

THE WULZEN CALCIUM DYSTROPHY SYNDROME IN GUINEA-PIGS¹

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In 1941 Wulzen and Bahrs (1) reported that guinea-pigs fed raw whole milk grew excellently and at autopsy showed no abnormality of any kind. Guinea-pigs on pasturized milk rations did not grow as well and developed a definite syndrome, the first sign of which was wrist stiffness. On pasteurized skim milk the syndrome increased in severity until the animals finally died. There was great emaciation and weakness before death. Upon autopsy the muscles were extremely atrophied and in most cases were streaked with closely packed, fine white lines of calcification running parallel to the muscle fibers. It is the calcium dystrophy syndrome developed by guinea-pigs on skim milk diet with which this review is mainly concerned. Prominent features of the syndrome are wrist stiffness, muscular dystrophy, arteriosclerosis, calcium deposits in soft tissues, teeth and bone pathologies, and loss of an auditory reflex.

THE PLANARIAN FACTOR

Wulzen and Bahrs were led to the calcium dystrophy syndrome in guinea-pigs from a study of planarian nutrition. In 1935 they (2) produced a deficiency disease in planarian worms (probably *Planaria agilis*) by feeding tissues from guinea-pigs given supposedly adequate diets in which vitamin C was furnished in the form of orange juice or tomato juice. Regular inspection of the planaria revealed the presence of abnormalities related to diet (3). When gliding freely, planaria had a very smooth outline, and the first sign of dietary disease was roughening of the smooth border. As the disease advanced, the surface of the worm shrivelled and became almost black. Irregular lumps developed, sometimes sufficient to bend the worms into abnormal shapes and render movement impossible. Lung and spleen of rats and guinea-pigs never gave rise to the planarian disease syndrome. (Possibly the guinea-pigs had been on the deficient diets for too brief a period.) However, liver, kidney cortex and heart constituted a group of tissues which usually gave rise to dietary disease in planarian worms, except that when fresh, green kale was included in the diet, these tissues did not lead to deficiency disease.

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Presented as part of the scientific program of the Third Medical Conference, sponsored by the Muscular Dystrophy Associations of America, Inc., New York, October 8 and 9, 1954.

The author wishes to thank the Oregon Medical Research Foundation, Armour and Company, and the Graduate Council General Research Fund of Oregon State College for financial support of this research. This paper is published with the approval of the Monographs Publication Committee, Oregon State College, as Research Paper No. 261, School of Science.

Received for publication October 9, 1954.

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LEE FOUNDATION FOR NUTRITIONAL RESEARCH

Milwaukee 3, Wisconsin

REPRINT NO. 81

Price - 10¢ each

TABLE 1. *Diet A (Diet 10, Wulzen and Bahrs (3), 1936)*

	Parts
Baked milk powder.....	25
Ground barley.....	42
Bran.....	23
Yeast.....	5
Cod-liver oil.....	3
NaCl.....	1
CaCO ₃	0.5
Ferric ammonia citrate.....	0.5
KI.....	0.002

The guinea-pigs were given individually, 1 cc. of cod-liver oil, 2 drops viosterol, twice weekly.

Vitamin C provided as orange juice, 15 cc., lemon juice, 15 cc., or fresh kale, 20 g. daily.

Rat tissues resembled guinea-pig tissues in their nutritional effects on planarian worms, but rats required more time than guinea-pigs on a specified diet before inadequacies for planaria became apparent. The existence of a nutritional factor, not one of the recognized vitamins, which must be present in the diet of guinea pigs if they are to produce liver, heart, and kidney cortex that are nutritionally adequate for planarian worms, was postulated by Wulzen and Bahrs.

When maintained on the supposedly adequate Diet A (table 1), one of the basic diets used by Wulzen and Bahrs in 1936, over several months, the guinea-pigs also developed a disease syndrome, which invariably ended in death (4). When the orange juice or tomato juice was replaced by fresh alfalfa or kale, or these green feeds were given in addition to the fruit juices, the guinea-pigs remained in excellent condition. The diseased guinea-pigs showed extreme degeneration of skeletal muscles, with immobilization of the entire body, and degenerative changes in liver, heart and kidneys. The diseased animals differed from the controls in that they had a lower body temperature and could not maintain their temperature as well in a cold environment.

The earliest indication that the guinea-pigs were abnormal was that planaria fed liver, heart or kidney from these guinea-pigs grew more slowly and ultimately developed a deficiency disease. The time required to develop deficient tissues was one month but at this time the guinea-pigs themselves were entirely normal in appearance and free from latent scurvy. At autopsy they were free from gross hemorrhages, and the teeth were always firmly set in the jaw. Hearing was very acute and the animals twinged at any sharp sound when normal controls gave no sign (4). It is also to be emphasized that white striae in the muscles (calcification) of the 1941 publication were not a part of the 1935-36 description.

DIETS DEFICIENT IN THE ANTI-STIFFNESS FACTOR (DASF DIETS) AND RELATED FOOD MIXTURES

Subsequently, among others, Diet B (table 2) was developed in Dr. Wulzen's laboratory and forms the basis for the majority of observations reported by Wulzen, Bahrs, van Wagtendonk, Krueger and their co-workers at the Oregon

TABLE 2. Diet B (Primary DASF diet of Wulzen)

A.	Milk powder	1800. gm.
B.	Water	9000. gm.
C.	Ascorbic acid	0.9 gm.
D.	Ferric chloride	0.361 gm.
E.	Copper sulfate	0.058 gm.
F-1.	Water soluble vitamin mixture	165.0 cc.
	Water	3.5 liters
	Choline hydrochloride	175.0 gm.
	Inositol	35.0 gm.
	P-amino benzoic acid	7.0 gm.
	Nicotinic acid	3.5 gm.
	Riboflavin	1.75 gm.
	Thiamin	0.70 gm.
	Pyridoxine hydrochloride	0.35 gm.
	Calcium pantothenate	0.35 gm.
	Folic acid	0.010 gm.
	Biotin	0.0075 gm.
F-2.	Fat soluble vitamin mixture	165.0 cc.
	Wesson oil	3.5 liters
	Carotene (7,500 I.U. per cc.)	70.0 cc.
	Vioosterol (4,000 I.U. per cc.)	35.0 cc.
	Alpha-tocopherol	0.35 gm.
	2-Methyl-1,4-naphthoquinone	0.35 gm.

Mixture A, B, C, D, E, F-1 was fed on odd days of the month and mixture A, B, C, D, E, and F-2 was fed on even days of the month.

Straw and iodized salt were available *ad libitum*.

TABLE 3. Diet C (Wulzen Stock Diet)

Rolled barley
Greens (fresh kale if possible)
Straw
Water
Iodized salt
Each <i>ad libitum</i>

State College guinea-pig laboratory. Diet B was presumed to be inadequate in some factor other than the substances listed because of the syndrome developing when the mixture is available to guinea-pigs *ad libitum*, and since the syndrome may, in part or *in toto*, be prevented by the addition of greens, raw cream or molasses to the diet. Guinea-pigs on Diet B were usually compared with guinea-pigs on Diet C, the diet taken as a standard for the stock guinea-pigs, and consisting of rolled barley, greens, straw, iodized salt cake, and water, all *ad libitum* (5, 6, 7, 8, table 3). Fresh kale was fed as the green food of choice when available. When kale could not be obtained, other greens or carrot roots were substituted. To avoid high mortalities guinea-pigs were gradually transferred from Diet C to Diet B. Grains were removed from the diet, and the guinea-pigs were trained to take the skimmed milk mixtures from a pipette. Straw was con-

tinuously available *ad libitum*. Greens were removed from the diet eight days later.

Mortality

About 1945-1946 Homberger and Reed (9) and Kon, Bird, Coates, Mullen and Shephard (10) failed to demonstrate wrist stiffness in guinea-pigs on a skim milk diet. They reported poor condition of the animals and a very high mortality. Dasler (11) reports that seven different laboratories (9-14) found, in contrast to the Oregon data, that the guinea-pigs suffered severe weight losses and high mortality when transferred to skim milk diets, even when gradual transfer from one diet to the other was employed.

If the guinea-pigs die within two weeks, and possibly even within a month, and control animals on a regime comparable to Diet C, do not die, it must be presumed that they are dying of acute starvation, in that they are not consuming Diet B or its equivalent. If guinea-pigs are properly trained to take the skim milk diet, there has been in the past, and is currently, no problem at Oregon State College of a high early mortality. The reasons for high early mortality in other laboratories are not clear. An effort is made to keep the temperatures at 72° F during the winter months. The guinea-pigs are housed in wooden pens, 4' x 8' (more than six guinea-pigs), 4' x 4' (3 to 8 guinea pigs), or 4' x 2' (1 to 4 guinea-pigs). The pens are one foot deep and have their floors protected by tarred wrapping paper covered with straw. Currently the straw is not autoclaved. The guinea-pigs are not considered adequately trained for the milk diet until each guinea-pig of a group has been observed at least twice drinking from the milk mixture. If one guinea-pig of a group drinks from the mixture, the others seem to learn rapidly, possibly by imitation. The difference in mortality on the skim milk diet in different laboratories may lie in group maintenance in the Oregon laboratories and single cage maintenance in other laboratories. Further observations on this point are required.

Wrist Stiffness

Laboratories other than Oregon State College have not succeeded in producing wrist stiffness in guinea-pigs on a skim milk diet. Presumably the failure lies in a high early mortality and death of the guinea-pigs before wrist stiffness appears.

In the Oregon laboratories guinea-pigs on Diet A eventually could not move about the pen at all but lay on their sides with their legs extended. After the animals were prostrate, death from inanition supervened.

Some animals were kept alive by pipette feeding. These gradually stiffened until both fore and hind limbs were thrust caudally. The toes showed a tendency to flex, the legs were very strongly adducted, and the posterior limbs could not spread apart without tearing the adductor muscles. The occiput was pressed against the shoulder blades. The whole body was so stiff as to be completely immobilized. Even these very stiff animals were able to eat well from pipettes and to swallow (4).

TABLE 4. *Diet D (Oleson et al., 14)*

Ground wheat.....	20 lbs.
Ground whole yellow corn.....	15 lbs.
Feeding oat meal.....	20 lbs.
Wheat bran.....	5 lbs.
Soybean oil meal (41% expeller).....	20 lbs.
Linseed oil meal.....	5 lbs.
Fish meal (60% vacuum).....	2 lbs.
Alfalfa meal (dehydrated 20%).....	10 lbs.
Bone meal (steamed).....	1 lbs.
Limestone (feeding).....	1 lbs.
NaCl.....	0.25 lbs.
Delsterol (900,000 I.U. vitamin D).....	0.125 lbs.
Ascorbic acid.....	12 g.

In 1947 Oleson, van Donk, Bernstein, Dorfman and SubbaRow partially confirmed the findings of Wulzen, Bahrs, van Wagendonk and their coworkers. Their guinea-pigs, on a specially devised solid diet, developed the wrist stiffness syndrome. Diet D is one of the DASF diets used by Oleson and his co-workers (14, table 4).

In 1948 further confirmation appeared from Petering, Stubberfield and Delor (12). Unable to maintain animals successfully on the skim milk diet of the Oregon workers, they devised another diet to produce the desired deficiency condition (casein, corn starch, brewer's yeast, salts, cottonseed oil with fat-soluble vitamins, ascorbic acid). They succeeded in producing stiffness with this diet. They found no tissue calcification, even though the guinea-pigs became very stiff.

Smith, Williams, Bauer, and Maynard (13) at Cornell University were able to produce both the wrist stiffness and calcification syndromes in guinea-pigs on various diets. They felt the manifestations of wrist stiffness and abnormal calcium salt deposition were two separate syndromes which could occur independently of each other and for different reasons. The different syndromes from diets A and B of Wulzen and Bahrs (1, 4) also indicate the possible separation of stiffness from the calcium deposits. Yet current evidence is insufficient to rule out the possibility that diets, other than B, provide for a more slowly developing calcium problem.

A very interesting contribution was that of Hogan, Regan and House (15). They reported the deposition of calcium phosphate on basal diets (E and F) having a content of phosphorus similar to that of the skim milk diets of Wulzen and van Wagendonk (table 5). They found that wrist stiffness can occur on diets which are either high or low in phosphorus, but that calcium phosphate deposition occurs readily only on a high phosphorus diet. The skim milk diet of Wulzen and van Wagendonk had 1.0 per cent P, and would be somewhat comparable to the 0.9 per cent high phosphorus diet of Hogan and Regan.

Using diets E and F, Hogan, Regan and House found no difficulty in confirming essential details of the Wulzen calcium dystrophy syndrome. According to their observations the disease may roughly be divided into three stages: 1)

TABLE 5. Diets E and F (Hogan, Regan and House, 15)

Ration No.	711 (E)	713 (F)
Casein	20%	20%
Sucrose	36%	35%
Cellu flour	15%	15%
Salts (mixture 5199)	4%	
Salts (mixture 5)		5%
Lard	10%	10%
Dried brewers' yeast	15%	15%
Final Ca content	0.8%	0.8%
Final Phosphorus content	0.5%	0.9%
Added fat-soluble vitamins per 100 gm. of lard		
Vitamin A	2,000 I.U.	2,000 I.U.
Vitamin D	283 I.U.	283 I.U.
Vitamin E	2.5 mg.	2.5 mg.
Vitamin K ₃	2.5 mg.	2.5 mg.

Pain in the wrist when the joint is manipulated; 2) the wrist, elbow, or both become stiff; 3) visible, or palpable, deposits of calcium phosphate. The three stages indicated varying degrees of severity of the same disease (15). While the data of Hogan and Regan suggest a problem involving only Ca and/or P dietary intake, it should be pointed out that Diets E and F lack greens, and hence would be lacking in the postulated anti-stiffness factor.

Variability of the Stiffness Syndrome

The development of the Wulzen syndrome is quite variable from guinea-pig to guinea-pig. Some do not survive a month on the diet; others will survive two years. Males are more susceptible than females. Pregnancy among the females delays the development of the syndrome. The surviving older deficient animals fall into two classes: 1) A small group of older, healthy, robust animals that seem to be highly resistant to deficiency of the anti-stiffness factor. They usually have stiff wrists without soreness. 2) A large group of older animals with varying degrees of abnormalities in growth, stiffness, body contour, calcification, and hearing (16). Some guinea-pigs survived for as long as 9 to 10 months without clinical effect other than failure to show optimal growth, whereas others became quite emaciated and showed severe injury within a few months. Some of the lesions to be described in the deficient animals were found also in the controls but their severity and incidence were much less (17).

Prevention and Cure of Wrist Stiffness

The 1941 publication of Wulzen and Bahrs (1) stated animals fed whole raw milk grew excellently and at autopsy showed no abnormality of any kind. Those on pasturized milk rations did not grow as well and developed wrist stiffness. On pasturized skim milk rations the guinea-pigs did not gain well, developed

wrist stiffness and showed calcification with emaciation and weakness before death. None of these symptoms developed when *ad libitum* supplements of kale or alfalfa were given in addition to the skim milk diet. If the disease had not progressed too far, administration of these greens restored the animals to a normal condition (8). Guinea-pigs very rarely developed wrist stiffness when raised on raw milk (5).

Stiffness alone has been cured many times by various substances such as raw cream, molasses, other sugar-cane products, and green vegetables. Even maximum stiffness has been cured by sufficiently prolonged treatment with molasses or raw cream (8).

Raw cream sterol. Since guinea-pigs fed *raw whole milk* grew well and showed no abnormalities, while guinea-pigs fed *heated whole milk* did not grow well and developed wrist stiffness, a heat labile sterol was sought and finally isolated from raw cream (5) by van Wagtendonk and later from sugar cane juice (18) which when administered in cottonseed oil was able to remove the symptom of wrist stiffness. Effective doses of the extract in presumably different states of purity were 0.1 $\mu\text{g.}$ and 0.002 $\mu\text{g.}$ daily for five days (5, 18). Active fractions were isolated having melting points near 82° and 164°C (19).

A sample of crystalline anti-stiffness factor, supplied by Caldwell, was tested by Oleson and his co-workers (14) at the Lederle Laboratories, and found active in doses of 1 to 5 $\mu\text{g.}$ daily when administered in cottonseed oil. Lower doses were not tested. Cottonseed oil was found to be inactive.

Ergostanol and ergostanyl acetate. Of 59 steroids tested, Oleson and his group (14) found ergostanol was active at the level of 5 $\mu\text{g.}$ daily and ergostanyl acetate at the level of 1 $\mu\text{g.}$ daily. Ergostanyl acetate was also effective in the hands of Petering, Stubberfield and Delor (12) for relieving wrist stiffness of the guinea-pig. However, levels of 100 $\mu\text{g.}$ were required. Smith and his group found no evidence of curative properties of ergostanyl esters. Likewise, Dasler (11) found that normal animals receiving daily doses as high as 5000 micrograms of ergostanyl acetate became stiff. They could find no curative properties in sugar cane juice nor in ergostanyl acetate. It should be emphasized that Wulzen, Oleson, Petering, Dasler and Smith were all using different diets.

Stigmasterol. In 1951 Kaiser and Wulzen (21) reported that guinea-pigs on the deficient diet for 8 months entirely recovered from stiffness on 25 $\mu\text{g.}$ daily of stigmasterol for 9 days. Control animals were treated with the solvent (Wesson oil and 6 per cent ethyl butyrate) for 2 weeks without showing any improvement in stiffness.

MUSCULAR DYSTROPHY

The skim milk diet produced muscular stiffness in all parts of the body, but for test purposes the forelegs proved most useful. Stock animals to be maintained in the limber condition, must receive abundant green feed. Difficulty in securing suitable green feed at certain seasons caused 20 per cent of the stock animals to show some degree of abnormal stiffness. In contrast, 95 per cent of animals fed 14 months on deficient diet showed abnormal stiffness (8).

Dasler (11) in an examination of the data of Oleson and his group (14) noted that 13 per cent of the negative controls showed marked and 17 per cent some improvement in wrist-stiffness, or spontaneous improvement in 30 per cent of the untreated animals. A possible explanation will be given later.

The earliest deformation in body contour is a flaring of the rib cartilages so that a rounded elevation can be plainly felt on each side of the thorax (8). After 14 months, 93 per cent of the deficient animals showed definite flaring of the rib cartilages. None of the stock animals showed this deformity.

Another early sign was the lowering of muscle tone, so that the animals remained flaccid when handled. The righting time was considerably prolonged and finally they could no longer right themselves when placed on their backs. They developed difficulty in moving about the pen, because the hind limbs could not be used properly.

As the deficiency advances, the neck assumes a sharp curve. The abnormal position of the neck affects the head, which is tilted upward with elevation of the chin and the occiput is drawn toward the shoulder blades. After 14 months on the deficient diet, 87 per cent of deficient animals showed abnormal curvature of the neck. In contrast, only 7 per cent of stock animals showed even a slight degree of this abnormality.

Another development in the advancing deficiency is a dorsal curvature of the spinal column. This results in a humped back, which can easily be seen in advanced cases and can be felt by running the finger along the back. At 14 months 65 per cent of the deficient animals were thus deformed against none of the stock animals.

A stiffening of the jaw muscles, possibly accompanied by similar changes in swallowing muscles, the malformation of the neck and a deformation of the teeth rendered the taking of food extremely difficult, even with pipette feeding, and in the end impossible. Final starvation appears to be a common cause of death among the deficient animals (8).

Histopathology

Harris and Wulzen (17) in 1951 made an intense study of the histopathology in the Wulzen syndrome. As a matter of convenience and uniformity they made sections routinely of only the triceps brachialis and quadriceps femoris, and only occasionally of other muscles. They were consequently unable to state whether certain muscles had a greater susceptibility to injury than others.

Necrosis of the triceps brachialis and quadriceps femoris muscle varied from damage of scattered fibers to involvement of the majority of fibers. Of 35 animals on the diet more than 300 days, the muscles of 9 were normal; for 8 animals only scattered fibers were necrotic; and moderate to extensive necrosis was present in only 8 of the 35 animals.

The slightly injured muscles appeared grossly normal, but more severe injury was manifested by slight pallor and opaque white grayish yellow, longitudinal streaks of various lengths and breadths. Some of the streaks were coarse and beaded. Such streaks were seen best and sometimes exclusively in the muscles

of the chest wall, notably the serratus anterior and pectoral muscles and the muscles attached to the scapula. In some guinea-pigs, they were clearly evident in the abdominal and leg muscles also.

Microscopically, there was necrosis with hyalinization, and sometimes with regeneration of the muscle fibers. In some instances injury was manifested by a slight to great increase in the number of nuclei in some fibers, with necrosis of only an occasional fiber. More commonly, however, the changes were characterized by fragmented muscle fibers with swollen, hyaline, eosinophilic, curdy cytoplasm, and loss of nuclei. Some fibers had lost their nuclei, but the transverse striations were maintained or even accentuated. Others showed loss of longitudinal striation. Some fibers were invaded by macrophages, sometimes aggregated to form foreign body giant cells. Frequently, although there was much necrosis, few or no macrophages were present. Sometimes calcification of the necrotic cytoplasm was accompanied by macrophagic infiltration with or without giant cells. In two animals the entire biceps brachii was calcified.

The reason for muscle necrosis was not apparent. Harris and Wulzen doubted that it was due to vascular changes, for no blood vessel encountered in sections designed to show muscle had its lumen diminished in any manner, although calcification of the walls of small arteries was seen very rarely (17).

Creatine

Based on the urinary creatinine ratio, van Wagtendonk felt that a deficiency of the anti-stiffness factor produced the characteristic wrist stiffness but had no significant effect upon the creatine excretion (22, 8). The skim milk diet was not used in these experiments, being replaced by a diet devised by Mackenzie in 1941. While it is possible that the difference may not be statistically significant, the range of creatine:creatinine ratios recorded for stock guinea-pigs was 0.17 to 0.61, while the ratios recorded for guinea-pigs on the deficient diet supplemented by alpha-tocopherol on the 79th, 86th, 91st and 97th days were 0.84, 1.03, 1.09 and 0.69.

In 1938 Reinhold and Kingsley (23) reported a decrease in the concentration of phosphocreatine and adenosine triphosphate in dystrophic muscles of guinea-pigs deficient in vitamin E. The low level of creatine in the muscle in vitamin E deficiency was recognized by Goettsch and Brown (24). Marked changes in the distribution of the acid-soluble phosphate in the muscle in guinea-pigs on the skim milk diet B were found by van Wagtendonk and Lamfrom (25). The concentration of the inorganic phosphate increased as compared with that found in animals on stock diet D. Some fluctuation in muscle creatine phosphate and adenosine triphosphate occurred in the early period of the deficiency. In the more advanced stages a significantly lower level of these phosphates developed.

Vitamin E Deficiency Not Identical with Wulzen Syndrome

According to Mackenzie (26), the most ubiquitous response to vitamin E deficiency is the development of muscle lesions. Creatinuria in E-deficient rabbits developed prior to loss of weight, loss of appetite and the appearance of physical

symptoms of muscle damage. Vitamin E-deficient muscles showed a reduction in creatine, an increase in chloride and widely scattered lesions.

The most striking microscopic change in the muscles of vitamin E-deficient rabbits was a coagulative or hyaline necrosis of the protoplasm, resembling the lesions of typhoid fever described by Zenker. Accumulations of dark staining nuclei were found in clefts in the hyaline masses. Nuclear proliferation was often present. Phagocytes appeared to remove the necrotic material. Later great masses of cellular elements dominate the picture. Calcification of the necrotic tissue was a variable and secondary phenomenon.

From the examination of sections prepared by the Cornell group (Hanlon, Foot, Travell and Rinzler), from *guinea-pigs* fed the diet described by Oleson and co-workers, Mackenzie felt that the lesions were indistinguishable from those encountered in E-deficient *rabbits*. In the experiments of the Cornell group, the muscle damage was not prevented by wheat germ oil, nor was it intensified by cod liver oil. The muscle damage was greatly reduced by ergostanyl acetate.

Mackenzie was of the opinion that the muscular dystrophy syndrome in guinea-pigs was a different deficiency disease from the E-deficient dystrophy of rabbits. He also suggested that wrist stiffness may not be a separate disorder, but rather is a gross manifestation of muscle degeneration. Mackenzie further felt that a decline in the creatine phosphate of muscle, but no creatinuria in the anti-stiffness deficient guinea-pigs, was seemingly contradictory and required clarification (26). There is the possibility, as indicated above, that guinea-pigs on the skim milk diet show a slight creatinuria. Muscular dystrophy develops rapidly in vitamin E-deficient rabbits and slowly in guinea-pigs on the skim milk diet. This may account for the absence of a high degree of creatinuria in guinea-pigs on a skim milk diet.

Harris and Wulzen also concluded that, although the muscular changes resembled those described for E deficiency, in view of the character and distribution of the other lesions observed, the etiology of the muscle injury resulting from administration of the skim milk diet was different. Vitamin E deficiency had not been found to produce vascular lesions like those described for the guinea-pig (17).

Local Reaction to Alpha-tocopherol in Guinea-pigs on Skim Milk Diet

Wulzen and Krueger (27) in order to investigate the relationship of alpha-tocopherol to the anti-stiffness factor, injected guinea-pigs, on Stock Diet C and Skim Milk Diet B, weekly intramuscularly for 3 months with 0.1 ml. of sesame oil containing 20 mg. alpha-tocopherol, or with 0.1 ml. of sesame oil alone. In all cases sesame oil alone left no palpable or visible trace except an occasional transitory slight swelling and redness. However, alpha-tocopherol produced pronounced swelling and hardening of the tissues in the vicinity of the injection site. Often hard and rough concretions, extending from the knee to the pelvis and from the elbow to the shoulder, were formed. These were opaque to X-rays and probably represented intense, locally induced calcified areas. The

more extensive and harder deposits were found among the animals on the milk diet.

Testosterone

About one-fourth of the guinea-pigs showed degrees of testicular atrophy ranging from cessation of spermatogenesis in some tubules to complete loss of spermatogonia from all tubules (17). This suggests the possibility that the loss of testosterone in male guinea-pigs may be a factor in the muscular dystrophy.

Testicular degeneration is the most prominent sign of E deficiency in the young male rat, but testicular damage was not encountered in Mackenzie's E-deficient rabbits. An important advance has been the discovery that alpha-tocopherylhydroquinone is a potent anti-dystrophic factor but not an anti-sterility factor in rats. The possible role of alpha-tocopherylhydroquinone in the Wulzen guinea-pig syndrome should be studied (26).

Oxygen Consumption

Muscular dystrophy is generally accompanied by increased *in vitro* oxygen consumption in the muscle. Per mg. of initial dry weight per hour, the oxygen consumption of excised muscle was 2.8 cu.mm. on diet C containing greens and 4.1 cu.mm. on the skim milk diet B. However, carbon dioxide production under anaerobic conditions decreased from 3.0 to 1.7 cu.mm. per mg. of dry weight per hour (8).

CARDIO-VASCULAR SYSTEM AND BLOOD

Autopsies showed the striated muscles to be finely streaked with calcium deposits running parallel to the muscle fibers (1). The tissues of guinea pigs on the skim milk diet are more opaque to X-rays than are those of normal guinea pigs. This leads to a much brighter roentgenogram from the deficient animals. Especially to be noted in the X-rays of deficient guinea-pigs are the fine lines in the abdominal area, indicating calcification of abdominal blood vessels (28). Calcification of the anterior wall of the stomach ranges in different deficient animals from small flecks to an incrustation of over two-thirds of the anterior surface of the stomach. Close inspection indicates that the calcification follows a vascular pattern (fig. 1).

Aorta

Frequently the aorta is found to be rough and calcified in the deficient guinea-pigs (17, 29). Lesions in the aorta or femoral arteries, or both, were found in about one-fifth of the animals dying before 150 days, in one-third of those dying after 150 days, and in nearly one-half of those dying after 300 or more days. Aortic injury was manifested grossly by the presence of small nodules in the intima distributed annularly. Microscopically, calcification of the media was sometimes found in association with foci of replacement of the elastica with collagenous tissue, calcification evidently having occurred in the necrotic tissue with replacement by rather cellular fibrous connective tissue without calcification. The underlying adventitia was usually normal, but occasionally showed

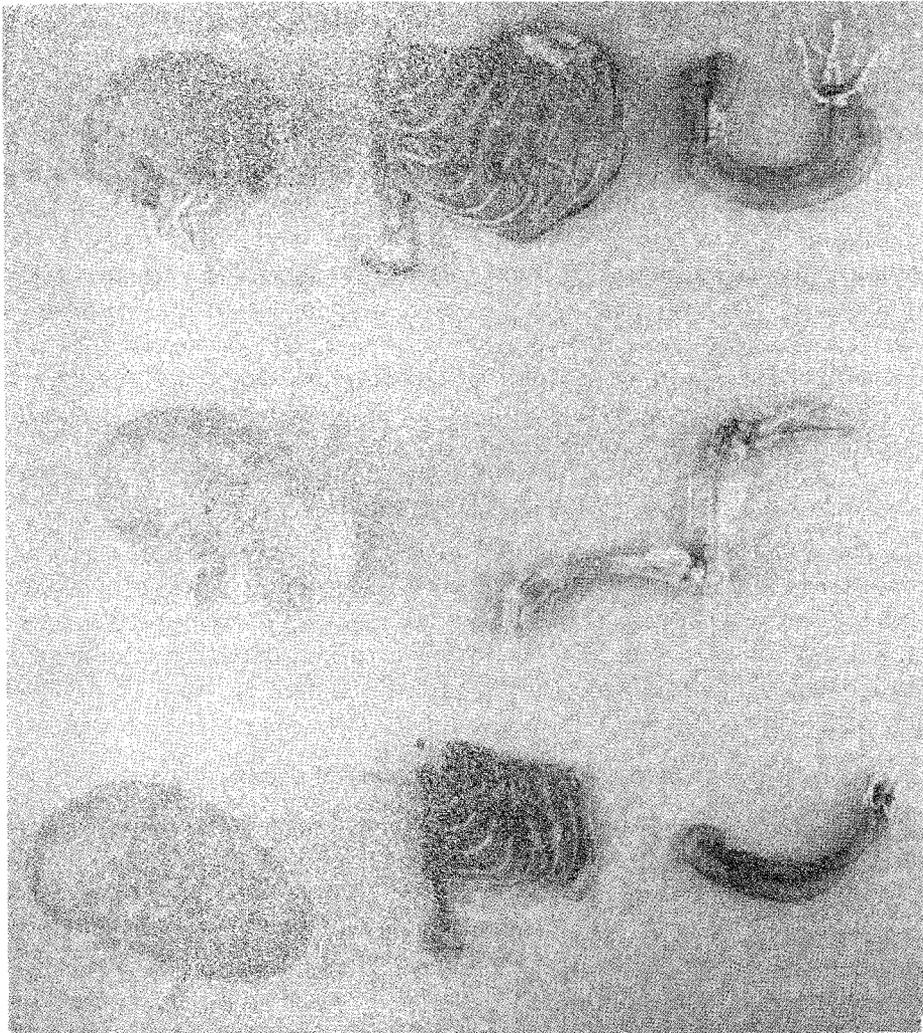


FIG. 1. Top line: Stomach, chest wall and cecal loop from guinea-pigs on the skim milk diet. Note calcification over fundus of stomach and just beyond flexure of colon. Also note white striae running parallel to fibers of the intercostal muscles. Middle line: left, stomach from guinea-pig on skim milk diet. Note vascular pattern of calcification. Right, leg from guinea-pig on skim milk diet. Note calcified lumps around knee and shoulder. Bottom line: Stomach, chest wall and colon loop from guinea-pig on a diet containing kale. Note absence of calcification.

some lymphocytic infiltration. The intimal nodules of rather dense collagenous tissue were invariably associated with scarring or calcification of the media, but medial calcification was not always associated with intimal reaction. In paraffin sections of the aorta there was no evidence that the intimal nodules contained cholesterol. Lesions began as medial necrosis with calcification, and subsequently collagenous tissue developed in the larger lesions. With the consequent weakening of the vessel wall there was formation of collagenous tissue in the intima.

TABLE 6. *Effects of skim milk diet on erythrocytes of guinea-pigs (51)*

	Green Diet	Skim Milk Diet
Red cell count per cu. mm.....	4,900,000	4,200,000
Hemoglobin.....	15.4 g.	10.9 g.
Hemoglobin coefficient.....	15.6 g.	13.3 g.
Red cell diameter: microns.....	7.35	7.70
Basophilic aggregations.....	2.06%	0.68%
Comparative sedimentation.....	2.35 mm.	3.02 mm.

The affected femoral arteries had a finely beaded appearance and microscopic examination revealed fragmentation, reduplication, and often, calcification of the internal elastic lamella. There was asymmetric intimal thickening, sometimes with focal calcification, and frequently, medial calcification (17).

Arterial Occlusion Pressure

Arterial occlusion pressure for stock animals averaged 30 mm. Hg. Animals maintained on the skim milk diet for eight months to two years had a mean arterial occlusion pressure of 42 mm. Hg. Therefore, the fully developed deficiency results in rise of blood pressure (Bahrs and Wulzen, 30).

Erythrocytes

The skim milk diet produced a mild to severe anemia (see table 6). The red cell count was reduced and 25 per cent of the deficient animals had counts between 3,500,000 and 2,200,000. Hemoglobin and hemoglobin per cell also decreased for the deficient animals. Red cell diameters were increased and sedimentation was more rapid. Since Pearlman and Limarzi found a correlation between basophilic aggregation and reticulocyte levels in several diseases, the decrease in basophilic aggregations indicated decrease of young erythrocytes in the circulating blood.

Leukocytes

Deficient guinea-pigs showed a leukocyte count of 16,700 as compared with 7,700 for non-deficient guinea-pigs. Males had abnormally high percentages of neutrophils and eosinophils, a decreased percentage of lymphocytes and a complete disappearance of Kurloff cells. Stiff male and female guinea-pigs showed a persistent eosinophilia (31).

TISSUE CALCIFICATION

The deficiency syndrome is characterized by palpable, X-ray-opaque deposits scattered widely throughout the body (29). Analyses by Hogan, Reagan and House, of the deposits of guinea-pigs maintained on Diet F, indicate that the deposits are calcium phosphate (15). The area of calcium deposition varies from animal to animal, but the regions of the foot pads, knees, scapulae and retro-occipital areas are frequently involved (29).

Foot Pads

The foot pads of DASF guinea-pigs usually become swollen and red and the position of the animal and the type of muscular movements are indicative of pain and an attempt to protect the pads from external stimulation. Roentgenograms indicate diffuse calcium deposition around and between the digits (32).

Abscesses often developed in the foot pads and were most numerous in the thinnest animals. In the earlier abscesses the pus had a mucoid appearance, but in the older ones it was thick. Ulceration over the abscesses was noted sometimes, but destruction of bones of the feet was never encountered. Abscesses were found also about the knees, elbows and shoulders, intramuscularly at the tip of the scapula, in the intercostal muscles and over the ribs laterally and dorsally, and deep within the muscles of the legs adjacent to the periosteum of the femur or humerus. Abscesses at first contained an opalescent, glary, white fluid that subsequently became inspissated and opaque, and ultimately underwent calcification. Foreign body giant cells often were seen at the periphery of the calcified abscesses. The abscesses about the elbows, shoulders, and knees were associated with proliferation of collagenous tissue about the joints, and periosteal new bone formation of the long bones near the affected joints. Usually, the joints were not invaded, but when they were, there was only slight, if any, erosion of the articular cartilage (17).

Stomach

Calcification was most common in the cardia and fundus near the greater curvature, and usually consisted of multiple, discrete, deposits that rarely caused much thickening of the wall (17). Calcification of the anterior wall of the stomach ranged in different deficient animals from small flecks to an incrustation of over two-thirds of the anterior surface of the stomach. Close inspection indicates that the calcification follows a vascular pattern (32, fig. 1).

Microscopically, the deposits were seen to be of irregular size and shape. Calcification of individual muscle cells was seen, but, in general, the masses appeared to have been deposited between the muscle cells, and frequently small groups of muscle cells extended about and between the deposits. Associated with the deposits was a variable amount of collagenous tissue. Slight calcification in the muscularis mucosae was sometimes present, but there was no calcification of the mucosa or submucosa (17).

Colon

Calcified areas are usually found in the deficient animals on the distal limb one cm. beyond the point of retroflexion of the colon. (About 10 cm. from the cecum the guinea-pig's colon bends back on itself with the distal limb of the colon on the lesser curvature of the flexure.)

Cartilage and Bone

In guinea-pigs on the skim milk diet many of the bones are altered by thickening, thinning, or the development of exostoses. Very often the thickenings or the exostoses involve an alteration of the contour of foramina. Thus, frequently the

shape of the foramen ovale of the sphenoid is altered and its area reduced. This suggests the possibility of nerve compression and subsequent interference with sensory and motor nerve function.

Rib, ear, and tracheal cartilages became calcified and opaque to X-rays. In the case of the rib cartilages the calcification makes the chest wall more rigid and a tendency for the ventral margins of the chest to flare outward develops (28, 29).

The severe anemia (table 6) often encountered in the deficient guinea-pigs is further evidence of a deep-seated bone pathology which must modify in many respects the muscular dystrophy. Histopathological studies of bone in the Wulzen syndrome are much to be desired.

Calcium and Phosphorus in Blood and Tissues

Average figures for blood calcium in the guinea-pigs on Diet C containing greens ranged between 9.5 and 10.8 mg. per 100 cc. In the guinea-pigs on the skim milk diet, blood calcium ranged from 10.2 to 12.4 mg. per 100 cc.

The average calcium content of the rectus femoris muscle in guinea-pigs on the green diet lay between 9.0 and 9.7 mg. per 100 gm. In guinea-pigs on the skim milk diet for two weeks, the rectus femoris yielded the unusually high calcium average 28.1 mg. per 100 gm. For guinea-pigs on the skim milk diet for 3 to 37 weeks average rectus femoris calcium values ranged from 16.7 to 19.9 mg. per 100 gm.

In guinea-pigs on the green diet inorganic phosphorus averages ranged from 3.0 to 3.3 mg. per 100 cc.; on the skim milk diet from 4.3 to 6.1 mg. per 100 cc. Inorganic phosphorus in the rectus femoris lay between 37 and 54 mg. per cent on the green diet; between 49 and 80 mg. per cent on the skim milk diet (7, 8).

Thus, inorganic phosphorus and calcium increased in blood and muscle of guinea-pigs on the skim milk diet as compared with guinea-pigs on a diet containing greens. The concentration of the inorganic phosphate in the liver and kidneys was also increased far above normal during the deficiency.

The normal distribution of the acid soluble phosphorus in the liver and kidneys was disturbed on the skim milk diet in the direction of a higher concentration of the inorganic phosphate. This simultaneous rise in the concentration of calcium and inorganic phosphate is favorable for the formation of colloidal calcium phosphate.

In guinea-pigs on the skim milk diet for a year or longer, a mean value of 97 mg. of calcium per 100 gm. dry lung tissue was found as compared to 46 mg. of calcium per 100 gm. in animals on Diet C containing greens.

TEETH

In guinea-pigs on a skim milk diet the teeth undergo many important modifications. Excessive excrescences are noted on the lateral surfaces of the mandible and maxilla in the vicinity of the molar alveoli. On the maxilla the excrescences encroach upon the orbital area. The upper molars extend laterally more than normally and are beveled at an angle of 135° , rather than 90° , to their axes.

The upper incisors have a blunt bevel instead of the normal double bevel yielding an angle into which the lower incisors fit. On the mandible the molars of the deficient guinea pigs are not beveled perpendicularly to their axes, and they extend further toward the midline than in normal guinea-pigs. Thus, the grinding surface has been increased but is not backed by an equivalent dental support. The lower incisors are longer and less curved in deficient guinea-pigs. In the later stages they overlap the upper incisors anteriorly.

The average length of lower incisors in stock animals 10 to 17 months old was 12.1 mm. for males and 10.4 for females. The length in guinea-pigs on the skim milk diet of same age span was 13.0 mm. for males and 12.0 for females. In some 10 to 17 month guinea-pigs of both sexes and in many older animals on the skim milk diet, a rapid increase in incisor length developed, and yielded lower incisors reaching 17 to 20 mm., whereas no stock male had lower incisors exceeding 15.2 mm. and female 11.7 mm. The average weekly rate of lower incisor growth was 2.2 mm. for stock guinea-pigs. The average weekly rate of growth for males on the skim milk diet was 1.9 mm. and for females 1.8 mm. Thus, increased length of lower incisors is due, not to increase in rate of growth, but to some other cause, possibly a decrease in grinding ability, due to dystrophy of the chewing muscles (33).

HEARING

Wulzen and Plympton reported that guinea-pigs fed the skim milk diet ultimately cease to respond with the ear-flick to auditory stimuli (34). Pathologies of the auditory complex were noted in a large number of guinea-pigs even on diets containing greens, but pathologies were more obvious and intense in the skim milk diet. In these guinea-pigs, the skulls, as obtained from NaOH digestion of the heads, had a chalky appearance and much of the detailed structure had disappeared from roentgenograms. Sometimes the skull bones were distorted by discrete external depositions and sometimes by thickening and enlargement of parts of the original bones. Sometimes the bones, particularly the cochlea, were thin and transparent.

Among the pathologies of the auditory complex noted in guinea-pigs on the skim milk diet were reduction and irregularity of the depression for the paraflocculus, reduction of the size of the internal acoustic meatus (exit of VIII) and of the facial canal, thickening of the wall of the bulla and of the cochlea, extensive obliteration of the tympanic cavity, and elongation of the external acoustic meatus by a second, or even a third, white hard incomplete annulus around the meatus. The auditory ossicles were often deformed and sometimes embedded in a hard, white amorphous papillated mass having the appearance of sugar candy concretions. In some of the bullae all possibility of movement of the ossicles had disappeared with their incorporation into a hard, brown concretion, best described as an obliterating petrification of the tympanic cavity.

Many of the pathologies noted would lead to a depression of auditory reflexes. Possible factors contributing to the loss of the auditory reflexes are reduction of the tympanic cavity, loss of mobility by the auditory ossicles, and a thickening

(sometime thinning) of the cochlea. Blood circulation difficulties and compression of the auditory nerve may also play a role (35).

KIDNEY FUNCTION

Harris and Wulzen (17) noted that the kidneys usually were pale, granular, somewhat enlarged, and heavier than normal, both absolutely and in proportion to body weight. The granularity resulted from cortical scarring and hypertrophy of tubules between the scars. Atrophic convoluted tubules were embedded in a small amount of collagenous tissue with a variable amount of lymphocytic infiltration and glomeruli were in the early stages of fibrosis. The glomeruli in these scars always seemed more nearly normal than the tubules. In badly scarred kidneys the intervening tubules were hypertrophic and dilated. Calcified masses of irregular size and shape were seen in convoluted and collecting tubules, and at times foci of calcification of the interstitial tissue were found in both cortex and medulla. Some of the masses obviously obstructed the tubules in which they lay. Calcification of the wall of the arteries was seen in no more than 1 per cent of the kidneys examined and in these the lumina were not reduced.

Water Excretion

Male guinea-pigs on a stock diet of barley and greens put out very little urine when placed in a metabolism cage with water *ad libitum* but with no food for 8 hours. The volume for the 8-hour period ranged from 0.0 to 12.5 ml. The average delay until the first bladder emptying was 5.3 hrs. The urine output for the 8-hour period increased from an average of 2.8 ml. in males approximately 3 months old to an average of 6.7 ml. for 15 months. Male guinea-pigs on skim milk mixture B put out 6.7 to 23.1 ml. with an average of 11.8 ml. The average time to the first sample was 2.8 hours, and the volume per bladder emptying, 8.4 ml. There were no clear relationships to time on the deficiency diet. The high urine output of the deficient males may reflect only the high water content of the skim milk diet, but the large volumes per bladder emptying suggest an altered physiological process (36). Comparison of urine output for stock and deficient guinea-pigs over a 36-hour period is given in figure 2 (37). (The average volume of urine per bladder emptying of males on the stock diet was 4.0 ml.)

Calcium Excretion

Guinea-pigs taken from the Stock Diet C containing greens and placed in metabolism cages with no food but water *ad libitum* excrete 4 to 22 mg. of calcium in a 36-hour period. Calcium excretion is usually higher in the earlier 6-hour intervals.

Guinea pigs on Skim Milk Diet B excreted much more calcium with a range of 9 to 73 mg. during the 36 hours and an average of 35.2 mg. Calcium excretion was exceedingly high in the first 6-hour period and over 50 per cent of the calcium excreted during the 36 hours was obtained during the first 6 hours in 72 per cent of the deficient guinea-pigs (38).

The total Ca in all the blood of a guinea-pig is only 5 mg. and Ca excretion

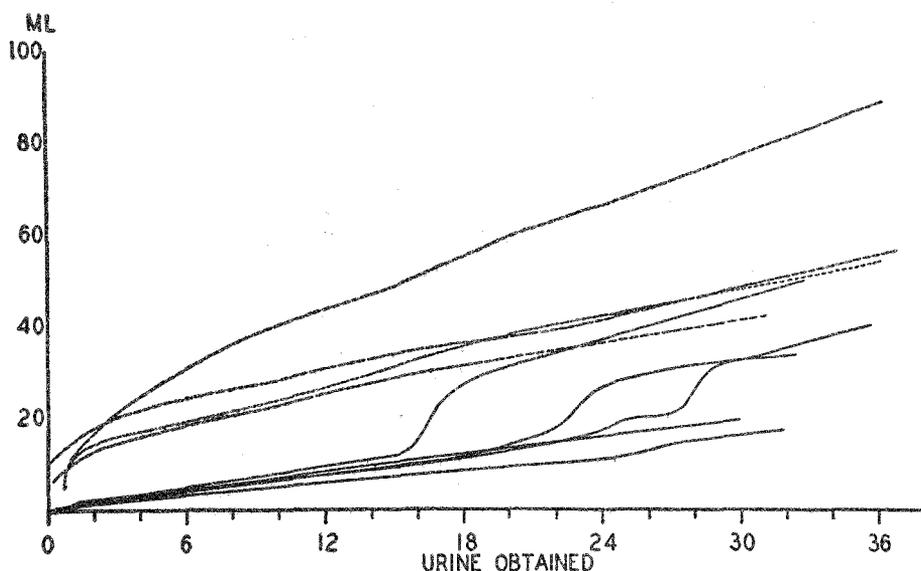


FIG. 2. Comparison of urine obtained from five guinea-pigs (solid lines) on a diet containing greens, and four guinea-pigs (broken lines) on the skim milk diet. The ordinates give the total ml. of urine obtained from a guinea-pig from the time of placement in the metabolism cage. The early urine output of the deficient guinea-pigs was high and that of the normal guinea-pigs low. From the 15th to the 30th hours the normal guinea-pigs had a period of increased output. Beyond the 30th hour rates of urine formation were similar for the normal and deficient guinea-pigs.

at the rate of 18 mg. in 6 hours means that the kidneys of the guinea-pigs on the skim milk diet were excreting daily 14 times as much Ca as was available in the blood. There is an amazing Ca turnover. Since this rate of excretion is carried on for months, there must be an unusual absorption of Ca from the gut and one of the basic problems in the Wulzen syndrome lies in establishing the cause of Ca absorption from the gut when the blood Ca is already high (see fig. 3 and 4).

Sodium Excretion

A comparison of sodium excretion in stock and deficient guinea pigs is given in figure 5. When stock guinea-pigs (solid lines) were placed in metabolism cages for 36 hours without food, little sodium was obtained in the urine until the 15th hour. Subsequently, sodium excretion was at higher rates. (Urine samples were collected immediately after each bladder emptying.)

Guinea-pigs on the Skim Milk Diet B (dotted lines) had very high initial rates of sodium excretion with smaller amounts subsequently. It should be noted that if twenty-four hour urine samples had been used, the differences in sodium excretion would not have been statistically significant (37).

Potassium Excretion

When guinea-pigs were placed singly in metabolism cages for 36 hours with water available but with no food, stock guinea-pigs excreted an average of 39 mg. of potassium in the first 6-hour collection with a range of 0.0 to 130 mg. In

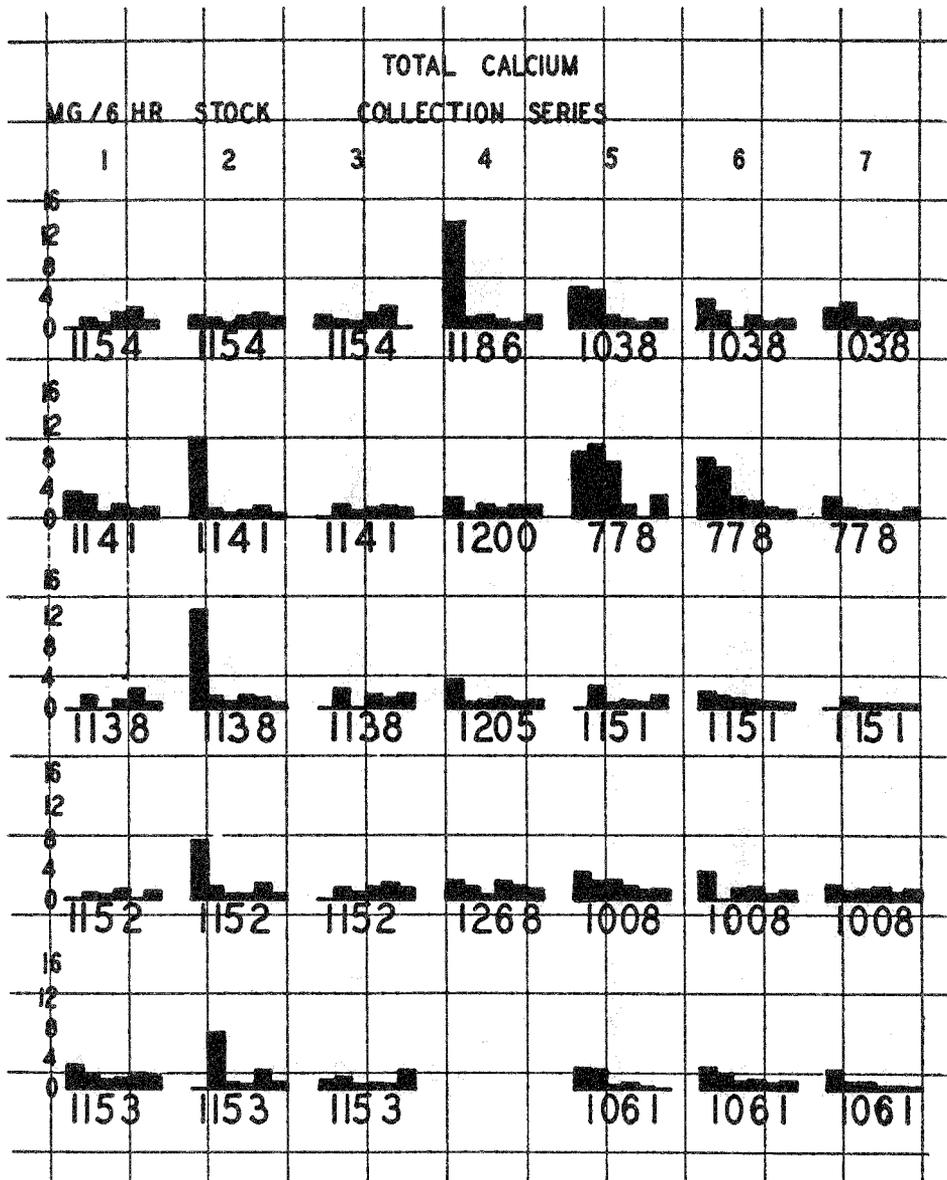


FIG. 3. Thirty-four graphs of calcium excretion during a 36-hour period are given. Each graph has time on the abscissa and mg. of calcium excreted in 6-hour periods on the ordinate. Each graph consists of six bars, each bar representing the calcium output during a 6-hour period. Under each graph is the number of the guinea-pig concerned.

The thirty-four graphs are distributed in seven columns and five rows. Each column represents data from four or five guinea-pigs on the same date. Columns one, two and three represent associated series of tests on the same guinea-pigs. Likewise, columns five, six and seven also represent associated series of tests on the same guinea-pigs. In general, the age of the guinea-pigs increases from series one to series seven.

the second 6-hour period average potassium excretion increased to 43 mg. while the extreme values were 0.0 and 91 mg. The third 6-hour period yielded only a 33 mg. average and ranged from 0.0 to 72 mg. Period four showed further decrease to an average of 31 mg. with a low of 13 mg. and a high of 63 mg. In the

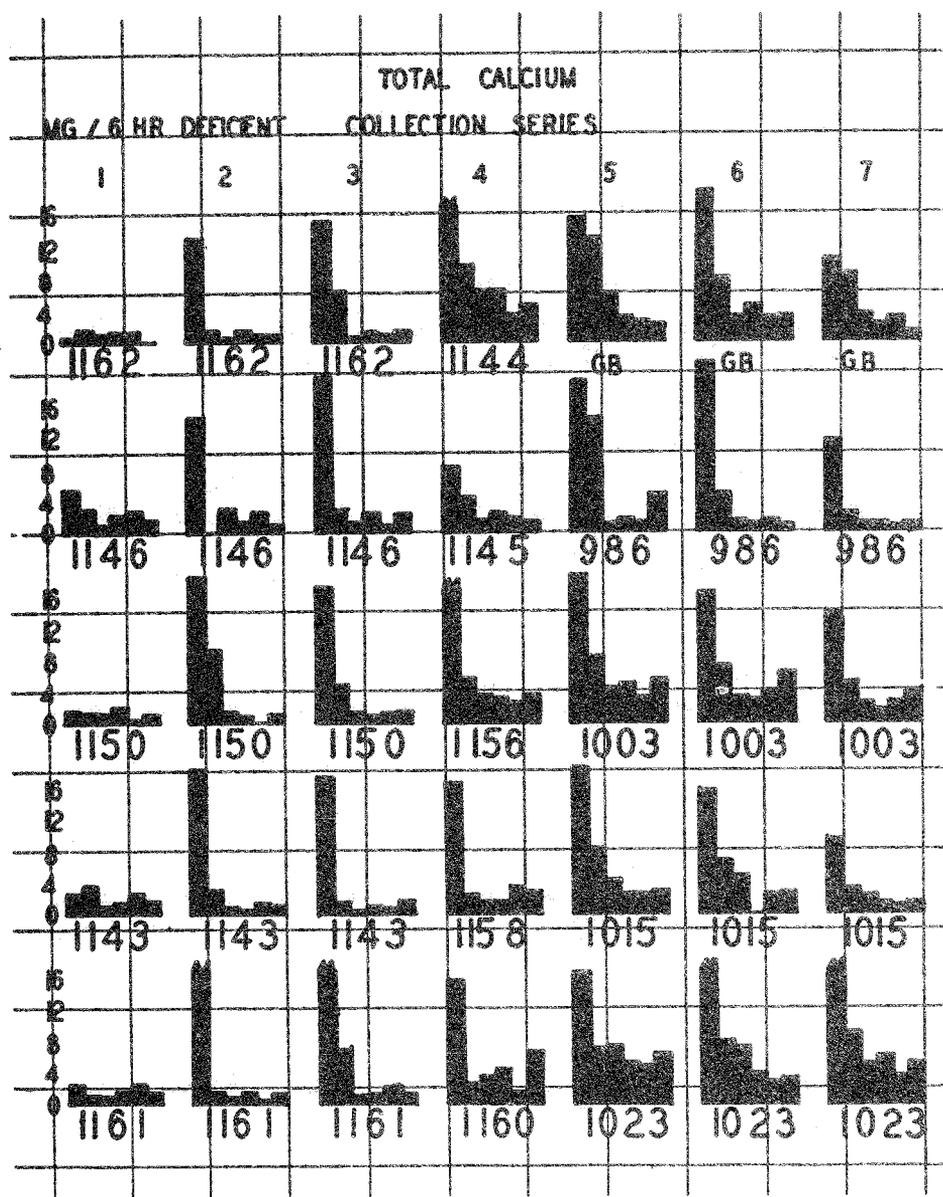


FIG. 4. Thirty-five graphs of calcium excretion during a 36-hour period are given. Each graph has time on the abscissa and mg. of calcium excreted in 6-hour periods on the ordinate. Each graph consists of six bars, each bar representing the calcium output during a 6-hour period. Under each graph is the number of the guinea-pig concerned.

The thirty-five graphs are distributed in seven columns and five rows. Each column represents data concerning five guinea-pigs on the same date. Columns one, two and three represent associated series of tests on the same guinea-pigs. Likewise, columns five, six and seven also represent associated series of tests on the same guinea-pigs. In general, the age of the guinea-pigs and time on the deficient diet increases from series one to series seven. (See text for details.) Calcium excretion was much greater in the deficient guinea-pigs than in normal guinea-pigs. From time to time normal guinea-pigs had a pattern of excretion similar to that of the deficient guinea-pigs.

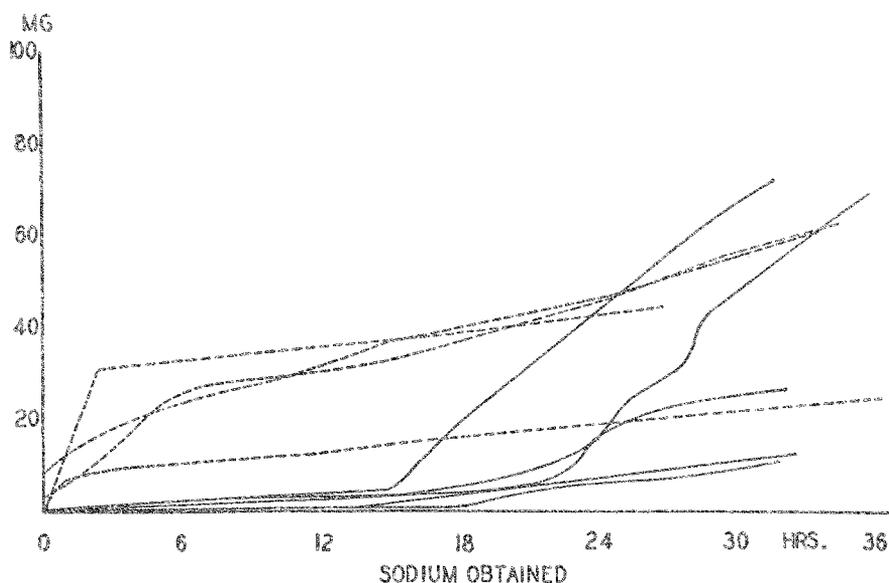


FIG. 5. Comparison of urinary sodium from five guinea-pigs on a diet containing greens (solid lines) and four guinea pigs on a skim milk diet (broken lines). The ordinates give the mg. of sodium excreted in the urine obtained from the time of placement in the metabolism cage. The abscissa gives the time in hours from the time of placement in the metabolism cage. Sodium excretion was high initially in the deficient guinea-pigs and low in the normal guinea-pigs. After the sixth hour sodium excretion was slow and similar in all the deficient guinea-pigs. Beyond the 12th hour sodium excretion in the normal guinea-pigs increased and attained rates equal to or above the corresponding rates in the deficient guinea-pigs. The data for figures 2 and 5 were derived from the same experiment.

fifth and sixth periods leveling off occurred so the averages were 21 and 20 mg. with lows of 0.0 and 0.0 mg. and highs of 56 and 50 mg.

Deficient guinea-pigs excreted potassium in a pattern that had a high peak in the first 6-hour period and a drop of almost 50 per cent in the second period followed by similar amounts in the third to sixth collection periods. The average potassium excretion for the six periods was 48, 25, 26, 27, 25 and 22 mg. The ranges were 5.2 to 81, 0.0 to 52, 13 to 67, 0.0 to 65, 0.0 to 61 and 0.0 to 39 mg. More variation was noted in the potassium excretion of the normal guinea-pigs than was found in the deficient guinea pigs (39).

Food Intake

Since the anti-stiffness factor is a dietary problem, the paucity of data on feed intake is disturbing. As far as the author is aware the only statement is that of van Wagtendonk (40) that the average daily food intake on the deficient diet was 280 gm. The guinea pigs on this regime gained as regularly in weight as the animals receiving the control diet, the average weekly gain in weight being 20 to 30 gm. for the first year. In the later stages of the deficiency a cessation of growth and loss of weight occurred (40). These data may be based on female or a combination of male and female guinea pigs. They are at odds with current data collected by Wulzen and Krueger (37) on male guinea-pigs. The weight

statement implies a minimum weight of 1200 gm. for guinea pigs at the end of a year and is at odds with a later statement (8) by van Wagendonk and Wulzen that 67 per cent of the deficient guinea-pigs were below average normal weight after 6 months and 83 per cent after 10 months on the diet.

The highest daily food intake we have recorded is 150 gm. of the skim milk diet daily. Additional data are given in table 7. These data were taken from an experiment designed to evaluate more clearly the role of the anti-stiffness factor. Wulzen and Krueger reasoned that a comparison of guinea-pigs on Diet B, fortified with sucrose, with guinea pigs on Diet B, fortified with molasses, should bring out differences due to the postulated anti-stiffness factor.

Standard tables indicated the caloric content of the skim milk mixture B to be 0.71 calories per gram, the skim milk mixture fortified with molasses 0.82 calories per gram and the skim milk mixture fortified with sucrose 0.82 calories per gram.

There were no clear cut differences in body weight or food intake on the diets fortified with carbohydrates. After six months on the skim milk diets, weight increases were a net of 60 gm. on the unfortified skim milk diet, 205 gm. on the skim milk diet fortified by sucrose and 128 gm. on the diet fortified with molasses. On the diet containing greens, the net weight increase was 305 gm. Food intakes on the three skim milk diets were similar. These data indicate that some other factor besides a calcium phosphorus imbalance is involved. The disastrous effects of the imbalance, if any, are ameliorated by the mere addition of carbohydrates to the diet. Molasses had no clear cut advantage over sucrose (37). The clinical improvement and disappearance of stiffness when guinea-pigs on the milk diet are fed 6 to 8 cc. of molasses may be partially due to the added calories. Tests should be run to see if sucrose alone, in caloric quantities equivalent to molasses, can eliminate wrist stiffness.

DISCUSSION

The cause of stiffness with abscess formation and connective tissue proliferation about the joints is obvious, but where only muscle necrosis was found possibly a functional contracture of the muscle was involved (17).

Every author who has reviewed the literature on the anti-stiffness factor is disturbed by the complexity of the contradictions. Some (8, 20, 11) have dismissed the matter with the very versatile criticism that the syndrome is brought about by high levels and imbalances of calcium and phosphorus in the diet. Yet the novice is tempted to ask, "If there is not a balance of Ca and P in milk, where or how can a ration balanced with respect to Ca and P be found?"

That the problem is much more complex is indicated by the data of table 7. The guinea-pigs on all three skim milk diets had similar mass intakes and hence similar intakes of Ca and P and in the same ratio. In spite of an identical Ca and P problem, added carbohydrate improved the condition of the guinea-pigs. The question arises as to whether some of the variability in the anti-stiffness assay may not be due to the variation in quantity of, and hence in the caloric content, of the solvents used for the steroids tested.

TABLE 7. *Body weight and food intake on fortified skim milk diets*

Days on Diet	-43	-11	10	30	80	110	140	170	Fatalities at Day 170
<i>Body weight</i>									
Skim milk diet B.....	574	768	803	776	712	818	833	828	4/8
Skim milk diet + sucrose...	661	814	876	908	917	993	992	1018	0/8
Skim milk diet + molasses...	704	844	913	980	972	971	985	972	1/8
Green diet C.....	599	793	878	941	1024	999	1080	1098	0/8
<i>Daily food intake</i>									
Skim milk diet.....			101	104	133	144	133	110	
Skim milk diet + sucrose...			96	108	125	145	126	125	
Skim milk diet + molasses..			109	122	108	147	114	147	

Growth and calcification data in guinea-pigs permanently on Diet B without and with added sterols (stigmasterol, ergostanol, cortisone, testosterone), and no added fat, should be obtained.

Dasler (11) comments that it is interesting that activated ergostanyl, which has had moderate success in the treatment of arthritis in spite of its dangers and limitations, that cortisone which has had signal success in the treatment of rheumatoid arthritis, and that the Wulzen factor, which apparently helps prevent abnormal calcification are all steroid substances. Christophle (41) of Lyon, France, in 1953 found stigmasterol having a marked relaxing (assouplissement) and analgesic action in polyarthritis and an amelioration of scleroderma.

Lansbury, Smith, Wulzen and van Wagendonk (42) point out that guinea-pigs on a skim milk diet develop a syndrome characterized by muscular degeneration and stiffness, calcinosis, and the general features of the collagen necrosis diseases (anemia, increased sedimentation rate, eosinophilia, and reversal of the albumin:globulin ratio), as well as the histologic lesions of collagen necrosis.

Treatment of 10 cases of scleroderma and calcinosis, with sources and concentrates of the anti-stiffness factor, indicates only a feeble beneficial effect on scleroderma and none at all on calcinosis. It was less effective than older treatments still in vogue.

The syndrome has, however, a number of features (clinical, pathologic, and radiographic) in common with calcinosis, dermatomyositis, and scleroderma, but it has also some differences, such as reversibility of the calcinosis in the experimental animals, a tendency to hyperextension deformity, and extreme lowering of the serum albumin.

The experimental production of a collagen necrosis disease with calcinosis by means of a deficiency diet is of interest to the rheumatologist, and suggests the possibility of a nutritional factor in the pathogenesis of some of the collagen diseases. No claim is made that lack of the anti-stiffness factor is the primary cause of the scleroderma group of diseases, or that the increase of it in the diet is effective in the treatment of these diseases (42).

The present writer feels that the relation between sterols, stiffness and cal-

cification remains an enigma. The syndrome developing on the skim milk diet is due to the prolonged action of many factors where cause and effect sequences are not clear. A 500 gm. guinea-pig taking into his gastro-intestinal tract 150 gm. daily may be close to the maximum load the tract can handle. The large quantities of calcium to be lost may place a load on the scybala forming mechanism. On the skim milk diet there may be a marked chronic state of starvation which over a long period of time can have only deleterious effects. Too little carbohydrate and too little amino acids will be delivered to the muscles or other tissues. Phagocytic cells may be among those damaged and they may become less active. Colloidal calcium phosphate would no longer be picked up as soon as deposited. Calcification of the vascular system would further impair nutrition. Faulty nutrition of bone marrow would lead to anemia and further impairment. The simple addition of carbohydrate to the diet could rapidly lead to improvement. These suggestions are sufficient to indicate the complexity of the problem. A consideration of the evidence indicates that the sterols play a role, but is this role a dominant one?

This summary is not a complete review of the many pertinent and valuable contributions bearing on the problem. Some additional details may be found in the reviews of van Wagtendonk and Wulzen, Dasler, Woods, and Cheldelin (8, 11, 20, 43).

SUMMARY

A pathological syndrome develops in guinea-pigs on certain diets and can be prevented by the addition of raw cream or cane sugar molasses to the diet. Correction of other phases is yet to be studied. When the syndrome has been well developed, muscles become dystrophic, and calcium is found to be laid down in many cartilages; the size of many bones or bony layers has been increased; much calcium has been deposited in striated muscles, in hair, along the blood vessels, and throughout the gastro-intestinal tract. Anomalies occur in the ear bones and these are associated with decreased response to auditory stimuli. Thus, deafness is a specific component of the deficiency syndrome. Certain phases, particularly stiffness of the wrist, can be corrected by proper diet.

Failure to respond to auditory stimuli by guinea-pigs on a deficient diet opens a field of study for the etiology of deafness. The rapidly developing pathologies of the teeth open the way for attacking important dental problems. The relations of the ear and teeth pathologies to new dietary factors is an extremely important nutritional concept. Continued study of the phenomena must lead to additional important contributions in the fields of muscle, dentistry, nutrition, and auditory physiology. Muscles and joint stiffness and calcium pathologies have marked similarity to the phenomena of arthritis and arteriosclerosis in man.

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