscular injection and lasts for forty-five to sixty minutes. This work has not been confirmed.

Surgery, Wound Healing, Burns. The importance of ascorbic acid for the healing of wounds has been previously mentioned (p. 410). Many workers have demonstrated that surgical patients often have low ascorbic acid reserves and that for some days after operation there is a considerable drop in the ascorbic acid in the blood and urine [786, 787, 788]. The stores of

ascorbic acid are likely to be low in patients undergoing operations for gastric lesions because of dietary restrictions. Lund [786] found that many gastric cases coming to operation were bordering on scurvy. When non-radical operations were performed more complications and deaths were observed among the patients with low reserves of ascorbic acid than among others. According to Bartlett and his co-workers [787] the magnitude of the surgical procedure is related to the post-operative ascorbic acid reserves; a parenteral dose of 1,000 mg. of ascorbic acid disappears from the blood stream far more rapidly after operation than before. Bartlett and his co-workers [852] have also shown that a sufficient depletion of ascorbic acid will decrease the tensile strength of healing wounds in the skin and fascia of human beings. According to them a fasting plasma ascorbic acid less than 0.2 mg. per 100 ml. is likely to interfere with wound healing, although Bourne [925] has been unable to confirm this. They state that in the presence of adequate ascorbic acid the ascorbic acid content of healing fascial scars is much greater than that of healing skin scars. The local application of ascorbic acid to wounds does not accelerate healing [85].

According to Hunt [77] ascorbic acid deficiency is a cause of wound eventration, the incidence of which was reduced in a series of surgical cases by seventy-five per cent. after saturating all the patients with ascorbic acid before operation. He also states that leakage from suture lines occurred only once in two and a half years after he adopted the practice of saturating all his patients with ascorbic acid, and that when wounds failed to heal in saturated patients gross infection, hæmatoma formation or ischæmic necrosis could often be incriminated as the cause. Hunt examined sections of abdominal incisions, sutured ulcers and gastro-intestinal anastomoses from patients who had succumbed after abdominal surgery. In those cases where collagen was poorly formed and cellular proliferation scanty there was a history of a low intake of ascorbic acid (Figs. 174 and 175). The microscopical appearances resembled those seen in guinea-pig scurvy (Figs. 176 and 177). Histological examination of the wounds of those patients whose intake of ascorbic acid was considered adequate showed normal collagen formation and cellular proliferation. Kraybill [909] observed seven cases of abdominal wound disruption; in each case the blood ascorbic acid was zero. Hunt also believes that the absorption of catgut ligatures may be delayed and that post-operative peritonitis may be more common among patients with low ascorbic acid reserves.

Lund and Crandon [813] studied the correlation between the ascorbic acid nutrition and the post-operative healing of abdominal wounds. While they state that post-operative wound eventration occurs more readily in patients with low ascorbic acid reserves, they think that mechanical factors are more important. Hypoproteinæmia, infection and bad suturing can also be causes. They consider that a daily intake of 20 mg. ascorbic acid adequate for wounds to heal easily and well. Pijoan and Lozner [873] consider that a daily intake of 12 to 25 mg. of ascorbic acid, which is adequate to maintain a level of 25 mg. per 100 ml. in the white cell layer (p. 470), is sufficient to ensure adequate wound healing and collagen formation. They base this view on observations on a subject whose daily ascorbic acid for twenty months averaged 16 mg. The plasma ascorbic acid was never more than 0.2 mg. per 100 ml. An experimental wound made in the back showed normal healing ten days after, when a biopsy was done. Carney [706] noted a very large scatter (0.1 to 2.4 mg. per 100 ml.) in the plasma ascorbic acid levels of one hundred surgical patients. Those who had repeated burst wounds or whose wounds failed to heal showed plasma levels varying from 0.5 to 1.44 mg. per 100 ml., with a mean level of 0.93 mg. He concluded that there was no relationship between plasma ascorbic acid levels and wound healing. This conclusion is probably correct because it is the level of ascorbic acid in the white cells that reflects the tissue reserves and not the plasma level (p. 470).

ASCORBIC ACID AND WOUND HEALING

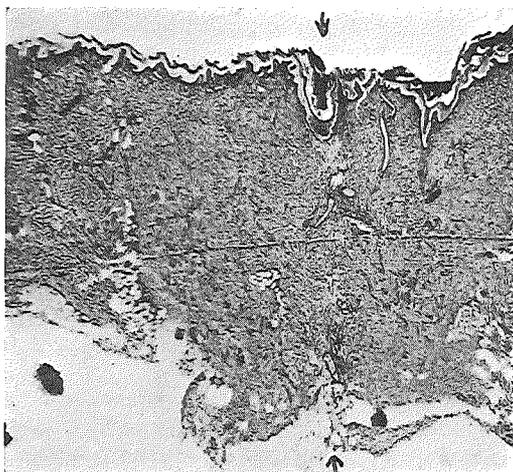


FIG. 174. Ascorbic Acid and Wound Healing. Cross section of a sutured skin incision of a patient given 2,600 mg. of ascorbic acid in divided doses after operation for perforated duodenal ulcer. Stain, silver impregnation. There is exact apposition of cut edges with good collagen formation. Little precollagen remains.



FIG. 175. Ascorbic Acid and Wound Healing. Cross section of a sutured skin incision of a patient grossly deficient in ascorbic acid. Stain, silver impregnation. The opposed edges of the corium are stretched apart and the gap filled with a mass of proliferative fibroblasts producing immature precollagen only. In other regions where the scar was more stretched the gap was filled with oedematous granulation tissue with little precollagen and no collagen.

ASCORBIC ACID AND WOUND HEALING

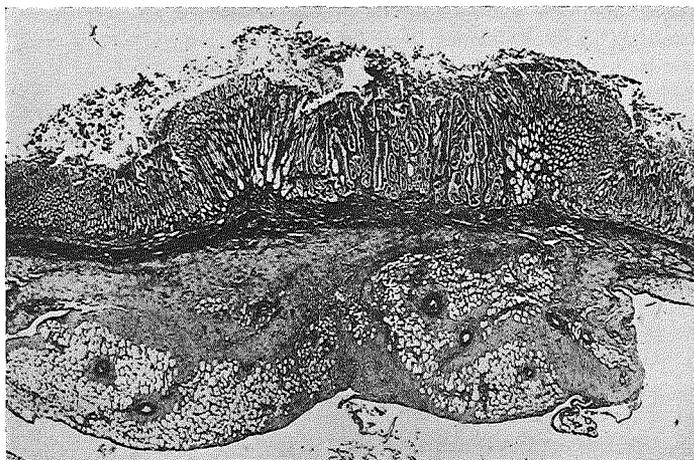


FIG. 176. Ascorbic Acid and Wound Healing. Control. Cross section of the gastric incision of a control normal guinea-pig killed twenty-one days after operation. Stain, silver impregnation. Note the uniform mature healing, and disappearance of catgut sutures (which disappeared on the seventeenth day). The epithelium is well healed and the only signs of the initial incision are the fusion of the layers of the muscularis and the substitution of the muscle by fibrous tissue.

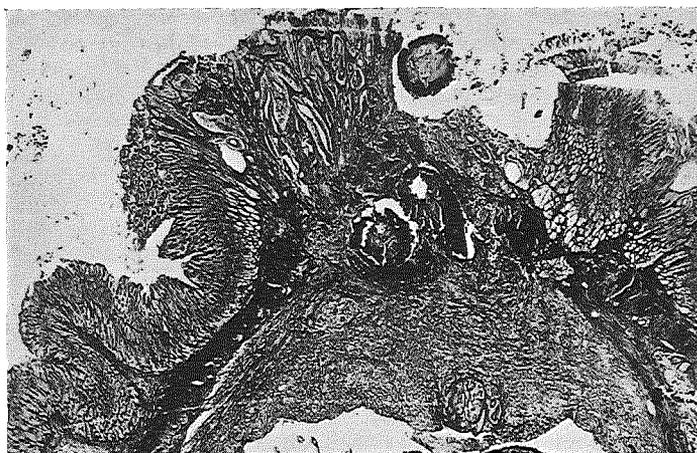


FIG. 177. Ascorbic Acid and Wound Healing. Subscorbutic guinea-pig. Cross section of the gastric incision of a subscorbutic guinea-pig killed twenty-one days after operation. Stain, silver impregnation. Note irregular delayed healing with a catgut ligature still not absorbed or extruded, and precollagen forming the intercellular substance in the subserous part of the scar.

According to Bartlett and his co-workers [852] normal wound healing may result, in spite of a low content of ascorbic acid in the plasma before operation, if adequate vitamin is given post-operatively. They have also shown that focal infection distant from the healing scar does not alter its ascorbic acid content or its tensile strength [954]. Local wound sepsis, however, reduces the tensile strength of a wound and interferes with its normal healing. The local use of sulphanilamide does not reduce the tensile strength of a wound scar nor does it retard healing. Analysis of scar tissue also reveals that sulphanilamide applied locally does not diminish its ascorbic acid content or increase the need of ascorbic acid in the production of a normal strong cicatrix. In the presence of considerable ascorbic acid deficiency local sulphanilamide does not promote wound healing nor does it adequately control wound infection. Bartlett and his co-workers [954] noted that in scorbutic animals the production of focal walled-off abscesses distant from the operative scar was followed by ultimate infection of the scar with the same organism. They call attention to the possibility that maximal tissue saturation with ascorbic acid may increase tissue resistance to infection as well as promote optimal conditions for wound healing.

From a study of thirty cases before and after operation Wildbolz [791] concluded that there was little evidence that ascorbic acid deficiency had any relation to post-operative complications. The administration of ascorbic acid to all operation cases during the course of a year reduced the incidence of post-operative complications from 24.2 to 19.7 per cent., but this reduction was not statistically significant.

On account of the necessity of ascorbic acid for wound healing massive doses of ascorbic acid are often given to surgical patients pre- and post-operatively, e.g. 1,000 mg. for nine to ten days [75]. It is possible that much less than this is adequate. Hunt [77] suggest 100 mg. daily, but 50 mg. daily is probably enough in view of the observations of Lund and Crandon [813] and Pijoan and Lozner [873]. If there is any reason to suspect impaired absorption or utilization the amount should be increased and given parenterally if necessary.

Hunt [77] suggests that the administration of ascorbic acid to surgical patients should be particularly considered under the following circumstances : (a) when clean and quick healing of a wound is desired ; (b) in major operations ; (c) when a hollow viscus has been opened ; (d) when post-operative complications are anticipated ; (e) when there is a history of insufficient intake of ascorbic acid ; (f) in all cases of serious injury ; (g) in patients with a history of vomiting over long periods ; (h) in cases of obstructive gastro-intestinal lesions and hypermotility of the small intestine ; (i) in syphilitics and alcoholics ; and (j) in patients receiving fluids such as glucose or saline intravenously or per rectum over a prolonged period.

The importance of an adequate intake of ascorbic acid for the healing of wounds is recognized by the Medical Division of the National Research Council, U.S.A., who recommend a daily oral intake of 75 mg. in all wounded and burnt men in the Services until recovery is complete [956].

According to Evans [953] skin grafts do not take readily in subjects with a low level of ascorbic acid nutrition.

Levenson and his co-workers [704] studied six patients with severe surgical conditions and observed very low ascorbic acid reserves. The patients were average hospital patients suffering from injury, hæmorrhage or infection. Beattie [706] also mentions the fall in ascorbic acid excretion after injury. A series of patients admitted for injuries or burns received 500 mg. ascorbic acid daily and no trace of the vitamin was found in the urine for five days.

Burns. Beattie [706] found that even when 500 mg. of ascorbic acid was administered daily to patients with burns no vitamin was excreted until the twenty-third day, in contrast to normal operation cases that excreted 50 to 100 mg. of ascorbic acid by the fifth day. It is probable that some part

of the increased demand for ascorbic acid in injury and burns may come from the adrenal cortex (p. 430). Certainly there is a fall in the ascorbic acid level in the adrenal cortex of burned animals [707, 708]. Lund and his colleagues [713] consider that large doses of ascorbic acid, e.g. 1 gram daily, thiamine, riboflavine and nicotinamide are needed by severely burned patients. A similar conclusion was reached in a study of patients with hæmorrhagic shock, traumatic injuries and infection.

Shock. Loss of hæmoglobin in acute hæmorrhage and circulatory failure in shock both lead to a considerable diminution in the oxygen supply to the tissues. In order to improve the transfer of oxygen from the blood to the tissues in cases of hæmorrhage and circulatory failure, Stewart and his associates [795] suggested the intravenous injection of ascorbic acid. Five control cats were bled until the blood volume was reduced to half; the blood pressure fell considerably and the animals died within an hour. Sixteen other cats were treated similarly, but they also received sodium ascorbate intravenously in doses from 200 mg. per kilo of body weight to a dose of 2 grams. In all cases the blood pressure rose and the survival period was much longer than in the control animals. In those animals that survived, a striking feature was the rapid reappearance of good pulse pressure and improvement in respiration after the injection of ascorbic acid. De Pasqualini [715] has also prevented hæmorrhagic shock in guinea-pigs by pre-treatment with ascorbic acid. The work of these investigators suggests the use of ascorbic acid in cases of accidents and injuries involving hæmorrhage. The dosage they suggest is 3 grams. of sodium ascorbate. The observations of Lucas [804] confirm Stewart's suggestion that ascorbic acid secures a more adequate supply of oxygen for the tissues. Lucas has shown that ascorbic acid administered intraperitoneally increases the resistance of mice and rats to low oxygen tensions. It has also been demonstrated that low concentrations of ascorbic acid increase the rate of respiration of isolated liver tissue [845].

Ungar [946], using a standard traumatizing technique in guinea-pigs, rats and mice, has shown that the injection of ascorbic acid within fifteen minutes after trauma reduces post-traumatic mortality in these animals. The minimum effective dose was 100 mg. per kilo of body weight. This effect is independent of the vitamin action of ascorbic acid, as it can be produced after oxidation; moreover rats and mice, the experimental animals used, do not suffer from ascorbic acid deficiency as they synthesize their own vitamin.

McDevitt and her co-workers [945] showed that scorbutic guinea-pigs are more susceptible to shock than normal animals, and that repeated trauma in animals with a normal intake of ascorbic acid "conditions" them to traumatic shock. It was found that massive doses of the vitamin immediately following trauma increase the survival time of animals, but do not lower the mortality rate.

Anæsthesia. Animal experiments suggest that tolerance to anæsthetics may be increased by the administration of ascorbic acid. The urinary excretion is increased in the dog, rat and guinea-pig after ether anæsthesia, and analysis of the adrenals, kidneys, liver and ovaries shows a fall in the ascorbic acid content of these organs after ether, chloroform or ethyl chloride anæsthesia [213, 796]. Guinea-pigs treated with 50 to 100 mg. of ascorbic acid show an increased tolerance to anæsthetics; they can withstand anæsthesia for a longer period than control animals that have not received the vitamin [213].

Beyer, Stutzman and Hafford [915] have shown that after anæsthesia with cyclopropane, ether, divinyl ether (vinesthine) and chloroform, the plasma ascorbic acid rises for the first seven hours, subsequently falling in twenty-four hours below the original level. This would account for the increased excretion after anæsthesia found by others. Beyer and his co-

workers have also shown that in animals suffering from ascorbic acid deficiency anaesthesia can be induced more rapidly, the animals are more depressed by a given dose, and they recover more slowly than healthy control animals treated with 30 mg. of ascorbic acid daily. If these data can be transferred to clinical anaesthesia, they suggest that large doses of ascorbic acid might be helpful before an anaesthetic is administered.

Psychiatry. In view of the dietetic idiosyncrasies and increased physical activity of some mental patients and the low ascorbic acid content of most institutional diets it is not surprising that a considerable degree of ascorbic acid deficiency has been found in such patients [495-498]. Some investigators have found low ascorbic acid levels in patients suffering from alcoholic and senile psychoses [495, 498, 503]. There is no evidence of any direct causal relationship between ascorbic acid deficiency and mental disorder. If a deficiency is found, it is usually due to malnutrition. One would expect, however, by improving the nutritional status of a patient to improve the general condition and even mental outlook of the patient. Minski and Constantine [797] found no correlation between the mental condition and the level of ascorbic acid in psychotic and psychopathic patients, nor did they observe any material change in the mental state of patients who had been treated with ascorbic acid. Stoltz [842] failed to observe any signs of ascorbic acid deficiency in patients with schizophrenia. Cranswick and Hall [501] gave ascorbic acid and desoxycorticosterone (DOCA) to thirty-seven patients with mental disorders. Those with a history of less than one year showed euphoric reactions and some beneficial effects, which were attributed to the liberation of a cortisone-like substance in the body. Similar observations were made by Bourne [774]

Cardiovascular Disease. Diuretic Action. Abbasy [801] has observed that ascorbic acid in doses of 700 mg. has a specific diuretic effect. This was noted during investigations on the excretion of the vitamin after giving test doses, and confirmed by using control subjects. It is claimed that the diuretic property of ascorbic acid may be of some use in cases where a slow and progressive dehydration of the body is desired, particularly as it is safe and unlike mercury diuretics does not damage the kidney. Evans [802] studied the diuretic effect of ascorbic acid in eight cases of heart failure and one of oedema of unknown origin. In doses of 500 to 300 mg. a day it produced a diuresis in all patients, as judged by excess of urinary output over fluid intake; its diuretic effect was greater than that of digitalis but less than that of theobromine, diuretin and ammonium chloride. The diuretic effect of ascorbic acid has also been observed by Lueg and Hammann [803], who state that it can be used to reinforce the mercurial diuretics, such as mersalyl, and actually increases their tolerance, since toxic effects on the kidney and liver are not so frequently observed when they are given with ascorbic acid.

Shaffer [944] noted that ascorbic acid in doses of 500 mg. produced a diuresis in patients with cardiac decompensation. The actual increase on a standard fluid intake of 1,500 ml. was from 250 to 1,000 ml. in seventy-two hours. When given intravenously it had no appreciable effect, possibly due to its rapid elimination by the kidney. In later publications with others Shaffer [179, 751] states that 100 mg. of ascorbic acid three times daily enhanced the diuretic effect of the organic mercurial drugs and diminished their toxicity. Anderson [752], using a self-retaining catheter, found that ascorbic acid had no diuretic action if the subject is receiving intravenous saline at the same time.

Croft, Jones and Richter [935] found that neither aneurine nor ascorbic acid deficiency is a significant factor in producing fatigue and other symptoms in patients with the effort syndrome.

Holtz [784] advanced the theory that histamine was formed *in vivo* and *in vitro* by the action of ascorbic acid on histidine, and Block and Pinösch [789] found that there was an increase of histamine in the lungs of guinea-pigs

following the administration of histidine and ascorbic acid. This suggested the use of histidine and ascorbic acid in the treatment of peripheral vascular disease, the continual and slow liberation of histamine producing vasodilation. Wirtschafter and Widmann [790] claimed that injections of histamine and ascorbic acid improved the blood supply in patients with peripheral vascular disease. Friedell and his co-workers [792] treated a group of twenty-five patients with impaired circulation of the lower extremities due to arteriosclerosis obliterans; they had been selected for amputation. Treatment consisted of injections of 5 ml. of four per cent. histidine every six hours or oral administration of 2 grams of histidine three times daily and 100 mg. of sodium ascorbate subcutaneously with each dose of histidine, and 600 mg. of ascorbic acid orally each day as well. These workers claimed that the pain of arteriosclerosis obliterans was relieved, and circulatory studies with radioactive phosphorus indicated an improvement. Weisman and Allen [793] failed to observe any improvement in digital blood flow after administering histidine and ascorbic acid to patients with occlusive arterial disease, nor were ischæmic pain relieved or the ischæmic lesions improved.

Ascorbic acid in large doses has a hypotensive effect on normal blood pressure in the rat, and on hypertension due to large doses of salt, renal compression and large doses of desoxycorticosterone acetate (DOCA) [743, 810]. It has not been used for this purpose clinically.

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CHAPTER VII

VITAMIN D

THE ANTIRACHITIC OR CALCIFYING VITAMIN

SEVERAL different substances are now known to be antirachitic : the term vitamin D is used to embrace them all. The most important are :

Vitamin D₂ or Calciferol. This seldom occurs naturally but is manufactured artificially by irradiating or "activating" the vegetable sterol ergosterol. Viosterol is a name chiefly used in the United States for unpurified irradiated ergosterol : it has little meaning, as the amount of calciferol in viosterol varies greatly, according to the method of irradiation employed.

Vitamin D₃. This is the most important naturally occurring vitamin, being formed in the skin by the action of the sun on the animal sterol 7-dehydrocholesterol.

Vitamin D₁ is a name which is no longer used, having been given to a substance which was later found to be an addition compound of calciferol and lumisterol.

HISTORY

The early history of vitamin D is the history of rickets. It is a depressing history : a history of perfect clinical observation on the cure of the disease being forgotten again and again for a century and a half.

Rickets suddenly became recognized as a definite disease by medical writers in the last half of the seventeenth century ; the poor had probably been familiar with it for many years. The name rickets, according to Skeat [1], is an old English word : the adjective rachitic was forced into our language by the mistaken desire of lovers of the classics to give rickets a Greek derivation.

The first description of rickets is given by Daniel Whistler, who, in 1645, published his "De Moro puerili Anglorum, quam patrio idiomate indiginæ vocant 'The Rickets.'" This was a thesis presented for his Doctorate of Medicine at Leyden, which has led some to say he was Flemish. He was, however, an Englishman educated at Thame and Merton College, Oxford. His description of rickets, written at the age of twenty-five, was followed four years later by another by Arnold Boate, and it was not until 1650 that Glisson [2], a Cambridge man, published his account, which for some obscure reason is generally said to be the first. J. Whitaker in 1646 and Thomas Fuller in 1647 had also briefly mentioned rickets in infants. A few much earlier descriptions of what were probably rickets or its adult equivalent, osteomalacia, have been collected [208, 245], though these were not recognized as an individual disease, but reported as curiosities.

The eighteenth century added little to the history of rickets until towards its end when cod-liver oil was first used in medicine, though apparently in Scotland and Northern Europe cod-liver oil had been popular for many years with the peasants as a cure for rickets and other diseases.

In 1782 Dr. Robert Darley [3] wrote to Dr. Thomas Percival an account of his use of cod-liver oil, which was so highly successful that the poor clamoured for it, though its smell and taste were loathsome as it was made by "heaping together the livers of the fish, from which, by gentle putrefaction the oil flows very plentifully." Percival [3] recommended peppermint to conceal the taste—advice which a century and a half of further experience has not bettered. But it must be admitted that the earliest account of the

value of cod-liver oil did not stress its value in rickets : in fact, only two children were reported, the other cases being arthritis or rheumatism.

After this the use of cod-liver oil for rickets seems to have spread rapidly in Germany and Holland and from there to France. By the middle of the last century Trousseau [4] was teaching that cod-liver oil was the well known and perfect cure for rickets, or when this was too expensive, large quantities of the best fresh butter—suitably concealed in a mixture so as not to shake the confidence of the patient by prescribing such a simple remedy. He also realized the uselessness of vegetable oils, the value of sun, the drawbacks of cereals, and identified osteomalacia as adult rickets.

The last century had also gone far in elucidating the nature of rickets by

animal experiments. Jules Guerin [5] in 1838 had produced rickets in puppies to support his theory that rickets was due to the wrong food, and Bland Sutton, fifty years later, had used cod-liver oil, milk and crushed bone to cure rickets in lion cubs at the Zoo.

At the beginning of the present century all this brilliant clinical observation appears to have been largely forgotten, even though Trousseau's book had been translated into English by the New Sydenham Society. In 1912 Sir William Osler was still only vaguely mentioning cod-liver oil as useful in rickets. Indeed, there was amazing confusion on the subject. There were three outstanding theories.

One theory was that it was a chronic infective condition like tuberculosis, this being still widely believed on the Continent as late as 1919.

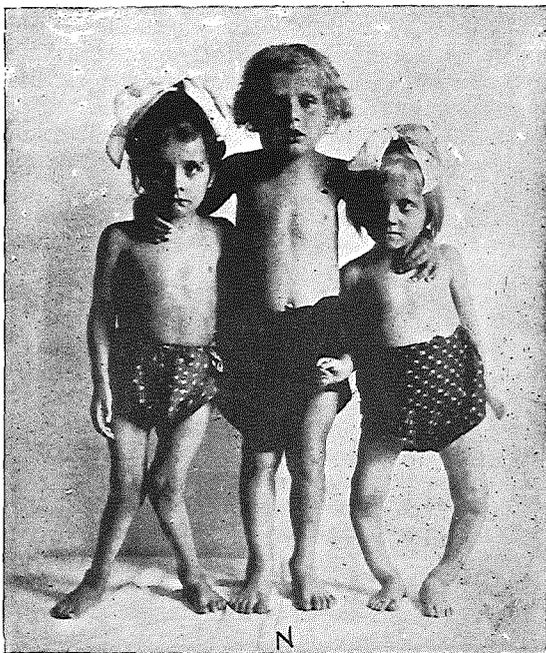


FIG. 178. Rickets in Vienna in the post-War famine of 1920. The children, six years of age, show severe rachitic deformities compared with the normally grown child of the same age in the centre. (See also Fig. 188.)

The second theory was Hansemann's "Domestication Theory" which was upheld by the "Glasgow School," so called from the observations of Fergusson and Findlay [7] on rickets in Glasgow in 1918. The theory is well summed up by Hutchison and Shah [8] in their observations on the effect of Purdah in India in 1922 : "The most important ætiological factor in the production of rickets is lack of fresh air, sunlight and exercise." But sunlight was never stressed as the one important factor in fresh air and exercise, though Palm [9] had emphasized this in 1890.

Thirdly, the theory was gaining ground that rickets was a deficiency disease. This was due to the work of Edward Mellanby [10], who by 1918 had produced experimental rickets in puppies by feeding them on diets which were deficient in some factor found in certain animal fats. At first this factor was thought to be the same as the growth factor described in 1913 by McCollum and Davis [11] and the "fat soluble A factor," which was necessary for the protection of the eyes—the antixerophthalmic factor—described in 1917 by McCollum and Simmonds [12].

But further work showed that the antirachitic factor and the anti-xerophthalmic factor were different. They did not always occur in the same proportion in all fats tested; one fat might give little protection against xerophthalmia but give good protection against rickets, while another fat might have the opposite effects. It was also found that heat and oxidation destroyed more of the antixerophthalmic than the antirachitic factor.

The importance of calcium and phosphorus in relation to rickets was shown by McCollum when extending Mellanby's work to rats, animals which only develop rickets if these two elements are badly balanced in the diet.

In 1919 Chick and her co-workers went to study rickets in Vienna during the post-War famine. The work was originally undertaken to follow up the clinical possibilities of Mellanby's work. It was extended to include the effect of sunlight, and exposure to the radiations from the mercury-vapour quartz lamp, because of the observations of Huldschinsky [13] and others, who had definitely proved that rickets was curable by these forms of light. Chick's final report in 1923 [14] showed that both cod-liver oil and sunlight, or the mercury-vapour quartz lamp, cured rickets; but improved hygiene with neither cod-liver oil nor sunlight was useless. In fact, the dietetic theory of rickets was completely confirmed, and the teachings of the Glasgow school in so far as they taught or implied the value of sunlight.

The end of the complicated story of rickets, food, and sunlight was unravelled by the work of Hume [15] and many others; though to the last there were unexpected complications, as when rats did not develop rickets because they were themselves supplementing their deficient diets by eating irradiated sawdust in their cages. Many experiments proved that ultra-violet irradiation of an animal or of food produced the antirachitic substance, the ultra-violet light activating a substance—"the provitamin"—found only in the unsaponifiable fraction of fat.

So it was finally shown that animals can either make their own vitamin D by the aid of the ultra-violet rays of the sun, or they can get it by eating other animals which have themselves already made it.

CHEMISTRY OF VITAMIN D

All the forms of vitamin D so far investigated are derived from sterols, generally by photochemical reactions. They are chemically, but not apparently physiologically, related to the sex hormones, the bufotoxin of toad venom, the digitalis and strophanthus alcohols, and the carcinogenic hydrocarbons [6]. The chemistry of the sterols is extremely complex: the following account of the formation and properties of vitamin D₂ and vitamin D₃ is largely taken from the excellent review and papers by Bills [16]. For an account of the early work on the chemistry of vitamin D₂ and its preparation in a pure form by English and German workers in the same year (1931), the reader should consult the Medical Research Council's "Vitamins: A Survey of Present Knowledge," published in 1932.

Vitamin D₂, or calciferol, was the first of the D vitamins to be fully investigated, though as will be seen when the physiology of vitamin D is discussed, it is as used in medicine an entirely artificial product made from ergosterol—a sterol only found in plants, especially fungi. It seldom occurs naturally, as opposed to vitamin D₃, which is the vitamin formed and used by animals [17] and the first and most important identified in fish liver oils [18].

Vitamin D₂ is made by exposing ergosterol to the action of ultra-violet light. The absorption spectrum of ergosterol gives bands of maximum intensity at 260, 270, 282 and 293.5 millimicrons. As would be expected from this it is wavelengths of about 230 to 305 millimicrons which effect the change of ergosterol to calciferol. But calciferol is not the final product which can be produced by irradiating ergosterol: for calciferol itself is changed by irradiation, especially by wavelengths shorter than 270 millimicrons.

Ergosterol can be irradiated either as a solid or in solution : the latter is the only satisfactory way, the products of irradiation on the outside of the solid ergosterol apparently protecting the rest of it from the effects of irradiation.

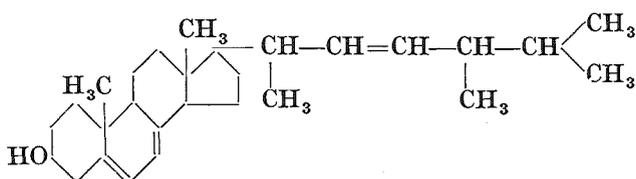
The solvent is important ; for instance, if alcohol is used it is difficult to avoid over-irradiation, with the result that vitamin D₂ or calciferol is destroyed with the production of the toxic toxisterol in its place. Ether, on the other hand, makes it relatively easy to control the irradiation and so to form calciferol with little or no toxisterol. This effect of the solvent is called the specific solvent effect ; it is not clearly understood.

The change from ergosterol to calciferol is purely a photochemical one, involving only a rearrangement of the molecular structure. It is almost unaffected by temperature, though the presence of more than minute quantities of oxygen should be avoided.

The usually accepted series of reactions which occur when ergosterol is irradiated are : ergosterol, lumisterol, tachysterol, calciferol, toxisterol (substance 248), suprasterol I and suprasterol II.

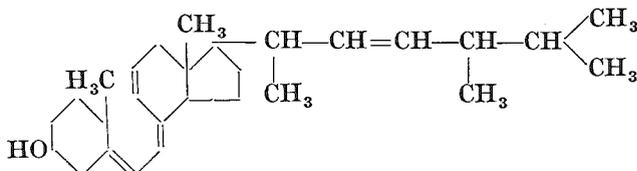
It must be remembered, however, that all these reactions are going on at the same time. It is necessary to stop irradiation when only about half the ergosterol has changed to calciferol, or the continued irradiation will destroy the calciferol which has already been formed. The result is that at the end of the irradiation large amounts of lumisterol and tachysterol with traces of toxisterol are still present. In the Whittier and other processes these drawbacks of making calciferol by irradiation are said to be avoided by activating ergosterol in various ways such as vaporizing it and then exposing it to an electrical discharge [19], but in spite of the often quoted claims for purity made by the manufacturers the resulting products—at least in the Whittier process—owe most of their activity to forms of vitamin D other than calciferol [35] and are no less toxic (p. 578).

Ergosterol has the formula :



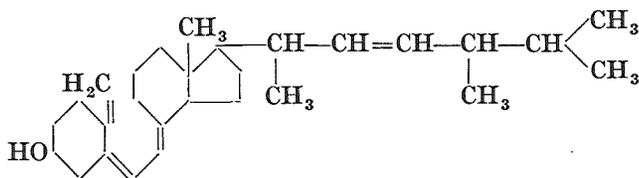
Lumisterol has exactly the same two-dimensional formula as ergosterol, but is not precipitated with digitonin, presumably only differing from ergosterol in the relative position of the hydroxyl and methyl groups. It has no antirachitic activity.

In tachysterol



a profound alteration in the molecule has occurred, one of the rings having been ruptured. Tachysterol, so called because of the rapidity with which it reacts chemically, has no antirachitic action.

Calciferol



is a white crystalline fat-soluble substance, melting at 115°–117° C. Its maximum absorption is at 265 millimicrons. It is only stable if kept in a refrigerator in amber coloured bottles, sealed without air; dissolved in propylene glycol or maize oil it is stable, even sealed with air, at room temperatures for at least three years [20]. It loses its antirachitic properties when heated to about 180° C. Its antirachitic potency is generally said to be 40,000 I.U. per mgm., though it may be ten to fifteen per cent. higher than this [20]; esterification greatly decreases it [21].

The estimation of calciferol in an irradiated solution of ergosterol, or in

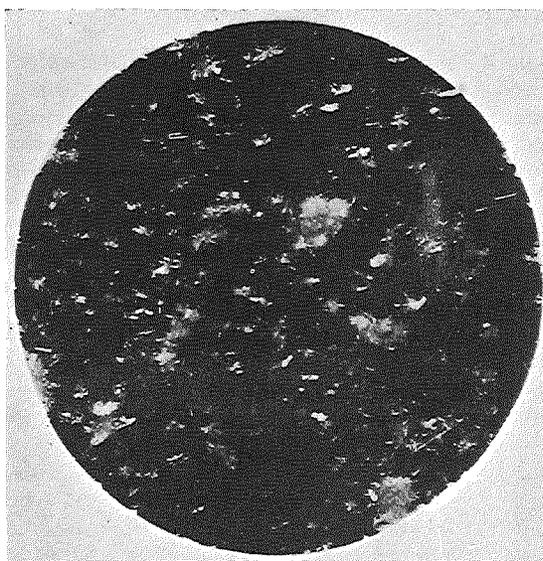


FIG. 179. Crystals of Vitamin D₂ (Calciferol).

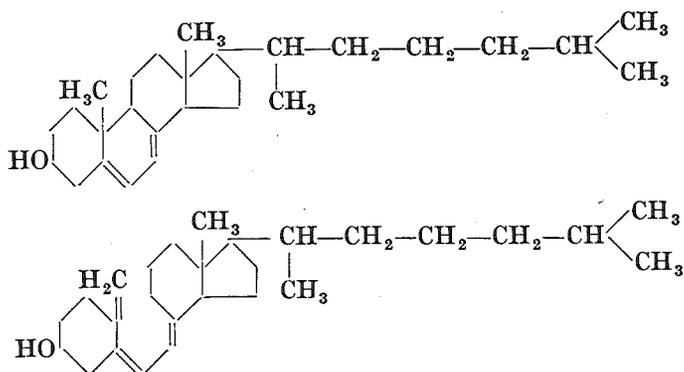
any other circumstances, is very difficult. Its absorption spectrum cannot be used to give accurate estimations, as other irradiation products blur the picture; the latter also prevent any chemical estimations, so that—apart from the use of colour reactions with antimony trichloride for the very inaccurate estimation of vitamin D₃ in fish liver oils [19, 22, 23]—biological assay is the only means at present available. This is discussed later on p. 523.

Calciferol, if irradiation is prolonged, turns to toxisterol (substance 248), about which little is known chemically. It has an intense absorption band with its maximum at 248 millimicrons. It probably has no antirachitic action, though it is toxic. With further irradiation it changes to the suprasterols. It is formed in the largest amounts when alcohol is the solvent.

Vitamin D₃ is formed in the same way as vitamin D₂, only its precursor, or provitamin, is 7-dehydrocholesterol, an animal and not a vegetable sterol. This provitamin is apparently made by the dehydrogenation of cholesterol in the wall of the small intestine [24] and can be converted *in vitro* to vitamin D₃ without irradiation [25, 26].

The absorption spectra both of vitamin D₃ and its provitamin are like the spectra of vitamin D₂ and its provitamin. This is the chief reason why for so long vitamin D₂ was thought to be the only vitamin D.

The formulæ for 7-dehydrocholesterol and vitamin D₃ are as follows :



The estimation of vitamin D₃ has the same difficulties as the estimation of vitamin D₂, and so to be accurate has to be biological (p. 523). The stability of vitamin D₃ is very similar to that of vitamin D₂; it has been very fully investigated under various conditions and with various solvents by Huber and Barlow [20]. The effect of saponification of fish liver oils on the biological activity of the vitamin D₃ which they contain has been investigated by Bailey [21].

Vitamin D₄ is made by irradiating 22-dihydro-ergosterol. It is an artificial vitamin of no practical interest.

There are many other D vitamins, of which about twenty have been more or less investigated. None as yet appear to be of more than theoretical importance. There is, however, evidence that some fish oils contain further new forms of vitamin D [27] which are especially antirachitic for the turkey [28, 29] and are also of clinical value [30]. A further complication is that fish liver oils enhance the biological action of vitamin D [31].

UNITS OF VITAMIN D

The International Unit of vitamin D is the vitamin D activity of 0.025 micrograms of the International Standard preparation of crystalline vitamin D₃, which has the following properties :

Melting point, 87°–89° C. (corr.).

$[\alpha]_{\text{D}}^{20} = +110^{\circ}$ (ethanol).

$E_{1\text{cm}}^{1\text{per cent.}}$ 265 $m\mu = 490$ (ethanol) corresponding to a molecular extinction coefficient of 18,800.

Coward's very interesting paper [32] should be read for her account of how the first standard of reference for any vitamin—originally thought of and used in England—has slowly evolved into the present International Standard. For all practical purposes this Standard has the same value as the calciferol one which it replaced, so the value of an International Unit in current and earlier work is the same. Coward [32] also gives the relative values of other units of vitamin D which were in common use up to 1952.

It must be remembered that as children (p. 538) and chicks [33] are less sensitive than rats to vitamin D₂ (calciferol), though equally sensitive to the vitamin D₃ of fish liver oils, the potency of oils intended for human or avian consumption must be assayed on chicks and not on rats, as otherwise any adulteration of the oil by vitamin D₂ will give it too high a value.

ESTIMATION OF VITAMIN D

Biological methods have to be used for accurately estimating vitamin D because chemical or spectroscopic methods are only of value for the very crude assay of fish liver oils (p. 523). For full details of the biological methods Coward's book [38] should be consulted. Four methods are commonly used, the results obtained being accurate to within \pm ten per cent.

The "Line Test." This is based on the cure of rickets. Two identical groups of rats are fed on a rachitic diet until rickets has developed. To the

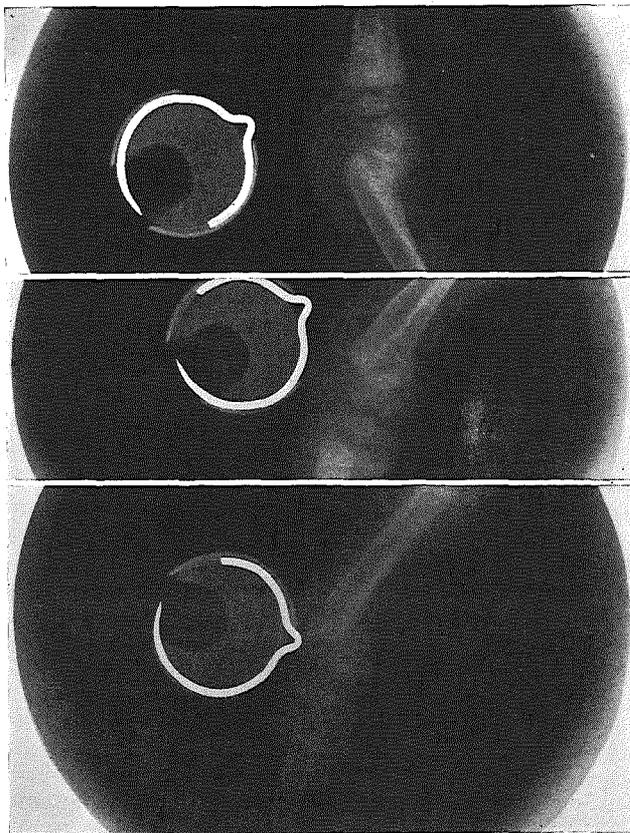


FIG. 180. X-rays of the tarso-metatarsal joints of five-weeks-old chicks used in Olssen's radiographic method of assay of vitamin D. The upper X-ray is from a chick receiving natural vitamin D₃ from cod-liver oil, the middle X-ray from a chick receiving the same number of International Units of synthetic vitamin D₂ (calciferol), and the lower X-ray is from a chick receiving no vitamin D. Note in the top X-ray the greater calcification and the narrower tarso-metatarsal gap. (See also Fig. 184.)

diet of one group is then added a standard preparation of vitamin D of known potency, and to that of the other group the substance to be assayed. This curative period requires about ten days. The rats are then killed and the distal ends of the radius and ulna in each rat are examined for calcification by splitting the bones and putting them in a solution of silver nitrate. The bone which has been deposited during the curative period is thus stained, showing as a black line. By comparing the "lines" of the two groups of animals the potency of the material being assayed can be deduced. Instead of staining with silver nitrate after death sodium alizarinate may be injected

into the animals during life. This stains newly deposited bone, thus making it easy to see [40]. The incisor teeth of rats, according to Irving [41], show a more rapid and more delicate response to vitamin D than do the epiphyses.

In every fresh assay the standard vitamin D preparation must be used as a control, since the response of a colony of rats to the same amount of vitamin D varies greatly at different times. The number of rats used depends on the material being investigated. If the potency of this is not already roughly known thirty-six animals will be required, so that the standard and the substance being tested may be compared at three different levels of intake. Where large numbers of estimations are being performed the construction of a "curve of response" as in vitamin A estimations saves time.

The X-ray Method. This is similar to the line test, but the degree of calcification is judged radiographically.

The Bone Ash Method. This method depends on the prevention of rickets. Groups of rats are fed for from four to six weeks with varying levels of the standard preparation and that being tested. They are then killed and the bones of the femora ashed, using the metaphyses alone being most satisfactory [42]. Comparison of the weights of the ash from the various groups of animals shows how much calcification has occurred. This method is more arduous than the others but gives more accurate results, and may be used with chicks [34].

Olsson's Radiographic Technique [34]. Here chicks are used instead of rats, and the antirachitic potency is gauged by radiographic measurement of the tarso-metatarsal gap (Figs. 180, 184). Baker and Wright [39] state that this method is as accurate as the bone ash method for chicks, and has the great advantage that records are easy to keep and that the birds are not sacrificed.

Other methods which have not yet been fully confirmed are based on the estimation of plasma phosphatase in chicks [36] and on the external "count" of the forepaws of rats given radioactive phosphorus [37]. It is important in all assays that the animals should eat all their diet, especially the phosphorus and calcium, and fat. When any of these are present in the substance being tested equivalent amounts must be added to the diets of the control animals.

PHYSIOLOGY OF VITAMIN D

Vitamin D₂ (calciferol) and vitamin D₃ have broadly the same biological effects and so will be regarded as one in the following pages, those differences not already mentioned being fully discussed as they arise, especially in regard to their relative toxicity (p. 534) and their relative antirachitic value for children (p. 538).

The Origin of Vitamin D of Animals. Animals gain their vitamin D in two ways: by devouring the tissues containing vitamin D of other animals, or by the direct action of the sun's rays on the provitamin in or on their own skins. Food as a source of vitamin D is generally not important; only man in temperate and cold climates and nocturnal animals and birds are driven to use food for a substitute for sun, as are stall-fed cows and calves reared, as they should be, in dim byres. Such cows [59] or calves [43, 44] gain sufficient vitamin D for calving and lactation or good bony development from hay or silage: the origin of this vitamin is presumably from sterols, activated by the sun during drying, in the grass or in fungi on the grass. Another rich vegetable source is cacao shells [16].

Vitamin D is sometimes deliberately increased in human food. This can lead to some confusion as to what vitamin is really being taken. Thus by irradiating a nursing mother or an animal vitamin D₃ is increased in their milk because their own animal sterol has been activated. The same result is obtained if the milk itself is irradiated. But if a nursing mother or animal is fed on irradiated yeast or any other form of irradiated ergosterol the increase

in the antirachitic power of the milk is due to vitamin D₂ being secreted in it.

The action of the sun on the skin is profoundly interesting. The formation of the vitamin appears to occur rather on the skin than in it. Thus birds when they preen themselves remove oil on their beaks from their preen glands and spread it over their feathers, where it is exposed to the sun and activated. It is then probably absorbed by the skin rather than scraped off the feathers by the beak and eaten [45]. The removal of the preen gland makes birds more susceptible to rickets, and prevents ultra-violet light from having any antirachitic effect unless the feet as well as the plumage are irradiated. The fur of animals in a similar way appears to be the place where the vitamin is formed: preventing rats from licking their fur destroys the antirachitic effect of irradiation, and owls and young carnivorous birds in captivity have to be given not only the flesh but also the fur of mice or rabbits if they are to thrive [46]. All this suggests that the incessant "washing" of cats and rabbits and the apparent hunt of monkeys for each other's fleas is really a method of gaining vitamin D. In man also activation appears to occur on, rather than in, the skin. Helmer and Jansen [47] found that the fat washed off the bodies of athletes who had been exposed to irradiation before taking violent exercise was antirachitic, while fat from the skin of athletes who had not been irradiated had only a trivial potency. Irradiation of this fat made it potent. Further, the ultra-violet rays of the sun penetrate only some 0.1 mm. [48] to 1.2 mm. [49] through the skin, so that activation must occur at least close to the surface. Possibly the old belief that too much washing makes babies fretful is due to the removal of their vitamin D leading to the fretfulness of rickets. After sunbathing it appears possible that swimming is a mistake, the activated fat being washed off the skin before it has had time to be absorbed.

The Origin of the Vitamin D of Fish. Nothing is definitely known about how fish acquire their vitamin D. The plankton in the sea which are the basis of the diet of the small fish, on which the larger fish feed, do not, according to Drummond and Gunther [50], make vitamin D, nor does it seem probable that the sun's rays activate a provitamin in the fish [51]. Thus the two ways in which animals acquire vitamins are not open to fish. One is driven back to the explanation that fish synthesize vitamin D, though why they do so, or what advantage it is to them to store so much is obscure. Apparently the vitamin in many cases is not only vitamin D₂ [75] but also one or more new vitamins of an even higher antirachitic value [27-30].

Assimilation of Vitamin D. In birds and furry animals we have already seen that the vitamin having been formed on the feathers and fur is, at least in part, swallowed during preening and licking. In man, whether the vitamin is made in or on the skin, it must be absorbed from the skin because irradiation will cure rickets. This, and the fact that vitamin D is active when injected, proves that no essential change has to take place in it during digestion before it can be used by the body. Polskin and others [187], from a review of the scant literature, find that over ninety per cent. of vitamin D is absorbed from concentrated preparations by man, though far less by animals and birds apart from the dog.

Bile is essential for the absorption of vitamin D. Taylor and his co-workers [52] found that dogs with biliary fistulæ did not absorb vitamin D when given by mouth unless bile salts were given at the same time. Other investigators, among whom is Heymans [53], have confirmed these results by very similar experiments on rats or dogs. No analogous work has been done on man, but there seems no reason to doubt that similar results would be obtained.

Fat does not appear to be essential for absorption since fat-free tablets of calciferol taken by mouth can cause vitamin D poisoning (p. 578), but vitamin D probably follows the same path as fat through the intestinal wall as impaired fat absorption in man may cause rickets (p. 561) or osteomalacia

(p. 566). Dissolving vitamin D in a fine emulsion of fat probably aids absorption, arguing both on analogy with vitamin A (p. 18) and because the vitamin D in milk has so high an antirachitic value for infants (p. 537).

Liquid paraffin, or mineral oil, hinders the absorption of vitamin D, probably because the vitamin is dissolved in the oil and so is excreted with it in the faeces. Smith and Spector [54] reported that rats on a non-rachitic diet develop rickets if as much liquid paraffin is added to their food as would correspond to the amount taken by man for constipation. Five times the normal requirements of cod-liver oil had to be taken by the rats to counter-balance the effect of this amount of liquid paraffin. The same workers [55] also found that the antirachitic value of irradiation is reduced by liquid paraffin, since when it is included in the diet of rats they require a larger amount of irradiation to avoid rickets. This, it is suggested, proves that vitamin D, formed by irradiation, has to be excreted into the gut to function. A more probable explanation is that liquid paraffin hinders the absorption of cholesterol and so reduces the amount of provitamin D which can be made from it in the gut wall [24] and then passed on to the skin for irradiation. Though nothing is known of the effect of liquid paraffin on the absorption of vitamin D in man, it would seem that the use of it and its emulsions as aperients should be avoided, especially as it also interferes with the absorption of vitamins A, E and K, and also damages the liver [57]. The advice given to patients to take liquid paraffin several times a day after meals therefore is bad.

Fœtal absorption appears to be excellent as long as the mother herself receives or has stored ample vitamin D. Thus in rats Embleton and Collings [58] report that a high maternal intake leaves the young resistant to a rachitogenic diet and, further, that it still confers such resistance four months after its replacement by a deficient diet. The blood of newly-born calves [59] only contains about half or a quarter as much vitamin D as does the maternal blood, the values for the latter varying from 79 I.U. per 100 ml. in winter to 347 I.U. in early autumn, while the values in calves range from 46 to 111 I.U., with liver stores of 0.09 to 0.21 I.U. per gram.

Storage of Vitamin D. The various tissues of the body store vitamin D for varying periods. The following times in weeks are given for rabbits by Heymann [56]: brain, 1-2; red blood cells, 5-6; small intestine, 5-8; large intestine, 6-8; lung, 6-9; kidney, 6-9; liver, 8-12; blood plasma, 8-12 or longer. Other reports mention shorter times, especially for the liver and the lungs; in the latter an enzyme was even thought to be present which destroyed vitamin D, but its disappearance was probably due to not keeping the lungs frozen until the estimation was made. There are only a few estimations of the amounts of vitamin D in the various tissues of the body—such as those mentioned in the previous section and the food tables—because lengthy biological methods have to be employed (p. 523). Warkany and Mabon [60] report that the normal level in the blood both of adults and children varies from 66 to 165 I.U. per 100 ml., with seasonal variations. When adults take from 50,000 to 500,000 I.U. of vitamin D daily the level in the blood rises to between 9,000 and 13,000 I.U. per 100 ml. [61]. Vollmer [62] gave three children dying from incurable diseases massive doses of vitamin D shortly before their death. After death no vitamin D was found in the tissues of one child; the second child had large stores in the brain, liver and skin; the third child had stores in the skin, thyroid and parathyroids, but none in any part of the brain, liver, thymus, or blood. The explanation of these confusing observations is not clear. Houet [102] examined an infant who died four days after an injection of 600,000 I.U. and found twenty-five per cent. of the vitamin still at the site of the injection, four per cent. in the cerebrosplinal fluid is said to contain no fat-soluble vitamins [103].

Excretion and Destruction of Vitamin D. The depletion of the stores of

vitamin D in the body are caused partly by its excretion into the gut and partly by its destruction in the body, though there is no knowledge about how or where the latter takes place. The former is said by Heymans [53] to occur in the upper third of the small intestine. Lawrie and others [63] state that no vitamin D is normally excreted in human or canine urine, but one patient with osteomyelitis and nephrosis excreted 20 I.U. daily when taking 9,000 I.U. by mouth, which is reminiscent of the urinary excretion of vitamin A (p. 30).

Rachitic Diets, apart from Lack of Vitamin D. The composition of the diet, apart from its vitamin D content, has a great effect in rickets. Thus it is necessary to consider briefly what backgrounds of diet may complicate investigations on vitamin D.

Calcium and Phosphorus. Not only the absolute amounts of these elements, but their proportions to each other are important. In rats, in spite of a deficiency of vitamin D, rickets does not occur unless the calcium and phosphorus intake is unbalanced.

In man and dogs rickets occurs on normal diets if vitamin D alone is deficient, but even here the calcium and phosphorus of the diet is a deciding factor in the amount of vitamin D which will prevent rickets. As they become more unbalanced in the diet, so does the amount of vitamin D required increase.

The usual ricket-producing diets contain an excess of calcium and act by forming insoluble phosphates in the bowel, which cannot be absorbed. Diets deficient in calcium or phosphorus really have the same ultimate effect—the inadequate supply of minerals for calcification.

Metals which form Insoluble Phosphates. Any metals, such as magnesium [64], lead [65], strontium [66], thallium [67], beryllium [68], and cadmium [69], which like calcium form insoluble phosphates, will accentuate rickets in the same conditions as will extra calcium.

Fluorine. Fluorine increases the deposition of calcium in the bones of rachitic rats and delays their death, though it hinders the antirachitic action of vitamin D [70, 71]. It also protects the teeth against decay (p. 570).

The "Acidity" of the Diet. Foods with an alkaline ash tend to decrease the absorption of phosphorus and calcium from the gut, but to increase their retention in the body, while foods with an acid ash have the opposite effect.

Organic Acids. Citric and tartaric acids and their salts may have a beneficial effect on rickets. This has nothing to do with altering the acidity of the bowel. Day [72] found that citrates only have an effect when there is phytic acid in the diet and a high calcium phosphorus ratio. He suggests this is due to the citrate forming a complex with the calcium, so enabling the phosphorus of the phytic acid to be absorbed instead of forming an unabsorbable compound itself with the calcium, a subject discussed in the next section. Lecoq [73], however, has shown that citric acid enhances the absorption of calcium when no phosphate or phytate complicates the position.

Phytate and Cereals. The rachitogenic action of cereals and especially of oatmeal was known by Trouseau [4] in 1865. Our present knowledge of the subject we owe entirely to Mellanby, whose paper [74] published in 1949 should be read in full. The main action of the phytate of cereals is to combine with the calcium in the food to form an insoluble compound which is lost in the faeces, thus robbing the body of calcium and also of the phosphorus of the phytate. Vitamin D can, but only to a limited extent, counteract the effects of phytate, decreasing the faecal excretion of both calcium and phytate. It is also possible that phytate more than inorganic phosphate uses up the reserves of vitamin D in the body. Green cereals are highly rachitogenic for sheep [96].

Phytate suddenly attracted considerable attention when the Government came in April, 1942, to the tardy decision that it was necessary to substitute

eighty-five per cent. extraction flour for the seventy per cent. extraction "white" flour which hitherto had been used for most of the bread and pastry in England. It is regrettable that this decision was made to save shipping space and not to improve the diet of the nation. Among the many protests of millers, politicians and literate gastric hypochondriacs against this dietetically wise legislation was the possibly valid objection that the increased phytic acid in eighty-five per cent. extraction flour would dangerously decrease the absorption of calcium, especially in view of the dearth of foods rich in calcium during war, such as milk and cheese. To compensate for this effect of phytic acid it was proposed that calcium should be compulsorily added to the flour. *The Times* and also Parliament and the medical press, throughout the winter and spring of 1942 and 1943, hotly argued about the proposal with common sense, bias, ignorance and science. One of these carried the day and 7 ounces of calcium carbonate was added to every 280 pounds of flour. For an excellent summary of and references to the spate of speeches and correspondence on this subject and also on the quaint earlier idea of the Government to add synthetic aneurine to white flour the reader should consult the fascinating review by Lepkovsky [75] on "The Bread Problem in War and in Peace." It is to be hoped that the actions of the Government as shown in this review will appear vacillating rather than venial. The outstanding papers on the effect of high extraction flour on mineral absorption in man are those by McCance and Widdowson [76], which strongly support the addition of calcium to such flour. The work, however, of Krebs and Mellanby [77] and of Cruikshank and others [78], suggests that this addition may be unnecessary and the work of Yudkin [79] shows that calcium phosphate might be a better salt to add than calcium carbonate, especially for young children [79, 80], and nursing mothers [81]. It would also seem possible that the efficiency of calcium absorption is largely governed by the needs of the body [82] so that investigations which show a negative balance on diets largely composed of high extraction flour may be invalid because the experimental subjects never lost sufficient calcium to bring about an increase in the efficiency of its absorption [84]. This endogenous factor for the control of calcium absorption appears, in animals at least [82], to be dependent on vitamin D and to be only present during growth.

Fat and Carbohydrate. The amount of fat in the diet is important. McDougall [83] counteracted the effect of cereals by a high fat intake, attributing this to the formation of calcium soaps which were rendered soluble by the action of bile, and so were easily absorbed. She later thought the cholagogue action of fat was also involved. Knudson and Floody [84] report that there is an optimum intake of fat for the prevention of rickets, larger or smaller amounts than this having a less beneficial effect, which may be due to the anticalcifying action of fat being reversed when the phosphorus of the diet is increased from low levels to high [85].

Outhouse and others [86] have shown that lactose, but not sucrose or starch, increase calcification and the retention of calcium, magnesium and phosphorus. This observation may be explained by the work of Laszt and others [87] who showed that in rachitic animals the phosphorus which is excreted into the gut for the absorption of glucose is poorly reabsorbed. This means that any sugars which cause the gut to excrete phosphorus will tend to be rachitogenic because the excreted phosphorus, if there is not sufficient vitamin D for its reabsorption, will be precipitated by calcium and magnesium and so all three will be lost to the body. Sugars like lactose which do not cause the secretion of phosphorus will, by contrast to sugars like glucose, appear to be antirachitic.

Yeast is rachitogenic when fed in large quantities to pigs. Why this is so is obscure, all the classical reasons for the occurrence of rickets having been ruled out [97]. Yeast also disturbs vitamin A metabolism (p. 49).

Effect of Vitamin D on Calcification : Rickets. Vitamin D regulates the metabolism of calcium and phosphorus (p. 535).

Since, by definition, vitamin D cures or prevents rickets, most physiological investigations have been on its effect on bone metabolism and the elements which chiefly form bone, calcium and phosphorus. It must, however, be admitted that the action of vitamin D is still very obscure. This obscurity is partly due to the complexity of the problem, partly due to the fact that too much attention has been paid to the bone changes in rickets and not enough to the wider metabolic disturbances. It should also be remembered that in spite of Mellanby using puppies for his original work and in spite of toads [221] being probably ideal for research and like infants in

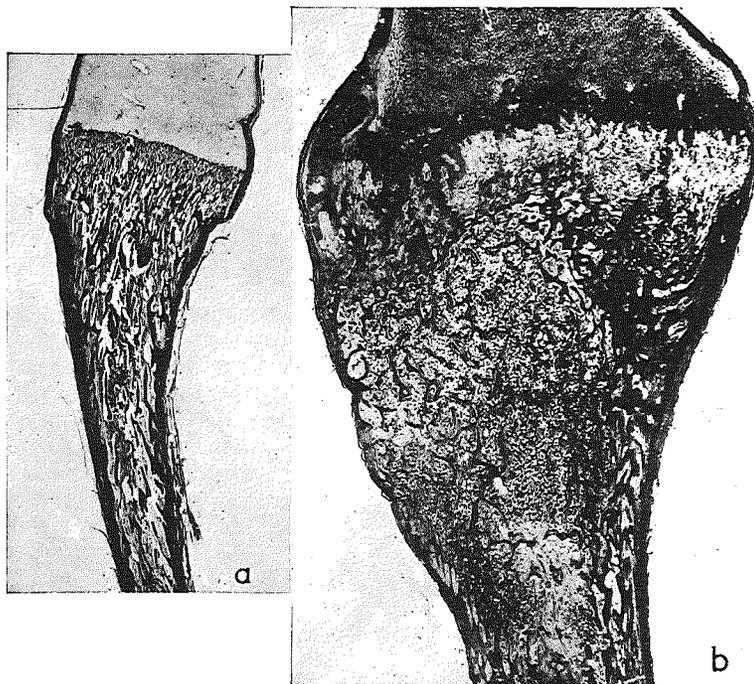


FIG. 181. Photomicrographs of costochondral junctions of a normal and a rachitic dog. The diet of both dogs contained a large amount of oatmeal, but the normal dog (a) received cod-liver oil while the rachitic dog (b) received olive oil. Note in (b) the swollen or "beaded" junction, and the uneven line of calcification.

their need for vitamin D, yet most later investigations have been carried out on rats. Rats, unlike puppies, are not good animals for this work as on a normal diet they are immune to rickets. They are only susceptible when their calcium and phosphorus intake is grossly unbalanced, which means that when rickets is produced it is not due to pure vitamin D deficiency, but to a deficiency grafted on to an already abnormal animal. The number of ways growing bone can develop abnormally is limited, so that rickets in rats is the "final common path" for a number of different metabolic disturbances of vitamin D, phosphorus or calcium, or of these combined together (p. 533). With these reservations as to the true meaning of much of the work done we may consider the importance of vitamin D for bone metabolism.

During growth the long bones of the body increase in length by the calcification of the cartilage between the diaphysis and the epiphysis. This cartilage on its epiphyseal side is continuously growing, while on its diaphyseal

side it degenerates, being then invaded by capillaries, and osteoblasts or bone forming cells. Bone salts are then laid down in the degenerating cartilage, but the formation of new cartilage keeps pace with its degeneration, so that growth in length occurs by the invading bone on the diaphyseal side of the cartilage always chasing the retreating new cartilage on the epiphyseal side. Growth ceases when no more cartilage is formed, so that the diaphyseal bone reaches and fuses with the epiphysis. The thickness of the bone is increased by osteoblastic activity underneath the periosteum.

In rickets the band of cartilage widens because it goes on growing but

ceases to degenerate. A large number of osteoblasts and capillaries appear between it and the diaphysis forming a mass of osteoid tissue, that is "the organic part of bone without the inorganic." No calcification takes place, since degeneration has ceased in the cartilage. The large amount of osteoid tissue and expanded cartilage causes the classical swellings, for instance, of beaded ribs and enlarged ankles and wrists. Under the periosteum of the bone calcification stops though there is an increase in vascularity and osteoblasts forming osteoid tissue. The finer structure of the bone and the trabeculae of the marrow cavity is affected, because decalcification also occurs. Clinically this results in weakening of the bones with bending and fractures.

The X-ray picture in severe rickets shows these changes. In normal growing bone the end of the diaphysis is smooth and there is a clear space between it and the epiphysis. But in rickets the end of the diaphysis is uneven and ragged because the degeneration of the cartilage and subsequent calcification has stopped unevenly. A faint curved shadow along the edge of the cartilage may sometimes



FIG. 182. Foetal rickets in a Chinese baby. A section of the distal end of the ulna stained with silver nitrate. Note the expanded cartilage and osteoid tissue, and the ragged diaphyseal end of the ulna. (See also Figs. 186 and 189.)

be seen extending towards the epiphysis: this is the faintly calcified perichondrium round the swelling caused by the uncalcified mass of osteoid tissue and overgrown cartilage. The shaft of the bone also shows rarefaction with a general coarsening of the structure, while the outline is irregular and blurred by the shadowless osteoid tissue under the periosteum (see X-rays in Figs. 183, 189 and 194).

When healing starts under the influence of vitamin D the first change (which can be shown histologically within twenty-four hours) is the resumption of degeneration of the cartilage cells nearest to the diaphysis. These cells lose their glycogen [99], and this is rapidly followed by capillary penetration and the laying down of bone salts. The result is that a line of preparatory

calcification is formed close to the ragged edge of the diaphysis which can be seen on X-ray examination, forming the "line test" for healing rickets. Bone salts are also laid down in the osteoid tissue at the ends of the bones and along the shafts, so that in both places denser shadows appear in an X-ray (Fig. 196).

In adult animals deprived of vitamin D the picture must be modified because growth is no longer taking place. The changes have to be limited to the decalcification of the bones, and the formation of osteoid tissue under the periosteum, with a resulting weakness giving clinically the picture of

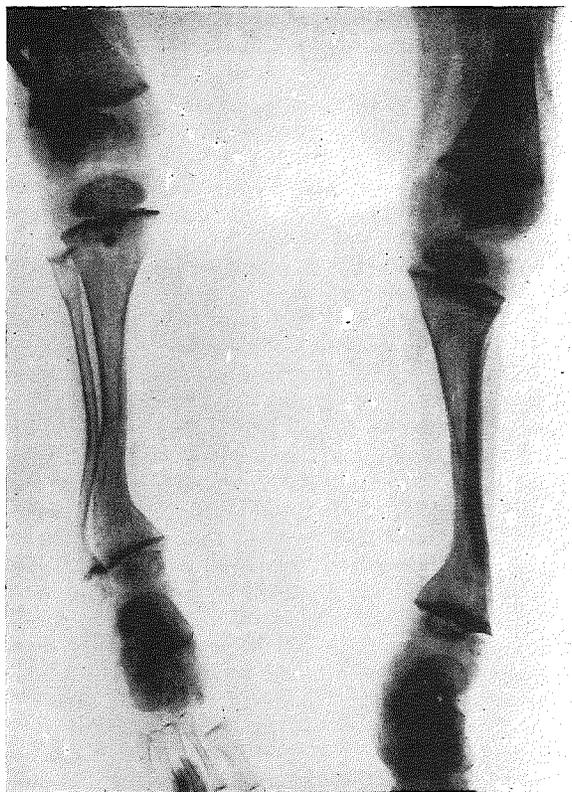


FIG. 183. Healing rickets with fractures in an English child. Note the lines of dense calcification at the ends of the diaphyses. Bands of newly formed bone can be seen at each end of the shafts, distinguished from the bone formed before therapy was started by their fine and homogeneous structure. Dense new periosteal calcification along the shafts is also visible.

osteoporosis or, in severe deficiencies, osteomalacia. The rôle of vitamin D in the calcification of the teeth is discussed on p. 570.

The other changes which occur in rickets are :

(a) The amounts of calcium and inorganic phosphorus in the serum are altered. In experimental rickets these elements largely mirror their content in the food, so that rickets can be produced with widely varying amounts and proportions of these two present in the serum. In human rickets (p. 559) the phosphorus is generally low, the calcium normal, but the latter may be grossly reduced. In osteomalacia the calcium is often reduced to tetanic levels, but it may remain nearly normal with very low phosphorus levels (p. 568).

(b) The plasma alkaline phosphatase (p. 558) is constantly raised in rickets

and, to a less degree, in osteomalacia. This rise, however, can be prevented in animals by depleting the diet of manganese [88]. The origin of this phosphatase is the bones [36].

(c) The calcium and phosphorus of the fæces is increased, of the urine decreased.

(d) The skeletal muscles lose their tone, and the ligaments become lax.

(e) The smooth muscle of the gut also loses its tone. This is important, as Yoder [89] reports that it may increase the time food takes to pass along the gut by as much as twenty-six per cent. He points out that this relative stagnation of the food means that chemical changes take place in the gut as the result of decomposition of the food, and not as the direct result of lack of vitamin D. He believes the alkalinity of the fæces in rickets is due to this decomposition, since the reaction can be restored to normal either by vitamin D or purgatives.

The Relation of Vitamin D to Growth. Stimulation of growth, as apart from the prevention of rickets, is an important function of vitamin D. Such clinical observers as Gardiner-Hill [90] and Still [91] state that a deficiency of vitamin D decreases growth quite apart from loss of stature due to rachitic deformities (Figs. 178, 188). This has been confirmed in rats [98] and lambs [96], while Mellanby [74] states that in puppies vitamin D above the amount needed to prevent rickets improves the architecture of the bones. Large-scale investigations on children also show that amounts of vitamin D above those necessary to prevent rickets lead to increased growth [92]. Norman's important work [93] on the difference in height between children of the richer and poorer classes also would suggest that vitamin D is related to growth, since it is one of the most deficient vitamins in the diet of the poor.

Whether it is an advantage to the individual to be taller is debatable. That children and experimental animals grow more on large amounts of vitamin D does not mean that such doses of vitamin D are the correct ones. It may equally well be held that large amounts of vitamin D stimulate excessive growth. Jeans and Stearns [94] found that when vitamin D is given in still larger doses growth is impaired, even though toxic symptoms are absent. Analogous results are reported by Speidel and Stearns [95], since giving infants 300 to 400 I.U. of vitamin D daily caused an earlier eruption of the first deciduous incisors than did larger or smaller doses. The body appears to have no power to regulate the amount of vitamin D it absorbs in the food; whether it can regulate that formed in the skin by irradiation is unknown. But as the latter appears to be the natural way of acquiring vitamin D experiments suggesting the value of a high consumption of the vitamin should only be accepted if the results are the same as are given by irradiation. Any results which appear to suggest feeding is better than irradiation may mean that they are not truly better, but are the effect of abnormal stimulation. If growth is taken as the criterion for measuring the correct dose of vitamin D we should know what is optimum growth. We do not.

The Relation of Vitamin D to the Endocrine Glands. *The Parathyroids* [104]. The parathyroids being so intimately connected with the metabolism of calcium and phosphorus must have some direct or indirect relationship to vitamin D. It has even been suggested that vitamin D only acts through stimulating these glands. This cannot be so, since the action of vitamin D and parathormone is quite different. With small doses the former keeps the serum calcium and inorganic phosphorus normal, and caused a positive balance of both in the body. The latter raises the blood calcium, after decreasing the inorganic phosphorus by diuresis, and causes a negative balance of both. Excessive amounts of the hormone and vitamin have different actions on bone: the former causes decalcification and replacement with fibrous tissue and giant cells, while the latter causes dissolution of the trabeculae with no fibrous replacement.

Clinically the difference is important, since rickets is cured by vitamin D but made worse by parathormone. Further, in parathyroid tetany the beneficial effect of parathormone wears off, but the effect of vitamin D does not [104, 112].

In rickets and osteomalacia the parathyroids often appear to be enlarged and over-active: vitamin D causes them to revert to a resting state [113]. In dogs [130] hypervitaminosis D decreases the size of the parathyroids.

Possibly vitamin D should be considered as regulating the metabolism of phosphorus and the parathyroids as regulating phosphorus excretion. Sometimes the hormone acts with and sometimes against vitamin D according to the needs of the body.

The Thyroid and Pituitary. The basal metabolic rate is decreased in rickets, but this is explained by Nicolaysen [105] as being due to decreased activity, since he found in narcotized rats that the carbon dioxide output was the same whether rickets was present or absent.

Vitamin D in doses on the threshold of toxicity raises the basal metabolic rate by stimulating the thyroid *viâ* the thyrotrophic mechanism of the pituitary [105, 107]; it seems doubtful if this can have any clinical importance. With toxic doses the thyroid appears to be over-active and there is an increase in the eosinophils of the pituitary [108]. Vitamin D₂ and calcium chloride, though not calcium carbonate, increase the size but not the iodine content of the thyroid glands of rats on a slightly goitrogenic diet [114]. The darkening of the feathers of rachitic chicks [100] may be due to some endocrine disturbance.

The Thymus and Pancreas. There is some evidence that the removal of the thymus in animals increases the severity of rickets and decreases the effect of vitamin D [109, 110]. Rickets in rats is said to give some protection against alloxan to the beta cells of the pancreas [101].

The Sex Glands. In osteomalacia the regularity of the menses and the disastrous recurrent pregnancies suggest that lack of vitamin D has no effect on reproduction in woman. It has been reported by Reed and his collaborators [111] that huge doses of vitamin D have occasionally increased sexual capacity and libido (even to an inconvenient extent) and also increased the regularity of the menses. Toxic doses decrease the size of the prostate and testes [130] and spermatogenesis [108] in the dog, but in the rat have no effect on this or ovulation [106], though lack of vitamin D in the latter animal causes diœstrus [115]. In mice the endosteal bone formation caused by œstrogens is not dependent on an adequate supply of vitamin D [116].

The Relation of Vitamin D to the Blood. Gray and Ivy [117], and McNealy and others [118] have shown that vitamin D prevents the hæmorrhagic tendency which is so common and dangerous in patients with jaundice and hepatic insufficiency (p. 576). Why the vitamin has this effect is obscure: it is not through any alteration in the blood calcium [117] or through correcting a prothrombin deficiency [120]. Vitamin D in man appears to have little or no effect on the formation of erythrocytes or leucocytes, or on the coagulation time or sedimentation rate [111]. In dogs large doses decrease capillary permeability [121]. The thrombocyte count in rats is said to be increased by large doses of vitamin D [119] and rachitic rats absorb iron and form hæmoglobin slightly less well than animals receiving vitamin D, especially when this is from fish liver oils [122].

The Relation of Vitamin D to Infection. During the last century in England cod-liver oil was greatly prized for its value in tuberculosis [123], which has been amply confirmed, at least as regards vitamin D in the treatment of some forms of tuberculosis, in recent years (p. 572). This, added to the commonness of catarrhal infections in rickets, and the clinical value of sunshine and ultra-violet light in improving the general condition of consumptive and convalescent patients, has led to a widely held belief that vitamin D increases the resistance of the body to infections. But the results of animal

experiments in which the formation of antibodies, etc., have been studied are indecisive and conflicting. Most probably the only value of vitamin D is the indirect one of avoiding the debilitating effect of a deficiency. It must be remembered that when cod-liver oil or ultra-violet light are used clinically the oil and vitamin A of the former and the general stimulation from the latter are valuable quite apart from any action of vitamin D itself. Reed and his collaborators [111] in 1939 summed up their excellent review of the literature on vitamin D and infections by saying ". . . there is no proof of a specific effect in any type of infection with the possible exception of tuberculosis." More recently Tomey [124], using monkeys, has shown that rickets reduces resistance to the virus of poliomyelitis when this is injected into the wall of the gut or into the suprarenals. Rachitic nerves ground up with the virus absorb it while normal nerves do not. Weaver and others [125], however, having inoculated cotton rats with the virus in every possible manner, could not confirm that lack of vitamin D had any effect on susceptibility or on the development of resistance. Tomey's findings are probably of no clinical importance since the seasonal incidence of poliomyelitis is not that of rickets, nor has poliomyelitis ever been found to select the rachitic and poorly fed. Young mice are also reported to be more susceptible to swine influenza virus when deprived of vitamin D [126]. The clinical results of treating infections with vitamin D have been too disappointing to consider.

Effects of Excessive Vitamin D. All forms and preparations of natural and synthetic vitamin D are toxic when given in sufficiently large amounts, though the belief still lingers that pure vitamin D itself is not toxic because the first workers with massive doses of the vitamin ascribed its toxicity wholly to the toxic impurities, such as toxisterol, which were present in the very impure irradiated ergosterol which they used. Actually pure or highly purified preparations of vitamin D₂ and concentrated fish liver oils are toxic for the rat [127, 128] and the dog [129, 130, 131] and man (p. 578). Vitamin D₂ is definitely more toxic than vitamin D₃ for rats [127] and dogs [131] and so it is reasonable to suppose it would be so for man, though no work has been done on this subject. Toxicity is reduced in animals by very large amounts of vitamin A [127, 131, 132]; this has not been confirmed in clinical work [111], though aneurine or yeast are often stated to be of value [111, 133].

Excessive doses of vitamin D mobilize the phosphorus and calcium from the tissues of the body, thus broadly having an opposite effect to normal doses. The soft tissues tend to become calcified, the bones to be rarefied—in growing bone the cartilage acts as a soft tissue. The soft tissues most affected are the tubules of the kidneys [129], the media of the arterioles of the kidney [134] and the media of the large blood vessels, though the bronchi, lungs, heart and stomach are also involved [130]. The aorta, for instance, in animals kept for some time on sublethal doses looks exactly like that found in old atheromatous men. This metastatic calcification takes place after the tissues have already been damaged by the excess of vitamin D; it is thus a secondary change, and not the primary one [43]. Casts composed of calcium salts are often present in the urine if this is not too acid. If the toxic doses of vitamin D are stopped the calcareous deposits may largely disappear [135]. The serum phosphorus and calcium tend to be grossly raised but not always—so that a raised blood calcium does not of necessity give a warning that the amount of vitamin being taken is toxic [111]. A diet rich in bone salts increases the metastatic calcification, but a diet deficient in salts does not decrease the fundamental damage to the tissues. Oppel [136] states that metastatic calcification is increased in rats when their renal function is impaired either by a diet deficient in vitamin A (p. 47) or by partial nephrectomy. In man [111] the blood pressure is not affected, but in dogs mild toxicity causes hypertension and severe toxicity hypotension [111], while in rats hypotension occurs with mild toxicity and severe toxicity has no effect [137]. With severe vitamin D poisoning animals develop severe

diarrhoea and loss of weight and may die in a few days. In dogs [130] there is atrophy of the testes and the prostate, while the parathyroids are smaller than normal with contracted nuclei (see also p. 532). The clinical picture and post-mortem findings in human cases poisoned by vitamin D are described on p. 47.

Fundamental Nature of the Action of Vitamin D. The effects of vitamin D are best explained, though it must be admitted not completely, by considering that its action is primarily on phosphorus metabolism throughout the whole body [138], essentially activating the alkaline phosphatases [244]. Thus vitamin D not only mobilizes phosphorus from the tissues, so aiding its combination with calcium [142] by converting organic phosphorus into an inorganic form [139], but it also has an effect on the metabolism of phosphorus during muscular work [143].

Nicolaysen [82, 144] and Harrison and Harrison [147] have shown, by most careful work on rats, that calcium absorption is increased by vitamin D, but that this effect is largely dependent on the needs of the body for calcium, especially in the young [82, 147]: in the adult both vitamin D and the needs of the body for calcium have much less effect on absorption, which has been confirmed for women [148]. Nicolaysen [144] did not find that the absorption of phosphorus from isolated loops of the intestine was different in rachitic and normal animals, though Laszt [87], using the same technique, reports that the absorption of phosphorus is decreased in rickets. But whether or no vitamin D directly affects phosphorus absorption it is probable that its effect on calcium absorption is really a secondary effect due to it rendering the phosphorus in the cells of the gut wall sufficiently labile for the calcium to combine with it and so be absorbed [149]. Congruous with this theory is the belief of Migicovsky and Emslie [150], who from experiments on chicks hold that the sole action of vitamin D is to prevent bone being dissolved away into the blood and so decreasing the vacuum for calcium in the cells of the gut wall. Greenberg's work with rats [151] can also be interpreted in a similar manner, though he himself adds to a direct enhancing action on the mineralization of bone a further direct enhancing action on the absorption of calcium.

Accepting the above theory of the action of vitamin D, calcification is started by the conversion of the organic phosphorus of the serum and bone to inorganic phosphorus. This so raises the concentration of the latter that it can combine with the calcium of the serum to form insoluble calcium phosphate, aided in the neighbourhood of growing bone by the action of Robison's bone phosphatase on hexose phosphoric esters [145].

It must be stressed that bone salts are not laid down because an increase in inorganic phosphorus in the serum automatically causes precipitation of calcium phosphate: they can only be laid down by the action of living cells [140] when the concentrations of salts bathing these are sufficiently high.

Rachitic bone and cartilage have no fundamental inability to lay down bone salts, since they do so if they are placed in normal serum or suitable salt solutions [140]. In living rachitic animals the injection of inorganic phosphorus and calcium also causes calcification [141], since the salts are artificially put into the fluid bathing the cells in a form which is only produced naturally by the action of vitamin D.

The poor tone of the skeletal and visceral muscles in rickets is possibly due to the effect vitamin D has on phosphorus metabolism in muscular work [143].

The damage done by excessive amounts of vitamin D (p. 534) may be due to an excessive mobilization and conversion of organic phosphorus to inorganic phosphorus not only in the soft tissues but also in the bones: a mobilization which is injurious in itself, only secondarily leading to metastatic calcification [129] when enough calcium is present to combine with the liberated phosphorus.

Another theory of the toxic action of vitamin D is that it is due to an impurity formed during irradiation, such as toxisterol (p. 520). In favour of this is that unit for unit the least purified products of irradiated ergosterol are the most toxic [146]. Toxic symptoms again often occur only after some intestinal upset which might have led to decomposition of vitamin D in the gut before absorption with the production of injurious substances [111]. Aneurine has a protective action against overdosage of vitamin D, which may be explained by the value of the former for the proper functioning of the gut, which would decrease any tendency to intestinal putrefaction [111]. As, however, very pure preparations of calciferol have a toxic action when injected one must also postulate that the cells of the body cannot completely destroy huge doses of vitamin D, but form from it some toxic substance like toxisterol. Here again adequate aneurine should aid the cells in the complete destruction of vitamin D. This theory does not run counter to the theory that excessive vitamin D upsets phosphorus metabolism, but only tries to explain further the underlying mechanism.

SOURCES OF VITAMIN D AVAILABLE TO MAN

Sunlight and Ultra-violet Light. Sunlight acting directly on the body should be the way in which vitamin D is obtained. But in northern climates and large cities clothes, window glass, smoke, and clouds cut off most of the active rays. Even so by letting infants and children be exposed as far as possible out of doors and by opening nursery windows much benefit is gained. In one hospital in the heart of London it is found that infants always thrive better if they are out on a roofed verandah whatever the weather. This raises the point that the action of sunlight and fresh air is not only due to the formation of vitamin D but also to a general tonic effect on the body; the fluctuating temperatures on the skin, for instance, cause a stimulation of the thyroid-suprarenal mechanism.

Sunlight in excess is a powerful, though delayed, poison which may cause not only severe sunburn, but also fever, headaches, general malaise and shock. As these symptoms do not appear for some hours after sun-bathing no warning is given at the time that it has been too prolonged. It should therefore be used with care, especially for infants. The eyes and head must be shaded by a wide-brimmed hat. As some children are more sensitive than others a ten-minute exposure of the arms and legs is enough for the first few days. The duration and amount of the body exposed may be gradually increased, but complete exposure of infants for more than half an hour twice a day is excessive. When the sunlight is very intense sun-bathing is best in the cooler parts of the day—early morning and evening. With adequate sunlight the amount of exposure recommended above will cure rickets as rapidly as the doses of vitamin D commonly used. For adults sun-bathing is of value, but the prolonged sudden exposure of city people on short holidays is harmful.

Artificial sunlight, given either by the carbon arc or mercury vapour quartz lamp, is an excellent substitute for sunlight, having the same value in the prevention and cure of rickets and also the same tonic effect. Hill and Laurie [48] found in a carefully controlled experiment that children benefited more from irradiation than cod-liver oil, their weight, appetite and sleep being improved, the number of colds decreased and nervousness lessened. But irradiation needs to be used with caution. Both the duration of exposure and the distance of the bather from the lamp must be carefully supervised. It is essential that the eyes are always protected with coloured glasses. Both adults and children may be unduly sensitive to irradiation, especially those who are fair. The first exposure should be of short duration to make certain there is no intolerance. A short transitory erythema should be produced, but

any other symptoms suggest the exposure is too prolonged. We have seen twenty-five people who had to go to bed with severe sunburn because of over-exposure at the christening party of a new lamp. Since the various lamps now sold vary greatly in the intensity of the ultra-violet rays they emit detailed instructions for their use cannot be given here, but must be obtained from a therapist or the makers.

Food. The dietetic sources of vitamin D can be divided into (a) normal foods, (b) fortified foods, and (c) concentrated natural and artificial preparations.

Normal Foods. Vitamin D is poorly represented in food. The only good sources are dairy produce, fatty fish, and dripping. Green vegetables, in spite of all belief to the contrary, do not contain vitamin D.

The value of all dairy produce depends on the diet and exposure to sunlight of the hens and cows. Hens' eggs contain nearly three times as much vitamin D in the summer as in the winter. The eggs of commerce produced in abnormal numbers by birds under the loathsomely cruel battery system may be almost valueless from the point of view of vitamins D and A.

English butter may only contain 3 I.U. of vitamin D in 1 ounce in the winter, though in the summer there may be 130 I.U. These figures are extreme values: butter from cows which are kept outdoors in the winter should never have such a low winter value. The importance of butter is shown by Friend's [152] report on the health of the boys at Christ's Hospital School. In 1918 the War made it necessary to give the boys less milk and unvitaminized margarine instead of butter. The average fracture rate till then had been 0.75 per cent. During the next four years the average fracture rate rose to 1.78 per cent., in 1922 even being as high as 2.38 per cent. Yet in 1922 all the War rationing had disappeared, the diet being as good as it was before 1918. The only difference was that margarine was still being eaten instead of butter. When butter was again given the fracture rate sank to normal. The explanation offered is that the vitamin D of the butter saved the boys from mild rickets or osteoporosis. But the amount of vitamin D in butter is small, so that it seems possible that the fat of the butter itself or some as yet unidentified factor in butter (p. 680) or the form in which it contains vitamin D was the important factor. In favour of this is the observation by Boer [155] that whole butter has eight times the antirachitic value of its unsaponifiable fraction, which suggests that butter, like milk, increases the potency of the vitamin D it contains.

Milk is an extremely valuable source of vitamin D. This is not due to the actual amount of vitamin D present, which is relatively small, but because its antirachitic value is greatly enhanced when it is given in milk. Thus Hess and Lewis [154, 156] reported that unit for unit "yeast" milk (p. 538) was five to ten times as antirachitic as calciferol dissolved in oil. Similar observations have been made by Drake [158], May and Wygant [157], and Eliot [159]. Supplee and his collaborators [153] have shown that the increased antirachitic effect of vitamin D when given in milk is due neither to better absorption of diluter concentrations nor to finer dispersion, but is the result of vitamin D forming a compound with the lactalbumin of the milk. This compound is more effective than vitamin D alone. It must also be remembered that the lactose of milk is of value in aiding calcification; cane sugar and starch have not this property [86]. Human milk is discussed on p. 543.

Sir John Russell [160] has pointed out that milk at present need not conform to any standards as regards its vitamin content. Legislation should be introduced to ensure that all milk sold has a certain amount of vitamins A and D, since the child population is largely dependent on milk for these vitamins. Legislation of this type has already been introduced in Finland [161].

Fatty fish are a valuable and relatively cheap source of vitamin D. Of special value are fresh or tinned herring, bloaters, pilchards, kippers, sardines,

salmon and eels. In 1939 the day's requirements of vitamin D could be bought as herring for three-halfpence, as tinned salmon for fourpence, and as eggs for tenpence [162], but, of course, by 1952 only the former remain freely available. Fish are also a good source of iodine for man, and of calcium and phosphorus [163, 164] if their bones are eaten, as they may be in the case of sardines, sprats, and tinned herring and salmon. The bones of salmon have been found to have a high antirachitic value for animals [163]. It used to be said in the days of plentiful cheap food that tinned salmon was the Londoner's sunlight, but even now, in the days of dearth, the still plentiful herring does not hold this title, even though the English Herring Fleet could land far more herring than are eaten at home [165]. In 1937 the average individual daily intake of vitamin D from herring, fresh or canned, was only 35 I.U. [166], though one fresh herring provides the day's needs of vitamin D.

Butcher's dripping bought in July in England is stated by Henry and others [167] to be as good a source of vitamin D as "summer butter," and, of course, it has a higher energy value than margarine.

Lindsay and Mottram [162] have explained how in the preparation of food additional vitamin D can be incorporated by the substitution of cod-liver oil for olive oil or butter in such things as white sauce, mayonnaise, and batter when these are for fish dishes where the faint taste of the oil is masked by that of the fish.

Fortified Foods. Cowell [168] in 1925 first irradiated food as a means of increasing its vitamin D. In spite of the fact that this pioneer work was English, its commercial applications have been largely ignored in England while widely used in the United States.

Vitaminized margarine is practically the only food in England to which vitamin D—as vitamin D₂—is added; all margarine must contain, by law, 90 I.U. per ounce with the exception of that doled out by the Ministry of Food to cake shops, etc., which contains none [171]. In any case margarine can never be a true substitute for butter; the only important point is to prevent the public from believing it is. But since margarine instead of butter is an economic necessity in many families, high praise is due to the manufacturers whose own private work on vitaminization has enabled the Government to insist on it officially.

Milk is widely reinforced, especially in America. This can be done by (1) irradiating the cow, (2) irradiating the milk, (3) giving the cow vitamin D in her food, (4) adding vitamin D to the milk. By the first two methods vitamin D₃ is increased in the milk, as it is if fish liver oil is given in her diet, or is added later to the milk. But in both the latter cases fish oils may give a fishy taste to the milk. Vitamin D₂ is free from this objection and can be added directly to the milk or fed to the cow as irradiated yeast, giving "yeast milk." Irradiated milk generally contains about 135 I.U. per quart, while "yeast milk" and milk to which vitamin D is added generally contains 400 I.U. per quart. The great advantages of giving vitamin D in milk are discussed on p. 537.

Cheese, or at least a substance which those with no palate could be deluded into thinking was cheese, can now be fortified with fish liver oils [242].

Concentrated Preparations of Natural and Artificial Vitamin D. Natural vitamin D₃, from the work discussed below, has about twice the potency of artificial vitamin D₂. This is important when deciding what concentrated preparation shall be prescribed: those widely available include good cod-liver oil and concentrated fish liver oils, both containing vitamin D₃; solutions of vitamin D₂ in vegetable oils; tablets of vitamin D₂; capsules of concentrated oils which may contain either vitamin; the Ministry of Food Cod-liver Oil Compound, containing a mixture of both vitamins in unknown proportions [171]; and foods like infants' cereals and sugars, which often have added vitamin D₂.

The superiority of vitamin D₃ over vitamin D₂ would appear to be

definitely proved. Thus May and Wygant [157] in a study of four hundred and fifty-seven infants decided that while 200 to 500 I.U. of vitamin D given daily as cod-liver oil were sufficient to prevent rickets, 400 to 800 I.U. of calciferol in oil were necessary to obtain the same effect. Hess and his collaborators [154, 156] from a study of sixty infants also give approximately the same figures, which are strongly supported again by Houet [172], who judged the relative efficacy of the two vitamins by their effect on mineral absorption—an investigation doubly important because it used another yardstick to those previously employed by other workers yet obtained the same result. Jeans [173] concludes that “vitamin D of animal source appears to be more potent for the human being than the vitamin D of vegetable source (calciferol).” Brockman, Rietschel and others [174, 175] state that vitamin D₃ is superior to calciferol when only one massive dose is given to protect children against rickets throughout the winter. Gerstenberger [176] comes to the same conclusion from experiments with monkeys. McBeath and Zucker [177] found the synthetic vitamin less valuable than the natural for increasing the immunity of children to dental decay (p. 570).

Most investigations [158, 159] which have purported to show that the two vitamins are of equal value are fallacious, because doses were used which were

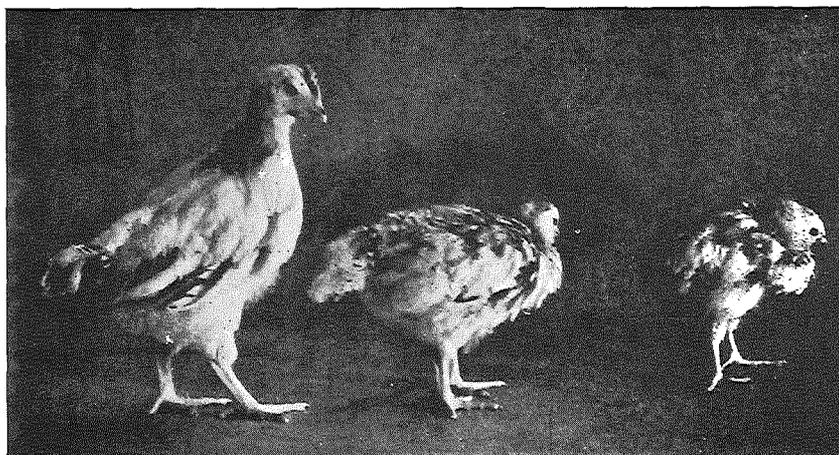


FIG. 184. Five-weeks-old chicks. The bird on the left received natural vitamin D₃ from cod-liver oil, the bird in the centre received the same number of International Units of synthetic vitamin D₂ (calciferol), and the bird on the right received no vitamin D. The average weight and mortality of the ten birds in each group were: with the natural vitamin 399 grams and no deaths; with the synthetic vitamin 346 grams and five deaths; with no vitamin 259 grams and six deaths. (See also Fig. 180.)

so large that the prevention or cure of rickets was inevitable by either vitamin and so, of course, no difference between them could be shown. An apparent exception is the work of Glaser and others [178], who gave small doses to premature infants and judged the effect by phosphatase levels and X-rays. But even here too small a number of infants was investigated and too small a proportion developed rickets for any definite conclusion to be drawn.

Further advantages of vitamin D₃ are that it is less toxic, at least for animals (p. 534); that given, as it generally is, in cod-liver oil may enhance its effect (p. 522); that the greater antirachitic value of the natural vitamin for chickens increases over that of calciferol as the doses of the two are increased [184]. This suggests that for human beings the superiority of the

natural vitamin might be enhanced at intakes above the minimum for preventing rickets. An analogy is found in the greater value of vitamin A than its provitamin (carotene) at levels of intake above the minimum for good growth.

Cod-liver oil is the best concentrated source of vitamin D for supplementing the diet of both children and adults. Its advantages are : (1) It provides the natural vitamin D₃. (2) It provides vitamin A. (3) It is nutritious. (4) A toxic dose of vitamin D cannot be given as ordinary cod-liver oil owing to its bulk. This was emphasized by Thatcher [179] in his account of a child who died from overdosage of a concentrated oil. (5) The work of Jeans and Stearns [80] suggests that concentrations of vitamin D higher than those found in ordinary cod-liver oil are not well absorbed. (6) Cod-liver oil is of value apart from its vitamins, containing, for instance, traces of iodine, and a high proportion of unsaturated fatty acids akin to those essential unsaturated fatty acids which sometimes are referred to as vitamin F (p. 671). (7) The cod-liver oil requirements of England can be supplied by the English fishing fleet [165].

The traditional objection to cod-liver oil is its taste. But poor children attending out-patient departments often ask for it, and one has the impression that richer children largely dislike it because their mothers tell them it is unpleasant.

Concentrated fish liver oils can be used in those rare cases where ordinary cod-liver oil cannot provide enough vitamin D, or where the taste is intolerable or the fat injurious as in coeliac disease.

Manufacturers of concentrated preparations of vitamin D should state on the bottle that there is a risk of overdosage if more is taken than is prescribed. We have seen one patient who drank a preparation of calciferol, merely because he liked it, in such quantities that he was showing the early signs of hypervitaminosis D (p. 578).

Effects of Cookery, Storage, Canning, Freezing and Drying on Vitamin D. Domestic cookery causes no loss of vitamin D, since it is not soluble in water nor easily destroyed by heat. Milk loses none by boiling or by pasteurization. Tinned and dried milks have the same vitamin D content as fresh milk, but the analyses of Bacharach and others [166] suggest that there is considerable loss when fish are canned or cured, since the average value of vitamin D in fifteen samples of fresh herring caught throughout the year was found to be 250 I.U. per ounce, and in five samples of canned herring only 50 I.U. per ounce. As, however, the fresh and tinned herring did not come from the same catch these differences may not be entirely due to the canning. Dried eggs lose a considerable amount of vitamins A and D when prepared by band drying but none by spray drying [170]. The value of butter is not impaired by its storage during transport over long distances, such as those between New Zealand and England [181].

AMOUNT OF VITAMIN D IN FOODS

This table has been chiefly compiled from the tables of Fixsen and Roscoe [181] and McCance and Widdowson [183].

The values of most of the fish, apart from salmon, lamprey and lampern, are only approximate, since research has been directed to the amount of vitamin D in the body oil of fish. To make this work of value in human diets, it has been assumed that the fat in the edible parts of the fish is body oil, and so since the amount of fat is known, the amount of vitamin D has been deduced.

Food	International Units of Vitamin D —probably D ₂ —in 100 grams or roughly 3½ ounces
<i>Butter</i>	
Danish. November–January	30–8
February–April	8–14
May–July	36–54
September–October	44–15
Dutch. General	21–48
English. November–March	10
April–June	15–40
July–August	55–97
September–October	40–15
New Zealand. General	30–57
Scotch. November–January	30–8
February–April	8–22
May–July	60–99
August–October	50–20
<i>Dripping</i>	
English butcher's. July	33–44
<i>Cacao Butter</i>	30,000 as D ₂
<i>Cheese</i>	Present. ? amount
<i>Cream</i>	
English cow's	50
<i>Eggs</i>	
Hen's. Whole	70
Yolks only. Summer	390
Winter	140
Whites only	0
Whole dried	220 (p. 540)
Duck's and other eggs	? as in hen's eggs
<i>Fish</i>	
Cod	52
Cod's roe	65–80
Cod's liver, (fresh or canned)	6,000
Eels	474
Herring. Canned, with Tomato	52–322
Canned, without Tomato	112–420
Fresh English, August	294–875
September	930–1,676
November	735
December	591–1,270
March	521
July	686
Kippers	590–744
Lampern	120
Lamprey. Sea	110–400
River	33
Mackerel	304–405
Oysters	5
Salmon. Canned. Chum and Chinook	200–300
Pink	600–700
Red	800
Sardine	1,800
Shrimp, flesh	147
Turbot	27

Food	International Units of Vitamin D —probably D ₂ —in 100 grams or roughly 3½ ounces
<i>Fish Liver Oils</i>	
Cod	8,100–30,000
Cod—minimum allowed in English and American medicinal cod-liver oil	8,500
Cod-Liver Oil Compound (Ministry of Food)	20,000 as D ₂ and D ₃
Halibut	20,000–400,000
Tunny (various kinds)	1,600,000–25,000,000
<i>Fungi</i>	
Edible	83–125 as D ₂
Mushrooms. Grown in dark	21 as D ₂
Grown in light	63 as D ₂
<i>Green Vegetables</i>	0
<i>Hay</i>	32–200 as ? D ₂
<i>Liver</i>	
English. Calf	0
American. Calf	10
Lamb	20
Ox	40–50
Pig	40–50
<i>Margarine</i>	
Vitaminized	315 as D ₂
Unvitaminized. Vegetable	0
<i>Milk</i>	
Human (see p. 543)	—
Cow's. Summer	2.4–3.8
Winter	0.3–1.7
Colostrum. Summer	8
Winter	4
Irradiated milk	Generally standardized at 12
Yeast milk	Generally standardized at 35 as D ₂
Fortified milk	Generally standardized at 35 as D ₂ or D ₃
Sow's	4.5
<i>Silage</i>	56–87 as ? D ₂
<i>Vegetable Oils</i>	
Arachis or Pea-Nut	0
Olive	0
<i>Whale Oil</i>	
Blubber	75
Liver	75

HUMAN REQUIREMENTS OF VITAMIN D

The dietetic requirements for vitamin D given in the following pages are for the inhabitants of temperate climates who are receiving little irradiation from the sun. During the summer, especially in the country, less vitamin D need be taken in the food. It is assumed that the diet is not grossly abnormal,

especially for calcium and phosphorus, and that no liquid paraffin, or laxatives containing liquid paraffin or mineral oils, are being given since these decrease the absorption of all fat-soluble vitamins. Such laxatives should never be given, especially to infants.

The British Pædiatric Association in 1942 stated that infants, children and mothers require the following amounts of vitamin D daily, expressed in international units, though these amounts, in the light of the work discussed below and of the recommendations of the U.S.A. National Research Council, are probably excessive, especially for premature infants.

Premature infants (under 5½ lb. at birth)	1,400
Full-term infants	700
Children, 5-14 years	500
Puberty and adolescence	300-600
Adults, male and female	300-600 ?
Pregnancy and lactation	700
Old age	300 ?

The U.S.A. National Research Council in 1948 recommended 400 I.U. daily for all infants, children, adolescents and pregnant and nursing mothers, while for night workers, the elderly and nuns a "small amount" is "desirable." The need of extra vitamin D for normal adults "seems to be minimum."

Breast-fed full-term babies often develop rickets. Premature babies and those which grow very rapidly generally do so unless they are given extra vitamin D. If surprise is felt that breast milk should ever be inadequate, it must be remembered—quite apart from the mother's diet being often deficient in the vitamin—that children should gain vitamin D from the sunlight on their bodies; their milk probably only supplements their sunlight rather than the other way round. Cow's milk or other artificial foods always require additional vitamin D. The problem is to decide how much vitamin D infants require, not only to avoid rickets, but also to give the best rate of growth (p. 532).

Human milk is reported by some workers to be a parsimoniously adequate source of vitamin D for the suckling, while others declare it to contain little or none. Thus Drummond and others [186] in England found an average of 68 I.U. per quart in random single samples from twenty-seven women living in towns during the first two months of lactation, though sometimes there was little more than 40 I.U. Values increased to 114 I.U. if an additional 200 I.U. daily were taken by the nursing mother; larger but still physiological additions caused no further improvement in the milk. In contrast to these figures Polskin and his collaborators [187] in the U.S.A. report that during the first seven to nine days of lactation eighteen of twenty-one women secreted in random single samples less than 10 I.U. per quart, while the other three had 17, 28 and 40 I.U. Vitamin D supplements during the latter half of pregnancy were probably almost wholly absorbed, but even when given as single weekly doses of 32,000 I.U., amounting in all to such grossly unphysiological figures as 256,000 to 480,000 I.U., the content of the milk never rose above 62 I.U. Taken during lactation, however, 40,000 I.U. daily raised the value to between 125 and 504 I.U. per quart—which is presumably merely an overflow and not a secretion. More in keeping with these U.S.A. figures are the further English figures of Kon and Mawson [188], who analysed the pooled milks from a large number of women, the samples being representative of the whole twenty-four hours, thus avoiding fallacies introduced by variations in fat secretion, etc., at different times of the day [188]. The average value per quart was 6 to 13 I.U. with, possibly, an increase in later lactation and during the summer.

Milk, even human milk, being so frequently an inadequate source of

vitamin D, some extra vitamin must be given. Jeans and Stearns [180] found that for three months old infants 70 to 100 I.U. of vitamin D₃ in cow's milk gave protection against rickets, but the maximum amount of growth—if this is really desirable (p. 532)—was given by 300 to 600 I.U. daily [92, 189], while doses of 1,800 I.U. definitely reduced the rate of growth [94] and delayed dentition [95], the first deciduous teeth erupting earliest on 300 to 600 I.U. [95]. Barnes [30] also considered that 600 I.U. provided by fish liver oils from whatever fish, is ample, since forty-eight infants with rickets were cured as rapidly with 600 as with 900 or 2,400 I.U., cure being assessed by blood phosphatase estimations. Corner [109], in her very careful investigations on town children living at home, found that a supplement of 400 I.U. daily had no effect on the incidence of rickets, which she considered meant either that this amount is inadequate or that it cannot overcome the other contributory causes of rickets among poor town children; this is largely confirmed by Krestin [191], who reported that the free distribution of oil, containing 400 I.U. per daily dose, to pregnant and nursing mothers and to infants had no beneficial effect. But it must be remembered that both these observers were dealing with the poor and so could not be certain the oil was being taken regularly. This is a strong argument in favour of providing free cod-liver oil with a higher content of vitamin D than would in theory be necessary in order that when a dose is forgotten it is compensated for by the next dose.

From the clinical point of view, therefore, breast-fed infants should be given two small teaspoonfuls of an ordinary cod-liver oil a day; this will contain about 500 I.U. For artificially fed infants another teaspoonful should be given. The oil is started about a month after birth in 5 drop doses thrice daily, dropped on the tongue, and increased slowly to the full quantity in about three months.

Premature infants suffer from two drawbacks—they grow very fast and they have not stored the bone minerals which are acquired in large quantities in the last weeks of foetal life. Faced with a hesitant digestion, enough minerals can seldom be absorbed for some months, so that slight osteoporosis is inevitable, but this can be improved and rickets avoided if relatively large doses of vitamin D are given. After the first month cod-liver oil should be taken in 5 drop doses thrice daily and rapidly increased if it does not cause indigestion. The oil can be dropped on the tongue. More concentrated preparations can be tried, but they are probably less well absorbed (p. 540). Not less than 600 I.U. appears to be the dose which should be aimed at as soon as possible [30, 192, 193, 194]; the 1,400 I.U. advised by the British Pædiatric Association would appear to be large enough to check growth and dentition [94, 95]. Mixing the oil with the milk in the feeding bottle is unsatisfactory, since it tends to float to the top and stick to the sides of the bottle.

Massive single doses of vitamin D are sometimes given orally or by injection to infants to confer prolonged protection against rickets both on the Continent and in America. This form of treatment should never be introduced into English medicine. The advocates of the single massive doses claim that it is safe, that the time of the mother is not wasted by frequent visits to baby clinics to obtain cod-liver oil she cannot afford to buy, and that the infant of the most feckless mother can be protected for several months against rickets, though he is only brought to a clinic once. Such arguments, granting the dubious assumption discussed below that one huge dose of vitamin D is not toxic, are only valid in backward countries in which, unlike England, there are few doctors and the poor are not educated to bring their infants regularly to clinics. In England every effort should be made not to curtail the number of visits to infant welfare clinics, but to increase them. Rickets is now one of the least of the ills against which the slum child needs protection: the value of a clinic lies not in doling out free cod-liver oil, but in guarding the health of the healthy infant by advice and those regular and

frequent examinations which alone can forestall illness. So in England one massive dose of vitamin D will not excuse regular attendance at a clinic nor even save the trouble of giving the infant cod-liver oil since this is still needed for its vitamin A and its very valuable fat.

There is only meagre work on the safety of massive single doses of vitamin D. Thus Palmén [195] gave 500,000 I.U. to seven non-viable infants with congenital defects: at autopsy no changes were found in the kidneys, adrenals, liver, spleen, aorta or heart muscle, but too little time may have elapsed between treatment and death for calcification to have occurred. Houet [196] gave 600,000 I.U. to five normal infants; during the short time they were under observation calcium retention was not affected but there was some increased excretion of phosphorus. In rachitic infants 600,000 I.U. given by injection caused the blood calcium to rise to normal in twenty-one days as against four days when given by mouth [197], presumably because of the slow absorption from the site of injection [102]. This suggests that it is safer to give large doses by injection—but only as long as the vehicle is a slowly absorbed oil [128]. But, in spite of these rather slight pieces of evidence all being in favour of the safety of single massive doses, we are still unhappy about their use. Apart from the *prima facie* case against giving large amounts of a toxic substance, there is the observation by Türk [175] that renal infections are more prone to occur just after large doses, while in puppies [198] single doses of 450,000 I.U. cause renal and pulmonary calcification and also abnormalities in the unerupted teeth, which is reminiscent of the delayed dentition and stunted growth seen in infants on daily doses of only 1,000 I.U. (p. 532).

Germany was the first country to use single massive doses of vitamin D both for the prevention and the cure of rickets, Harnapp [199] coining the phrase "Vitamin D-Stoss" therapy for this. From a study of one hundred infants he decided that protection against rickets for at least four months was given by 300,000 to 600,000 I.U., though thirteen of sixty infants given 600,000 I.U. by Brockman [174] developed craniotabes within four months. Türk [175] prevented rickets in thirty premature infants for six months by oral or intramuscular doses of 200,000 to 400,000 I.U., while most of his untreated controls did develop rickets. In Scandinavia Palmén [195] treated two hundred premature infants with 500,000 I.U.—none developed rickets and none appeared to be harmed. In America Rambar and others [200] successfully treated twelve infants with a single oral dose of 600,000 I.U. or a monthly dose of 100,000 I.U. for seven months; no controls were studied and their results were only confirmed by X-rays. Wolf's first observations [201] on the effect of large oral doses were partly controlled, since of his sixty-two infants, eighteen had rickets at the beginning of treatment and none had rickets nine months later, after having had two doses of 600,000 I.U., with a three to six months' interval between. In a second paper, Wolf [201] advises 50,000 I.U. at one and two months of age and a third dose of 600,000 I.U. at three months of age. Of twenty-one infants given these doses, two developed rickets, radiologically confirmed, just before the third dose—and this at an age when X-rays are more prone to miss than to disclose rickets. As Wolf ignores craniotabes and mild beading of the ribs, and did no phosphatase estimations, the incidence of rickets in his cases may have been considerably higher than he states (p. 548). He also includes breast-fed babies in his series "because it has been shown that vitamin D is not secreted by the breast."

Krestin [202] is the only English worker who has used single massive doses. He gave 300,000 I.U. by mouth to forty-three infants under one year of age. Of these three developed rickets within six months. In a comparable group of fifty-six infants, whose mothers were supposed to be giving them 1,500 I.U. daily, five developed rickets. The single massive dose had no adverse effect on growth, gain in weight or liability to infection.

Children between the ages of two and twelve should have two teaspoonfuls of cod-liver oil in the winter when sunlight is scarce. If the parents have already warned the children it is unpleasant or if the children genuinely dislike the taste, the classical method of floating it on peppermint is excellent, or of taking a pinch of salt on the tongue before the oil. Concentrated fish-liver oil capsules may be taken as a last and expensive resort. Of course ultra-violet irradiation can be given instead of cod-liver oil, though with more trouble and cost (p. 536).

Boarding schools—both boys' and girls'—often give very bad diets, especially from the point of view of the costly foods rich in vitamin D at ages when they are most needed. In examining children who live at a board-

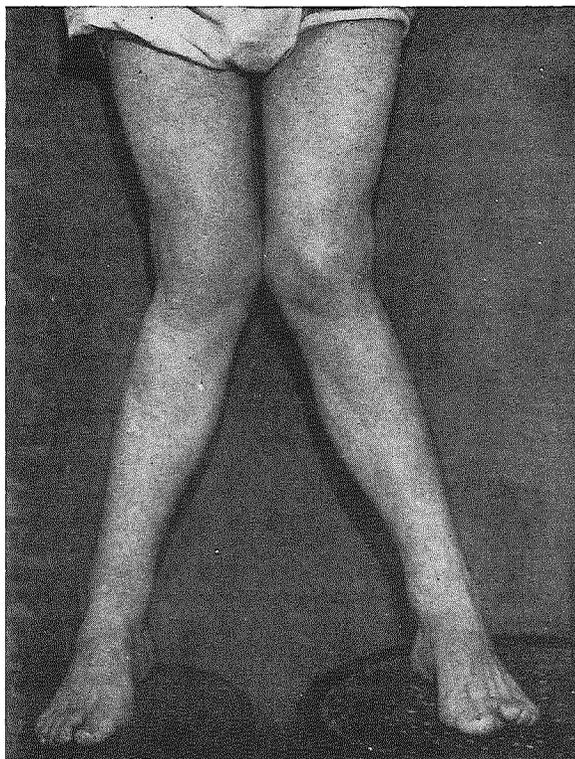


FIG. 185. A severe case of adolescent rickets in England.

ing school for two-thirds of the year, the probability of cod-liver oil being needed must be remembered.

Puberty and adolescence, with its sudden growth impulse, especially needs cod-liver oil, since mild rickets or osteoporosis (Fig. 185) may occur at this period.

During pregnancy and lactation vitamin D is required both for the mother and for the child. The results of a deficiency are well seen in osteomalacia. McLennan [203] suggests that mild osteomalacia may explain why some women who have had no difficulty in earlier labours may appear in later ones to have a slight contraction of their pelvis. About 600 to 800 I.U. daily appear to be ample to allow the pregnant or nursing mother to maintain her own mineral reserves [205, 206, 207]. If the nausea of pregnancy is increased by concentrated fish-liver oil capsules, then calciferol may be necessary since in tablets it has no taste. Children born of mothers deficient

in vitamin D have been reported to have unduly soft skulls and poorly calcified bones [204] and to develop rickets, or even to be born with rickets [208, 209]. On the other hand Finola and his colleagues [210] give the warning that too much vitamin D during pregnancy may cause excessive calcification of the foetal bones of the head, thus making birth difficult. This has been emphasized to us by London gynæcologists who also complain that unduly large babies may be caused by the current craze for large doses of vitamin D.

The adult needs are uncertain—most adults apparently get enough from their food and the sun to prevent obvious deficiencies. But it seems probable that most diets lack some vitamin D : breast milk often contains very little

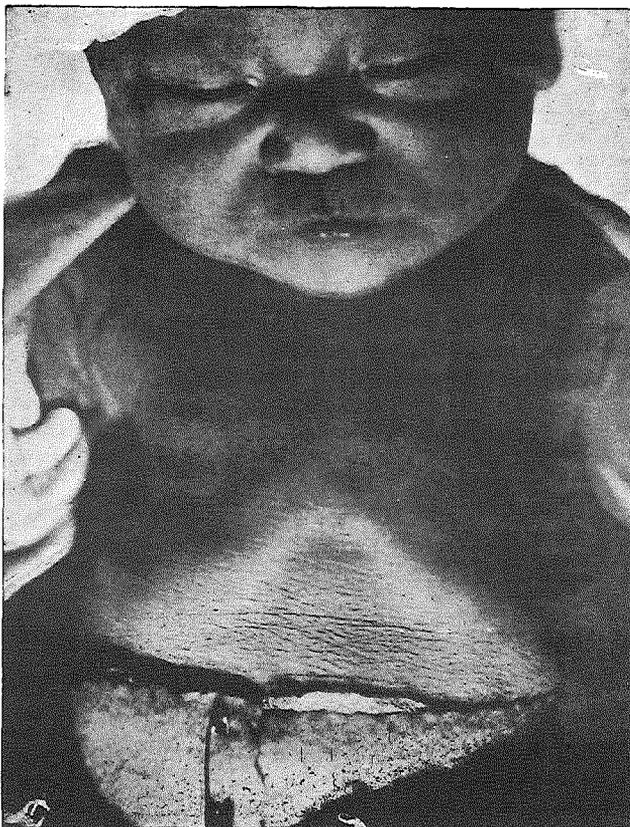


FIG. 186. Fœtal rickets in a Chinese baby photographed on the day of birth. Note the rickety rosary and vertical grooves. (See also Figs. 182 and 189.)

(p. 543), while senile osteoporosis is fairly common and appears to be due to a mild vitamin D deficiency [211]. Reed and his collaborators [111] have reported that huge doses—150,000 to 200,000 I.U. daily—given to men doing sedentary work increased their general well-being, weight, and muscular power. The men previously had been taking only about 100 I.U. daily, and most were underweight before the investigation started. Of course 200,000 I.U. a day is a fantastic amount if it is taken in the food, and it seems unlikely that “in a state of nature” irradiation would enable the body to form so much. The doses employed were, in fact, unphysiological, but this does not detract from the implication that more vitamin D is desirable in the adult’s diet. Probably 300 to 600 I.U. a day should be taken.

In old age the value of sunlight is often lost, since the old keep indoors, and the appetite may be small. According to Meulengracht [211] supplementing the diet of the elderly with cod-liver oil should prevent osteoporosis and the brittleness of the bones which so frequently leads to serious fractures, which, in the femur, for instance, may end fatally.

DISEASES DUE TO DEFICIENCY OF VITAMIN D RICKETS

Lack of vitamin D is the cause of rickets.

The grotesque deformities of the rachitic dwarfs of a century ago are now so rare that one is apt to forget that mild rickets is still one of the commonest deficiency diseases. Probably between one-third and one-half of all the infants and children in England have or have had rickets. Reports on the incidence of rickets give such different figures because the different authors have based their diagnosis on different criteria. Rickets is now so mild a disease that its clinical recognition is often difficult, especially after infancy. This has led not only to an unjustified belief that clinical reports on the frequency of rickets are quite valueless, but also to undue reliance being placed on radiological reports. Actually the only completely reliable reports are those based on the level of the serum phosphatase and confirmed by clinical or radiological or post-mortem examinations. Such confusion, however, now exists about the incidence of rickets that some recent reports need to be discussed in full.

X-rays (p. 559) are not so valuable as is generally believed. They were the basis of the widely quoted survey on rickets, published in 1944, which was carried out in Great Britain during the first two months of 1943 on 4,818 urban and rural infants between the ages of three and eighteen months by the British Pædiatric Association for the Ministry of Health [212]. Each infant was examined clinically and also had one wrist X-rayed. Clinically thirty-three and one-third per cent. of all the infants had active or healed rickets, while in some towns the figure was above sixty per cent. These figures were, however, completely ignored when assessing the results of this investigation on the grounds that they were quite unreliable both because only five per cent. were confirmed by X-rays and because half of the 1.7 per cent. of infants who had positive X-rays were not diagnosed clinically as having rickets. But mild rickets in infants shows itself only in the skull and the costochondral junctions, the wrists not beginning to be affected until toward the end of the first year. Since only the wrists were X-rayed most of the younger infants, and many of the older inevitably appeared to be radiologically normal however clinically definite was their craniotabes or the beading of their ribs. In the final summary no attention was drawn to such considerations nor to the uselessness of the figures which were given. "The (calculated) rate of incidence of rickets diagnosed radiologically for children between three and eighteen months of age was two and a half per cent. before six months of age, four per cent. during the first year of life, and negligible over this age." The clinical finding that thirty-three and one-third per cent. of infants had active or healed rickets is, on the other hand, probably correct, agreeing with the other reports discussed below and most of the investigations made during the last ten years; these are summarized in the report which has just been discussed.

An analogous investigation to the above was carried out by Krestin [191], who during the autumn, winter and spring months of 1942 and 1943 examined clinically and radiologically one thousand healthy children between the ages of two months and five years who were in nursery schools in Preston. Of five hundred and eighteen infants below the age of two years eleven per cent. were diagnosed as having active rickets by X-rays and 36.5 per cent. by clinical examination. After the age of eighteen months rickets steadily declined till

between the ages of four and five years it was clinically present in only 16.4 per cent. of seventy-seven children. The post-mortem examinations discussed below suggests a higher incidence in this last age group and also confirm the uselessness of X-rays for the diagnosis of mild rickets.

Estimations of plasma phosphatase (p. 558) give the most reliable figures for the frequency of rickets. The outstanding work on this subject, and also on many aspects of the diagnosis and contributory causes of infantile rickets, is that of Corner [190] who in 1944 published the results of her investigations on infants in Bristol which lasted from September 1939 to May 1941. Eight hundred and twenty infants between the ages of two months and two years, being a representative sample of the healthy and sick population, were examined clinically and the plasma phosphatase was estimated in seven hundred and ninety-seven. X-rays and post-mortem examinations were also carried out in a few cases. Rickets was not diagnosed unless the clinical findings were confirmed by an increase in the plasma phosphatase or by X-rays or by autopsy. Definite rickets was present in 31.4 per cent. of all the infants and a further 12.2 per cent. showed doubtful or very mild evidence of rickets. At six to nine months of age 52.0 per cent. of infants had rickets and at twelve to eighteen months 48.0 per cent. Below six months the diagnosis was definite in only 26.2 per cent., but in another 17.3 per cent. the serum phosphatase was either definitely raised or slightly raised and accompanied by doubtful clinical signs: these figures may be unduly high since Corner included some infants whose phosphatase levels were probably within normal limits [215].

Clinical diagnosis (p. 552) used alone for assessing the frequency of rickets in infants under two years of age has already been discussed, while reviewing surveys based on other forms of diagnosis. The British Paediatric Association Report [212] gives the percentage of infants affected as 33.3, Krestin [191] as 33.6 and Corner [190] as 45.9. The close agreement of these figures and, especially, the agreement of Corner's clinical finding of 45.9 per cent. with her confirmed finding of 31.4 to 43.6 per cent., does most strongly support the validity of investigations on infants based solely on clinical observations.

Post-mortem examinations for the estimation of the frequency of rickets have only, in recent years, been used by Follis, Jackson, Eliot and Park [217] who in 1943 published the results of consecutive post-mortem examinations on two hundred and sixty children between the ages of two and fourteen years who died from whatever cause in the Harriet Lane Home of the Johns Hopkins Hospital in America. Histological studies, the validity of the technique being carefully confirmed, were made of the shafts and costochondral junctions of the middle ribs. In these two hundred and sixty children active rickets was present in roughly forty per cent. at all ages between four and fourteen years, and in sixty per cent. during the third year of life. Half the children of this whole series who died from an acute illness, lasting at most two weeks, had rachitic changes which were too extensive to have developed during their illness. So it seems probable that rickets is as common in healthy as in sick children.

It is of considerable interest that X-rays were taken of all the ribs which were removed and only five were positive though one hundred and seven showed, histologically, active rachitic changes: the five positive X-rays were all of cases below five years of age.

The importance of very mild rickets, such as that dealt with in the above surveys, may be thought to be slight since nearly half the healthy adult population of England must have had rickets and yet do not appear to have permanently suffered. But mild rickets is of great importance. Firstly it is a sign that our infant and child population is still badly nourished. Secondly the rachitic infant is one who is apt to develop tardily, is prone to infection, is not going to grow to his full stature, and in adult life will join that mass of patients whose chests and legs are mildly deformed, and whose pelvis are

sufficiently contracted to make child-birth difficult. In fact, if the importance of the diagnosis and treatment of very mild rickets is not immediately obvious, yet taking the long view it is of the greatest value.

Contributory Causes of Rickets. In temperate climates the lack of sun and the expense of food rich in vitamin D often cause the milk of nursing mothers and the diet of children to be deficient in vitamin D (p. 542). It is not, therefore, surprising that the time of year at which rickets is most common depends entirely on the amount of sunlight. Thus rickets shows a sharp increase in late winter and early spring when the reserves of vitamin D are low both in nursing mothers and in food, and have not yet been replenished. Rickets, however, may occur with ample sunlight especially in dark-skinned people suffering from severe malnutrition [218, 219].

An inherited disposition to rickets is generally accepted, though, of



FIG. 187. Early rickets in a Brahmin who is almost in the "Budha position."

course, accurately to disentangle all the factors which ameliorate or intensify the disease in man is impossible [208]. Still [220] points out that sometimes the osseous, sometimes the nervous or respiratory systems are most affected in early rickets, this predilection of the disease for a special system often being found in all the children of the same family. It seems probable that the negro requires more vitamin D than natives of northern countries [217].

Turning to the individual infant, by far the most important personal factor in the causation of rickets is the rate of growth. It is so important that the position has been summed up as "no growth, no rickets."

Breast feeding during the first six months of life reduces the incidence of rickets but increases it after this age compared to infants weaned on to a good diet. A poor

antenatal diet has an adverse effect which lasts at least until the end of the second year, this prolonged effect being probably due to the antenatal diet reflecting the diet which is given to the weaned infant. It makes little difference whether artificially fed babies are given milk, or dried milk or sweetened condensed milk [190].

Premature infants and twins are very prone to develop rickets, partly because they grow fast, partly because they start life deficient in bone salts and vitamin D, with a digestion which cannot easily make up the deficiency.

Infections, chronic and acute, are generally believed to make rickets worse. However it is an old observation that children with tuberculous glands seldom develop rickets [208] and in Corner's study [190] of eight hundred and twenty infants rickets, which was present in 31.4 per cent. of these, was commonest in the healthy and lowest in the severely ill. In post-mortem studies on children, between two and fourteen years of age, rickets was not found associated with any particular disease apart from lead poison-

ing [217]. Presumably the healthy infant is more prone to rickets than the sick because he grows faster.

Congenital syphilis is frequently associated with rickets, or, rather, causes or exaggerates the deficiency. Craniotabes, which for so long has been regarded as either rachitic or syphilitic in origin—if not due to osteogenesis imperfecta or hydrocephalus—is discussed on p. 553. There it will be seen that probably it is purely a rachitic condition, though it may be an example of the predilection of an infection for certain tissues being determined by the especial susceptibility of such tissues to a deficiency of a particular vitamin, such as is seen in the occurrence of bronchitis in infants suffering from lack of vitamin A.

The age of onset of rickets varies slightly in different countries, due most probably to the maternal diet, which starts the infant off either with an

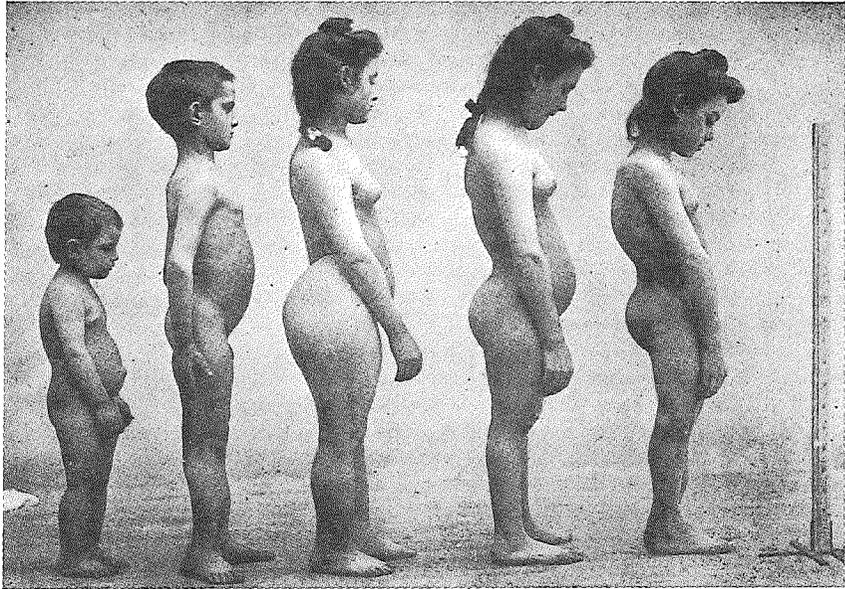


FIG. 188. Five French children in one family with rickets, aged $6\frac{1}{2}$, $11\frac{1}{2}$, $13\frac{1}{2}$, $14\frac{1}{2}$ and 17. Note bossing of the forehead, bent tibiae, curvature of the spine, and protuberant abdomen. (See also Fig. 178.)

adequate or poor supply of vitamin D and bone salts. In the post-war famine in Vienna where the mothers themselves were poorly nourished, rickets in infants only three months old was a common finding, while in England rickets before the age of six months is generally thought to be fairly rare, though this is not confirmed by Corner's figures which have been given in the previous discussion on the incidence of rickets (p. 548). After six months rickets increases until the middle of the second year and then declines as the first wave of the growth impulse is spent and the child tends to eat a wider diet and to spend more time outdoors.

Rickets, however, can occur at any age. Infants (Figs. 186 and 189) have been born with rickets [208, 209], and "late rickets" or "juvenile osteomalacia" may occasionally be found in older children (Fig. 185), especially during periods of rapid growth, such as at the second dentition and puberty; a wartime example in England is described on p. 537. At this age the picture hovers between rickets, osteoporosis and osteomalacia, depending on how much active growth is still going on, the minerals in the

diet, and the severity of the deficiency (Fig. 185). After growth has finished a deficiency of vitamin D causes osteomalacia, which is only adult rickets, modified by the absence of growth (p. 564). Girls are slightly more susceptible to rickets than boys, and dark children more than blond [208].

The General Symptoms of Rickets. The clinical picture of rickets is too often painted as if the bone changes alone were important ; but rickets is a disease which affects the whole body so that even if the osseous system were ignored a very definite disease picture would still remain.

The rachitic infant is a tired, restless, unhappy creature ; by day he is fretful, by night he sleeps badly, throwing off his bedclothes as he twists and turns. He impatiently rolls his sweating head on his pillow, even to the

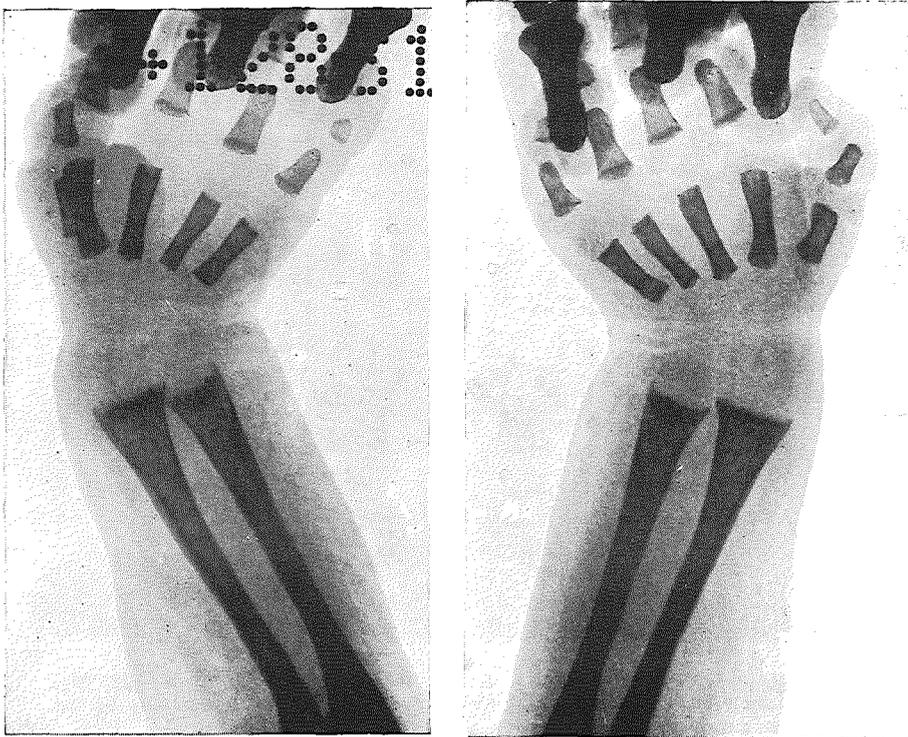


FIG. 189. Fœtal rickets in a Chinese baby, X-rayed on the day of birth. Note the uneven frayed diaphyseal ends of the radius and ulna, and the cupping of the latter. (See also Figs. 182 and 186.)

extent of wearing away his hair. His bowels are often constipated with sudden attacks of diarrhœa. His stomach is distended, partly because the intestines lack tone and are blown up by carbohydrate fermentation from his faulty diet, partly because the liver and spleen are forced down by the deformed chest, and partly because the abdominal muscles are flabby. This flabbiness affects all the other muscles of the body while at the same time the ligaments of the joints are lax, so that the limbs may be twisted into bizarre positions. The Buddha-like position commonly seen in statues of the holy men of the East, with the soles of the feet lying upwards, is probably the result of rickets and osteomalacia. A very mild tenderness of the bones is common, causing the child to cry if roughly handled. But any marked tenderness is more suggestive of scurvy.

Catarrhal infections are common ; they are not due to the rickets, but

rather to the unhealthy life and poor diet of these children, a diet which, among other things, is probably grossly deficient in vitamin A.

Spasmus nutans is a rare complication: when it occurs it is generally with mild rather than severe rickets. The child slowly nods or shakes his head when it is unsupported, and at the same time has a rapid, very slight or more obvious nystagmus, which is almost unique in often being unilateral. When the two eyes are affected their movements may bear no relation to each other and move in any direction and manner. Hippus—rhythmic contractions of the pupil—is also sometimes seen. Spasmus nutans is generally found in the first year of life, starting in the winter, and disappearing in a few months. Its cause is obscure. It is not due to the infant living in the twilight of a cellar or dark room, such as would cause miner's nystagmus, because it is found in infants who live in well-lit rooms.

Anæmia is common, being generally of the normocytic type; it seems to be rather the result of general ill health or bad food than an essential part of rickets, though in animals vitamin D, especially from fish liver oils, slightly increases the absorption of iron and the formation of hæmoglobin [122]. The liver and spleen may be just palpable. This has no significance, being due to the deformed thorax pushing down the viscera. A really enlarged spleen suggests syphilis. Von Jaksch's anæmia, or pseudo-leukæmia infantum, is often associated with rickets for some still obscure reason.

The usual infantile milestones are passed late. The teeth are often not cut until after the first year (p. 570), crawling and walking are delayed, or if walking has started the onset of rickets "takes the child off his legs."

Mental development is unaltered, though severe cases may show the precocity which is common in invalid children who of necessity are too much in the company of adults.

The Bony Changes of Rickets. The bony changes which may occur in rickets are craniotabes, a large square-shaped head, late closure of the fontanelle, beading of the ribs and deformity of the chest, bending of the long bones, enlargements at the epiphyses, and fractures.

To understand these changes it must be remembered that:—

- (1) Rachitic changes are greatest where growth is most rapid.
- (2) Deformities are the result of the combined action, on the bones, of gravity and the pull of muscles.

Craniotabes is the earliest finding in rickets and is diagnostic of the disease when associated with beading of the ribs [190]. While it is very common in rachitic infants under nine months, it is often completely absent in severe cases in older children. This is partly because the growth of the skull is extremely rapid in the first months of life, and partly because when children start to sit up there is no longer continuous pressure from the pillows on the skull.

In typical craniotabes round or oval unossified areas are found in the skull which yield like parchment under the pressure of one's fingers, giving a crackling feeling. These areas vary in size from those which can be just felt with the tip of a finger to those which appear to fit the entire ball of the thumb. They occur in the bones on which the weight of the head rests as the infant lies in his cot; the upper occipital, the posterior parietal, and sometimes in one upper temporal bone if the infant lies much on one side. They do not join with the sutures, but often lie close to them.

Confusion may arise either from the soft feeling of the skulls of some infants in whom calcification is delayed, or because craniotabes is generally stated to be a finding common not only in rickets, but also in congenital syphilis. Probably craniotabes is always rachitic in origin, so that when it occurs in syphilitic infants they are suffering from two diseases. Still [220] points out that the condition in syphilitics will clear up in seven or ten days with vitamin D and no anti-syphilitic treatment, and also that craniotabes is frequently associated with laryngismus stridulus, which is a rachitic and

not a syphilitic condition. Also syphilis tends to make the bones harder, not softer. In osteogenesis imperfecta the whole of the vault of the skull, and not only small areas in its posterior half, is largely membranous, while in hydrocephalus the size of the head prevents confusion.

The persistence of the anterior fontanelle is common and should suggest rickets if it still measures an inch each way at the end of the first year, or persists after the age of two.

The enlargement of the skull is difficult to explain. The four crosses over the frontal and parietal eminences which give the square hot-cross-bun effect are due to the heaping up of osteoid tissue in these regions.

The chest also suffers in early infancy when the ribs are growing rapidly and are very soft. The pull of inspiration drags in the sides of the chest so

that the sternum is left sticking forward like the keel of a boat. At the same time the lower ribs flare out, being supported from collapse by the liver and heart, leaving "Harrison's sulcus" above them. These deformities of the chest are serious. Full expansion of the lungs is impossible so that even mild catarrhal infections are liable to end fatally because the flaccid cage of the ribs collapses more and more with any obstruction to inspiration.

Beading of the ribs, the "rickety rosary," is a nearly constant finding after the age of six months or even earlier, but not one on which much reliance as a diagnostic sign can be placed in mild cases. Corner [190] in her extensive study of eight hundred and twenty infants found that one-third of all infants with mild beading and one-eighth with definite beading had not got rickets, judged by the level of the plasma phosphatase; beading associated with craniotabes was diagnostic. The "beading" is the expanding osteoid tissue at the junction of the rib and its cartilage, being most obvious over the fifth, sixth and seventh ribs. Some slight swelling at the costo-chondral junctions in young



FIG. 190. "Rickety Rosary."

infants is normal, and has no rachitic origin. The ribs in severe cases may be displaced backwards, so that no beading is felt. "Posterior beading" is caused by greenstick fractures at the angles of the ribs.

The arms become deformed when the child starts to crawl or sit upright. In the latter position he appears to fall into himself, and as the weak muscles of his back cannot hold his back straight, he curls forward supporting himself on his arms. This causes them to bow under the strain, while the already enlarged radial and ulna epiphyses broaden still further. The clavicle is bent upward and forward at its inner third by the pull of the neck muscles at one end and the drag of the arms at the other. The fingers sometimes show sausage-like enlargements due to the shafts of the phalanges swelling from osteoid tissue, while the joints remain a normal size.

The femur develops a forward curve from the weight of the legs when the child is sitting in his mother's lap, while later on, if he manages to walk, the

lower third of the tibia tends to bend outward and forward and the femur to bend outward. But at this stage any deformity of the legs may occur, increased by the lax ligaments of all the joints.

Pelvic distortion, is, however, the most serious result of rickets. It may make child-bearing in later life impossible except by Caesarean section, so that in girls every effort must be made to insist on thorough treatment, and above all complete rest from walking until the pelvis is firm enough not to bend under the weight of the body.

Greenstick fractures are common and may be overlooked, being often



FIG. 191. Rickets in an English infant. Note the deformity and enlargement of the epiphyses at the wrist. (See also Fig. 192.)

painless and giving no more deformity than may have already arisen from the twisted bones.

Dental caries is not caused by rickets (p. 572).

Atrophic rickets is the name given to rickets where osteoporosis is profound, due to the diet being grossly deficient in calcium as well as vitamin D. It is commonest in the first year of life, and in premature babies.

Hypertrophic rickets occurs in older children, especially when the rickets is mild and chronic. It causes great thickening of the bones due to masses of osteoid tissue in which only some slight calcification can be seen.

Spasmophilia. The nervous symptoms of rickets are so important that they are often discussed as if they were a different condition, and it is true that some of the worst cases, those with generalized convulsions or prolonged attacks of tetany, show only very slight signs of the underlying rachitic condition. Indeed, it may be the convulsions which first cause a suspicion that rickets is present.

The proportion of rachitic children who suffer from spasmophilia varies greatly in different places. The underlying mechanism is a low blood calcium;

where the calcium of the diet is high the tendency to spasmophilia should therefore be slight, but as yet this has not been investigated. Boys are affected more often than girls and the condition may occur at any age, even in infants a few weeks old.

The irritability of the nervous system may show itself in many ways.

The most trivial manifestations are sudden starts with a cry as if the child were suddenly pricked ; or for a moment he turns his eyes up ; or he cries out in his sleep, rolls over and is quiet again. Facial stiffness is also quite common. The muscles of the child's face are in spasm, so that when he

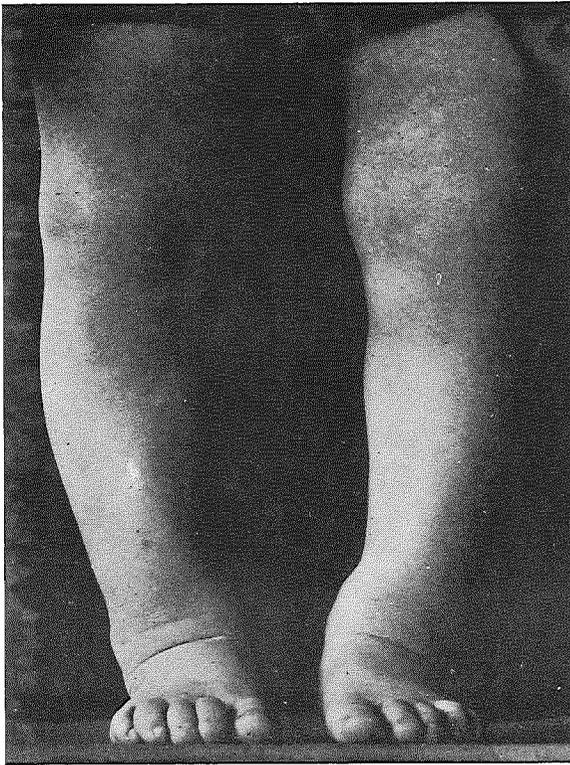


FIG. 192. Rickets in an English infant. Note the bowing of the tibiae, and the enlargement and deformity of the epiphyses at the ankles. (See also Fig. 191.)

cries the face gives an impression of being stiff. Change of expression is limited, lacking the finer shades.

Or, again, true tetany may occur, the hands and feet being drawn into the typical position, with the thumbs pressed against the fingers, which are held rigidly straight though flexed at the carpo-metacarpal joints with the wrist slightly bent. The feet are extended while the toes are bent down and bunched together. These spasms may last for seconds or hours. They appear to be only painful when they start, so that children will often go on clumsily playing with their toys during an attack. If the spasm lasts for some hours both the hands and feet may swell from static congestion.

Laryngismus stridulus is, however, a more frightening symptom. The child on waking, or when he starts to cry, or indeed for no very obvious cause suddenly gets a spasm of his glottis. He holds his breath, appears terrified, and goes blue in the face. Then suddenly the spasm passes and he draws in

his breath with a long whoop. Sometimes the child does not recover so simply but goes into general convulsions.

General convulsions may occur for no reason, but usually the child has had an attack of diarrhoea, or a mild feverish upset. The attack starts with the child going into tonic stiffness which passes into clonic convulsions. After a minute or two the attack passes, the child being again normal but exhausted. Generally these attacks occur at long intervals, but they may follow each other so rapidly that the child does not regain consciousness between each one.

There is a definite danger to life in infantile convulsions so that it is important to recognize and treat the early mild symptoms of spasmophilia before generalized convulsions occur. The treatment is discussed on p. 562.

Examination of the child may disclose any of the classical signs of latent tetany due to an unusual excitability of the motor nerves—Chvostek's sign, or twitching of the muscles of the face when the facial nerve in front of the ear is lightly percussed; Trousseau's sign, or compressing the arm or leg by encircling it with the hand and squeezing, when the hand or foot goes into the position assumed in attacks of tetany; Erb's sign, or undue excitability

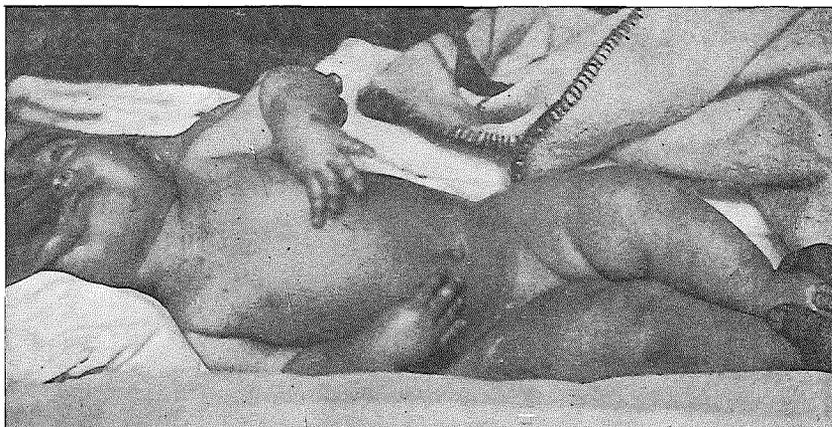


FIG. 193. Tetany in an English infant with rickets. Note the position of the hands, and their swelling from static congestion.

of the motor nerves to electrical stimulation—but the necessity for electrical apparatus and experience in its use vitiates the value of this test.

Other causes besides rickets may cause infantile convulsions. Acute fevers and infections of the brain and spinal cord often start with convulsions. In these conditions the child between its convulsions is still ill while in spasmophilia it is well. Trivial causes like constipation or indigestion may cause doubt for a moment, and middle ear disease must be remembered. Any infection may flare up a latent tendency to true spasmophilia.

Idiopathic epilepsy is rare at this age, and will not yield to the treatment for spasmophilia.

Lead encephalopathy may closely simulate spasmophilia, the earlier symptoms being vomiting and convulsions. Mild cases often occur in the spring when the increased supply of vitamin D from sunlight enables children to absorb more lead from the paint of their toys, etc., since lead absorption is increased by vitamin D [222, 223]. Lead poisoning is said to cause rickets [217], and so presumably would poisoning by any of the metals mentioned on p. 527 which form insoluble phosphates.

Aids to Clinical Diagnosis of Rickets. In severe cases of rickets the diag-

nosis is simple, but in early cases doubt may be felt over the significance of mild beading of the ribs, dubiously enlarged epiphyses, late teething, delayed closure of the fontanelle, mild spasmophilia or convulsions with no obvious bone changes, recurrent attacks of bronchitis, weakness and disinclination to walk, and general poverty of well-being and health. In all ailing children it is wise to remember that mild rickets may be the cause of many rather vague symptoms. Rickets is a disease of general metabolism.

Whenever there is hesitation over the clinical diagnosis of active rickets the alkaline serum phosphatase should be estimated, this definitely confirming or disproving the diagnosis since other generalized bone diseases and hepatitis which may increase the serum phosphatase are of academic rather than

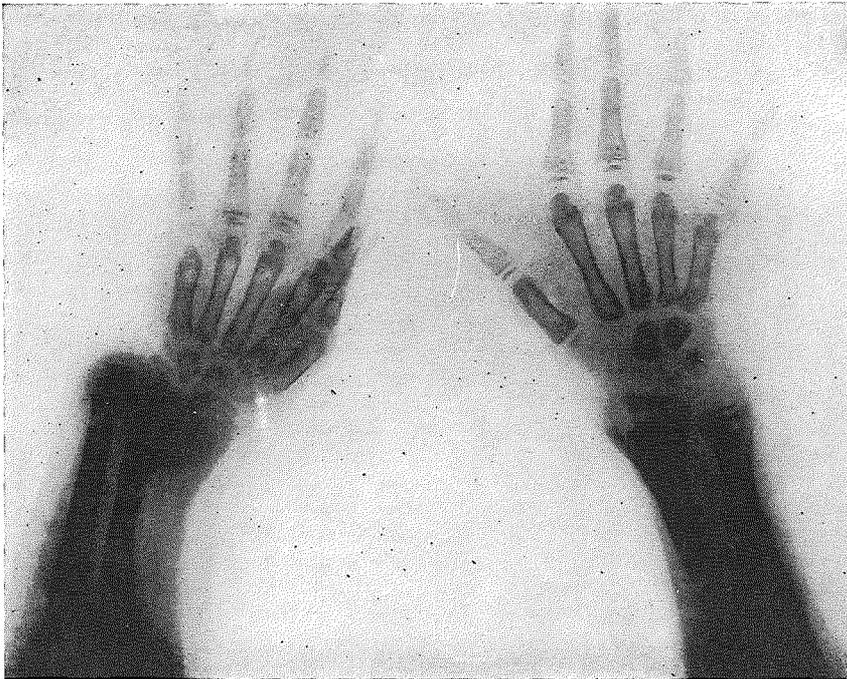


FIG. 194. Active rickets in an English child. Note the enlargement and deformity of the epiphyses with lightly calcified masses of osteoid tissue in one wrist, and in the other the uneven ragged diaphyseal ends of the radius and ulna, with slight cupping of the latter. (See also Fig. 195.)

practical interest. Two methods of estimation may be employed. That which is used in England [213] gives "King-Armstrong Units": it takes about twenty-five minutes and requires only 0.1 to 0.3 ml. of serum or plasma; it can be modified to give as well the level of inorganic phosphorus [214]. According to Gray and Carter [215] normal values during the first three years of life are 11 to 20 units; 17 units being the average and 20 units the upper limit of normal. Between three and five years the average is 12 units, with a range of 8 to 18 units. In adults values vary from 0 to 10 units. Corner [190], however, put the average at all ages as being 3 to 10 units, 15 units and over being diagnostic of rickets. But from the results of investigations on apparently normal children, from the changes with age in Bodansky units and, especially, from the results of giving vitamin D to children with levels of about 20 units, it seems definite that the figures of Gray and Carter are the more correct. In early rickets levels of 30 to 40 units are usual, but the same

levels may be found in more severe cases, so that high phosphatase levels are diagnostic of rickets but not of its severity. Levels in severe rickets under treatment fall, then rise and then fall again, while in less severe rickets there is a steady fall from the beginning [215], but normal values may not be reached for three months or longer. In the U.S.A. "Bodansky Units" are generally employed, measured by a technique which requires 2 ml. of serum and takes over an hour to complete [216]. It is said that 20 Bodansky units is the average for premature infants [178], while for normal infants the average is 7.1 units on the third day of life, rising to 13 units at the third or fourth month with a fall to 11.5 units at the age of three years and a further fall to 1.4 to 4 units in adult life [215].

The blood calcium in infants is normally 10 to 11 mg. per 100 ml., and in the usual type of rickets is little if at all below this level. In the "low calcium" type of rickets associated with spasmophilia this calcium may be as low as 4 mg. but is more often between 5 and 7 mg.

The inorganic phosphorus is normally 4.5 to 5.5 mg. per 100 ml., which commonly falls in rickets to 2 to 4 mg., 4 mg. being generally taken as the dividing line between the normal and the rachitic. But a low level of either phosphorus or calcium is by itself no proof of rickets; when, however, the product of the two is below 40, this is definitely suggestive of active rickets.

The typical X-ray appearances of the bones in active and recently healed rickets have been described on p. 530. For the diagnosis of mild rickets, however, X-rays have several drawbacks. Generally the only rachitic changes in infants under one year of age are found in the skull and ribs, but the surrounding tissues prevent these being satisfactorily X-rayed. As a consequence the wrist has to be examined and changes here are uncommon before the end of the first year. Even after this age a negative X-ray does not prove that rickets is not present. In a positive X-ray (Figs. 189 and 194) the distal end of the ulna shows "cupping" while in both it and the radius the smooth



FIG. 195. Healed rickets in the child shown in Fig. 194. The X-ray, taken five years later, is of the wrist which was most affected.

end of the diaphysis appears irregular and rough. The increased width of the cartilage is difficult to recognize with certainty, though spreading up along its sides the faint shadows of the slightly calcified perichondrium may be visible. The early changes in rickets must be diagnosed by experts, and even these are liable to differ in their opinions. Much confusion is caused by the ends of rapidly growing bones showing slight irregularities which suggest rickets though they are really normal, being due to calcification being so rapid that it is not quite uniform [224, 225].

Differential Diagnosis of Rickets. In congenital syphilis the X-ray changes are moth-eaten epiphyses and periostitis while clinical stigmata are probably present. Of course rickets, being common in congenital syphilis, may complicate the radiological and clinical picture.

In osteogenesis imperfecta confusion will be avoided if the difference in the changes in the skull is remembered (p. 554) and the blue sclerotics.

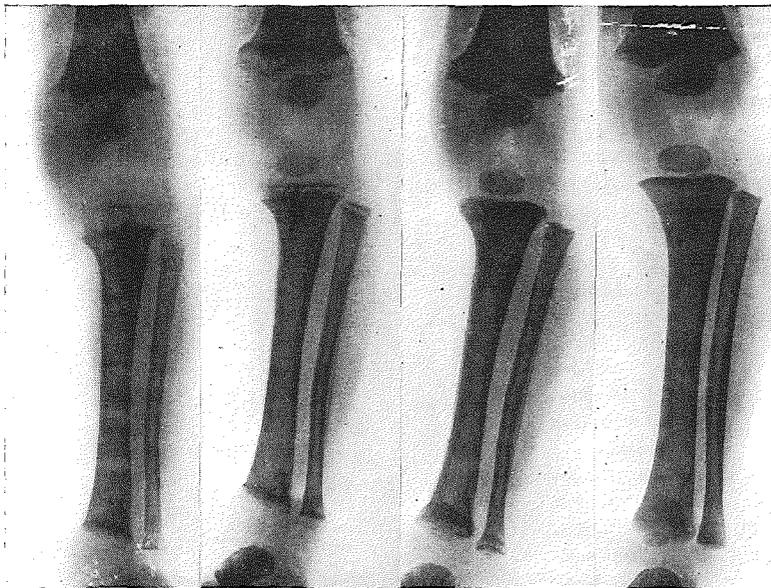


FIG. 196. Rickets cured by cod-liver oil.

There is a frequent family history. The X-ray appearances are not those of rickets, and the blood calcium, phosphorus, and phosphatase are normal.

Chondro-osteo-dystrophy in older children will not be mistaken for rickets if an X-ray is taken, since the epiphyses are fragmented and the lumbar vertebræ are deformed.

Achondroplasia leads to an unduly long body compared to the short legs and arms: the hands are like star fish: the head is domed. X-rays and the usual biochemical investigations confirm the diagnosis.

Scurvy causes acute pain when the child is gently moved and there is bruising, bleeding gums if the teeth are erupted, red blood cells in the urine, and subperiosteal hæmorrhages and epiphyseal separations—the latter never occur in rickets, while pain is never severe and there is no hæmorrhagic tendency. Rickets and scurvy can, of course, occur together.

Renal rickets and other forms of "rickets" are discussed on p. 562.

Treatment of Rickets. The amount of vitamin D required for the treatment of most infants with moderately severe rickets is about 1,200 I.U. a day. Individual needs vary greatly. The correct dose is that which cures

the rickets, some children with "resistant rickets," a strange condition discussed on p. 562, requiring enormous doses.

The various sources of vitamin D have been discussed on p. 538 so that here it is only necessary to point out that up to 1,200 I.U. daily can be given as cod-liver oil, but that higher doses need the use of one of the concentrated fish liver oils.

Some physicians advise very large amounts of vitamin D, by that means hurrying the cure. There seems no advantage in most cases over a lower dosage and slower recovery. The only exception is in infants where the weakness and collapse of the ribs is threatening death from pulmonary infections [226]. Here doses of 50,000 units a day may be given in order to calcify the ribs with the greatest rapidity; X-ray and blood examinations must be used to check the results. If the infant is too marasmic to take a normal amount of milk, calcium phosphate gr. 10 should be given daily to supplement the mineral intake.

The use of artificial sunlight as an alternative or aid to cod-liver oil has been discussed on p. 536.

Mineral salts given without vitamin D are valueless and calcium preparations containing neither magnesium [227] nor phosphorus [79, 80, 81] are definitely injurious.

Improvement is hard to judge clinically. The changes in the bones fade away too slowly to be of value in gauging the success of treatment, except where craniotabes is present, when healing should be obvious in two or three weeks. The general condition of the child furnishes some evidence. He is better in himself, happier, and his muscles appear stronger.

X-ray examination is a simple and generally available method of assessing treatment. After three weeks on average curative doses of vitamin D a faint line of preliminary calcification should appear running across the clear epiphyseal cartilage just beyond the end of the diaphysis. The further X-ray changes due to healing have been described on p. 530.

Phosphorus and calcium estimations are of value; within two weeks they should be approaching normal.

The blood phosphatase levels approach the normal more slowly than those of the minerals, falling in two to three weeks, but not becoming normal for about three months or even longer [215]. In mild cases the fall toward normal is continuous from the beginning but in severe cases this fall may be interrupted by a brief rise [215]. A fall in the phosphatase is the best guide that healing has started [30], and in doubtful early cases is proof that the diagnosis of rickets was correct [215].

Celiac Rickets. Celiac rickets is only rickets complicating celiac disease: a common complication because the inability to absorb fats reduces the amounts of both vitamin D and calcium acquired by the body. Treatment consists in giving calcium lactate in large doses by mouth and also vitamin D. Irradiation is ideal (p. 536) but if it is too costly a highly concentrated fish liver oil containing little fat or even tablets of calciferol or of synthetic vitamin D₃ must be used.

Scurvy—Rickets. This is a combination of scurvy and rickets, the usual treatments for each condition being required.

Treatment of Rachitic Deformities. Rest is the essential treatment both for preventing and curing rachitic deformities, since only at rest is the weight of the body taken off the soft bending bones.

In mild cases carefully controlled exercise and insistence on regular rest after lunch is sufficient. Where deformities have already occurred splinting may be necessary to prevent the child crawling or walking until the bones have become recalcified. This is absolutely essential in girl babies, as their pelvis must be protected from distortion. General massage is invaluable when no exercise is taken; a masseuse can rapidly teach the mother how to give it.

The body's power of remoulding deformed bone is remarkable, but where the deformity is gross, surgical correction may be necessary. The suggestion that the bones should be deliberately decalcified to such an extent that they may be bent back to a normal position is bad.

The prognosis in deformities is excellent, except in the pelvis which in girls may cause serious trouble over childbirth in later life. Some abnormality of the chest is liable to persist, and deformities acquired at puberty when growth is nearly over will not disappear of themselves.

Treatment of Spasmophilia. The immediate domestic treatment of convulsions is to put the child in a hot bath at a temperature of 100–105° F.

Calcium deficiency being the immediate cause of spasmophilia the level in the blood should be raised rapidly. Calcium chloride gr. 15 thrice daily in milk may be tolerated and is ideal, or calcium lactate gr. 30 may be given instead. In continuous convulsions 5 ml. of a ten per cent. solution of calcium lævulate or gluconate should be given intravenously if possible, but if not into the buttock. Calcium chloride, 1 ml. of a two per cent. solution, is excellent, but it causes ulceration unless it is given into a vein, and for this reason can only be used in an emergency when no other calcium preparation is available.

Sedatives should be given to all children who show any signs of spasmophilia. Vitamin D acts slowly and until it acts there is always the risk of general convulsions, which may even end fatally.

Rickets Due to Deficient Renal Tubular Reabsorption of Phosphorus. In this group of rare diseases the essential abnormality [228, 229, 234, 235, 243] is a failure of the renal tubules to reabsorb phosphorus. This causes a hypophosphatæmia which in its turn causes a chronic insufficiency of phosphorus for the calcification of bone. Since in ordinary rickets there is a similar hyperphosphaturia, etc., which is corrected by normal doses of vitamin D, and since in the rare forms of rickets correction may also be largely or wholly achieved by huge doses of vitamin D, it would seem that in essence both types of rickets are the same, only differing in the amounts of vitamin D required by that enzyme system of the kidney [235] which is concerned with phosphorus reabsorption.

The great general interest of this group of diseases lies in their being the only definite known examples of deficiency diseases which (a) are often inherited and (b) are not cured when the deficient nutrient is supplied in ample amounts, judging not only by its intake but also by its level in the blood. These two facts, bearing in mind that these diseases are curable by enormous doses of vitamin D, mean that a disease cannot be dismissed from the ranks of deficiency diseases and diseases which are curable merely because it is familial or, which is more important, because there is no response to treatment which judged by customary criteria should be successful.

Raised Resistance to Vitamin D (R.R.D.) or Resistant Rickets or Refractory Rickets. This condition is in its clinical picture ordinary rickets which fails to respond to vitamin D until the dose is a hundred or more times as large as that usually required. McCance [228], who coined the name Raised Resistance to Vitamin D or R.R.D., has written the best of several excellent reviews and papers on this subject [228–235].

The familial tendency in R.R.D. is very strong, siblings of both the patients and their parents frequently being found to be affected, though isolated cases occur. An onset in infancy is usual, but it may be delayed until early adolescence [228]—an age, oddly enough, at which spontaneous improvement often occurs [233].

The cause, as stated above, is a failure of the renal tubules to reabsorb phosphorus. There is no inability to absorb vitamin D from the gut, since blood levels may be over a hundred times the normal before there is healing of the rickets [230].

The diagnosis of R.R.D. depends on recognizing that what would be

ordinary rickets were it responsive to normal doses of vitamin D is in truth rickets, but rickets responsive only to abnormal doses of vitamin D. All the biochemical investigations which are of help in the diagnosis of ordinary rickets (p. 537) are just as helpful in the diagnosis of R.R.D. and unmask the same abnormalities. The X-ray changes [236] are also those of ordinary rickets, but in spite of all this easily gleaned evidence, which in sum can only mean rickets, a diagnosis is often made of chondrodystrophy or osteogenesis imperfecta or hyperparathyroidism or even of muscular dystrophy, though these have few or no biochemical similarities with rickets.

Treatment consists in giving as much vitamin D—which may be even a million or more I.U. daily [232]—as is necessary to control the rickets, though this, in sharp contrast to ordinary rickets, may fail to correct entirely the hypophosphatæmia [229]; further, and again in contrast to normal rickets, vitamin D must be given daily, no effect being produced by very large doses at long intervals. The dose may vary considerably from time to time and may fall abruptly at puberty. A serious danger [230, 231, 233, 236], which means that the child must be under constant supervision, is that there may be little margin between the curative and the toxic dose, Mackay and May [233], for instance, describing two children in whom there was virtually no margin, one of them even developing the osseous changes of vitamin D poisoning (p. 583). Some pædiatricians urge that the urine should be tested weekly with Sulkowitch's reagent because an increase in urinary calcium occurs not only in those cases with hypercalcæmia but also in cases with normal calcæmia which are nevertheless toxic or on the verge of toxicity [231]. But probably the well-being and symptoms (p. 579) of the child are the surest guide as to whether the dose must be reduced or temporarily stopped. A further danger is sudden hypercalcæmia with serious renal damage when such children are put to bed after osteotomies or for any other reason, since added to the tendency of all young patients to develop hypercalcæmia when they are immobilized is the hypercalcæmic effect of vitamin D. The danger unfortunately is only slightly abated by stopping treatment a short time before any operation, because the effect of vitamin D and a raised blood calcium may persist for weeks.

The Fanconi and Other Syndromes [237–244]. These conditions are in essence the same as R.R.D. but the urine also contains glucose and often amino acids, other abnormalities in its secretion may occur. From the point of view of rickets the only germane excretion is the excessive phosphorus, but inevitably the rickets of patients with these abnormal kidneys have been labelled with particular names though “R.R.D. with renal glycosuria, etc.” would have been clear and logical. Of these names the “de Toni-Fanconi Syndrome” and “Cystine Rickets” are the best known, the former implying rickets, hyperphosphaturia, renal glycosuria and often the excretion of amino acids—probably cystine—in the urine, while the latter is the same. To this group may be added the further abnormality of failure to acidify the urine and, perhaps, failure to form ammonia. The same failure over acidification and formation of ammonia and with hyperphosphaturia but with no other renal abnormality is found in the Butler-Albright Syndrome (renal acidosis or nephrocalcinosis). All these conditions save the last are often familial. They would, as regards the rachitic abnormality, probably respond to vitamin D in sufficiently large doses [228] in spite of the belief of earlier workers, who used too small doses, that they would not; indeed Stevenson [243] has reported one case with sugar and amino acids in the urine which had not only the rickets but also apparently the urinary abnormalities controlled by 100,000 I.U. daily.

Renal Rickets. This condition, also known as renal infantilism, renal dwarfism, renal osteitis fibrosa cystica and renal osteodystrophy, is the exact opposite of R.R.D. being due to damaged renal function preventing the excretion of phosphorus, with the result that it rises to such high levels

in the blood that calcium ionization is depressed and little calcification can occur. Renal rickets is commonest in mid-childhood. It is characterized by rickets, dwarfism, albumin in the urine, a high blood phosphorus and impaired renal function. Vitamin D makes the condition worse by further mobilizing phosphorus from the tissues. A high calcium low phosphorus

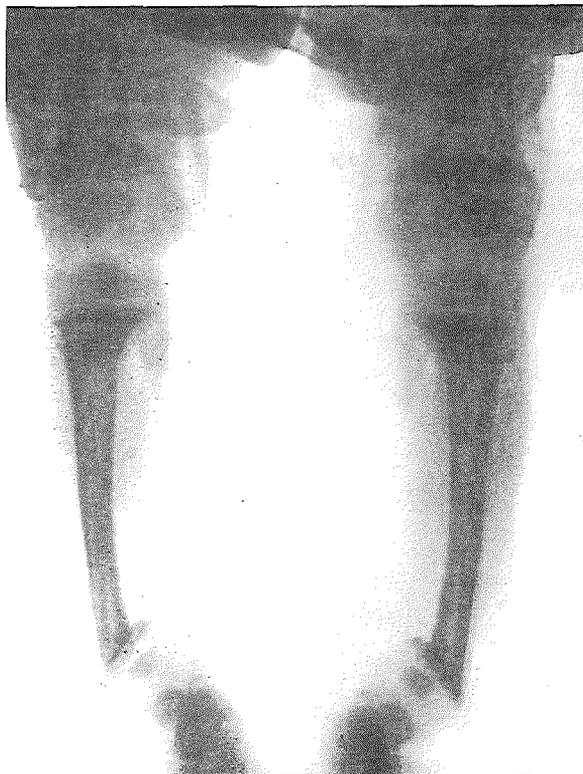


FIG. 197. Renal rickets in an English infant. Note the displacement of the distal tibial epiphyses, and the slight calcification of the expanded osteoid tissue.

diet may delay the hopeless march of the disease by aiding the bowel in the excretion of the excessive phosphorus.

OSTEOMALACIA (MOLLITIES OSSIIUM).

Osteomalacia of pregnancy, hunger osteomalacia, and probably many cases of senile osteoporosis are fundamentally all the same, being due to a deficiency of vitamin D. In the past they were not recognized as identical because the obvious cause appeared to be repeated pregnancies, famine, or old age rather than a common vitamin lack. They are, indeed, the adult equivalent of low calcium rickets modified by the absence of growth.

Osteomalacia is such a dramatic disease that sufferers have taken their place in myths and legends almost as if they were giants. The French of the last century besides making the best clinical study of the condition [4] have collected many historical accounts [208, 245, 246]. The best known was a dwarf who in the sixth century lived to be three hundred years old, but appears to have been more like a jelly fish than a man so soft were his bones.

He could not move, and was plagued by the teasing of dogs and children : his compensation was his fame, since he could be twisted into any position at will. Then there was a woman called Supiot who attracted much attention in 1700, and another nameless, who "Ayant été d'une taille tres elevees, devint, avant de mourir plus petit qu'un nain." Trousseau's account [4] of Madame Rehbin, however, is still the only perfect description of the disease, and the perfect example of possibly the greatest physician's mastery of prose.

Osteomalacia of Pregnancy. This is now a rare disease in Western civilization, though cases are still occasionally seen [203, 247, 248, 249], and MacLennan [203] suggests that mild osteomalacia in England may be the reason why some women who have had no difficulty in earlier labours may appear in later ones to have a slight contraction of their pelvises. When

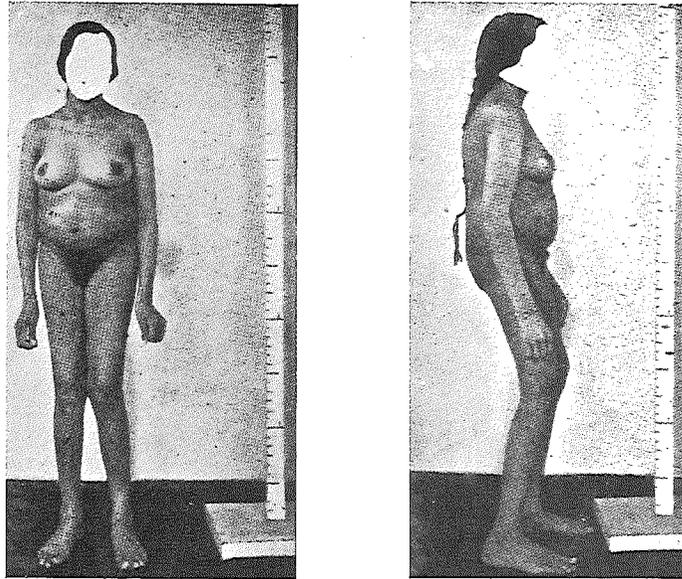


FIG. 198. Osteomalacia in a Sikh woman. The condition, due apparently to poverty and pregnancy, started in 1939 while living in England. Note the absence of the normal lumbar lordosis, the adduction of the thighs, the left genu valgum, and the abnormal height of the fundus at the third month of gestation. (See also Figs. 199 and 200.)

osteomalacia was more common it was generally among young women who had had frequent pregnancies on bad food and no sunlight. But it was also found during first pregnancies, and then the mother was often over thirty.

With each child the condition takes a step forward as the body is further drained of its minerals and vitamins. And with each child the condition becomes more piteous : the mother is more imprisoned in the house by pain and deformities ; her chances of earning more money, of gaining more food, more sunlight, are curtailed ; her pelvis collapses further and further, making her next confinement even worse than the last. Sometimes her downward progress halts between her pregnancies, sometimes she may even improve if fortunately she does not suckle her infant in the vain hope of thus warding off her tragic fertility.

The gradual extinction over five hundred years of the Norse colony founded by Eric the Red in Greenland at the end of the tenth century was apparently due to the pelvic deformities of osteomalacia hindering childbirth.

It is thought that the Norse colonists did not eat the local diet of fish and fish oils which is necessary to supplement the scant sunlight in Arctic regions [209].

Hunger osteomalacia. This is found quite apart from pregnancy during periods of chronic famine. Both sexes suffer and the elderly more frequently than the young, who are more exposed to the sun. The condition is endemic in areas where the diet is poor, or custom keeps women indoors, as in some parts of China and India. There is a seasonal variation, fewer cases being seen in the sunny part of the year when sunlight helps to eke out the deficient diet. Rarely hunger osteomalacia is found in large cities where solitary old people live in proud self-respecting poverty rather than apply for charity. We have seen such an old woman, who could only hobble along with difficulty and had lived for years alone in London.

Steatorrhœic osteomalacia. This is probably the commonest type seen in

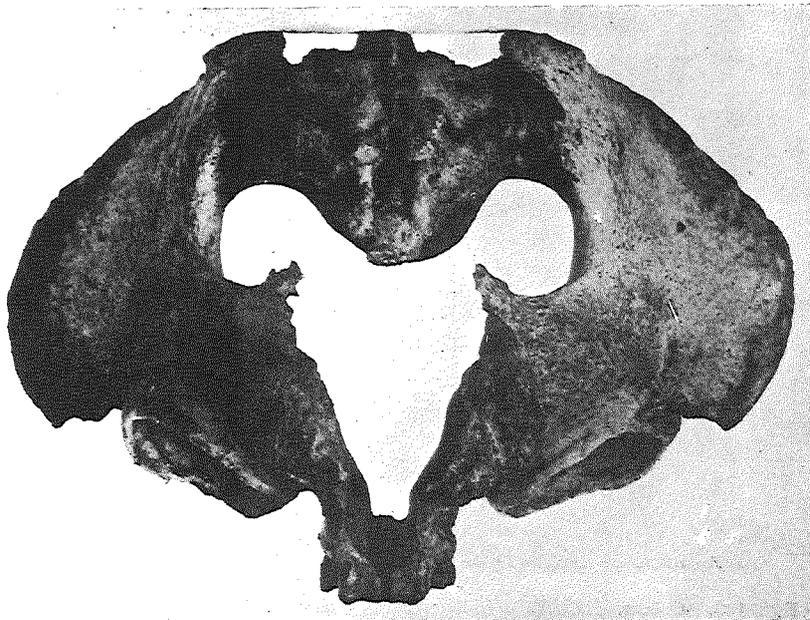


FIG. 199. Osteomalacia. The Shansi pelvis presented by Professor Maxwell to the Royal College of Surgeons. Note the diminished size of the pelvic outlet, which makes childbirth impossible (see also Figs. 198, 200 and 201).

England to-day [250, 251, 252], being the adult equivalent of coeliac rickets. It is caused by the impaired fat absorption hindering the body from acquiring sufficient vitamin D and calcium. Since the bowels are often virtually normal in steatorrhœa [250, 251, 252], the diagnosis may never be made until the secondary osteomalacia becomes so severe that even the rheumatologist is forced to seek some definite reason for his patient's increasing pain and general misery. Or the primary diagnosis may be made because of a megalocytic anæmia which fails to respond fully to liver therapy or to vitamin B₁₂ [252].

Senile Osteoporosis. This appears to be often a mild form of hunger osteomalacia, and not, as was formerly held, a senile atrophy of bone [211].

Symptoms, Diagnosis, and Treatment of Osteomalacia. The earliest symptom in osteomalacia is a vague tenderness or aching pain in the bones of the lumbo-sacral region and hips, which is worse when the patient walks. It is generally called "rheumatism." As the condition progresses the tender-

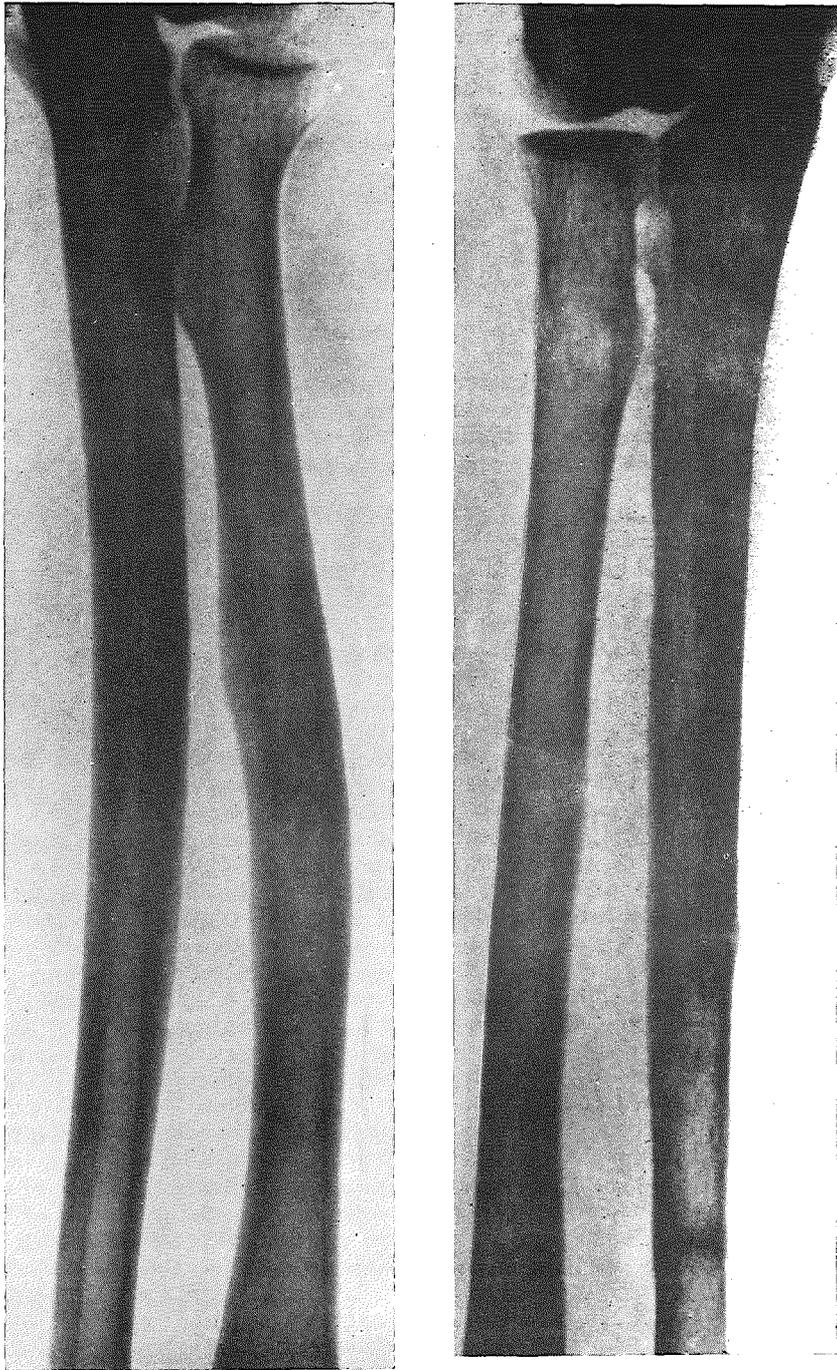


FIG. 200. English Osteomalacia FIG. 201.
X-ray of bones of forearm : (*right*) showing Looser's zone ; (*left*) control normal forearm.

ness and aching of the bones spreads so that percussing the chest may cause the woman to cry out. She may even refuse to be touched at all and wince when approached.

Bony deformities often occur with surprising rapidity. The woman appears to shrink. She loses height partly by the bowing of her legs, chiefly by the softening of her spine, which may become twisted and always bows forward, so that the ribs pile up over one another like the spokes of a fan, even coming to rest on her pelvis. The pelvis itself becomes grossly distorted until childbirth, from being difficult, becomes impossible. Trousseau [4] found a woman for whom a sound had to be used instead of digital examination, the pelvic outlet was so small. The legs twist and bend in any direction. Generally in the old it is the spine, in the young the pelvis and legs which are most affected (Figs. 198 to 201).

Fractures from trivial falls, from the normal pull of the muscles, or from no apparent cause, are common. They heal slowly or not at all, false joints forming instead.

Muscular weakness and flaccidity analogous to that of rickets occurs; on getting up the patient climbs up herself like a child with muscular dystrophy. The adductor muscles of the thighs tend to go into spasm due to the aching of the bones (Fig. 198). The walk becomes a wide based waddle compounded of weakness, pain, and deformity.

Cataract, according to Maxwell and Pi [247], is found in at least fifteen per cent. of marked cases. The condition develops slowly, and in its early stages can only be diagnosed with a slit lamp. It is important to consider whether cataract in young and middle-aged women may not be caused by undiagnosed osteomalacia which is still only causing vague pain in the back and limbs. Treatment of the underlying deficiency of vitamin D may improve the cataract.

The teeth are not affected. Taylor and Day [253] studied twenty-two Indian women with severe osteomalacia and found only thirty-four cavities in five hundred and sixty-five teeth. Eight of the women had no dental decay whatsoever. These observations are of great importance as confirmation that dental caries is not due to a deficiency of vitamin D (p. 570).

Menstruation and fertility in women are not, unfortunately, affected. The ovaries have been said to contain an abnormal number of follicles.

X-rays may show in the early stages little beyond a doubtful rarefaction of the bones of the pelvis or spine, but in the late stages the picture is unmistakable with the extreme osteoporosis of all the bones miming the skeleton of a ghost seen through frosted glass; with the kyphotic spine and its wedge-shaped, fish-tailed, collapsed vertebrae; with the trefoil pelvis (Fig. 199); with the multiple fractures and deformities. *Looser's Zones* [254] or translucent ribbon like areas of symmetrical decalcification may appear before there is any definite osteoporosis in any condition where there is general decalcification such as occurs in R.R.D. or resistant rickets [228]; in osteomalacia and in cadmium poisoning [69]. Indeed the first indication of a serious disease is often the discovery of these zones. They occur most commonly in the axillary borders of the scapulæ and also in the necks of the femora, the pubic and ischial rami, the ribs and the proximal third of the ulnæ (Fig. 200). Their cause is obscure; they may be the result of impoverished bone reacting to mechanical stress or to the proximity of arteries. *Milkman's syndrome* [255] or "Multiple spontaneous idiopathic symmetrical pseudofractures" is only another and later eponym, better forgotten, for the condition first described by Looser.

In all forms of osteomalacia the blood calcium is generally low, often to tetanic levels, but it may be normal if the parathyroids, which are often enlarged, succeed in mobilizing enough calcium from the skeleton; when this occurs the blood phosphorus may be low. The phosphatase is slightly [244] or markedly [251, 252] increased. The startlingly light bones, which

are often so soft they may be cut with a knife or bent like cardboard, largely consist of osteoid tissue; their enlarged cavity is filled with hæmorrhagic osteoid tissue or fat; their ash contains little calcium, though the phosphorus is, by comparison, high. Magnesium is increased. The urine on standing may form a phosphatic scum.

The diagnosis rests on the dietetic history, the tenderness, weakness and deformities, X-ray examinations, the levels of the serum calcium, phosphorus, and phosphatase, and the response to treatment. Sometimes the pain in the ribs is so acute that this, combined with the bronchitis which is so common, suggests pleurisy; a suggestion which is only discarded when the pain is found to be acute all over the ribs, which not only are exquisitely tender but in their weakness crepitate beneath the light pressure of the hand.

Death, unless treatment is given, is from pulmonary infections and the toxæmia from bed sores.

The outlook as regards life is excellent with treatment; some recovery of stature and amelioration of the deformities may be hoped for.

Treatment is with large doses of vitamin D. Probably about 3,000 I.U. daily will be enough, but much larger doses may be required. To supply the necessary minerals for the recalcification of the bone calcium phosphate gr. 30 thrice daily should be given, as well as plenty of milk, cheese and fish. Radiography and calcium estimations should, if possible, be used to make certain adequate vitamin D and calcium and phosphorus are being given.

Extra calcium may have to be given if tetany persists (see p. 562) or if treatment causes the blood calcium to sink further because it is diverted to the bones, thus bringing on tetany.

In the past ovariectomy has been strongly advocated [245]. It is quite unnecessary and unjustifiable.

DENTAL DECAY

There is such a widespread belief that lack of vitamin D is the chief cause of dental decay that it seems important to consider the subject in some detail. Dental decay, or dental caries, is due to the destruction of the enamel and dentine of the teeth by saprophytic organisms. Their growth and the progress of decay may be assisted by any one of several different factors. The more important are (a) the use of refined foods, especially white flour and white sugar, (b) the use of heated milk instead of raw milk, (c) the failure of the individual to produce "immune saliva," and (d) possibly, but improbably, a diet deficient in vitamin D.

Refined Foods. The bad effects of refined foods have been observed for many years. The most classical observations were made on the island of Tristan de Cunha. When the island was visited in 1932 caries was practically non-existent, especially among people of under fifty. No food was imported, the islanders living entirely on the unrefined produce of the island. In the next five years, however, a large amount of refined flour and sugar was left by visiting ships, so that the inhabitants suddenly had added to their diet foods which they had never had before save in minute quantities at long intervals. The result from the point of view of dental decay was tragic. Barnes [256] observed that in only five years caries from being almost unknown in children was common, while in adults between forty and fifty years old it had increased by fifty per cent.

If further proof is required of the effect of refined foods it is furnished by the teeth of the Bantu [257]. These people had exceptionally fine teeth, but when they started eating refined European foods caries became as common as in England. In one generation perfect teeth, which have often been considered to be an inherited racial characteristic, were utterly lost. That it was the fault of their new foods appears certain because there was no other change in their manner of life which appeared to have any possible

significance. The increase in dental decay in the island of Lewis also tells the same story [258].

It is generally stated that white flour and sugar cause decay by clinging round the teeth while coarse fibre-containing flours and sugarless foods being less sticky are easily removed by the natural self-cleansing action of the mouth—the “detergent diet” of Sim Wallace. The sticky white flour and sugar are decomposed by bacteria, thus producing acids which erode the enamel. This appears to be true, but is not the whole truth about the bad effects of refined foods.

The work of Osborn and Noriskin [259] showed that unrefined sugars and cereals have a protective action against the destruction of enamel. When they incubated human teeth with saliva and white flour, or refined sugar, the enamel was dissolved. If, however, wholemeal flour or unrefined sugar were used the enamel was hardly affected, especially with the unrefined sugar.

The protective substance present in unrefined food has not yet been isolated, but its action is not dependent on any change of acidity in the saliva. It explains the puzzling observations that dental decay may be absent in the mouths of native children whose teeth are always coated in sticky sugar from eating raw sugar cane all day [257].

Heated Milk. Raw milk, that is unpasteurized and unboiled milk, has a very marked protective action against dental decay. This was found to be so by Sprawson [260], who noticed a sudden startling fall in caries in a children's institution. The only change which had been made was giving each child daily a pint of raw milk. Further enquiries among dentists confirmed that children brought up on raw milk—either cow's or goat's—were free of caries. Sprawson [261] also points out that in the island of Pitcairn caries was very common, while in Tristan de Cunha it was very rare. The only difference between the diets was that in the latter island raw milk was drunk though probably less vitamin D was taken.

In the first edition of this book the contentious subject of pasteurization was discussed in its wider aspects. As, however, one of the authors (F. B.) believes pasteurization is unwise while the other (F. P.) believes in its value, it seems better to omit this wider discussion, referring the reader to the sections in each chapter for the narrower issue of how pasteurization alters the individual vitamins of milk.

Immune Saliva. Fish [262] has pointed out that some human saliva prevents decay and some does not. Dog's saliva always does so, while monkey's saliva varies as in man. When Fish placed carious human teeth in a dog's mouth in a few days they were sterile; the saprophytic organisms which cause decay had been destroyed. The same occurred if a carious tooth was incubated with human saliva from a man with no active caries. But saliva from a mouth with progressive caries did not sterilize the tooth nor did that from a monkey with caries. There is, therefore, a quality in immune saliva which protects teeth from the organisms of decay. This immune saliva is most frequent after adolescence and is commoner in men than women [263]. If it is the product of a healthy general metabolism it would explain why a good diet gives protection against caries in children, without having to postulate the unsatisfactory theory that the protection apparently given in some cases by vitamin D is directly due to improved dental calcification [264].

Traces of fluorine in the drinking water cause mottling of the enamel of the teeth. Such teeth are very resistant to decay, even when the mottling is so slight as not to be aesthetically objectionable [265–268]. It is possible that the fluorine, excreted into the saliva, acts by inhibiting the growth of saprophytic organisms in the mouth: in other words a form of immune saliva is produced.

Vitamin D. In rachitic children the teeth tend to erupt late and decay early. The late eruption appears to be a direct effect of too little vitamin D, since Speidel and Stearns [95] report that there is an optimum intake of

vitamin D above or below which eruption is delayed. The early decay is probably due to the refined carbohydrate diet such children generally eat. The teeth of rachitic children, may, however, be perfect. Both Taylor and Day [218, 219] and Wilson [269] have reported many Indian children with severe active rickets who had no caries, even when born of osteomalacic mothers. Their diet was very coarse; this coarseness appeared to be the reason for the absence of decay.

In osteomalacia, as has been pointed out on p. 568, the teeth do not decay unduly though here the lack of vitamin D and calcium is profound.

Vitamin D is therefore not *necessary* for good teeth in either the foetus or the child or the adult. But the question arises as to whether vitamin D is *valuable* in helping to protect the teeth. To give the answer before the evidence: the addition of extra vitamin D to the diet appears in some cases to decrease dental decay, but we believe this to be due to the improvement in general health brought about by removing a mild deficiency of vitamin D.

The evidence that vitamin D is valuable for the teeth is based firstly on animal experiments and secondly on human investigations. The animal experiments were chiefly done by Lady Mellanby on dogs [270]. She showed that the teeth of rachitic puppies had poor thin enamel and the dentine was what she called hypoplastic. These teeth did not decay, since dogs have a naturally immune saliva (p. 570). One also wonders whether part of their poor structure was due to the puppies never chewing or biting anything hard. "Function creates structure," and in these puppies the teeth had no function—the food was pap, they were too ill from their rickets to gnaw their cages as did the healthy control puppies, and when they were allowed outdoors they were muzzled.

However, having shown that rachitic puppies have poor teeth which do not decay, the next step was to investigate the shed milk teeth of children which had decayed. Those teeth which had most caries were found to have the poorest structure, reminiscent of the rachitic puppies' teeth. This was held to show that the teeth decayed because they were bad, and bad because they were rachitic. This argument only contains two possible fallacies: firstly, bad teeth may be associated with decay because chronic bad health—from whatever cause—might hinder both the proper formation of the teeth and the production of immune saliva; secondly, there was no reason to believe the children who had bad teeth were rachitic [271] except that they had bad teeth—in fact the argument having gone in a circle gets nowhere.

The clinical value of vitamin D was tested by giving it as a supplement to groups of children and comparing their teeth to those of children who had had no such supplement. Many investigations [271] both on a large and a small scale in England and America showed that by increasing the amount of vitamin D in the diet not only was the number of teeth which subsequently developed caries reduced, but that caries which had started tended to be arrested. It must, however, be noted that full protection was not given. Thus in one investigation over a period of about eight months the children receiving "abundant fat soluble vitamins" had on an average 1.4 teeth showing fresh or increased caries, against 5.1 teeth in children who had little vitamin and some oatmeal. So that though the teeth were far better preserved with extra vitamin D, yet the degree of caries was still deplorably high. McBeath and Zucker [177] found the natural vitamin more valuable than the synthetic.

The arguments against vitamin D being directly responsible for this improvement are:—

(a) Some investigators have not found that vitamin D has any effect, even on recently erupted teeth, when given for over a year to large groups of children [272].

(b) Fish [273] found experimentally that there was no evidence that vitamin D had any effect on formed dentine or enamel, so that it is difficult

to understand how the enamel, and so the resistance to decay, could be improved by vitamin D.

(c) The faults in the rachitic teeth of puppies are not like the faults where caries starts in human teeth [263].

(d) The teeth which should show most hypoplasia, if this is due to lack of vitamin D, are those which are formed during the first year and a half of life when rickets is most common. The incisors should thus be affected most; they are affected least. Then the most affected in order are the second molars, first molars, canines, though they erupt in the order of first molars, canines, second molars [274, 275]. Thus there is no relation between hypoplasia and the teeth formed at the period when vitamin D tends to be most deficient.

(e) Children with extensive progressive caries do not commonly show any signs of rickets [271], and children with severe rickets, even when born of osteomalacic mothers, may have no caries (p. 570).

(f) Dental caries in rats is not prevented by vitamin D [278].

We are, then, left with the fact that vitamin D decreases caries to some extent in some children, but that caries is not dependent on a deficiency acting directly on the teeth themselves. It appears most likely that vitamin D improves caries only indirectly by improving the general health and nutrition of the child—both factors which have an effect on caries [218, 219, 264, 276].

Immunity to dental decay depends on eating coarse unrefined food—especially unrefined sugar and stone ground flour—raw milk and a good diet. There is no simple specific against decay such as vitamin D, and cleaning the teeth cannot compensate for a bad diet.

The absolute necessity for vitamin A at ages when the enamel is being formed, and the importance of vitamins A and C for the health of the gums, and so indirectly for the preservation or rather fixation of the teeth, must be remembered.

Dental Decay and Pregnancy. The old saying that “every child costs a tooth” appears to be only explicable on the spread of paradontal disease during pregnancy. Actual dental decay during pregnancy does not appear to increase even under the added strain of osteomalacia (p. 570). Fish [277] states: “There is no active evidence as yet of any definite loss of calcium from the dentine under any circumstances,” including pregnancy, low calcium diets, excessive vitamin D, and parathormone injections. Protection during pregnancy depends on the dietetic factors already mentioned, on cleaning the teeth, and not simply on taking more vitamin D.

TUBERCULOSIS

Lupus Vulgaris. In May, 1848 Devergie [279] described the treatment of lupus vulgaris with large doses of cod-liver oil—the “l'huile brune” variety, because it was more fishy. In September of the same year Emery [280] also advocated cod-liver oil, increasing the dose to about two pints daily. Though he gives detailed instructions as to how to overcome vomiting, etc., and though he claims to have cured twenty-eight of forty-three cases with improvement of another twelve, one is left with the feeling that he was probably lying and plagiarizing Devergie whose treatment, with a maximum of only one-third of a pint of oil daily, is at least possible while two pints seems impossible as a dose. Devergie states that: “Aujourd'hui je n'hésite pas à déclarer que c'est de tous le plus efficace: je vois plus loin, et j'avance qu'administré seul il guérit. Cette assertion est tellement vraie, qu'à partir du moment où ce moyen à été mis en usage par quelques-unes de nos malades, les autres m'ont successivement prié de le leur faire prendre.”

The oil had to be taken for three to six months or longer. He started with “une cuillierée à bouche matin et soir.” Every two or three days he increased

the dose by another spoonful. After the dose had been increased to two to three spoonfuls at one time the patients overcame their first nausea, and then the dose could rapidly be increased to the twelve to fourteen spoonfuls daily which he found were necessary. The patients felt very well; their appetite went down and their weight up.

He had been using this treatment for eight years. He gives no figures of the numbers of patients who recover, but from his previous accounts of other treatments and his statement that general and local treatment must be combined, it seems probable that he was not getting a very large proportion of cures.

Until the Second World War this French treatment, like all French work of the last century on vitamin D, was completely forgotten. Then Dowling and Prosser Thomas [281] in England, Fanielle [282] in Belgium and Charpy [284] in France all independently reported that calciferol in large doses was a highly successful treatment for lupus vulgaris. In Belgium such treatment is called after Fanielle while on the rest of the Continent it is called after Charpy. The value of calciferol has now been confirmed in nearly every country in the world.

The dose of vitamin D used in England for adults is 150,000 I.U. daily given by mouth as tablets; 100,000 I.U. is less certain in its effects and 50,000 I.U. is useless [282]. An ineffective dose remains ineffective however long it is continued [282]. For children the dose has generally been 100,000 I.U. daily [287]. There is no certainty as to whether treatment should be given

continuously, possibly for a year or more, until a clinical cure is achieved or whether short courses each of two or three months are equally good and less dangerous. In England treatment is stopped when there is a clinical cure. If there is a relapse it is better to wait until the condition has developed, rather than to restart treatment immediately [287]. Local treatment in the form of Finsen therapy, caustics, etc., must also be used [284, 287]. On the Continent the general custom is to give the Fanielle or Charpy form of treatment, that is 600,000 I.U. twice weekly by mouth for a month, followed by the same dose weekly for two years, irrespective of clinical cure [285, 286]. Calcium or milk is also given and a diet rich in everything except fat and salt. However, workers in Scandinavia [300], Holland [290], and Denmark [289] have reported excellent results on doses similar to those used in England and without any extra calcium or special diet. Though emphasis has been



FIG. 202. One of the first cases of lupus vulgaris treated in England with calciferol: extensive lesions of the face and neck almost completely healed after 150,000 I.U. calciferol daily, later reduced to 100,000 I.U., for one year.

laid by Charpy on giving calciferol dissolved in propylene glycol [285], results are just as good when the solvent is oil [289], or no solvent at all is used but dry tablets instead, as in England. Lightbound [294] claims that giving calciferol by intramuscular injection reduces its toxicity and gives a more rapid cure, while Moguer [292] found that infiltrating the lesions with weekly injections of 100,000 I.U. brought about a cure in seven to nine weeks. In resistant cases injections twice weekly of 500 mg. of unirradiated ergosterol in oil [293] or 50,000 I.U. of vitamin A by mouth daily [291] have been said to be of value, though ergosterol in enormous oral doses is valueless [242]. Feeny [243] has given an excellent account of the use of calciferol combined with chemotherapy.

Improvement may occur in a couple of weeks and a clinical cure in two months, though often it is delayed for over a year. A Herxheimer-like reaction is frequently present in the first few weeks of treatment, with an



FIG. 203. Lupus vulgaris before and after treatment for eight months with 100,000 I.U. daily of calciferol.

increase in the redness and swelling of the lesions and even ulceration [287]. As in the first English case ever treated [281, 282], great improvement may be caused by a violent toxic reaction [287] though many patients are cured without any toxic symptoms. Such symptoms and the biochemical effects of calciferol are discussed on p. 578.

Microscopical examination of the lesions shows that under the influence of calciferol a quantity of young connective tissue develops about the giant-cell systems which are invaded and disintegrated, so that in the end there is only an area of fibrosis and lymphocytic infiltration [287]; before this the histological picture resembles sarcoid [286, 287]. However Ruiter and Groen [290] found that of fifteen clinically healed cases six had microscopic lesions from one of which tubercle bacilli were grown, and tubercle bacilli were also grown from another three cases which had no microscopic lesions. Leuggenhager and others [295] even report that three-quarters of their clinically cured cases still showed microscopic infection. Healed lupus skin contains more calcium than normal skin from the same patient [299].

Clinical cures vary from virtually 100 per cent. at the end of treatment

[285, 287, 289, 300] to only 30 per cent. [290]. Relapses are less common than would be expected from the persistence of infection in "cured" cases, ranging from none [285] to about one-quarter [287, 300]. Lesions of mucous membranes respond excellently [284, 300].

The reason why calciferol acts is obscure; it is not due to elevation of the total serum calcium since this may remain virtually normal in cases which are cured [282, 287] though Anning and others [288] have from a small number of cases suggested the possibility that the rise in the diffusible calcium is important; Charpy [285] believes that mobilization of phosphorus is the key action, which is congruous with the action of vitamin D (p. 535); tubercle bacilli themselves [290] are not sensitive to calciferol since they have been grown in Dubos' liquid medium containing 10 to 1,000 I.U. per 100 ml. from lesions which both did and did not respond to treatment. Further, human and bovine tubercle bacilli are equally insensitive to calciferol [290], nor does this have any greater inhibiting effect in solid media [287].

Scrofuloderma [296, 297, 308] may be virtually cured or may flare up [296] while the results are only moderately good in *erythema induratum* [284, 297], the condition sometimes being made worse [296, 308]. *Papulonecrotic tuberculide* [297] is said not to be affected.

The contraindications to the use of calciferol in large doses are discussed on p. 579.

Other Forms of Tuberculosis. Calciferol used in the same doses as for lupus vulgaris is excellent for tuberculous adenitis both in children and adults, causing a rapid healing of sinuses and a shrinking and calcification of the glands which leaves them mobile and easy to excise [283, 284, 287]. Tuberculous peritonitis [287, 301], cystitis [287, 301], and epididymitis [284], have not been extensively treated, though cases have responded in a miraculous manner [287, 301], while infections of bones and joints [287] may be benefited. Pulmonary tuberculosis may be made worse or better (p. 579).

Boeck's Sarcoidosis was not benefited in eight cases compared to eight control cases by Nelson [302] and Pascher and his colleagues [308] only mildly improved one of seven cases but single cases have responded [303, 304, 305], especially when there was a toxic reaction to calciferol, and Curtis and others [306] have added another six cases of improvement. It is, however, the skin which improves, the lesions in the lungs and bones remaining unaffected or becoming worse [305].

Leprosy though so akin to tuberculosis does not react in the same dramatic manner to calciferol, Floch and Destombes [307], after the treatment of twenty-one cases, only being able to say that further work is worth while, while De Castro and Haedo [297] obtained no results in six cases.

FURTHER CLINICAL USES OF VITAMIN D

Endocrine Disorders. Cretinism. Cretins when they start taking thyroid often grow amazingly fast. Rickets as a result may occur, so the possibility must be kept in mind and ample cod-liver oil given as a prophylactic measure.

Thyrotoxicosis. The action of vitamin D in very large doses on the thyroid has been discussed on p. 533. The possible value of cod-liver oil, as far as its vitamins are concerned, is probably due to its vitamin A.

Parathyroprivic Tetany. The use of vitamin D in controlling the tetany due to damage of the parathyroid glands is most valuable, since it raises the low blood calcium and decreases the high blood phosphorus. It also stabilizes the calcium and phosphorus metabolism so that there is less tendency to sudden tetany [111], but as the effect of vitamin D in relieving tetany takes several days, it is useless in post-operative emergencies. In prolonged

treatment it has the great advantage of not losing its effect, while parathormone, on the other hand, does so after repeated administration.

There is the danger that large doses of vitamin D may have toxic effects, but by using the natural concentrated fish liver preparations, by keeping the dose at the lowest level and stopping it for short periods where possible, harmful effects are unlikely to occur (p. 578).

The dose of vitamin D must depend entirely on the individual case. With a diet high in calcium and low in phosphorus 20,000 units should be given daily by mouth and the amount adjusted from the clinical results. Sevringhaus and St. John [112], however, state that the diet need not be carefully controlled since they have successfully treated six women who ate as much meat, milk and eggs as they liked. Four of the women were observed for over two years and one of them had an uneventful pregnancy. Vitamin D, combined with calcium salts, was given orally in doses of 150,000 to 400,000 I.U. daily, the correct amount being adjusted partly by blood calcium estimations and partly by subjective symptoms, of which tingling in the arms and hands was the earliest and most reliable indication for increasing the dose. Implants of vitamin D tablets have been advocated [309], to our minds most unwisely since the amount absorbed cannot be adjusted to meet the needs of the individual patient.

Dihydrotachysterol, one of the products of the prolonged irradiation and hydrogenation of ergosterol called commercially A.T.10 (anti-tetanic preparation number 10), has been used instead of vitamin D for the treatment of tetany by Snapper [310], Himsworth and Maziels [311], and many others. It has no appreciable antirachitic effect [312], but raises the blood calcium more rapidly than vitamin D. It is, however, more toxic, and in some cases has to be given in increasing amounts. It also has a more erratic effect on the level of the calcium in the blood. It appears to have no advantages and severe drawbacks compared to vitamin D, apart from its more rapid action in raising the blood calcium.

Sexual Disorders. An occasional increase in libido of both sexes and also in sexual capacity, and an improvement in the rhythm of the menses, has been reported in a small proportion of cases who were taking very large doses of vitamin D [111]. The therapeutic use of vitamin D for sexual disorders, however, is ruled out by the dangerously high amounts which must be given, and by the small proportion of patients who will benefit.

The Bleeding Tendency in Jaundice and Hepatic Insufficiency. It is very surprising that vitamin D is seldom used for preventing the hæmorrhage which is so common and so disastrous in patients with jaundice, or hepatic insufficiency, who have to undergo an operation. In a very large series of carefully controlled cases Gray and Ivy [117], and McNealy and others [118] found that when the "Ivy" bleeding time was prolonged the operative outlook was bad because of hæmorrhage during or after the operation. But vitamin D given thrice daily by mouth, in doses of about 7,000 I.U. for four to fourteen days before operation, almost invariably reduced the bleeding time to normal, and prevented any hæmorrhagic complications. When there was no bile in the stools bile was given by mouth to aid the absorption of the vitamin. Where the hepatic insufficiency was very profound vitamin D did not improve the bleeding time, nor did it decrease a hæmorrhagic tendency in any condition not associated with liver damage. Why vitamin D only has an effect in one kind of hæmorrhage is obscure: it does not act through changing the blood calcium [117], or through correcting the prothrombin deficiency [120] which is often found in jaundice owing to poor absorption of vitamin K.

Lead, Cadmium and Radium Poisoning. Lead behaves like calcium from the point of view of storage in the body [65, 104, 222, 223, 313]. During the acute stages of lead poisoning when it is important to decrease the ionization of the lead in the blood, and to remove it rapidly from the circulation, 300 I.U.

of vitamin D a day should be given to aid the deposition of the lead in the bones. Calcium lactate, 180 grains daily, should be given at the same time, both to decrease the ionization of the lead and also to ensure that lead and calcium are being deposited and not mobilized in the bones.

After the acute symptoms of lead poisoning are over it is necessary to "de-lead" the bones. Vitamin D in large doses is not so certain in its results as parathormone, and both need careful supervision. Ammonium chloride must be used when patients cannot be carefully guarded against excessive mobilization of lead bringing back the acute symptoms of poisoning. In any case the diet should give a low calcium high phosphorus intake—the latter to depress the ionization of the lead in the blood [65, 104]. Cadmium poisoning [69], on the other hand, only needs to be treated like osteomalacia (p. 569), being really the same disease.

Radium is so toxic that it must be eliminated from the body as quickly as possible, vitamin D being of less value than the other measures mentioned for "de-leading" [104].

The Healing of Fractures. About 600 I.U. daily of vitamin D should be given to patients with fractures. This ensures that the calcification of the callus shall not be prevented by lack of vitamin D which is especially important in the old [108, 211] where mild osteoporosis is common. Massive doses of vitamin D delay calcification [314]. A.T. 10 (p. 576) has also been extensively, and to our mind unwisely, used to hasten calcification. Irradiation has also been suggested [315]. The use of ascorbic acid in the healing of fractures is discussed on p. 407.

Arthritis. Reed and his collaborators [111] sum up the effect of using vitamin D in arthritis thus: "A group of forty-five cases of atrophic arthritis, seven cases of hypertrophic arthritis and three mixed cases were treated with 150,000–200,000 units of vitamin D daily with definite measurable improvement in 74.5 per cent. of cases as manifested by reduced swelling and pain, increased mobility (active and passive), improved muscular tonus and weight gain." The authors point out that there is a definite risk from such large doses, though apparently less than one per cent. of cases showed toxic symptoms. Small doses should be given at first to avoid risk in unduly susceptible patients (p. 578). Treatment has to be continued for many months or even years. No claim is made for any physiological reason for this form of treatment. Good results have also been reported by other workers [316, 317, 318]. But Freyberg [319] from a most careful study, in which he used the reputedly pure "activated" vitamin D, reports that six out of thirty-six patients had to give up treatment because of toxic symptoms and that "this form of therapy should not be relied on as the only form of treatment, for seldom is the course of the disease favourably altered." The general impression is left that other forms of treatment over such a long period would have as good results without the very real danger of toxic damage.

Skin Diseases. Tuberculous infections of the skin, sarcoidosis and leprosy have been discussed on pp. 572 to 575. Many other dermatological diseases have been treated with large or toxic doses of vitamin D; probably it is useless in all of them, though there are unsatisfactory and unconfirmed reports that it has occasionally benefited scleroderma [320, 321], acne [321, 322], leukonychia totalis and pemphigus [321], eczema and epidermolysis bullosa [323], granuloma annulare which may on the other hand be made worse [308] and lichen planus [308]. Chilblains are neither prevented [325] nor cured [284], and in our experience cod-liver oil—discussed fully on p. 533—and vitamin D ointments have been valueless in diabetic ulcers, bed sores and varicose ulcers.

Psoriasis has been very fully investigated but again it would appear that treatment with vitamin D is quite ineffective. In the most thorough investigation yet carried out, which was reported by Clarke [324] and carried

out by twenty-two doctors on one hundred and forty-one patients, only twelve per cent. improved ; spontaneous improvement should have given a better figure. Kindler [326] reviewed the literature in 1949 and from his own treatment of thirty-one cases, of whom twelve were virtually cured, believes vitamin D is on the whole worth trying, while Wright [323] after treating forty-five cases believes it is useless and Madden [327] from his experience with twenty-four patients states that improvement is caused only when the patients are made toxic by vitamin D ; when it is stopped the psoriasis recurs.

VITAMIN D POISONING

All preparations of synthetic vitamin D₂, however pure they are reputed to be, are toxic for man in large doses [288] and so are synthetic vitamin D₃ [342] and concentrated fish liver oils, two children dying from drinking large quantities of cod-liver oil while also being exposed for long periods to the sun.

The dose of vitamin D which, when repeated daily, is toxic varies greatly with the individual. From the great numbers of patients with lupus vulgaris (p. 572) who have received 100,000 to 150,000 I.U. daily or 600,000 I.U. weekly it is definite that such doses are always on the verge of toxicity and, in about twenty per cent. of cases, cause definite toxic symptoms and in a larger percentage of cases abnormally high levels of total serum calcium or of diffusible serum calcium [288]. Adult cases have been reported in whom only 25,000 I.U. daily of a very pure "activated" preparation of vitamin D have caused toxic symptoms [319]. Reports seldom mention how soon toxic symptoms may appear but on daily doses of 700,000 I.U. they have occurred in ten days [129], and the same time appears to have elapsed in some of Anning and his collaborators' patients [288]. Toxicity, however, may not appear for about a year and after a very large total intake such as 35 million I.U. Toxicity is said to be precipitated by emotional strain, indigestion and constipation [111], though the last two conditions may really be the result and not the cause of the toxicity.

Children may be given single very massive doses with no obvious ill effects (p. 545) but repeated large doses may be disastrous. Debré [328] in 1948 saw or collected from the literature thirty-one children who developed acute poisoning, of whom twelve died. Delayed toxic symptoms may be and doubtless generally are completely overlooked since doses of 1,800 I.U. daily in infants only cause slight retarding of growth and delayed dentition (p. 532). Larger doses may, however, have most serious delayed effects. Thus Briskas and Maret [329] saw one infant of eight and a half months and two children of ten years whose growth appeared to have been severely perverted in different ways, depending on the age when the excessive vitamin D was given. The infant had received 4 million I.U. in the preceding two months : sixteen teeth were present, the centres of ossification were those of a child of eighteen months, growth was excessive with wasting and hypotonia. One of the children had received 20 million I.U. over several years : he was pale, thin and very hypotonic with the appearance of a boy of four or five and carpal centres of ossification of a boy of five and a half ; his liver was not enlarged and his blood calcium and phosphorus were normal. The other boy of ten had had 10 million I.U. over three months when he was seven years old : he was mentally retarded, very thin and profoundly hypotonic ; his height and centres of ossification were normal. Though the evidence is obviously dubious that excessive vitamin D was responsible for the condition of these children, yet they have been described so fully to emphasize that the serious effects of poisoning may be covert and delayed.

The method of administration alters the toxicity, the more rapidly vitamin D is absorbed the more toxic it becomes. Thus when given by mouth in oil it is said to be less toxic than when given in alcoholic solution [330]

and, at least in dogs, rapidly absorbed injections are highly toxic while oily injections are not [128], which appears to be broadly true in man as regards the relative degree of toxicity of different types of injections [128, 294].

Indications for Using Large Doses of Vitamin D with Caution. Fat patients, as their fat is largely inert from the point of view of metabolism, should not be given very large doses to begin with. Patients confined in bed may also metabolize vitamin D slowly, since two with fractures rapidly developed serious toxic symptoms and the healing of the fractures was delayed [331]; while the risk in young patients of renal calculi is severe (p. 563).

Personal sensitiveness is important and can be avoided only by beginning with small doses. One patient has been reported who showed toxic symptoms on 25,000 I.U. daily [319] and one infant, who was frequently outdoors in the sun, died from taking a concentrated cod-liver oil which only gave him about 1,500 I.U. daily [179].

Nephritis and cardiovascular degeneration are contraindications for the use of high doses of vitamin D, since both may be exacerbated. Two elderly men with such conditions have been reported by Steck and others [129] as dying from poisoning with vitamin D.

Children require watching. One child we saw in hospital died from both being exposed to the sun, and also drinking not only his own cod-liver oil but also that of several other children in his ward.

Pulmonary tuberculosis is a reason for giving large doses with the greatest caution since Ridderbos [334] made all of nine severely ill patients worse and eighteen of fifty-six patients who had less extensive pulmonary lesions. Other workers [286, 296] have also reported bad effects, though when used with chemotherapy it may be of value, possibly by causing a local vascular reaction which enables chemotherapy to reach the infection [344].

Symptoms of Poisoning. The symptoms of chronic poisoning in children have been discussed above. In acute poisoning, that is in poisoning which produces overt symptoms during treatment, the adult patient may suffer in a great variety of ways almost all of which have been collected with references to the relevant papers, by Anning and others [288]. General well-being and a good appetite is often the first symptom of poisoning. In this lies a danger for doctors who buy large bottles of concentrated irradiated products for dispensing and drink them, often in huge doses, for the tonic effect that they give.

Loss of appetite follows quickly on the sense of well-being, and loss of weight greater than would be expected from the loss of appetite. After this intestinal symptoms such as nausea, vomiting, constipation or diarrhoea increase rapidly. Abdominal pain may be so severe as to inveigle surgeons into performing laparotomies.

Thirst may be intense and the urine is increased and voided frequently by night and day. Shortly before death dehydration occurs with scanty urine.

Weariness and weakness, and more rarely profound mental depression come on, and may be early symptoms. The memory occasionally is confused. Restless excitability may also occur and photophobia and dislike of noise.

Headaches are usual, and one special form has been noticed which may be the first symptom to arouse suspicion. This is a tightness across the back of the head which goes on to acute sensitiveness of the scalp, so that the patient cannot rest his head on the pillow. The same tenderness at the back of the head has been found in dogs. Pain along the jaws and tender teeth have been noticed, and pains in joints and muscles. Profuse sweating may occur. The hands and feet occasionally feel numb and tingle, and polyneuritis [332] has been reported. Epileptiform fits are a rare complication, which cease when treatment is stopped [333].

Aphrodisiac effects to the extent of social inconvenience have been occasionally noted without other toxic symptoms.

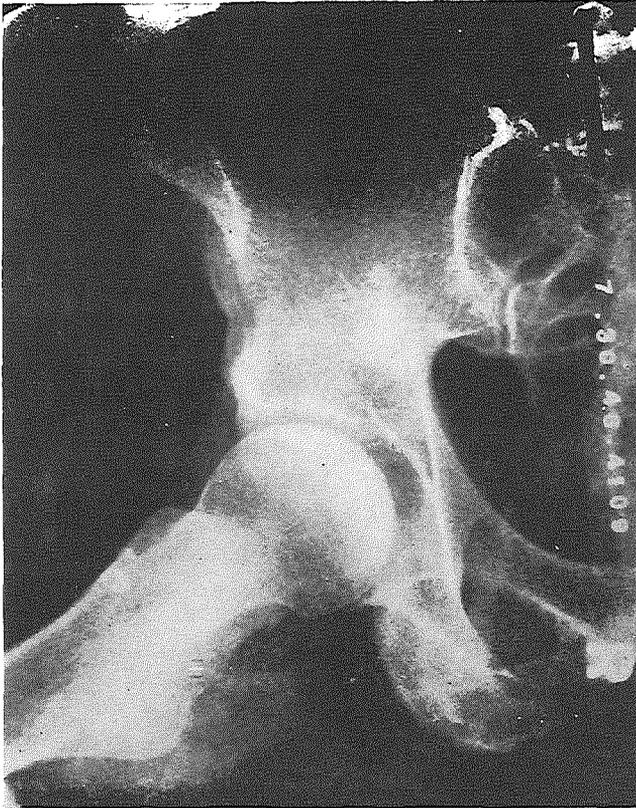


FIG. 204. Multilobular calcifications of hip in a man of fifty-six who had taken 150,000 I.U. of vitamin D daily for two and a half years.

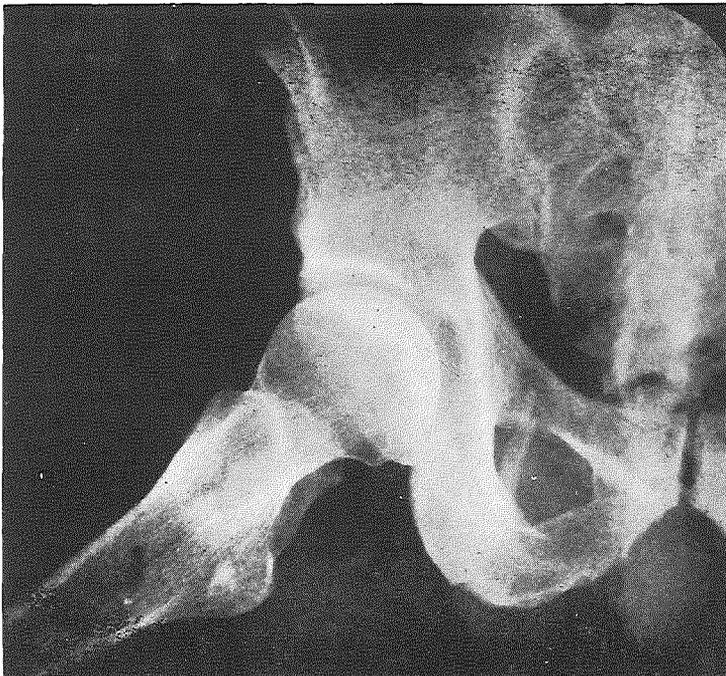


FIG. 205. The same as Fig. 204, showing resolution of calcification fifteen months after vitamin D was stopped.

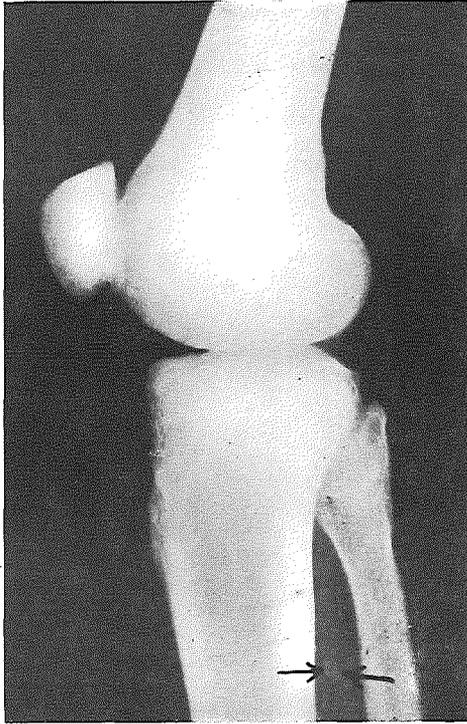


FIG. 206. The same patient as in Fig. 204, showing calcification of posterior tibial artery.

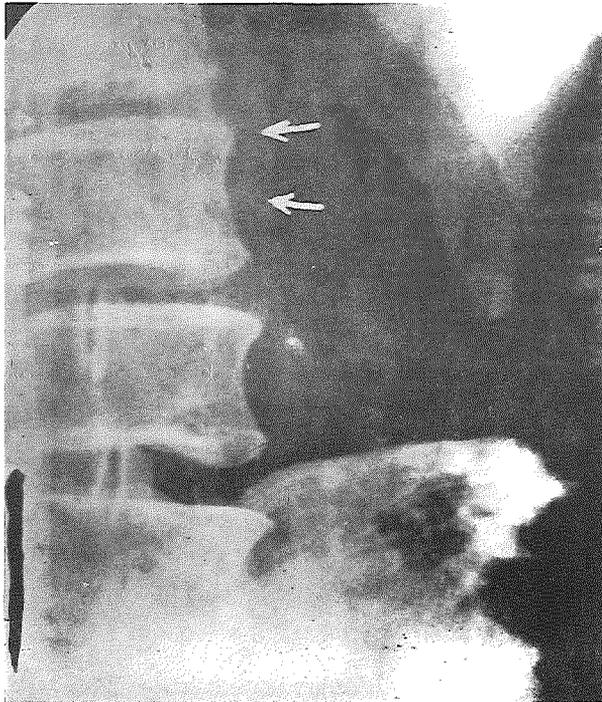


FIG. 207. The same patient as in Fig. 204, showing calcification of the abdominal aorta.

In fatal cases the general picture is that of uræmia which, indeed, it generally is.

In children [179, 328] sudden loss of appetite is the constant first symptom. This may come and go in so marked a manner as to cause a diagnosis of mental deficiency. Sudden insistent and persistent vomiting may occur and thirst and polyuria may be extreme; a child of two passing as much as six pints a day. Intense constipation is common, interrupted by brief attacks of diarrhœa. Pain may be absent or may be occipital or frontal or in the

abdomen, joints or muscles.

The child is very thin, wan and sallow with a dry pale skin, though he is seldom anæmic. His blood pressure is raised. From being irritable and depressed he slips into stupor without meningeal signs or stiffness, and with a normal cerebrospinal fluid. He lies dying curled up on his side, his breathing heavy and deep, his pulse perhaps slow, his temperature normal or high. Sometimes generalized convulsions interrupt the stupor [179, 328, 340].

Biochemical and Pathological Changes in Poisoning. The urine is loaded with calcium and phosphorus and may contain calcium casts if it is not too acid. It is said that Sulkowitch's reagent may show this excessive calcium excretion even when the total serum calcium is normal, and so gives a valuable warning of impending toxicity (p. 563), as does the low specific gravity in the morning. Albumin occasionally and sugar and blood rarely are

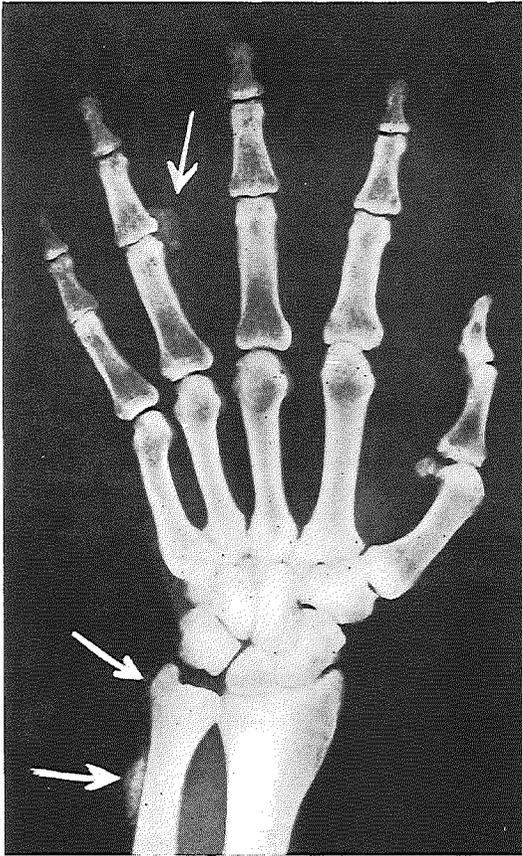


FIG. 208. The same patient as in Fig. 204, showing calcification in the soft tissues of the wrist, interphalangeal joint and tip of the ring finger.

found in the urine. Renal function is generally impaired and the blood urea rises [288].

The total serum calcium need not be raised though the patient is frankly toxic, or conversely it may be very high and the patient in the best of health [282, 287, 328]. Anning and his co-workers [288] found that the diffusible calcium was definitely raised in all their thirty toxic cases, 7.5 mg. per 100 ml. being taken as a warning that the patient was verging on the toxic, especially if there was no parallel rise in the total serum calcium. The inorganic serum phosphorus is not constantly affected and the alkaline phosphatase remains normal [288, 328]. The plasma protein content is increased [288] which may be the cause of the increased erythrocyte sedimentation rate. The latter rises at the beginning of the treatment of lupus vulgaris and then falls unless

toxicity supervenes when it rises again [287]. The blood pressure and electrocardiogram remain normal [288].

X-rays may show calcification of the larger arteries [288, 335] and in children of the lungs and soft tissues [330]. There may be generalized osteoporosis and when this is accompanied by large smoothly lobulated masses of metastatic calcification, confined to periarticular structures, the diagnosis lies between vitamin D poisoning, chronic nephritis and hyperparathyroidism [337]. In children there is increased density of the zone of provisional calcification with an area of rarefaction proximal to this and also periosteal thickening with a dense outer layer and a rarefied inner zone [336]. Metastatic calcification visible to the naked eye [338] or by a slit lamp [328,

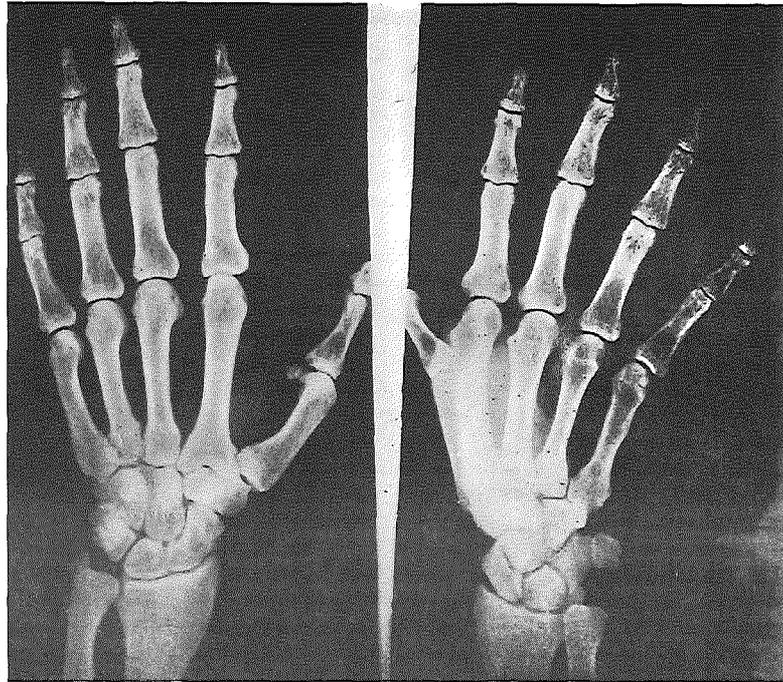


Fig. 209. The same hand as in Fig. 208 fifteen months after stopping vitamin D. All the deposits of calcium have been absorbed.

339] may be seen in the sclera, cornea and conjunctiva : it appears as small glass-like particles beneath the conjunctival basement membrane.

Post-mortem examinations [288] of fatal cases show almost constantly calcification of the renal tubules and less constantly general calcification of the kidneys, of the larger and smaller arteries, of the myocardium, of the lungs, of the bronchi and of the pancreas and in children [328, 336], in addition, of the dura, tracheal cartilage and gastric glands. Death may be caused by subarachnoid hæmorrhage [336].

Treatment. Stopping the vitamin D generally relieves the symptoms in a few days, but in severe cases, especially in infants, intravenous normal saline should be given, since the cause of death is the loss from the body of its electrolytes [327]. This loss is due to the diuresis caused by the kidney's efforts to secrete the excessive calcium and phosphorus.

Prognosis. The outlook is good. As has been said on p. 534 the damage to the tissues and even the calcification disappears in animals and may in man [335] (Figs. 205, 209). In man there is no evidence as to the ultimate

effect of prolonged overdosage. No bad chronic effects have been noted in patients who have had toxic symptoms, apart from a persistent impairment of renal function which may continue for many months [288] or only an inability to secrete a concentrated urine [331]. In children who have recovered from convulsions, the prognosis must be guarded as there may be a relapse after several months [328] or later impairment of development (p. 578).

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CHAPTER VIII

VITAMIN E

THE ANTISTERILITY OR ANTIDYSTROPHIC VITAMIN. ALPHA-, BETA-, GAMMA- OR DELTA-TOCOPHEROL

VITAMIN E is the name generally used when speaking of the vitamin in general or as it occurs in foods. Alpha-tocopherol is the most biologically active of several very similar substances, all of which have the properties of vitamin E. Thus while alpha-tocopherol means one distinct substance, vitamin E may mean either alpha-tocopherol, or a mixture of this and other similar substances. To avoid confusion vitamin E should be only used in the latter sense, and not as a synonym for alpha-tocopherol. The name tocopherol is derived from the Greek *τόκος*, childbirth, and *φέρω*, to bear.

HISTORY

Herbert McLean Evans, of California, will always have his name associated with vitamin E, partly because he and Bishop [1] in 1922 demonstrated the existence of an antisterility vitamin and partly because of the monograph on vitamin E written by himself and Burr [2] in 1927. This work remains to the present day the foundation of our knowledge of vitamin E. The authors showed that the foods richest in the vitamin were green leaves and the germ of seeds. Wheat germ and wheat-germ oil were found to have a remarkably high content of vitamin E, and remain to this day the best source. Rats were the experimental animals used, and for these animals it was proved that a deficiency of vitamin E leads to sterility in the male and abortion, though not failure to conceive, in the female.

The existence of the vitamin, however, had been foreshadowed in 1920 by Mattill and Conklin and confirmed in 1922 by Mattill and independently in 1923 by Sure. This early work is summarized by Evans and Burr [2].

Until 1928 vitamin E was thought to be entirely concerned with reproduction, but in this year Evans and Burr [3] reported that young rats suckled by vitamin E deficient mothers became paralysed, while Goettsch and Pappenheimer [4] in 1931 showed that guinea-pigs and rabbits when deprived of vitamin E developed a primary muscular dystrophy histologically identical with the progressive muscular dystrophies of man. From Denmark Ringsted [5], in 1935, and Einarson and Ringsted [6], in 1938, published careful and extensive research on the effects of lack of vitamin E on the central nervous system of adult rats. They pointed out that the neurological degenerations which were produced resembled those of amyotrophic lateral sclerosis and *tabes dorsalis* in man.

It is a depressing demonstration of the lack of co-ordination between research workers and clinicians that it was not until nine years after the discovery of vitamin E that Vogt-Möller [7] in Denmark first put it to any useful purpose by treating sterility in cows. In the same year he treated two women with habitual abortion with success [8], and six years later Young [13], in England, and Shute [14], in Canada, reported good results in the treatment of threatened abortion and pregnancy toxæmias.

Bicknell [9] in 1938, seven years after the possible value of vitamin E in human muscular dystrophy had been implied by animal research, started to treat cases of muscular dystrophy and neurological degeneration with vitamin E, or, rather, wheat germ. The improvement in his cases was

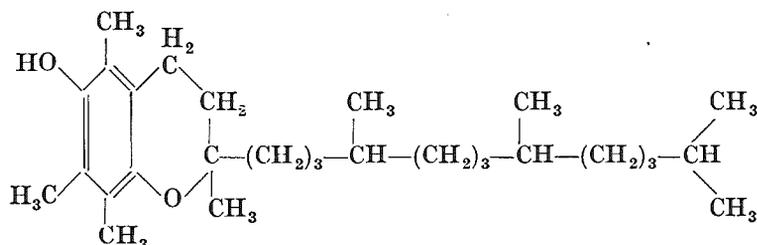
reported in 1940. Stone [10] and Wechsler [11] in 1940 reported cases of muscular dystrophy and amyotrophic lateral sclerosis successfully treated with vitamin E, but it was only in 1941 that the subject began to arouse wide clinical interest. The early promise, however, of the value of vitamin E in the treatment of muscular and nervous diseases has not been confirmed by later work, which at best only suggests that these diseases are caused by some complicated failure in the metabolism of the vitamin, which can only be corrected in rare cases by simple treatment with the vitamin alone. Many other conditions—especially those due to cardiovascular degenerations—have also been treated, but the results have been disappointing. Indeed, the clinical value of vitamin E is still obscure in spite of the great volume of experimental work during the last ten years, which shows how essential is the vitamin for many physiological processes, probably acting both in a non-specific manner as the body's chief antioxidant and also specifically in some enzyme systems.

The elucidation of the chemical structure of vitamin E was rapid after 1936. In this year Evans, Emerson and Emerson [12] isolated from wheat-germ oil two alcohols, alpha- and beta-tocopherols, and from cotton-seed oil gamma-tocopherol, all of which had vitamin E activity.

Further work by many investigators, including Todd, Bergel and Drummond and their collaborators in England, Fernholz in America, and Karrer and John on the Continent, finally led to the synthesis of alpha-, beta- and gamma-tocopherols and the elucidation of their chemical structure [7], though only in 1946 were the existence and the properties of delta-tocopherol discovered by Stern, Robeson, Weisler and Baxter [15].

CHEMISTRY OF VITAMIN E

Vitamin E occurs as alpha-, beta-, gamma- and delta-tocopherol, and there are also various synthetic compounds which may [7, 16, 17] or may not [208] possess some slight activity. Alpha-tocopheryl hydroquinone and alpha-tocopherylquinone, the first oxidation products of alpha-tocopherol, are said to prevent dystrophy in the rabbit [18, 206], but not foetal resorption in the rat [22]. Indeed, in the mouse [207] foetal resorption is actually caused. The chemistry and the physical properties of the tocopherols is extremely complex; the papers by Todd [7], Karrer and Bergel [7] and Stern, Robeson and others [15, 20, 21, 41, 42] and Rosenberg's book [19] should be consulted for discussions on the subject. The formula of alpha-tocopherol is:



Beta- and gamma-tocopherols are isomers, identical with alpha-tocopherol except that they possess two methyl groups in the aromatic nucleus, while in delta-tocopherol there is only one [15].

The tocopherols are oils at room temperatures, giving maximum absorption at 290 to 294 millimicrons. They are soluble in all lipid solvents, and the phosphate is moderately soluble in water, which is the reason why it is used in preference to the other esters, which are insoluble in water, for studying the effect of vitamin E on enzyme systems. The tocopherols are stable to strong alkali and to strong hydrochloric and sulphuric acids, but they are very easily oxidized, which is the basis of chemical methods of

estimation and is also the reason why they are such important biological antioxidants for fats and why *they are so rapidly and disastrously destroyed by rancid fats* (p. 615). They are unstable in air and when exposed to ultraviolet light, though their esters are not. The stability of vitamin E in foods is discussed on p. 627, and the great differences in biological activity between the various tocopherols and also their esters are discussed below.

UNITS OF VITAMIN E

The International Unit of vitamin E is the amount which, when administered orally, has the same specific activity as 1 mg. of synthetic racemic alpha-tocopheryl acetate in preventing gestation resorption in rats deprived of vitamin E.

The different forms of alpha-tocopherol have different biological and antioxidant potencies. Judging by the prevention of gestation resorption in rats the following are the values in I.U. per milligram [205] :

Synthetic racemic alpha-tocopheryl acetate . . .	1.00
" " " tocopherol . . .	0.68
Natural dextrorotatory alpha-tocopheryl acetate . .	1.36
" " " succinate . . .	1.21
" " " tocopherol . . .	0.92

The greater value of the esters is probably due to their greater stability in the gut before absorption, but the allophanates have no activity [19, 210, 211].

The four tocopherols vary greatly in their activities. For preventing gestation resorption in rats the relative activities of alpha-, beta-, gamma-[210] and delta-[15] tocopherols are 100, 40, 8.3 and 1, while comparing the first three racemic synthetic tocopherols to each other [27] values are 100, 25 and 19. Judging by the cure of muscular dystrophy in rabbits [212] values for the first three natural tocopherols are 100, 30 and 20, and for synthetic alpha- and gamma-tocopherols the values are eighty-two and thirty per cent. of their natural forms. Judging by resistance to hæmolysis [226] the relative potencies of the four natural tocopherols compared to racemic alpha-tocopherol are 1.33, 0.17, 0.07 and 0.04. Storage of the tocopherols in the body and their transfer by the hen to the egg are discussed on p. 600.

For a long time the *in vitro* antioxidant potencies of the different tocopherols appeared to be exactly the opposite of their different biological potencies, which was difficult to harmonize with the belief that the tocopherols owe their biological activity to their antioxidant properties. But the excellent review and work of Kunkel [213] in 1951 discusses and solves this difficulty : in the conditions which presumably prevail in the living body alpha-tocopherol has the greatest antioxidant potency. It also has the advantage of being stored to a far larger extent in the body (p. 603). Alpha-tocopheramine is said to be as potent as alpha-tocopherol in the cure of muscular dystrophy [209]. Other substances which may have vitamin E potency are referred to on p. 593.

FUNDAMENTAL OR BIOCHEMICAL ACTION OF VITAMIN E

Vitamin E probably acts as a non-specific general antioxidant in all the tissues of the body. It may also possibly act as part of a specific enzyme system, but of this there is as yet no clear proof. If all its action is merely due to its antioxygenic activities, an explanation must be found for why some tissues suffer more than others from a deficiency. But this question can be easily answered by postulates which are largely based on observed facts : firstly, that different amounts of vitamin E are stored in or are made available

to different tissues, so that some are more protected than others ; secondly, that the metabolism of different tissues differs in its need for antioxidants. Investigating the action of vitamin E is difficult, partly because the biochemical changes which occur in the living deprived animal are only the end result of an already far-advanced biochemical chaos, and partly because the addition of vitamin E to tissues or isolated enzyme systems does not reproduce its action in the intact animal. In the following discussion the biochemical action of vitamin E will be considered as it appears, firstly from studies on living animals, secondly from studies on isolated tissues, and thirdly from studies on isolated systems.

The living Animal. The antioxidant activity of vitamin E starts within the lumen of the gut, where it protects vitamin A and carotene (p. 23) and biotin and the vitamin B complex [184, 185] against destruction by rancid unsaturated fatty acids. This protection for vitamin A is continued within the body (p. 23), though apparently it is not necessary for the preservation of biotin in the tissues [186]. Vitamin E is the only antioxidant present in body fats [195], and it probably plays the same role in fish liver oils [196, 284].

Oxygen consumption greatly increases in the young rat deprived of vitamin E before there are any signs of muscular dystrophy [187]. When muscular dystrophy appears the oxygen consumption falls in rats [187] and remains normal in guinea-pigs [188], but probably this is only because partial inanition counteracts the effect of lack of vitamin E. The increase in oxygen consumption in the intact animal can be wholly accounted for by the increased oxygen consumption of the dystrophic muscles [117, 187, 189] and so does not necessarily involve an increase in any other tissues, which in the liver at least does not occur [187]. Injections of alpha-tocopheryl phosphate reduce the oxygen consumption of muscles removed at biopsy almost to normal within four hours, though the same effect is only achieved by oral doses of alpha-tocopherol in twenty-seven hours [117]. In normal children vitamin E decreases the basal metabolic rate and the specific dynamic action of glycine [190].

Phosphorus turnover in all the tissues of the body is stimulated by lack of vitamin E. This is thought by Weissberger and Harris [191] not to be a direct effect of the deficiency but a secondary result of the increased oxidation in muscle, since here oxidation and phosphorylation are coupled, so that what affects one will affect the other. It is considered that this increased turnover in muscle leads to the increased turnover which occurs in both sexes equally in the bone, kidney, blood, uterus, ovary, testicle and muscle [191]. It is of profound interest that no greater change in phosphorus metabolism occurs in dystrophic muscle than in bone or blood, and also that the change in the genital tissues—which in the rat are the first to be damaged by a deficiency—is no greater than that in other tissues. Doses of vitamin E of at least one hundred times the normal requirements also cause an increase in the turnover of phosphorus, but this is not similar to that caused by a deficiency, since it is greater, it occurs more rapidly and it is never associated with pathological changes in muscle, though, on the other hand, osseous rarefaction occurs [191].

Creatine is decreased in dystrophic muscle [117, 131, 182] and is excreted in large amounts in the urine even before there are any clinical signs of a deficiency of vitamin E [29]. Again, this abnormal creatine metabolism may be held to be a secondary effect of the uncontrolled and excessive oxygen and phosphorus metabolism of the muscle. Alpha-tocopheryl phosphate injections reduce still further the creatine content of biopsy specimens of dystrophic muscle for two hours, after which time it slowly rises, presumably because the muscle's thirst for creatine has been slaked. Hottinger [190] claims that giving 12 to 18 mg. of alpha-tocopherol daily to healthy children immediately decreases their normal creatinuria and also that caused by glycine, though that caused by creatine is not affected. In deficient

rabbits hepatic creatine is increased ; Heinrich and Mattill [194] suggest that this is due to increased synthesis by the liver and failure of phosphorylation by the muscles. Allantoin excretion is increased six-fold in deficient monkeys [92].

Fat and protein metabolism are both discussed later on p. 620, and blood clotting on p. 622.

Tissues from vitamin E deficient animals show a multitude of changes. In calf muscle [131] potassium is decreased and sodium increased, presumably because of the oedema (p. 619). There is a true decrease in the total nitrogen with an increase in stroma protein and a decrease in muscle albumin. There is only an apparent increase in fat, since, while the total and free cholesterol and the lipid phosphorus fraction are increased, the proportion of these to the total fat remains normal. Roughly similar observations have been made on rat and guinea-pig muscle [177, 192], and, further, glutamine is grossly decreased in rats and slightly in rabbits [186] ; but all these investigations are not truly helpful in understanding the true nature of the metabolic disaster, since by the time a muscle has become dystrophic its analysis only reveals the sum of the compounds which occur in an uneven mixture of normal, degenerating, necrotic and calcified muscle fibres, and wandering cells and connective tissue. The same might be said about the fall of glycogen, which is stated, on rather slight evidence, to occur in the muscles, heart and liver [193], though it is of interest to note that vitamin E may be necessary for the formation of the glycogenic hormone of the pituitary (p. 611). Butturini [407] states that vitamin E in animals and diabetics increases the difference between the levels of sugar in arterial and venous blood, causing storage of glycogen in the muscles, liver and heart. Vitamin E has been reported to raise the blood sugar curves of normal children [190]. The very important part vitamin E may play in warding off senility is discussed on p. 622.

Isolated Tissues. The oxygen consumption is increased in dystrophic muscles [117, 186, 187, 189] and in muscles from the deprived hamster before the onset of dystrophy [186] : adding glucose or diphosphopyridine nucleotide to the nutrient medium makes no difference [186], while some workers claim [117] and others deny [197] that the addition of alpha-tocopheryl phosphate reduces the oxygen consumption. Homogenates of muscles from deprived rabbits and guinea-pigs show decreased activity of aspartic glutamic transaminase, which is not increased by the addition of pyridoxal phosphate [198]. However, no decrease in six enzymes in the muscle and two in the liver from dystrophic rabbits was found by Jacobi and others [203].

Isolated Enzyme Systems. Minute amounts of alpha-tocopherol stimulate acetylcholine synthesis [199], but generally tocopheryl phosphate is used for investigating enzyme systems because it is water soluble and so can be added to aqueous preparations. However, Ames and Risley [200] have pointed out that "the *in vitro* effects of alpha-tocopheryl phosphate probably bear no relationship to the biological functions of vitamin E." This is partly because the phosphate inhibits practically every enzyme on which it has been tested, partly because alpha-, gamma- and delta-tocopheryl phosphates have the same inhibiting effect on succinoxidase systems [200], and partly because the phosphate combines with protein [200] and therefore most probably combines non-specifically with an active enzyme, blocking the active centres. Similar conclusions have been reached by others also working with succinoxidase systems [202], though Jacobi and others [203] in 1950 claimed to have shown that part at least of the inhibition of this enzyme is a specific effect. They also report no inhibition of several other enzymes. References to and brief reviews of the various enzyme systems which have been investigated up to about the end of 1949 have been given by Ames and Risley [200] and Ames and Harris [201]. When an alpha-tocopherol-protein conjugate is

added to an enzyme system it probably can exercise its normal biological function ; in the case of a succinoxidase system it gives protection, probably merely acting in its classical rôle of an antioxidant [200]. In an important paper Miller and Dessert [204] suggest that vitamin E may regulate hyaluronidase activity and that the immunity of the immature testis to deprivation of vitamin E is due to it not yet having hyaluronidase, while the mature testis suffers because in the absence of vitamin E the activity of its hyaluronidase is unchecked. One man had no hyaluronidase in his semen as long as he was given vitamin E.

ESTIMATION OF VITAMIN E

The cumbersome and costly biological method of estimating vitamin E by its effect in preventing resorption-gestation in rats has been largely supplanted by highly complex but more concise physico-chemical methods, though it must remain the basic yardstick for checking the accuracy of all types of estimation.

Biological Estimation. The foundations of the biological method were laid by Evans and Burr [2], the criterion of vitamin E activity used being

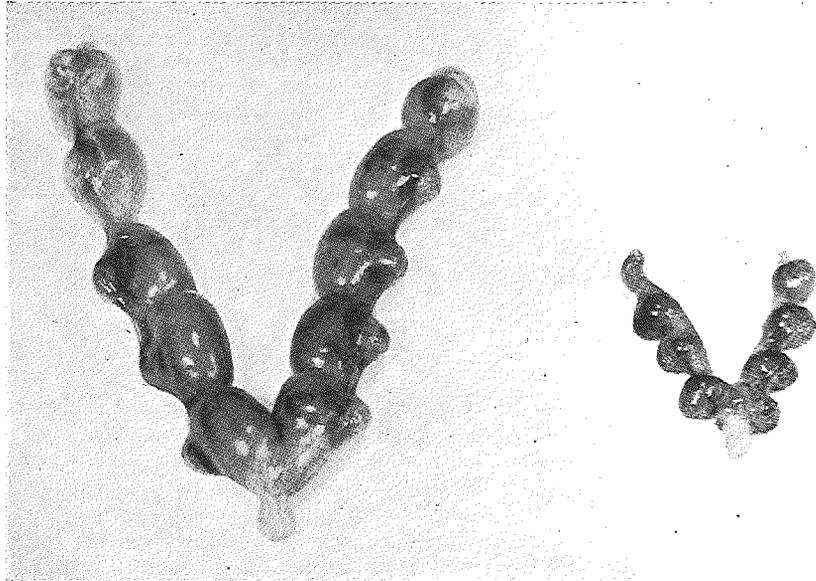


FIG. 210. The uterus on the left is from a pregnant rat on a normal diet, while that on the right is from a pregnant rat on a diet deficient in vitamin E. Note the smaller number of embryos within the latter, and their arrested development.

the cure or prevention of the particular kind of sterility brought about by deprivation of vitamin E (p. 606). These authors pointed out that the only certain method of knowing that a doe on a vitamin E deficient diet was really depleted of vitamin E was for her to have a typical resorption pregnancy. Only then was such a doe fit to be given the dietetic supplements whose vitamin E content was being examined. It was necessary to mate her with a buck of proved fertility and to make certain that both positive mating and implantation had occurred. Then if a litter was born it was good proof that the dietetic supplement contained vitamin E. Time could not be saved by omitting the preliminary absorption gestation, because the animals varied so much in their initial stores of vitamin E that even in litter mates, kept on

the same deficient diet, some rats might show "first litter fertility," and some might not.

Bacharach [23], however, has perfected a technique by which the preliminary absorption gestation can be omitted, with great saving of time. He stresses that there are "very large and unpredictable variations in the average response of groups of animals at different periods even though the pre-experimental and experimental conditions have, as far as possible, been kept constant." He found, for instance, during one series of experiments that the mean fertility dose of a substance appeared to increase threefold [7]. This emphasizes the importance of using racemic alpha-tocopheryl acetate as a control during all estimations. Bacharach [24] has also worked out a dosage-response curve for vitamin E which shows that in comparing two substances for their vitamin E activity accurate comparisons are only possible when the fertility rate of the animals is about fifty per cent.

Originally it was necessary to adopt the "all or none" law for fertility; rats either did or did not produce a litter. This meant that a graded response to vitamin E supplements in the diet was not provided. But Mason [7] overcomes this difficulty by killing all assay animals on the sixteenth day of pregnancy and examining the number of living and dead fetuses and resorption sites. From this he works out the "uterine index," which shows a graded response to graded supplements of vitamin E [25]. A somewhat similar technique is used by Homrich [26], who emphasizes the importance of a fat-free diet, of giving the test substance after mating instead of before, of discarding animals which have less than seven or more than fourteen implantation sites and of not using bucks with a poor breeding record.

Gottlieb, Quackenbush and Steenbock [27] have found in rats that, with a diet low in fat, the increase in weight during pregnancy is, within limits, in direct proportion to the amount of vitamin E supplied. This method of assay is economical, since the animals can be used again and few are needed because of the graded nature of the response. It is also claimed to be more sensitive than other methods, enabling amounts of vitamin E to be determined which are too small for the production of litters.

Other biological actions of vitamin E have been suggested for use in its estimation: thus Herraiz and Radice [28] have done preliminary work with the testicular degeneration which occurs in deficient young rats (p. 610); Mackenzie and McCollum [29] have drawn attention to the rapid decrease in urinary creatine of rabbits, rendered dystrophic by lack of vitamin E, when the vitamin is added to their diets (p. 615); Dam and Glavind [30] believe that the alimentary exudative diathesis which occurs in deficient chicks (p. 619) would be suitable; Rose and György [31] have shown that the red blood cells of deficient rats are far more sensitive than those of normal animals to hæmolysis with dialuric acid—though according to Cater [32] this hæmolysis is much less marked with the red blood cells from deficient chicks, possibly because they are nucleated. Using hæmolysis, when it has been further investigated, promises to be of great value not only for easy and rapid assay work, without killing the animals, but also for indicating both in animals and man a deficiency which gives no overt signs.

Physico-chemical Estimations. Physical and chemical methods are generally combined when estimating the tocopherols, the former including molecular distillation, chromatography, photometry, spectroscopy, etc., while the chemical methods are most often based on the reactions first described by Emmerie and Engel [33] or Karrer and Keller [34] or Furter and Meyer [35]. In the first of these ferric chloride is reduced by the tocopherols, the reduction being measured by the photometric estimation of the red-complex formed with α, α' -dipyridyl, while in the second method the reduction of the gold chloride is measured electrometrically. The third method is based on the red colour given by tocopherols with nitric acid.

Total tocopherols in oils and animal tissues, in blood and in foods may be

assayed by the methods described by Cuthbertson and others [36], by Tošić and Moore [37] and by Quaife, Dju and Harris [38, 39], whose papers should be read for the full and complicated details of the various techniques employed. The first of these, and by far the least sensitive, is the only one based entirely on spectroscopic methods, the others being in essence chemical.

Different foods contain different proportions of the four tocopherols, and as these vary greatly in all aspects of their biological activity (p. 594) it is necessary to measure them separately if the value of foods is to be assessed with any accuracy. The problem is simplified by beta-tocopherol only occurring, at least as regards the commoner vegetable oils so far investigated, in wheat-germ oil [40, 41, 42]. Delta-tocopherol apparently occurs in all oils [15, 41], and so measuring it and gamma-tocopherol together [40, 43] may introduce a slight but definite error when assaying the biological potency of a food. To measure gamma- and delta-tocopherols separately in a food also containing alpha-tocopherol, *o*-dianisidine is employed, which gives no colour with the latter and different colour intensities with the two former in different alkaline solutions [41]. All four tocopherols may be measured in a food by the method described by Quaife [42], which in essence depends on estimating the total tocopherols and then making the nitroso derivatives of beta-, gamma- and delta-tocopherols, separating them chromatographically and measuring each photometrically. Paper chromatography is excellent for qualitative and possibly for quantitative assay of all the tocopherols [413].

PHYSIOLOGY OF VITAMIN E

Nothing is known of how plants synthesize vitamin E, though it is possible its origin is closely related to that of vitamin K [16]. All animals so far investigated appear to be dependent on plant tissues for the vitamin, not forming it themselves. Most probably the bacteria of the gut—at least of the rat [44] and cow [45]—do not synthesize vitamin E. For insects the vitamin is important or perhaps essential [46, 47].

Absorption. Whether vitamin E, like carotene, undergoes any change within the wall of the intestine during absorption is not clear, though the matter may be important since Milhorat and Bartels [48] have suggested that the fundamental metabolic error in human muscular dystrophy is failure to convert vitamin E during absorption into its active form. Many workers, among them Evans and Burr [2] and Knowlton and others [49], have reported that vitamin E is active when injected in an oily solution for the prevention and cure of both sterility and muscular dystrophy. In the latter condition, however, Mattil [50] and others [51, 52] found alpha-tocopherol more active when given by mouth than by injection. There is no rise in the blood level unless it is dissolved in a "Tween" before injection, which may be dangerous for man [311]. Wechsler and his collaborators [53] report that the level of vitamin E in the blood of three patients actually fell after intramuscular injections of 100 mg. of alpha-tocopherol, though the level rose when the vitamin was given by mouth.

There is strangely little information about how complete is the intestinal absorption of vitamin E. Some escapes absorption and appears in the faeces of rats when the diet contains large amounts [36, 44, 54]; the amount lost may be a quarter of that eaten, males excreting more than females [24]. The amount excreted by rats is proportional to the amount consumed at intakes between 1 and 100 mg., age making no difference, nor whether the tocopherol is in the natural or the racemic form [44]. On the other hand, absorption is said to be more efficient in deficient animals [44]. Hens excrete about three-quarters of a single dose of 1,000 mg. [62]. Man also excretes part of the tocopherol he consumes [44], which on normal diets is said to be about two-thirds of the intake [414].

Fat and the normal absorption of fat may be important for the absorption of vitamin E; a possibility which should be investigated now that fat-free forms are almost wholly used in medicine. But no direct work has been done on this, so that evidence must come from conditions where fat is poorly absorbed. Thus in sprue [55, 65, 71] and the steatorrhœas [71] the level of vitamin E in the blood is low, and five men have been reported [56, 61] who, after dying from "sprue" or chronic and profound digestive disorders, were found at post-mortem to have the tissue pigmentation, muscular dystrophy and testicular atrophy typical of experimental vitamin E deficiency. Two cases of steatorrhœa [57, 58] are said to have shown a remarkable clinical improvement when given vitamin E. Therefore, even if fat is not itself an important aid to the absorption of vitamin E, yet any conditions which hinder fat absorption are liable to affect vitamin E in the same way. But it must be remembered that rancid fat destroys vitamin E during digestion (p. 23).

Bile appears to be necessary for the absorption of vitamin E. Brinkhouse and Warner [59] report that dogs with chronic biliary fistulæ develop within seven to nine months both the nutritional muscular dystrophy and testicular degeneration typical of a deficiency of vitamin E. In one dog the muscular dystrophy was arrested and improved when bile was fed by mouth. Greaves and Schmidt [60] have obtained similar results in rats. The effect of hepatic disease in man on the level of vitamin E in the blood is discussed on p. 602.

Liquid paraffin probably hinders the absorption of vitamin E as it does that of vitamins A, D and K.

The placenta appears to offer no barrier to the passage of vitamin E, since the level of vitamin E in the plasma of women and in the *venous* cord blood plasma of their infants is virtually the same [63, 64]. As the level in the arterial cord blood plasma is only a half or a third of that in the venous cord blood plasma [63, 64], it would appear that the new-born infant destroys or stores vitamin E with amazing rapidity; so estimations of the level of vitamin E in "cord" blood plasma, without any care being taken to collect only arterial blood, are valueless. Not realizing this has led several investigators to the belief that vitamin E passes the placental barrier with difficulty, merely because "cord" blood plasma has been found to contain less vitamin E than the maternal plasma. Fœtal plasma levels and fœtal storage are discussed below.

The hen [62] transfers vitamin E to the egg in considerable amounts: on a commercial laying mash the vitamin E content may be as high as 4 mg. per 100 grams of egg, of which about eighty-five per cent. is alpha-tocopherol. The average content, however, is about 1.2 mg. per 100 grams, which drops to somewhere between 0.16 and 0.64 mg. before a vitamin E deficient diet stops laying, at which time the plasma level has fallen to about 0.1 mg. At intakes of 100 to 200 mg. weekly the efficiency of transfer to the egg is for alpha-, gamma- and delta-tocopherols 16.5, 2.9 and 1.35 per cent.

Plasma Levels. The level of vitamin E in the blood is virtually always estimated and given as the level in the plasma, even when it is called the "level in the blood," though Bratzler and others [93], working with pigs, have shown that when the plasma contains no vitamin E the whole blood may yet contain about 0.087 mg. per 100 ml., and when the plasma contains 0.67 mg. the whole blood may contain 0.73 mg. This difference between plasma and whole blood might just conceivably be important, especially when considering levels in anæmic patients or those with polycythæmia, such as new-born infants.

The average level of vitamin E in the plasma of healthy human adults is about 1 mg. per 100 ml., but there is a wide variation. In England [415] the mean value for 64 healthy subjects was 1.31 mg. with a range of 0.60 to 2.91 mg. and for 175 mental patients 1.15 mg. with a range of 0.56 to 3.25 mg. Engle [65], from an investigation of 122 normal Dutch adults, found a

range of 0.3 to 1.3 mg., while Harris and others [66] in the U.S.A. consider 0.88 to 2 mg. normal, and below 0.88 mg. subnormal, basing this opinion on the blood levels of seventy normal adults and 350 who had a background of malnutrition and vague symptoms without any abnormal physical signs. Similar levels have been reported by many other workers in North America [68, 69, 70]. Sex [68, 69] and negroid colour [68] make no difference, though there is a tendency for higher levels in old age [68, 71], especially in men [69]. During pregnancy levels tend to rise; in one extensive investigation [71] the average values for successive trimesters being 0.84, 1.07 and 1.24 mg. per 100 ml., falling to 0.96 mg. six weeks after delivery. Other workers [70, 75] give very similar figures for pregnancy and the puerperium, with an average blood level at term of 1.7 to 1.8 mg. There is no change during the menstrual cycle [71]. Venous cord blood has roughly the same value as the mother's, and arterial cord blood considerably less [63, 64], while the new-born infant has low levels of about 0.26 mg. [72, 73], though there are very wide variations. Prematurity and weight at birth make no difference [72], but after five days the average level for full-term infants has risen to an average of 0.36 mg., while in premature infants no rise occurs for two months, after which levels reach about 0.5 mg. at three to six months [72]. In nine children, aged five to fifteen years, the level was 0.72 to 1.12 mg. [85].

Poor nutrition, such as that which generally results from hospital diets [68] and poverty [66], tends to show itself in blood levels of 0.9 mg. or less, while during the German occupation and starvation of France [75] in 1943 the average values were 0.2 mg. The German starvation of Holland had the same effect [76].

Daily oral doses of synthetic racemic alpha-tocopheryl acetate may raise the plasma level very considerably or may have little effect, presumably depending on individual differences in absorption, storage and destruction. Thus 600 mg. daily for one month raised the levels of twenty-one cases from an average of 1.2 mg. to an average of 2.95 mg.—values ranging from 1.64 to 4.69 mg.—which fell after a month to 1.66 mg. [67]. With doses of only 150 mg. daily the average level reached was 1.76 mg., with a range of 1.28 to 2.73 mg. High values and wide individual differences after dosing have also been found in premature infants who, with an average level of 0.25 mg., may show no increase with daily doses of 150 mg. or may have levels above 4 mg. [73].

Tolerance curves after single oral doses again show marked variations; thus Klatskin and Krehl [77], using 500 mg. doses, found in twenty-one subjects that the maximum rise occurred any time between three and thirty-six hours after the dose, the increase in some cases being only 0.04 mg., while in others it was 3.63 mg. The average rise was 1.31 mg., and this occurred most frequently between six and nine hours after the dose. Levels were still slightly elevated in many cases after seventy-two hours.

The cerebrospinal fluid contains no vitamin E [76, 78].

Illness has strangely little effect on the level of vitamin E in the blood in spite of the spate of claims that many diseases are benefited by vitamin E (pp. 632-661). Indeed the only two groups of diseases which appear to have any effect on the level are, firstly, those associated with chronic steatorrhœa (p. 600), where the level is low due, presumably, to impaired absorption, and, secondly, those associated with hypercholesteræmia, where the level is often high [71], which may be the reason why the disease of old age tends to have high levels [68, 71], especially in men [69]. But there is no absolute relationship, since in hepatitis the cholesteræmia and vitamin E levels do not always fluctuate together [77]. Whether a high level of vitamin E causes a high level of cholesterol or *vice versa* is obscure; in favour of the latter are observations on diabetics, in whom a high level of cholesterol appears to be always accompanied by a high level of vitamin E [82], while raising the level of vitamin E does not raise the level of cholesterol [83]; nor

does it in rats [288], though it does so in rabbits [84] and is said to do so in schizophrenics [375].

Of the conditions for which vitamin E has been most frequently advocated, the muscular dystrophies and neurological degenerations tend to have normal [65, 85] or low levels [53]; cardiovascular diseases, normal [68] or high [67, 71, 79] levels; diabetics [82, 83], cases of primary fibrositis [153] and infants with retrolental fibroplasia [73], normal levels; while in the complications of pregnancy Scrimshaw and his colleagues [80] compared the blood levels of 197 normal women at various stages of pregnancy with the blood levels at the same stages of pregnancy of sixty-nine pre-eclamptic women, another twenty-five who had premature infants and a further sixteen whose pregnancy was complicated by essential hypertension. They found that none of these women had abnormal levels. In contrast Rauramo [81] reported that, of thirty-two women with levels below 0.6 mg. per 100 ml., forty-four per cent. developed toxæmia, nineteen per cent. had hyperemesis and forty-one per cent. gave a history of previous abortions or premature delivery; on the other hand, women with levels above 0.8 mg. had respective percentages of 8, 14 and 14. The part played by vitamin E in human pregnancy is further discussed on p. 633.

Hepatic disease gives a flat tolerance curve [77, 79], but in the very thorough investigation carried out by Klatskin and Crehl [77] "the plasma tocopherol level could not be correlated with the degree of hepatic dysfunction, the type of liver damage, the presence of jaundice, the serum cholesterol concentration or age." Levels are high in nephritis [65, 71, 79], normal in nephrosis [79] and are said to be low in Leber's optic atrophy and hyperplastic uterus [65]. A few cases of most medical conditions have been investigated [71, 77, 79] and levels appear to be within normal limits in virtually every disease not already discussed, including pneumonia [77] and infections [71].

Animals vary greatly in their levels, the following average plasma values being milligrams per 100 ml.: *macaca rhesus* monkeys [86] 0.58; horses and mares [88] 0 to 0.4 and 0.15 to 0.34, whether stall fed or at pasture, with very slightly lower levels during pregnancy and normal levels in sterility; cows [88] 0.1 to 0.2 when stall fed and 0.8 when at pasture, the levels falling slightly with age but not altering with pregnancy, parturition, *Brucella* infections with or without abortion, sterility, nymphomania and anaphrodisia, though other workers [89, 102] state—probably correctly—that there is a fall shortly before pregnancy such as occurs in women (p. 601); new-born calves [89] 0.04; lambs [90] 0.02; kids [90] 0.016; farrows [90] 0.12; pigs [93] plasma 0.63, and whole blood 0.69; sheep [94] 0.33; rats [31] on stock ration 0.38, and on deficient ration 0.27; chickens [91] at 1.0 have complete protection against cod-liver oil, and at 0.1 cease laying [62]. Supplementing the maternal diets raises the levels in new-born calves, kids and lambs but not farrows [89], which suggests that, as the last animals are the only ones whose diets are very rich in vitamin E, all the others were really receiving an insufficient diet.

Storage. In the tissues of a man of thirty and a woman of forty-three, killed in an accident, the following values respectively in milligrams per 100 grams were found [39]: muscle, 0.62 to 1.32 and 1.56 to 3.8; liver, 2.49 and 2.19; fat, 24.7 to 29.2 and 39.2 to 49.5; heart, 1.11 and 1.28; kidney, 0.8 and 3.32; pancreas, 5.49 and 10.6; spleen, 1.88 and 4.7; testis, 2.83; uterus, 1.47. The fat of the testis and the uterus, with 1,210 and 1,160 mg. per 100 grams, was the richest in the body, fat from adipose tissue containing only 297 to 359 mg. and 605 to 626 mg. An odd finding was that in the man gamma- and delta-tocopherols were only present in the subcutaneous fat, while in the woman they formed twenty to forty per cent. of the tocopherols in most tissues. Very similar values for muscle have been found by other workers, the highest level of 4.8 mg. being found in a case of myasthenia gravis [95]. In the livers of two patients who died from heart disease and

carcinoma of the colon the values were 3.37 and 2.64 mg., while values of only 0.44 and 0.94 mg. were found after death from cirrhosis and obstructive jaundice [79]. The average content of the placenta of ten women was 0.41 mg., which for eight women rose to 1.04 mg. after dosing for three to thirty days with 90 mg. daily of synthetic alpha-tocopherol [64]. Other workers report values of 0.56 to 1.07 mg. on normal diets [96].

Animals can store vitamin E for a considerable period, Evans and Burr [2] finding that a single dose of vitamin E might suffice the rat for three or even four normal pregnancies. Biological assays by Mason [98] on the relative amounts of vitamin E in various tissues of the adult rat have shown that the liver stores large amounts when the intake of natural mixed tocopherols is high, and less than any other tissue when the intake is low; storage is never as great as that of vitamins A and D. Active mammary tissue has the highest content of all organs, and the heart, lungs and spleen are twice as rich in the vitamin as the other viscera, muscles and body fat.

Chemical assays by Hines and Mattill [54], which they suggest fail to estimate all the vitamin in the tissues, do not agree with Mason's findings, since in rats, whatever their intake, the liver always contained large amounts of vitamin E compared to the muscles. The values—expressed as milligrams of tocopherol per kilo of tissue—in animals receiving (1) a vitamin E concentrate equivalent to 100 mg. of tocopherol daily, (2) a "normal" diet which probably contained very little vitamin E, and (3) a vitamin E deficient diet, were: for liver and for muscle, 42.3 and 11.9; 22.1 and 7.5; 22.6 and 4.8. For rabbits the values were 86.8 and 28.1; 9.2 and 8; 9.4 and 5.7. Further figures for rabbits [97] are: liver, 9 to 14; muscle, 1.3 to 3.3; brain, 5 to 18; lungs, 10 to 19; spleen, 10; kidney, 5 to 7; intestine, 4 to 7; stomach, 6 to 12; skin, 2.6; and fat, 6. Hines and Mattill [54] have suggested that the high and very slowly depleted hepatic stores of the rat may explain why this animal is more resistant to lack of the vitamin than the rabbit, whose hepatic stores are low, especially compared to those in the muscles.

Lundberg and others [99], basing their assays on the effect of alpha-tocopherol on the induction period [195] of abdominal fat, found that storage in the fat of rats did not reach a maximum until seven or ten days after a single dose of 50 mg., after which the amount stored fell to half its maximum in two months. With an intake of 50 mg. daily for ten days the maximum amount stored was 97 mg. per kilo. Alpha- and beta-tocopherols [100] were deposited in equal amounts, the latter only reaching its maximum in fifteen days. Much less gamma- than alpha-tocopherol was deposited in the abdominal and ham fats, but not in the skin fat, which is reminiscent of the deposition in male human skin mentioned above. From these results it is suggested that the biological activity of the different tocopherols is not related to their antioxygenic activity but to the amount of each deposited in the tissues.

The pig [93], from studies on only five animals, has the following stores respectively of total tocopherols in milligrams per 100 grams when fed a diet deficient in vitamin E and the same diet supplemented with 55 mg. per kilo daily: liver, 0.16 and 42.3; muscle, 0.37 and 4.16; spleen, 0.46 and 5.34; kidneys, 0.25 and 2.79; heart, 0.1 and 5.6; while the content of the various fat depots varied from 1.36 to 35.3 for ruffe fat to 0 and 17.6 for ham facing fat. But all the above figures varied very greatly from animal to animal. Figures reported for the ox [97] are: posterior pituitary, 0.9 to 1.1; anterior pituitary, 2.6 to 3; brain white matter, 2.3; and grey matter, 1.2.

The fetus has small stores of vitamin E, since the young born of rats and rabbits on a deficient diet develop muscular dystrophy only slightly sooner than do the young born of normal animals when both are suckled by a vitamin E deficient doe [3, 101]. The tissues from newly born rats of mothers

on a normal diet contain only small amounts of vitamin E [2], while livers of newborn lambs contain 2.5 mg. per 100 grams, kids 1.04 mg. and farrows 2.47 mg. [90]. Supplementing the maternal diets of the last three animals did not appreciably increase foetal hepatic stores. The newborn animal appears therefore, from the figures given below, to depend for its vitamin E on colostrum (p. 629) and not its own stores.

Excretion of vitamin E in the faeces has been discussed on p. 599, while its excretion in the urine of rats only occurs when it has been consumed in very large amounts [36], and it has not been found in human urine [76]. In human bile it is probably present in the same amounts as in the plasma [79]. The amounts of vitamin E in colostrum and in milk are given in the food tables and the subject is also discussed on p. 609, so that here it is only necessary to emphasize the high levels of vitamin E in colostrum.

Destruction. The depletion of stores of vitamin E was shown by Evans and Burr [2] to continue at the same rate whether rats became pregnant or not, though vitamin E is excreted in milk (p. 629). It thus appears that vitamin E is used in the normal processes of the body, pregnancy not appreciably increasing the consumption. When large amounts of vitamin E are taken they are rapidly destroyed; where this occurs is obscure [36], though it may be in the muscles, partly because vitamin E plays an important part in muscular metabolism (p. 612) and partly because Pappenheimer and others (p. 618) have shown that the vitamin is only essential for the integrity of working muscle, and therefore is possibly metabolized and destroyed during muscular work.

It is improbable that the first stage in the destruction of vitamin E is its oxidation to tocopherylhydroquinone (p. 593), though this has been suggested because the blood of dogs contains 0.46 mg. per 100 ml. and that of man 0.31 mg. per 100 ml. [152]. Hines and Mattill [54], however, found none in the liver, muscles and urine of rats consuming 100 mg. of vitamin E daily, which is a strong argument against vitamin E being oxidized to this substance.

Requirements. The need for vitamin E varies greatly with the composition of the diet; highly unsaturated fatty acids, for example, increase requirements (p. 615), while protein has the opposite effect (p. 620). Therefore requirements are never a fixed amount but must always be considered against the whole dietetic background. Hyperthyroidism [154] and old age (p. 607) both increase the needs for vitamin E. Further, Harris [107] has suggested that requirements depend on "physiological" weight, which for many activities of the body [103] is only seventy per cent. of the physical weight. This relationship can very roughly be used to deduce the needs of one animal from the known needs of another; thus per kilo. of body weight the lamb should, and in practice does, require seventy per cent. of the vitamin E required by the mouse [107].

In the rabbit, in which muscular dystrophy is the dominant effect of a deficiency (p. 614), requirements depend on body weight—but not on age or sex [52]—being, according to Mackenzie and McCollum [29], from 0.7 to 1 mg. daily of alpha-tocopherol per kilo. of body weight. Much smaller amounts allow of growth over a long period and ward off muscular dystrophy, so that such mild dystrophic lesions as do occur give no clinical symptoms [103]. Eppstein and Morgulis [52] state that doses as low as 0.32 mg. daily per kilo. of body weight are sufficient for all needs.

In the rat, in which loss of fertility is the dominant symptom of a deficiency (p. 606), the male requires more than the female. Evans and Emerson [104] gave rats of both sexes from weaning 0.1, 0.25 and 0.75 mg. of alpha-tocopheryl acetate daily. The smallest dose protected both sexes from muscular dystrophy and enabled the females to have three litters, though ultimately there was uterine discoloration (p. 607) and the suckling young all developed muscular dystrophy. Daily doses of 0.25 mg. had a

slightly better effect, but even doses of 0.75 mg. did not completely prevent dystrophy in the young of the third pregnancy. In the males doses of 0.75 mg. preserved fertility for at least sixteen months, while doses of 0.25 and 0.1 mg. preserved it for nine and five months. Post-pubertal testicular degeneration is considerably delayed, but not prevented, by a single dose of 0.5 to 5 mg. when this is given on the fifteenth day of life; when given two weeks later or one week earlier, there is little or no effect [105]. Similar protection is provided by giving the nursing mother 51 mg. [106]. Gottlieb and others [27] give the total requirements for a normal pregnancy with normal offspring as 0.85 to 1.5 mg. when the diet contains no fat, and double this when the diet is rich in fat, while Rose and György [31], judging by the resistance of red cells to hæmolysis (p. 598), state that the rat needs 3 mg. daily per kilo of body weight.

Guinea-pigs require 3 mg. daily [109], lambs 0.23 to 0.37 mg. per kilo. [110], while Harris [107] at the end of 1949 collected the references to the scant literature on the needs of mice, dogs, calves and goats.

Ducklings [124] require 2 to 4 mg. daily to prevent muscular dystrophy; laying hens [62] a minimum of 1.2 mg. daily for egg production, which will not give an egg rich in vitamin E; while chicks (p. 619) vary in their needs with the composition of their diets.

THE FUNCTIONS OF VITAMIN E

There is a remarkable variation in the response of different species to a deficiency of vitamin E, though fresh work is continually showing that what at first seemed a variation in kind is really only a variation in degree: different tissues in different species may suffer first from a deficiency, but the same tissues probably all suffer in the end if death due to the involvement of some essential organ does not supervene. In the rat [2] the earliest sign of a deficiency is sterility in the male and failure in the female to carry pregnancy to term. If the deficiency is prolonged changes occur in many organs, showing that vitamin E is not solely concerned with reproduction, but with most activities of the body. The endocrine system and kidneys may be affected (pp. 610, 623) and the central nervous system degenerates (p. 617). There is also a primary degeneration or dystrophy of voluntary and involuntary muscle (p. 612) and extensive deposition of "ceroid" pigment (p. 621). The mouse appears to differ from the rat: according to current work [111] only the female requires vitamin E for reproduction—though this has been questioned [136]—only the young develop muscular dystrophy, the central nervous system and kidneys do not degenerate and less "ceroid" pigment is formed. In the guinea-pig dystrophy of voluntary muscles dominates the picture [4], though failure to carry pregnancy to term [113] and testicular degeneration [114] can occur in animals rendered dystrophic but not killed by diets only partially deficient in vitamin E.

A similar muscular dystrophy with or without "ceroid" is the earliest and as yet the only recognized symptom of deprivation of vitamin E in the monkey [86, 87, 92] and rabbit [29, 115], both of which also suffer myocardial damage, and in the hamster [117, 118] and tree kangaroo [116]. The rabbit [137] and hamster [136] also suffer in time from testicular damage. The dog develops muscular dystrophy [119] and probably testicular degeneration [59], and there is some evidence that foxes [120] also require the vitamin. Cats [121] are said not to require vitamin E, but this may be due to the deprivation not having been long enough to produce symptoms [119]. "Steatitis," a condition which attacks minks, appears to be muscular dystrophy with "ceroid pigment" [129].

The duck [124] develops dystrophy of voluntary muscle, while in the turkey [125] it is the smooth muscle of the gizzard which is affected. The chick suffers from encephalomalacia or an oedematous condition of the body

(p. 619), and possibly muscular dystrophy [126]. The laying hen ultimately ceases to lay [62]. The testes of cockerels [127] degenerate and the development of the embryo in the egg is arrested [127, 128].

All farm animals, so far as they have been investigated, require vitamin E, though there is little information about the goat [107, 177] and horse [88], the paralytic myoglobinuria of the latter being possibly muscular dystrophy [130]. In calves Blaxter, Watts and Wood [131] have produced typical muscular dystrophy on a diet of dried skim milk, arachis oil, lard, glucose, vitamins A and D and minerals. The dystrophy is prevented by 50 mg. daily of alpha-tocopherol, unless 16 ml. daily of cod-liver oil is given instead of the arachis oil—an interesting observation, since the Ministry of Agriculture and Fisheries advises giving calves four times this amount of cod-liver oil. The cow, as Gullickson and others [45] have shown, may continue in perfect health for four generations on diets which cause resorption-gestation in rats. But in spite of normal reproduction without abortions, in spite of normal growth and lactation, in spite of all this, the animals may suddenly die, generally in the latter half of pregnancy or shortly after calving. Post-mortem examinations show no cause for death, though electrocardiograms during life suggest it is due to myocardial degeneration [132].

Cotchin [130] has given an excellent review of "stiff lamb disease," or muscular dystrophy of lambs, and has described his findings in a flock in Berkshire. The condition may attack large numbers of lambs when the ewes have been fed a poor ration during a hard winter. Besides typical lesions in the voluntary muscles the myocardium is often involved, but the central nervous system is spared. The ewes themselves are not affected, though there is slight evidence that a very prolonged deficiency may attack their muscles. At least 50 mg. of alpha-tocopherol are required to cause a slow recovery; death is generally due to pneumonia or to starvation, the lambs being too weak to suck. Sows [135] suffer from resorption gestations, and farrows from muscular dystrophy, and probably there is testicular atrophy.

Tadpoles require vitamin E for growth [133], but the hastened metamorphosis induced by thyroxine is abolished [134]. Insects probably require vitamin E [46, 47], though a report [122] that the vitamin is present in the queen bee's royal jelly has not been confirmed [123].

In man a deficiency of vitamin E possibly causes abortion and muscular dystrophy, but whether or no the nervous system is affected is still obscure (pp. 632, 635, 647).

From all these different manifestations of a deficiency of vitamin E it can be seen that the name "antisterility vitamin" is due purely to the chance use of rats in the first investigations. Had guinea-pigs, for instance, been first used the name "antidystrophic vitamin" would have been as widely adopted.

In the following pages the functions of vitamin E will be considered separately in relation to the various systems of the body and to various metabolic activities; but in order to avoid repetition some subjects which are relevant to several aspects of vitamin E are only discussed in detail once; therefore the reader should consult the index to find the whereabouts of any particular subject.

The Relation of Vitamin E to Reproduction. Rats are the animals which have been chiefly used in studying the part played by vitamin E in reproduction. The results of a deficiency of vitamin E in the female and male are so different that they need to be discussed separately.

Reproduction in the Female. Evans and Burr [2] stated that: "In (female) animals reared on E-free diets the processes of oestrus, ovulation, fertilization, migration and implantation take place in normal fashion, but the young are never born, resorption occurring instead." This sterility, unlike that of the male, could always be promptly cured by giving vitamin E. Later work, however, has modified these statements. Martin and Moore [138]

report that "after prolonged deprivation of the vitamin the œstrous cycle became abnormal, and it was impossible to render the animals pregnant." This failure to induce pregnancy is only due to failure of implantation [139], which is not surprising since there is in the uterus after prolonged deprivation a degeneration of the smooth muscle and brown discoloration due to small yellow granules in the muscle cells [138]. Further the uteri of rats which had been pregnant were often large and misshapen and metritis, salpingitis, and ovarian cysts were common. Hessler [140] also noted similar changes in the uterus which occasionally spread to the vagina and ureters. The reaction of the uterine muscle was normal both to drugs acting on it directly and to those acting through its nerve supply. Barrie [141] has recorded pigmentation apparently increased by pregnancy, and also fibrosis of the uterine muscle, and, in some cases, fibromyomata. The longer animals were deprived of vitamin E, the larger the amount they required for a normal pregnancy. A resorption pregnancy also increased the requirements of vitamin E. Females deprived of the vitamin tended not to mate. Vitamin E did not reduce the discoloration of the uterus unless pregnancy occurred — presumably because the increased uterine circulation of pregnancy is necessary to remove the pigment. The pigment is further discussed on p. 621.

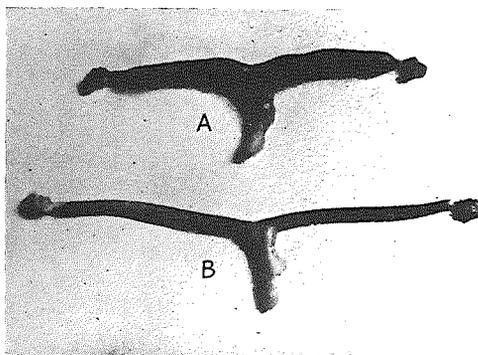


FIG. 211. Uterus A was taken from a virgin rat about fifteen months old which had received a diet deficient in vitamin E for about a year. Note the discoloration which contrasts with the normal colour of uterus B, taken from a control of the same age and history, which had received a supplement of two drops weekly of a preparation of the unsaponifiable matter of wheat germ oil.

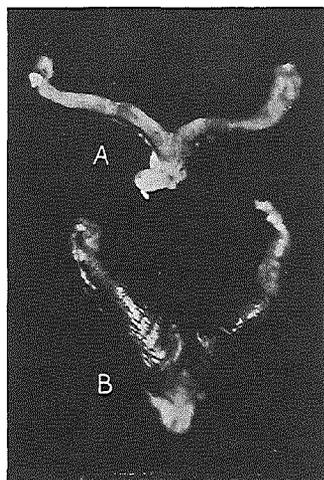


FIG. 212. Uterus A was taken from a normal rat which had been used for breeding purposes while receiving an adequate mixed diet. Uterus B was taken from a rat which received a diet deficient in vitamin E and which had undergone pregnancy during routine vitamin E tests over a prolonged period. It is not only discoloured but permanently enlarged and misshapen.

the sexual mechanism in the female, and not merely on the foetal tissues as was originally held by Evans and Burr [2].

Bacharach [142] has emphasized the increased requirement of vitamin E after a resorption pregnancy, and has also noted that in such animals the implantation rate is very low. The presumable explanation of the latter is the change brought about in the uterus itself from lack of vitamin E and foetal resorption. The latter is the important factor, since a moderately prolonged deficiency in virgin rats has no such affect.

Age itself also increases the need of vitamin E for reproduction: about tenfold from the first half year of life to the second year [145], while the prolonged œstrous cycle of old rats is largely due to lack of vitamin E [146].

From all this it can be seen that lack of vitamin E exerts a profound influence on all

The following account of the pathology of resorption gestation is chiefly taken from the classical work of Evans and Burr [2].

Until the eighth day of gestation no abnormality can be demonstrated by examination of the uterus or its contents, but some irreversible change has already taken place by the fifth day, since vitamin E deficient rats cannot produce living young if a supplement of vitamin E, however large, is given later than the fifth day of pregnancy.

From the eighth day onwards foetal development lags behind the normal by about one day. Mesodermal structures, especially, develop slowly. The hæmopoietic system is greatly affected, the mesodermal blood islands of the yolk sac being few in number. They also disappear early before the liver has adequately taken over the formation of the blood cells. This causes in most embryos, though not in all, a gross anæmia.

In the yolk sac there is a marked reduction in the size and number of the entodermal villi, and the growth and differentiation of the allantois is delayed. The foetal capillaries are generally small in number and only carried a short distance by the allantoic projections. The maternal placenta remains relatively normal apart from being small and having enlarged blood vessels. There is also in the early stages some placental hæmorrhage which appears in vaginal smears.

After the death of the foetus, which occurs about the thirteenth day, but may be later if the diet contains some vitamin E, complete resorption of the foetus slowly occurs. The placenta, however, continues to grow for a few days. Barrie [143], by giving small amounts of vitamin E, has prolonged gestation and delayed resorption until the twenty-eighth day.

The death of the foetus appears to be due to starvation and asphyxia brought about partly by the failure of the blood-forming organs and yolk sac, partly by failure of the allantois.

The female mouse also is very sensitive to lack of vitamin E [147], and is said to differ from the rat in that foetal death is in part due to decreased histiotrophe secretion by the uterine mucosa [148], though this might well be part of the reason for the failure of implantation in the rat. Further the ovaries in the mouse [112] are affected by vitamin E, an excess causing an increase in primordial ova, primary follicles, follicular cells and inter-follicular tissue with a decrease in the number but an increase in the size of the mature follicles and corpora lutea. Ultimately the ovary decreases in size and contains degenerating follicles filled with blood, and neutral fat is increased. A deficiency of vitamin E causes the opposite to an excess and there is a complete absence of neutral fat. Ultimately the ovary, comparatively large, is hard, yellowish and contains only cells filled with lipids other than neutral fat. Vitamin E, therefore, appears to stimulate the development of tissues derived from germinal epithelium and to influence fat metabolism in and around the ovary so that neutral fat increases and the formation of other lipids decreases.

In eggs from vitamin E deficient hens the embryo develops slowly and dies toward the end of the first week because its nourishment is cut off by a lethal ring in the blastoderm formed by cellular proliferation in the mesoderm [127, 128].

Toxæmias of Pregnancy. Most of the work on the effect of vitamin E on pregnancy toxæmias has been done on man (p. 633). Barrie [144], however, has reported thirty-eight cases in rats in six hundred and sixty-seven pregnancies. All these cases occurred in vitamin E deficient rats, and nearly three-quarters in rats which had been only partially deprived of vitamin E. Barrie states that the toxæmia was "associated with the resorption of foetal material brought about by the inability of the animal to continue the gestation owing to the lack, but not the complete deficiency, of vitamin E." This is of clinical interest, as in pregnant women a deficiency of vitamin E is unlikely to be ever complete.

Lactation. Loosli [149] and Gullickson [45], among many others working with cows, have shown that vitamin E has no effect on the quantity or fat content of milk. Loosli has also briefly reviewed the relevant literature.

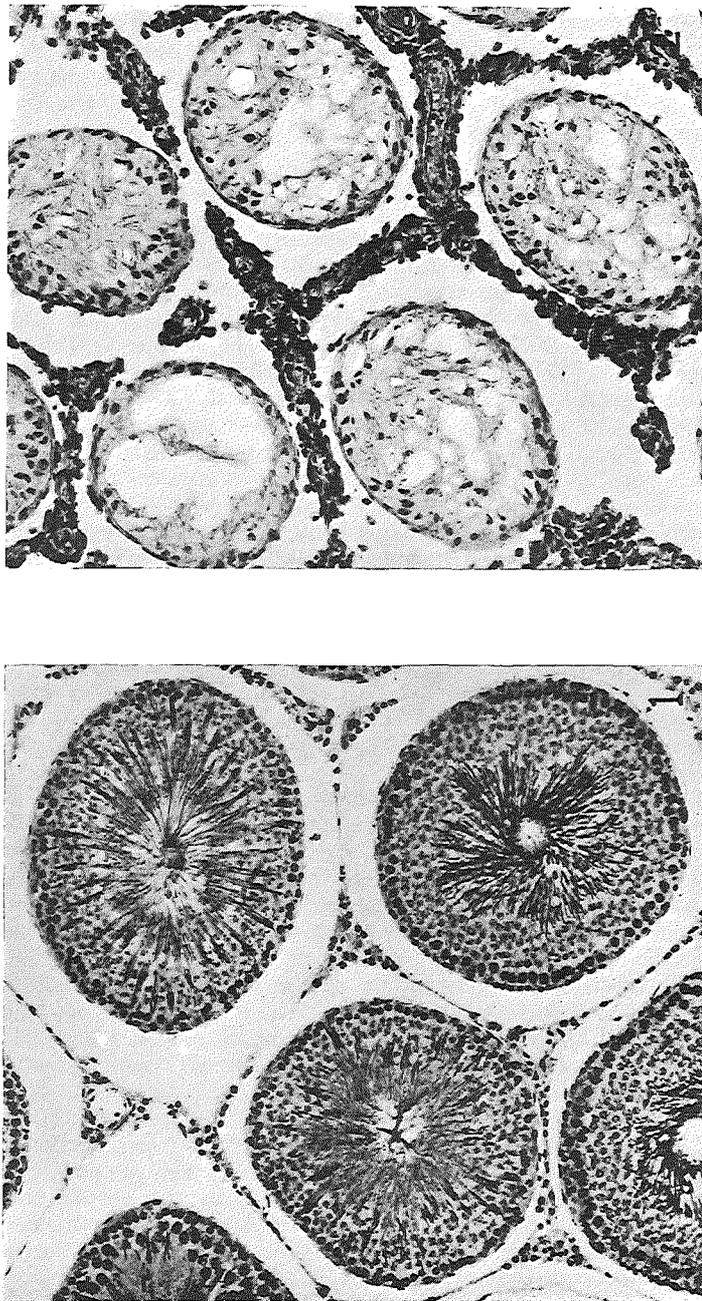


Fig. 213. Photomicrographs depicting the testes of litter mate brothers 277 days old fed on an identical vitamin E low diet, save that one brother (photograph 1) also received 0.75 mg. of alpha-tocopherol six times weekly. (For details see text, p. 610.)

In rats, also, the balance of evidence is against vitamin E being necessary for milk production [141]. Of course the amount of vitamin E in milk (p. 629) depends on how much is eaten.

Reproduction in the Male. The following account is taken from the work of Mason [150] and Evans and Burr [2]. In male rats born of mothers on normal diets, and then at weaning placed on deficient diets, sterility occurs when the rats are fifty to one hundred and fifty days old. If, however, such rats are also suckled by mothers on a deficient diet they never become fertile (see also p. 605).

In the first stage of the sterility the sperm appear completely normal both as regards their morphology and motility. Nor can any changes be seen in the testes. Even at this stage, according to Mason [150], the sterility cannot be cured nor complete degeneration of the testes averted. Evans and Burr [2], however, cured about one-quarter of their rats when testicular changes were already marked (Fig. 213) and Mason [136] has reported similar cures in the hamster.

As the degeneration of the testes progresses the sperm lose their motility and then begin to fuse together, or appear as fused cytoplasmic masses with fused sperm tails. After this no more sperm are produced, and the germinal epithelium of the testes continues to degenerate. This degeneration is first seen in the most mature cells, leading to fusion of the mature sperm. Then the spermatids and secondary spermatocytes show nuclear changes with liquefaction and segregation of the chromatin material. This is followed by the cells fusing together to form giant cells containing as many as forty nuclei. These giant cells tend to slough into the lumen of the tubule. Similar changes occur in the spermatogonia, but the Sertoli tissue undergoes little change. There is a marked difference in the rapidity of the degeneration in different tubules. The testicular changes brought about by deprivation of vitamin E are typical and never seen in any other condition [150]. Sexual interest is preserved for a long period after the onset of sterility, but is at length lost [2, 150, 151] as it is in the female (p. 607). Cater (p. 625) states that in chicks the first effect of deprivation is precocious stimulation of the germinal epithelium, which is the cause of the ultimate atrophy.

Other animals in which testicular degeneration has been reported are mentioned on p. 605 and Mason [150] believes he has seen it in human testes as do others in sprue (p. 600). Hyaluronidase (p. 597) may play an important part in testicular degeneration.

The Relation of Vitamin E to the Endocrine Glands. Drummond, Noble and Wright [151] reviewed the work on the relationship between vitamin E and the endocrine glands in 1939 and reported the results of extensive investigations of their own. They stated that "the experimental results which have been obtained do not indicate that the endocrine system is primarily responsible for the changes observed after feeding rats on an E deficient diet."

Testis. The testicular degeneration described previously was again confirmed by the above authors, who found that its progress could not be arrested with vitamin E concentrates alone or in combination with injections of extracts from human pregnancy urine or pregnant mare serum. The latter when injected into vitamin E deficient hypophysectomized rats also had no effect on the germinal epithelium, though the interstitial tissue responded. That the interstitial tissue of vitamin deficient animals continued to function is shown by the weight of the prostate and seminal vesicles remaining relatively normal. This is further shown by the normal reaction to drugs of the smooth muscle of the *vas deferens* and seminal vesicles from vitamin E deficient animals, which is quite different from the reaction of the smooth muscle from castrated animals [155]. Sexual interest, however, is ultimately diminished [2, 150, 151]. Mason [150] agrees that the testicular damage is not a secondary effect from the pituitary, since the latter's removal does not cause the typical degeneration seen after deprivation of the vitamin. Spermatogenesis in immature rats is not stimulated by vitamin E [160]. Castrated rats survive longer than normal rats when both are deprived of

vitamin E [156], but this is probably merely an example of decreased metabolism decreasing the need for vitamin E, even though testosterone has been reported to delay the onset of muscular dystrophy in vitamin E deficient rabbits [159].

Adamstone [157] found that vitamin E reinforces the action of testosterone in caponized male fowls: this could well be due simply to the vitamin protecting testosterone from oxidation in the same way that it appears to protect progesterone [158].

Estrogen. Bacharach and Chance [161] found alpha-tocopherol, when given by mouth or by injection, had no oestrogenic effect in rats. Beerstecher [162] reports that the oestrogens in the urine of vitamin E deficient rats is normal during their pregnancy until resorption occurs, when it drops sharply. Drummond and others [151] observed no effect, from giving vitamin E, on the genital organs of young rats or those of adult hypophysectomized rats. The uterus in spayed animals responds equally to injections of an oestrogen, whether or no the animals are deprived of vitamin E [164]. "Ceroid pigment" (p. 621) does not develop in the uterus of the deficient spayed animal—as long as the diet is low in unsaturated fatty acids—unless an oestrogen is also given [165], presumably because the metabolic activity of the infantile uterus is so low. The absorption gestation of vitamin E deficient rats cannot be prolonged to term by oestrogen [151, 166]. The effect of vitamin E on oestrus in old or deficient rats is discussed on p. 607, and the effect of a deficiency on the ovary of the mouse on p. 608. Minot and Dodd [163] have reported that the urines of seven boys with muscular dystrophy all contained oestrogens while the urines of normal boys did not.

Progesterone. The resorption gestation of vitamin E deficient rats cannot be prolonged to term by progesterone [151, 166] which is not surprising as the *corpora lutea* only degenerate after the death of the foetuses [166]. In normally menstruating women pregnanediol excretion is not affected by 50 to 150 mg. daily of alpha-tocopherol for one month [167], but when 60 mg. are given daily for one week to postmenopausal women before an injection of progesterone the subsequent excretion of pregnanediol is considerably larger than that following an injection without any previous tocopherol: this is almost certainly due to the tocopherol preventing the oxidation of progesterone [158].

Thyroid hypoplasia has been reported by Barrie [52] and Singer [55] in rats, and by Anderson and others [44] in dogs. Singer [55] found the hypoplasia did not respond at all to iodine and so little to injections of pituitary extract that she did not think it a pure anterior pituitary effect. Mason and Bryan [56], on the other hand, could not find any changes in the thyroids of young deficient rats, nor could Telford and his collaborators [57] in rats at any age. There appears to be no explanation of these contradictory reports. In chicks [154] the need for vitamin E is increased by thyroxine while in tadpoles [134] minute amounts of vitamin E abolishes the stimulating effect of thyroxine on metamorphosis.

Pituitary. The pituitary both in function and structure has been reported to be affected by lack of vitamin E. Barrie [143] and Underhill [7] state that the anterior pituitaries of both young and old rats show degranulation of the acidophils, a spongy appearance of some of the basophils, and considerable numbers of empty cells which are presumably acidophils. They think that a deficiency of vitamin E hinders the formation of gonadotropic, thyrotropic, and galactotropic hormones. This would explain the condition of their young rats which had very soft, poorly ossified skulls and cretinism with hypoplastic thyroids; it also explains the difficulty adult rats may have in suckling. Singer [168], however, failed to prevent the thyroid hypoplasia with pituitary extracts and mammary regression occurs only after foetal resorption [166]. Most workers also stress the normal appearance of young rats even when these have muscular dystrophy.

Nelson [169] and Mason [150] have reported "castration changes" in the pituitaries of vitamin E deficient male rats, leading to an increase in the factor for stimulating the female genital system in young rats. Since no such changes were found in the pituitaries of female rats, those of the males were thought to be secondary to the testicular degeneration. Rowlands and Singer [170], however, tested the activity of pituitary extracts from female deficient rats on rabbits in oestrus, instead of young rats. From this they deduced that lack of vitamin E caused a decrease in the luteinizing or ovulation producing substance but not in the follicle stimulating substance. These results have been largely confirmed by Drummond, Noble, and Wright [151] using hypophysectomized rats as the test animals. Pan and others [171], however, report that in deficient animals of *both* sexes gonadotrophin is increased when hypophysectomized young males are used as the test animals.

Kepinov [172] has observed that in frogs vitamin E is necessary for the formation of the glycogenic hormone of the anterior pituitary. In starved or hypophysectomized frogs injections of adrenaline cause no mobilization of glycogen from the liver, but after vitamin E is given adrenaline produces glycogenesis in the starved but not in the hypophysectomized frogs. Sugar metabolism is further discussed on p. 594.

The resorption gestation which always occurs in vitamin E deficient rats cannot be prolonged to term by giving injections of whole pituitary glands, by implanting two fresh pituitary glands every other day or by injections of extracts of pregnant mare serum or human pregnancy urine [151].

The weights of the pituitary, adrenals, ovaries, and uterus of deficient animals remain normal, while those of the prostate and seminal vesicles are not greatly reduced compared to the diminution of the testes [151]. A longer list of organs is given by Copping and Korenchevsky [7], who also report that the thymus is larger in both male and female vitamin E deficient rats. Tonutti [172] has reported involution of the adrenal cortex.

"From a consideration of the results obtained it appeared very unlikely that the effects of E-deprivation could be explained by an alteration in function of the anterior pituitary gland, the slight changes recorded being in all probability related to a secondary effect on the pituitary" [151].

Clinical work on vitamin E and the sex hormones is discussed on p. 632.

The Relation of Vitamin E to Growth. Impaired growth is one of the earliest symptoms of a deficiency of vitamin E both in the embryo (p. 608) and the young animal. Evans and Burr [2] noted that the growth of rats was affected by the amount of vitamin E in the diet, and Copping and Korenchevsky [7] state that "the final body weight, total gain in weight and amount of fat deposition were less in the rats deprived of vitamin E than in the corresponding controls." Barrie [143] has emphasized the poor growth of her young rats, and Nelson and others [174] have reported that on diets deficient in vitamin E rats stop growing, to begin again when alphatocopherol is given. Emerson and Evans [175] bred four generations of rats on diets low in vitamin E. Each successive generation grew less than the last.

Rabbits may cease to grow days before any other symptom of a deficiency appears (p. 614). Foxes [120], dogs [119], insects [46, 47], tadpoles [133] and children (p. 647) also probably require vitamin E for proper growth. The relation of vitamin E to fat and to protein metabolism is discussed on p. 620.

The Relation of Vitamin E to Muscular Dystrophy. A degeneration or dystrophy of the voluntary muscles occurs in virtually all animals (p. 605) which have been deprived of vitamin E for a sufficiently long period, and also possibly in man (p. 635). This degeneration is a specific result of deprivation of vitamin E, never following deprivation of aneurine, riboflavine, pantothenic acid, pyridoxine, vitamin A or protein [255]. However, lack of any of the last three added to lack of vitamin E greatly intensifies the degeneration, though lack of the first three makes no difference [225].

How far the muscular dystrophy is a *primary* effect of lack of vitamin E and how far it is a *secondary* effect brought about by changes in the nervous system is obscure. The subject is discussed on p. 617.

Muscular dystrophy in animals will be discussed under the headings of (a) the clinical picture, (b) the muscular degeneration, (c) cardiac failure, (d)



FIG. 214. Photomicrographs of muscle removed for biopsy from the calf of an English boy aged seventeen with pseudohypertrophic muscular dystrophy. In the top fibre transverse striations are barely evident and the left-hand portion shows well wrinkling and longitudinal fraying. In the second fibre these changes are more marked: transverse striations have completely vanished, wrinkling and fraying are very extensive and there is considerable sarcolemma nuclei proliferation. The other fibres show varying degrees of similar change.

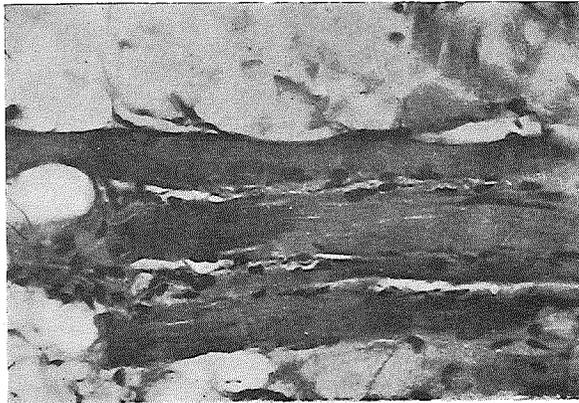


FIG. 215. Further photomicrograph of the muscle in Fig. 214. The middle fibre shows on the left a portion of healthy muscle fibre with well-marked striations, a very rare finding. The right-hand portion of the fibre shows swelling and hyaline change with complete loss of the striations.

the changes in metabolism, (e) rancid fats: their destruction of vitamin E and their relation to muscular dystrophy, (f) the cure of muscular dystrophy.

The Clinical Picture. Evans and Burr [3] first reported a mysterious paralysis which appeared in the suckling young of rats deprived of vitamin E. Suddenly, towards the end of weaning, the young, who till then had appeared normal, became weak, lost weight, and sometimes died in a few hours. A few animals escaped altogether, and some spontaneously recovered without treatment. Others again did not die but remained in good health

though partially paralysed. In adult rats muscular dystrophy (Fig. 216) develops very slowly [5, 6, 138]. But the rat is not a satisfactory animal to use when investigating muscular dystrophy because a nervous degeneration may obscure the picture (p. 617).

Guinea-pigs and rabbits, on the other hand, develop muscular dystrophy without any neurological complications (p. 617). The condition appears within a few weeks on a deficient diet and, unless vitamin E is given, always progresses rapidly to death. Generally the onset of muscular dystrophy prevents deficient rabbits from reaching sexual maturity, but in a few dystrophic animals Pappenheimer [116] has reported the birth of living young, and here muscular dystrophy was present at birth. It is interesting to note in passing that Mackenzie and McCollum [29] point out that experiments purporting to show rabbits do not need vitamin E for reproduction must be fallacious as the mothers would die of dystrophy before complete depletion of vitamin E could prove it unnecessary for reproduction.

The Muscular Degeneration. Goettsch and Pappenheimer [4] in 1931 first described what they termed "nutritional muscular dystrophy" in guinea-pigs and rabbits when these animals were deprived of vitamin E. They wrote "... in the more chronic cases ... these muscles with replacement fibrosis and lipomatosis closely resemble those of progressive muscular dystrophy in man." In 1940 Pappenheimer [116] in an excellent review stated: "The disease may run a chronic course. In such animals comparatively few fibres are destroyed at any one time, but their gradual loss and replacement by fat and fibrous tissue brings about a picture which is identical with that of an advanced case of human muscular dystrophy." Olcott [178] has described the pathological changes in the muscles of young rats (see Figs. 214 and 215).

Not all muscle fibres degenerate at the same time, some remaining normal while others rapidly disintegrate. The first change in the affected fibres is loss of their transverse striations, and a hyaline change, sometimes going on to complete degeneration. Polymorphs and histiocytes appear, the latter often fusing into giant cells. Many fibres make attempts at regeneration; in some the nuclei increase and the muscle substance splits longitudinally, making fresh small fibres; in others the nuclei multiply rapidly under the sarcolemma, so that a tube of nuclei is formed. On the surface new myofibrils start to reform new muscle fibres. These efforts at regeneration go on even in the midst of the most intense degeneration [4, 116, 138]. The pigment which occurs in dystrophic muscles is discussed on p. 621. Hoagland and others [179] have described the changes in human dystrophic muscles shown by ultra-violet photomicrography.

Cardiac Failure, in spite of the absence of pathological changes in the cardiac muscle of rats [178] or in their electrocardiograms [180], appears to be the reason for the sudden death of animals with muscular dystrophy; a death which it is hard to explain by the condition of the voluntary muscles. In rabbits with muscular dystrophy foci of acute myocarditis may occur together with electrocardiographic changes [115], while Houchin and Smith [181] have shown, by well controlled work, that dystrophic rabbits die from doses of posterior pituitary extracts which are harmless to normal rabbits. On the other hand dystrophic rabbits not only tolerate doses of digoxin and ouabain which are normally lethal, but also have their lives prolonged by such doses. These findings appear to be only explicable on the assumption that the heart of the dystrophic rabbit is so severely damaged that the decrease in the cardiac circulation brought about by the pituitary extract precipitates cardiac failure, while the different toxicity of the cardiac glucosides in the normal and dystrophic animal is that which would be expected if they were acting upon a normal or upon a failing heart. Houchin and Smith [181] suggest that lack of vitamin E may be a factor in the sudden cardiac failure of beriberi, and may also be of importance in the nutrition of the human

heart, a subject discussed on p. 657. In deficient cows (p. 606) sudden cardiac failure occurs, preceded by electrocardiographic changes, though there is little structural damage to the heart, while in dystrophic lambs (p. 606) there may be gross damage. In deficient monkeys there is virtually no damage [87] but there are changes in the electrocardiogram [86, 92]: the amplitude of the R and T waves is reduced with inversion of the latter [86], and also there is shortening of the time for the initiation of ventricular ejection [86]. The effect of vitamin E on the vascular system and blood is discussed on p. 622.

Changes in Metabolism. The metabolic changes brought about by lack of vitamin E are discussed on p. 594, so that here it is only necessary to draw attention to the way in which the creatine in the urine reflects the disturbed metabolism of the muscle. Just as dystrophic changes may be found in the muscles while the animal yet appears normal [103, 214], so may the creatine rise in the urine before any clinical change can be seen. Ni [182] was the first to apply to research in muscular dystrophy the well-known fact that urinary creatine is increased when the volume of functioning muscle is decreased [183]. He and Mackenzie and McCollum [29] noted the warning rise in the creatine of animals deprived of vitamin E. The latter authors have very fully investigated the subject and report that the best guide to the approach of muscular dystrophy is the rise in urinary creatine. This may occur while the increase in weight and the appetite are still satisfactory. The drop in creatine brought about by giving vitamin E is dramatic and precedes clinical improvement in strength, appetite and weight. It occurs in from twenty-four to forty-eight hours [29, 103]. There is increased destruction and increased formation of myoglobin, the former predominating [289]: this is of considerable interest as paralytic myoglobinuria in horses [130] has much in common with "stiff lamb disease" (p. 606), which is due to lack of vitamin E.

Rancid Fats: their Destruction of Vitamin E and their Relation to Muscular Dystrophy. Rancid fats rapidly destroy vitamin E by oxidation. This destruction is most liable to occur when vitamin E is in the form of the synthetic vitamin or concentrated preparations, since then it is no longer protected against oxidation by the anti-oxidants found associated with it in such natural sources as whole wheat germ [215].

It is important to realize that as vitamin E itself is an anti-oxidant it will be destroyed by fats before they become rancid through auto-oxidation. In other words a fat need not be rancid, certainly not smell rancid, before all its vitamin E is destroyed. Further, rancid fats can destroy vitamin E during digestion as long as both are fed together or within a short time of one another [50, 103, 216]. Claims that rancid fat given by mouth or by injection destroys vitamin E already absorbed into the body [217, 218] require further confirmation, probably being incorrect [99, 219]. If substantiated they are an argument against using ketogenic diets in any medical treatment.

The destruction of vitamin E by fats, due to their auto-oxidation, was investigated by Cummings and Mattill [220], who state that "the oxidation of unsaturated fats by atmospheric oxygen causes the formation of substances which impart to those fats a characteristic acrid odour, usually described as rancid." Rancidity, however, is difficult to define: some of its products (p. 671) are free fatty acids, aldehydes, ketones, and peroxides. Some substances having hydroxyl groups, such as wheat germ and other vegetable oils, retard rancidity and so protect vitamin E, but ultimately wheat germ oil itself becomes rancid. The fats commonly used in food are auto-oxidizable in the following order: cod-liver oil, lard, butter. Margarine is compounded of sundry fats and hydrogenated oils: far from destroying vitamin E it may even possibly be a good dietetic source, though it has many nutritional disadvantages.

From the point of view of the diets used to produce muscular dystrophy

this destructive action of fats is most important. Not realizing this has led to much confusion. When Goettsch and Pappenheimer [4] first reported nutritional muscular dystrophy, they did not consider a deficiency of vitamin E was the cause because wheat germ oil supplements failed to prevent the dystrophy. It can now be seen that this was because their diet contained so much cod-liver oil and lard that the vitamin taken in the wheat germ oil was destroyed during digestion [103].

Ni [222] also made a perplexing observation, when by adding Chinese donkey skin gelatine (ah-chiao) to a vitamin E deficient diet he prevented and cured muscular dystrophy. This not only lent support to, but also gained support from, the treatment of human muscular dystrophy with gelatine (p. 646). The explanation, however, according to the further work of Ni [224], is that the ah-chiao, being added to the diet as a powder, adsorbed pro-oxidants and so prevented the destructive oxidation of vitamin E.

The most serious result of not realizing the effects of rancid fat was that Morgulis and Spencer [221] were led to postulate that two factors were necessary to prevent or cure muscular dystrophy. One was fat soluble and probably vitamin E, the other was water soluble being found in wheat germ and lettuce. The evidence for this came from observations that wheat germ oil alone could not prevent dystrophy but wheat germ oil and lettuce or whole wheat germ could do so. Much work has been devoted to investigating this "dual theory" of muscular dystrophy, which has now been proved wrong.

The most thorough proof that deprivation of vitamin E is the sole cause of muscular dystrophy is given by Mackenzie and others [103] using the same diet as Goettsch and Pappenheimer. Alpha-tocopherol given separately from the rancid food, so that no destruction of the vitamin could occur, prevented muscular dystrophy. Wheat germ from which vitamin E had been extracted (defatted wheat germ) had no effect. Further, alpha-tocopherol cured animals who had had no "water soluble factor," which proved that the cure was not due to stores of the latter in the body. To complete the destruction of the "dual theory" of muscular dystrophy it was shown that animals who received wheat germ oil mixed with the rancid ration became dystrophic, but if defatted wheat germ was added as well dystrophy did not occur: the defatted wheat germ protected the vitamin E from oxidation. This rounds off the proof that vitamin E is the only vitamin necessary to prevent muscular dystrophy and that the superiority of wheat germ over vitamin E alone, or as wheat germ oil, is solely due to its protecting vitamin E from destruction by rancid fats. This, of course, does not mean that the other vitamins and protein which are synergistic with vitamin E from the point of view of muscular dystrophy and are also contained in wheat germ do not assist in preventing, though they cannot prevent, muscular dystrophy. The animals discussed above were all on diets containing an abundance of all the vitamins except vitamin E.

The Cure of Muscular Dystrophy. Vitamin E cures and prevents muscular dystrophy in all the many animals mentioned on p. 605, but it can only prevent and not cure dystrophy in adult rats (p. 613). In the previous section it has been seen that the vitamin must be given so that it does not come in contact with rancid fat either in the food or in the stomach. Where this may, however, occur the vitamin should be given as whole wheat germ, because in this form it is given some protection against oxidation. Apart from this it is unimportant what preparation of vitamin E is used—whole wheat germ, wheat germ oil as long as it itself is not rancid, or alpha-tocopherol. The latter may also be given by injection, but is less potent than when given by mouth (p. 599). The value of the other tocopherols and the esters is discussed on p. 594. The importance both of protein and of vitamin E synergists which are of course present in wheat germ must not be forgotten.

Vitamin E acts with amazing rapidity. Within twenty-four hours the creatine in the urine may drop, and in a couple of days may be normal. The appetite and weight of animals improve as the creatine falls, and a normal rate of growth is re-established in one or two weeks. Even when the animals are so weak they cannot stand or feed themselves vitamin E leads to a rapid return of strength in one or two days, and by the end of a week few symptoms of the dystrophy may be left. The cure of such animals is permanent and complete. All trace of even severe dystrophic changes may vanish from the muscles in a week [29, 103, 116]. In children, however, recovery, if it occurs, may take years (p. 641).

The Relation of Vitamin E to Nervous Degenerations. In rabbits and guinea-pigs, as has been seen in the previous sections, lack of vitamin E

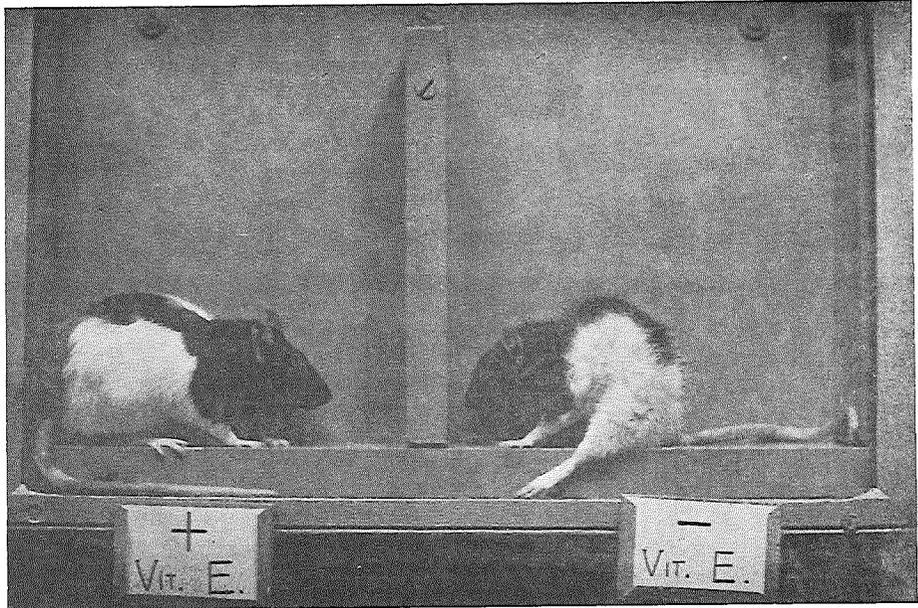


FIG. 216. The rat in the right partition is a virgin female which received a diet deficient in vitamin E for about thirteen months. It is emaciated, rough haired and has paralysed hind legs. The rat in the left partition is a control animal which received 3 drops weekly of the unsaponifiable matter of wheat germ oil.

causes the rapid onset of a muscular degeneration which can be rapidly cured by the vitamin. It is highly improbable that this degeneration is secondary to any nervous lesions because no changes have been found in the central nervous system [116] and the terminal neurites or end plates in the muscles remain normal even when the muscle fibres have completely degenerated [227]. Further the rapidity of the recovery when vitamin E is given is far greater than could be accounted for by regeneration of nervous tissue. In rabbits and guinea-pigs, therefore, it seems most probable that lack of vitamin E causes a primary muscular dystrophy uncomplicated by any neurological degeneration, though it must be admitted that some workers (p. 618) have reported functional or anatomical changes in the nerves of these animals.

In rats, on the other hand, lack of vitamin E probably affects both the muscular and the nervous systems so that a primary muscular dystrophy and a secondary muscular degeneration occur together. The paralysis of

rats, however, is a complicated problem, partly because some observers report extensive degeneration of the nervous system while others do not, partly because young deficient rats may spontaneously recover without vitamin E and partly because adult rats, though they develop paralysis very slowly, cannot be cured by vitamin E nor even have the progress of their paralysis checked. This inevitable progress of the paralysis is more typical of a neurological than a muscular disorder and is entirely unlike the primary muscular dystrophy of the rabbit and guinea-pig, which can be rapidly cured.

The outstanding work on the nervous system in deficient adult rats is that by Ringsted [5] in 1935 and Einarson and Ringsted [6] in 1938. At the date of their experiments pure synthetic alpha-tocopherol was not available so wheat germ oil had to be given to their control animals. Apart from this probably unimportant point, their work and their histological technique appear to be beyond criticism. They reported that the initial lesion is a degeneration, in the lumbar cord, of the proximal parts of the posterior roots and the proprioceptive paths in the posterior tracts and probably in the uncrossed tactile paths. The amount of neuroglial reaction is inconstant, depending apparently on the rapidity of the degeneration. The cells of the spinal ganglia are not affected. Degeneration of the anterior horn cells of the lumbar cord generally begins shortly after the degeneration of the posterior columns and progresses until it is more or less complete. With the death of the motor cells the motor fibres of the peripheral nerves degenerate and the muscles atrophy. The pyramidal tracts might be expected also to be constantly affected instead of only slightly and rarely, but these tracts are of very recent origin in the evolution of the nervous system, being unimportant in the rat and not really analogous to those in man. As it is, "We have, on the whole, a pathologic-anatomical picture that resembles closely a combination of two of the most important systemic degenerations occurring in man, namely: tabes dorsalis and spinal progressive muscular atrophy" [6]. If the rat had true pyramidal tracts, the picture would be complete. Einarson and Ringsted [6] failed to prevent the progress of the nervous degeneration by giving vitamin E, except possibly in its earliest stages. An outstanding review of the whole subject was made by Einarson [416] in 1952.

Luttrell and Mason [228], Malamud, Nelson and Evans [229], Monnier [6] and Gutierrez-Mahoney [231] have confirmed that lack of vitamin E causes a widespread degeneration of the central nervous system of adult rats, though their descriptions of the lesions differ and also do not exactly tally with those given by Einarson and Ringsted. These discrepancies between the reports of various workers do not invalidate their essential agreement, since differences in diet, and so in the rapidity of the neurological degeneration, will considerably affect the final condition of the nervous system. This was originally investigated and emphasized by Einarson and Ringsted [6] and has been confirmed by others [228, 229]. Lipschutz [233] has described similar changes in the nervous system of the young paralysed rat.

On the other hand, Olcott [178], Pappenheimer [116] and Wolf and Pappenheimer [232] have all failed to find any changes in the nervous system of young or old paralysed rats. Differences in diet, the varied length of the deprivation and the different ages of the animals offer an adequate explanation [6, 228, 229] of these negative findings.

The motor end plates in the muscles of paralysed rats have been found to be normal by Pappenheimer [116] and few in number by Telford [214]. Confirmation of the latter's work comes from the observations of Hines and others [234], who showed that both in dystrophic rats and guinea-pigs the tension in muscle developed after direct electrical stimulation was greater than that after stimulation of the motor nerve. De Castro and others [235] claim that in rabbits changes in the central and peripheral synapses precede the muscular degeneration.

Cutting the motor nerve of a muscle protects it from the onset of

dystrophy [116, 234]. This effect cannot be due to any removal of an abnormal nervous stimulation since it also occurs when the tendon is cut [116]. This strongly suggests that vitamin E is necessary for the active but not the resting muscle.

Neuromuscular regeneration after crushing of a motor nerve is not delayed by a deficiency nor hastened by a super-abundance of vitamin E [234].

Wheat germ oil is reported to give protection against the neurological symptoms of distemper and diphtheria.

Davison's extremely important investigations (p. 654) on human amyotrophic lateral sclerosis also strongly suggest that vitamin E is intimately connected with the central nervous system. He treated ten cases with wheat germ oil and alpha-tocopherol, and in six of these post-mortem histological studies (Figs. 225 to 238) showed that, in comparison with untreated cases, there was less destruction of axis cylinders and myelin sheaths and considerably less gliosis. There was no difference in the nerve cells of the bulbar nuclei and anterior horn cells.

Vitamin E Deficiency in Chicks. In chicks lack of vitamin E causes two quite separate conditions—nutritional encephalomalacia and the "alimentary exudative diathesis."

Nutritional Encephalomalacia. This was first described by Pappenheimer and Goettsch [290] in chicks fed on diets deficient in vitamin E. The chicks thrived for about three weeks and then suddenly or slowly developed weakness, ataxia, tremors, retraction of the head and other symptoms pointing to some nervous disorder. Death was common, but about two-fifths of the chicks never developed the disease and others recovered without treatment, in the same way that young rats with no treatment may recover from paralysis (p. 613). As the birds grow older the condition becomes rarer, never being seen in the adult [116].

All parts of the brain may be affected, but the severest lesions are generally found in the cerebellum. There is extensive ischæmic necrosis with œdema, small hæmorrhages, and hyaline thrombi in the small vessels in and about the necrotic areas. The brain elements are disrupted, with degeneration and necrosis of the cells. Pappenheimer [116] states that while the arrest of the circulation is the cause of the condition it is impossible to tell whether the closure of the vessels was originally functional or caused by the hyaline thrombi. The cholesterol content of the brain is said to be decreased [157], but this is probably incorrect [293]. Adamstone [291] has compared the neurological degenerations caused by lack of vitamin A and vitamin E.

Alimentary Exudative Diathesis. This is the name given by Dam and Glavind [30] to a condition which appears between the sixth and thirtieth day in chicks reared on a diet low in vitamin E.

Not all chicks are affected; those that are die in six to eight weeks. Protection is given by synthetic vitamin E. There is a generalized œdema with fluid of the composition of blood plasma collecting in the subcutaneous and subfascial tissues, especially those of the breast and abdomen. The fluid also collects in the pericardial and peritoneal cavities. The brain and lungs are œdematous, and there is coronary and intestinal hyperæmia. Deposits of urates are found in the kidneys and ureters. This œdematous condition is reminiscent of that found by Pappenheimer [116, 216] in young mice and rabbits born with muscular dystrophy.

Dam [293] in 1944 reviewed his own work and that of others on these two deficiency diseases of chicks, especially from the point of view of what factors decide which disease shall develop. The amount and kind of fat in the diet is extremely important. On fat-free diets exudates seldom develop and encephalomalacia never. Both conditions are accentuated by highly unsaturated fats; fresh cod-liver oil, linseed oil and lard especially causing

exudates while the fatty acids from hog liver cause encephalomalacia. Oleic acid and thoroughly rancid cod-liver oil have no effect, but a mixture of rancid and fresh cod-liver oil has the same effect as fresh cod-liver oil. This shows that it is the unsaturation of the fats and not their rancidity which is important. An increase in the sodium chloride of the diet increases exudates and so does a certain carbohydrate protein ratio and also cholesterol, though the latter gives protection against encephalomalacia. Inositol [293] and methylene blue [292] give partial protection against both conditions: partial protection against exudates only is given by antabuse, thionine, *nordi-hydroguaiaretic acid*, thiodiphenylamine and ascorbic acid [292]. Methylene blue, antabuse and thionine cause higher levels of vitamin E in the depot fat and decrease peroxidation [292]. Ni [224] reports that large amounts of vitamin A and carotene, and possibly vitamin D, increase the incidence of encephalomalacia, presumably by increasing the intestinal destruction of vitamin E.

The Relation of Vitamin E to Hepatic Necrosis and to Protein Metabolism. Rats deprived of *both* vitamin E and sulphur-containing amino acids develop acute massive hepatic necrosis [275-278]. Casein, however, confers more protection against such necrosis than would be expected from its content of thio-amino acids, so that there would appear to be some additional protective factor in first-class protein [275, 277]. Confusion in the past has been caused by some investigators failing to produce necrosis owing, as Lindan and Himsworth [277] have shown, to their not realizing that adult animals reared on stock diets, or any animals previously given a high protein diet or large amounts of vitamin E are all protected for a long period against the effects of a dual deficiency of vitamin E and protein [277]. Young rats, especially males, very frequently also develop massive lung hæmorrhages and distension of the subcutaneous blood vessels [275].

The reason for this hepatic necrosis may be the inability of the doubly deprived liver to detoxicate poisons or toxins. Thus György and others [278] have shown that necrosis is delayed from about forty days to about one hundred days by oral aureomycin, terramycin and streptomycin. The probable reason for this is that all these drugs destroy bacteria in the gut and so prevent the absorption of their toxic metabolites and their subsequent injury of the liver. That these antibiotics do not directly aid the liver is proved firstly by their effect not being permanent, owing, presumably, to the appearance of resistant strains of bacteria in the gut; secondly by the effect of streptomycin which is not absorbed and which has its protective effect enhanced when its antibacterial effect is enhanced by mixing with pectin; thirdly, poorly absorbed sulphaguanidine also has some protective effect, though the easily absorbed succinylsulphathiazole hastens the symptoms of vitamin E deficiency [279]. Penicillin, polymyxin and chloromycetin have no effect, presumably because they alter the flora of the bowel in a different manner from the other antibiotics.

Protein appears to be better utilized in the presence of vitamin E, but in a very inconstant and confusing manner [272, 273]: for instance with a diet containing ten per cent. of casein utilization is better but it is worse with a diet containing fifteen per cent. of wheat gluten [273]. Vitamin E is also stated to cause a hyperproteinæmia when fed to rabbits and rats daily in 30 mg. and 10 mg. doses [274], while lack of vitamin E has the opposite effect [280].

The Relation of Vitamin E to Fat Metabolism. The destructive effect of the polyunsaturated fatty acids on vitamin E and the rôle which vitamin E may play in preventing the conversion of such acids into intra-cellular pigments has been discussed on pp. 615 and 621, while the influence of fat on the symptoms of vitamin E deficiency in the chick is described on p. 619. Vitamin E also plays a direct part in the metabolism of ordinary fat. Thus mice [263] fed on a diet rich in fat but deficient in vitamin E become lean,

while their liver cells contain abnormal, insoluble, pigmented "lipo-proteic" globules, but no neutral fat. On the other hand mice on the same diet with 2.5 mg. of vitamin E daily become very fat and their liver cells contain excessive neutral fat, though very little of the "lipo-proteic" substance. Similar changes occur in the ovaries of mice [112] and Copping and Korenchevsky [7] long ago noted that deprivation of vitamin E reduced the amount of fat laid down by rats. The changes in the muscles of dystrophic calves are discussed on p. 596. Both cholinesterase and lipase are said to be reduced in the plasma and brain of vitamin E deficient rats [264]. Hickman [265] reports that vitamin E does not inhibit lipoxidase but does inhibit chain and side reactions. The other changes which occur as a result of a lack of vitamin E are a decrease in cholinesterase activity in the liver, brain, serum [372] and muscle [374], an increase in the cholesterol content of dystrophic muscles and the blood [177] and brain [157, 373]—though this has been denied for chicks [293]—and some increase in other lipoids in the muscles [373], but not in the heart or other organs [177]. It is interesting to note that alpha-tocopherol is said to raise the level of cholesterol and fatty acids in the blood of schizophrenics [375]. The relation between vitamin E in the blood and cholesterol is described on p. 601.

The Relation of Vitamin E to Pigmentation. Martin and Moore in 1936 first observed a brown fluorescent discoloration of the uterus of rats deprived of vitamin E. Later work by Martin and Moore [138], Moore and Wang [256], Barrie [141] and many others [257-260] has shown that this fluorescent discoloration is due to small yellow-brown, acid fast, iron-free insoluble granules within the muscle cells of the uterus. The uterus, however, is only the first tissue of the rat to be affected, nearly every other tissue becoming affected if the deficiency is sufficiently prolonged. Thus in the rat pigment also appears ultimately in voluntary muscles, the diaphragm apart from the area round the oesophagus, the heart, kidneys, ovarian stroma and Graafian follicles [256]; and in the fallopian tubes, vagina, trabeculae and capsule of the spleen, seminal vesicles, prostate, vas deferens, ureter, bronchi, small intestine and the walls of the pulmonary and uterine blood vessels [257]; in the interstitial tissue of the testis and the motor ganglion cells of the cord and medulla [258]; and within the fat cells [259]. The appearance of pigment in the lymph nodes is probably due to its transport there by the macrophages which can be seen in the affected tissues packed with the pigment [257, 259]. The foetal rat from a brown uterus remains unpigmented [256].

The pigmented uterus of the rat responds normally to drugs (p. 607). The pigment does not appear, or only very slightly in the infantile uterus (p. 607) and can be reduced by vitamin E together with a normal pregnancy [141] though vitamin E alone has no effect [138]. In the mouse [111] the pigment is said to be only in the reticulo-endothelial cells of all organs save the heart, liver and adrenal cortex, where it appears within the parenchymal cells. In guinea-pigs pigment has been reported in the testes [258] but not in dystrophic muscle [256]; in dystrophic calves no mention is made of pigment [131] while it appears to be readily produced in the hamster and cotton rat and dog [87]. In the monkey [87] pigmentation of the smooth muscle of the blood vessels and intestine is very pronounced and less pronounced in the muscle of the urinary bladder, gall bladder, bronchi, bronchioles and ducts of the epididymis.

The nature of the pigment is still in doubt. Moore and Wang [256], from very thorough chemical studies on the extracted pigment, believe it to be oxidized and partially deaminated protein. Most other workers [259, 260], however, hold that it is formed by the polymerization of the peroxides of poly-unsaturated fatty acids, basing this belief on its histochemistry [260] and on the observation that the peroxide value of fat increases as the pigmentation increases [259, 261]. The pigment may be the same as the

“ceroid” pigment, thoroughly described by Endicott and Lillie [262] in regard to its histochemistry, which occurs in the livers of rats rendered cirrhotic by a deficiency of protein. Since such cirrhosis-producing diets generally have been deficient in vitamin E and when rich in vitamin E have been reported to cause cirrhosis without “ceroid” [258], it would seem very probable that “ceroid” and the pigment induced by lack of vitamin E are the same: a probability increased by their very similar properties [260, 262]. If they do differ slightly it could well be that this is only because the cell labouring under lack of vitamin E produces slightly different abnormal waste products according to whether there is too little protein or too much fat, in the diet. There is also a second fat soluble pigment caused by lack of vitamin E about which nothing is known [259].

In human tissues “ceroid,” with the distribution of the pigment found in vitamin E deficient animals, has been found in conditions where the absorption of vitamin E has probably been grossly impaired [56, 258] and where other changes also indicative of lack of vitamin E, such as muscular dystrophy, have occurred. Pigments similar to or identical with ceroid have been found in many tissues—especially in atrophic testes and in association with hæmochromatosis [258].

The practical implications of this production of pigment by a dietetic deficiency are fascinating. Old age is associated with the appearance of various pigments within the cells of the body. They appear to represent an accumulation within the cell of waste products which cannot be excreted—in fact “metabolic clinkers.” It is possible that the necessity for sexual reproduction, for recurrently rebuilding the animal from a single cell, is that old cells become too choked with unexcretable pigments to live. As lack of vitamin E is one reason for the accumulation of such pigments within the cells of animals and as a similar or identical pigment is found in many human tissues—especially when deprived of vitamin E—it well may be that a mild deficiency of vitamin E when prolonged for many years greatly increases the choking of the cells with “clinkers” and so is one of the reasons, and probably the only avoidable reason, for senile degeneration.

Dental Depigmentation of the perpetually growing incisor teeth of rats deprived of vitamin E was first observed by Davies and Moore [266] in 1941, who later showed that piebald rats are more resistant to this bleaching than are albino rats [267]. The pigment of the teeth is not a lipochrome, porphyrin or melanin but does contain inorganic iron in the ferric form [268]. In the depigmented teeth iron is greatly reduced both in the enamel and dentine, while manganese is greatly increased, and magnesium is slightly increased in the enamel but decreased in the dentine; calcium and phosphorus are unaltered [268]. The alkaline phosphatase activity in the enamel organ is not altered [269], as might have been expected from the rise in manganese. Manganese, *nordihydroguaiaretic acid* [270] and protein [270, 272] give some protection against depigmentation, but iodine and copper and castration [270] and iron [268] give no protection, nor does lack of iron cause depigmentation [267]. Pigmentation of fat and depigmentation of teeth do not always run parallel with each other [270].

Irving [271] has described changes in the incisors of rats which he believes only occur from lack of vitamin E. In the centre of the middle third of the tooth there is a sudden premature and abnormal degeneration of all layers of the enamel organ, which is replaced by fibrous tissue. This is reminiscent of the depigmentation and damage to the enamel organ caused by lack of vitamin A (p. 35).

The Relation of Vitamin E to the Blood and Blood Vessels. Lack of vitamin E decreases the resistance of red blood cells to hæmolysis by dialuric acid (p. 598) and several investigators have reported anæmia in deficient animals, but here the very low protein intake [272] or the very high cod-liver oil intake [280] blur the picture. Megakaryocytes are reported to be greatly

increased in the bone marrow, with myeloblasts and myelocytes in the lymph nodes and early forms of granulocytes in the spleen [230]. In dystrophic rabbits there is increased hæmoglobin formation [289]. Anæmia cannot well be a major result of lack of vitamin E or it would have attracted far more attention than it has. In monkeys lack of vitamin E causes a polymorpho-leucocytosis [92]. Vitamin E is said to be antithrombic *in vivo* at concentrations normally present in the blood and so may help in preventing intravascular clotting [281]; this is supported by clinical work (p. 658). The purpura caused in dogs by stilbœstrol is stated, on account of very unconvincing work [283], to be prevented and cured by oral daily doses of 200 mg. of vitamin E.

A great and rapid increase in the collateral circulation round post-traumatic venous thromboses in dogs, and decreased inflammatory and degenerative changes in the walls of the veins is caused by 140 mg. daily of vitamin E, according to very excellent and well controlled work by Enria and Ferrero [282]. Holman [285] reports that vitamin E in daily doses of 2.5 to 4 mg. per kilo. of body weight protects dogs on a high cod-liver oil diet from the arterial lesions which develop after the kidneys have been damaged by agents such as uranium. The arterial lesions are very like periarteritis nodosa and rheumatic arteritis, consisting of œdema, swelling, fragmentation and ultimately necrosis of collagen, with fibrin and intense leukocytic reactions. In rabbits Manuel and others [84] state that vitamin E gives a high degree of protection against arteriosclerosis induced by cholesterol, though Dam [286] denies this both for rabbits and chicks and Bruger [287] even found in rabbits given extra cholesterol that the cholesterol content of the aorta was raised by vitamin E. In the rat [288] large doses of vitamin E are said to cause sclerotic patches and cholesterol deposits in the aorta, even though no extra cholesterol is given in the diet; lack of vitamin E on the other hand merely causes some fatty infiltration. Clinical work is discussed on p. 657.

The Relation of Vitamin E to other Tissues : Neoplasms : Resistance to Infection and Poisons. *Fur and Skin.* The fur of rats on a diet low in vitamin E is reported to remain infantile and unusually white in the young [143] and to become rough [230], while in the adult it becomes coarse, sparse, and discoloured [175], as can be seen in Fig. 216. In young pigs the coat becomes rough [135]. Skin sores have often been reported in rats [138] and in dogs [59]. We have often found wheat germ most valuable for the coats and health of dogs.

Osseous Tissues. Very soft skulls with little ossification have been observed in the suckling young of rats deprived of vitamin E by Barrie [143], and a similar condition in adult rats has been reported in a personal communication by Wright. Very high intakes of vitamin E cause decalcification [191]. The changes in the mineral content of the teeth of deficient rats also suggest that vitamin E plays an important part in mineral metabolism (p. 622).

Eyes. The eyes of adult vitamin E deficient rats have been said to be unduly protuberant by Demole and Pfaltz [294] and this has also been observed in the young by Lecoq and Isidor [230]. Demole and Knapp [295] report that in adult rats a deficiency of vitamin E causes clouding and vascularization of the cornea, keratoconus, iridocyclitis, opacities of the lens and serous retinal exudates. Though such changes have never been reported in any of the vast numbers of rats which have been observed by other workers, yet they are of interest in view of the clinical work reported on p. 658, and the protuberant eyes seen in some children with muscular dystrophy (p. 635).

Renal Degeneration. Martin and Moore [138] have observed a slow progressive parenchymatous degeneration of the kidney unaccompanied by any inflammatory reaction or by any change in the blood vessels. The first

renal changes occur after deprivation of vitamin E for three or four months ; after ten months nearly all the convoluted tubules are destroyed, so that it is surprising that the animal yet survives. The degeneration starts in the cells lining the convoluted tubules, which become granular and detached

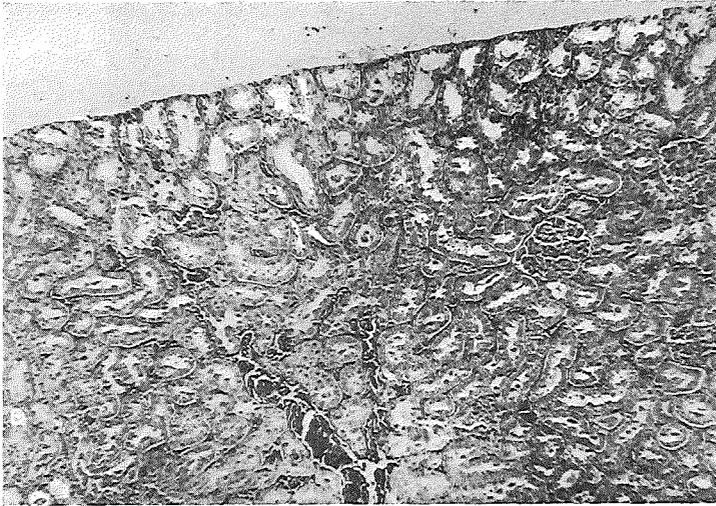


FIG. 217. Photomicrograph of normal kidney from a rat given a vitamin E concentrate.

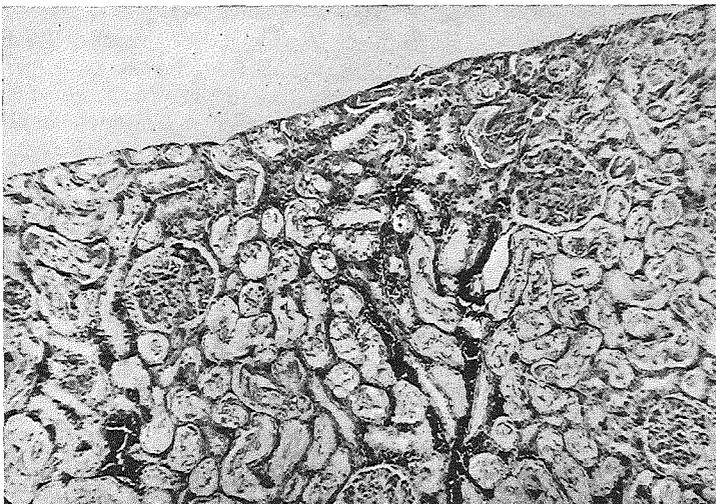


FIG. 218. Photomicrograph of kidney from a rat on a diet deficient in vitamin E. Note the extensive degeneration and detachment of the epithelial lining of the urinary tubules.

from their basement membrane. In time the degeneration may spread to the loops of Henle and the collecting tubules. The glomeruli show little change (Figs. 217, 218).

Neoplasms. Early work on the relation of vitamin E to neoplasms was complicated by lack of any pure preparation of vitamin E, so that wheat germ oil had to be used. Nothing but contradictory reports without any

clear cut results emerged from this period of research, though those who are interested will find references to it in the paper by Telford [296] and in the second edition of this book. The current use, however, of pure alpha-tocopherol has not added greatly to our knowledge. Telford [296] induced lung tumours in mice by subcutaneous injections of 1, 2, 5, 6 dibenzanthracene. Mice deprived of vitamin E developed tumours less often and in smaller numbers than mice given 2 mg. of alpha-tocopherol every other day. But there were only twenty-four mice in the deprived group and seventy-five per cent. of these as against thirty per cent. of those receiving vitamin E developed cysts which often broke down at the site of the injection of the carcinogen, so that probably much was never absorbed. In other words this work at best only suggests vitamin E may stimulate neoplastic growth, and it is contradicted by a report [297] that alpha-tocopherol causes regression of spontaneous mammary tumours in female mice, though the same report states that in male mice vitamin E may even stimulate the growth of tumours.

Cater [32], investigating Rous sarcoma in chicks, has contributed the most important work on vitamin E and neoplasms. He found that the largest tumours relative to the weight of the chick occurred in deficient chicks and the smallest tumours in chicks given large amounts of vitamin E. This is important as it is usual for tumours to grow badly when the host is suffering from general malnutrition, such as inevitably occurred in the vitamin E deficient chicks. Cater suggests that vitamin E acts as a controller of mesenchymal growth while Rous sarcoma produces a growth promoting substance. Therefore the vitamin controls the exuberant growth caused by the sarcoma. In support of this he reports that Rous sarcoma and lack of vitamin E both stimulate the activity of the preen gland and both cause a precocious development of the testes: the gland and the testes finally atrophy as a result of this excessive stimulation. This theory, though startling, is in harmony with the way in which the muscles in muscular dystrophy burn themselves away.

Resistance to Infections and Poisons. The work on vitamin E and infections [298-301] is too slight and indefinite to be worth considering. The rôle played by some antibiotics and sulphonamides in giving partial protection against massive hepatic necrosis of dietetic origin is discussed on p. 620.

Poisons and vitamin E appear to be often intimately linked together. Thus Daft and others [279], from well controlled work on young rats, have shown that succinylsulphathiazole accelerates the development of muscular dystrophy in vitamin E deficient animals, though the poorly absorbed sulphaguanidine has no such effect. Meunier and Chenavier [302] found that while normally fed rabbits given di-*o*-cresyl succinate died within seven days from diarrhoea, loss of weight and paralysis, similar rabbits given 50 mg. of alpha-tocopherol completely recovered. Akin to these observations are those of Hove [303] who found that carbon tetrachloride poisoning in rats on a low protein diet could be cured by vitamin E. Hove states that the poisoned rats developed symptoms identical with those induced by lack of vitamin E. The theory which best accounts for all the above observations is that vitamin E plays an important or, when protein is scarce (p. 620), an essential part in destroying various poisons and is itself destroyed in the process. It would be of great practical value to know what effect vitamin E has on the toxicity caused by flour treated, as it always is in England, with nitrogen trichloride or "agene."

VITAMIN E IN FOODS

There is startlingly little vitamin E in English food: some 4 to 8 mg. of alpha-tocopherol would seem, from the food tables given later, to be the

average amount consumed daily in 1952. In the U.S.A. [43] about 14 mg. daily is the average while in the Netherlands [65] it is about 15 mg. The serious consequences of the poverty of English food in vitamin E are discussed on p. 631.

Bread should hold a pre-eminent place as a source of vitamin E. In the Netherlands [65] it provides more than one-third of the total, but in England [236] bread is to all intents valueless because it is made of eighty-one per cent. extraction home-milled flour and seventy-two per cent. extraction foreign flour, with potato flour when this is not too costly. The miserable bread made from this flour should have about 0.05 mg. per slice but the Government in its infinite lunacy continues to permit the agenizing of flour, and as this destroys about three-quarters of the vitamin E in the flour [237, 238] we are left with possibly 0.013 mg. in one slice. Since agene may be toxic—its use being banned in the U.S.A., France, Holland and Germany—and since vitamin E is probably essential for many of the detoxifying activities of the body (pp. 620, 625), the Government is indeed to be congratulated on managing at one and the same time to introduce a probable poison and remove the vitamin possibly essential for its destruction.

“ Bleached flour does more than Malthus can
To sterilize the Englishman.”

The best way by which the citizen can avoid eating this malnutritious flour and bread is by buying eighty-one per cent. extraction stone ground flour and making his own bread : a surprisingly easy occupation. The bread is a light cream in colour, contains the germ but no bran, and keeps excellently. Being as bland as white bread, it is suitable for patients with gastritis or “ weak digestions.” Some “brown breads ” contain the germ, but often this has been removed during milling, specially treated, and then returned again to the flour. Such treatment may well destroy some or all of its vitamins. There are no reasons against using stone ground flour in domestic cookery, since it is as good as white flour for pastry and soufflés.

A further great advantage of stone ground flour is that it contains less phytic acid than brown flours, so that there is less danger of calcium, iron, and the other minerals present in the diet forming insoluble phytates in the gut with their consequent loss to the body (p. 527).

Brown rice, oatmeal and indeed all the cereals from which the germ has not been removed are excellent sources of vitamin E, but too often porridge is replaced by facilely prepared breakfast foods of no value whatsoever.

Green vegetables are a good source of vitamin E and so are some root vegetables, while potatoes are almost valueless : values however, as can be seen from the food tables, vary greatly. They appear to be much higher in the Netherlands [65] than in the U.S.A. [43], while the values of English vegetables are unknown. Possibly, however, the absorption of vitamin E from vegetables is very poor [65]. *Fruit*, were it eaten in large amounts, would be a valuable source, and rose hips are very rich in vitamin E [30] but probably the seeds and not the pulp contain the vitamin, so rose hip jam will not add to the consumption of those who have the patience to make it.

Meat could be a good source of vitamin E, but as chilling or freezing (p. 627) appears to destroy vitamin E, and as most meat eaten in England has been frozen for weeks or months and in any case is eaten in minute amounts, the contribution of meat to our intake of vitamin E is negligible.

Fish, or at least sardines, cod's roe and haddock, is an excellent source.

Dairy produce depends for its value on the diet of the cows and hens. Milk, and so butter, from pasture fed cows is richer than milk from those stall fed, and the milk from Guernsey's is about three times as rich as the poor milk of Holsteins [239]. The colour of the milk is a good guide to the amount of vitamin E it contains, since carotene and vitamin E fluctuate together [239]. Eggs reflect the diet of the hen, containing per 100 grams as little as

0.16 mg. or as much as 4.0 mg. [62]: it is probable the colour of the yolk is again a guide to the value of the egg.

Margarine *may* be an excellent source, depending on what oils have been used in its manufacture.

Effect of Rancidity, Staleness, Storage and Cooking. Rancidity rapidly destroys vitamin E (p. 615). This is probably of importance in human diets. Most human foods containing fat are eaten stale, and even if they do not smell rancid may well have lost all their vitamin E, since this will be destroyed before frank rancidity can develop. Conversely an "off flavour" in meat, poultry and milk is often a sign that the animals lacked vitamin E in their diets [239, 240, 241].

Storage of meat and presumably of all foods except whole grains, destroys vitamin E even at low temperatures [242, 243]. For instance Dju and others [243] report that twenty-two per cent. of the vitamin E in animal fat is destroyed after only one month's storage at -20°C ., thirty-nine per cent. after ten weeks and forty-six per cent. after twenty weeks. For the English whose meat and fish and even vegetables are often only "chilled" at -2°C . or frozen at only -9°C . for many weeks before they rest for days in the domestic refrigerator, the loss of vitamin E must be very high indeed.

Domestic cooking probably causes no appreciable loss, though little vitamin E remains in fat recurrently used for deep fat frying [43]. There is some evidence that dried and pasteurized milks may contain no vitamin E [244], so artificially fed babies may receive inadequate supplies. From the single morsel of evidence that vitamin E is not destroyed in the canning of cod's roe [245], it seems possible that canning is not deleterious.

Preparations of Vitamin E Available for Medical Use. Vitamin E may be given as the pure natural or synthetic tocopherols, as wheat germ oil or as wheat germ. The latter is by far the best when only small amounts are required.

Pure Tocopherols. The relative values of the different natural and synthetic tocopherols have been discussed on p. 594, so here it is only necessary to point out that as yet there are no preparations suitable for injection (p. 593). It is also important to remember that alpha-tocopherol is so much more active than the other tocopherols that the content of the latter in any mixtures can be ignored. The advantage of the pure tocopherols is that enormous oral doses may be given, though most of such doses are excreted (p. 599). Probably it is wise to give tablets of the tocopherols mashed up in butter or after a fatty meal as they are fat soluble and probably require fat to aid their absorption (p. 600).

Wheat Germ Oil. The vitamin E in wheat germ oil is unusual in being composed of about seventy per cent. of alpha-tocopherol and thirty per cent. of beta-tocopherol: the latter is only present in very small amounts in oils and foods not derived from wheat, but as it has a low biological potency compared to alpha-tocopherol (p. 594) it would appear to have no particular value unless it is held that naturally occurring combinations of the tocopherols are liable to have some value not as yet shown by investigation. Since the vitamin E of wheat germ oil is dissolved in the oil it is possible that it is well absorbed (p. 600). Wheat germ oil has been shown [246], by work which appears to have been well controlled, to have a marked stimulating effect on the growth of the ovaries, uterus and adrenals of rats. If this endocrine effect should be confirmed, it would raise very complicated problems not only about the medicinal use of wheat germ oil but also might still further emphasize the importance of leaving the germ in flour and bread.

Whole Wheat Germ. This is undoubtedly the best method of giving vitamin E. All the tocopherols are present and also the vitamin B complex and many other valuable substances, some of which have a marked effect in enhancing the activity of vitamin E. The anti-oxidants in the germ give considerable protection to vitamin E against destruction by rancid fats

during digestion. Further, the germ keeps excellently if a reliable preparation is used : germ obtained from mills, however, may become rancid in a couple of days. It is difficult to understand why wheat germ oil should ever be given in preference to whole wheat germ. It is certain the oil cannot contain anything not present in the parent germ, and it is equally certain that much of value may be lost. It is, indeed, without doubt lost in as far as the vitamin B complex and minerals are concerned.

The trace elements, whose great importance in relation to the vitamins is only slowly being realized, should also be remembered when considering the value of wheat germ since it is one of their richest sources, notably for zinc [247] and manganese [248]. The trace elements are intimately connected with the proper use by the body of the vitamins [249, 250, 251] and so should be supplied when treating deficiency diseases, especially as the distribution of the trace elements in foods largely follows that of the vitamins, so that when there is a deficiency of the latter there is also most probably a deficiency of the former [247, 252]. The use of whole wheat germ ensures that the value of the vitamins it contains is not limited by the absence of those minerals which are essential for the full physiological activity of the vitamins and which are absent in purified and synthetic vitamin preparations.

Rarely children refuse to take wheat germ : wheat germ oil and the vitamin B complex should then be given instead [10, 253], and this combination may be used to give children a short rest from wheat germ during prolonged treatment.

Bicknell finds that wheat germ very occasionally causes an urticarial rash in children who are being treated for muscular dystrophy. It appears, like other forms of urticaria, most often after the child has had a hot bath or is warm in bed. After a few months it disappears ; in the meantime it is best treated with the usual lotions and not with drugs. Donovan [254] has also seen a similar rash in a boy with muscular dystrophy who was taking wheat germ. Stone [253] using wheat germ oil for children with muscular dystrophy, and Shute [7] using it for adults with sterility or threatened abortions have reported occasional skin rashes. Newman [255] using wheat germ and wheat germ oil caused a rash resembling lichen planus. Apart from these rare effects wheat germ and wheat germ oil are well tolerated, and do not cause any digestive disturbances.

Vitamin E should not be given close to a meal which has contained any food which may be on the verge of rancidity. In children cod-liver oil, and cod-liver oil concentrates, should be given at different meals to the wheat germ. All foods which are not fresh and all cheese should be avoided.

AMOUNTS OF VITAMIN E IN FOODS

In the following table—which is largely derived from those of Emmerie and Engel [33] and Harris, Quaife and Swanson [43]—all the values for vitamin E are based on chemical estimations, since no accurate biological estimations have been performed owing to the enormous labour these involve. Of the various tocopherols which together make up the vitamin E activity of a food, alpha-tocopherol is by far the most biologically active (p. 594), so only the alpha-tocopherol and total tocopherol values are given. The difference between these values is for most foods made up almost entirely of gamma- and delta-tocopherols : the activity of these is so slight (p. 594) it may be ignored. Wheat and its products, however, are exceptional in having about thirty per cent. of their tocopherols as beta-tocopherol and sixty-five per cent. as alpha-tocopherol : the former has roughly one-third the value of the latter. Soya beans are the opposite of wheat, the almost inert delta-tocopherol forming about thirty per cent. of the total tocopherols [15] and alpha-tocopherol about ten per cent. Only the total tocopherol content of some foods is known : this has been given though it is of scant help in

estimating the vitamin E value of a food since the active alpha-tocopherol may form as much as seventy per cent. or as little as ten per cent. of the total tocopherols.

Food	Milligrams in 100 grams or roughly 3½ ounces	
	α-Tocopherol	Total Tocopherols
<i>Cereals</i>		
Barley	? 1.6-2.6	3.2-5.2
Agenized Germ	0.4-0.6	0.8-1.3
“ Bemax ”	? 14	14
Bread, 100% extraction flour	? 0.75	1.3
80% extraction flour	? 0.12	0.23
English agenized (p. 626)	? 0.03	? 0.06
Cassava (East Indies)	—	0.2
Flour (wheat) whole	? 1.5	2.2
80% extraction	? 0.84	1.2
English agenized (p. 626)	? 0.21	? 0.3
Groats	—	1.2
rolled	—	1.5
Cornmeal, yellow	0.84	1.7
Oatmeal	1.94	2.1
Panicum viride	—	4.0
Rice, brown	1.2	2.4
polished	0.35	0.57
bran	—	3.0
Rye	? 1.1-1.7	2.2-3.5
agenized	? 0.3-0.4	0.6-0.9
Spaghetti	? 0.6	1.20
Wheat germ	18.9	27
<i>Dairy Products</i>		
Butter, Dutch, U.S.A.	—	2.1-3.5
Cheese, American	—	1.00
10% fat	—	0.3
20% fat	—	0.6
Eggs (p. 626)	1.2	1.4
Milk (p. 626), cow's	—	0.02-1.2
summer	—	0.17
winter	—	0.08-0.15
pasteurized (p. 627)	—	? None
powdered (p. 627)	—	0.3-0.5
skim (p. 627)	—	0.05
ewe's milk	—	1.5
colostrum	—	4.7
goat's milk	—	1.4
colostrum	—	5.9
sow's colostrum	—	18.6
woman's, early	—	0.13-3.6
late	—	0.11-1.64
<i>Fish</i>		
Cod, liver oil (fresh)	—	13-14
roe (fresh or canned)	—	5.25-7.5
Haddock	0.35	0.30
Herring	—	0.5
Liver oils (pp. 595, 616)	—	?
Mackerel	—	1.8
Salmon	—	1.6
Sardines	—	4.5
Shark liver oil	—	10-? 40

Food	Milligrams in 100 grams or roughly 3½ ounces	
	α-Tocopherol	Total Tocopherols
<i>Fruits</i>		
Apples	0.72	0.74
Banana	0.37	0.4
Capsicum	—	2.4
Coconut	—	0.2
Grapefruit	0.25	0.26
Oranges	0.23	0.24
<i>Meat (p. 627)</i>		
Beef, liver	1.4	1.4
steak	0.47	0.63
brain	—	1.2-2.3
Chicken	0.21	0.25
Lamb chops	0.62	0.77
Pig, bacon	0.41	0.53
pork chops	0.63	0.71
lard	2.3	2.7
<i>Oils and Fats, Vegetable</i>		
Arachis or peanut oil	24 11 15.6-21.6	48 22 26-36
Beechnut	—	100
Cocoa fat	—	12.5
Coconut oil	3.6	—
old	—	3.6-5
hydrogenated	—	0
Corn oil	7 10-13	87 90-102
Cottonseed oil	43	250
refined	56	86 90
Linseed oil	—	60 23
Maize oil	—	70-250
Margarine	—	—
Olive oil	—	?
Palm oil	—	3-8
red	30	56
Rice bran oil	—	110
Sesame oil	55	91
—	—	5
—	11.9	40
Soya bean oil	10	168
—	—	140
—	9-12	92-120
Wheat germ oil, crude	—	100-420
medicinal	—	320
<i>Vegetables</i>		
Beans, dried navy	0.1	3.6
kidney	—	1.2
white	—	4
Beetroot	—	0.2
Brussels sprouts	—	1.7
Cabbage	0.06	0.11
red	—	0.2
white	—	0.7
Carica papaya, leaves	—	36

Food	Milligrams in 100 grams or roughly 3½ ounces	
	α-Tocopherol	Total Tocopherols
<i>Vegetables—continued.</i>		
Carrots	0.45	0.45
	—	1.5
Celery	0.46	0.48
		2.6
Endive	—	2.0
Belgium	—	0.2
Ipomœa, leaves	—	8.1–11.8
Kale	—	8.0
Leek	—	1.9
Lettuce	0.29	0.43–0.54
Onions	0.21	0.26
Parsley	—	5.5
Peas, green	0.1	2.1
	—	5.4–6.4
Potatoes, boiled	—	0.1
white, peeled	—	0.06
sweet	4.0	4.0
Radishes, black	—	0.04
Scorzonera	—	0.6
Soybean	—	18.8
Spinach	—	1.7
Tomatoes	0.27	0.36
Turnip	—	0.02
greens	2.24	2.3
<i>Various</i>		
Chocolate, unsweetened	5.3	11.1
Cocoa powder	—	3.1
Fungi	—	0
Peanuts	4.6	9.3
	—	6.3–11.9
Yeast	—	0

HUMAN REQUIREMENTS OF VITAMIN E

Nothing is accurately known of the human requirements of vitamin E, though from the figures given in the preceding table many of the English must consume less than 5 mg. a day yet still continue in what in England is regarded as good health. Indeed vitamin E has been considered to be of no importance in human nutrition. But it is fantastic to believe that while mice and monkeys, ducks and dogs, beetles and barrows all need vitamin E, yet a beneficent all wise biological providence has excused man from this irksome necessity. In most animals complete deprivation of vitamin E leads to rapid and overt illness, but when some small amounts of vitamin E are given in the food over long periods—as they are in the food of the English—the symptoms are covert: merely a disturbance of the reproductive system in old rats (p. 607) or slight myocardial damage in the monkey (p. 615) or apparently complete health in the cow for years until there is sudden death (p. 606). There is also the probability (p. 621) that insufficient vitamin E is one of the reasons why senility pigments or “metabolic clinkers” collect in the body’s cells over the long course of the years, first to damp and finally to smother the bright flame of life.

It is impossible to believe that vitamin E is not essential for man, but it well may be that the symptoms of a prolonged mild deficiency only show

themselves after many years, and then are so blurred by the changes of senility which they themselves helped to produce, that they are difficult, impossible, to recognize. It is grievous that our food loses so much of what may be an essential protection against senility merely because the vision of science is just as long as the life of a rat.

DISEASES DUE TO A DEFICIENCY OF VITAMIN E ABORTION AND OTHER SEXUAL DISORDERS

The tendency for the birthrate to decline among industrial or "civilized" communities is a serious problem. There are probably many causes for this among which a deficiency of vitamin E has often been considered of importance. Drummond and Wilbraham in their book "The Englishman's Food" have drawn attention to the possible relationship between our falling fertility and the fall in our consumption of vitamin E with our increasing consumption of white flour from which the wheat germ has been removed. Maurice [304] has pointed out that the agricultural labourer of to-day has apparently a better diet than he did fifty years ago, but he eats white flour where he used to eat stone ground, and his family is smaller. To explain this low fertility by the spread of contraception ignores that it and white flour are both benefits of civilization. It also ignores that contraception is not likely to be efficiently used by most labourers.

Hogbin [305] mentions reports that the spread of refined foods low in vitamin E has been one of the causes of the falling fertility in some Pacific Islands, while Young [13] suggests that racial fertility is connected with diet, and that though the number of abortions due to a faulty diet is unknown, "we may, however, safely assume that it constitutes a considerable proportion of spontaneous cases." The importance of abortion, as apart from a decline in fertility, is shown by the fact that in England and America one-fifth of all pregnancies end in spontaneous abortion [310], while in France abortions from all causes are believed to be higher than the birth rate [306]. The level of vitamin E in the blood during pregnancy, etc., is discussed on p. 601, and experimental work with animals on the endocrine glands on p. 610.

Recurrent Abortion. Vitamin E has never gained much popularity with gynaecologists in the treatment of recurrent abortion. Browne [7, 307], for instance, has pointed out that as good results are obtained with endocrine preparations, simple rules of conduct, or indeed with no treatment save placebos. This rather hopeless attitude towards the problem may be criticized firstly on the grounds that Bacharach's [308] statistical examination of the published clinical results show quite definitely that vitamin E is of value in recurrent abortions. Secondly, because one form of treatment gives good results is no reason for doubting the value of another. In those women who tend to abort there may well be several mildly injurious factors—endocrine, dietetic, psychological—which added together terminate the pregnancy. The removal of any one will enable the pregnancy to proceed. The relation of abortion to the level of vitamin E in the blood is discussed on p. 602.

In treatment wheat germ oil has generally been used as the source of vitamin E: should the endocrine activity of wheat germ oil (p. 627) be confirmed it is possible that its vitamin E accounts for only a part of its effect.

Vogt-Möller [8] in 1931 first used wheat germ oil in the treatment of habitual abortion. He only reported two cases: treatment was successful in both though one woman had previously had four successive abortions and the other five. In the next five years he treated seventy-two women with no demonstrable reason for their recurrent abortions with 3 grams of wheat germ oil daily. Fifty-five living children were born, all of whom were well developed. In 1939 he summed up his own experiences and

reports in twelve other papers. "Since 1936 my evidence from such cases has increased considerably. Taken as a whole, favourable results were obtained in about eighty per cent. of cases. Many investigations have confirmed my observations. The records of treated cases of habitual abortion now amount to some hundreds with a mean value of seventy-five to eighty per cent. favourable results" [7].

Currie [7] in 1939 reported his results from giving about 6 mg. of tocopherol daily in the form of concentrated wheat germ oil. The length of treatment varied from three to thirty-two weeks and was often not begun until the middle weeks of pregnancy. Treatment with luteinizing hormone did not give such good results. In all, eighty-one women were treated who had had two hundred and seventy-four previous pregnancies of which only forty-seven had gone to term. With treatment the next eighty-one pregnancies resulted in sixty-two viable infants being born. Isles [7] using the same dose of vitamin E as Currie treated eight women, who had had only five successful pregnancies out of twenty-nine, with complete success, eight viable infants being born.

Watson [309] had successful results in twenty-one of twenty-eight women who had had two or more abortions, and in eight of nine who had had one previous abortion. Lubin and Waltman's figures [319], from giving 3 mg. of synthetic vitamin E daily, were respectively five out of seven and eight out of ten.

Malpas [310], however, criticized the value of wheat germ oil on the grounds that it gave no better results than those which would have been expected had no treatment been given. From his own investigations, and those of others, he concluded that eighteen per cent. of all pregnancies end in abortion, and of these abortions only one is due to recurrent causes. From this he deduced that in one hundred pregnant women treatment could at best only lead to eighty-three live births and better results than this proved too much. But why he should assume that lack of vitamin E could only cause recurrent abortions and not contribute to the seventeen per cent. of non-recurrent abortions is obscure. Many reasons might lead to a transitory deficiency of vitamin E which only was present during one conception, and so treatment with vitamin E might well lead to more than eighty-three per cent. of live births. In fact his figures suggest, if anything, that lack of vitamin E may be one of the causes for so many women aborting once for no obvious reason. However, going on the assumption that seventeen per cent. of all pregnancies must end in abortion, he states that the expectation of living children in untreated cases of recurrent abortion is: after two successive abortions sixty-two per cent., after three twenty-seven per cent., and after four six per cent.

Bacharach [308] has examined the figures of Malpas and applied them in a slightly modified form to the published results of the treatment of habitual abortion. He points out that treatment has given far better results than would be expected from Malpas's figures were it valueless. In fact the probability of chance alone accounting for the apparent success of treatment is: after four or more consecutive abortions 1 in 10^{40} , after three or more 1 in 10^{10} , after two or more 1 in 200. This analysis of Bacharach's appears definitely to confirm the value of wheat germ oil in the treatment of recurrent abortions.

Threatened Abortion and Toxæmias of Pregnancy. Shute [311] observed that when rats became sterile from lack of vitamin E the resistance of their blood serum to proteolysis was increased. Treatment with vitamin E removed this antiproteolytic factor from the blood. He then investigated women who were threatening to abort and found that their serum was similar to that of vitamin E deficient rats in its resistance to proteolysis. Giving these women vitamin E returned the blood serum to normal.

Work by Jeffcoate [315], Shute [312], and others suggests that this

antiproteolytic factor is of an oestrogenic nature. Its presence hinders the proteolytic erosion of the placental villi into the uterine wall. This in turn leads to a weak attachment of the placenta and so to termination of the pregnancy.

From all these observations Shute [311] was led to believe that "... vitamin E and œstrin, or a substance very much like it, exist in a sort of equilibrium during pregnancy. If there is too much of the œstrin-like substance the pregnancy is interrupted. An excess of vitamin E appears to have no effect on the pregnancy." Therefore in cases of threatened abortion with premature partial separation of the placenta and a high antiproteolytic factor in the blood vitamin E should be of value in treatment. Shute has treated a considerable number of such cases with wheat germ oil, and claims in one series success in sixty-eight per cent. of one hundred and eighteen cases [7]. Young [13] also found wheat germ oil of value in cases of threatened abortion, and so have others using synthetic vitamin E [319]. Again it will be noticed that wheat germ oil with its possible endocrine activity (p. 627) and not pure vitamin E has been mainly used in treatment.

An objection raised to preventing threatened abortion occurring is that it may be a sign of an abnormal foetus which would be better dead. But a study of all the available reports shows that only five out of eighty-nine children born after a threatened abortion had congenital anomalies [7].

Shute [7] emphasizes that women should have their blood examined for the antiproteolytic factor early in pregnancy. If it is high they will require wheat germ oil throughout the whole pregnancy. If this is not continued or is given in inadequate amounts toxæmia and premature placental detachment will occur later in the pregnancy, such as was observed by Young [13], who thought vitamin E given during pregnancy might unearth a later toxæmia.

Pregnancy toxæmias may be divided, according to Shute [313], into those produced by too little vitamin E and those produced by too little oestrogen. In the former type there is a raised blood pressure, œdema and albuminuria, and premature detachment of the placenta which gives rise to the name "hæmorrhagic toxæmia." This type responds to treatment with vitamin E. The second type is the true eclamptic with low oestrogens, and must on no account be given vitamin E, as this further depresses the oestrogens and so causes convulsions. Instead oestrogen therapy is required, though too much will convert the eclamptic to the hæmorrhagic type.

The amount of wheat germ oil advocated by Shute [7] is large and should be supplemented with the vitamin B complex. An initial dose of 1½ ounces of fresh oil are given, and then 1-2 drachms daily till the end of pregnancy. More may be needed to control the symptoms, especially towards the end of pregnancy and in patients with hypothyroidism, who tend to have high blood oestrogens. In the summer the higher content of vitamin E in the food decreases the amount of oil required.

Krieger [317], however, after very careful work could not confirm the presence of Shute's antiproteolytic factor, in the blood serum of cases of abortion, premature labour, accidental hæmorrhage or normal pregnancy. Cuthbertson and Drummond [316], using a slight modification of Shute's test [318] for the susceptibility of blood serum to proteolysis, failed to find any difference in the blood of rats reared on vitamin E deficient diets, normal diets, or diets rich in vitamin E. These authors also criticize the biochemical theory of the test. Drummond, Noble, and Wright [151] hold that Shute is wrong in believing that lack of vitamin E causes an excess of oestrogens in the blood, chiefly because if this were so lack of vitamin E would produce symptoms of an oestrogenic excess in animals, such as failure to conceive, irregular œstrus, and testicular changes unlike those observed from a deficiency of vitamin E. Shute [314] has answered these objections to his test and theory by saying his test was incorrectly carried out, and that the

presence of small amounts of oestrogens in the blood of animals need not have the effects Drummond and his collaborators mentioned. In our opinion Shute's test is valueless.

Progesterone combined with vitamin E has been used, chiefly by German gynaecologists, for the treatment of threatened abortion. One possible reason for the good effects which are claimed for this dual therapy is that the vitamin protects the hormone from destruction in the body (p. 594). Winkler [320] reports that 30 mg. of alpha-tocopherol daily raises the low urinary excretion of pregnandiol which he has observed in cases of threatened abortion. He and Bach [321] advise giving progesterone with the vitamin E during the first five days of treatment in order to tide over the period before the vitamin has had time to stimulate the corpus luteum. In seventy-four untreated cases there were twenty-four abortions, in twenty-seven cases treated with progesterone there were five abortions, in ten cases treated with 30 mg. of alpha-tocopherol there was one abortion, and in sixteen cases treated with both the hormone and the vitamin there were two abortions. Schäfer [322] successfully treated forty-five out of fifty-three cases with vitamin E alone or combined with progesterone. He does not state what dose of vitamin was given. Silbernagel and Patterson [412] gave a wheat germ oil concentrate to 825 women, starting between the third and sixteenth week of pregnancy, using as controls 1,973 untreated women. The respective results for the untreated and treated were, in percentages, threatened abortion 16.6 and 10; abortion 15 and 3; prematurity 7.1 and 3.7; toxæmia 10 and 2.1; stillbirths 2.3 and 0.4.

Primary Sterility. In both men and women primary sterility is probably not cured by vitamin E. This is not surprising since deprivation of vitamin E causes an irreversible testicular change in male animals, while in female animals vitamin E is not necessary for conception (pp. 606, 610). Farris [323] found no change in the sperm count of fertile and subfertile men treated with vitamin E and it has been suggested that such treatment may decrease the essential hyaluronidase in the semen (p. 597). Some human testes apparently show degenerative changes due to lack of vitamin E, especially in those conditions such as the steatorrhœas where there is probably prolonged impaired absorption (p. 600). In such conditions prophylactic treatment before degeneration has occurred should be of value.

The Menopause. Menopausal flushes may be greatly benefited by vitamin E. Thus McLaren [324] in an excellent paper on the treatment of forty-seven severe cases with 500 mg. daily of synthetic alpha-tocopheryl acetate, states that in about five weeks, on an average, there was complete cure in twenty-three cases, marked improvement in seven cases and no benefit in seventeen cases: the latter did not respond to still heavier doses of vitamin E but did respond to stilbœstrol. A year later of seventeen cases who had been cured by vitamin E, twelve had relapsed. A careful and well controlled investigation by Finkler [325] shows that out of sixty-six women excellent results were obtained in thirty-one, fair in sixteen and none in nineteen. Only 30 mg. daily of alpha-tocopheryl acetate was used over periods of ten days to seven months. No toxic effects were noticed by either of the above authors and they emphasize the value of vitamin E for these frequent cases for whom stilbœstrol is contraindicated. Both authors report that there are no changes in the lower genital tract, though McLaren found a gradual improvement in half his cases of senile vulval or vaginal lesions.

MUSCULAR DYSTROPHY (PSEUDOHYPERTROPHIC MUSCULAR DYSTROPHY: PROGRESSIVE MUSCULAR DYSTROPHY: PRIMARY MYOPATHY)

Muscular dystrophy is probably caused by an inability of the muscles to use vitamin E. This is thought to be so because:—

(a) The peculiar muscular degeneration of muscular dystrophy in animals is caused and is *only* caused by lack of vitamin E [225].

(b) Human muscular dystrophy shows identically the same peculiar degeneration.

(c) Muscular dystrophy can be induced in the monkey and in all other animals (p. 605).

(d) Typical early muscular dystrophy has been found in the muscles of severely malnourished children [326] and in adults suffering from chronic intestinal conditions where absorption of vitamin E is presumably impaired (p. 600).

(e) Muscular hypotonia in children may be remedied by vitamin E (p. 647).

(f) An analogous failure to use a fat soluble vitamin is seen in children and adults suffering from "resistant rickets" (p. 562). This too, like muscular dystrophy, may be familial.

(g) A superabundance of vitamin E may check or even improve the muscular degeneration (p. 643).

The reason why the muscles are unable to use vitamin E is obscure. It is not due to a simple dietetic deficiency or a failure of absorption, since the diets of many dystrophic children have always been excellent and the level of vitamin in the blood is normal (p. 602). Milhorat and Bartels [327] in 1945 suggested that during intestinal absorption vitamin E was acted upon by an enzyme and converted into the form which, probably as a compound with inositol, is active in the body. Bicknell [328] in 1949 failed to confirm this theory. He fed children who had not responded to wheat germ alone, with a mixture of wheat germ, wheat germ oil, synthetic alpha-tocopheryl acetate, inositol and various preparations of dried hog's stomach or dried duodenum or dried heart muscle. There was no change in the creatine and creatinine excretions measured daily for several months. This of course did not disprove the theory, but only showed that if such an enzyme existed it was not present in the preparations used or was not active under the conditions of the experiment.

The lack of a second vitamin or some common constituent of food is not the reason for the failure of the muscles to use vitamin E: Bicknell has given large doses of every available vitamin, together with vitamin E, without any extra benefit. One boy had his daily creatine and creatin excretion measured almost continuously for two years while his diet was accurately recorded. But no relation was found between the customary violent fluctuations in excretion and any article of his very excellent, varied and fresh diet. In passing it is interesting to note that in one boy on delta-tocopherol both the daily creatine and creatinine excretions were increased, so that the two curves remained in the same relative positions to each other but at a higher level.

We are, therefore, left with a strong logical case for believing that the key to the cure of muscular dystrophy is vitamin E, but we have as yet no knowledge of how to fit the key to the lock.

History. Muscular dystrophy was first separated from other forms of paralysis by Duchenne of Boulogne, in 1861. The three types of muscular dystrophy generally recognized are the common pseudohypertrophic type found in children, which was first described fully by Duchenne [329] in 1868; the juvenile or scapulo-humeral form of Erb first recognized by him [331] in 1884; and the facio-scapulo-humeral type which bears the name of Londouzy and Dejerine [330] from their account of it in 1884. Intermediate forms are seen, and it must be stressed that all forms of dystrophy are only different manifestations of the same disease. The part which heredity plays has been exhaustively studied by Bell [332].

Pseudohypertrophic Muscular Dystrophy. This is the most common form of dystrophy seen in England, though it appears to be less common than the others in the United States judging by the relative numbers reported in

recent papers. It occurs in all European countries and also in India, Ceylon and Japan, and there seems no reason to suppose any race is exempt.

Boys are affected far more often than girls. In the latter the condition tends to start later, often at the age of puberty, and to progress more slowly (Fig. 219).

A familial tendency is very marked, so that it is common to find in one family two or three brothers in different stages of the disease. Generally the girls are spared even when their brothers are not, and older boys seldom develop the disease after their younger brothers have done so. It is, in fact, the boys born after the one who develops dystrophy who require careful watching. Heredity appears to play no part in the disease, possibly because those affected have in the past died before the age of marriage.

The mothers of boys with muscular dystrophy, especially when the condition has apparently been present from birth, often give a history suggesting that during pregnancy and lactation they themselves were deficient in vitamin E. Thus it is not uncommon to find that before the dystrophic infant was born there were recurrent abortions, and recently two mothers attributed the dystrophy of sons born in the middle of long and normal families to starvation during these particular pregnancies. Prolonged nausea and vomiting has also been mentioned by mothers as a possible reason. The number of cases investigated, however, is too small to make these observations more than suggestive.

The age of onset is commonly between five and ten, but many children give a history of crawling late and never walking properly, while others, especially girls, may show the first symptoms at puberty or even later. In these late cases the condition tends to be less typical and to progress more slowly.

Infections often appear to unmask or emphasize the weakness so that the disease is ascribed to such complaints as scarlet fever or pneumonia.

The important changes occurring during the disease are increasing muscular weakness, increasing contractures, and a rare primary osseous atrophy.

Muscular Weakness. The picture of the disease is unique. It is that of a child fighting in an unsympathetic world against an increasing but unrecognized weakness of his legs. It is often surprising how weak the child becomes before he is taken to his doctor. Boys at school may be blamed for months for being lazy and clumsy before any medical advice is sought. Usually the earliest symptoms are slowness in running, frequent falls so that the knees are never free from bruises, and hesitancy about going up, and especially down, stairs. Careful use is made of the banisters, and the children go down one foot at a time. In some children the frequent falls cause fear of slippery floors and loose mats.

If the weakness first shows itself in the lumbar muscles, and those joining the pelvis to the thighs, the typical low lumbar lordosis and protuberant stomach will appear. The waddling wide-based gait will also be seen as the child dips from side to side in his painful efforts to overcome his inability to lift up his legs by swinging them outward and forward with each step. The weak dorsiflexors, evertors and external rotators of the feet cause the child to trip over his own dragging turned-in toes.

When the child falls down he has difficulty in getting up again, so that he "climbs up himself." This way of getting up is often thought to be pathognomonic of muscular dystrophy, but it is displayed in all cases where the muscles of the lower trunk and legs are weak. If the child is laid on his back and told to get up he first rolls over on to his stomach. Then he gets into a crawling position and slowly walks his hands backward toward his toes at the same time trying to straighten his knees and hips. The latter, however, he cannot do without the help given by his arms as he walks his hands up his legs (Fig. 224).

The shoulder girdles and upper arms are mostly affected long before the forearms, though after the legs. This leads to winging of the scapulæ and an inability to raise the arms above the head. Children whose shoulder girdles are weak are described as "slipping through" the hands of anyone trying to lift them by holding them under the arms. The flexors and extensors of the neck are often preserved to the end.

The face is commonly spared in this form of dystrophy, but the eyes in many cases appear to be unduly prominent, or to show more of the sclerotics than usual, giving a fleeting impression of hyperthyroidism. During sleep

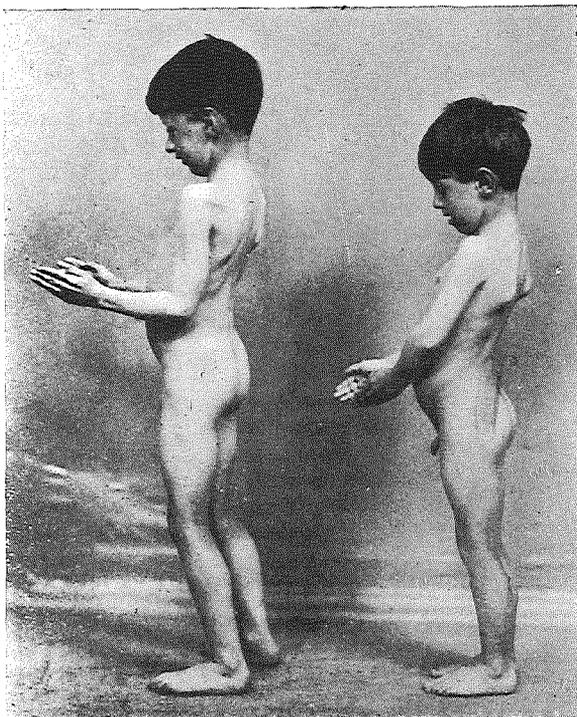


FIG. 219. Two English brothers, aged eight and six, with pseudohypertrophic muscular dystrophy. An elder brother is more seriously affected. Note the winging of the scapulæ; the weakness of the shoulder girdles preventing the arms from being raised further; the low lumbar lordosis and the pseudohypertrophy of the calves and thighs.

the eyes often remain open, the gruesome look of the face being most distressing to the mother.

The involvement of the muscles of respiration is generally late, but when it occurs is extremely serious, since the inability to cough turns a trivial bronchitis to a rapidly fatal pneumonia.

Swallowing is never, or very rarely, impeded, and the sphincters of the bowel and bladder always remain normal.

The nervous system is never affected, but the tendon reflexes are often lost early in the disease from the loss of tone in the muscles.

Irony is better shown in pseudohypertrophic dystrophy than in any other disease, since while the legs grow weaker their muscles tend to grow larger. This pseudohypertrophy often causes mothers to take a pathetic pride in their son's fine calves, and is also the reason why they realize so late that the legs are weak. Though the calves are most frequently, almost

constantly, affected, any other muscles may enlarge. The extensors of the knees commonly undergo pseudohypertrophy at some stage, though the enlargement is often localized to one part of the quadriceps, or may be so circumscribed that it is only obvious when the muscle contracts causing a sudden ball of muscle to rise up. This "ball" enlargement is also often seen in the muscles of the upper arm, the biceps, triceps, and less often the deltoids, giving the impression of hard but minute bunched up muscles, rather as if a pygmy boxer had given his muscles to a giant. Where the whole muscle is enlarged, as in the calves, the feel is typical. It has been described as woody or rubbery, but it is more like handling a piece of pickled pork. The lumbar muscles may be enlarged along their whole length, standing out each side of the spine. On the other hand any group of muscles may waste from the beginning of the disease, especially in girls (Fig. 220). In general, however, the muscles of the calves and thighs enlarge and the muscles of the upper arms and shoulder girdles waste.

Contractures. The importance of contractures in muscular dystrophy cannot be over emphasized. Indeed, they often appear to be as much a symptom of the disease as the weakness of the muscles themselves. They are generally stated to occur late in the disease when the child has become too weak to move, so that by sitting all day in a chair his thighs and knees and feet become largely fixed in one position. This, however, appears too simple an explanation. It is quite common to see children who have had their Achilles tendon lengthened, because it was too short, months or even years before there was any suspicion that they were dystrophic, and years before they ceased walking. On the other hand an occasional child is seen with no trace of any contractures who has been confined to his chair for months with no massage or treatment of any kind. One is indeed forced to wonder whether the inconstant but often dominant contractures are due not to the primary muscular weakness, but to a primary change in connective tissue. Why this change is not found in all cases is obscure, but an analogy is seen in the rare primary involvement of the osseous system described below. The clinical relation of vitamin E to collagen is discussed further on p. 660.

Probably so little notice has been taken of contractures because the muscular weakness has been held to explain all the child's disabilities. Actually, however, the contractures are a major cause of his disabilities, especially through their effect on the feet and hips.

The feet, as has already been mentioned, may be drawn down by the contractures of the Achilles tendons, so that the child in the early stages tends to stand or walk on tip-toe, wearing out the toes of his shoes. As the deformity gets worse it is a very real hindrance to walking, even where the muscles of the legs are still moderately strong.

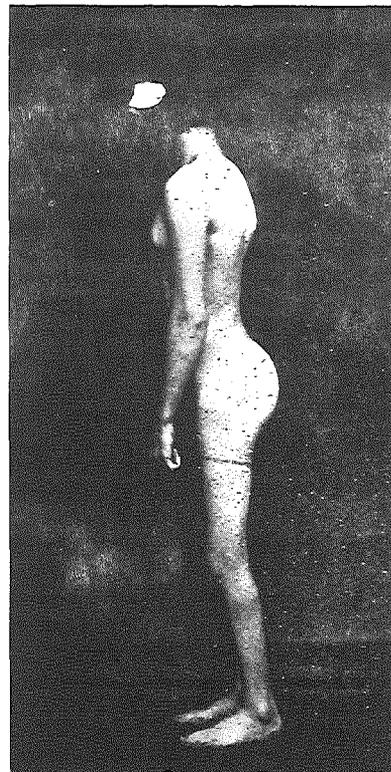


FIG. 220. English girl, aged thirteen, with muscular dystrophy. Note the low lumbar lordosis, and wasting of calves and thighs without any pseudohypertrophy. Sexual development is, if anything, precocious.

Contractures of the hips are seldom recognized, yet it is they as much, or more, than the weak pelvic muscles which lead to the grotesque lumbar lordosis of these children. To show that this is so it is only necessary to lie the child flat on his back, when he will be unable to keep his lumbar spine on the floor without flexing his thighs. If he is laid on his face flexing his knees will cause his buttocks to rise in the air, because the shortened muscles from his pelvis to his legs are thus stretched and so flex his body on his thighs. Kneeling in an upright position is also impossible for many children who can still stand, because in this position the shortened anterior thigh muscles pull the body out of the vertical as they are stretched by the flexed knees.

Contractures of the knees are seldom an early complication of muscular dystrophy, though of course when they occur they are an added difficulty

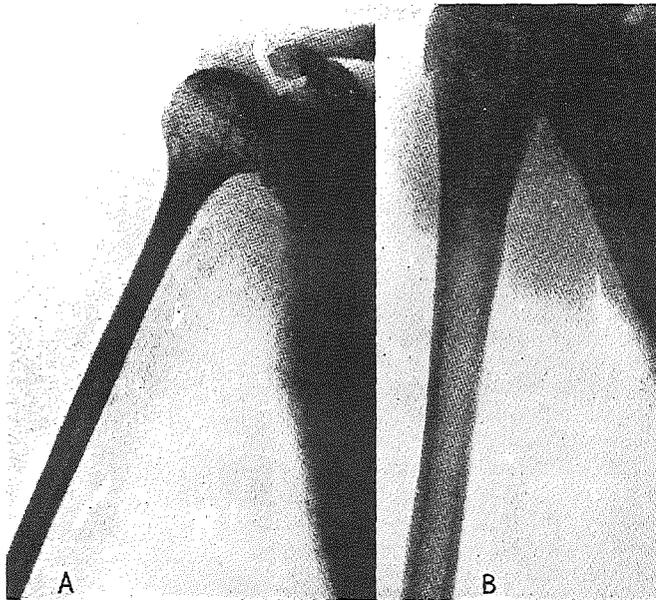


FIG. 221. X-ray A is of an Italian boy in America, aged sixteen, with muscular dystrophy and external concentric osseous atrophy (see text, below). X-ray B is of a normal boy of the same age.

in walking. Some limitation of movement at the elbows may occur early in the disease, but it causes little disability.

Osseous Atrophy. For many years it has been recognized that in a very few cases a primary osseous atrophy may occur at the same time as the primary muscular atrophy. Bramwell [333] believed that both these atrophies were manifestations of a change in the endocrine or sympathetic nervous system, and Maybarduck and Levine [334] also hold both conditions are independent processes of the same disease. The latter authors and Ashby and others [335] have each reported one case of their own, and very fully reviewed the literature.

The bone atrophy has been described as an external concentric progressive atrophy.

The shafts of the long bones become progressively thinner, though the thickness of the cortex is not greatly reduced. Apparently as the outside of the cortex is removed the inside is reinforced with further bone, so that the slimness of the shaft chiefly occurs at the expense of the medullary

cavity. The length of the bones remains normal. Cases are reported where the femora were no thicker than a little finger. The epiphyses of the bones are not decreased in size, so that the thin shaft connects two normally grown ends, giving a dumb-bell effect. The ends, however, may show considerable rarefaction. The flat bones of the pelvis and scapula may undergo some rarefaction as well as the bones of the feet and patellæ. Whether the small size of the winged shoulder blades seen in some cases is due to this atrophy or is secondary to the muscular weakness is uncertain. The fingers are thin and tapering.

Rarely the mandible is involved, developing a short vertical ramus and an obtuse angle, so that only the molars can be made to meet.

It appears definite that these bony changes are one of the primary results of the disease and are not merely a secondary disuse atrophy brought about through the weak muscles. For no bone changes may be seen in very advanced cases, and when they do occur they may be present where the muscular power is still unimpaired. It is also significant that a case is recorded where there was both osseous atrophy and hypertrophy at the same time. Further, a boy with muscular dystrophy and osseous atrophy had a sister whose muscles were normal but whose bones showed the same typical atrophy as her brother's. Indeed it appears as if lack of vitamin E may sometimes only cause osseous atrophy, sparing the muscles completely. It is interesting to recall that vitamin E deficient rats may show osseous atrophy (p. 623).

Other Symptoms. Pituitary dysfunction is said by Bramwell [333], and hypothyroidism by Wechsler [337], to be a common complication of muscular dystrophy, as indeed it may be in vitamin E deficient rats. However, sexual development in boys often appears to be precocious, a point noticed both by Bicknell and by Armstrong [336]. Minot and Dodd [163] report that the urine of dystrophic boys contains oestrogens. Most children with muscular dystrophy have unusually good teeth. Mental precocity is common in muscular dystrophy, since the children are too much in the company of adults; added to this precocity is often a lively intelligence which never suffers any change.

Diagnosis. When the disease is fully established it is unlikely to be confused with any other. The diagnosis, however, is difficult in very young children who have been late in walking and then not walked properly, or in children whose early symptoms all appear to be merely the result of short Achilles tendons. In such cases the estimation of the creatine and creatinine of the urine is of value, since the former is increased in muscular dystrophy and the latter diminished. Creatine tolerance is also lowered. These changes are found in all conditions where muscular function is impaired, so that they are really only of value in confirming that the child's symptoms are due to muscular weakness, where this is uncertain clinically. In children up to about the age of seven some creatine is normally present in the urine, but after this age it should disappear.

A biopsy on the most affected muscles will confirm a doubtful diagnosis (p. 614). The simpler electrical reactions of the muscles are of no interest, remaining normal as long as any contractile muscle is left.

Course. Without treatment the child steadily gets weaker, generally dying from a respiratory infection somewhere in his teens. Even with treatment if the chest expansion is bad when the child is first seen the outlook is very uncertain, as the most trivial bronchitis may end fatally in a few hours. This is due to the child being so weak that he cannot cough up any mucus, with the result that bronchial obstruction and pulmonary collapse may rapidly occur. The expansion of one side of the chest may also be hindered by the extreme scoliosis which develops in children left all day slumped in a chair.

Some children become very fat: apparently this is not entirely due to

lack of exercise, since they grow thinner with treatment even before they begin to move more freely. Most very advanced cases become extremely thin.

Facio-Scapulo-Humeral Type of Landouzy and Dejerine [330]. This form of muscular dystrophy is sometimes referred to as progressive muscular dystrophy. Intermediate forms, however, between it and the pseudohypertrophic type are common, and the same changes in the muscle fibres occur in both. It seems, therefore, that the two diseases are fundamentally the same, though they differ in certain aspects.

Thus in the facio-scapulo-humeral type both sexes are equally involved

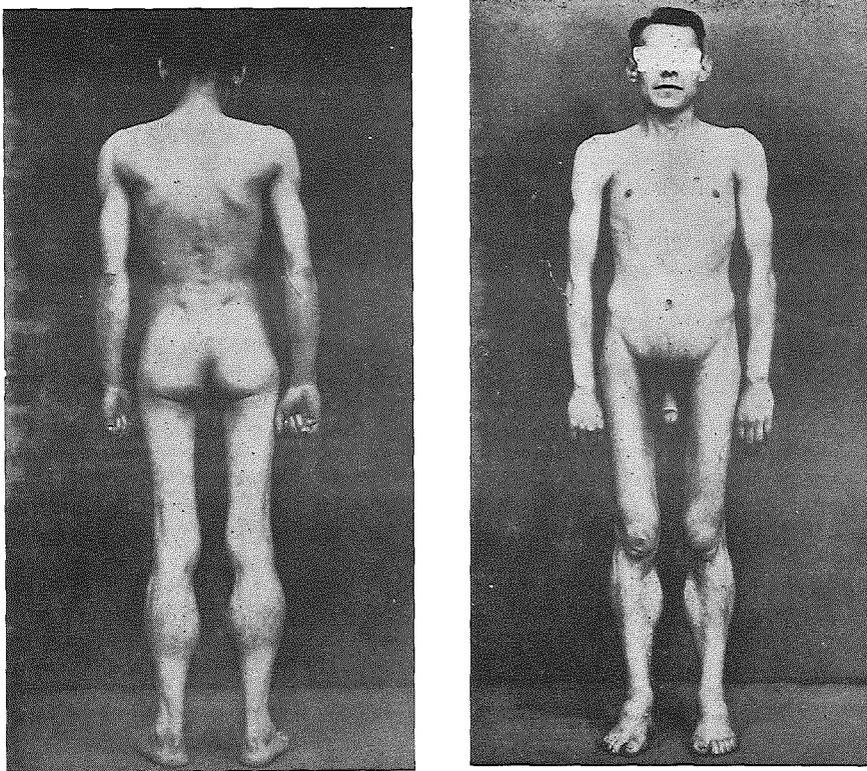


FIG. 222. English man, aged twenty-three, with pseudohypertrophic muscular dystrophy dating from early life, and progressing with unusual slowness. Note the pseudohypertrophy of the calves and possibly the triceps, the wasted thighs and buttocks and the low lumbar lordosis. Sexually he is very well developed.

and there is a strong hereditary tendency. The age of onset is also different, being commonly in later childhood, though infants and young adults may develop the disease. Progress is often so slow that the expectation of life is hardly diminished. One of the first cases to be described developed symptoms when he was twenty and died at sixty still pushing a hawker's barrow.

As the name implies it is the muscles of the face, shoulders and upper arms which are chiefly affected. The facial muscles first become weak causing the myopathic facies, with its prominent lips, transverse smile and inability to close the eyes or frown. The expression has been called stupid and sanctimonious [330]. With the involvement of the shoulder girdles the scapulæ become winged, and as the weakness spreads to the upper arms

many movements become impossible. Lastly, the thigh muscles become affected. Pseudohypertrophy is rare, and tends to be of the "ball" variety (p. 639).

The Scapulo-Humeral or Juvenile Type of Erb [331]. This variety tends to occur at adolescence or in early adult life and often progresses slowly. The upper arms and shoulder girdles are affected first and many years may pass before the legs become weak, though progress is sometimes rapid.

Treatment of All Forms of Muscular Dystrophy. Bicknell [9] in 1940 first treated muscular dystrophy with vitamin E, using whole wheat germ. He claimed better results than were justified, owing to his failure to realize

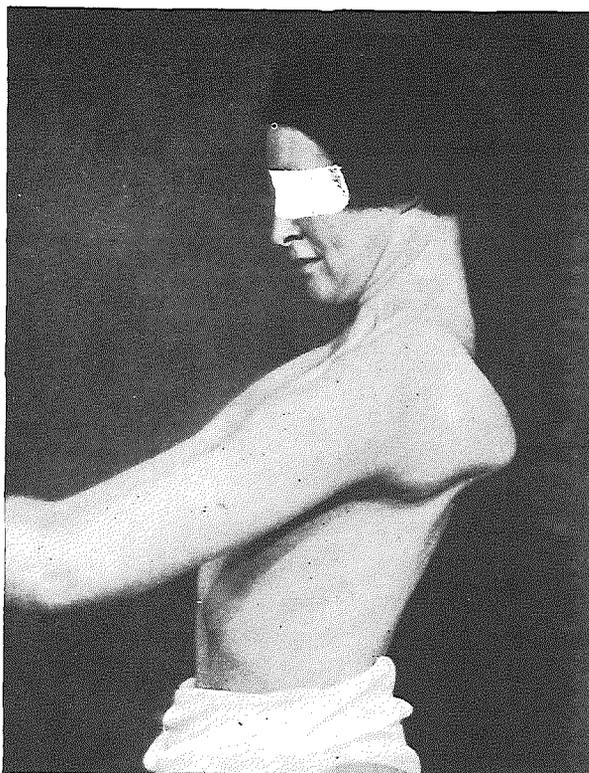


FIG. 223. English woman, aged forty, with the facio-scapulo-humeral type of muscular dystrophy. There is some difficulty, not shown in the photograph, in closing the right eye and showing the teeth. Note the winged scapulae and weakness of the shoulder girdles preventing the arms from being fully raised.

that encouragement alone can greatly increase the apparent muscular power of a child who has hitherto been left unstimulated and neglected in his bed or chair. But over the last twelve years, treatment in essence still being based on whole wheat germ, a few, a very few, children have improved in a dramatic manner; a few have improved and have then remained stationary from before puberty to the twenties, even marrying and having children; a few have remained stationary for years; many have continued to deteriorate. Of those who have not deteriorated, most have had as a background excellent and fresh food and no complicating diseases or chronic sepsis. One observation to which it is easy to attach too much importance is that bread made at home from stone ground flour has been eaten by the majority though not all of the children who have not got worse. Synthetic

racemic alpha-totopheryl acetate in doses ranging from 20 mg. to 200 mg. daily has been valueless.

By chance, owing to the circumstances of the patients, only those children who have not responded to treatment with wheat germ alone have had their creatine and creatinine excretions measured. But arrest or improvement on wheat germ is at best so slow and fluctuations in excretion from day to day, and even from week to week, are so great in all cases, however treated [328], that significant alterations in excretion could only be demonstrated were they estimated almost continuously for many months. Therefore until a rapid cure is discovered such investigations are of less value than the clinical progress of the child.

Stone [345] in America has reported twenty-five children treated with fresh wheat germ oil daily. All improved, and one completely recovered. Progress was hastened by adding the vitamin B complex to the oil, thus giving a mixture very similar to wheat germ, and also ascorbic acid. Minot and Dodd [163], giving "large amounts" of vitamin E, improved two of five cases. Donovan [254] treated a boy with dystrophy with three-quarters of an ounce of wheat germ four times a day for six weeks with very marked success.

No improvement, however, has been reported by the following investigators. Harris [338] observed three dystrophic children for about nine months while they were taking up to 200 mg. of alpha-tocopherol daily by mouth. He found no change in the excretion of creatine and creatinine or in their physical condition. Minot and Frank [85] also failed to reduce the creatinuria in eight boys, five to nineteen years old, with varying doses of synthetic vitamin E or wheat germ oil. In these boys the level of vitamin E in the blood (p. 602) was normal before treatment and rose after. Shelden and others [339] report eight cases of muscular dystrophy, aged 5, 19, 28, 28, 30, 34, 38, 58. Treatment was with either 100 mg. of alpha-tocopherol injected twice weekly, or 50 mg. given daily by mouth. In every case 3 tablespoonfuls of wheat germ oil were taken after each meal. The duration of the treatment is vague. Ferrebee and his collaborators [340] treated twenty cases, of whom thirteen were aged fifteen or less. Treatment was continual for from four to fifteen months. It consisted of the daily consumption by mouth of two tablespoonfuls of wheat germ, 110 to 190 mg. of alpha-tocopherol, and 10 to 30 mg. of vitamin B₆. Injections of 100 to 400 mg. of alpha-tocopherol were also given each week. Pohl and Baethke [341] gave a wide range of oral doses of all forms of the natural and synthetic vitamin to fifteen cases for nearly two years with negative results. In Spain Alemany Soler [343] and Allué Horna [344] have reviewed the results of many workers, the great majority of whom failed to obtain any benefits from vitamin E, though a few reported cures.

Two criticisms of all these negative results can be made. Firstly, adult cases, in whom the disease has lasted for many years, cannot be expected to improve as the greater part of their weakness must be due not to muscle fibres which are still degenerating but to fibres which have completely disappeared.

Secondly, many of the young cases whose muscles should have been capable of some regeneration had large, in most cases huge, doses of alpha-tocopherol. In fact, most of them were taking as much vitamin E as would be contained in a daily consumption of 50 lb. of wholemeal bread. Such quantities would appear to be far beyond any physiological requirements, and to be thirty to forty times as large as those given to those cases which have been arrested or improved. Nothing is known of where or how excess vitamin E is destroyed, but since it is mostly stored in the muscles it well may be that they not only utilize vitamin E but also destroy any excess. If this is so muscular metabolism might be overburdened by the effort of destroying large amounts. The stimulating effect of large quantities of

vitamin E on phosphorus metabolism in muscle (p. 595) may also be overwhelming. Indeed, some of the American cases give the impression that they deteriorated faster when given massive doses of vitamin E.

Along such lines as these probably lies the explanation of why small amounts of wheat germ, or wheat germ oil and the vitamin B complex, may cause a slow and continuous improvement in cases of muscular dystrophy, while at best no lasting effect is gained by large amounts of alpha-tocopherol insufficiently balanced by the vitamin B complex and the other constituents of wheat germ.

From the above discussion it appears that the most satisfactory way of administering vitamin E is to give whole wheat germ and fresh foods rich in vitamin E. About 1 ounce a day of wheat germ is ample. Most children prefer it taken like a "breakfast food" before breakfast, mixed with cold milk. It is essential that the wheat germ should not be stale or rancid.

Wheat germ oil and the vitamin B complex may be given to those unusual cases who dislike wheat germ. Apart from the greater expense of this form of treatment there is always the uncertainty as to how fresh is the wheat germ oil, and so how much of its vitamin E potency is retained. In England wheat germ oil, and wheat germ oil concentrates, are usually sold in capsule form, and are reported not to deteriorate. The unconcentrated oil is probably best. About 6 mgs. daily of alpha-tocopherol in this form should be sufficient.

Wheat germ, wheat germ oil, and synthetic vitamin E all may cause an irritating urticaria.

Rancid fat destroys vitamin E, so that any food like rancid butter, sour milk, stale meat, or cheese must be avoided (p. 615).

The diet should also contain as much food rich in vitamin E as possible, especially stone-ground bread, and green vegetables in large amounts. The former is well worth the trivial trouble of making it at home. Such a diet unsupplemented with any preparation of vitamin E is reported to have cured two children of muscular dystrophy and to have improved three others. Bile salts should be given by mouth with the vitamin if there is any intestinal history suggesting that bile may be deficient, since it is necessary for proper absorption (p. 600).

Liquid paraffin, or paraffin emulsions, should not be used as aperients, as they probably hinder the absorption of vitamin E (p. 600). Constipation may be a severe trial to many patients and their families: no single aperient suits every child, so each type must be tried in turn, senna pods or salines often being found the best.

Sepsis delays or stops progress, even if it is mild, such as carious teeth, sinusitis, otitis media, or ingrowing toe nails. It cannot be overemphasized that sepsis must be treated.

Infectious diseases such as scarlet fever tend to make the child worse for a time, but, on the other hand, they may have no effect. Children should be got up as soon as possible after illnesses. Treatment with the sulphonamides and penicillin has no adverse effect on the muscular condition, and has even been reported to be beneficial. Since the mildest bronchitis may be fatal within a few hours (p. 638), it should be immediately treated as if it were severe pneumonia.

Treatment of Contractures, Massage and Exercise. Contractures in the majority of cases greatly increase the disability (p. 639). Almost constantly there is a shortening of the Achilles tendon which, added to the usual extreme weakness of the flexors and evertors of the feet, makes walking difficult. At the same time weak external rotators of the thighs may allow the feet to turn in so that the child trips up over them.

Adjustable light splints worn at night will help to prevent further shortening of the calf muscles and also will tend to passively lengthen them. Where splints are tolerated badly and the child sleeps on his back, pressure

can be kept on the outer front of the sole by arranging that the feet press on a padded board pushed upward from the end of the bed. Raising the head of the bed slightly can also be used for pushing the feet against their support.

Very simple exercises should be given several times a day to make the child use the dorsiflexors and evertors of the feet and the rotators of the thighs. It is remarkable how a child who has stopped using a muscle will not begin to use it again as it grows stronger, unless he is taught to do so.

The contractures of the thighs and knees are partly checked if the child sleeps flat on his back, but it is not worth sacrificing sleep in trying to make children stay in a position they dislike. For at least an hour a day all children, especially those with contractures, should lie flat on their backs on the floor or table with only a small cushion under their heads. If the adductors of the thighs are so weak that the knees flop apart the legs should be tied loosely together with a scarf. This rest is good for all children, and also tends to pull out the flexion contractures of the thighs and knees.

When sitting the feet should be drawn under the child to increase dorsiflexion, but sitting is always a very bad position because it favours contractures of the thighs and knees. Children instead should, as far as possible, recline so that the angle between their body and thighs is increased. When children are too weak to change their own position it should frequently be changed for them. Lateral curvature of the back must be avoided.

Gentle passive stretching of the contractures should be carried out several times a day. Laying the child on his back on a table so that his thighs hang unsupported over the end is an excellent method of stretching contractures of the thighs.

Boots with high heels and the outer side wedged may be used to help in walking. The boots must be light with flexible soles, and as the calf muscles stretch, the heels must be cut down. Spinal jackets and other forms of support must never be employed.

Surgical treatment of contractures is seldom necessary, being generally corrected by the above methods. In any case the longer an operation is postponed the stronger will be the muscles and the less the likelihood of a recurrence.

General light massage is valuable for children who can move but little. The great value of a masseuse, however, is to teach the child to use his muscles again as they grow stronger. Very simple, varied, and amusing exercises must be given which the child and his mother can do several times a day. Ideally every joint and muscle should be fully used every day. Faradism should not be employed.

Exercise is good as long as it is not forced. Children will not over-tire themselves when playing alone, and such games as "Red Indians" make them use combinations of muscles which are difficult with set exercises. A puppy is an excellent playfellow for a dystrophic child. Swimming or playing in the water is often possible when walking is not, because the body is supported by the water. On the other hand it is so important to avoid infections that public baths should never be used. If the child gets cold he will for a few hours be considerably weaker.

Glycine in the Treatment of Muscular Dystrophy. Armstrong [336] has given a thorough review of the use of glycine in the treatment of muscular dystrophy, and has reported its effects in eighteen cases of his own, many of whom were treated for more than three years. He also followed the effects of treatment with biopsies on muscles. It appears that glycine may give a temporary improvement in some cases, but it is certainly of no lasting value in males, and probably does not influence the ultimate course of the disease in females. The experimental work of Ni, which Armstrong mentions as supporting the use of glycine in dystrophy, is discussed on p. 616, where it will be seen that Ni himself now does not consider that his experiments showed that glycine itself influenced the nutritional dystrophy of animals.

The transitory clinical benefit of glycine is probably due to its increasing the power of normal muscle [342].

Other Diseases of Muscle. *Amyotonia congenita*, according to Stone [253, 345], is the result of a very severe intra-uterine deficiency of vitamin E. Treatment with 2 to 4 ml. of fresh wheat germ oil and the vitamin B complex gave excellent results. Bicknell [9] also reported improvement in one case treated with whole wheat germ.

Development and Growth. Stone [253, 345] treated a large group of children between the ages of one and five who were seen because of poor muscular development, late standing and walking, and inability to hold up their heads until they were two or three. Treatment with the vitamin B complex had little effect, but rapid improvement occurred when 8 to 12 minims daily of fresh wheat germ oil were given as well. He believes that young children with muscular hypotonia are very mild cases of a vitamin E deficiency resulting from poor placental supplies.

In eleven of seventeen premature infants Widenbauer [346], using wheat germ oil, caused a rapid increase in weight after it had been stationary for some time. His results were carefully controlled, and appear important; they are supported by one Spanish case [347]. The malnourished children of Blackfan and Wolbach [326] appear to have had muscular degeneration due to lack of vitamin E.

Scleroderma and Dermatomyositis. Whole wheat germ has been reported as decreasing the creatinuria in three of five patients with this condition [348] and one case is said to have been cured by alpha-tocopherol [349].

Fibrositis and Rheumatic Diseases. Steinberg [370] in 1949 stated that he had treated three hundred patients with generalized primary fibrositis: a condition commonest in middle age where there is soreness in one or more groups of muscles, often with bursitis, which comes on after chilling or unusual muscular movements or stresses. The twenty-four hour creatine excretion is above 100 mg. but otherwise all investigations give normal results and the patient is in good health. "The vast majority of such cases" are cured by 300 mg. daily of mixed tocopherols, after which 50 to 150 mg. daily are needed as a maintenance dose. No controls are mentioned. Steinberg states in an earlier paper that other forms of fibrositis do not respond, but Ant and Cyan [371] claim, again without controls, that vitamin E is of value in all rheumatic diseases from rheumatoid arthritis and osteoarthritis to muscular rheumatism.

VITAMIN E IN THE TREATMENT OF NEUROLOGICAL CONDITIONS

The experimental work of Einarson and Ringsted (p. 618) raised high hopes that vitamin E would be of value in certain degenerations of the central nervous system—notably amyotrophic lateral sclerosis and tabes dorsalis. The weight of evidence, however, is now against this, though it must be admitted that the position is not yet clear. From the clinical results discussed below it appears that alpha-tocopherol used alone and in large amounts is at best of uncertain value in progressive muscular atrophy and amyotrophic lateral sclerosis. But there are too many reports of vitamin E having had a transitory or prolonged effect on nervous degeneration for them to be completely ignored.

The subject is extremely complex. There is firstly the experimental evidence of Einarson and Ringsted that once lack of vitamin E has initiated a nervous degeneration, the addition of vitamin E to the diet will not check further degeneration if this is at all advanced. Secondly, the experimental work of Wintrobe and his colleagues [350] has shown that a degeneration of the central nervous system in pigs can be produced by food deficiencies, though the deficient factors have not been identified. Thirdly, synthetic vitamin E may be toxic in large amounts and may require the vitamin B

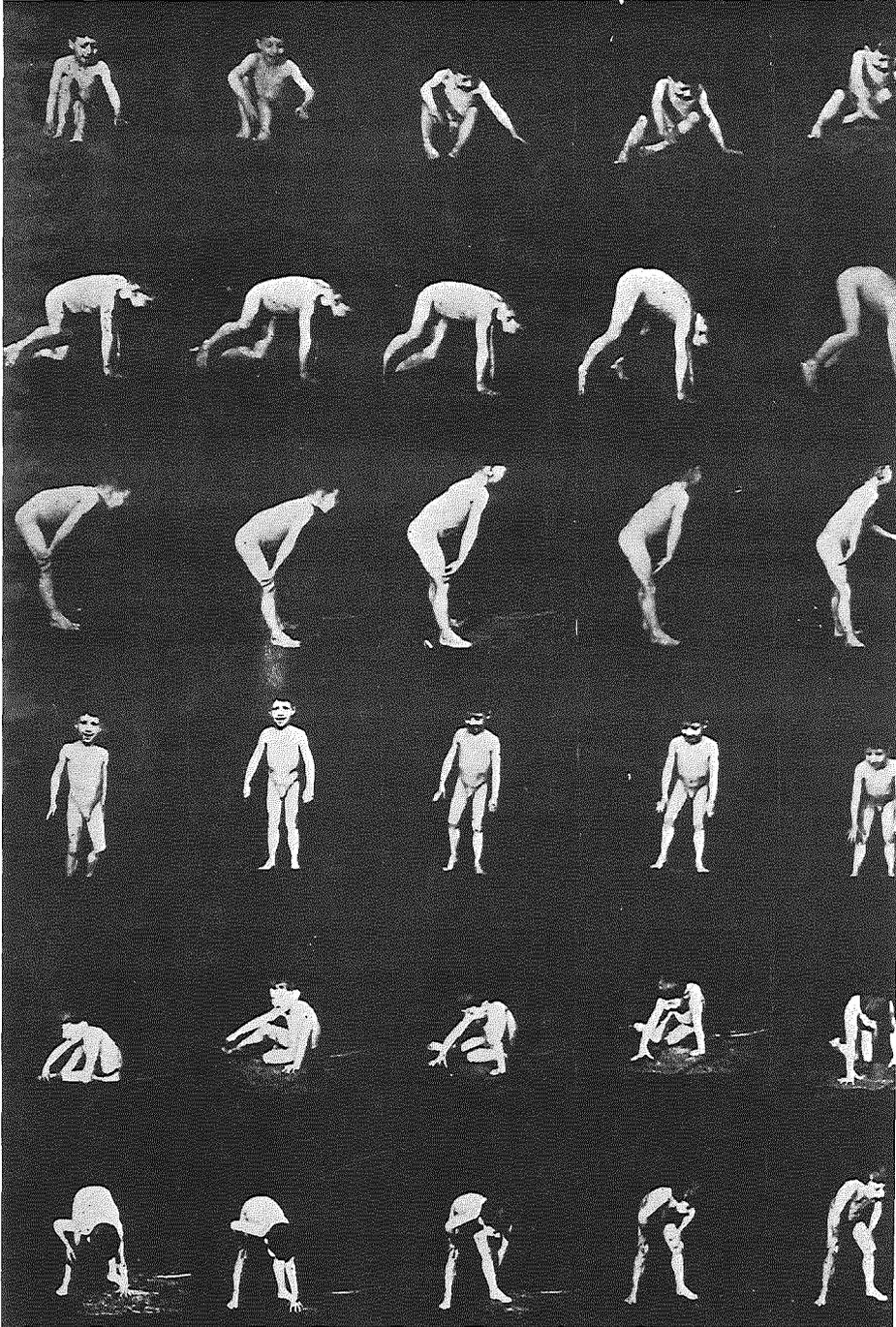
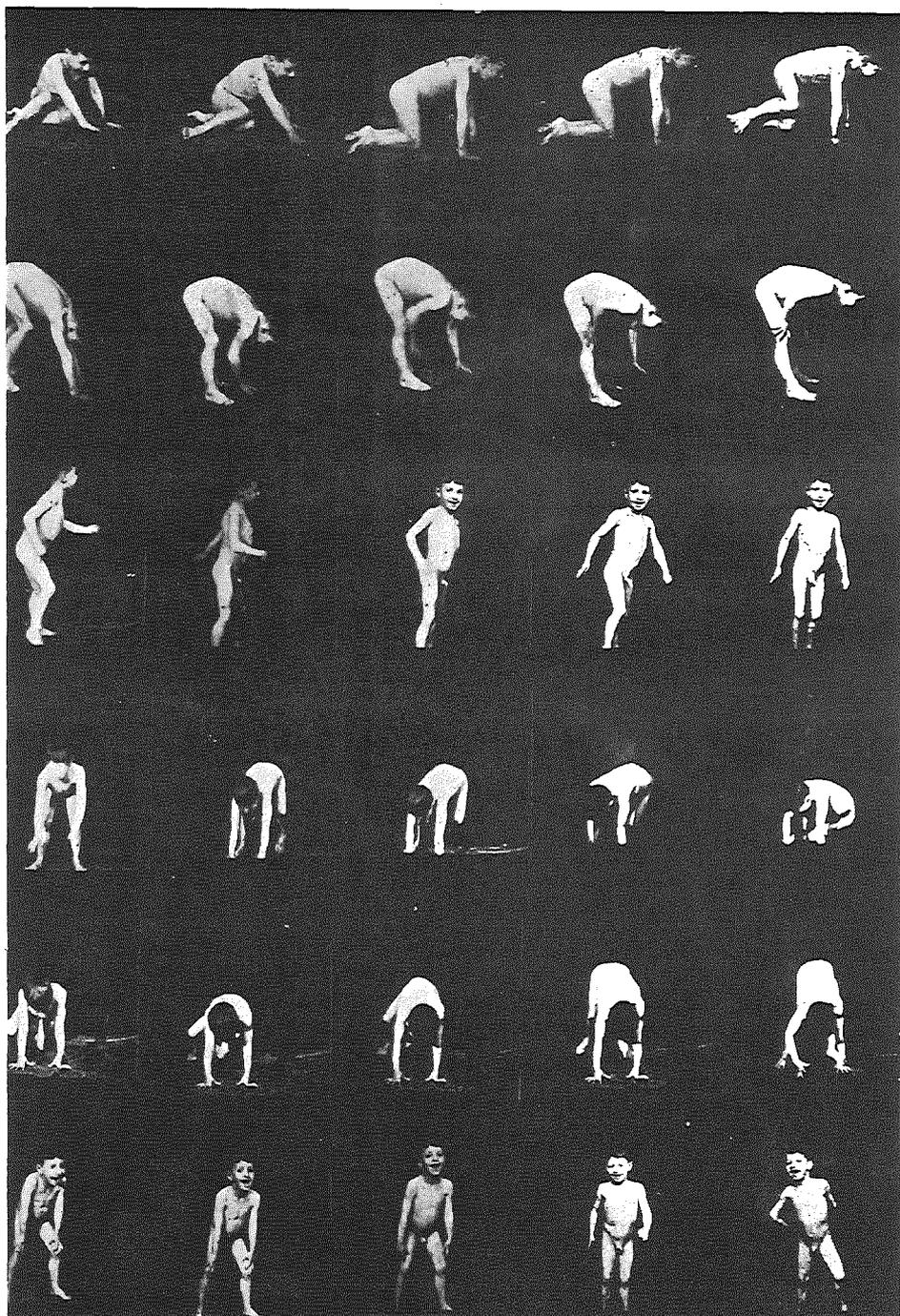


FIG. 224. Typical movements of an English child



hypertrophic dystrophy. (See text, p. 637.)

complex for its proper utilization or destruction by the body. Fourthly, as has been pointed out by Wechsler [351], similar nervous degenerations may, in different people, have different causes—thus some cases of amyotrophic lateral sclerosis may be due to a food deficiency, some to a vascular degeneration, and some to other causes. Lastly there is Davison's outstanding work, to be discussed later, which strongly suggests that vitamin E profoundly modifies pathological changes in the central nervous system, even if it cannot prevent them.

The impression left from the clinical reports is that we are still fumbling in the dark close to a solution of many of the nervous degenerations, but that until more light is thrown on the subject the patient's best chance is to be treated with natural vitamin E and the B complex, and a high intake of foods rich in the other vitamins. This does not necessarily imply that the degenerations are caused by a food deficiency, but it does mean that any



FIG. 252. Case I. Lack of visible demyelination in the pyramidal pathways of a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Fig. 226. Myelin sheath stain.

deficiency which is hampering the nervous system in its recovery is removed. The level of vitamin E in the blood is discussed on p. 602.

Amyotrophic Lateral Sclerosis and Progressive Muscular Atrophy. Bicknell [9] in 1940 reported good results in two of four patients treated with whole wheat germ, but later cases treated with either wheat germ, or synthetic vitamin E have only shown, at best, a mild objective and subjective improvement for a few weeks before continuing to deteriorate. In three cases, and one reported from South America, synthetic vitamin E has caused extremely painful cramps or spasms of the legs, occurring chiefly at night. Two patients regulated their own dose of vitamin E by these cramps, which came and went as the dose was increased or diminished. In one case the pain was so severe that a fatal collapse was feared, though not more than 20 mg. of alpha-tocopherol was being taken. A case of disseminated sclerosis also developed cramps whenever the daily intake rose about 9 mg. The vitamin B complex appeared to increase the tolerance for vitamin E in these cases. One patient who had marked euphoria gave up wheat germ oil because it changed his euphoria to depression.

Wechsler [352] has reported sixty cases whom he treated with synthetic vitamin E in 50 mg. doses daily either by mouth or injection. He also

gave wheat germ oil, the vitamin B complex, foods rich in vitamin E, and bile salts to aid the absorption of the latter. Ten of his cases showed varying degrees of improvement, two completely recovering. The longest period of treatment was two years. His paper [351] should be read for an excellent discussion on the general problems and implications raised by considering such cases are due to food deficiencies. Some of his cases improved or got worse as the vitamin was given or withheld [351]. He and others [53] report that in seventeen cases the average tocopherol content of the blood was 0.67 mg. per 100 ml. compared to 0.96 mg. in twelve normal subjects, the level in the latter varying between 0.59 and 1.62 mg. Transitory clinical improvement with oral doses did not occur unless the level was raised by at least 0.4 mg., this being only achieved by doses of at least 200 mg. daily.

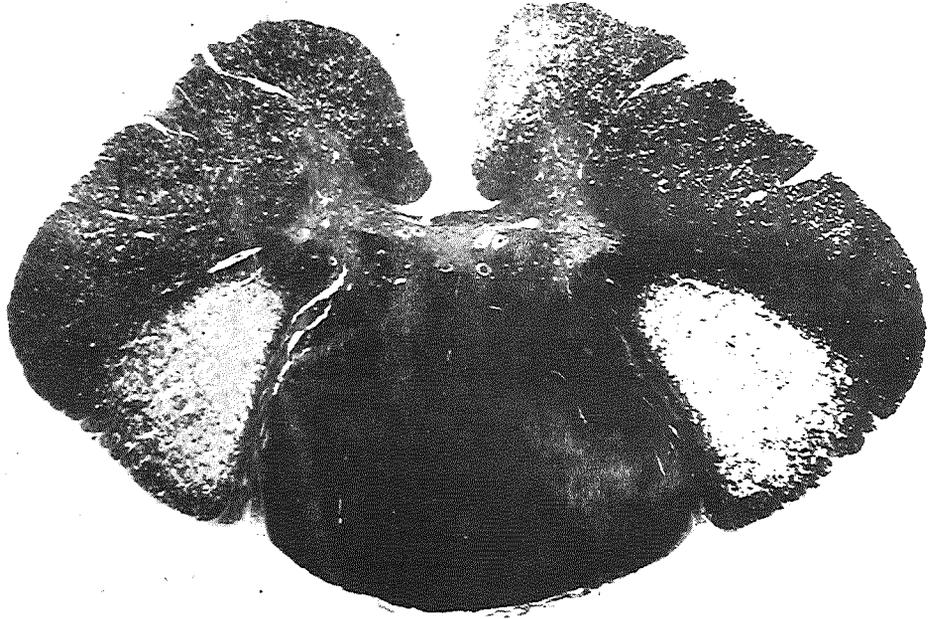


FIG. 226. From a non-treated case, showing extensive demyelination of the crossed pyramidal and left direct pyramidal tracts. Compare with Fig. 225. Myelin sheath stain.

Injections of vitamin E caused a fall in the level in the blood, which is analogous to the decrease in the level of vitamin A after it is injected.

Einarson, Ringsted, and their collaborators [352] state that with 90 mg. of alpha-tocopherol their results from eight cases "furnish some basis for believing that vitamin E may have an effect at least on neurogenic muscular atrophy."

Meller [353] treated fourteen cases with 100 to 250 mg. of alpha-tocopherol daily by mouth or by injection for periods of three to seven months: three recovered and seven improved. Rosenberger [354] relieved some of the symptoms in eight of nine patients with a diet rich in vitamin E, synthetic aneurine and 100 to 200 mg. of alpha-tocopherol given orally. His patients were observed for about a year. Donzallaz and Monnier [355] in Switzerland treated an interned Alsatian soldier for three months with 30 mg. of alpha-tocopherol daily by mouth. There was a considerable degree of recovery, writing and other activities becoming possible. During a short period with no treatment his weakness returned. Gotten [356] reports good results in

AMYOTROPHIC LATERAL SCLEROSIS



FIG. 227. Case I. Insular myelin sheath destruction from a case treated with vitamin E. Compare with Fig. 228. Myelin sheath stain. $\times 240$.

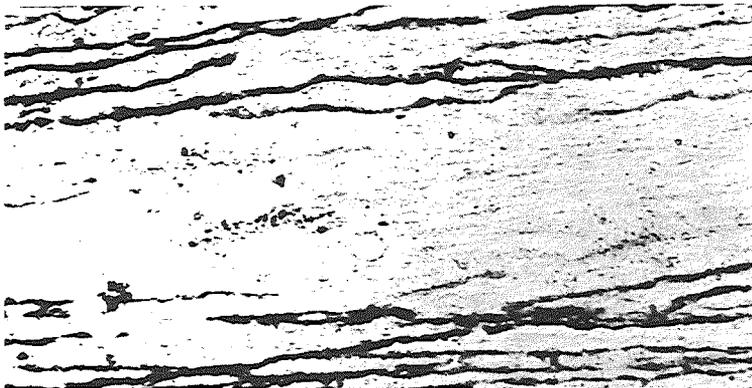


FIG. 228. Extensive myelin sheath destruction from an untreated case. Compare with Fig. 227. Myelin sheath stain. $\times 240$.

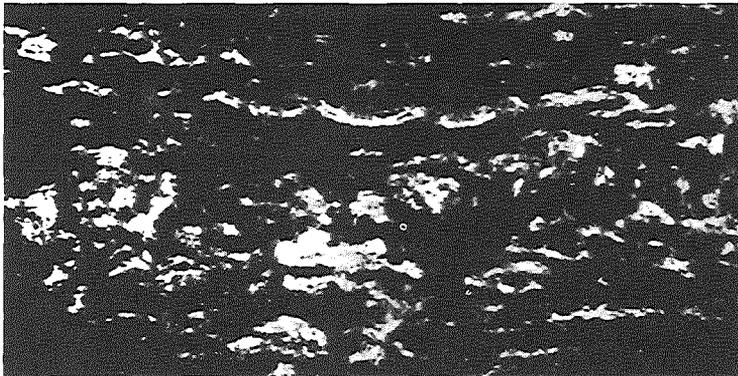


FIG. 229. Case I. Slight disintegration and swelling of single myelin fibres in parts of the pyramidal tracts that appeared uninvolved, from a case of amyotrophic lateral sclerosis that received vitamin E. Compare with Figs. 227 and 228. Myelin sheath stain. $\times 480$.

AMYOTROPHIC LATERAL SCLEROSIS

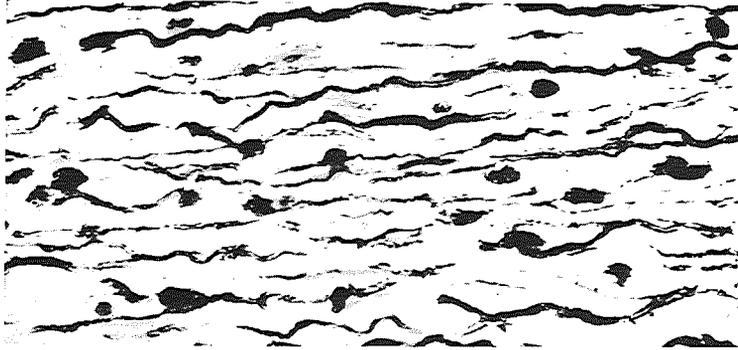


FIG. 230. Case I. Axis cylinders from the crossed pyramidal tract of a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Fig. 231 from a normal case, and Fig. 232 from an untreated case of amyotrophic lateral sclerosis. In Fig. 230 there is a slight diminution in number, swelling and slight tortuosity of axis cylinders when compared with the normal in Fig. 231 and the severely diseased, fragmented and swollen axis cylinders of the untreated case of amyotrophic lateral sclerosis in Fig. 232. Bielschowsky stain. $\times 480$.

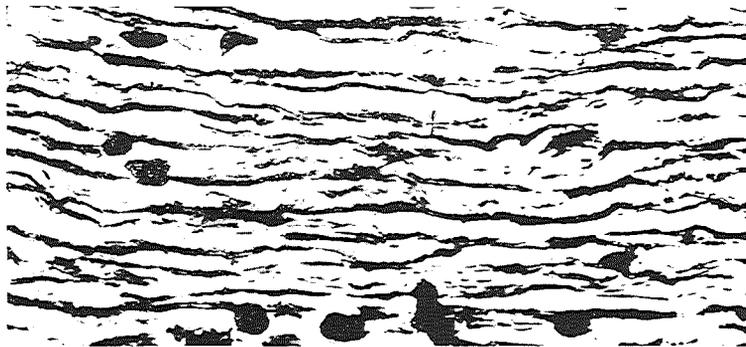


FIG. 231. From a normal control case, for comparison with Figs. 230 and 232. Bielschowsky stain. $\times 480$.



FIG. 232. From an untreated case of amyotrophic lateral sclerosis. For comparison with Figs. 230 and 231. Bielschowsky stain. $\times 480$.

two patients, Viets [352] improvement in one patient among twenty-one, and Slaughter and Cleckley [357] one who completely recovered on 1 drachm of wheat germ oil daily.

Pakenham-Walsh in a personal communication states that of three cases treated with wheat germ one definitely improved and the others did so for a short time, and then continued to degenerate. Gutiérrez-Mahoney [231]

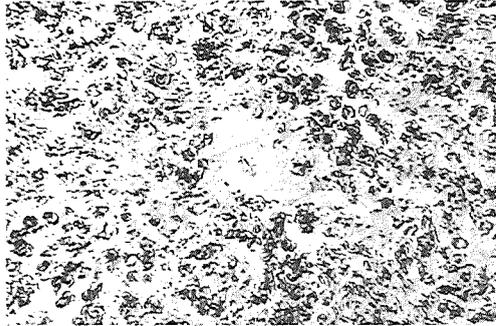


FIG. 233. Case I. Almost complete absence of fat in the pyramidal pathways from a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Fig. 234 from an untreated case showing lipoid deposits throughout and in the perivascular spaces. Sudan III stain. $\times 100$.

cured six of nine cases. Three relapsed when they ceased to take wheat germ oil concentrate, but again recovered when they resumed. One man, who could only walk with a stick, after two years' treatment ran well and returned to work.

Weinberg [358] gave wheat germ oil to one man who had developed signs suggestive of amyotrophic lateral sclerosis while taking sulphathiazole. The neurological condition was cured though the concentration of the sulpha-

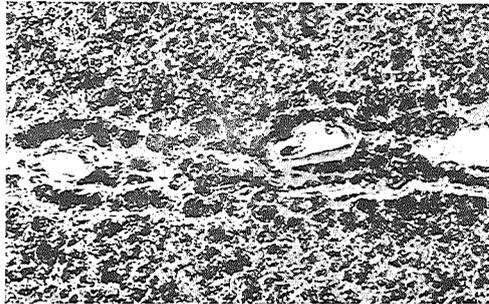


FIG. 234. From an untreated case of amyotrophic lateral sclerosis, for comparison with Fig. 233. Sudan III stain. $\times 100$.

thiazole in the blood remained unchanged. Other vitamins failed to prevent the recurrence of neurological signs each time the drug was given. This is reminiscent of the effect of sulphathiazole in accelerating the development of muscular dystrophy in vitamin E deficient animals (p. 625), though not in man (p. 645).

Davison [359], who has very kindly allowed us to reproduce his most important and unique microphotographs in Figs. 225 to 238, treated ten cases—six men and four women—in the manner advised by Wechsler. After death histopathological examinations were made, forty untreated cases being

AMYOTROPHIC LATERAL SCLEROSIS



FIG. 235. Case I. Lack of gliosis in the pyramidal tracts from a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Fig. 236 from an untreated case of amyotrophic lateral sclerosis showing dense gliosis in the crossed pyramidal tracts. Holzer stain.

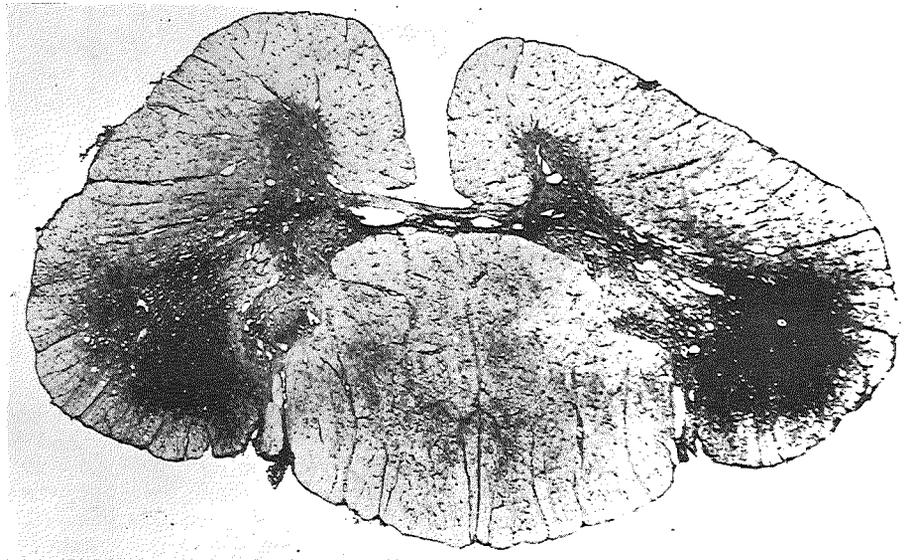


FIG. 236. From an untreated case of amyotrophic lateral sclerosis, for comparison with Fig. 235. Holzer stain.

used as controls. In the six cases who were considered to have had adequate treatment for a sufficient period of time—five weeks to over seven months—the degeneration of the cells of the bulbar nuclei and anterior horns was not affected. But the other pathological changes were checked or reversed. The demyelination of the crossed pyramidal tracts was not one-tenth of

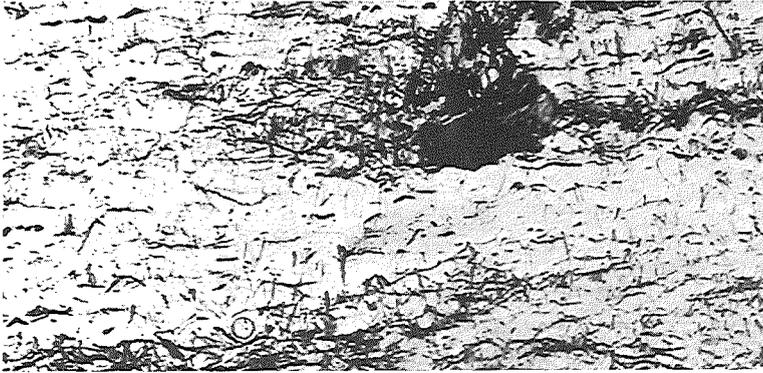


FIG. 237. Case I. Slight insular gliosis from a treated case of amyotrophic lateral sclerosis. Compare with Fig. 238 from an untreated case. In Fig. 238 the gliosis is dense; Holzer stain. $\times 200$.

that in the untreated cases and, indeed, was hardly visible to the naked eye in stained preparations. The changes in the axis cylinders were also far less pronounced. The dense gliosis, plainly visible in stained preparations from the untreated cases, was virtually absent and in most cases the fatty deposits were also greatly reduced. This outstanding work suggests, like that on the

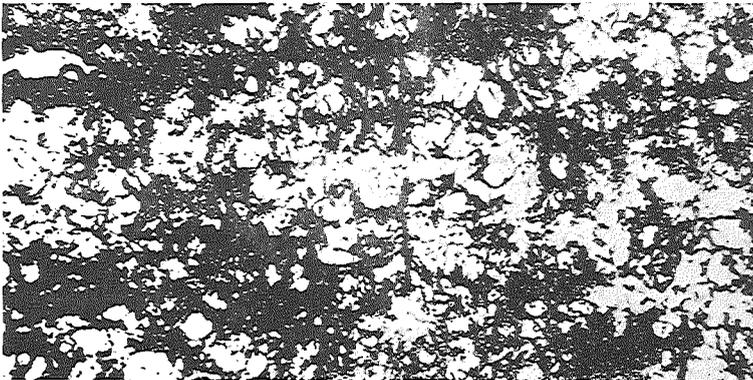


FIG. 238. From an untreated case of amyotrophic lateral sclerosis, for comparison with Fig. 237. Holzer stain. $\times 200$.

muscular dystrophies, that while vitamin E may play an essential part in treatment, it is not in most cases the whole answer to the problem.

Worster-Drought and Shafar [360] have reported that synthetic vitamin E in 30 mg. doses daily had no effect in twelve cases, apart from one who appeared slightly better. They noted that mental depression was relieved. Doyle and Merritt [362] gave eight patients an excellent diet, the vitamin B complex, cod liver oil and 7 ml. of wheat germ oil daily all by mouth, and synthetic vitamins E (60 mg. daily), B₆, aneurine, and liver extract by injection. The results were negative in both the late and early cases. Shelden

and his colleagues [339] also failed to improve ten cases treated with 45 ml. of wheat germ oil with each meal, supplemented daily by 50 mg. of synthetic vitamin E by mouth or 100 mg. twice weekly by injection. A later report following more intensive therapy on further cases is equally negative [363].

Denker and Scheiman [361] have reported that eleven patients, including one vegetarian, did not respond to about 100 mg. daily of synthetic vitamin E given by mouth or by injection. Four further patients given 250 mg. daily and 100 mg. of vitamin B₆ did not improve. There was no evidence, either from the histories, gastric analyses, or radiography, that intestinal disease was hindering absorption. Ferree, Klingman and Franz [340] gave wheat germ and synthetic vitamins E and B₆ by mouth and also synthetic vitamin E by injection to six patients with no results. About 200 mg. of vitamin E were being taken daily.

Goodhart, of the Montefiore Hospital of New York, tells us that twelve cases treated with synthetic vitamins E and aneurine showed no improvement. De Jong [352] has only noted at best a fleeting subjective improvement in twenty-six patients treated for upwards of a year with wheat germ oil, yeast, and injections of alpha-tocopherol up to 240 mg. daily. In the discussion following De Jong's paper Moersch and Viets reported, without giving details of treatment, another thirty-eight cases among whom only one improved. Furtado and De Carvalho [364] also failed to benefit twelve patients treated with wheat germ oil or alpha-tocopherol for one to twenty-seven months.

Disseminated Sclerosis and Other Conditions. Meller [353] and Couperus [76] found that large amounts of alpha-tocopherol were valueless for *disseminated sclerosis* but Dowd [365] believes that they may have a dramatic effect when combined with extensive vitamin and symptomatic therapy. Stone [366, 367], using wheat germ oil for children and 50 to 150 mg. daily of mixed tocopherols for adults, both in conjunction with other therapy, has reported excellent though uncontrolled results in *tabes dorsalis* and *congenital non-obstructive hydrocephalus* and in *psychiatric disorders*, especially those with depression, anxiety and fatigue; benefit in the last of these has also been reported by Michael and Ruggles [368]. Vitamin E is said to raise the level of cholesterol and fatty acids in the blood of schizophrenics [375]. For *Sydenham's chorea*, between the ages of six and nineteen years, Dowd [369] gave every alternate patient 90 to 225 mg. daily of natural alpha-tocopherol. In only two of the seventeen untreated cases was there improvement, while all the treated cases were symptom free in a month. Subsequent treatment of the untreated cases abolished their symptoms. Before treatment all the patients had had routine rest in bed and sedatives, but in almost all choreiform movements, a rapid pulse and joint pains had persisted.

FURTHER CLINICAL USES OF VITAMIN E

Cardiac Diseases. Vitamin E is valueless in cardiac diseases. The subject evoked much interest in the later nineteen-forties because the Shute brothers and others [376, 377] claimed that cardiac degenerations and, especially, angina of effort were greatly improved by massive doses of vitamin E. But all their work is remarkably vague without any controls, and when, rarely, objective findings such as electrocardiographic changes are reported, these again are uncontrolled and indefinite.

The Council on Pharmacy and Chemistry of the American Medical Association [378] early in 1950 repeated their earlier statement that "vitamin E is of no value in coronary heart disease, hypertension or rheumatic heart disease." This statement is borne out by the most carefully controlled work of many investigators [67, 378-386]. Further, the level of vitamin E in the blood of patients with cardiac disease is normal (p. 602) and the experimental reasons advanced for using vitamin E appear com-

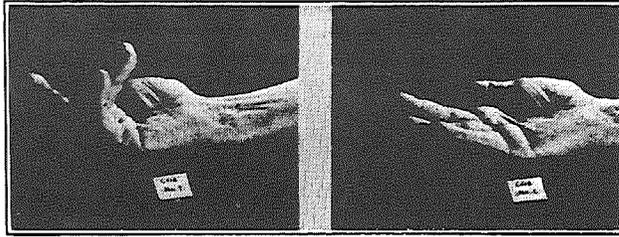
pletely fallacious : bits and pieces have been taken from various papers which, when read in their entirety, do not justify the use of vitamin E. Again the cardiac condition in animals deprived of vitamin E (p. 614) is not analogous to human degenerations which, by the time they are noticeable, have progressed far beyond the trivial changes induced by the severest experimental deficiency. If it is claimed that daily doses of 200 to 500 mg. of natural mixed tocopherols or alpha-tocopherol confer benefit not by remedying a deficiency but by alleviating an anoxia, then the simple answer is that clinically they do not.

Peripheral Vascular Diseases, etc. Here vitamin E may be of value. In intermittent claudication the only properly controlled work is that of Ratcliffe [387] in England who gave 400 mg. of racemic alpha-tocopheryl acetate daily for three months and judged the effects with a walking machine. Of forty-one patients with intermittent claudication who at most could only walk 500 yards, thirty-four improved to the extent of being able to walk at least 880 yards with little or no pain, while of twenty-five control patients only five improved to a similar degree. Boyd and his colleagues [388] treated seventy-two patients with intermittent claudication comparable to that of Ratcliffe's patients, using the same dose of vitamin E. Fifty-nine improved—some walking a mile with no pain—and thirteen were not benefited. In four very severely affected patients the nutrition of the feet improved though the pain was not relieved. Treatment must be continued for three months before abandoning it as valueless. The experimental evidence on arteritis is given on page 623, where it can be seen that vitamin E has only been shown to prevent perhaps arterial degeneration caused by grossly abnormal conditions.

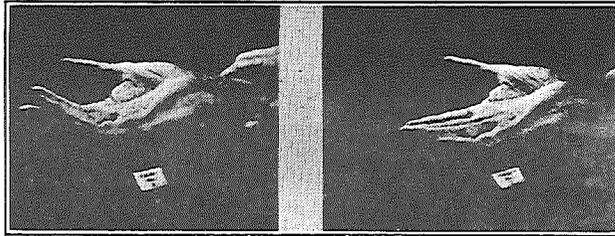
Ulcers of the Legs. Since from the above reports vitamin E might be expected at least to improve some types of ulceration it is disappointing to find that reports claiming benefit [389] are vague and uncontrolled and have not been confirmed in a small number of cases [390].

Thrombosis, Capillary Permeability, etc. Kay and others [391] state that nineteen patients out of two hundred and thirty-eight suffered from post-operative thrombosis following major operations such as intestinal resection, gastrectomy and pneumonectomy. But there were no thrombotic complications in a similar group of one hundred and seventy-five patients who were given post-operatively 200 mg. of alpha-tocopherol every eight hours by mouth, combined with intravenous injections every twenty-four hours of 10 ml. of 10 per cent. calcium gluconate. This report deserves to be considered seriously. The experimental work described on p. 623 suggests that vitamin E might well be of value in post-traumatic thrombosis.

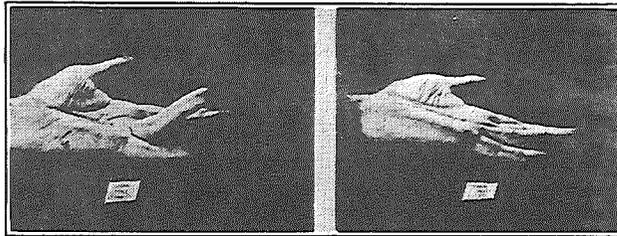
Capillary Permeability, etc. Minkowski [392], from very careful work which merits attention, claims that giving 400 to 600 mg. of alpha-tocopherol during labour increases capillary resistance and decreases hæmorrhage in the newborn and premature infant. This receives some slight support from the beneficial effect which 200 mg. daily of vitamin E is said to have in dogs rendered purpuric by stilbœstrol [283] and from claims that five adults with thrombocytopenic purpura, three women with menorrhagia and bruising, and patients with a terminal purpura all had their purpura relieved within one or two weeks by daily doses of 200 mg. to 400 mg. of alpha-tocopheryl acetate [283]. Akin to this is the reputed good effect of vitamin E in sclerœdema in infants [393] which is also reminiscent of the œdema in vitamin E deficient animals (p. 619). The work of Owens and Owens [73] on retrolental fibroplasia in premature infants again appears to be possibly related to vascular permeability. Each alternate infant was given thrice daily 50 mg. of a water miscible preparation of racemic alpha-tocopheryl acetate. Of eleven infants so treated not one developed retrolental fibroplasia, while of fifteen untreated infants five were affected. Of the next twelve infants given vitamin E only one was affected and he was so ill he



Case 1.



Case 2.



Case 3.

FIG. 239. Three hands with Dupuytren's contracture before treatment and after the patients were given 200 mg. daily of alpha-tocopheryl acetate by mouth, for three to five months.

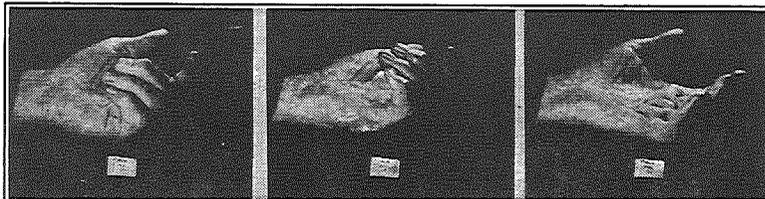


FIG. 240. Dupuytren's contracture before treatment, after treatment with alpha-tocopheryl acetate and later after surgical treatment.

only started treatment eleven days after birth. Of sixty-three infants born before vitamin E therapy was introduced, twelve were affected. The condition was even arrested—which is most unusual—in five of nine infants. On the other hand Laupus and Bousquet [394] report that of sixty-five infants treated with the same dose of vitamin E, eleven were affected. The incidence of retrolental fibroplasia varies greatly in different hospitals; possibly it has several causes and so vitamin E will only be of value in those hospitals which in some manner are giving their expectant mothers or the infants a faulty diet.

Retinal degenerations in adults are said to benefit from vitamin E [395], as is syphilitic interstitial keratitis [396].

Dupuytren's Contracture, Penile Fibroses and the Collagenoses. Russell Thomson [397] in Scotland has reported that 200 mg. daily of racemic alpha-tocopheryl acetate causes a slow softening and stretching of the fibrous bands of Dupuytren's contracture (Fig. 239). He treated twelve cases, all with success, though four hands required operative treatment to complete the cure which generally started in six to eight weeks, though dramatic improvement even occurred in a fortnight in two further cases only treated with 30 mg. of wheat germ oil weekly. At the beginning there may be swelling and acute pain in the palmar fascia. This work is supported by Steinberg [153] who used 300 mg. daily of mixed natural tocopherols: of forty patients only three did not benefit. Treatment must be continued for more than three months before being abandoned as useless. Negative [418] and positive [419] results have been reported by other workers, who gave adequate doses for several months. Possibly the general dietetic background is the deciding factor in the response to vitamin E. King [398] gave 300 mg. of racemic alpha-tocopheryl acetate daily to thirteen patients and reported that six abandoned the treatment because of nausea, giddiness, headaches, tinnitus, swelling of the tongue, etc. Of the seven who continued for one or two months, only one may have been slightly better. This paper is incomprehensible since vitamin E has been frequently given in doses far larger than these with no ill effects.

Penile Fibroses. Scardino and Scott [400] treated twenty-seven cases of Peyronie's disease with 300 mg. daily of mixed tocopherols and obtained good results in thirteen, fair in nine and none in five. Treatment in some cases lasted for eighteen months. No relapses were observed a year after treatment was stopped. There were no control cases. Scardino and Hudson [401] report that urethral strictures, whatever their ætiology and however long they have been present, often respond to mixed tocopherols in daily doses of 200 to 1,200 mg. Of twenty-two cases response was good in fifteen, fair in four and absent in three. There were no controls and the duration of the treatment is vague.

The Collagenoses. Burgess [399] in a paper giving no clinical details states that daily doses of 100 to 600 mg. of mixed tocopherols generally benefit *dermatomyositis* (p. 647), *lichen sclerosis et atrophicus*, *morphea* and *granuloma annulare*. Thirteen cases of the latter condition were treated by Cochrané [402] with daily doses of 300 to 600 mg. of racemic alpha-tocopheryl acetate: after six to nine weeks, nine patients were cured, two probably cured, one better and one unaffected. In *lupus erythematosus* Burgess and Pritchard [403] have reported excellent results: twenty-five cases were treated with very large doses of mixed tocopherols both orally and by mouth, and only one failed to improve. Morgan [404] however, found vitamin E useless for this condition and Sweet [405] found it useless for all the collagenoses.

Miscellaneous Conditions. Diabetics have been said to benefit dramatically from vitamin E [406, 407] but careful work [408, 409, 410] shows this is not so. The level of vitamin E in the blood of diabetics is normal (p. 602) and there is no experimental evidence (p. 596) suggesting that this form of

treatment is warranted. In a few cases of neural leprosy, 20 mg. daily of alpha-tocopherol for two months, combined with sulphones, has given remarkable results [417].

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and subcutaneous tissue contains traces of the polyunsaturated acids while the total content of the carcass fat is still more than half of what it was at weaning. Even after a further six months on the deficient diet there is little further depletion and the liver contains about 0.3 per cent. of these acids, probably as dihydroarachidonic acid [28]. In complete starvation arachidonic acid is spared until about seventy-five per cent. of the fat reserves have been used up; then there is a rapid fall as the rat becomes seriously ill [29].

Little is known about what activities of the body lead to depletion of its stores of the essential unsaturated fatty acids, though probably growth is the most important. Thus when deficient rats are given arachidonic acid, so that growth is resumed, a large proportion of the acid cannot be recovered from the animals [13, 17], and in mature rats deficiency symptoms only occur during rapid gain of weight (p. 672). In symptomless chronically deficient mice a slight loss of blood or X-ray trauma will precipitate deficiency symptoms [37]. Rapidly growing tumours decrease storage—though they grow normally even when storage is very low [30]. During lactation there is some loss through secretion in the milk. The various physiological and pathological conditions which have been stated to alter the amount of unsaturated fatty acids in the serum have been reviewed by Hansen (p. 677).

Activity of Fatty Acids. "Fat-deficiency disease" is an unfortunate name since the disease is not due to a deficiency of fat in general, but to a deficiency of the essential unsaturated fatty acids. The disease can neither be prevented nor cured by saturated fatty acids [31, 34], by oleic acid [32], by isomeric oleic acid [32] with the double bond at 12:13, by alpha-elæostearic acid [33], by erucic acid [32], by chaulmoogric acid [32, 34], by ricinoleic acid [32, 34], or by some of the oxidation products of linoleic and linolenic acids [34].

Cod-liver oil [13], salmon egg, pilchard and herring oil [14] have little or no effect, though one of the highly unsaturated acids of cod-liver oil promotes growth [34]. Cod-liver oil is said to cause storage of tetraenoic, pentaenoic and hexaenoic acids in the rat [35] and dienoic acid in the chick [18].

Prevention and cure of the fat-deficiency disease is brought about by linoleic acid, linolenic acid, some of the oxidation products of these [34], and arachidonic acid. The activity of the first two of these appears to depend on having double bonds at 9:10 and 12:13, but not on their carboxyl group, since this may be changed for an alcohol group [32]. Arachidonic acid is about three times as potent as linoleic acid for promoting growth [32] and linoleic acid is about six times as potent as linolenic acid [19, 34]. For normal gestation linolenic acid is less effective than the other two acids [36]. For curing the skin symptoms of the fat deficiency disease, however, linoleic and arachidonic acids are equally efficacious [13], while linolenic acid has very little effect [19]; all this, combined with the fact that docosahexaenoic acid from cod-liver oil promotes growth but has little or no effect on the skin [34], has led Hume and others [34] to the conclusion that the essential fatty acids have not identical actions. Greenberg and his co-workers [19], however, state that linolenic acid when given with linoleic acid is as efficient as the latter for promoting growth and the cure of the skin symptoms: linoleic acid "sparks" linolenic acid. Widmer and Holman [16] agree with Hume, believing that the "fat-deficiency disease" is really two diseases, one due to a deficiency of linoleic or arachidonic acid and the other due to a deficiency of linolenic or hexaenoic acid.

The optimum daily intake of linoleic acid for weanling rats is for females 10 to 20 mg. and for males probably 100 mg. or less [19, 38], arachidonic acid being about three times as effective [32]. The needs of some bacteria for unsaturated fatty acids can be met by linoleic or linolenic acids [54].

Symptoms of the Fat Deficiency Disease. Weanling rats have chiefly been

used for studying the effects of a deficiency of the essential unsaturated fatty acids, though isolated reports suggest that the mouse [37, 39] and the dog [40, 64] respond to a deficiency in the same way as the rat. The chick responds in a different manner, discussed at the end of this section. Observations on man are described on p. 676.

The symptoms and signs of the fat deficiency disease in weanling animals are retarded and ultimately arrested growth—accompanied by a raised metabolic rate—altered fat and water metabolism, changes in the skin and hair, renal degeneration and impairment of the sexual functions. Adult rats [15] and adult mice [37] develop no obvious symptoms unless under the strain of growth [15] or trauma [37].

Growth. During the first three or four months of the deficient diet growth continues though at a diminished rate; after this the weight remains stationary or declines slightly though the animals may survive for many months [2, 27, 32, 34]. The consumption of food, however, remains as high as that of animals on a normal diet [2], so that the basal metabolic rate is considerably increased [41].

Fat and Water Metabolism. Burr and Burr [2] originally believed that deficient animals could not synthesize fat, but it is now known that fat metabolism remains virtually normal. Fat is synthesized from carbohydrate since after a carbohydrate meal the respiratory quotient rises above unity [41] and this strong evidence for the synthesis of fat is confirmed by Smedley-Maclean and others [27] finding that the proportion of fat in the subcutaneous tissues and, to a lesser extent, in the carcass is actually higher in deficient than normal animals while in the liver it remains normal [27] or raised [28, 37]. That fat is burnt normally as shown by the low respiratory quotient of fasting deficient rats [50]. The rate of synthesis [42, 43] and breakdown [42] of phospholipoids remains normal in the liver [42, 43] and kidney [42] of the deficient animal, while it is increased by about one-third in the muscles [42]. On a high fat diet fat storage is impaired, but less in females than in males [55].

The sparing action of fat on aneurine (p. 197) is not affected by the degree of unsaturation of the fat [44].

The lipotropic effect of choline depends, according to Engel [45], on the presence of linoleic acid. But this is probably a secondary effect due to the acid permitting growth and so fat consumption [30].

About twice the normal amount of water is drunk by the deficient animals although there is no increase in the amount of urine [2], and this may be the only symptom in adult mice [37].

Skin and Hair. Both Burr and Burr [2] and Hume and her collaborators [34] report that dryness and scurfiness of the fore and hind paws is the earliest and most constant symptom of the deficiency, occurring while the animals are still growing, after only ten days to a few weeks on the deficient diet. The condition of the paws returns to normal within three to five weeks when linoleic or arachidonic acid are given. The skin over the rest of the body also becomes dry and scurfy and the hair thin, especially over the face and round the eyes [32]. The tail may be hairless and corrugated or annulated or even necrotic, but these changes are inconstant and irregular and heal very slowly [34]. Some observers [32] even find all skin changes are slight and unreliable indicators of the deficiency—a finding possibly due to the climate of different laboratories, since the type of weather which causes chapping in man accentuates the skin changes in the deficient rats [41, 46]. Vitamin B₆ has been thought to have some relationship to the essential unsaturated fatty acids, since its deficiency also causes somewhat similar changes in the skin of rats, but the only relationship is the obvious one that a deficiency of both will cause grosser skin changes than a deficiency of only one [47]. The same applies to pantothenic acid. Vitamin B₆ does not enable the animal to synthesize linoleic or linolenic acids [48].

Williamson [49] reports that the epidermis of the fat deficient rat becomes thicker and more differentiated, the *stratum granulosum* being especially distinct and the horny layer thick. Cells showing mitosis may be four or even eight times as numerous in rats kept on the deficient diet for nine weeks as in normal rats [3].

Renal Degeneration. Hæmaturia was a frequent finding in the rats originally described by Burr and Burr [2], indeed these workers have since reported renal lesions in all their animals [46]. Other workers, however, have frequently failed to find any hæmaturia in rats [32, 34], and no renal lesions in dogs [40]. The lesion in the kidney is said to be calcification in the cells of some renal tubules and necrotic areas in the renal medulla [51]. Burr and Burr [2] consider the renal lesion, which is made worse by a high protein diet, is the cause of death.

Sexual Functions. Ovulation, though rarely it may remain normal [32], is generally grossly disturbed or completely absent [2, 32]. When it is absent it will start again within five days of giving the essential unsaturated fatty acids [2]—this response is as rapid as that of spayed rats to œstrone. Mating may take place if ovulation occurs, but gestation is prolonged and ends in resorption in one-fifth of the animals [52], or in protracted labour with excessive hæmorrhage [36]. The litters are undersized and often so weak that they soon die [3, 52]. In mice no litters are born and it is even doubtful if conception occurs [37]. Lactation is possible if the mothers are given the missing fatty acids, but is poor unless other fat is also added in reasonable amounts to the diet [52].

Males show a loss of the normal sexual responses and will seldom mate ; when they do they are sterile [2]. This sterility can be cured [53]. After nine months on the deficient diet most of the tubules of the testis are lined with spermatogonia and one or more layers of spermatocytes, no maturer cells being present. Considerable numbers of tubules have no epithelium and multinucleated giant cells may be found in the lumen [53].

Chicks cease to grow and become so intensely œdematous that they may appear almost transparent [18]. The relation between the unsaturated fatty acids and vitamin E in the nutrition of the chick is discussed on p. 619.

Distribution in Foods and the Effects of Storage and Hydrogenation. Vegetable and seed fats, though not margarine, may contain large amounts of linoleic and linolenic acid, but for all practical purposes contain no arachidonic acid. The highly unsaturated acids of fish oils appear not to contain the essential fatty acids in the forms found in land plants and animals. cod-liver oil and other fish oils having little effect on the cure of the fat deficiency disease (p. 671), though one of the highly unsaturated acids of cod-liver oil does bring about a resumption of growth (p. 674). Animal fats, according to the diets of the animals, may contain large amounts of the essential unsaturated fatty acids (p. 672), and human milk [56], in contrast to cow's milk, is an excellent source, providing as well higher unsaturated fatty acids.

The ease with which the essential unsaturated fatty acids are destroyed by oxidative rancidity in the presence of air (p. 671) means not only that they themselves may be destroyed when food is not fresh, but also that their rancidity may destroy other essential constituents of the diet (p. 671) besides the vitamins (p. 671). The hydrogenation of the vegetable fats of margarine, while it has the advantage of enabling manufacturers to sell a product which delights shopkeepers by remaining tasteless for many months in a warm room, has, of course, the drawback that much of the essential unsaturated fatty acid is converted to saturated fatty acid.

The following figures have been largely taken from *The Chemical Constitution of the Natural Fats* by Hilditch [4] to which the reader should refer for further information.

THE VITAMINS IN MEDICINE

Food	Per Cent. of Essential Unsaturated Fatty Acid.
<i>Animal Products</i> (p. 672);	
Butter	1.9-4.0
Beef Fat	1.1-5.0
Lard	5.0-11.1
Mutton Fat	3.0-5.0
Liver Fat	3.0-7.0
Milk (Cow)	0.15-0.23
(Ewe)	0.36
(Goat)	0.22
(Human)	0.39-0.40
(Mare)	0.69
Fish Oils	Traces (p. 675)
Margarine	2.0-5.0
<i>Vegetable Fats</i> :	
Barley Germ Oil	63
Cocoa Butter	2.0
Coconut Oil	6.0-9.2
Corn Salad Oil	70
Cotton Seed Oil	35-50
Ground Nut or Arachis Oil	13-27
Linseed Oil	72-83
Maize Germ Oil	42
Oat Germ Oil	31
Olive Oil	4.0-13.7
Palm Oil	2.0-11.3
Rice Bran Oil	29-42
Rye Germ Oil	48
Soya Bean Oil	56-63
Sunflower Seed Oil	52-64
Wheat Germ Oil	44-52

Human Requirements of the Essential Unsaturated Fatty Acids. Nothing is known about the human requirements of these acids though the Food and Nutrition Board and The National Research Council of the U.S.A. [61] stated in 1948 that "in spite of the paucity of information . . . it is desirable . . . that the fat intake include essential unsaturated fatty acids to the extent of at least one per cent. of the total calories." Only about half this amount has been available in England since 1945. One human volunteer [58] has lived for six months on an almost fat-free diet, which produced the fat deficiency disease in rats, with no ill results, though, as might have been expected, he lost weight, his respiratory quotient altered and there was a marked fall in the level of linoleic and arachidonic acid in the blood. The frequent attacks of migraine from which he suffered were permanently cured. Of seven infants reported in the literature [8] who were given diets very low in fat, two developed eczema which was then cured by fat and there is some clinical evidence, discussed below, that eczema, especially in infants, is associated with abnormally low levels of unsaturated acids in the blood and may be improved by adding unsaturated fatty acids to the diet. The condition of the skin and especially of the hair in coeliac disease and other diseases where fat absorption is impaired [59, 60], in which the level of the unsaturated fatty acids of the serum is low [62, 63], may also possibly point to the unsaturated fatty acids being necessary in human nutrition.

But when it is remembered that rats show few definite symptoms of the fat deficiency disease until they have been several months on the deficient diet and when it is also remembered how high are the stores of the essential unsaturated fatty acids in man (p. 672), it is hardly surprising that neither deliberately planned deficient diets nor diets low in fat such as those in

England, cause any definite symptoms clearly ascribable to lack of the essential fatty acids.

While, however, there appears to be no frank deficiency of these acids in human nutrition, it is relevant to remember that in processed and stale food they may be deliberately destroyed to improve the keeping quality of the food or accidentally destroyed by rancidity, while changes from the traditional feeding of cattle and poultry may alter the amounts in animal fats and dairy produce [4].

Essential Unsaturated Fatty Acids in Medicine

Eczema. The only condition where these acids appear to have some definite value is eczema. Hansen and his colleagues [65] have carried out the best and most extensive investigations on the part played by the essential unsaturated fatty acids in human disease, and have reviewed the changes in the iodine number of the serum lipoids in various conditions [63], among which a rise in temperature and possibly dermatitis are of particular interest in their effect on lowering the level of unsaturated fatty acids: fever has the same effect on the level of vitamin A (p. 30) but not of vitamin E (p. 602). An important point is that the iodine number should be estimated in serum or plasma and not whole blood, as the latter, at least in dogs [64], varies far less than the former when a deficiency of fat is present.

One hundred and seventy one eczematous patients and one hundred and one controls, taking the same type of diet, had the iodine number of their sera estimated [65]. Of the infants under two years of age, eighty per cent. had iodine numbers below the controls, and so did seventy-five per cent. of children between the ages of two and fifteen years and over half the adults. When lard, in amounts up to three ounces daily, or vegetable oils were given the response was "good to excellent" in sixty of one hundred and forty-eight patients and "fair to good" in fifty-one. Most of those who did not respond were in the older age group. Clinical improvement generally coincided with a rise in the serum iodine number. This rise was also caused by crude coal tar ointments, which is an argument in favour of it being the result and not the cause of the improvement in the condition of the skin, but on balance it would seem that this is the wrong explanation. Among the other investigators Cornbleet [66] gave eighty-seven patients with allergic eczema, most of whom were adolescents or young adults in whom the condition had been present for many years, four tablespoonfuls three times a day of maize oil. Cure took twelve to eighteen months, and followed an erratic course; concomitant asthma also often improved. Faber and Roberts [67] confirmed the low iodine number in the serum lipoids of infants with eczema, but could not confirm the curative properties of unsaturated oils. Taub and Zakon [68] treated eczematous patients with raw linseed oil and reported that some were worse after the treatment, while Ginsberg and others [69] failed to improve the eczema of infants and of adults with corn oil and linseed oil, and found no difference in the iodine number of their serum lipoids compared to that of normal subjects. The work of Finnerud and his colleagues [70] may explain these contradictory results since only half of forty-seven eczematous patients had low iodine numbers and it appears that it was especially in all these patients that definite improvement occurred after taking three tablespoonfuls of lard daily, which apparently were administered by being used in the cooking. In the patients with normal serum values improvement was less constant. The level of arachidonic acid in the blood was never low, even when the iodine number was low.

Burns and Wounds. Cod-liver oil is the oil which has been most extensively used in the treatment of burns and wounds, but though it contains a large proportion of highly unsaturated fatty acids, these are not, to any marked degree, the "essential" unsaturated fatty acids (p. 671). Löhr and Zacher [71] first drew attention to the use of cod-liver oil ointments for treating burns owing to the excellent results which they obtained in a

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FIG. 241. Infant with very severe eczema before and after treatment by mouth for eleven weeks with 200 mg. daily of the ethyl esters of linoleic and linolenic acids, combined with local applications of a two per cent. ointment of these acids.



FIG. 242. Hands of a child before and after the same treatment for fifteen weeks as that given to the infant shown in Fig. 241. He had had eczema for two years on the back of the hands and behind the knees.

large number of cases; they especially stressed the rapid cleaning and epithelization. Steel [72] confirmed these results both for burns and wounds, being particularly impressed by the stimulation of indolent areas. Dann and her collaborators [74] have carried out the most thorough experimental work yet published, and have given an extensive bibliography to German clinical work. They report that cod-liver oil, arachis oil and linoleic acid all stimulate granulation tissue, though only the last stimulates epithelial regeneration. Arachis oil causes excessive collagen formation. Puestow and his colleagues [73], from excellent experimental work on pigs and rabbits, concluded that burns treated with fish-liver oils healed twenty-five per cent. more rapidly than burns which received no treatment or olive oil. The effect was not due to the vitamins in the fish-liver oils, and it is interesting to note that the "essential" linoleic acid of the olive oil had no effect. The bacteriostatic action of cod-liver oil is said to be enhanced by its distillation at 250° C. and its subsequent iodination [75]: a procedure which must destroy the unsaturated fatty acids, whatever unknown substances are produced.

Tuberculosis. Here again it is cod-liver oil and not the unsaturated vegetable oils which has won for itself a wide reputation in treatment. It is difficult to understand why cod-liver oil has this reputation, it is more difficult to ignore it. For over half a century physicians have been impressed by it both in England [76] and in America [77], and though at the present time it receives scant attention in medical journals, yet when it is mentioned it is praised, McConkey [77], for instance, reporting after well-controlled work that it gives remarkably good results in preventing laryngeal and intestinal tuberculosis in cases of pulmonary tuberculosis. Emmerie and others [112] have isolated from cod-liver oil an unsaturated fraction which, at least *in vitro*, inhibits the growth of tubercle bacilli. The early French work on cod-liver oil and lupus vulgaris and the present day work on large doses of vitamin D₂ and tuberculosis are discussed on p. 572.

OTHER MINOR FAT-SOLUBLE VITAMINS

Butter Growth Factor. The review by Burr and Barnes [8] in 1943 gives an excellent brief summary of, and references to, the earlier conflicting evidence that butter fat contains a factor which promotes growth. There can be little doubt that butter fat for the calf is superior to all the common vegetable oils and, to a lesser degree, to animal depot fats [78]. For the rat, which is the animal that has been most extensively studied, Boutwell and his collaborators [79] in their first papers reported that butter is superior to maize, coconut, cotton seed or soya bean oils, this superiority apparently being due to the presence of long chain saturated fatty acids. But in later papers these workers stated that the superiority of butter vanishes when mixed carbohydrates instead of lactose alone were given in the diet [80, 81]. On the lactose diets the flavour of the butter was not the factor which was responsible for the better growth and food consumption, because removing the diacetyl—the substance which largely gives butter its distinctive flavour—from the butter did not decrease the effect of the butter nor was the relative effect of corn oil reversed by flavouring it with diacetyl [81].

Deuel and his collaborators [82] have confirmed that young rats prefer and consume far larger quantities of diets containing butter than diets containing vegetable oils. They have also shown that vegetable oils flavoured with diacetyl are preferred to oils without this flavour, but they have not confirmed or denied the crucial work of Boutwell and others [81] that even when vegetable oils are flavoured they are inferior to butter. Their experiments [83] in which they showed that rats consuming *the same amounts* of diets containing butter or other fats grew equally well and had the same carcase composition, are of little value since these by ensuring that the same

amount of food was eaten by all the rats, ignored the essential fact that stimulation of appetite is one of the most important properties of food and of vitamins such as aneurine. On the balance of evidence it would appear that butter, at least for calves and young rats, is superior to vegetable fats for promoting appetite and growth when lactose, the normal carbohydrate for young animals, is the only carbohydrate in the diet.

The factor in butter which stimulates growth is not [84, 85], as was once thought [86], vaccenic acid—an isomer of oleic acid with the double bond in the 11 : 12 position instead of the 9 : 10 position. But there is work [84, 87] which strongly suggests that some of the liquid fractions of butter contain the factor, especially when the pasture of the cow is good [84]. The physiological importance of the branched-chain fatty acids of butter [111] has not yet been investigated.

Margarine's value for man compared to that of butter is of course of very great importance. So it is unfortunate—as it is in all work on nutrition—that the rat should be our guide when it is quite unsuitable to be so by its very capacity to thrive in abnormal laboratory conditions on grossly distorted diets. To some it might seem unwise to advise an army to march on a rat's stomach. On the other hand satisfactory human experiments are almost impossible : the only work [88] purporting to show that butter and margarine are equally valuable are completely vitiated because the two groups of children who eat the two different fats were living in two different institutions. The sole truly important evidence about margarine is given by the Ministry of Labour's investigation carried out before the war on the food consumption of nearly eleven thousand industrial and agricultural families [89]. An average of only three ounces per head per week was eaten compared to over six ounces of butter, though the latter was nearly three times as costly. In other words man seldom can stomach more than three ounces of margarine a week unless he is compelled by dire necessity.

Anti-Stiffness Factor. This factor, whose lack is reputed to cause chiefly stiffness of the wrists of guinea-pigs and calcification of soft tissues, does not exist. The evidence against its existence is :—

(a) Some workers cannot confirm that it exists [90, 91].

(b) Stiff wrists in guinea-pigs are common in normal animals, the condition waxing and waning for no known reason [92, 93].

(c) The calcification found by some [92, 96, 97] but not all [94] workers is probably due to poisoning by calcium or vitamin D, caused by the excessive amounts of these included in the experimental diets.

(d) Even those who agree that the factor exists do not agree over what diets can demonstrate its deficiency [92].

(e) More than twenty-four widely differing steroids are said to cure the deficiency [93], including small amounts of vitamin D₂ though the experimental diets contain an abundance.

(f) There is complete disagreement as to what substances contain the anti-stiffness factor [92].

(g) Judging if animals are deficient by the stiffness of their wrists is a very crude and fallacious method [92] and not the delicate one it is claimed to be [95].

(h) A factor said to be active in doses of 0.00002 micrograms [105] is non-existent.

However, since some workers go on producing and discussing evidence about the action of the anti-stiffness factor it is necessary to discuss it as if it were no myth.

Bahrs and Wulzen [96] in 1936 noticed that planarian worms became diseased when fed on the tissues of guinea-pigs whose vitamin C requirements had been supplied by tomato juice, orange juice or synthetic ascorbic acid. When, however, fresh green kale was used to provide vitamin C the tissues of the guinea-pigs caused no disease in the worms.

Following up this indication that kale contained a new vitamin, Wulzen and her collaborators [96, 97] showed that guinea-pigs develop a definite deficiency disease when fed on a diet of grain and the "necessary" vitamins, or on a diet of skim milk supplemented with straw, orange juice, carotene and a salt mixture, in both cases very large amounts of vitamin D as irradiated yeast being given.

The dominant symptom of the disease is stiffness of the wrists which gradually grows worse and also in time involves the elbows. At autopsy the muscles are extremely atrophied and like those caused by lack of vitamin E [99], though they may be streaked with closely-packed fine white lines of calcium deposits running parallel to the muscle fibres [96, 97]. The heart muscle is normal [99]. There are often lumps of calcium phosphate deposited under the skin, in the region of the joints, between the ribs and indiscriminately in many organs including the heart and aorta [96, 97]. This deposition of calcium phosphate appears to be due to the increase in the blood of both calcium and inorganic phosphorus [102]. The serum phosphatase is decreased [102] and there is an abnormal distribution of the acid soluble phosphorus in the kidney and liver [103] and muscle [104]. This disease is quite different to the muscular dystrophy caused by lack of vitamin E, since in animals suffering from both diseases either one can be cured separately and, further, the "stiffness" disease, which does not cause creatinuria, is cured without reducing the creatinuria of the muscular dystrophy [98]. The islets of Langerhans may be greatly enlarged [100]. Deafness is common [101].

Cod-liver oil accelerates the onset of the symptoms and aggravates the condition. Neither the grass juice factor of Kohler, Elvejhm and Hart, nor vitamin E, in the form of wheat germ oil or synthetic alpha-tocopherol, cures or prevents the condition [98]. Methyl vinyl ketone, in spite of a report to the contrary [96], also has no curative effect [97]. More than twenty-four steroids have been reported to be curative [93], ergosterol acetate being the most effective, though some workers give the necessary daily dose as 5 micrograms [93] while others [94] state 100 micrograms or more is needed. Calciferol though present in superabundance in the diet is said to be curative in small doses [93].

Fresh kale or alfalfa and fresh raw cream, but not pasteurized, and sugar cane juice are the foods *par excellence* for the cure of the stiffness of the wrists according to Wulzen and her collaborators, the discoverers of the anti-stiffness factor. Thus from raw cream a crystalline substance was extracted which was fat soluble, containing one carbonyl group and had a molecular weight of about 200 : it was curative in doses of 0.1 micrograms daily [97]. But other workers [90] have found cream ineffective. From cane juice a factor active in 0.002 microgram [95] or even 0.00002 microgram [105] doses was extracted, yet cane juice in the hands of other workers is valueless [92]. Stigmasterol has been acclaimed as the factor by two groups of workers [109].

Clinically the anti-stiffness factor is valueless in scleroderma [106], and experimentally has no relation to the adrenal steroids and so probably would have no effect on rheumatoid arthritis [107].

Vitamin U or Anti-Peptic Ulcer Factor. Cheney [108] is the only worker who has investigated this factor, but it appears probable that it exists. Judging by the protective action against histamine produced peptic ulcers in guinea-pigs, vitamin U is present in the fat of cabbage and probably in parsley, lettuce and celery, and eggs and raw milk. It is very thermolabile and is rapidly destroyed by the wilting of vegetables.

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AND OTHER MINOR FAT-SOLUBLE VITAMINS

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CHAPTER X
VITAMIN K
HISTORY

BETWEEN 1929 and 1933 several observers described a hæmorrhagic disease in chicks fed on diets poor in fats. The existence of a vitamin deficiency as a cause of the hæmorrhage was suspected in 1929 by Dam [1] of Copenhagen, and later in 1931 by McFarlane and his collaborators [2, 3] in their work on the fat-soluble vitamin requirements of the chick. At first it was thought that the hæmorrhagic syndrome was related to scurvy, although it is known that the chick synthesizes its own ascorbic acid. Cabbage, which contains this vitamin, was found to cure the hæmorrhagic condition [4], but supplements of lemon juice, pure ascorbic acid, cod-liver oil and wheat-germ oil failed to do so, showing that the condition was not due to deficiency of ascorbic acid or to any of the then known fat-soluble vitamins [5]. In 1934 Dam [5] suggested that this hæmorrhagic tendency in chicks was a definite dietary deficiency disease due to lack of a fat-soluble factor, and in the next year he proposed that this factor be called vitamin K (Koagulations-Vitamin), a name that has been generally adopted.

The first definite proof of the fat-soluble nature of vitamin K was supplied in 1931 by McFarlane and his co-workers, who noted that fish meal cured the hæmorrhagic disease in chicks on basal diets, but did not do so if the meal was first extracted with fat solvents. The fat-soluble nature of the vitamin was conclusively proved by Dam [5], Almquist and Stokstad [6], who obtained crude concentrates of the vitamin by the extraction of certain foodstuffs with ether.

The relationship between the new vitamin and the clotting of blood was established when McFarlane [3] and Schönheyder [7] observed that the blood of chicks suffering from the nutritional hæmorrhagic syndrome had a prolonged clotting time. In 1936 Schönheyder [29] first suggested that the condition resulted from hypoprothrombinæmia. This was proved by Dam, Schönheyder and Tage-Hansen [9], who were unable to isolate prothrombin from the blood of vitamin K deficient chicks, although it was found to be present in the blood of normal chicks. They also noted that an aqueous emulsion of a vitamin K concentrate failed to restore clotting time to normal when added to a blood plasma-thromboplastin mixture, thus showing that the prolonged clotting time was due to a low plasma prothrombin level in the vitamin K deficient chicks, and that vitamin K itself had no thrombin-like activity. Quick [10] in 1937 observed a progressive fall in the prothrombin level in the blood of chicks on a vitamin K deficient diet. A distinct hæmorrhagic tendency appeared when low prothrombin levels were reached, and both the low prothrombin and the hæmorrhagic tendency were cured by the administration of foodstuffs rich in vitamin K.

Finally in 1937 it was shown that mammals and man may develop vitamin K deficiency with its associated hypoprothrombinæmia and hæmorrhagic tendency, not by feeding on a diet deficient in the vitamin, but in conditions associated with the absence of bile in the intestine. In the dog and rat this was achieved by an experimental biliary fistula [11, 12]; in man it was observed in cases of obstructive jaundice and biliary fistula [13].

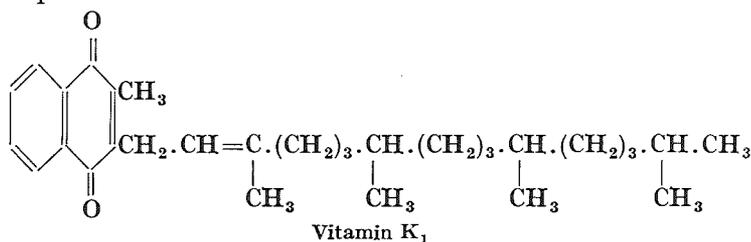
CHEMISTRY OF VITAMIN K

The early workers obtained concentrates containing crude vitamin K by extracting alfalfa and fish meal with fat solvents. Long before it was isolated the general chemical properties of the vitamin were known. Thus it was found to be fat-soluble, alkali-labile, inactivated by light in a few hours, and destroyed by oxidizing agents and strong acids. Vitamin K in concentrates of alfalfa or of hog liver fat resists temperatures of 100°–120° C. for twenty-four hours. It can be concentrated by adsorption on a number of substances, including acidic fuller's earth, synthetic zeolites (permutit), calcium sulphate and activated carbon, from which it may be eluted with fat solvents such as petroleum ether. Crude preparations can be concentrated by molecular distillation or chromatographic adsorption using dehydrated magnesium sulphate and then zinc carbonate.

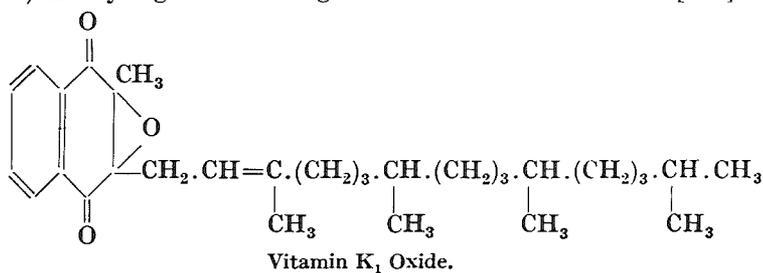
Following Almquist and Stokstad's discovery in 1935 that alfalfa (lucerne) meal is a potent source of vitamin K [6], numerous attempts were made to isolate it from this substance by utilizing certain properties of the vitamin, such as differential solubility, adsorption and elution, and molecular distillation. The isolation of vitamin K in pure or almost pure form was first achieved in January, 1939, by Karrer, Dam, and their associates [16], who obtained it as a yellow oil. Binkley [20] and his co-workers obtained vitamin K in the form of yellow rosettes with a melting-point of -20° C.

By the spring of 1939 it became apparent that more than one compound possessed vitamin K activity, and that there existed a number of analogues closely resembling the vitamin K isolated from alfalfa. Thus MacCorquodale [17], McKee [18] and co-workers isolated two different compounds from alfalfa meal and putrefying fish meal respectively. They named the vitamin obtained from alfalfa vitamin K₁, and that from fish meal vitamin K₂. Almquist and Klose [19] also found that phthiocol, a compound originally isolated from tubercle bacilli, had a slight vitamin K action.

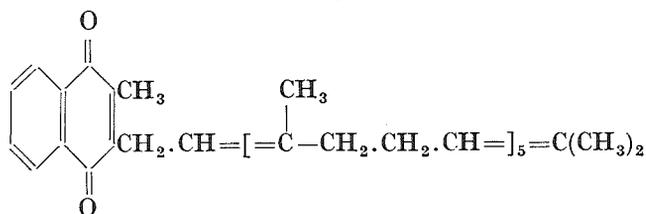
Within a short time of the isolation of vitamin K₁ from alfalfa its structure was identified, as a result of the work of Doisy [20], Almquist and Klose [21], Fieser and his collaborators [22]. It is 2-methyl-3-phytyl-1:4-naphthoquinone—



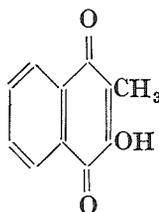
The final step in the proof of this structure was the synthesis of the vitamin in three laboratories practically simultaneously a few months later [23–25]. Vitamin K₁ oxide, unlike the parent substance, is unacted upon by light, and is as active. It has been used clinically. It is insoluble in water, but a suspension may be injected by drawing up a solution in alcohol (10 mg. in 3 ml.) in a syringe and diluting with 10 ml. of normal saline [268].



Vitamin K₂, which was isolated from putrefying fish meal by methods similar to those used for the isolation of vitamin K₁, is a light yellow crystalline solid melting at 53.5° to 54.5° C., with a biological potency of about two-thirds that of vitamin K₁. It is a 2-methyl-3-difarnesyl-1:4-naphthoquinone with an unsaturated hydrocarbon side chain [26].

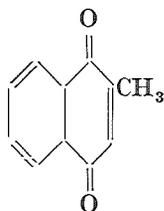
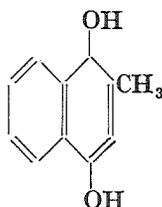
Vitamin K₂

Synthetic Analogues of Vitamin K. Many compounds related to vitamin K₁ are known to have an antihæmorrhagic action. The first vitamin K analogue to be discovered was phthiocol, or 2-methyl-3-hydroxy-1:4-naphthoquinone.



Phthiocol

Unlike vitamins K₁ and K₂ it is water soluble, and forms yellow prismatic crystals melting at 173° C. Most of the analogues are derivatives of 1:4-naphthoquinone or 1:4-naphthohydroquinone. A synthetic naphthoquinone that has been exhaustively studied is 2-methyl-1:4-naphthoquinone which is official in the B.P.C. as menaphthone and in the U.S.P. as menadione.

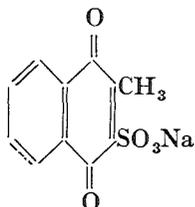
2-Methyl-1:4-naphthoquinone
(Menaphthone, Menadione.)

2-Methyl-1:4-naphthohydroquinone

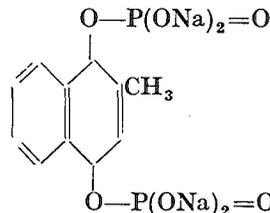
This has a high antihæmorrhagic potency, and weight for weight it is in man stated to be more potent than vitamin K₁ [176], although according to Quick [418] it is not as effective in the dog. 2-Methyl-1:4-naphthoquinone is only slightly soluble in water. Its activity is impaired by exposure to light or by sterilization with steam for any length of time. It is absorbed when applied cutaneously in a fatty base [189], but is not suitable for intravenous use because of its oily character.

A number of derivatives of 2-methyl-1:4-naphthoquinone have been prepared. 2-Methyl-1:4-naphthohydroquinone diacetate (acetomenaphthone, B.P.C.) is more stable than 2-methyl-1:4-naphthoquinone, but has the disadvantage of not being water-soluble. The sodium salts of 2-methyl-1:4-naphthohydroquinone diphosphoric acid ester [28] and 2-methyl-1:4-naphthoquinone-3-sulphonic acid [107, 169, 246], and menadoxime, the ammonium salt of 2-methyl naphthoquinone-4-oxime O-carboxymethyl

ether, are water-soluble and stable. They are used for intravenous injection.



Sodium salt of 2-methyl-1 : 4-naphthoquinone-3-sulphonic acid.



Sodium 2-methyl-1 : 4-naphthohydroquinone diphosphoric acid ester.

It has been shown in both the experimental animal and in human beings that water-soluble vitamin K analogues are absorbed from the gastrointestinal tract without the aid of the bile salts originally given with natural vitamin K and its fat-soluble analogues [50, 51]. Smith and Owen [51] have shown that 4-amino-2-methyl-1-naphthol (vitamin K₅), which is water-soluble, is physiologically active without bile salt medication. 4-Amino-2-methyl-1-naphthol and 1-amino-2-methylnaphthol hydrochlorides are stated to be more active physiologically than 2-methyl-1 : 4-naphthoquinone [64, 65]. The former has marked antibacterial activity against many gram positive and negative organisms [269]. Other water-soluble analogues that have been used clinically are 1 : 4-dehydroxy-2-methyl-3-naphthaldehyde [148], 2-methyl-1 : 4-naphthohydroquinone bisulphite and 2-methyl-1 : 4-naphthohydroquinone dibutyrate [332].

These compounds are all of comparable activity. The following table shows the activity of some of them in terms of that of menaphthone (1,000) based on a biological assay.

Menaphthone	1,000
Acetomenaphthone	450
Vitamin K ₁	300
Vitamin K ₂	240
2-Methyl-4-amino-1-naphthol hydrochloride	470
Sodium salt of 2-methyl-1 : 4-amino-naphthohydroquinone diphosphoric acid ester	490

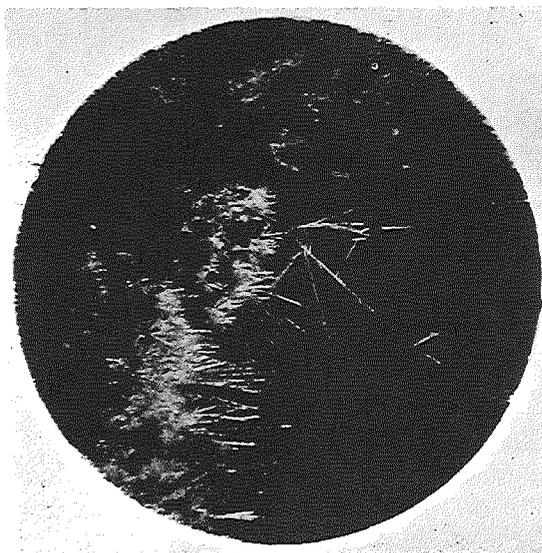


FIG. 243. Crystals of Menaphthone (2-Methyl-1 : 4-naphthoquinone).

An intact benzene ring in the vitamin K molecule is essential for activity ; if it is substituted activity is lost. The 2-methyl group is also essential and cannot be replaced by hydrogen or by other groups without serious loss of activity. The quinone structure is not essential, e.g., the hydroquinones are almost as active, and the quinone oxygen can be replaced by other groups such as amino- and aldehyde. The side chain in the 3-position can be eliminated without loss of activity.

Natural vitamin K and its synthetic analogues have been estimated by : (a) reduction and titration with 2 : 6 dichlorophenolindophenol, using phenosafranine as an indicator, or with ceric sulphate, with *o*-phenanthroline as indicator [238, 239] ; (b) direct titration with excess bromine in carbon tetrachloride, the excess bromine being titrated by means of potassium iodide and sodium thiosulphate solution [239]. A colorimetric test using sodium diethyl dithiocarbamate has also been devised [240].

UNITS OF VITAMIN K

The methods used for the assay of vitamin K and its analogues are based upon changes in the clotting time or upon the prevention of hæmorrhage. Units were devised by Schönheyder [29], Dam and Glavind [30], Thayer and Doisy [31], Ansbacher [32], and Dann [33]. Almquist and his co-workers [34, 35] also developed a method of assay. These units are now obsolete owing to the use of menaphthone and its analogues in both biological and clinical work. No adequate experimental data are available from which the various units can be correlated. The following equivalents are approximate only :—

- 1 Ansbacher unit = 20 Dam units.
- 1 Thayer-Doisy unit = 30 Dam units.
- 1 Almquist-Stockstad unit = 37.5 Dam units.
- 1 Dann unit = 25 Dam units.
- 1 gram vitamin K₁ = 12,000,000 Dam units.

This table is given because in much of the earlier work dosage of vitamin K is given in units. 1 mg. of pure vitamin K₁ is equipotent with 450µg. of menaphthone.

DISTRIBUTION OF VITAMIN K IN FOODS

Green plants are the richest sources of vitamin K, although moderate amounts are found widely distributed in the animal body. Alfalfa (lucerne) and spinach are rich sources of the vitamin. Other plants with a fairly high vitamin K content are cauliflower, cabbage, carrot tops, kale, soya bean, chestnut leaves, pine needles and seaweed. It is also present in tomatoes, hemp seed, bran and orange peel. The green parts of the plant contain more than the fruits, seeds and roots. Most fruits, except tomatoes, are poor sources of vitamin K. Mountain ash berries and honey contain the vitamin, or at any rate an anti-hæmorrhagic principle [15, 313]. Vitamin K is also found widely distributed throughout the animal body. Dam [39] and his colleagues examined chicks on a normal diet and found that the vitamin was distributed in relatively large amounts in all tissues ; liver and lung contain the least. Apparently increased stores of vitamin K are found in the tissues of animals receiving a diet rich in the vitamin. The best animal sources of vitamin K never contain more than ten per cent. of that present in alfalfa. Egg yolk contains small but variable amounts of the vitamin. According to Dam [40] human urine contains no vitamin K, even after the consumption of diets rich in the vitamin.

Bacteria can synthesize vitamin K. It has been shown that 0.6 to 2.0 gm. of dried bacteria per kilo of basal diet will protect chicks against vitamin K

deficiency ; some bacteria are as potent in their vitamin K content as alfalfa. Fæces are rich in the vitamin, its production being attributed to bacterial action, although Andrus [153] from experiments on isolated intestinal loops disputes this. Moulds, yeast and fungi contain practically no vitamin K.

The vitamin K content of some materials, based on dry weight, is given below :—

	<i>Dam Units per 100 grams.</i>
Alfalfa	20,000 to 40,000
Algæ	13,000 to 17,000
Cabbage leaves	40,000
Cauliflower	40,000
Chestnut leaves	80,000
Fæces	30,000
Liver (pork)	5,000 to 10,000
(poultry)	300
Maize (leaves)	1,400 to 1,800
Nettle leaves	40,000
Pine needles	20,000
Putrefied fish meal	90,000
Spinach leaves	55,000
Tomato, green	10,000
ripe	5,000
Carrots	1,000
Cereals	500 to 4,000
Eggs from chickens fed on diets rich in alfalfa	1,000
Fish meal	500
Milk, cows'	very little
Milk, human	0 to 200
Parsley	200
Peas, fresh	3,500
Potatoes	1,000
Rose hips	2,800
Strawberries	2,250

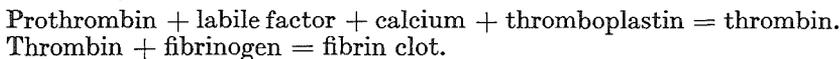
PHYSIOLOGY OF VITAMIN K

Function. Vitamin K is essential for normal blood coagulation. The term "anti-hæmorrhagic vitamin" for vitamin K is misleading. It does not arrest hæmorrhage in normal persons or in hæmophilia, purpura or bleeding diseases. As far as is known vitamin K has only one function in the body. It participates in an enzyme system in the liver to form prothrombin, the precursor of thrombin, a water soluble glycoprotein present in plasma to the extent of 15 to 20 mg. per 100 ml. [386] ; it is ineffective *in vitro* if added to prothrombin deficient plasma or blood. Vitamin K does not form part of the prothrombin molecule but probably serves as the prosthetic group which complements the apo-enzyme in the enzyme system synthesizing prothrombin [418]. According to Quick [270] prothrombin is composed of calcium and two separable components, one of which (A) is stable and appears to be related to the oxidation-reduction systems of the blood, the other (B) being heat labile and inactivated by dicoumarol (p. 716). Prothrombin A, which is stated to be present partly free and partly combined in plasma, is said to be diminished in vitamin K deficiency and in stored plasma [390]. A "labile factor" that is destroyed at 58° C. and disappears from stored plasma due to slow oxidation is also stated by Quick to be essential for prothrombin activity. It is not inactivated by dicoumarol [392]. Quick's prothrombin A is probably identical with his "labile factor," and with

Ac-globulin (p. 692) and the Factor V of Owren [271]. Lack of Owren's factor produces a hæmorrhagic diathesis termed *parahæmophilia* [271] which differs from true hæmophilia in that blood coagulation does not return to normal by the addition of thromboplastin. Parahæmophilia manifests itself by hæmorrhage into the skin, epistaxis, menorrhagia and hæmaturia. Owren [343] has recently prepared another factor, Factor VI which is formed from V. Seegers and co-workers [263] reject the multiple component hypothesis of Quick and Owren as they have prepared prothrombin that is electrophoretically homogeneous. They state it is a pure protein and that it can be activated to thrombin by sodium citrate. It has been suggested that vitamin K takes part in an oxidation-reduction system in which SH-groups are oxidized to -S-S-, which has been postulated to occur in the transformation of fibrinogen to fibrin [20]. Stefanini [414] has devised a method for determining the "labile factor" and finds that it is depleted in liver dysfunction, the thrombocytopenia of acute leukæmia, the hypoprothrombinæmia of terminal carcinoma and the immediate post-operative period.

The coagulation of the blood results from a series of complex reactions involving the interaction of prothrombin, thromboplastin (thrombokinase), calcium, thrombin and fibrinogen.* There is also an opposing mechanism, the inhibition of coagulation, in which plasma antiprothrombin, plasma antithrombin and heparin play a part. Heparin interferes with the conversion of prothrombin to thrombin, lessens the tendency of platelets to agglutinate, catalyses the antithrombic activity of plasma and may inhibit the activity of Factor V. It is generally accepted that when blood clots the soluble plasma protein, fibrinogen, is converted into fibrin, the basis of the clot, by the action of thrombin. Thrombin does not occur preformed in the blood, but is only produced when blood is shed by the interaction of prothrombin, calcium ions, and thromboplastin. Calcium and prothrombin are both normally present in blood plasma; thromboplastin probably exists in circulating plasma in an inactive form, thromboplastinogen, which in the presence of an enzyme derived from platelets changes to active thromboplastin. According to Quick [161] the action of calcium is stoichiometric and not catalytic.

The clotting of blood has been formulated as a two-stage process for about half a century :



The reaction between these factors is stoichiometric according to Quick, and catalytic according to Ware and Seegers [8]. A number of recent studies suggest that there are several other new factors involved in the conversion of prothrombin to thrombin. Ware and his co-workers [187] have prepared a blood globulin (accelerator globulin or Ac-globulin) that accelerates the conversion of thrombin to prothrombin. It decreases in experimental liver damage, in dicoumarol therapy, and slowly in citrated human plasma after ten days' storage. It is increased in patients taking large doses of aminophylline, and in patients with thrombo-embolic disease showing evidence of intravascular clotting [409]. Ac-globulin may be identical with Owren's Factor V, Quick's labile factor, and the "accelerator factor" of Fantl and Nance [413]. These factors are collectively referred to by Stefanini [417] as "plasma prothrombin conversion factor" (P.P.C.F.). Alexander and others [404] have described a serum factor they call S.P.C.A. (Serum prothrombin conversion accelerator) which accelerates the conversion of prothrombin to thrombin. It has been isolated and may be identical with thrombokinase [406]. A lipid substance also seems to be essential for clotting since fat free plasma will not clot on the addition of a fat-free thromboplastin and calcium ions.

* The complexity of the subject may be judged by the fact that 10,000 papers on this subject have been written.

Ware and Seegers [265] have formulated the following scheme to explain the reactions occurring during the clotting of blood.

First Phase

Prothrombin + Thromboplastin + Calcium = Thrombin (in small amounts).

Plasma Ac-globulin + Thrombin (in small amounts) = Serum Ac-globulin.

Prothrombin + Thromboplastin + Calcium + Serum Ac-globulin = Thrombin (large amounts).

Second or Active Phase

Thrombin + Fibrinogen = Fibrin.

It was formerly thought that calcium in ionized form was indispensable for the clotting of blood, but Quick [270] has recently produced evidence that the calcium is intimately linked with the prothrombin molecule. The reaction between prothrombin, thromboplastin and calcium occurs between pH 6.2 to 8.7 [373]. According to Loomis and Seegers [373] calcium can be replaced by related metals such as strontium.

In mammals prothrombin is present in large excess over minimal needs for efficient clotting. Thus in dogs the prothrombin may be reduced to one-fifth of the normal level without prolonging the clotting time or bleeding time. In human beings normal plasma only contains about twice as much prothrombin as is needed to prevent the development of a hæmorrhagic tendency. The speed at which blood clots not only depends on the level of prothrombin in the blood, but also upon its "convertibility." Increased convertibility of prothrombin may compensate for a relative deficiency in amount [42].

The liver is the site of prothrombin formation [110]. Extensive hepatic damage in the human being is associated with hypoprothrombinæmia [43], and in the experimental animal hepatic toxins, such as phosphorus, chloroform, carbon tetrachloride, hepatic tumours [374], and hepatectomy cause a fall in the blood prothrombin [44, 318]. If the hepatic injury is severe enough the administration of vitamin K is not effective in correcting the prothrombin deficiency [45]. With regeneration of the liver, the blood prothrombin returns to normal levels. Chloroform, which is a well-known hepatic poison, produces a fall in the plasma prothrombin level of human beings [46]. Adrenaline on the other hand [191] causes a rise in the prothrombin level [191].

Busing and Zuzak [345] have shown that the complement titre in young chicks parallels the vitamin K intake. On a low intake the titre is consistently low; it rises with increased vitamin K intake and is higher in chicks receiving an excess of the vitamin than in normal birds.

The fate of plasma prothrombin after its formation in the liver and release into the circulating blood has been the subject of experimental work [47]. Certain studies point to the lungs as the site of disappearance of plasma prothrombin. A possible explanation of the rôle of the lungs is thought to be the production of blood platelets in this organ [48]. Platelets undergo disintegration, and initiate the first stage of the clotting process by releasing thromboplastin, which, in the presence of calcium, changes prothrombin to thrombin. Prothrombin is present in the bone marrow [135].

Absorption of Vitamin K. Bile is essential for the proper absorption of natural vitamin K. Synthetic vitamin K analogues, particularly the water-soluble ones, do not need bile salts for their absorption, if given in adequate dosage (p. 703). The exact point of absorption in the gastrointestinal tract is not known for certain. Butt and Snell [179] believe that it is not absorbed through the colon or lower part of the ileum, but from the upper part of the small intestine. The taking of mineral oil (liquid paraffin) inhibits the proper utilization of vitamin K. The oil is not absorbed and

dissolves the vitamin K, most of which is voided in the oil with the faeces [172, 190, 212, 213].

Storage of Vitamin K. Vitamin K is stored in the liver in small but definite amounts [49]. The storage cannot be appreciable because fatal hypoprothrombinæmia can occur in a week. Studies with menaphthone containing radio-active carbon (C^{14}) show that only traces are present in the blood [435].

Excretion of Vitamin K. Vitamin K is present in relatively large quantities in the faeces, being mostly derived from the bacterial flora inhabiting the intestine. These are known to synthesize vitamin K, e.g., *E. coli* contains 750 to 1,600 Dam units per gram of dry weight, and it can synthesize the vitamin from a simplified medium containing asparagin, glucose and citrate. It is not known to what extent vitamin K taken in the food is excreted.

Pharmacology. No toxic effects have been observed clinically after the administration of vitamin K or its analogues in therapeutic doses. Doses up to 200 mg. of 2-methyl-1 : 4-naphthoquinone are well tolerated [277] and 40 mg. of 2-amino-2-methyl-1-naphthol hydrochloride have been given intravenously for a period of nineteen days without any untoward effects [179]. Eight mg. of menaphthone has been administered daily for thirty months without untoward effects [277].

Excessive doses of vitamin K or its analogues are toxic to the experimental animal. The oral lethal doses (L.D.₅₀) of phthiocol and 2-methyl-1 : 4-naphthoquinone for mice are 200 and 500 mg. per kilogram respectively. The figures for the sodium salt of 2-methyl-1 : 4-naphthohydroquinone diphosphoric ester and 2-methyl-1 : 4-naphthohydroquinone sodium bisulphite are of the same order [275]. Vitamin K₁ is not lethal in doses of 25 mg. per kilogram [58]. The oral toxicities of the vitamin K analogues are approximately one-third to one-fifteenth of their subcutaneous toxicities. Chronic toxic effects are due to injury to the circulating red cells [58, 274], and aplastic anæmia [275]. In toxic doses the compounds produce respiratory depression and acute vascular congestion. As the maximum single therapeutic dose of any of the vitamin K analogues appears to be about 20 mg. there is no danger of untoward reactions from doses of this order.

Russell and Page [214] have shown that 2-methyl-1 : 4-naphthoquinone is absorbed through the skin and that the application of 10 mg. of the substance in an ointment protects newborn infants from hypoprothrombinæmia. Vollmer and co-workers [276] have shown that the rate of percutaneous absorption is enhanced by decreasing the viscosity of the solvent. They find doses of 0.1 to 1 mg. effective in four hours. This method of administration, which is of academic rather than practical interest, is not without danger as it may lead to dermatitis [215].

Menaphthone intramuscularly produces a lowering of the blood pressure in hypertensive rats [356] and in patients with hypertension [36]. In this respect it resembles the action of some other quinones.

If added to whole blood menaphthone causes marked methæmoglobin formation [357].

Massive doses of vitamin K, e.g., 20 to 75 mg., produce hyperprothrombinæmia in animals and man, which is also said to be produced by theophylline, theobromine and caffeine [380, 381], although the latter statement has been questioned [41]. There is no evidence that the use of such drugs clinically, appreciably alters the coagulation time of blood [370].

The vitamin K analogue, tetrasodium 2-methyl-1 : 4-naphthohydroquinone diphosphate produces mitotic inhibition in chick fibroblast cultures and in some human carcinomata [236].

REQUIREMENTS OF VITAMIN K

The human requirements of vitamin K are not known. Bacterial synthesis of the vitamin can occur in the large intestine (p. 690), but whether adult

man needs an exogenous source is unknown. It is assumed that he does, as dietary vitamin K deficiency in man has been reported [52, 53]. According to Poncher [360] the vitamin K produced in the intestine by bacterial action is an important endogenous source in the human being, and is probably more important than exogenous sources. Bile is essential for the absorption of vitamin K formed in this way. The newborn is considered to require an exogenous source as the prothrombin level falls after birth and only returns to normal levels after breast or artificial feeding. Normally the newborn infant gets from almost nothing to 400 Dam units of vitamin K₁ (= 33 micrograms) from mothers' milk daily [298]. Hardwicke [299] found the minimal effective dose of a vitamin K analogue (tetrasodium 2-methyl-1:4-naphthoquinone diphosphoric ester) necessary to prevent the development of hypoprothrombinæmia in the newborn infant. This was 0.5 to 5 micrograms daily. In established hypoprothrombinæmia of the newborn a dose of 1.25 mg. was sufficient to restore the prothrombin level to normal. Sells, Walker and Owen [38] consider that the vitamin K requirement of the newborn is 1 to 2 micrograms daily, an amount provided by the milk. If adults require an exogenous source of vitamin K this would be something less than 0.1 mg. daily on the basis of the figures of Sells, Walker and Owen.

According to Mills, Cottingham and Mills [300] a rise in environmental temperature increases the requirements of vitamin K in the experimental animal. Animals adapted to tropical heat seem more prone to severe manifestations of vitamin K deficiency than controls kept under temperate conditions.

VITAMIN K DEFICIENCY

Vitamin K deficiency, which is detected by a lowering of the blood prothrombin level (hypoprothrombinæmia), may occur in any of the following circumstances.

Inadequate Supply of Vitamin K. This may be due to the following causes.

(a) *Nutritional Deficiency of Vitamin K.* That a nutritional vitamin K deficiency can exist is well supported by the experimental production of low prothrombin levels in chicks, rabbits, rats, and mice by the administration of diets deficient in vitamin K [54-56]. Apparently the vitamin formed by bacterial synthesis in the intestine is insufficient for normal requirements.

Kark and Lozner [52] observed four patients with a lowered blood prothrombin level unassociated with disease of the liver or biliary tract, and due apparently to a simple dietary deficiency. Three had scurvy and one, an alcoholic, suffered from pellagra and subclinical scurvy. Vitamin K without bile salts was given by mouth, and the day after the plasma prothrombin level returned to normal, showing that a nutritional deficiency of vitamin K was the cause of the hypoprothrombinæmia.

Scarborough [53] has reported eighteen cases of nutritional deficiency of vitamin K in patients without any clinical evidence of hepatic or biliary tract disease. An examination of the diet and laboratory tests showed that all patients were deficient in one or more vitamins.

Vitamin K deficiency is not common in pellagrins, but complicating factors, such as prolonged diarrhœa, may result in a serious deficiency of this vitamin according to Warner and his co-workers [164]. They believe that many chronically debilitated persons have a moderate hypoprothrombinæmia, which is probably due to inefficient utilization of vitamin K, rather than to a lack of it.

A deficiency of vitamin K has been recorded in anorexia nervosa [301].

(b) *Conditioned Deficiency of Vitamin K.* A conditioned deficiency of vitamin K may be produced by certain drugs. Thus sulphaguanidine and succinylsulphathiazole and other sulphonamides, such as sulphadiazine,

sulphathiazole and sulphaquinoxaline [146], administered to rats produce a fall in the blood prothrombin, probably by interfering with the bacterial synthesis of the vitamin in the gut of the animal [302]. The hypoprothrombinæmia can be prevented by vitamin K analogues [379]. Hæmorrhage responding to vitamin K has been recorded in patients given succinylsulphathiazole [306] and phthalylsulphathiazole [388]. It is also stated that the prothrombin level is significantly decreased in patients taking sulphonamides [316], although Kove and Benton [405] observed no change in the prothrombin levels of infants given sulphadiazine. According to Pirk and Engelberg [359] quinine in therapeutic doses causes a fall in the blood prothrombin level, reversed by large doses of vitamin K, although this effect of quinine is denied by Quick [221]. The discrepancies are probably due to differences in the procedures employed for determining prothrombin.

The administration of propylthiouracil for hyperthyroidism is stated to have caused hypoprothrombinæmia, due to deficiency of accelerator globulin; a case described responded to treatment with serum and blood [419].

Huebner and Link [305] have shown that dicoumarol, which produces a hypoprothrombinæmia, can be degraded to salicylic acid. It has been shown that salicylic acid and aspirin can induce hypoprothrombinæmia, which can be prevented by vitamin K [303, 304]. It is now considered that the prolonged prothrombin time resulting from the administration of salicylates, as in rheumatic fever, is of no clinical significance in most patients and only approaches a critical level likely to precipitate hæmorrhage in very few patients [231]. Approximately 1 mg. of menaphthone counteracts the prothrombinopenic activity of 1 gram of aspirin in man [319]. Jaques and Lepp [244] consider that salicylates and aspirin might be converted into dicoumarol in the gut by bacterial action.

Some drugs, e.g. penicillin, streptomycin, aureomycin, the mercurial diuretics (e.g. mersalyl), the anti-syphilitic arsenicals, amphetamine and methylamphetamine accelerate the coagulation of the blood [426].

(c) *Idiopathic Hypoprothrombinæmia.* Idiopathic hypoprothrombinæmia has been described [228, 307]. In one case described by Rhoads and Fitz-Hugh [228] the subject was an eighteen-year-old male with a hæmorrhagic diathesis, due to hypoprothrombinæmia. There was no evidence of dietary vitamin K deficiency. Giordano [307] has recorded a family incidence of idiopathic hypoprothrombinæmia with evidence that it may be associated with some functional liver defect, and Quick [372] has described a congenital and familial hypoprothrombinæmia due to lack of component B, and which is refractory to treatment with vitamin K. Another type of congenital hypoprothrombinæmia due to lack of component A and Factor V has also been described [390, 399]. Hagen and Watson [188], who observed a case over a decade, describe the occurrence of epistaxis, subcutaneous hæmatomata, hæmorrhage into joints, menorrhagia and metrorrhagia. The only agents effective in preventing the hæmorrhage were purified prothrombin and human plasma.

(d) *Hypoprothrombinæmia of the Newborn.* In the newborn infant and during the first few days of life there is a deficiency of prothrombin in the blood, and an increased tendency to hæmorrhage [57]. This subject is further discussed on p. 708.

Inadequate Intestinal Absorption. Inadequate intestinal absorption of vitamin K may result from:—

(a) Lack of bile in the intestine due to defective secretion of bile salts, as in infective jaundice, or due to biliary fistula and operations on the biliary tract.

(b) Obstructive jaundice of all types (e.g., due to stones, cancer, stricture). Bile fails to reach the intestine, and ingested vitamin K is not absorbed.

(c) Pyloric and intestinal obstruction [63].

(d) Pancreatic Insufficiency. Pancreatic achylia and pancreatectomy in

the cat are followed by a reduction in the prothrombin level [309]. Clinically a lowered prothrombin level is stated to occur in acute pancreatic disease [382]. It may be due to trypsin entering the blood stream and acting on the prothrombin and fibrinogen.

(e) Inadequate absorption due to various intestinal lesions and short-circuiting surgical procedures. Absorptive difficulties may result from excessive vomiting and loss of succus entericus from a drainage tube. In conditions associated with chronic and severe diarrhoea, food is hurried through the intestine, so that absorption of vitamin K and other vitamins is impaired [59]. Hypoprothrombinæmia has been observed in cases of ulcerative colitis, regional enteritis, intestinal obstruction, Banti's disease, gastrocolic fistula, intestinal neoplasm, polyposis coli, and those diseases in which fat absorption is particularly impaired, such as tropical sprue, idiopathic steatorrhœa and cœliac disease. Page and Bercovitz [310] found that nearly thirty per cent. of a group of patients with ulcerative colitis suffered from hypoprothrombinæmia. They consider that this may account for the bleeding from the rectum and mucous membranes; this is reduced by giving vitamin K.

It has always been thought that bile is essential for the absorption of vitamin K. This view has been challenged since Kark and Souter [169] showed that fat soluble 2-methyl-1:4-naphthoquinone can be absorbed without the administration of bile salts even in complete biliary obstruction. Sanford and Shmigelsky [308] have also shown that in infants with an imperforate œsophagus and congenital absence of the gall bladder and bile ducts the plasma prothrombin may remain normal until death occurs. This contradicts the view that in infants vitamin K is synthesized from the food by bacterial action and that it requires bile salts for its absorption. This work is at variance with that of Macpherson [340], who fed hydrocephalic infants with glucose saline; there was a continued fall in the prothrombin level until death. Bile undoubtedly helps the absorption of vitamin K even if it is not essential.

Mineral oil (liquid paraffin) prevents the proper absorption of vitamin K [172, 212, 213].

Injury to the Liver. Even if bile is being produced and finds its way into the intestinal tract, prothrombin deficiency may still occur if the liver is damaged. The proper treatment of a jaundiced patient must therefore include restoration of liver function as well as vitamin K therapy.

Hepatic injury in the experimental animal by trauma or toxins causes a fall in the blood prothrombin level. The effect is noticeable in a few hours [233]. Clinically primary hepatic disease in man, such as cirrhosis, acute yellow atrophy, chronic hepatitis and generalized carcinomatosis is frequently accompanied by hypoprothrombinæmia [61], and after the administration of any anæsthetic in any major procedure the plasma prothrombin level also decreases [46]. Even manipulation of the liver at operation is said to lower the plasma prothrombin [166]. Borgström [317], who investigated the prothrombin level of a number of patients post-operatively, states that the most important factor affecting it is the anæsthetic. After spinal or local anæsthesia there is little change, but a significant fall occurs after operation. This work is questioned by Steggerda and Richards [311], who state that in the rat anæsthetic doses of chloroform and pentothal do not markedly affect the prothrombin level. Levy and Conroy [136] state that in man ether causes a considerable fall in prothrombin, and that sedation with barbiturates or morphine also causes a fall.

In rats primary hepatic tumours due to butter yellow (*p*-dimethyl-aminoazobenzene) cause hypoprothrombinæmia [374].

A fall in the prothrombin level is said to occur in patients given massive doses of arsenicals for the treatment of syphilis [312]. This is taken as evidence of liver damage by arsenicals. Elliott and co-workers [312] noted

a hæmorrhagic encephalitis following massive arsenotherapy in syphilitics, but the prothrombin level of the blood was not recorded.

In a series of patients with liver disease, studied by Herbert [314], sixty-eight per cent. had hypoprothrombinæmia. If, however, the hepatic parenchyma is damaged or there is extensive liver disease, vitamin K is ineffective in restoring the prothrombin level. Herbert also states that patients with a normal plasma prothrombin level may develop hypoprothrombinæmia and bleed a few days after operation.

Infarction of the liver is associated with hypoprothrombinæmia [315].

Infection, particularly of the Respiratory Tract. There is increased destruction of prothrombin in artificial fever therapy, and prolonged fever, particularly pneumonia [167].

Tocantins and Hause [225] note that hypoprothrombinæmia is observed regularly in pneumonia, especially in the first stages of the disease. This diminution in prothrombin no doubt results from a disturbance in hepatic function and may account for the delayed blood coagulability and the moderate hæmorrhagic manifestations observed in pneumonia. There are other evidences of liver dysfunction in pneumonia, such as hyperurobilinuria and diminished response to liver function tests. The slowness of the return of the prothrombin level to normal after the acute stage of pneumonia and the poor response to vitamin K therapy are also evidence of liver dysfunction. Hypoprothrombinæmia in pneumonia may also be due to increased utilization of prothrombin in the fibrinous exudates of the lung. Nja [92] has described a case of allergic purpura with hypoprothrombinæmia associated with pneumococcal infection, which showed a reduction in prothrombin time from five minutes to twenty seconds after treatment with sulphathiazole.

A number of observers have recorded that patients suffering from active and chronic pulmonary tuberculosis generally show low prothrombin levels [226, 227, 237, 320-322]. Patients with active disease or hæmorrhage show a more marked diminution in the prothrombin level than chronic or healing cases. Hæmoptysis cannot, however, be predicted from the prothrombin level [320]. The prothrombin time in tuberculous patients is restored to normal by giving vitamin K, unless the condition has so progressed that there are signs of hepatic damage. Vitamin K has no effect on pulmonary hæmorrhage [235, 320]. These conclusions are contested by Plum and Poulsen [323], who state that the prothrombin level of tuberculous patients, even in those with hæmoptysis, is within the normal range.

As in the case of pneumonia the lowered prothrombin level is probably related to the toxæmia resulting from tuberculous infection.

Hæmorrhage. It is possible that the prothrombin level might fall after a massive internal hæmorrhage, particularly if fluids and not blood are given to the patient to restore blood volume. These would dilute the blood and some time might elapse before sufficient prothrombin is made in the liver. Coller and Farris [62] have recorded a case of massive gastric hæmorrhage in which a low prothrombin level was found after the intravenous administration of 7.5 litres of fluid.

It is stated that the prothrombin time is well below normal in patients during an attack of acute coronary occlusion [371] and in heatstroke [143].

TESTS FOR VITAMIN K DEFICIENCY

Vitamin K is not estimated as such, but the prothrombin level of the blood is found by a clotting method. The prothrombin level should be determined as soon as possible after the blood is obtained from the patient, as the value changes with storage. Page and De Beer [324] have shown that at a given temperature the prothrombin time increases as a straight line function of the logarithm of the storage time; Kove and Benton [405],

however, state that it decreases, the decrease following a hyperbolic curve. The rate of increase of prothrombin time is also directly proportional to the temperature at which the blood is kept [324].

Prothrombin Determination. One-Stage Method. Two methods have been devised, one using oxalated plasma, the other whole blood.

Plasma Prothrombin Time. This was developed by Quick [68] and modified later by a number of investigators. Quick's method is a measurement of the time necessary for the conversion of prothrombin into thrombin and for the subsequent conversion of fibrinogen into fibrin by this thrombin. It estimates the continued activity of prothrombin and the labile factor (p. 691). Briefly the method consists of determining the clotting time of oxalated plasma at 37.5° C. after the addition of an excess of a preparation of thromboplastin and a fixed amount of calcium chloride. With a given thromboplastin preparation the normal plasma prothrombin time is practically constant for any one species; for normal human beings, using Quick's original method, it is 11 to 12.5 seconds, which represents one hundred per cent. concentration of prothrombin. The thromboplastin preparation used by Quick was cephalin obtained from rabbit brain; others have used an extract of beef lung [178]. Most workers use dried extracts of brain. A standardized rabbit-lung thromboplastin preparation is now available commercially [416]. Owen and Toohey [193] simplify the process by using a saline extract. The prothrombin time for plasma containing as little as sixty per cent. prothrombin is nearly the same as for normal plasma, so that a normal prothrombin time does not necessarily mean that the plasma contains 100 per cent. prothrombin. This difficulty has been overcome by determining prothrombin times not only on whole plasma, but also after serial dilutions [52, 69]. Non-soapy detergents, e.g. "Teepol," should not be used for cleaning glassware as traces interfere with the estimations [428].

Various modifications of the Quick method have been made. Thus Pohle and Stewart [70] studied the effect of adding varying amounts of calcium chloride over a wide range and found that which gave the lowest clotting time. With optimal recalcification this was ten seconds for human plasma. Heparin has also been used to prevent coagulation instead of calcium chloride. For clinical purposes the use of tissue extracts as a source of thromboplastin has several disadvantages, since such extracts are tedious to prepare, their potency varies, and they may interfere with observations on the formation of the fibrin web when the plasma clots. Fullerton [71], Page and others [162, 192] have therefore used a preparation of Russell-viper venom (e.g., Russven, Stypven) in place of other thromboplastin preparations. Normal prothrombin time using viper venom varies from eighteen to twenty-five seconds. When viper venom reagents are used to estimate prothrombin in plasma from patients receiving dicoumarol, the results are much higher than those obtained by Quick's original method. The addition of lecithin appears to potentiate the action of viper venom so that normal prothrombin times are from six to ten seconds [72, 192]; the activity of the lecithin depends upon the presence of a fat soluble factor [241]. The one- and two-stage methods do not give the same results, although if Russell viper venom is used in the one-stage method, the results of the two techniques are more in agreement [375].

Further modifications of the Quick method have been made by Holmboe [73], using a stable thromboplastin preparation from sheep brain, by Abramson and Weinstein [242] and by Reid [178], who uses lung extract as a source of thromboplastin. Reid employs a mechanical shaker for the plasma to increase the accuracy of the end point of the coagulation time. Owren [182] has modified the one-stage method by using prothrombin-free ox plasma to supply a surplus of factor V (p. 692) in the coagulation mixture.

Shapiro [325] and Brambel and Loker [326] have shown that greater

sensitivity is obtained in the Quick method if the plasma is diluted 12.5 per cent. Rosenfield and Tuft [79] use plasma to which barium sulphate is added as a diluent and standardize the thromboplastin by determining prothrombin times on a plasma pool from at least five normal subjects. Frommeyer [217] uses lyophilized prothrombin-free human or bovine plasma stored at -20°C . for ten to twenty weeks as a diluent, the material being adjusted to $\text{pH } 7.3$ before use.

A method utilizing a drop of blood in a loop coagulometer has been devised by Ulin and Barrows [328]. On rotating the loop the drop of blood moves readily on the wire; the clotting point is indicated by cessation of this movement.

Control determinations of prothrombin time are essential as Page and De Beer [329] have shown that periodic fluctuations may occur in the same individual over a period of a few weeks.

Using the Quick technique and assuming twelve seconds for a normal plasma prothrombin time prothrombin activity can be expressed as a percentage of normal from the expression:—

$$C = \frac{k}{p.t. + a}$$

in which C = prothrombin concentration expressed as per cent. of normal, $p.t.$ = prothrombin time, and k and a are constants with a value of 303 and 8.7 respectively, provided twelve seconds is the value for normal plasma.

Blood and Serum Coagulation Times. Smith, Ziffren and their co-workers use whole blood instead of plasma [42, 76]. This method is suitable for the bedside. 0.1 ml. of a standard thromboplastin solution prepared from rabbit or beef lung is placed in a small serological tube, which is filled with venous blood up to a 1 ml. mark. The contents of the tube are mixed and the tube tilted every second or two to observe when clotting occurs. Clotting activity (per cent. of normal) =

$$\frac{\text{Clotting time of normal control}}{\text{Clotting time of patient}} \times 100$$

A modification of the Smith method has been made by Huber and Shrader [194] as a simple method for examining the coagulation time in the newborn. Micro-methods have also been elaborated to overcome the objection of venipuncture [74, 75, 155, 162, 327, 398].

Schwager and Jaques [78] determine the prothrombin time of whole blood by adding the latter to thromboplastin at the time the blood sample is drawn. The thromboplastin, prepared from desiccated rabbit brain according to Quick's method, is dispensed in capillary tubes and stored frozen, so that it is available for each determination. The blood is drawn with syringe, needle and glassware treated with silicone, which avoids use of an anti-coagulant.

Fullerton and Anastasopoulos [84] have emphasized the necessity of a lipid factor in the coagulation of blood. They obtain values of from 9.3 seconds to 25.4 seconds for normal prothrombin times using various methods with and without lecithin, which is a lipid.

<i>Thrombokinase</i>	<i>Prothrombin Time</i>
Venom	22.5 secs.
Brain powder	24.1 "
Venom + lecithin	9.3 "
Brain powder + lecithin	25.4 "
Brain powder + venom	10.8 "
Brain powder + venom + lecithin	10.2 "

They point out that since optimal concentrations of thrombokinase and the lipid factor are assured using venom and lecithin to determine the pro-

thrombin time it would seem preferable to the method using only brain extract, the lipid content of which is variable. Fullerton and Anastasopoulos obtained accelerated clotting times using the venom method if the test subject consumed a meal rich in fat before the test was carried out.

They have also shown that slight hæmolysis produces considerable shortening of the accelerated clotting time when the venom method is used, but not with the venom and lecithin and brain extract tests. Hence when the venom method is used the greatest possible precautions must be taken against hæmolysis, *e.g.*, the syringe used for withdrawing blood must be dry and oxalate solution, not crystals, must be used as an anticoagulant. It does not matter what technique is employed in a given laboratory provided conditions are scrupulously standardized and repeated for each patient.

Because of the differences in potency of the various thromboplastin preparations employed, and variations in technique, the use of the prothrombin time is not a satisfactory method of expressing prothrombin activity. The various thromboplastin preparations give different prothrombin values. Thus various workers using Quick's method and various sources of thromboplastin give normal values of from eleven to thirty seconds. Preferably the prothrombin level of an unknown plasma is expressed in terms of the percentage of normal concentration as derived from a prothrombin dilution curve. This curve, which is hyperbolic, should be redrawn for each lot of thromboplastin employed. The rate of formation of thrombin in plasma after the addition of thromboplastin is accelerated or retarded by factors (*e.g.*, Quick's labile factor, Ware's globulin factor and Owen's factor V) other than prothrombin itself. Stefanini [402] has devised a new one-stage procedure for the estimation of both prothrombin and labile factor in plasma.

Although the one-stage method is not as accurate as the two-stage for estimating prothrombin, it gives a better estimate of the chance of bleeding in a patient. Allen and Vermeulen [197] have pointed out that not only the prothrombin level, but the vitamin K reserves of the body are important in assessing a hæmorrhagic tendency. Unfortunately there is no method of measuring the vitamin K reserve. Apparently a patient may be at the point of depleting his reserve and still have a normal plasma prothrombin level.

Prothrombin Determination. Two-Stage Method.

This method, originally devised by Warner, Brinkhouse and Smith [66], is accurate but difficult to perform. It is a more accurate measure of the available prothrombin but the one-stage method is a more accurate gauge of the likelihood of bleeding in a patient [397]. In the first stage the prothrombin of the blood is converted to thrombin with an optimal amount of calcium and an excess of thromboplastic substance. In the second, or clotting stage, the amount of thrombin formed is measured by the time required for the clotting of a standard fibrinogen solution. It is assumed that the amount of thrombin is a measure of the amount of prothrombin originally present and that the clotting time depends upon the amount of thrombin. One unit of thrombin is defined as that amount which under specified conditions makes 1 ml. of a fibrinogen solution clot in fifteen seconds at 28° C. One unit of thrombin is formed from one unit of prothrombin. Normal human blood plasma contains approximately 300 units of prothrombin per cubic millilitre. The manipulations in the two-stage method ensure conversion of inactive component A (p. 691) to the free form, so that this method estimates total component A.

The two-stage technique is carried out by defibrinating oxalated plasma, which is then serially diluted and incubated with calcium chloride, a preparation of thromboplastin, and acacia. After this a standard fibrinogen solution is added and the clotting time determined. The dilution which will give a final concentration of 1 unit of prothrombin per cubic millilitre is determined, and the dilution is then an exact measure of the number of prothrombin

units of the plasma. Thus normal human plasma must be diluted 300 times before it contains 1 unit of prothrombin per cubic millilitre. If a plasma contained 100 units of prothrombin units per cubic millilitre, the prothrombin concentration would be 33.3 per cent. of normal. The two-stage method is the one of choice in anticoagulant therapy.

The two-stage method has been improved by Stewart and Rourke [67], and Herbert [154]. Ware and Seegers [265] have shown that prothrombin conversion is both retarded and incomplete when accelerator globulin is decreased or absent, and this may occur under certain pathological conditions in which hypoprothrombinæmia has been observed. Ware and Seegers [157] have accordingly modified the two-stage method by adding supplements of accelerator globulin. Other factors have also been described (p. 692) which accelerate the conversion of prothrombin to thrombin.

Shinowara [391] has devised a technique for the estimation of prothrombin by an isolation method. Cohn's fraction I is removed with alcohol and the prothrombin precipitated iso-electrically. It is then activated with calcium and placental thromboplastin and titrated with a fibrinogen solution. Sternberger [393] estimates prothrombin by removing fibrinogen from oxalated plasma with thrombin, which is inactivated after standing ten minutes by the antithrombin present in blood. The antithrombin activity is then suppressed with alcohol, and the prothrombin converted to thrombin with milk (a source of thromboplastin) and calcium. The amount of prothrombin is estimated by adding the resulting preparation to normal plasma and recording the thrombin-fibrinogen clotting times. The method is independent of the activity of the reagents used.

At present it appears to be difficult if not impossible to compare prothrombin levels reported from one laboratory with those obtained in another. There is often considerable discrepancy in the two methods, for example in the prothrombin of infants' blood, stored blood, and blood from patients treated with dicoumarol. Mawson [77] has compared the various one-stage techniques with the two-stage method and concludes that when plasma from patients treated with dicoumarol is used the two-stage method gives results in fair agreement with those obtained by the one-stage method in which Russell viper venom and lecithin are used as the source of thromboplastin. When rabbit brain or ox lung is used, the prothrombin concentration is lower than that found by the two-stage method.

The prothrombin activity of stored or "bank" blood increases during the first four or five days, probably due to disintegration of platelets. After the seventh day it falls and reaches forty-eight per cent. of its initial value by the end of three weeks [174]. Stored blood a week old is quite suitable for transfusion to correct a lowered prothrombin level. Stored frozen and dried citrate plasma is also an adequate source of prothrombin for transfusion purposes. Even after long storage the prothrombin activity is only slightly diminished. After fifteen months storage the activity is still seventy to ninety-five per cent. of normal, and after four to six years it is still almost as active [141]. Oxygenation of blood causes prolongation of the prothrombin time, possibly by inactivating the labile factor [388].

CLINICAL MANIFESTATIONS OF HYPOPROTHROMBINÆMIA

A hæmorrhagic tendency due to a reduction of prothrombin only occurs when the level has fallen considerably. Quick [168] puts the critical level at fifteen to twenty per cent. of normal (11 to 12.5 secs.). Later he stated that a prothrombin time of nineteen to twenty secs. indicates a potential hæmorrhagic state [372]. Kark and Souter term the hæmorrhagic condition resulting from marked hypoprothrombinæmia hæmorrhagic hypoprothrombinæmia, and divide it into two classes, latent and spontaneous [170].

Latent hæmorrhagic hypoprothrombinæmia occurs at the sites of obvious

trauma when the prothrombin level has fallen to about thirty-five per cent. of normal. Operation wounds begin to ooze or bleed, and the gums bleed if the teeth are vigorously brushed. Hæmatomata appear if the skin is pricked or a vein is punctured.

A spontaneous hæmorrhagic diathesis appears according to Kark when the blood prothrombin has fallen to fifteen to twenty per cent. of normal, and is seen particularly in the newborn, in idiopathic steatorrhœa, obstructive jaundice and severe parenchymatous hepatic disease. Large hæmatomata may appear on the back, thighs and other pressure areas, and hæmarthrosis, hæmatemesis, epistaxis, hæmaturia or melæna may occur. Menorrhagia, retinal hæmorrhage and in the case of infants intracranial hæmorrhage have been noted. Intractable hæmorrhage from trivial wounds and in infants umbilical hæmorrhage have been described. Bleeding may manifest itself by a slow oozing from the gums, nose and post-operatively from wounds. The blood coagulation time is prolonged but capillary fragility is unaltered.

VITAMIN K THERAPY

The need for treatment with vitamin K depends on the clinical detection of vitamin K deficiency or on the estimation of the prothrombin time.

Vitamin K concentrates obtained from alfalfa or from cereal grass were originally used for treatment and dosage was specified in Dam units (20,000 to 50,000). One to four grams of bile salts or sodium desoxycholate were also given at the same time to facilitate absorption. The natural concentrates have been replaced by synthetic analogues which are all derivatives of 2-methyl-1 : 4-naphthoquinone (menaphthone, menadione). The dosage employed is 1 to 10 mg. orally or intramuscularly, although 2 mg. daily by mouth or every few days parenterally is probably effective in most cases [223, 232, 331]. In the case of infants a dose of 1 mg. is sufficient. Menaphthone is oil-soluble and is therefore injected in oil intramuscularly. It can be prepared in a form suitable for intravenous use by dissolving in alcohol and diluting with ten per cent. glucose solution [230], although this is unnecessary since water-soluble derivatives such as the phosphate and bisulphate of 2-methyl-1 : 4-naphthohydroquinone and menadoxime are available (p. 688). An intravenous injection of one of these will control hæmorrhage due to hypoprothrombinæmia in one and a half to three hours and produce a normal prothrombin level in twenty-four to forty-eight hours [169]. Treatment must be maintained by intramuscular injections of the oil or water-soluble compounds or by means of oral preparations. Kark and

Compound	Solubility	Dosage	Route of Administration
Vitamin K ₁ Oxide . . .	Insoluble in water.	10 mg. daily.	Intramuscular or intravenous. (Solubilized by dissolving 10 mg. in 3 ml. alcohol and diluting with 10 ml. saline.)
Menaphthone (Menadione) .	Oil soluble.	1-5 mg. daily.	Oral or intramuscular.
Acetomenaphthone . . .	Oil soluble.	2-10 mg. daily.	Oral.
Menaphthone bisulphite (2-methyl-1 : 4-naphthohydroquinone-3-sodium sulphonate, Hykinone).	Water soluble.	5 mg. every 12 hours. 2.5-5 mg. daily.	Oral. Subcutaneous, intramuscular or intravenous.
4-Amino-2-methyl naphthol hydrochloride (Synkamin).	Water soluble.	4 mg. every 12 hours. 1-5 mg. daily.	Oral. Subcutaneous, intramuscular or intravenous.
Tetrasodium 2-methyl-1 : 4-naphthohydroquinone diphosphoric acid ester (Synkavite).	Water soluble.	5 mg. every 12 hours. 5-10 mg.	Oral. Subcutaneous, intramuscular or intravenous.

Souter [169] have shown that bile salts are not necessary for the absorption of vitamin K analogues by mouth although it is claimed that they increase their effectiveness [350]. The following is a list of synthetic vitamin K preparations that have been used with their dosage and route of administration. If menaphthone or the water soluble analogues fail, the more potent vitamin K₁ oxide should be tried [429].

The treatment of hypoprothrombinæmia with just sufficient vitamin K to raise the prothrombin level of the blood to normal will not ensure against hæmorrhage except for very short periods. Treatment must be continued beyond this point to build up a reserve in the body before operation and the administration of the vitamin continued post-operatively. Unless this is done the prothrombin level will fall when treatment is withdrawn, with danger of subsequent hæmorrhage. In hæmorrhage due to hypoprothrombinæmia a blood transfusion is the first necessity, not vitamin K therapy, which can be given later. The danger of post-operative bleeding is not passed until the tenth day after operation. In patients with severe hepatic damage hypoprothrombinæmia cannot be corrected even with large amounts of vitamin K. Blood transfusions from donors given large doses of vitamin K are also ineffective in such cases [14].

There appears to be no danger from overdosage with vitamin K or its analogues. Unger and Shapiro [381] have induced hyperprothrombinæmia in normal subjects with very large doses of a water-soluble analogue, but it was only transitory and lasted twenty-four to forty-eight hours. Stewart [232] records only three cases in which the plasma prothrombin was raised to more than 110 per cent. of normal by vitamin K; one patient was dehydrated and the other two suffered from recurrent thromboses of peripheral veins. He has given up to ten times the effective dose of vitamin K without untoward effects and without elevating the plasma prothrombin above normal.

Hyperprothrombinæmia has been recorded in untreated acute thrombophlebitis, in the initial stage of embolism, and in multiple myeloma [330] and in the late night hours and early morning in arteriosclerotic patients [60].

CLINICAL USES OF VITAMIN K

In Conditions not Associated with Jaundice or Liver Disease. Vitamin K cannot be used to check bleeding, irrespective of its origin, nor has it any appreciable effect on the prothrombin level of subjects not suffering from hypoprothrombinæmia [267]. It is not of value in hæmophilia, purpura, and diseases of the blood-forming organs; although there is a tendency to bleed in these conditions there is no deficiency of prothrombin. Nor is the vitamin effective for the control of hæmorrhage in the normal individual.

It is now being realized that prothrombin deficiency may be encountered in other conditions, particularly those associated with an abnormal state of the gastro-intestinal mucosa, which interfere with the absorption of the vitamin. Patients with gastro-intestinal disease also consume diets poor in vitamins. Thus Snell and his co-workers [82, 88, 138] have reported occasional instances of vitamin K deficiency in ulcerative colitis, polyposis of the colon, regional ileitis, pyloric obstruction, sprue and gastro-jejuno-colic fistula. A hæmorrhagic diathesis in sprue is not common, but it has been reported by several observers [169, 176]. Bercovitz and Page [310] have shown that some thirty per cent. of patients with ulcerative colitis have a lowered prothrombin level, and that the administration of vitamin K analogues causes a marked diminution in the mucus membrane bleeding seen in this condition.

The plasma prothrombin levels of thirteen patients suffering from intestinal lesions were examined by Butt, Snell and their co-workers [82, 94]. The cases included ulceration, external and internal fistulæ, intestinal obstruction, diarrhœa and collapse of the ileum. A marked hypoprothrombinæmia was observed in many of the patients, and in three hæmorrhage

occurred. The vitamin K deficiency in these patients was considered to be due to an insufficient amount of normal intestinal mucosa for adequate absorption. Butt and Snell suggest that the blood prothrombin should be determined as a prelude to therapy if bleeding occurs in any medical or surgical case in which a diminution of the absorptive surface of the intestine is probable or suspected, or in which biliary obstruction or infection is present. Although there may not be spontaneous hæmorrhage before, if patients with a low prothrombin level are submitted to surgical procedures the hæmorrhage may become serious.

Others have recorded low prothrombin levels in severe diarrhœa, intestinal obstruction, fistulæ, mesenteric obstruction, hæmorrhagic retinitis, and in pseudo-hæmophilia hepatica of childhood [89]. Stewart [196] and his colleagues have reported prothrombin deficiency in patients with peptic ulcer, malnutrition and cachexia. According to Warner and Owen [264], patients with pernicious anæmia show prothrombin levels of only forty to sixty per cent. of normal; the hypoprothrombinæmia is not rectified by vitamin K therapy, unless liver is given simultaneously.

Moderate reduction in the prothrombin level in such diverse conditions as duodenal ulcer, melæna, lung abscess and rectal carcinoma has been observed by Stewart and Rourke [96]. Treatment with vitamin K and cholic acid resulted in a gradual rise in the prothrombin level. Rawls [234] has also observed a hypoprothrombinæmia in patients suffering from rheumatoid arthritis, hepatitis due to cincophen, hæmorrhage from gastric ulcer, myelogenous leukæmia, aplastic anæmia, thrombocytopenia, hyperthyroidism, malignant endocarditis, chronic intestinal diseases, and toxic reactions due to the administration of gold. In most of these the prothrombin level returned to normal after administering vitamin K. Thordarson [335] noted a low prothrombin level in ten out of fourteen patients with myeloid leukæmia. The administration of vitamin K₁, however, failed to raise it to normal.

It has been claimed that vitamin K is effective in the treatment of menorrhagia [336], although another worker investigating three hundred gynaecological cases found the prothrombin time within normal limits [91].

Low prothrombin levels are present in Banti's syndrome and in the early stages only is vitamin K effective in restoring prothrombin [257]. It is recommended as part of the treatment in preparation for splenectomy.

Vitamin K has failed to control pulmonary hæmorrhage (pp. 698, 714).

In the treatment of hypoprothrombinæmia resulting from intestinal disorders vitamin K analogues should be given parenterally as they may not be effectively absorbed by mouth. Five to ten mg. of a water-soluble analogue is given parenterally daily or 1 mg. to 2 mg. menaphthone in oil intramuscularly. Hepatic function if impaired should be treated and blood transfusions given if necessary.

In Jaundice and Liver Disease. *Incidence.* The hæmorrhagic tendency in patients suffering from obstructive jaundice and diseases of the liver has long been known, and constitutes a distinct hazard in the case of patients submitted to operation. Thus in nearly four thousand operations on jaundiced patients sixty-one of the four hundred and forty-two post-operative deaths (13·8 per cent.) were attributed to hæmorrhage [104], a figure which agrees closely with the twelve per cent. given by Sir John Fraser [105]. A much higher figure is given by Butt [82]. Before it was realized that such hæmorrhage was due to a vitamin K deficiency, conditioned by lack or escape of bile salts, the patient was prepared for operation by giving large doses of glucose and by calcium therapy. A review of the older literature reveals that the incidence of bleeding in patients with obstructive jaundice was higher before the development of modern pre-operative and post-operative care. Absence of bile salts in the intestine may occur without obstruction of the common bile duct, as it has repeatedly been shown that bile from the liver following obstruction or severe liver

damage may contain no bile salts. All patients with obstructive jaundice should have pre-operative prothrombin determinations and operation deferred if possible until the prothrombin level is raised to normal. The value of vitamin D in preventing hæmorrhage in jaundiced patients is discussed on p. 576.

Causes. Suggested causes for this considerable fall in the prothrombin level are : (1) increased utilization of prothrombin for the formation of exudates, (2) defective absorption of vitamin K due to deficient secretion of bile salts, (3) liver damage resulting from anæsthesia (p. 697), infection and operative trauma, (4) lack of adequate vitamin K reserves.

Prothrombin Level. Bleeding may occur when the prothrombin level falls below thirty to forty per cent. of normal [80]; values below this may be dangerous. No sharp prothrombin level can be considered a bleeding level as some patients with a plasma prothrombin as low as twenty per cent. of normal may show no evidence of hæmorrhage. Other factors such as trauma, infection, operative hæmorrhage, vitamin K reserves, and exudate formation play a part in determining the hæmorrhage level. The danger of post-operative bleeding in patients suffering from diseases of the liver or biliary passages may persist for as long as ten days after operation; massive and fatal hæmorrhage may occur in a patient with severe liver damage. The lowest level is reached between the first and fourth days after operation [106].

It has been suggested that anæsthetics might contribute to the post-operative hypoprothrombinæmia in patients with jaundice or disease of the biliary tract. Although chloroform produces a hypoprothrombinæmia, Allen and Livingstone [200] have concluded from prothrombin studies on a hundred and six patients who underwent operations under ether, vinesthene, nitrous oxide, ethylene, bromethol (avertin), nupercaine and spinal and local anæsthesia, that these anæsthetics have no effect on the prothrombin level. They suggest that some form of storage of vitamin K or prothrombin occurs in the body and that failure to replenish this store in the patient with obstructive jaundice or biliary fistula accounts for the post-operative hypoprothrombinæmia.

For many years it has been noted that there is liver impairment in hyperthyroidism. Andrus and Lord [223] have shown that there is an immediate post-operative fall in the plasma prothrombin level, averaging fifteen per cent. and amounting to forty per cent. or more in some cases. The values were similar to those obtained after chloroform anæsthesia (p. 693). It would, therefore, seem advisable to improve the hepatic function of the hyperthyroid patient pre- and post-operatively.

Treatment. Despite the fact that patients with liver disease may not appear to need vitamin K as shown by their prothrombin levels, it should be given to all such patients as a routine, particularly if surgery is contemplated, whatever the prothrombin level or the presence or absence of bleeding [197]. A normal prothrombin on the day of operation is no guarantee that post-operative hæmorrhage will not occur. Where possible, the dosage of vitamin K should be controlled by repeated prothrombin estimations. In conditions in which there is damage to the hepatic parenchyma, as in cirrhosis of the liver, acute yellow atrophy, Wilson's disease (hepatico-lenticular degeneration), multiple liver abscesses, fatty infiltration of the liver, acute hepatitis and carcinoma of the liver, neither the administration of vitamin K, nor blood transfusions will raise the prothrombin level [107, 144, 314].

Pre-operatively 2 to 5 mg. of menaphthone or 5 to 10 mg. of a water-soluble derivative is given orally every day for at least four days or until the prothrombin approaches 70 per cent. of normal. In the presence of jaundice it is advisable to give bile salts with menaphthone. These preparations may be given parenterally if the patient is vomiting or defective absorption is suspected. The dosage of menaphthone is 2 mg. in oil intramuscularly ;

that of the water-soluble analogues 5 to 10 mg. by any parenteral route. Treatment should be continued, regardless of the post-operative prothrombin level, for two weeks and as long as there is any evidence of liver damage, obstructive jaundice or biliary fistula. Therapy should be controlled by frequent prothrombin estimations. These should be done daily for four days and then every other day. If bleeding occurs or surgical interference is considered 10 to 25 mg. of a water-soluble vitamin K analogue should be given intravenously as well as whole blood, which will provide additional prothrombin for about eight hours. Stored or "bank" blood is only satisfactory if it is used within a week of its withdrawal from the donor [174]. The prothrombin level of stored blood slowly falls after this time (p. 702). The administration of vitamin K to the donor before bleeding has not been found to be of value if the recipient is suffering from severe hepatic damage [14].

Liver biopsy is not a safe procedure unless the prothrombin level is within seventy per cent. of normal. If it is below this 10 mg. of a water-soluble vitamin K analogue should be given daily intravenously and continued for forty-eight hours after the biopsy.

Liver Function Test using Vitamin K. Attempts have been made to use the prothrombin response to vitamin K as a test of liver function, since hypoprothrombinæmia associated with severe liver damage does not respond to treatment with vitamin K [186, 198, 266].

Lord and Andrus [223] and Allen and Julian [337] have suggested that the response of plasma prothrombin to intramuscular injections of 2-methyl-1 : 4-naphthoquinone may be used to differentiate between intrahepatic and extrahepatic jaundice. Lord and Andrus found that there was a rise of from ten to sixty-two per cent. in the plasma prothrombin following the intramuscular injection of 2-methyl-1 : 4-naphthoquinone in extrahepatic jaundice (common duct stone, cholangitis, carcinoma of the head of the pancreas, stricture of the common duct), whereas in cases of intrahepatic jaundice the response was ten per cent. or less. The cases of extrahepatic jaundice were confirmed at operation or autopsy. Kark and Souter [224] also observe that in patients with intense jaundice the return of a low prothrombin level to normal after vitamin K therapy favours a diagnosis of obstructive or extrahepatic jaundice. They recognize five types of response to vitamin K therapy in patients with liver disease : (1) Rapid response in obstructive jaundice ; (2) no response in gross liver disease ; (3) partial response, with the level still slightly subnormal after vitamin K therapy in cases of acute or subacute parenchymatous hepatic damage of a moderate degree ; (4) a gradual rise in the prothrombin level with treatment, coincident with clinical improvement, in cases of infective cholangitis, infectious hepatitis, acute or toxic hepatitis and obstructive jaundice complicated by infection ; (5) a fluctuating prothrombin level, above the threshold for hæmorrhage, in patients with chronic and long-standing hepatic disease, usually unassociated with jaundice.

Begtrup and Hansen [83] found that the response to vitamin K confirmed the diagnosis of extrahepatic jaundice in seventy-seven per cent. of one hundred and fifty-two cases and of intrahepatic in eighty-six per cent. The corresponding figures for the Takata-Ara test were sixty-five per cent. and sixty per cent. respectively ; for the urobilin excretion test seventy-nine per cent. and thirty-two per cent., and for the galactose tolerance test fifty-five per cent. and fifty-eight per cent. In chronic liver impairment the figures for the various tests were eighty-five per cent., seventy-seven per cent., thirty-three per cent. and twenty-two per cent. Unger and Shapiro [338] have shown that the response to vitamin K gives excellent correlation with other liver function tests and with the clinical findings.

Owren [272] has shown that in intrahepatic jaundice a concentration of factor V (p. 692) under fifty per cent. of the normal value carries a bad prognosis. A steadily falling factor V concentration indicates malignancy

with a fatal outcome, while persistently subnormal values suggest a chronic hepatitis. According to Owren factor V may be normal or increased in obstructive jaundice.

Allen [384], in a critical review of liver function tests, states that the prothrombin response to vitamin K is as good as the bromsulphthalein excretion test or the serum albumin-globulin ratio for detecting liver damage. In the jaundiced patient, where the diagnosis lies between obstructive and intrahepatic jaundice, the character of the prothrombin response to vitamin K may enable a diagnosis to be made, when all other methods, short of surgery, have failed. Allen gives the following conditions for performing the prothrombin response test. There must be a significant prothrombin reduction (less than seventy-five per cent.); the patient must be eating well and afebrile; a sensitive prothrombin method should be used; and the vitamin K must be given parenterally as a water-soluble analogue in two doses of 10 mg. several hours apart. In obstructive jaundice there is at least a twenty-five per cent. increase in the prothrombin, whereas in cases of extensive liver damage there is little or no response. These observations have been confirmed by Stein [383], who claims to have achieved an accuracy of over ninety-five per cent. in differentiating between intra- and extrahepatic jaundice using the prothrombin response to vitamin K test. Of course the interpretation of the test may be difficult in cases of combined intra- and extra-hepatic jaundice, *e.g.*, carcinoma of the liver with metastases in the portal glands, and biliary cirrhosis following chronic obstruction of the bile ducts.

A summary of the results of various workers using the response to vitamin K test is given in the table opposite.

The response to vitamin K test appears to be a reliable indicator of hepatic disease and impaired liver function and may be used with some success to differentiate extrahepatic from intrahepatic jaundice. The usefulness of the test is limited by the fact that jaundice is not always accompanied by prothrombin deficiency. The test is of no prognostic value as it does not differentiate between obstruction due to neoplasms and other lesions.

Hypoprothrombinæmia in the Newborn. It was originally observed by Brinkhous [111] and it has been repeatedly confirmed since that the prothrombin level of the newborn and in early infancy is lower than normal. Values from ten to forty per cent. of normal have been recorded [111, 120]. According to Waddell and his co-workers [113] the period of most marked deficiency occurs from forty-eight to seventy-two hours after birth; the prothrombin level returns to normal at the beginning of the second week [194]. It is of interest that according to the Mosaic law circumcision was delayed until the eighth day because of the risk of bleeding if done earlier. If vitamin K or an analogue is administered within twenty-four hours of birth this fall in prothrombin is prevented [203]. It is also prevented if the mother is given an intramuscular injection of vitamin K or an analogue shortly before delivery, which shows that vitamin K must pass through the placenta. Normally the cord blood prothrombin is much lower than that of the maternal blood, indicating that natural vitamin K does not pass readily from the mother's blood through the placenta.

It has been suggested that the hypoprothrombinæmia of the newborn is due to a low food intake just after birth and to the low vitamin K content of milk. The gut of the newborn is also sterile so that bacterial synthesis of vitamin K, which normally occurs in the gut, does not take place. Salmonsens and Nygaard [121] observed that supplementary feeds of cows' milk—which was probably contaminated with vitamin K producing organisms—helped to prevent the fall in the prothrombin level after birth. Cellis and Lyon [249] also noted that supplements of evaporated milk and corn syrup, which form a good bacterial medium, had a similar effect. Macpherson [340] believes that the prothrombin level in the newborn is dependent on the mother's diet, although this has been denied by others [159, 203, 344]. In his series the

PROTHROMBIN RESPONSE TO VITAMIN K TEST

Author	Method of Estimating Prothrombin	Technique of Test	Interpretation (24 hours later)	Accuracy of Test
Begtrup and Hansen [83]	Thordarson, Begtrup and Hansen's method [83].	2 mg. of 2-methyl-1:4-naphthohydroquinone disuccinate by mouth.	Increase of 30% or more indicates obstructive jaundice; less than 30% suggests parenchymatous jaundice.	77%–86%
Lord and Andrus [223]	Two stage (p. 701), expressed as percentage of normal prothrombin concentration.	2 mg. of menaphthone i.m.	Increase of 10% suggests extrahepatic jaundice; less than 10% intrahepatic. Repeat 48–72 hours later; less than 15% rise indicates intrahepatic jaundice.	94%
Allen [384]	Modified Quick, serial dilutions, expressed as percentage of normal.	Two 10 mg. doses of water soluble preparation i.v. several hours apart.	Original level less than 75%. Rise to normal in 24 hours in obstructive jaundice. Response of less than 25% indicates intrahepatic jaundice.	99%
Stein [383]	Modified Quick, expressed as percentage of normal.	5–10 mg. menaphthone i.m.	Original level less than 75%. If rise exceeds 10%, dose repeated. In obstructive jaundice returns to more than 85% after 5–10 mg. If less than 85% indicates intrahepatic jaundice.	95%
Althausen [97]	Quick, expressed as percentage of normal.	1 mg. menadione bisulphite parenterally.	Original level 70% or less. If increase 20% or more indicates obstructive jaundice.	94%
Unger and Shapiro [338]	Modified Quick, using dilution to 12.5% (normal 39.5 sec. \pm 2.5 sec.).	76 mg. Synkayvite i.v. daily for 3 days.	Failure to return to normal prothrombin time by third day indicates impaired liver function.	100% in cases confirmed by biopsy. 91% in cases confirmed clinically.

prothrombin levels of the blood of newborn infants of adequately fed mothers was considerably higher than those of infants whose mothers subsisted on inadequate diets. Some of the children of the poorly fed mothers suffered from hæmorrhagic disease and three showed signs of cerebral irritation, which was attributed to cerebral hæmorrhage resulting from a lowered prothrombin level. Macpherson also considers that prolonged labour and the use of anæsthetics causes a fall in the prothrombin level of infants' blood sufficient to cause hæmorrhage. It is not certain whether the anæsthetics and barbiturates used in labour affect the prothrombin level of the newborn. According to Fitzgerald and Webster [339] barbiturates such as sodium pentobarbital and sodium amyl bromoallyl barbiturate administered to the mother produce not only a lowered prothrombin level in the infant but increase hæmorrhage. They state that this can be prevented by administering

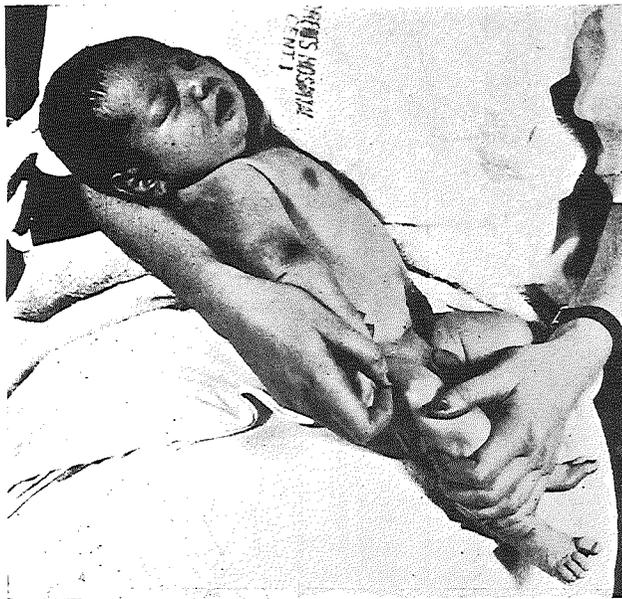


FIG. 244. Typical hæmorrhagia hypoprothrombinæmia neonatorum in a three-day-old infant. Hæmorrhage into the skin of shoulder and upper arm and from the umbilical cord (omphalorrhagia) can be seen. The infant responded to treatment with menaphthone (2-methyl-1 : 4-naphthoquinone).

vitamin K to the mother in labour. These views have been challenged [299].

The work of the Randalls [195] suggests that more than a deficiency of prothrombin is involved in the apparent low prothrombin levels of the newborn, as measured by standard methods, because the plasma of infants, normal by the one-stage test, is less effective than adult plasma in restoring the prothrombin level of deficient infants. According to Quick [270] newborn infants' plasma is deficient in component A (p. 691). If this were so the plasma of patients receiving dicoumarol or suffering from liver disease should restore the prothrombin time of the plasma of these infants, but this is not so according to the Randalls. They also state that Quick's labile factor (p. 691) does not seem to be the basis of the prothrombin deficiency of the newborn, because stored plasma, in which this factor is absent or diminished, is as effective as fresh plasma for restoring prothrombin times. Perhaps most significant is their finding that stored serum, from which prothrombin, thrombin and fibrinogen would have disappeared, is also effective in bringing the prothrombin times of newborn infants to normal. It would appear from

the work of the Randalls that the prolonged prothrombin times seen in the newborn, as measured by the one-stage method, cannot be adequately explained in terms of a deficiency of prothrombin itself or of any of the classical clotting factors. A possible explanation is that the newborn infant is unable to convert prothrombin to thrombin with the same speed and efficiency as the normal adult.

Hæmorrhagic Disease of the Newborn. Clinical. This is characterized clinically by hæmorrhage into the gastro-intestinal tract (melæna, hæmatemesis), bleeding from the cord (omphalorrhagia), nose, palate, the genito-

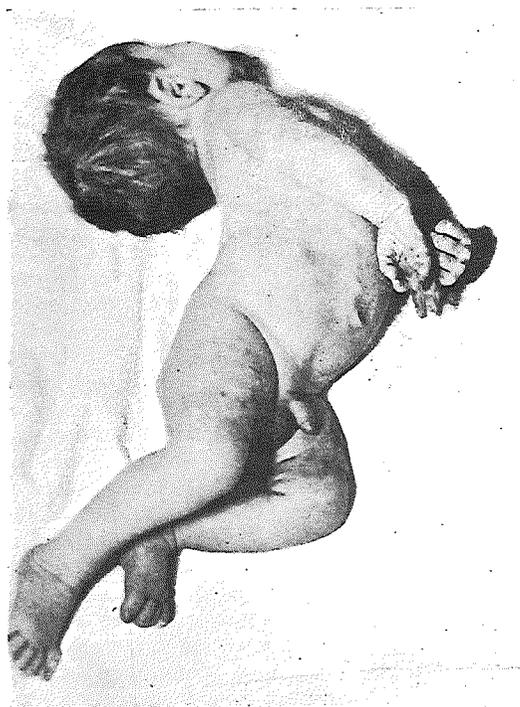


FIG. 245. Delayed hæmorrhagic disease of the newborn, with intracranial hæmorrhage superimposed on a previous interventricular hæmorrhage. The infant, who shows marked opisthotonus, also had severe diarrhœa and oral hæmorrhage. Subsequent therapy with vitamin K and a blood transfusion failed to save the infant's life. Autopsy revealed petechial hæmorrhage in the brain and massive hæmorrhage into the ventricles. The diarrhœa undoubtedly contributed to the hæmorrhagic state and depressed the prothrombin level so low that a poorly formed clot at the site of a previous hæmorrhage was dislodged and further hæmorrhage resulted. It is probable that hæmorrhage into the ventricles had continued since birth, and that when they were distended opisthotonus developed.

urinary tract, vulva, and the suprarenals. The commonest first symptom is the passage of a large tarry stool, but the condition may be ushered in by hæmatemesis, bleeding from the umbilicus (Fig. 244), pallor due to internal hæmorrhage, or the rapid enlargement of a cephalhæmatoma. The child may pass enormous quantities of blood in the stools and if the hæmorrhage is not arrested it may die on the fourth or fifth day. In the differential diagnosis of hæmorrhage of the newborn the following must be considered: birth trauma, infection and sepsis, congenital thrombopenia, constitutional fibrinopenia, hereditary hæmophilia and pseudo-hæmophilia. The incidence of hæmorrhage of the newborn is stated by various authorities to be from 1 in 150 to 1 in 500 births [122, 361].

Although it is generally considered that hæmorrhagic disease of the newborn occurs between the second and sixth days after birth, it has been stated that in a third of one series studied it occurred on the first day [118]. Neonatal hæmorrhage *in utero* has also been reported by Javert [119], who suggests that uterine contractions increase the intracapillary pressure of the foetus and tend to produce hæmorrhage, which becomes pathological when the clotting mechanism is disturbed.

Intracranial Hæmorrhage. It is believed by some workers that intracranial hæmorrhage in the new-born is associated with hypoprothrombinæmia and that obstetric trauma may only play a secondary rôle [113, 115, 122, 124]. It is conceded that the trauma of birth may in many instances cause the appearance of small bleeding points in the intracranial cavity. However, even in normal births and in babies born by cæsarean section such bleeding points have been demonstrated, and death from cerebral hæmorrhage has been reported after cæsarean section [204]. With an abnormal clotting mechanism, such as will result from a temporary hypoprothrombinæmia, no clot will form and slow oozing will continue. With a normal clotting mechanism bleeding from small vessels would cease. This slow oozing from the bleeding points might explain why the symptoms of intracranial hæmorrhage often only reveal themselves on the fourth to sixth day after birth (Fig. 245). Thus out of sixteen cases of intracranial hæmorrhage at the Children's Hospital, Washington, D.C., the average date of onset was 6·3 days after birth [125].

Treatment of Hæmorrhage of the Newborn. The general consensus of opinion is that hæmorrhage of the newborn is due to hypoprothrombinæmia and that the administration of vitamin K or one of its analogues restores the prothrombin to normal levels and controls the hæmorrhage [112, 116, 124, 127, 130]. From a study of 31,000 infants, Lehmann [341] concluded that the prophylactic administration of a water-soluble vitamin K analogue reduced the mortality rate from hæmorrhage by 1·6 per 1,000. Hellman and Shettles [255], of the Johns Hopkins Hospital, Baltimore, recorded a neonatal mortality of 1·9 per cent. among 1,042 infants whose mothers received vitamin K prophylactically during labour; the mortality among a comparable control group whose mothers did not receive vitamin K was 3·9 per cent. Statistical analysis shows that the difference between the figures is significant.

These figures, however, are challenged by Sanford and his colleagues [253] and by Potter [378]. Sanford and his colleagues from a study of 1,693 newborn infants were unable to find any correlation between the prothrombin level and the frequency of what they term "hæmorrhagic manifestations." These occurred in 6·59 per cent. of 711 newborn infants given vitamin K and in 6·6 per cent. of 982 untreated controls. The mortality was the same in the two groups. Parks and Sweet [351] and Hay, Hudson and Rodgers [410] were also unable to observe any reduction in the incidence of neonatal hæmorrhage by administering vitamin K to the mother before birth. These conclusions have been criticized by Waddell [254]. Hay and his co-workers [410] observed hæmorrhagic disease of the newborn in 0·24 per cent. of 4,602 births after giving the mother vitamin K before birth, and in 0·19 per cent. of 12,131 control births. The difference is not statistically significant. Potter states that there is no real proof that prolongation of the prothrombin time is a direct cause of neonatal hæmorrhage. She found that many infants with an excessively prolonged prothrombin time show no evidence of hæmorrhage, while others with relatively little prolongation bleed severely. According to her, true hæmorrhagic disease is exceptionally rare, the cause of most neonatal hæmorrhage being trauma. Potter bases her views on a study of 6,560 infants whose mothers received vitamin K before birth and 6,630 untreated controls. The foetal mortality in the two groups was 29·8 and 25·8 respectively. Both Sanford and Potter state that evidence of bleeding is not a justification for the diagnosis of true hæmorrhagic disease,

in which there is a prolongation of the prothrombin time and vitamin K is of value. They believe that conditions other than prolonged prothrombin time and vitamin K deficiency are responsible for the majority of hæmorrhagic manifestations in the newborn. The explanation of these anomalous results depends upon the interpretation of the expressions " hæmorrhagic manifestation " and " hæmorrhage of the newborn." In a small group of premature infants it was observed that there were just as many hæmorrhagic manifestations in premature infants given vitamin K as in untreated controls, and the mortality and frequency of cerebral hæmorrhage as shown by necropsy findings was actually higher in the treated group [394].

A voluminous literature that is unnecessary to detail has arisen on the prophylactic treatment of the hypoprothrombinæmia of the newborn with vitamin K analogues. Shettles and his colleagues [129, 255], at Johns Hopkins Hospital, were the first to give vitamin K to the mother before birth. They gave it daily for several weeks before delivery ; it is now known that a single dose a few hours before delivery is sufficient to produce a normal prothrombin in the infant after birth. A dose of 20 mg. by mouth given to the mother early in labour or 10 mg. of a water soluble analogue parenterally late in labour prevents hypoprothrombinæmia in the newborn. If the infant is given 1 mg. of a vitamin K preparation intramuscularly daily for the first few days after birth the prothrombin is restored to normal adult levels. Should, however, the infant be bleeding a blood transfusion is essential and should be given in addition to vitamin K [348, 352].

It has been stated that healthy newborn infants do not need vitamin K as their prothrombin level returns to normal spontaneously and that it should only be given to those with birth injuries or in cases of hæmorrhage in addition to blood transfusion [333].

Gastro-Intestinal Diseases of Infancy and Childhood. Diseases characterized by chronic or subchronic gastro-intestinal lesions often develop hypoprothrombinæmia with or without bleeding. The literature contains reports on post-operative hæmorrhage in the pyloric stenosis of infants after Ramstedt's operation [293].

Cases of congenital obstruction of the alimentary tract show hypoprothrombinæmia, partly because no food, and hence exogenous vitamin K, is consumed and partly because there is no bacterial synthesis of the vitamin. Since surgery is imperative to save life, the hypoprothrombinæmia should be corrected with vitamin K before any operative procedure. Grossman [258] quotes a case of congenital jejunal obstruction in which hæmorrhage occurring after operation was controlled with vitamin K, and one in which a child with duodenal stenosis was operated upon without vitamin K therapy and died from hæmorrhage.

Celiac disease in children, like sprue and idiopathic steatorrhœa in adults, is sometimes associated with hæmorrhagic manifestations. Fanconi [89] suggested that this was due to vitamin K deficiency caused by malabsorption of fats. Other authors have confirmed the presence of hypoprothrombinæmia in these conditions [95, 170].

Pseudohæmophilia Hepatica and Hereditary Pseudohæmophilia. Pseudohæmophilia hepatica is a rare hæmorrhagic syndrome accompanying acute destruction of liver tissue in infectious and toxic states such as syphilis, acute yellow atrophy, and poisoning by such drugs as arsenic, chloroform and the sulphonamides. The bleeding is due to a decreased production in the liver of prothrombin or fibrinogen or both, with a resultant prolongation of clotting time. Kugelmass [126, 137] describes the treatment of a case of infective jaundice with hæmorrhagic manifestations that responded favourably to the administration of vitamin K. Another case of hæmolytic anæmia, due to the toxic effects of sulphanilamide, also improved when given vitamin K in the same dosage.

Hereditary pseudohæmophilia is a syndrome appearing in both males

and females characterized by a hæmorrhagic tendency, prolonged bleeding time, normal clotting time and a normal platelet count. It may be present at birth or latent for many years, the hæmorrhage developing as a result of an inherent qualitative defect in the blood platelets. A case of this condition with a low prothrombin level was satisfactorily treated with vitamin K by Kugelmass [126]. He is careful to point out, however, that since the hæmorrhage is primarily determined by defects in the platelets, vitamin K therapy is only indicated when there is a coincident decrease in the prothrombin level.

Retinal Hæmorrhage. There have appeared in the ophthalmological, obstetrical and pædiatric literature numerous theories explaining the ætiology of retinal hæmorrhage in the newborn. Maumenee, Hellmann and Shettles [163] attribute it to vitamin K deficiency. They state that newly born infants with retinal hæmorrhage show lower prothrombin levels than normal infants and they believe that the incidence of the condition is reduced by administering vitamin K to the mother before labour. Two milligrams of menaphthone were given by mouth daily for at least four days before delivery. They point out that the vitamin has very little effect if given during labour. By giving expectant mothers 2 mg. of menaphthone daily for four days before the onset of labour the incidence of retinal hæmorrhage was reduced to four per cent. compared with twenty-five per cent. in controls [255].

Pray and his co-workers [259] also report a reduction in the incidence of retinal hæmorrhage in the infants of mothers treated with 10 to 20 mg. of menaphthone during or before labour. They obtained the best results by treating the mother before the onset of labour. Wille [202] stresses that vitamin K must be given to the mother every day in the last months of pregnancy to obtain any reduction in the incidence of retinal hæmorrhage in the newborn.

Falls and Jurow [250] were unable to observe any reduction in the incidence of retinal hæmorrhage in the newborn within two days of birth after giving the mother vitamin K during labour or giving it to the infant after delivery. They believe that trauma and anoxia are important factors in producing retinal hæmorrhage in the newborn and point out that at birth, when the hæmorrhages can be seen, prothrombin levels are usually normal.

This reduction in the incidence of retinal hæmorrhage in newborn infants by means of vitamin K therapy is significant because of the possible relationship between retinal and intracranial hæmorrhage.

Effect of Vitamin K on Thrombosis. Morton, Shearburn and Burger [273] have investigated the effect of vitamin K on thrombus formation. The post-partum incidence of thrombosis was 0.14 per cent. in a group of seven hundred pregnant women given vitamin K; the incidence in a group of 5,728 controls studied over a ten-year period, who had not received vitamin K, was 0.48 per cent. In animal experiments the leg veins of two groups of dogs, one of which received vitamin K, were traumatized and sections later removed and examined microscopically. There was no significant difference in the incidence of thrombosis in the two groups. Doles [371] states that in acute coronary occlusion there is a hypoprothrombinæmia and that the prompt administration of 50 to 72 mg. of a soluble vitamin K analogue every six to eight hours intravenously or intramuscularly diminishes pain and prevents the formation of thrombi.

Hæmoptysis. Levy [321], Bauer [237] and Sheely [226] state that the intensity and duration of hæmoptysis in tuberculous patients—who often show low prothrombin levels—is considerably diminished after vitamin K therapy. On the other hand Kaplan [354], Harrell and Ray [355], Farber and Miller [320] report that vitamin K is of no value in the control of hæmoptysis.

Abortion. Moore and his colleagues [353] observed that does fed a diet deficient in vitamin K to produce hypoprothrombinæmia and then mated invariably aborted from the tenth to the fourteenth day, and at autopsy

retroplacental hæmorrhages were seen. They suggest that because hæmorrhage occurred at this site the placenta is unduly susceptible to vitamin K deficiency since the blood prothrombin level did not fall to critical hæmorrhagic levels. Javert and Stander [346] believe that a deficiency of vitamins C and K is a factor in certain cases of threatened and spontaneous abortion. They observed that in seventy-nine patients with threatened, spontaneous or habitual abortion ascorbic acid deficiency was found in sixty-nine per cent. and hypoprothrombinæmia in seventy-two per cent. The patients usually complained of skin ecchymoses, epistaxis, bleeding gums, and vaginal bleeding. A clinical study of thirty-three patients with threatened or habitual abortion showed that after treatment with vitamins C and K, progesterone, minerals and vitamin E the incidence of abortion was twenty and seven per cent. in the two groups, while in controls the abortion rate was one hundred per cent. in two groups of forty-six. As so many preparations were given it is difficult to evaluate the precise rôle of vitamin K in diminishing the incidence of abortion.

King [385] studied a hundred cases of abortion, and observed that in cases of inevitable and complete abortion the prothrombin level was lower than in pregnant and non-pregnant controls. In cases of threatened abortion the prothrombin level was within normal limits.

Drug Hypoprothrombinæmia. Certain drugs, such as organic arsenicals, sulphonamides, aspirin and salicylates are known to produce hypoprothrombinæmia (p. 696). It has been recorded that vitamin K therapy can raise a lowered prothrombin level when this is due to sulphonamide [306], aspirin and salicylate therapy [319]. Shapiro [319] has shown that approximately 1 mg. of menaphthone will counteract the prothrombinopenic activity of 1 gram of acetylsalicylic acid (aspirin) in patients receiving prolonged therapy with this drug. Hypoprothrombinæmia has been recorded in syphilitic patients given massive doses of organic arsenicals [312], but there is no record of this being corrected with vitamin K.

The clinical significance of the hypoprothrombinæmia following the administration of aspirin has been exaggerated. Livingstone and Neary [109] found practically no change in the prothrombin level after administering 21 grams of aspirin for a week.

Other Uses of Vitamin K

It has been suggested that vitamin K might be of value in the treatment of hypertension [36], as it has been found to be active in lowering the blood pressure in experimental renal hypertension [356], although this has been denied [184]. Black [260] observed a diminished prothrombin level in sixty-five per cent. of one hundred and fifty-six patients with urticaria who had obtained no relief from elimination diets, search for infections and allergens and avoidance of drugs. Sixty per cent. obtained relief with menaphthone given 2 mg. three times a day before meals. Unfortunately the results were not submitted to statistical analysis.

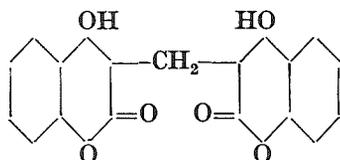
Mitchell [132, 236] observed that the vitamin K analogue, tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate, produces a small but measureable improvement in the palliative results of X-ray therapy in some cases of advanced cancer. The rationale of the treatment was to block the synthesis of the nucleic acids in the rapidly growing cancer cells, which the above analogue may do as it inhibits mitotic division in chick fibroblast cultures and in some human carcinomata. Many other quinones have an antimitotic action.

VITAMIN K ANTAGONISTS

A number of compounds are known, with a composition similar to vitamin K, that antagonize its action. When administered to animals on a normal

diet they produce the symptoms of vitamin K deficiency, i.e., hypoprothrombinæmia. These include 2:3-dichloro-1:4-naphthoquinone [243], 3-hydroxy-1:4-naphthoquinone [261], phenylindandione [279], dihydroxystearic acid [180], 2-methyl-2-methoxy-4-phenyl-5-oxodihydropyrano-benzopyran, also known as "No. 63" and cyclocoumarol [279, 396, 430, 431]. The subject is reviewed by Brown and Douglas [434].

Dicoumarol. Dicoumarol, or 3,3'-methylene-bis-(4-hydroxycoumarin), is



Dicoumarol.

a compound present in spoiled sweet clover which produces the condition known as the hæmorrhagic disease of cattle [10] by diminishing the prothrombin level [208]. It was first isolated from sweet clover and synthesized in 1941 by Link and his associates [209] of the University of Wisconsin. Since then a considerable literature on the pharmacology and clinical applications of this compound has appeared.

Mode of Action. The oral administration of dicoumarol to animals or human beings results in a reduction of the prothrombin level of the blood, as shown by a prolongation of the prothrombin time [211]. There is a latent period of some twenty-four to seventy-two hours before the prothrombin time is affected. This is because it is slowly absorbed and largely combined with the plasma proteins, with the result that it is slowly eliminated by the kidneys [417]. It is largely stored in the liver as shown by tests with dicoumarol containing radio-active carbon [407]. It has been suggested that dicoumarol inhibits the formation of prothrombin in the liver and that the prothrombin in the blood is reduced before the prothrombin time is prolonged [278]. Quick [358] believes that it acts by diminishing or inactivating the B component of the prothrombin complex (p. 691), probably by inhibiting an enzyme mechanism which produces prothrombin [427]. Other workers believe that dicoumarol not only inhibits prothrombin but another factor associated with the plasma pseudoglobulins [158]. Dicoumarol also prolongs clotting time and clot retraction time; the bleeding time is not appreciably affected. Dicoumarol decreases the adhesiveness of platelets, which play an important part in blood coagulation [368], and in large doses lowers the plasma fibrinogen concentration [377]. Experimental work on animals has shown that if the prothrombin time is prolonged by dicoumarol, artificially induced intravascular thrombosis is inhibited [279]. For any subject there is a threshold plasma level of dicoumarol which must be reached before a detectable prothrombin response is elicited; this varies from person to person, but is generally between 5 and 10 mg. per litre [417].

Pharmacology. The principal toxic effects produced by excessive dosage are pulmonary œdema [281] and bleeding, which may occur from the nose, stomach, lung, bowel, kidney, bladder, skin or beneath the conjunctiva. The hæmorrhage is both gross and microscopic. In addition there is acute glomerular swelling, a toxic lymphoid tissue reaction [282], rapid respiration, general vasodilatation, irregular heart beat, congestion of the liver, and after death rapid rigor mortis [283]. If given in adequate dosage vitamin K, and its oxide, but not vitamin K analogues, can inhibit the action of dicoumarol, although there is a long latent period of about five days before the hypoprothrombinæmia is corrected [256, 284]. This effect of vitamin K is denied by Boyd and Warner [151], who claim that there is a recovery phase after the initial fall in prothrombin caused by dicoumarol. They state that the results

of clinical studies are confused by other factors, such as withdrawal of the drug and blood transfusion. The protective action of vitamin K against the hypoprothrombinæmia produced by dicoumarol is lessened if there is liver damage, e.g., by tumours [374].

Dicoumarol is effective orally, parenterally and rectally. A single dose will prolong the prothrombin and coagulation times for two to three weeks. Its rectal absorption, however, is irregular and the effect given by this route is uncertain [285]. It is more effective in animals in which pyrexia has been induced, and care should therefore be exercised when it is used clinically in febrile patients.

Dicoumarol produces no changes in the white cell count, erythrocyte sedimentation rate, percentage hæmoglobin, blood sugar, icterus index, liver function tests, non-protein nitrogen, or in the urine [369].

Clinical Uses and Indications. Clinical evidence shows that the carefully controlled and individual administration of dicoumarol is effective in the prevention of post-operative venous thrombosis and pulmonary embolism [285-286], and in the treatment of venous thrombosis, thrombophlebitis [288-292] and the spread of thrombosis after embolism. These include cavernous sinus thrombosis, puerperal thrombosis [279], mesenteric thrombosis, the thrombosis of arteriosclerosis (in cerebral and coronary vessels) and thromboangiitis obliterans, and retinal thrombosis. Dicoumarol administered prophylactically diminishes the incidence of puerperal thrombophlebitis without increasing the danger of post-partum hæmorrhage [279]. Brambel and Loker [367] used dicoumarol in post-traumatic arteriosclerosis and gangrene due to diabetes and frostbite. They concluded that it prevented the extension of established gangrene and prevented it developing in traumatic cases. MacLean and Brambel [363] have administered dicoumarol for periods up to six months to patients with central retinal venous occlusion, and diabetic, degenerative and central serous retinopathies. They claim to have improved visual acuity.

A committee sponsored by the American Heart Association has investigated the use of anticoagulants in the treatment of coronary thrombosis with myocardial infarction and they conclude from a study of eight hundred cases that the death rate and incidence of thromboembolic phenomena during the six weeks following an attack of the disease was markedly lower in those treated by anticoagulants than in those treated solely by conventional methods [156]. Allen and his colleagues [287] have reviewed 2,307 cases of acute vascular thrombosis treated at the Mayo Clinic with dicoumarol. They conclude that its use constitutes the greatest contribution to the successful treatment and prevention of intravascular thrombosis and embolism. A survey of the prophylactic use of dicoumarol against thromboembolic complications after surgery has been made by Wise, Loker and Brambel [387] in 3,300 cases. They have shown a statistically significant reduction in the incidence of venous thrombosis and fatal embolism following major abdominopelvic surgery when dicoumarol was used prophylactically. This has been confirmed by Borgström [395] who states that with early ambulation and dicoumarol the risk is reduced to a quarter.

Putnam and his co-workers [362] treated forty-three patients with disseminated sclerosis with doses of dicoumarol sufficient to raise the prothrombin time to thirty seconds (Quick) for periods varying from six months to four years. They were, however, unable to attribute any improvement to this form of therapy which was given on the assumption that disseminated sclerosis might result from thrombosis of small cerebral venules.

The advantages of dicoumarol over heparin are that it is relatively cheap, it can be given by mouth or intravenously, and its effect is prolonged. The cost of heparin is prohibitive and it must be given intravenously over a long period. However, dicoumarol cannot be regarded as completely safe as many cases of severe and alarming bleeding have occurred, and twenty-one fatalities

have been reported [280]. The increased prothrombin time following its use persists for several days after its withdrawal. Crawford and Nassim [364] describe a case of retinal thrombosis in which there was a delayed response to dicoumarol followed by a severe reaction to the drug. The reactions, which included hæmatemesis, hæmaturia, albuminuria and a prolonged increased prothrombin time after the drug was withdrawn, necessitated blood transfusion. Wasserman and Stats [294] record such well-marked individual variations in the response to dicoumarol that they are unable to fix a standard dose. Douthwaite [365] and Evans [85] describe cases resistant to dicoumarol. Barker and his colleagues [90] at the Mayo Clinic believe that the risk of bleeding after giving dicoumarol has been exaggerated. They state that in 1,000 cases on the drug, only 3.9 per cent. showed evidence of minor hæmorrhage and 2.5 per cent. major hæmorrhage. Major bleeding was controlled by an intravenous injection of 60 mg. of menaphthone bisulphite. They state that if the proper contra-indications are observed and the dosage carefully regulated, the danger of severe bleeding is slight.

There are definite contra-indications to the use of dicoumarol. Absolute contra-indications are: renal insufficiency; purpura; hæmorrhagic diathesis; prothrombin deficiency, e.g., in cases of jaundice, hepatic disease and malnutrition; blood dyscrasia with tendency to bleed; and subacute bacterial endocarditis. Relative contra-indications are: ulcerative lesions, open wounds, bleeding surfaces; possibility of operation within two weeks; operations on the brain or spinal cord; and vomiting due to gastric or intestinal drainage. Dicoumarol should not be used as a routine without control after operation because of the danger of hæmorrhage.

When given in doses producing an unsafe level of prothrombin in pregnant rabbits dicoumarol is stated to cause death of the fœtus *in utero* [366]; care should therefore be exercised when administering the drug in pregnancy. Hypoprothrombinæmia can be induced in suckling animals if the mother is given dicoumarol, and can be corrected by giving vitamin K to the mother [376]. Dicoumarol can be administered to nursing mothers without the hazard of hypoprothrombinæmia in the infant [279, 403].

Dicoumarol Therapy. Dicoumarol should never be used unless daily and consistently comparable prothrombin time tests are done, as it is impossible to predict the dose for any one patient. The Quick prothrombin time is often used, but there have been objections to this method in patients receiving dicoumarol on account of the numerous variables. Hæmorrhage following the administration of dicoumarol is not directly proportional to the "concentration of prothrombin" [216]. The two-stage method is the method of choice [222, 262]. It probably does not matter what technique is used, provided the test is carried out with scrupulous attention to detail and the significance of the findings clearly understood. The Quick method, using viper venom does, however, appear to give false low clotting times, with danger of overdosage [193].

Two hundred to three hundred milligrams of dicoumarol are given daily until the prothrombin time is thirty seconds and reduced to 50 to 100 mg. daily if this is between thirty and thirty-five seconds. The drug is withheld if the prothrombin time is thirty-five seconds or more and no more is given until the prothrombin time is down to thirty seconds or less, after which the drug is again given continuously in 100 mg. doses [363]. These prothrombin times are in terms of the Quick or Link-Shapiro method [325]. It should be remembered that effective prothrombin levels may not be reached until twenty-four to forty-eight hours, and even longer, after giving dicoumarol. The drug varies in its activity in different people, some, particularly the elderly and frail, being susceptible to small doses. When given for prophylactic purposes after operation, treatment is started on the third post-operative day. The prothrombin time is kept at about thirty-five seconds several days or a week after the patient has become ambulatory. After stopping treatment

it takes two to ten days for the prothrombin time to return to normal. It may take longer than this in cases of liver or kidney damage or in patients with a hæmorrhagic tendency. The prothrombin time may be kept elevated for months or even years by dicoumarol [362]. Rice and others [401] have given 66 grams of the drug over a period of forty months without any mishap.

To get an immediate anti-coagulant effect dicoumarol has been combined with heparin, the latter being discontinued when the prothrombin time reaches thirty-five seconds [296].

Facilities for blood transfusions should always be available. Hæmorrhage is treated by a blood transfusion of 500 c.c. of fresh (not bank) citrated blood, repeated several times if necessary, as there is a tendency for the prothrombin time to increase again after several hours. Large doses of a soluble vitamin K analogue, e.g., 75 to 150 mg. are given intravenously every four to twenty-four hours [411, 415], or vitamin K₁ oxide (180 to 250 mg.) [412], or a single dose of 500 mg. of vitamin K₁ orally [421]. A five per cent. emulsion of vitamin K₁ given intravenously in doses of 5 to 20 mg. per kilogram of body weight is also effective in overcoming dicoumarol hypoprothrombinæmia [422, 425]. It is claimed that the protective action of vitamin K is increased by simultaneous administration of vitamins C and P [349].

An analogue of dicoumarol, bis-3 : 3'-(4-oxycoumarinyl) ethyl acetate ("Tromexan") has recently been claimed to be more potent and safer to administer since it is excreted and destroyed more rapidly [247, 424]. After withdrawal of the drug the prothrombin level is stated to return to normal in thirty-six hours [424]. It has a shorter lag period than dicoumarol [423], producing its maximum effect in twenty-four hours [4, 24]. The pharmacology of the drug has been described by Stirling and Hunter [424].

Dicoumarol is now being displaced by safer anticoagulants such as "Tromexan," Cyclocoumarol [279, 396, 430, 431] and phenylindandione, which is intermediate in action between heparin and dicoumarol [400]. The initial dose of phenylindandione is 200 mg. and the maintenance dose 50 to 100 mg.

Poisoning has occurred from the unauthorized oral use of "Warfarin," 3 (α -acetylbenzyl)-4-hydroxycoumarin, which has an anticoagulant action and is used as a rodenticide [432].

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CHAPTER XI

VITAMIN P

HISTORY

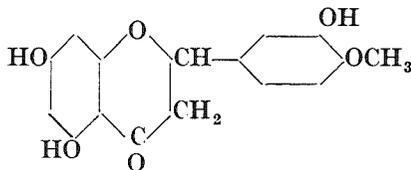
THE existence of vitamin P was postulated in 1936 by Szent-Györgyi [1] and his co-workers, who claimed that extracts of Hungarian red pepper and lemon juice contained a substance which was more effective in the clinical treatment of increased vascular fragility than ascorbic acid. Later it was reported that fractionation of these extracts yielded an active preparation consisting of a flavone or flavone glucoside, which was called *citrin* or vitamin P [2]. This was stated to be effective in the treatment of increased capillary permeability in three patients suffering from vascular purpura, but it had little effect on four patients with thrombopenic purpura. A moderate effect was observed on the capillary fragility in seven patients suffering from infectious disease, myxœdema and diabetes.

Szent-Györgyi [2] also stated that citrin decreased the number of hæmorrhages in scorbutic guinea-pigs and prolonged the survival period from 28.5 days for the negative control group to forty-four days for the animals given 1 mg. of citrin daily [3]. It was suggested that the full clinical syndrome of scurvy in the guinea-pig was produced by a combined deficiency of ascorbic acid and vitamin P.

Efforts to repeat the observations of Szent-Györgyi and his co-workers in experimental animals yielded conflicting results. Thus Zilva [5], Moll [6], Hiramatsu [7], Detrick [8], Bensath and Das [9] and McHenry and Perry [33] were unable to confirm them. Zilva administered a vitamin P preparation (0.66 mg. hesperidin and 0.33 mg. eriodictyol daily) to guinea-pigs on a scorbutic diet, but he failed to observe any delay in the onset of scurvy or in the time taken for the animals to succumb. He claimed that the administration of a daily dose of 0.1 to 0.2 mg. ascorbic acid to such animals produced a condition resembling that obtained by Szent-Györgyi and his colleagues by administering a daily dose of vitamin P. Szent-Györgyi [10] has since reported his failure to repeat his original experiments upon which the existence of vitamin P was based. More recent work shows that vitamin P does affect capillary fragility in the experimental animal.

CHEMISTRY

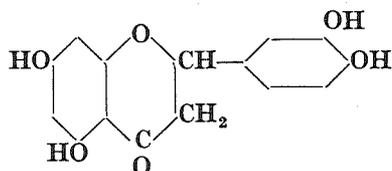
The chemistry of "vitamin P" was investigated by Szent-Györgyi [4], who considered it to consist of mixed crystals of two related flavones, the glycoside hesperidin and the glycoside of eriodictyol. According to him the former constitutes the major part of citrin; the latter is responsible for the chemical reactivity and yellow colour. Hesperidin is the rhamnoglucose glycoside of 5 : 7 : 3'-trihydroxy-4'-methoxyflavone or hesperetin :—



Mager [58], however, states that it is the L-rhamnose glycoside. According to Scarborough [65], hesperidin is not vitamin P, as there is an active material

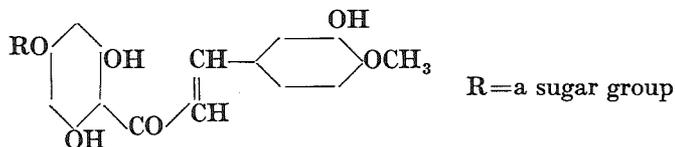
in rose hips at least two and a half times more active. Moreover, hesperidin is practically insoluble in both water and lipoids.

Szent-Györgyi believes that eriodictyol glycoside is the glycoside of 5 : 7 : 3' : 4'-tetrahydroxy-flavonone :—



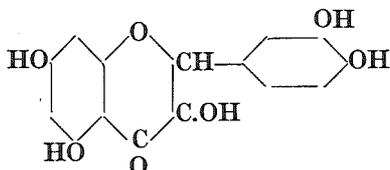
This lacks confirmation.

Wawra and Webb [46] claim to have discovered a new oxidation-reduction enzyme containing "vitamin P" as the prosthetic group. They believe that the eriodictyol of Szent-Györgyi is the chalcone of hesperidin :

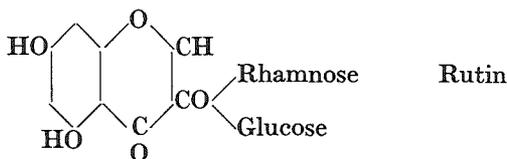


and that it is capable of being reversibly transformed by reduction and ring closure to the flavonone glycoside. Preliminary experiments showed that the chalcone decreases capillary fragility and prevents hæmorrhage. This has not been confirmed by Higby [68].

Robeznieks [69] by means of chromatography showed that citrin contains a quercitrin-like substance. Quercitrin is 3 : 5 : 7 : 3' : 4'-pentahydroxy-flavonone :—

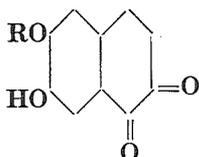


The discovery that rutin, a rhamno-glycoside of quercitrin, has vitamin P-like activity was made in 1944 by Griffith and his colleagues [66]. They first isolated rutin :—



from tobacco and later found that the best source is buckwheat, some Japanese varieties of which contain as much as six per cent.

Epicatechin (p. 735) is also a flavonol with a vitamin P-like action. Esculin :—



is a flavone with an activity several hundred times that of citrin,

ESTIMATION AND UNITS OF VITAMIN P

A method of estimating vitamin P based on the measurement of capillary resistance has been developed by Bacharach, Coates and Middleton [41]. They apply a suction cup with a diameter of 12 mm. to the greased and shaven area on the back of a guinea-pig, the pressure being gradually reduced by 5 mm. stages, and maintained at each stage for three to five seconds. The pressure is noted at which petechiæ are first seen when the area under suction is viewed through the cup. This is called the critical petechial pressure. If this is plotted against the logarithm of the dose of vitamin P administered to the animals, a straight line graph is obtained. A similar technique is used by Bourne [48], who uses a suction cup 20 mm. in diameter, the pressure being rapidly reduced to the desired level for ten seconds and then released. The capillary resistance is then taken as the negative pressure required to produce so many petechiæ that the area under test becomes uniformly reddish-purple in colour. Animals on a scorbutogenic diet show a steady and significant fall in capillary resistance not corrected by ascorbic acid. It is abolished by hesperidin and the citrus glycosides. Much confusion in the literature has resulted from the discriminate use of the two types of capillary fragility tests, i.e., the positive and negative pressure tests (p. 464). There is no significant correlation between the two tests [121].

Bacharach and Coates [50] express the activity of a vitamin P preparation under test in terms of a standard water-soluble calcium-containing glycosidic complex derived from citrus peel, which is similar to Szent-Györgyi's citrin. One "provisional unit" (P.U.) is defined as the activity of 1 mg. of this preparation. Recrystallized hesperidin had an activity of 100 P.U. per gram. Bacharach and Coates [64] have prepared a vitamin P standard of potency 160,000 units per 100 gram. Black-currant concentrates of 1,060,000 units per 100 gram have been obtained.

Scarborough [79] using a negative pressure method to assay vitamin P activity has determined the vitamin P content of a number of foodstuffs. He uses a water-soluble preparation obtained from orange pulp and peel by a modification of Szent-Györgyi's method [10] as a standard. This appears to have the same activity as Bacharach and Coates' preparation, 100 mg. of it being equivalent to 100 units of "vitamin P activity" or to 1 gram hesperidin or 150 mg. of Szent-Györgyi's citrin.

Field and Rekers [113] have devised a "screening" test for substances with vitamin P activity depending upon protection against irradiation hæmorrhage (p. 743). Other suggested methods of assay depend on the reduction of bleeding time in normal guinea-pigs [125] and the increased resistance of erythrocytes to hypotonic saline [126], both of which are affected by vitamin P. Chemical methods of assay are inadmissible as they are group specific and include flavone derivatives that are devoid of vitamin P action.

DISTRIBUTION OF VITAMIN P IN FOODSTUFFS

Fruits are the richest source of vitamin P, followed by green leaves. There is very little vitamin P in roots and seeds, although there is an increase in the latter on germination. Little vitamin P is lost in the processing of commercial fruit concentrates and syrups, but some occurs on storage. Although vitamin P and C are associated in fruits and vegetables there is no correlation between ascorbic acid content and vitamin P activity in the same food [64], nor between vitamin P and the anthocyanins.

There is complete loss of potency if the food is boiled in air. If a solution containing potent vitamin P be kept in the light but without contact with air, and in a cool place its activity is maintained for one to two months. At room temperature a preparation of blackcurrant with a potency of 600 units falls to about 200-300 units in three months, and to 150-200 units in six months, while after nine months the potency is negligible [79].

THE VITAMINS IN MEDICINE

The figures in the following table, taken from Bacharach [41] and Scarborough [79], are very approximate, the authors themselves admitting that they should be accepted with reserve.

VITAMIN P CONTENT OF FRUITS AND VEGETABLES

Food	Description	Part Tested	Vitamin P Content in "Units" per 100 grams
Apple	Bramley's seedling	Fruit	60
Apricot	Dried	Whole fruit	75-100
Beetroot	—	Root	15
Bilberry	—	Whole fruit	100
Blackberry	—	Fruit	60-100
Blackcurrant	Purée = 65%	Raw fruit	200-500
"	Fresh	Fruit	75
Cabbage	Spring (April)	Leaves	60
"	Summer (October)	"	100
Carrot	April crop	Root	10
"	August crop	"	40
Cauliflower	—	Flower	40
Cherry	Black	Fruit	60-100
"	White	"	50
Dandelion	—	Leaves	30
Dock	—	"	20
Grape	Black	Whole fruit	500-1,000
"	White	"	500-1,000
Grapefruit	—	"	100
Lemon	Fruit	Peel	500
"	"	Juice	450-750
Lentil	—	Seed	0
Lettuce	Round (May)	Leaves	80
"	" (September)	"	100
Maple pea	Dried	Seed	10
Orange	Fruit	Whole fruit	300-500
"	"	Juice	300-600
"	"	Peel	200-300
Parsley	—	Leaves	130
Parsnip	—	Root	40
Pea	Dried	Seed	40
"	Germinated	"	80
" maple	Dried	"	10
"	Germinated	"	10
Plum	Blue	Fruit	50-200
Prune	—	Whole fruit	300-400
Potato	Old (April)	Tuber	25
"	New (July)	"	40
Rhubarb	—	Stalks	20
Rose hips	—	Fruit	240-680
" " syrup	Fresh	"	350-500
" "	Commercial	"	0-100
Rowan	—	"	300
Spinach	—	Leaves	130
Swede	—	Root	20
Tomato	—	Fruit	50-70
Turnip	—	Root	20-30
Walnut	—	Kernel	100
Watercress	April crop	Shoots	10
"	October crop	"	70
<i>Concentrates</i>			
Water soluble concentrate	—	From blackcurrant	300,000
"	—	From whole orange	100,000
Citrus	From lemon by Szent-Györgyi's method.	From whole lemon	65,000
" Hipexa "	—	From rose hips	20,000-30,000
Hesperidin	—	M.P. 265°-266°	9,500-10,000
Hesperetin	—	M.P. 223°-224°	2,500-5,000
Rutin	—	—	5,000

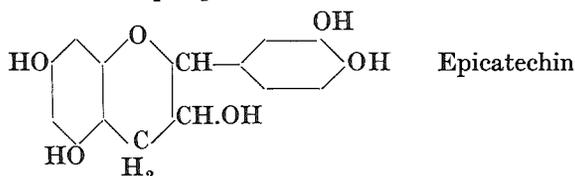
HUMAN REQUIREMENT OF VITAMIN P

Very little is known about the vitamin P requirements of man. From dietary studies Glazebrook, Scarborough and Wokes [75] concluded the daily requirement to be not less than 33 units daily, and possibly considerably more. Scarborough [79] found that the daily intake of vitamin P needed to maintain a high level of capillary resistance in two scorbutic subjects was of the order of 300 units, which would be present in 50 to 100 ml. of orange juice and would be provided by three grams of hesperidin. This is of interest as Göthlin, fourteen years earlier, concluded that 50–70 ml. of orange juice was the minimum daily dose to protect against scurvy in man (p. 441).

Seasonal variations in the human intake of vitamin P occur. In a group of boys studied by Glazebrook, Scarborough and Wokes [75] the vitamin P increased from 7 units daily between February and March to 15 units in August and September. This was probably below the normal daily requirements of vitamin P.

THEORY OF ACTION OF VITAMIN P

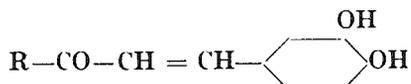
In 1941 Lavollay and his colleagues in France [52] put forward the view that vitamin P controls capillary resistance and permeability by retarding the oxidation of adrenaline, sympathin or one of their metabolic products such as adrenochrome. These are then able to exert a vasoconstrictor effect on the capillaries and diminish capillary fragility. They tested D-catechin, or D-3 : 5 : 7 : 3' : 4'-pentahydroxy benzodihydropyran and found this to be inactive, but further investigations led to the isolation of D-epicatechin a related compound, which was found to be extremely active. Wilson [81] confirmed Lavollay's observation that flavonols such as rutin prolong the effect of adrenaline in isolated organ experiments, and it has been shown that rutin diminishes the amount of adrenaline required to produce a vasoconstrictor effect on blood vessels [138].



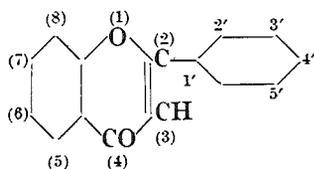
According to Haley, Clark and Geissman [70] the catechins are highly active vasoconstrictors in doses of 0.0001 microgram or less. They have tested the effect of substances with a vitamin P-like activity topically on mammalian capillary bed preparations, observing microscopically vasomotor responses of the vessels. They found that rutin and hesperidin were inert in the concentration used, although the catechins including the D-catechin and L-epicatechin were highly active. Lavollay and his co-workers found D-epicatechin the most active vitamin P-like compound. It is possible of course that intravascular contact with the compounds under test may differ from the topical effects. The theory of Lavollay and his followers and their method of assay of vitamin P-like substances is not accepted by some workers [73]. Using adrenergic blocking agents Schiller [141] was able to show that although rutin prolongs the vasoconstrictor action of adrenaline and noradrenaline it also has a strong cutaneous vasoconstrictor action of its own; adrenaline and rutin do not compete for the same receptors.

It is clear that "vitamin P" is not an entity in the sense that aneurine, riboflavine or ascorbic acid are. Such diverse substances as rutin, quercitrin and epicatechin and some unidentified factors in lemon peel infusions possess a vitamin P-like activity. Clark and Geissman [71] have tested numerous compounds for their potentiating effect on the action of adrenaline on excised

smooth muscle and conclude that for high activity the following structure is necessary :—



They could not, however, correlate this potentiating effect on adrenaline with the effect on capillary fragility and reject it as a method of assaying "vitamin P." Wilson and De Eds [72] tested a number of flavonols and flavonones, both natural and synthetic for their ability to protect adrenaline from destruction, using an isolated strip of intestine as the test object. Using the notation indicated in the general formula of the flavones given below, Wilson and De Eds conclude that a glycosidal linkage



on C - 7 increases, while methoxylation (OCH_3) of this carbon atom decreases activity ; a double bond between C - 2 and C - 3 increases activity, and two free hydroxyl groups, ortho to each other on the phenyl ring also increase activity.

One of the difficulties encountered in evaluating the vitamin P potency of flavone derivatives has been the confusion between fragility and permeability changes in the capillaries and the variety of methods used for detecting changes in these (cf. pp. 415, 464). There is no correlation between the positive and negative pressure methods, and the effects of age, sex, season, time of day, emotion and menstruation further complicate the picture. The rôle of adrenaline in controlling capillary permeability, as suggested by Lavollay, is by no means clear since Clark [74] has shown that adrenaline actually causes dilatation of muscle capillaries. Changes in capillary permeability have been followed by observing changes in cutaneous lymph flow by noting the spread of dyes, such as Evans blue, after injection into the antecubital space. The application to the skin of an irritant, such as chloroform, or the intracutaneous injection of histamine, results in the accumulation of intravenously injected trypan blue in the areas of inflammation [76]. Ambrose and De Eds [77] have used this technique as a method of estimating capillary fragility and of assaying the vitamin potency of various flavones ; the method is only applicable when the substance under test can be injected, and a complicating factor is that the rate of escape of the dye from cutaneous capillaries is probably influenced by changes in the blood pressure. Wilson and his co-workers [81] claim that rutin inhibits the extravasation of dye given intravenously into histamine wheals, although this cannot be confirmed by Clark and MacKay [128]. Bohr, McIvor and Rinehart [80] criticize the dye technique and ascribe the changes produced by the flavones to a decrease in capillary blood flow in the skin, rather than to changes in capillary permeability. The potency of flavonoid compounds has also been estimated by an air blast method, which ruptures the lung capillaries [131] ; the method does not give statistically significant results [132].

In scorbutic human beings the positive pressure test for measuring capillary resistance (p. 464) fails to show any change after the administration of vitamin P preparations ; there is a slight improvement using the negative pressure method [78]. The capillary resistance of a group of 100 allergic children was studied by Rapaport and Klein [29]. Twelve with a low capillary resistance were treated with 100 to 150 mg. of a vitamin P preparation daily

for six months, when the capillary resistance became normal. The capillary resistance of normal persons is not influenced by vitamin P [63].

It has been suggested by Levitan [67] that hyaluronidase may be a factor in controlling tissue and capillary permeability and that hyaluronidase is inhibited by vitamin P-like substances. He states that rutin markedly inhibits the spreading activity of intradermally injected hyaluronidase. Rodney and her co-workers [127] observed that some flavonoid compounds inhibited the action of hyaluronidase, but only those capable of orthoquinone formation. They did not regard the effect as specific. Elster [85] failed to observe any inhibiting effect of rutin on the increased diffusion across capillary membranes caused by hyaluronidase. Levitan [86] believes that the effect of vitamin P on the capillaries is due to a non-specific increase in the resistance of the connective tissue ground substance in the pericapillary sheath. In vitamin P deficiency, induced by feeding an antivitamin P factor, the permeability of connective tissue was increased; this was partially reversed by aesculin, a flavonol, but not by ascorbic acid.

PHYSIOLOGY AND PHARMACOLOGY OF VITAMIN P

The work of Zacho [11], Bacharach [41, 50, 64] and Bourne [48], suggests that vitamin P-like compounds play a part in the control of capillary permeability and resistance in the experimental animal. The low capillary fragility of scorbutic guinea-pigs rises after giving ascorbic acid, but still keeps at a subnormal level. When citrin is added to the diet, however, the capillary fragility reaches normal levels. The intestinal hæmorrhages of scorbutic animals also clear up when vitamin P is added to the diet. Rusznyak and Benko [31] found that lowered capillary resistance in rats could be raised to normal levels in ten to fourteen days by subcutaneous injections of 3 to 4 mg. of citrin. Todhunter and his co-workers [15] report that scorbutic guinea-pigs receiving supplements of lemon juice show fewer hæmorrhages than do animals receiving equivalent amounts of synthetic ascorbic acid. They observed that on a scorbutic diet reinforced by synthetic ascorbic acid the capillary fragility decreased considerably, but only rose to normal levels or above after the administration of vitamin P or citrin.

Sokoloff and Redd [73] induced scurvy in animals by means of glucoascorbic acid, an ascorbic acid antagonist, and produced complete recovery by treatment with ascorbic acid and vitamin P. Crampton and Lloyd [82], using the odontoblast method for the assay of ascorbic acid, state that rutin enhances the biological effect of ascorbic acid. Cotereau and others have reported that catechin permits the storage of ascorbic acid in the tissues when the latter is fed to guinea-pigs in minimal amounts; they consider it to exert a sparing action on the oxidation of ascorbic acid [83]. This is confirmed by the observation of Ekman [84] that a number of flavonols, including quercetin and citrin, stabilize ascorbic acid in solution and protect it against oxidation. They also cause increased ascorbic acid excretion [49]. Flavonoids obtained from citrus fruits can diminish increased capillary fragility due to leukotaxine, a substance obtained from inflammatory exudates, and bacterial polysaccharides [142, 143].

Citrin and the flavones with vitamin P activity cause a slight fall in blood pressure when administered intravenously; a dose of 100 mg. of citrin causes a fall of 10 to 15 mm. after about three to four seconds [39]. This fall is apparently due to vasodilatation. On the other hand the catechins produce capillary vasoconstriction [70]. In the scorbutic guinea-pig there is a twenty to thirty per cent. increase in the reticulocytes within a few days of giving citrin [40]. Rutin *in vitro* and *in vivo* reduces increased sedimentation rates.

According to Plungian, Munch and Wolffe [87] rutin decreases the coagulation time of the blood and antagonizes the effect of dicoumarol. This has been confirmed by Martin and Swayne [88] for rutin and D-catechin, but not

for hesperidin. Clark [89] could not confirm the protective action of rutin against dicoumarol. D-Catechin reduces the bleeding time in man from a mean of seven and a half minutes to one and a half minutes [94].

Rutin, hesperidin and other flavonoids have been stated to give some protection to guinea-pigs from anaphylactic and histamine shock, although the effect is not spectacular [47, 81, 140] and is even denied by some workers [124, 128]. Wilson, Mortarotti and De Eads [91] suggest the protection against histamine shock is due to the potentiation of adrenaline in the tissues by the flavonols. There is some evidence that citrin protects guinea-pigs against the action of diphtheria toxin, although it is not as effective as ascorbic acid [38]. Flavones have a detoxicating effect on benzene, phenol [49] and thiocyanates [38] and with ascorbic acid protect against the toxic effects of organic arsenicals such as dichlorophenarsine (Chlorarsen) [57].

Ungar [129] claimed that hesperidin methylchalcone and epimerized D-catechin diminished bleeding time by suppressing the formation of histamine. This could not be confirmed by Clark and MacKay [128]. Rutin prevents the leucocytosis that can be produced by irritating the skin [136], and the anaemia and leucocytosis following splenectomy in the albino rat [139].

Vitamin P has a moderate inhibitory effect on the growth of mouse sarcoma and carcinoma [146]. It prolongs the life of adrenalectomized rats [146].

Certain flavonoids inhibit the action of choline acetylase *in vitro* [130]. Whether this occurs *in vivo* and the significance of effect are not known.

Hartzell and Stone [54] have shown that vitamin P, unlike ascorbic acid plays no part in the healing of wounds.

Vitamin P is absorbed when given orally, and it is active when administered parenterally. It is excreted in the urine [37]. Apparently vitamin P is not stored to any appreciable extent in the tissues [36]. After the administration of test doses of 50 to 100 mg. of citrin or eriodictyol intravenously about fifty per cent. is excreted in the urine [37]; twenty per cent. of a dose of epicatechin can be recovered from the urine [90]. Saturation of the tissues is reached in normal persons in from two to six days, but in disease a much longer period is required. In vascular purpura saturation cannot be attained [37].

Substances with a vitamin P action, such as hesperidin and rutin, are virtually non-toxic even in large doses, *e.g.*, 15 grams daily [92, 93]. Patients have tolerated 120 mg. rutin for nine months without any side effects [95]. The growth rate of rats is uninfluenced by incorporating one per cent. rutin in their diet [93].

HUMAN VITAMIN P DEFICIENCY

A number of clinical studies suggest that vitamin P is an essential factor in human nutrition. The observations of Scarborough [12-14, 65] appear to establish with some certainty the existence of a dietary factor essential for the maintenance of capillary resistance in human beings. He observed an increased capillary fragility in a number of patients suffering from multiple vitamin deficiencies of varying degrees of severity. In two patients with scurvy the daily administration of 3 grams of hesperidin and a rose hip preparation containing 550 units produced a significant rise in capillary resistance [65]. Previous treatment with ascorbic acid had produced a fall. Scarborough observed that orange and lemon juice and extracts made from them produced an increase in the capillary resistance, even when ascorbic acid had failed to produce any effect. He concluded that at least two forms of subcutaneous bleeding may develop as the result of nutritional deficiency in man [14]. One form due to ascorbic acid deficiency is characterized by the large subcutaneous hæmorrhages (ecchymoses) seen in the scorbutic state and involving considerable areas of subcutaneous tissue and muscle. Gingival bleeding is also common. Vitamin P does not control these hæmorrhages, which are only arrested by large doses (500 mg.) of ascorbic acid. Scarborough also reported that vitamin P has no effect on the other important manifesta-

tions of the scorbutic state, but apparently it can increase the capillary resistance of scorbutic subjects either before or after treatment with ascorbic acid.

Cameron and Mills [55] gave vitamin P but not ascorbic acid to a patient with classic scurvy. The hæmorrhagic features promptly disappeared although the other symptoms of the disease were unaffected until ascorbic acid was given. Ambrose and De Eds [116] state that supplements of rutin and ascorbic acid prolong the life of scorbutic guinea-pigs longer than either substance alone. Lazarus, Munro and Bell [96] do not consider the association of capillary resistance and scurvy to be clear cut. In a series of fourteen cases the capillary resistance was within normal limits. There was no change in capillary strength after treatment with either vitamin P or ascorbic acid. Although the patients were cured clinically they consider that scurvy is more liable to develop in subjects who already have some form of capillary weakness, which is probably present in a small proportion of the population, e.g., they noted five medical students with a high capillary fragility that did not respond to ascorbic acid or vitamin P.

Scarborough believes that a deficiency of vitamin P may exist in man even after dosage with large quantities of ascorbic acid. The clinical manifestations of vitamin P deficiency that he describes include pains in the legs on exertion, pain across the shoulders, weakness, lassitude and fatigue. It is invariably associated with a much decreased capillary resistance and may be characterized by the development of spontaneous petechial hæmorrhages, especially in areas exposed to pressure (e.g., of tight clothes). It has not been found to be accompanied by any hæmatological abnormality, and it responds to treatment with vitamin P (50 mg. doses). The hæmorrhages developing as a result of vitamin P deficiency are always small (petechiæ) and take place in the skin. They are often circumpilar. Hæmorrhage is more severe in parts exposed to the pressure of clothing and in the legs because of the higher venous pressure in the latter. Scarborough considers that some forms of purpura may have a nutritional basis, although he states that vitamin P is ineffective in its treatment [56]. He has described an experimentally induced clinical syndrome in two human subjects attributable to a deficiency of vitamin P [32]. The major features of this syndrome are petechial bleeding, low capillary resistance, and a slightly prolonged bleeding time.

CLINICAL USES OF VITAMIN P

Various flavones with vitamin P activity have been used clinically, e.g., citrin (20 to 60 mg. daily), hesperidin (0.25 to 1 gm. daily), and rutin (60 to 180 mg. daily). The latter is readily obtained from buckwheat and is now the preparation that is generally used. Preparations of lemon of unknown potency have also been employed so that caution is needed in interpreting results. Vitamin P preparations have been used mainly in those conditions with decreased capillary resistance. These include diabetes, hypertension, rheumatic fever, allergic conditions, bacterial toxæmias and toxic manifestations from drugs. Unfortunately much of the work has been done on small numbers and not controlled statistically, and no standardized method has been used for determining capillary resistance. Many of the statements made are suggestive but not conclusive.

Vascular Purpura. There have been numerous reports on the treatment of purpura with vitamin P preparations. It has not been established that any form of purpura is primarily due to an increased fragility of the capillary walls. Scarborough [27] points out that the presence of extravascular blood, either in the tissues or in the alimentary canal, markedly increases the capillary resistance and so makes petechial counts as a measure of the latter very inaccurate. Thus after the development of a purpuric eruption resulting in the extravasation of more than 4 ml. of blood into the tissues, the capillary

resistance will be high for two to four days and the individual may show a decreased tendency to the development of further purpuric spots during this period. Failure to recognize this means that entirely fallacious conclusions may be drawn from observations on the therapeutic value of vitamin P in purpura. Some of the conflicting results may also be due to differences of technique used by various workers. It is known for example that there is no correlation between the negative and positive pressure methods (p. 464) for measuring capillary fragility [63, 121]. Kugelmass [16] claims to have satisfactorily treated four cases of purpura of nutritional, allergic and infectious origin with vitamin P in doses of 150 mg. daily by mouth. A case of purpura following scarlet fever showed improvement, but traumatic conjunctival petechiæ, resulting from attacks of whooping cough and epilepsy showed no change, nor did purpura caused by the pressure of plaster casts. Miller [28] records a case of purpura developing during the convalescent stage of measles that responded to treatment with vitamin P in eight days. In a single case such as this natural recovery cannot be excluded.

Jersild [17] treated a case of Schönlein-Henoch purpura, refractory to ascorbic acid, with 50 mg. of a vitamin P preparation. Petechiæ disappeared, but reappeared when this was withdrawn, and disappeared again when the vitamin was re-administered. Hiramatsu [25] treated three cases of Schönlein-Henoch's purpura, one of purpura following rheumatic fever, and one case of purpura simplex with vitamin P. All showed an increased capillary resistance, the effect being potentiated by ascorbic acid. Vacek [34] states that in thrombopenia vitamin P causes the disappearance of petechiæ, although it does not affect the platelet count. Scarborough [32] records two cases of senile purpura that responded to vitamin P.

These observations have not been confirmed by others. Vaughan [98], Rudy [62] and Franke [26] failed to observe any improvement in cases of purpura treated with vitamin P. Davis [97] as a result of analysing 1,200 cases of Schönlein-Henoch purpura concluded that neither vitamin P nor ascorbic acid were of any value in treatment. Madison and Pohle [43] examined the effect of rutin in fourteen cases of purpura—six allergic, two toxic, one endocrine, one associated with hypertension and four associated with malignant disease—and were only impressed with the results in the four patients with malignant disease. The results in the allergic group were not convincing and in the remainder they were questionable. Vitamin P does not affect the thrombocyte count in purpura and analysis of the published work reveals that vitamin P is of doubtful value in raising the capillary resistance unless lowered by infection and nutritional deficiency; the results in allergic purpura are not convincing.

Several investigators have reported that purpura due to drug toxæmia is favourably affected by vitamin P. Scarborough and Stewart [12] showed that the diminished capillary resistance that sometimes occurs as a complication of arsenic and bismuth therapy in the treatment of syphilis can be checked by giving hesperidin in doses of 1 gram daily. Horne and Scarborough [19] have also observed that the toxic erythema and dermatitis sometimes resulting from the treatment of syphilis with arsenic is associated with decreased capillary resistance, and that this can be prevented by administering vitamin P. Gorrie [20] reported a case of acute hæmorrhagic purpura occurring after injections of neoarsphenamine that rapidly improved after treatment with 1 gram of vitamin P daily. Capillary damage is more likely to occur in the rapid massive dose treatment of syphilis with arsenicals. Goldstein, Stolman and Goldfarb [60] suggest that this might be minimized by administering vitamin P without inhibiting the treponemicidal effect of the arsenical. They administered 10 to 30 mg. per kilogram of hesperidin methyl chalcone daily for seven days to rabbits before treatment with toxic doses of mapharsen. In the treated group the survival rate was ninety per cent.; in the untreated group fifty-seven per cent. Friend and Ivy [59] observed that hesperidin

methyl chalcone and ascorbic acid had a similar protective action against the toxic effects of another arsenical, dichlorophenarsine.

Hæmorrhagic Telangiectasia. Hereditary hæmorrhagic telangiectasia, or Rendu-Osler-Weber disease, also known as the Sturge-Weber syndrome, is a rare condition characterized by multiple telangiectasia, hereditary transmission, and widespread hæmorrhage. The latter may result in epistaxis, hæmatemesis, melæna, hæmaturia and small cerebral hæmorrhages which may cause paralysis. Telangiectases can appear in the skin, mouth, alimentary canal and brain. Kushlan [18] has reviewed 1,000 cases in 175 families and records the treatment of one case with 40 mg. of rutin daily after five blood transfusions had failed to stop a severe hæmatemesis, epistaxis and bleeding gums. Improvement was stated to have occurred within twenty-four hours of administering rutin. Cope and Grover [114] also record improvement in a case treated with 120 mg. of rutin daily. Petch [119] and Glass [135] failed to observe any benefit in cases treated with rutin; the cases mentioned by Glass showed no change in capillary fragility.

Nephritis and Hæmaturia. Vitamin P has been used in the treatment of hæmorrhagic nephritis and hæmaturia due to food allergy, drug idiosyncrasy and bacterial toxins [1, 20, 21]. One observer, however, administered vitamin P to seven patients with hæmorrhagic nephritis and obtained results that were no better than those obtained by rest in bed, careful nursing and dieting [22]. Gorrie [20] reports the case of a patient with acute hæmaturia occurring after several injections of neoarsphenamine; the hæmaturia ceased forty-eight hours after commencing treatment with vitamin P. Raunert [23] treated thirty cases of hæmaturia due to nephritis, renal tumours, cystoscopy and prostatectomy with 50 to 100 mg. of vitamin P every four hours and claimed that the hæmaturia ceased within a few hours. Hæmorrhage in a case of hæmaturia due to congenital polycystic disease of the kidneys is stated to have stopped after administering rutin [99].

Hypertension. Rutin can prevent experimentally produced malignant hypertension in dogs [145]. Griffith and Lindauer [100] observed that in eighteen per cent. of 265 cases of hypertension there was a decrease in capillary resistance, which they supposed predisposed to retinal and cerebral hæmorrhage; capillary resistance was further decreased by administering potassium thiocyanate to correct the hypertension. In a later communication Griffith [101] reported on a group of 1,600 hypertensive subjects. Nineteen per cent. showed increased capillary fragility, as measured by Göthlin's test (p. 464) modified by Griffith and Lindauer [52], and an additional eleven per cent. increased capillary permeability, as evidenced by increased cutaneous lymphatic flow measured by the patent blue method of McMaster. Patent blue is a dye which when injected intracutaneously is absorbed through the lymphatics which it colours and renders visible. According to Griffith the incidence of cerebral and retinal hæmorrhage was greater in patients with capillary defects (nine per cent.) than in the controls (two per cent.). Rutin in a dose of 20 mg. three times daily was given to the patients for periods up to four years. Capillary tests became normal in seventy-five per cent. of those treated; they remained constantly or intermittently abnormal in the remaining twenty-five per cent. In the majority of cases rutin had no beneficial effect in lowering blood pressure. The incidence of cerebral and retinal hæmorrhage was, however, reduced to about that of the normal group. The administration of rutin enabled thiocyanate to be given without further increase of capillary fragility. It must be pointed out that vitamin P preparations do not have any direct effect on hypertension. Griffith's observations have been repeated, although on a much smaller number of subjects by Zfass [52] and Shanno [51], who reports that rutin is effective in controlling pulmonary hæmorrhage. As only two cases of the latter were treated and the condition often ceases spontaneously it is difficult to make any comment on this. Barishaw [122] states that a combination of hesperidin

and ascorbic acid reduces abnormal capillary fragility in hypertensive patients. It is claimed that rutin reduces increased capillary fragility in hypertension occurring in pregnancy [134]. Soloff and Bello [102] did not observe any change in the Rumpel-Leede test for capillary fragility after administering rutin to hypertensive patients, nor did they observe any correlation between retinal hæmorrhage and a positive Rumpel-Leede test.

Glaucoma and Retinal Hæmorrhage. In 1942 Schmidt and Saubermann [57] reported that intraocular pressure in hydrophthalmic rabbits is reduced by intravenous injection of vitamin P. According to Stocker rutin has had no effect on the permeability of the blood-aqueous barrier in rabbits [103]. The same observer administered rutin in doses of 20 mg. three times a day to twenty-six patients with glaucoma for an average period of eight months. In seventeen the intraocular tension fell; in four the results were equivocal and in five there was no change. Shanno, Griffiths and Lamotte [104] consider that some cases of retinal hæmorrhage may be due to a capillary weakness throughout the body. From a study of seventy-nine patients they state that there is an abnormal response to capillary fragility and permeability tests in most subjects with recurrent retinal hæmorrhage. The administration of 60 mg. of rutin daily resulted in a return of capillary fragility and permeability to normal levels in fifty to seventy per cent. of the patients investigated and a reduction in the subsequent incidence of retinal hæmorrhage in many of these. Mathewson [61] describes an extensive case of retinal hæmorrhage, associated with hæmorrhage in other parts of the body, that ceased on administering vitamin P. Another case of recurrent hæmorrhage into the anterior chamber after cataract extraction responded to treatment with vitamin P. Wolffe and Danish [35], however, report the occurrence of subconjunctival hæmorrhage in two out of sixty patients receiving rutin in doses up to 150 mg. daily.

Rheumatic Fever and Rheumatoid Arthritis. Rinehart [105] treated thirty-nine cases of rheumatic fever with vitamin P and claims that it reduced the sedimentation rate within a month. Warter and his co-workers [106] state that hesperidin reduces abnormal capillary fragility in patients with rheumatoid arthritis, but they do not report on its effect on the general condition of the patients. Similar observations were made by Barishaw [122], using hesperidin and ascorbic acid. Rudy and others [62] observed no change in the capillary fragility of patients with rheumatoid arthritis treated with vitamin P preparations.

Diabetes. Many diabetics with hæmorrhagic retinitis show increased capillary fragility, which, it has been claimed can be reduced to within normal levels by administering rutin in doses of 20 to 60 mg. three times a day or by hesperidin [137]. Increased capillary fragility occurs in diabetic patients as an early evidence of generalized arterial disease and is closely correlated with the development of retinitis and later nephritis. There is no evidence that rutin alters the course of the disease in diabetic retinitis [111, 144]. Some workers admit that there are so many variable factors influencing diabetic retinitis that any improvement in the small numbers studied cannot be attributed to rutin or ascorbic acid therapy [111]. Rudy and his co-workers [62], while they confirm the increased capillary fragility in diabetes, state that it is uninfluenced by vitamin P therapy. Palmer and his colleagues [147] consider that the results obtained with rutin in the treatment of diabetic retinitis were no better than would be expected from good diabetic control with diet and insulin.

Skin Diseases. Vitamin P has been used in the treatment of some skin diseases with inconclusive results. It has been used in the treatment of eczema [24, 44] and psoriasis [30]. Goldfarb [30] claimed to have obtained improvement in thirty out of forty-five cases of psoriasis treated with citrin preparations made from lemon, but Niedelman and Horoschak [112] obtained no benefit from giving hesperidin and ascorbic acid in this condition.

Irradiation Sickness. Exposure to heavy but sublethal doses of X-rays or other forms of radiation causes purpura with severe and often fatal hæmorrhage from mucous membranes. On the assumption that the intrac-table capillary oozing is due to excessive capillary fragility Field and Rekers [113] administered a number of flavonols to dogs submitted to X-radiation. Rutin was first tried, in a large dose of 150 mg. daily, and although it failed to stop the leucopenia and thrombocytopenia it did diminish the hæmorrhage ; sixty per cent. of the control irradiated animals died, whereas only eleven per cent. of those treated with rutin succumbed. Clinical signs and post-mortem evidence of a hæmorrhagic diathesis were most marked in the untreated dogs. Similar observations have been made in rats and guinea-pigs [117, 118, 142]. In Field and Reker's dogs the flavonols had little effect unless they were given before exposure to irradiation. Other flavonols which appeared to be approximately equally as active as rutin were hesperidin, epimerized D-catechin and homoeriodictyol ; but quercetrin, quercetin, naringin and hesperidin methyl chalcone were inactive. Ascorbic acid alone was also inactive but with quercetin did afford protection to ten per cent. of the dogs. Field and Rekers propose to use the protective effect against irradiated dogs as a " screening " test for vitamin P activity. Other workers have failed to observe any beneficial effect in animals submitted to X-radiation [120, 148].

Mathewson [61] reported a case of myeloma treated by X-ray " spray " therapy, which caused an acute hæmorrhagic diathesis. This was checked after the administration of vitamin P. Madison and Pohle [43] describe four advanced cases of cancer treated by irradiation that benefited by the administration of rutin. Sokoloff, Eddy and Redd [142] administered 300 to 600 mg. of a flavonoid preparation from citrus fruits to ninety-two patients receiving radiation therapy ; skin erythema was diminished considerably, but the incidence of nausea and vomiting was unchanged.

The subject requires further investigation in view of the irradiation effects likely to be experienced in atomic warfare.

Frostbite. Fuhrman [115] induced frostbite artificially in guinea-pigs and states that if they are previously treated with rutin they suffer less tissue damage. Vitamin P has not been used clinically for frostbite.

Allergic and Vasomotor Rhinitis. On the assumption of Lavollay that vitamin P retards the oxidation of adrenaline, the antagonist of histamine, Saylor [123] treated allergic and vasomotor rhinitis with hesperidin chalcone in doses of 100 to 600 mg. daily. He claims that thirty-five per cent. of ninety-nine patients obtained complete relief from symptoms, thirty-four per cent. obtained partial relief and twenty-five per cent. experienced no relief. Five per cent. showed side reactions such as urticaria, nausea and aggravation of bronchial asthma.

Clark and MacKay [133] have assembled evidence to show that these effects of flavonoid compounds are not due to any vitamin-like action or that they exert any specific chemical or therapeutic effects. They have shown that the compounds are not absorbed from the human gut and are in fact destroyed either by enzymes or organisms in the stools. When injected they may cause a decrease in adrenal ascorbic acid and thus may elicit a non-specific stress or " alarm reaction " (Selye), which may explain some of the physiological and pharmacological effects.

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