

THE PATHOLOGIC CHANGES RESULTING FROM VITAMIN DEFICIENCY

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Descriptive accounts of the pathologic changes resulting from each vitamin deficiency would make a voluminous report, dry and nonstimulative. The subject will be presented with the attitude that each deficiency causes distinctive functional disturbances and is accompanied by distinctive morphologic changes which together may reasonably be regarded as primary effects. In consequence, it is assumed that some of the general disturbances in nutrition, blood formation and growth, common to several of the vitamin deficiencies, are in all probability secondary nonspecific effects. The primary morphologic effects, as far as known, when analyzed all prove to be manifestations of retardation or suppression of normal processes. In recovery from a vitamin deficiency following restoration of the vitamin to the diet, normal morphologic sequences are resumed and proceed for a brief period at a rate exceeding the normal, until repair is completed. Possibly physiologic activities of certain types may be suppressed without demonstrable tissue or cytologic effects occurring. In the search for initial specific morphologic changes the pathologist has opportunity to make his humble contributions to the goal common to all biologic sciences—the understanding of the chemistry of living cells.

The pathologic or morphologic characterization of a vitamin deficiency involves a dual basis: (a) the changes in consequence of the deficiency, and (b) the changes accompanying recovery from the deficiency ending in restoration to normal structure.

VITAMIN A DEFICIENCY

Specific Pathology.—The primary effect of vitamin A deficiency is on epithelial structures. The sequences are atrophy of the epithelium concerned and the substitution for it of a stratified keratinizing epithelium, identical in appearance in all locations, and arising from focal proliferation of basal cells. These sequences have been carefully worked out in rats¹ and guinea-pigs.² Since replacement by keratinizing epithelium in many organs has also been found in human infants,³ in the monkey (*Macacus rhesus*)⁴ and in the albino mouse,⁵ and since gross changes indicate the same histologic changes in swine,⁶ in dogs,⁷ in rabbits,⁸ in cattle,⁹ and in the

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1. Wolbach, S. B., and Howe, P. R.: Tissue Changes Following Deprivation of Fat-Soluble A Vitamin, *J. Exper. Med.* **42**:753 (Dec.) 1925.

2. Wolbach, S. B., and Howe, P. R.: Vitamin A Deficiency in the Guinea-Pig, *Arch. Path. & Lab. Med.* **5**:239 (Feb.) 1928.

3. Wilson, J. R., and Dubois, R. O.: Keratomalacia in Infant with Postmortem Examination, *Am. J. Dis. Child.* **26**:431 (Nov.) 1923. Blackfan, K. D., and Wolbach, S. B.: Vitamin A Deficiency in Infants, *J. Pediat.* **3**:679 (Nov.) 1933.

4. Tilden, E. B., and Miller, E. G.: The Response of the Monkey (*Macacus Rhesus*) to Withdrawal of Vitamin A from the Diet, *J. Nutrition* **3**:121 (Sept.) 1930.

5. Wolfe, J. M., and Salter, H. P.: Vitamin A Deficiency in the Albino Mouse, *J. Nutrition* **4**:185 (July) 1931.

6. Hughes, J. S.; Aibel, C. E., and Lienhardt, H. E.: The Importance of Vitamin A and Vitamin C in the Ration of Swine, etc., *Kansas State Agric. Coll. Techn. Bull.* **23**, 1928.

7. Steenbock, Harry; Nelson, E. M., and Hart, E. B.: Fat-Soluble Vitamins: The Incidence of an Ophthalmic Reaction in Dogs Fed on a Fat-Soluble Deficient Diet, *Am. J. Physiol.* **58**:14 (Nov.) 1921. Stimson, A. M., and Hedley, O. F.: Observations on Vitamin A Deficiency in Dogs, *Pub. Health Rep.* **48**:445 (April 28) 1933.

8. Nelson, V. E., and Lamb, A. R.: The Effect of Vitamin Deficiency on Various Species of Animals, *Am. J. Physiol.* **51**:530 (April) 1920. Nelson, V. E.; Lamb, A. R., and Heller, V. G.: The Effect of Vitamin Deficiency on Various Species of Animals, *Am. J. Physiol.* **59**:335 (Feb.) 1922.

domestic fowl,¹⁰ the conclusion seems unavoidable that vitamin A is essential in most vertebrate species and that its withdrawal is followed by a common effect on epithelial structures. For convenience this change will be called keratinizing metaplasia, regardless of exact connotations of the term. The detailed studies by Howe and myself¹¹ have led us to the conclusion that the absence of vitamin A creates a starvation specific for many epitheliums. The consequence is atrophy, which progresses to a state wherein the cells, although having the appearances of viability, become inert in physiologic activities and in their rôle of covering membranes. An invariable sequence in pathology is that a break in the continuity of a tissue is followed by reparative proliferation. In vitamin A deficiency the basal cells normally concerned in maintaining the integrity of epithelium respond by active mitotic division. As the basal cells have a focal distribution in all nonstratified epitheliums, the next effect of the deficiency after atrophy is the appearance of scattered areas of proliferative activity beneath the original epithelium. The new (reparative) cells by their continued growth undermine and replace the original epithelium and, regardless of previous function and morphology of the region, develop into a stratified keratinizing epithelium. An important feature in explanation of much of the gross pathologic change is the fact that this replacement epithelium is identical in all locations and comparable in all its layers with epidermis. It has a continuous layer of basal cells—a stratum germinativum—in continuous proliferative activity while superficially it is continuously casting off keratinized cells. The striking gross pathologic condition of vitamin A deficiency in animals and in human infants is the outcome of the accumulation of keratinized epithelial cells in many glands and their ducts and in other organs. In glandular organs, cysts of considerable size are formed, filled with yellowish cheesy masses of keratinized cells. In the lungs of human beings as well as of experimental animals this process leads to cyst formation, bronchial occlusion, and consequences such as bronchiectasis and atelectasis. Early students of vitamin A deficiency, chemists and physiologists, neglected to study these cysts and believed them to be abscesses. Hence, for many years vitamin A was believed to protect against infection. As a matter of fact, the plugs of desquamated epithelial cells in ducts, bronchi and trachea, opening as they do on regions normally infected with bacteria, provide a culture medium for their growth. In spite of the fact that in both human beings and laboratory animals such bacterial growths were frequently found by us, very rarely was there invasion of the tissues, presumably because of the protection afforded by the stratified epithelium.

Distribution of the Keratinizing Metaplasia.—In the rat, the order of response to vitamin A deficiency by metaplasia is:

1. Salivary glands, including submaxillary, parotid and all accessory glands of the tongue, buccal cavity and pharynx.

9. Jones, I. R.; Eckles, C. H., and Palmer, L. S.: The Rôle of Vitamin A in the Nutrition of Calves, *J. Dairy Sc.* **9**:119, 1926. Guilbert, H. R., and Hart, G. H.: Storage of Vitamin A in Cattle, *J. Nutrition* **8**:25 (July) 1934.

10. Beach, J. R.: Vitamin A Deficiency in Poultry, *Science* **58**:542, 1923. Guerrero, L. E., and Concepcion, I.: Xerophthalmia in Fowls Fed on Polished Rice and Its Clinical Importance, *Philippine J. Sc.* **17**:99 (July) 1920. Emmett, A. D., and Peacock, G. J.: Does the Chick Require the Fat-Soluble Vitamins? *J. Biol. Chem.* **56**:679 (June) 1923.

11. Wolbach, S. B., and Howe, P. R.: Epithelial Repair in Recovery from Vitamin A Deficiency: An Experimental Study, *J. Exper. Med.* **57**:511 (March) 1933; footnotes 1 and 2.

2. Respiratory tract, including nares, maxillary sinuses, Jacobson's organ, trachea and bronchi.

3. Genito-urinary tract, including the renal pelves, ureters, bladder, epididymis, prostate, seminal vesicles, coagulating glands, uterus, oviducts and accessory sex glands of the vulva.

4. Eye and parocular glands, including the corneal and palpebral conjunctiva and the harderian, intra-orbital and extra-orbital lacrimal glands and the meibomian glands.

In the guinea-pig, lesions of the conjunctiva and parocular glands did not develop in our experiments even though advanced lesions (keratinizing metaplasia and its consequences) were found in all other locations as recorded for the rat. A marked difference in behavior as compared with the rat was early and extraordinary degrees of metaplasia in the uterus and bladder.

In human infants, keratinizing metaplasia has been found in the conjunctiva, mucosa of the nares, accessory sinuses, trachea, bronchi, pancreas, renal pelves, ureters, salivary glands, uterus, and periurethral glands. The commonest and earliest appearance of the metaplasia is in the trachea and bronchi. Next in frequency and sequence is the pelvis of the kidney. The early effect of the deficiency on the respiratory mucosa is a satisfactory explanation of the frequency, severity and persistence of the pneumonias that have been in most instances responsible for death in vitamin A deficient infants.

In the human being, as in the rat, involvement of the eye occurs late. The first change is metaplasia of the epithelium of the cornea and of the conjunctival sac. Atrophy and metaplasia of ducts of the parocular glands contribute to the consequences of the accumulation of keratinizing cells in the conjunctival sac. The cornea becomes vascularized, edematous and infiltrated with leukocytes. Infection of the cornea, favored by excessive accumulation of keratinized cells, may lead to ulceration of the cornea and hypopyon.

The Teeth in Vitamin A Deficiency.—The continuously growing incisor teeth of rodents—rats and guinea-pigs—are profoundly affected owing first to atrophy and metaplasia of the enamel forming organ and subsequently to atrophy and cessation of or irregular functioning of odontoblasts. Enamel formation is suppressed, and striking deformities of the dentin result.¹² May Mellanby¹³ has summarized her work on teeth in an excellent review and presents convincing proof that absent or defective enamel and dentin formation are consequences of vitamin A deficiency.

Boyle¹⁴ has described in the tooth germ of a human infant with vitamin A deficiency enamel organ changes comparable to those we have studied in rodent incisor teeth. In all probability, vitamin A deficiency, during the formative period of teeth, outranks all other vitamin deficiencies in importance.

Secondary Effects of Vitamin A Deficiency.—While the keratinizing metaplasia produces impressive gross lesions which are often the immediate cause of death, as in the lungs of infants and in experimental animals leading to complete suppression of urine by occlusion of ureters and renal pelves, it must be remembered that it is a late effect of the deficiency; in fact, the

consequences of attempted repair following atrophy of essential epitheliums. The effects on the organisms as a whole which follow reduced activities of many epitheliums in earlier periods of atrophy preceding metaplasia replacement must be considered.

The secondary effects, common both to infants and to laboratory animals, are (1) loss of weight due largely to loss of fat in all storage depots, but also to atrophy of musculature and many organs which do not undergo keratinizing metaplasia, (2) anemia, (3) cessation of growth of bones, (4) degenerative lesions of skeletal muscle, and (5) lymphoid hypoplasia of the spleen.

The loss of fat in experimental animals in the absence of vitamin A takes place in spite of abundant fat in the diet, but this is also the case with other vitamin deficiencies accompanied by emaciation, notably the B₂ complex. The anemia in infants and experimental animals is accompanied by hemosiderosis in the spleen and liver and, finally, by atrophy of the spleen and bone marrow.

In animals, restoration of vitamin A to this diet is followed by regeneration of the bone marrow, disappearance of hemosiderin from the spleen and liver, and in the rat also in the spleen by an active hyperplasia of lymphoid tissue and an outburst of erythroblastic activity. Again, the anemia and hemosiderosis are not peculiar to vitamin A deficiency. Both occur in striking degree in animals in vitamin B₂ complex deficiency and in long continued partial vitamin C deficiency.

The cessation of growth of bone is due to cessation of proliferative activity of the epiphyseal cartilages. A narrow band of atrophic cartilage results, which becomes bounded by a thin plate of bone on the diaphyseal side. In recovery from vitamin A deficiency the cartilage regenerates, and blood vessels from the diaphyseal marrow penetrate the limiting bony plate, and normal endochondral bone formation is resumed. The effect on bone is that common to any atrepsia, of vitamin deficiency origin or otherwise.

The degeneration of skeletal muscle in vitamin A deficiency, while often very striking and even terminating in calcification, is also common to deficiencies of diverse causation.

Lesions of the nervous system, evidenced by degeneration of the myelin sheath, have been described by several workers, notably in swine, fowls and cows by Hughes and his associates,¹⁵ in rats by Zimmerman¹⁶ and Aberle.¹⁷

Recently, Edward Mellanby,¹⁸ on the basis of studies of the trigeminal and a few other nerves by the capricious Marchi technic, suggests that the epithelial responses to vitamin A deficiency are secondary to lesions of afferent nerves.

Degeneration of the myelin sheath is common to so many disorders of man and animals as the result of several vitamin deficiencies and divers causes that very careful work will be required to establish a specific relationship of its presence to vitamin A deficiency.

15. Hughes, Aubel and Lienhardt.⁶ Hughes, J. S.; Lienhardt, H. F., and Aubel, C. E.: Nerve Degeneration Resulting from Avitaminosis A, *J. Nutrition* 2: 183 (Nov.) 1929.

16. Zimmerman, H. M.: Lesions of the Nervous System in Vitamin Deficiency: I. Rats on a Diet Low in Vitamin A, *J. Exper. Med.* 57: 215 (Feb.) 1933.

17. Aberle, S. B. D.: Neurological Disturbances in Rats Reared on Diets Deficient in Vitamin A, *J. Nutrition* 7: 445 (April) 1934.

12. Wolbach, S. B., and Howe, P. R.: The Incisor Teeth of Albino Rats and Guinea-Pigs in Vitamin A Deficiency and Repair, *Am. J. Path.* 9: 275 (May) 1933.

13. Mellanby, May: The Influence of Diet on the Structure of the Teeth, *Physiol. Rev.* 8: 545 (Oct.) 1928.

14. Boyle, P. E.: Manifestations of Vitamin A Deficiency in a Human Tooth Germ, *J. Dental Research* 13: 39 (Feb.) 1933.

18. Mellanby, Edward: Xerophthalmia, Trigeminal Degeneration and Vitamin A Deficiency, *J. Path. & Bact.* 38: 391 (May) 1934; *Nutrition and Disease: The Interaction of Clinical and Experimental Work*, Edinburgh and London, Oliver and Boyd, 1934.

For the present it seems logical to regard the lesions of the nervous system as among the late secondary consequences of vitamin A deficiency.

Repair in Recovery from Vitamin A Deficiency.—The lesions of vitamin A deficiency, uncomplicated by destruction of tissue by infectious process, disappear rapidly after restoration of the diet. In rats, gain in weight, resumption of growth in bones and teeth, and regeneration of the bone marrow and spleen become apparent in from six to eight days. Reparative changes in the metaplastic epithelium begin as early as the fifth day. The initial changes are separation of superficial keratinized cells and vacuolization of cells of intermediate layers. The epithelium becomes divided into two zones by further vacuolar degeneration and leukocytic infiltrations. The superficial zone of cells degenerate, the deep zone, consisting of the lowermost layers of cells corresponding to the stratum germinativum of the epidermis, survives and the cells proceed to differentiate into the type of epithelium originally present. On the whole, the change back to the normal epithelium is an abrupt one and affords further evidence that the primary consequence of vitamin A deficiency is epithelial and not of nervous origin. This reparative sequence of the epithelium has its normal counterpart in changes in the vagina of rodents during that part of the estrous cycle in which the cornified vaginal mucosa returns to the mucous type.¹⁹

VITAMIN C DEFICIENCY

Of vitamin C deficiency, only the specific pathologic lesions will be considered. The pathology of scurvy is well known and covered in the books by Aschoff and Koch,²⁰ Hess²¹ and others.

The requirements of all animals for vitamin C are not known. Scurvy, duplicating that in man, can regularly be produced in guinea-pigs and monkeys. The cow, rat, mouse, prairie dog, pigeon and domestic fowl do not require vitamin C in the diet; nevertheless it is found in their livers—evidence that these creatures can synthesize this vitamin.

The gross and microscopic pathologic changes of human scurvy, as seen in the infant and experimental scurvy in the guinea-pig, are so nearly identical that no reasonable doubt can be entertained with regard to applying to the human being the facts ascertained from the experimental studies.

In 1926 Wolbach and Howe²² characterized the condition of scorbutus as the inability of the supporting tissues to produce and maintain intercellular substances. The effect is therefore on cells of mesenchymal origin in contrast to the ectodermal and endodermal effects of vitamin A deficiency. These conclusions were reached through histologic studies of human infantile scurvy and through studies of the histologic sequences, in growing guinea-pigs, of progressive scurvy and of the repair following administration of vitamin C in natural forms. Subsequently²³ further verification that vitamin C was the only missing factor in scorbutus

concerned in the inability of tissues to produce intercellular material was obtained through the study of reparative processes following administration of crystalline cevitamic acid, orally and parenterally.

Further experimental proof of the correctness of this pathologic characterization was obtained²⁴ by a comparison of isolated fibroblasts during the organization of blood clots in the complete deficiency with those during the progress of recovery induced by the administration of vitamin C.

The intercellular substances concerned in vitamin C deficiency are the collagen of all fibrous tissue structures, the matrices of bone, dentin and cartilage, and all nonepithelial cement substance, including that of the vascular endothelium. The reparative proliferative powers of epithelial cells, endothelium, fibroblasts and osteoblasts are not impaired, while there is evidence of increased proliferation of osteoblasts, which in the periosteum and at the sites of endochondral bone formation undergo a striking change in morphology, taking on the appearance of young fibroblasts.

Aschoff and Koch²⁰ anticipated in part these conclusions from their studies of human material. They explained some features of the pathology of bone as due to failure of osteoblasts to form osteoid tissue and, by inference, the hemorrhage of scurvy as due to a failure of cement substance in blood vessels. Mechanical factors were emphasized as determining the sites of hemorrhages in bone, muscle, skin and alveolar processes of the jaws.

Soft tissue changes in vitamin C deficiency, human, and experimental in guinea-pigs, are hemorrhages in regions determined by mechanical stresses and trauma; also anasarca (human) and degenerations of skeletal and cardiac muscle. Hypertrophy of the heart occurs particularly in infants and children, attributed by Erdheim²⁵ to difficulties in respiration in consequence of the lesions of the ribs at the costochondral junctions. No such explanation can be applied to the cardiac hypertrophy of beriberi or B₁ deficiency. In long continued partial vitamin C deficiency in guinea-pigs, degeneration of skeletal muscle fibers becomes widespread. Affected fibers become completely necrotic. We have found this lesion common also in vitamin A and vitamin G (B₆ + lactoflavin) deficiency.

Goettsch and Pappenheimer²⁶ describe a diet with adequate vitamin content which leads to a progressive fatal degeneration of the voluntary muscles. The lesion of the individual fibers is identical with that of vitamin deficiencies A, G and C. Furthermore, it was shown by Pappenheimer and his associates²⁷ that the muscle degeneration was not associated with demonstrable changes in peripheral nerves or their motor terminals. Accordingly, the striking degeneration of muscles in long continued partial experimental scurvy²⁸ as in acute scurvy must be regarded as secondary effects of vitamin C deficiency. Anemia is another secondary effect of importance. In long continued partial vitamin C deficiency in guinea-pigs, large regions of bone marrow

19. Long, J. A., and Evans, H. McL.: The Oestrus Cycle in the Rat and Its Associated Phenomena, *Memoirs of the University of California*, vol. 6, 1922.

20. Aschoff, L., and Koch, W.: *Scorbut, Eine Pathologisch-Anatomische Studie*, Jena, Gustav Fischer, 1919.

21. Hess, A. F.: *Scurvy, Past and Present*, Philadelphia, J. B. Lippincott Company, 1920.

22. Wolbach, S. B., and Howe, P. R.: Intercellular Substances in Experimental Scorbutus, *Arch. Path. & Lab. Med.* 1: 1 (Jan.) 1926.

23. Menkin, Vally; Wolbach, S. B., and Menkin, Miriam F.: Formation of Intercellular Substance by the Administration of Ascorbic Acid (Vitamin C) in Experimental Scorbutus, *Am. J. Path.* 10: 569 (Sept.) 1934.

24. Wolbach, S. B.: Controlled Formation of Collagen and Reticulum: A Study of the Source of Intercellular Substance in Recovery from Experimental Scorbutus, *Am. J. Path. (suppl.)* 9: 689, 1933.

25. Erdheim, J.: Ueber das Barlow Herz, *Wien. klin. Wchnschr.* 31: 1293, 1918.

26. Goettsch, M., and Pappenheimer, A. M.: Nutritional Muscular Dystrophy in the Guinea-Pig and Rabbit, *J. Exper. Med.* 54: 145 (Aug.) 1931.

27. Rogers, W. M.; Pappenheimer, A. M., and Goettsch, M.: Nerve Endings in Nutritional Muscular Dystrophy in Guinea-Pigs, *J. Exper. Med.* 54: 167 (Aug.) 1931.

28. Bessey, O.; Boyle, P., and Wolbach, S. B.: Unpublished research.

become devoid of blood forming cells and the seat of a deposit of homogeneous amyloid-like material.²⁸

The important gross features of scurvy at all ages are hemorrhages and changes in the bones. The most striking lesions are the subperiosteal hemorrhages and those in the epidiaphyseal junctions of growing bones. All the pathologic features of scurvy are understandable only on the basis of the characterization as a condition of cessation of formation and maintenance of intercellular substances. Calcium metabolism is not primarily affected. In the first period of scurvy, existing cartilage trabeculae at epidiaphyseal junctions become more densely calcified than normally.²⁹

In advanced scurvy, calcium salts are liberated through the resorption of bone matrix, which is the process of the osteoporosis of scurvy.

In general, the pathologic picture of scurvy is produced by the resorption of intercellular materials and by the absence of formation of intercellular materials in growth and reparative reactions. Hemorrhages are due to mechanical weakness, occasioned by the lack of collagenous material in fibrous tissue structures and in bone. Diminished cohesion of endothelial cells contributes to the ease with which moderate stresses occasion bleeding in skin and muscles. In the hemorrhages from the gums, the resorption of alveolar processes and the loosening of teeth, the two factors of stresses and loss of intercellular materials are apparent, the first affecting the second.

At the epidiaphyseal junctions in growing bones, the first demonstrable change in experimental scurvy is an increase in the number of osteoblasts applied to the cartilage columns. Formation of cartilage and bone matrices ceases, and the osteoblasts become elongated, assume the shapes of fibroblasts and migrate toward the diaphysis. Here these cells become surrounded by liquid, presumably a deficient product of continued activity toward matrix formation, and give rise to an apparent region of edematous connective tissue at the ends of the diaphysis, the gerüst mark (framework marrow) of German authors.

Marked changes in the epiphyseal cartilage occur because of resorption of matrix. Union between epiphysis and diaphysis becomes severed because the trabeculae of the spongy bone no longer communicate with epiphyseal cartilage, owing to the cessation of formation of periosteal bone. This disunion permits movement of the epiphysis in relation to the diaphysis, and traumatic fragmentation occurs, producing the trümmerfeld zone or zone of disorganization between gerüst mark and epidiaphyseal line. Hemorrhage complicates the picture. This accounts for the complete separation and dislocation of epiphyses so often seen in scorbutic infants and rarely at costochondral junctions in adults. Proliferation of cells, osteoblasts, of the periosteum in contact with the cortical bone is continued in growing bone and is a conspicuous histologic feature of scurvy in infants. A layer of cells without intercellular material thus results and separates periosteum from bone; hence the massive subperiosteal hemorrhages so characteristic of scurvy in infants. These sequences have been worked out in guinea-pigs, yet each stage has been seen in human material.

In growing teeth (incisors) of guinea-pigs, formation of dentin ceases and the pulp becomes separated

from the dentin by liquid, which may be interpreted as due to the continued production by the odontoblasts of a liquid product. Presumably, teeth in process of formation in infants may be similarly affected in scurvy, but direct demonstration is lacking.

Repair of Scurvy.—Histologic repair following administration of vitamin C in natural foods or as cevitamic acid is dramatic in character and promptness, and in the small amounts of the remedy required. Newly formed dentin, collagen and bone matrix can be seen after twenty-four hours. Osteoblasts, disguised in morphology as fibroblasts, resume their rôle in formation of bone matrix. New capillary formation becomes possible, so that repair by granulation tissue formation proceeds in organization of blood clots and subsequent callous formation where the hemorrhages were in contact with bone. All normal processes of repair are resumed, infractions and fractures heal and subperiosteal hemorrhages become in part organized and ossified. In guinea-pigs, dentin formation is resumed in volume and rapidity suggestive of the jelling of a liquid material between pulp and old dentin.

VITAMIN D DEFICIENCY OR RICKETS

The physiologic rôle of vitamin D, whether or not identical with the pure substance calciferol or viosterol, in the metabolic processes concerned in the deposition of calcium phosphate in bone is not clear, particularly in relation to the experimental production and cure of rickets in white rats. In the absence of vitamin D, proper amounts and ratios of calcium and phosphate in the diet prevents rickets, yet vitamin D cures rickets produced in the rat by dietary methods. Likewise, restoration of a proper calcium and phosphate intake is curative.

Experimental rickets can be produced also in swine,³⁰ rabbits,³¹ the monkey³² and the domestic fowl.³³

Important landmarks in the progress of knowledge of the pathology of rickets are the publications of Pommer,³⁴ Schmorl,³⁵ Erdheim³⁶ and Pappenheimer.³⁷ An adequate bibliography is contained in Hess's³⁸ book.

In white rats, experimental rickets, in all phenomena amenable to study, duplicates human rickets. This applies with particular force to the histologic sequences in the development of the lesions and in the repair.

The pathologic conditions of rickets arise from retardation and suppression of sequences primarily concerning cartilage in the endochondral formation of bone and from failure of bone matrix or osteoid to calcify in all locations.

Briefly stated, the sequences disturbed in endochondral bone formation in rickets are as follows:³⁹ The epiphyseal cartilage during normal growth exists as a narrow plate, supported by bone on the epiphyseal side

30. Elliot, W. E.; Crichton, A., and Orr, J. B.: Rickets in Pigs, Brit. J. Exper. Path. 3: 10 (Feb.) 1922.

31. Goldblatt, H., and Moritz, A. R.: Experimental Rickets in Rabbits, J. Exper. Med. 42: 499 (Oct.) 1925.

32. Christeller, E.: Die Formen der Ostitis Fibrosa der Säugtiere, etc., Ergeb. d. allg. Path. u. path. Anat. 20: 1 (part 2) 1922.

33. Nonidez, J. F.: Studies on the Bones in Avian Rickets, Am. J. Path. 4: 463 (Sept.) 1928.

34. Pommer, G.: Untersuchungen über Osteomalacie und Rachitis, Leipzig, 1885.

35. Schmorl, G.: Die pathologische Anatomie der rachitischen Knochenkrankung mit besonderer Berücksichtigung ihrer Histologie und Pathogenese, Ergeb. d. inn. Med. u. Kinderh. 4: 403, 1909.

36. Erdheim, J.: Rachitis und epithel Körperchen, Vienna, 1914.

37. Pappenheimer, A. M.: Experimental Rickets in Rats, J. Exper. Med. 36: 335 (Sept.) 1922.

38. Hess, A. F.: Rickets, Including Osteomalacia and Tetany, Philadelphia, Lea & Febiger, 1930.

39. Shohl, A. T., and Wolbach, S. B.: Rickets in Rats: XV. The Effect of Low Calcium-High Phosphorus Diets at Various Levels and Ratios upon the Production of Rickets and Tetany, J. Nutrition 11: 275 (March) 1936.

29. Park, E. A.; Guild, H. G.; Jackson, D., and Bond, M.: The Recognition of Scurvy with Especial Reference to the Early X-Ray Changes, Arch. Dis. Childhood 10: 265 (Aug.) 1935.

and uniformly penetrated by blood vessels of capillary dimensions on the diaphyseal side. Very little evidence of growth is present on the epiphyseal side, where bone is closely applied in the form of transverse trabeculae or a thin fenestrated plate. Growth is accomplished by continuous proliferation of cartilage cells, arranged in columns, on the epiphyseal side, and simultaneous degeneration of the matured cells on the diaphyseal side. The cavities occasioned by the degeneration and disappearance of the cartilage cells at the diaphyseal end of the columns are entered by capillaries accompanied by cells (osteoblasts) which deposit osteoid on the exposed cartilage matrix; hence the first formation of bone is within spaces previously occupied by cartilage cells.

This behavior of the epiphyseal cartilage may be regarded as an ingenious device of nature in order to maintain a continuously retreating gap in the continuity of tissues, which is responded to by vascular outgrowth from the diaphyseal side, comparable to repair of any defect of tissues by the process of organization or granulation tissue formation. In normal growth there is, on the diaphyseal side of the narrow epiphyseal cartilage, a continuous layer of clear or empty cartilage cells, one or two cells deep. In normal growth, calcification of the cartilage matrix lateral to the columns of cells (so-called zone of provisional calcification) extends toward the epiphyseal extremity of the bone only as far as the cartilage cells are markedly degenerated. The first histologic evidence of rickets is the absence in whole or in part of the layer of clear (degenerated) cells and the consequent absence of ingrowth of capillaries. The matrix between the non-degenerated cartilage cells does not calcify. Slight degrees of rickets are manifested by a moderate increase in width of the epiphyseal cartilage presenting an irregular border on the diaphyseal side. This irregularity is due to the fact that the cessation of degenerative sequences in the cartilage cells does not take place simultaneously over the diaphyseal border. The width of the epiphyseal cartilage continues to increase because of the continued activity of the proliferative zone and the survival of the cells on the diaphyseal side. After cessation of the degenerative sequences of the cartilage cells and failure of cartilage matrix to calcify, osteoblasts in relation to the blood vessels in the diaphysis adjacent to the cartilage continue to deposit bone matrix or osteoid that does not calcify.

The degree or measure of severity of rickets may be gaged by the volume of the epiphyseal cartilage and amount of osteoid accumulated in the adjacent diaphysis, both expressions of degree and duration of retardation of two normal processes, one the cartilage sequence and the other, calcification of matrices.

In advanced rickets the noncalcified cartilage at the diaphyseal border becomes transversely stratified in places, evidently a mechanical effect of weight bearing. Osteoid material increases in amount with the duration of the deficiency and, being noncalcified, is molded by the stresses of weight and muscular efforts. Finally, there is disappearance of the cancellous bone of the diaphysis, marked resorption of cortical bone and deposition of subperiosteal osteoid. These are the factors that account for the deformities and fractures of bones in rickets.

Repair of Rickets.—The first histologic evidence of repair following corrections of the diet, either by amount of calcium and phosphorus or by administration

of vitamin D, is the presence of cleared or degenerated cells on the diaphyseal border of the cartilage. Simultaneously, calcium is deposited in the cartilage matrix lateral to these cells. These effects are demonstrable at the end of twenty-four hours and are accompanied by extensive vascular responses, as shown by ingrowth of capillaries into empty cell spaces within forty-eight hours.

The first osteoid to be calcified in repair is that laid down in the resumption of normal sequences. The osteoid in the diaphysis that has accumulated during the deficiency subsequently becomes calcified and then is largely removed by osteoclasia. Repair proceeds rapidly in rats, even after severe rickets. From five to seven days suffices to restore the appearances of normal growth almost completely, though there is not complete removal of excess bone.

The osteomalacia of adults, when advanced, presents striking deformities of the skeleton and complicated microscopic pictures, all to be explained by loss of calcium salts in bone, secondary resorption of the matrix and new formation by way of reparative response of cells producing osteoid that does not calcify.

VITAMIN B COMPLEX

Present knowledge does not permit a rational account of the pathologic consequences of the deficiencies of what in the past has been called vitamin B or B complex. Vitamin B complex consists of a heat labile fraction, designated as B₁ and recently isolated in pure form, and a heat stable group. Claims by various authors have been made for as many as six different vitamins in the heat stable group, which is called vitamin B₂ or G. It is fairly certain now that this heat stable group contains probably no more than three vitamins: (1) lactoflavin, already isolated in pure form; (2) vitamin B₆, the absence of which in the diet of rats is responsible for a characteristic dermatitis affecting the extremities, nose, eyes and ears (this dermatitis has also been referred to as rat pellagra and B₆ as the antirat pellagra vitamin); (3) the pellagra preventing factor, which also prevents and cures black tongue in dogs.

It is not known whether pellagra and black tongue are due to the same deficiency, although the evidence is very strong that this is the case.

In the human being beriberi is a disease resulting from lack of vitamin B₁, but in all probability some of the features of this disease as commonly seen are due to other deficiencies. Those pathologic features which may be reproduced in pigeons by means of a diet adequate in all respects except in vitamin B₁ which are common also to human beriberi are some degree of enlargement of the heart, edema, atrophy of muscles, and degenerations of the nervous system. Manifestations of disorder of the nervous system are so striking that the condition in experimental animals is usually called polyneuritis. The conspicuous lesion is degeneration (Marchi degeneration) of the myelin sheaths of peripheral nerves; less certain are degenerative changes in ganglion cells of the brain, cerebellum, spinal cord and dorsal root ganglions. It must be remembered, however, that nervous symptoms appear late in the deficiency, and rats kept on vitamin B₁ deficiency may die without developing nervous lesions, so that a number of workers have questioned the specificity of degenerations of the myelin sheaths and have claimed to have produced the same degree of degeneration in

animals on a starvation diet in the presence of abundant vitamin B₁.⁴⁰ The consequences of starvation on the maintenance of myelin must be determined before the myelin sheath degenerations of this deficiency and other vitamin deficiencies (vitamin A and the heat stable fractions of B) can be attributed to a primary effect on the nervous system. Also of great importance in this connection is the fact that recovery from the nervous manifestations of experimental beriberi takes place in a very short time, and physiologic recovery results in a period of five or six hours following the administration of vitamin B₁.

I⁴¹ could find no differences in the nerve lesions present in pigeons allowed to succumb with polyneuritis and those in which functional recovery had been induced by treatment. On the whole, it seems best to regard the primary pathologic effects of vitamin B₁ deficiency as not demonstrable at present and to regard all the pathologic changes thus far recorded, including the myelin sheath lesions, as secondary effects. The profound functional disturbances of the nervous system and speedy recovery, with treatment, do indicate that vitamin B₁ is directly concerned in the physiology of neurons. Also among the secondary consequences presumably due to disturbance in carbohydrate metabolism is enlargement of the islands of Langerhans in the pancreas, which I saw in experiments in 1925 and which have been reported by Ogata⁴² and by Bierry and Kollmann.⁴³

Similar difficulties are encountered in the consideration of the pathology of those conditions and diseases presumably resulting solely from deprivation of heat stable components of vitamin B. There is, for consideration, pellagra in the human being, black tongue in dogs, and rat pellagra or rat dermatitis or, as it is probably now preferably called, as suggested by Birch, György and Harris,⁴⁴ "rat acrodynia."

In pellagra, lesions of the nervous system are found but they are not pathognomonic in character. They are degenerative in type, in nerve cells and in myelin sheaths. In pellagra of long standing, lateral and posterior column degenerations in the spinal cord occur. Whether or not such extensive cord lesions have a pathogenesis (atrophy of the gastric mucosa) in common with those occurring in pernicious anemia remains to be solved. In dogs maintained on a diet deficient in the heat stable vitamin B complex (vitamin B₂ or G) extensive degeneration of the myelin sheaths also develop in peripheral nerves and tracts of the spinal cord.⁴⁵ Howe, Bessey and I²⁸ have been unable to obtain degeneration of the myelin sheaths in spinal cord or peripheral nerves in white rats maintained on a similarly deficient diet, even though they were carried to the point of death in the complete deficiency and for very long periods in partial deficiency. It must be concluded that the rat responds differently. The rats in our experiments did develop, as was to be expected,

the skin lesions, or "rat acrodynia." That the factor preventing dermatitis in rats is different from that preventing the lesions of pellagra and black tongue has been proved.⁴⁴ It has been shown that diets producing pellagra in man and black tongue in dogs will cure the vitamin B₂ complex deficiency dermatitis of rats. Also, the dermatitis of rats is not influenced by light. We have found it to progress just as rapidly in rats kept in absolute darkness as in those kept in strong light. The distribution is also different and yet we are confronted with the paradox that histologically the lesions of the skin are very similar to those of pellagra and to the lesions of mucous membranes in black tongue of dogs. It is not advisable to describe in detail the histology of the skin lesions of rat dermatitis and of human pellagra. Careful study of rats made during the progress of the deficiency and in repair has failed to throw light on the initial effect in the skin, whether in the epidermis or in the underlying supporting tissues.

Until experiments on animals can be conducted with diets deficient only in single B factors, it would seem not worth while to attempt to characterize, pathologically, these deficiencies.

Until it is proved that the myelin sheath degenerations of the deficiencies are not simply starvation effects, the conclusion is warranted that the demonstrable nervous lesions of all the deficiency diseases are secondary effects and that no one vitamin is concerned in the maintenance of myelin. Lesions of skin and mucous membrane, such as have been described for pellagra, black tongue and rat dermatitis, are not common to deficiencies other than those found in the heat stable fraction of vitamin B or the vitamin B₂ complex, but it does not seem probable that the chemical mechanisms disturbed by the absence of the respective vitamins concerned will be found to occur primarily in the skin and mucous membranes. It may be said that the pathologic histology of pellagra, black tongue and vitamin B₂ or G deficiency in the rat, although presenting many interesting and suggestive features, is at present not more illuminating in regard to the mechanisms involved than are the sequences observed with the naked eye.

COMMENT

Our knowledge of the morphologic consequences of vitamin deficiencies is limited and has not extended beyond obvious tissue and cytologic changes.

The pathology of vitamin A deficiency indicates that the seat of the physiologic disturbances is in the epithelial cells. Chemical rôles are suppressed but proliferative powers are not inhibited; neither are the potentialities of cells lost, as is shown by the return to normal physiologic function when vitamin A is restored to the animal.

Vitamin C deficiency affects mesenchymal tissues, the most obvious morphologic consequence being the effect on the formation and maintenance of intercellular materials.

Vitamin D deficiency, besides the well known effect on calcium and phosphorus metabolism, suspends the cartilage cell cycle essential to endochondral growth of bone, though not impairing multiplication of the cartilage cells.

Vitamin B₁, as is indicated by recovery phenomena from the deficiency, must be more directly concerned in the physiology of the nervous system than is any

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other vitamin. The sudden return to normal function after administration of vitamin B₁, before morphologic repair has been initiated, warrants this conclusion.

A reasonable working hypothesis is that each vitamin is necessary in certain cells for one type of chemical mechanism the suppression of which is compatible.

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NEW FORMS AND SOURCES OF VITAMIN D

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Certain facts about vitamin D are widely known. It occurs only rarely in foodstuffs. It is formed in the skin by exposure to ultraviolet rays from the sun or from artificial sources. It is developed in some foodstuffs by their being briefly irradiated. It is produced by irradiating ergosterol, the sterol of fungi. From irradiated ergosterol it can be isolated and crystallized.

The fact that vitamin D is not a single chemical substance has only recently been recognized. The erroneous view still commonly held is that ergosterol is the parent substance, or provitamin, from which all vitamin D arises. Ergosterol exhibits four spectral absorption bands in the ultraviolet region. These bands were observed in the unsaponifiable fraction of the various materials that become antiricketic on irradiation. Since it is rare that even one band, not to mention such a series of bands, is exactly duplicated by different chemical substances, the evidence was misleadingly good that ergosterol was the one and only provitamin D.

I have reviewed elsewhere¹ in considerable detail the history of our knowledge of vitamin D, giving special attention to the experimental work on which its multiple nature is established. In a brief space, this can be recapitulated only in outline and brought up to date. So rapidly has investigation progressed that several new forms of vitamin D have been discovered within the past few months. One of these I shall describe for the first time.

The most thoroughly investigated form of vitamin D is that which results from the irradiation of ergosterol. This is known to physicians under trade names such as viosterol. The chemical name of the pure substance is calciferol. This particular form of vitamin D is also the form that is produced by irradiating yeast, the provitamin of which is ergosterol. It is thus the form that is present in the milk of cows to which irradiated yeast has been administered—the so-called yeast milk of commerce.

In our laboratory, in 1930, Massengale and Nussmeier² made the important discovery that the vitamin D of irradiated ergosterol and the vitamin D of cod liver oil act differently on rats and chickens. They found that, rat unit for rat unit, irradiated ergosterol is far less effective than cod liver oil for preventing rickets in chickens. Or, in other words, irradiated ergosterol is far more effective than cod liver oil, chick unit for chick unit, in preventing or curing rickets in rats.

This discovery, like several others in the field of vitamin D, was made simultaneously in more than one laboratory. Mussehl and Ackerson³ and Hess and Supplee⁴ came to the same conclusions from slightly different avenues of approach. It is recalled that Hess⁵ and Steenbock⁶ announced the discovery of activation within a few weeks of each other, that the provitamin concept was promulgated by three groups of investigators⁷ Dec. 10, 1926, and that the high relative efficacy of irradiated 7-dehydro-cholesterol for chickens was reported by two independent groups⁸ on the same day a few weeks ago.

The device of employing two species of test animals for assay purposes has come to be an extraordinarily useful tool in studies on the multiple nature of vitamin D. The knowledge that one species responds better to one form of this vitamin while another species responds better to another form has prompted investigators to compare the effectiveness of cod liver oil and irradiated ergosterol, rat unit for rat unit, on human beings. Differences have been claimed and denied. There is agreement to the extent that the difference, if any, is not large. Most of this work has been of poor quality, with groups of children so few in number and so diverse in age and background that the recorded observations are unimpressive to one who is familiar with the proper use and grouping of animals for the conduct of a biologic assay. Birds other than the common fowl, for example turkeys, respond like the common fowl, and mammals other than the rat, e. g., man, respond at least somewhat like the rat to these two kinds of vitamin D. It is therefore reasonable to expect that, if the number of species put to the test is extended, mammals in general will be found to respond well (per rat unit) to either irradiated ergosterol or cod liver oil, and birds in general will do poorly with irradiated ergosterol but will do well with cod liver oil.

The chemical constitution of the sterols is fairly well understood. The conventional numbering of the sterol ring, and the probable formulas of cholesterol, ergosterol and calciferol are shown in the accompanying structural formulas. From these concepts, the organic chemist has built several new vitamins D.

Windaus and Langer⁹ added two atoms of hydrogen to the side chain of ergosterol at position 22. The new compound, 22-dihydro-ergosterol, gave by irradiation a new vitamin D, presumably 22-dihydro-calciferol. McDonald¹⁰ has recently found that this slight molecular alteration increases by several times the effectiveness of the vitamin, per rat unit, for chickens.

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