SCIENCE

VOL. 86 FRIDAY, DECEMBER 24, 1937 No. 2243

SCIENCE: A Weekly Journal devoted to the Advancement of Science, edited by J. MCKEEN CATTELL and published every Friday by

THE SCIENCE PRESS

New York City: Grand Central Terminal
Lancaster, Pa. Garrison, N. Y.

Annual Subscription, $6.00 Single Copies, 15 Cts.

SCIENCE is the official organ of the American Association for the Advancement of Science. Information regarding membership in the Association may be secured from the office of the permanent secretary, in the Smithsonian Institution Building, Washington, D. C.

VITAMIN DEFICIENCY EXPERIMENTATION AS A RESEARCH METHOD IN BIOLOGY[1]

By Dr. S. BURT WOLBACH

HARVARD MEDICAL SCHOOL

VITAMINS are organic substances, not related chemically to one another, indispensable to normal functioning of some one or more animal species. They are effective in small amounts, do not furnish energy, are not structural materials as the fats, carbohydrates and proteins, but are necessary for the chemistry of cells. Our knowledge of them came through discoveries that substances of plant origin—the vitamins or provitamins of to-day—are essential for the well-being of many animals. Species not requiring a given vitamin in their diet may have the power of synthesizing it from elementary compounds, as has been proved for the rat in the case of vitamin C. Absence of a vitamin results in the suspension, in all probability, of a single type of intracellular chemistry necessary for the tissue concerned, and indirectly, for the organism as a whole. One of the outstanding results of the attempts of Howe and myself to achieve morphological characterizations of the vitamin deficiencies was the discovery that cells deprived of a function essential for the organism as a whole may, nevertheless, survive and multiply.

We have endeavored to find the initial tissue or cellular responses to each vitamin deficiency with the belief that the cells first to exhibit changes would be those in which the vitamin was necessary for the performance of an essential chemical process. In some instances we have succeeded for the requirements of a morphological characterization, but with all members of the B group, B_1 and the B_2 components, we have failed, possibly because the chemistries involved are common to many tissues and concern energy processes.

[1] DeLamar Lecture, the Johns Hopkins University, School of Hygiene and Public Health, May 11, 1937.

not involving structural maintenance and hence unaccompanied by distinctive morphological changes. Thus far we have not resorted to intensive cytological studies, beyond investigating the behavior of mitochondria in several of the deficiencies (A, C and the G complex) in each instance with negative results.

The steady progress in understanding of the biochemistries of the vitamins now obtainable in pure form is a challenge to the cytologist because in some instances it should be possible to determine the loci, within cells, of vitamin activities. The opportunity of associating chemical activities or functional rôles with nuclear and cytoplasmic structures appears to be at hand.

My endeavor to-day is to review our material in exposition of the possibilities of the vitamin deficiencies and related repair phenomena as a technique of research of fairly wide interest in morphological fields.

VITAMIN A

Observations made on many vertebrate species indicate that vitamin A is presumably indispensable to all vertebrates. "So far as known, no vertebrate can synthesize carotenoids *de novo*."[2] The first observable consequences of its absence is the atrophy of epithelial structures and, in many locations, replacement by stratified keratinizing epithelium identical in appearance in all locations and arising from proliferation of basal cells.[3, 4, 5] This occurs in the ducts of many glands, in glands themselves and in many mucous membranes. Not all epitheliums show this effect. The mucosa of the stomach and intestines and the renal tubules show practically no change. When it occurs, the atrophy progresses to a state wherein the cells, although having the appearances of viability, become inert in physiological activities and in their rôles of covering membranes. An invariable sequence in pathology is that a break in continuity of a tissue is followed by reparative proliferation, hence the basal cells normally concerned in maintaining the integrity of epithelium respond by active mitotic division. As the basal cells are focally distributed in all non-stratified epitheliums, the next stage in the progress of the deficiency is the appearance of scattered foci 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 keratinized epithelium. This replacement epithelium is identical wherever it occurs and comparable in all its layers with epidermis. Apparently mature epithelial cells which possess power to multiply in addition to their physiological activity in behalf of the organism as a whole, do not respond to vitamin A by keratinizing metaplasia. The best examples of this are liver and renal tubule epitheliums. One explanation may be that such cells have no activity dependent upon a supply of vitamin A. Another may be that these cells can adapt to the deficiency by virtue of potentialities retained with the power of division. Why the reparative activities of basal cells of many different epitheliums in vitamin A deficiency lead to a common product—an epidermis-like structure—can not be answered unless a keratinizing epithelium represents a more primitive type than those having secretory activities. The fact that in vitamin A deficiency certain stratified epitheliums, the transitional epithelium of the urinary tract and the corneal epithelium become hyperkeratotic indicates that these covering membranes have unknown functions which, when suppressed, are accompanied by a diversion of energy into another channel—a more rapid growth with the simpler chemistry of keratinizing epithelium.

In recovery which follows restoration of vitamin A to the diet, in spite of the complete morphological masking, the epithelium in each region returns to its normal type.[6] By instituting repair, we can start at will the differentiation of the stratum germinativum towards the normal epithelium of the region. The minute cytological changes which may accompany this shift have not been adequately searched for. Early in the reparative process, a line of demarcation appears separating the cell layer where differentiation toward keratinization has progressed to an irreversible stage from cells below which have retained the complete potentialities of the region. It is apparent that the potential characteristics of the masked epithelium reside only in the cells with power to divide. This line of demarcation in repair is produced by vacuolar degeneration of the cells accompanied by infiltration with leucocytes. The cells above are either cast off or disintegrate. The cells of the lower stratum proceed to differentiate directly into the normal type and, because desquamation has ceased, with a lower rate of division.

The only normal process which involves a mechanism similar to those in recovery of epitheliums in A deficiency is that of the changes in the vagina of rodents during the estrous cycle. Here the sequences involved in the periodic cornification of the vaginal mucosa bear some resemblance to those of vitamin A

[2] G. Wald, *Jour. Gen. Physiol.*, 19: 351, November, 1935.

[3] S. B. Wolbach and P. R. Howe, *Jour. Exper. Med.*, 42: 753, December, 1925.

[4] S. B. Wolbach and P. R. Howe, *Arch. Path. and Lab. Med.*, 5: 239, February, 1928.

[5] K. D. Blackfan and S. B. Wolbach, *Jour. Pediat.*, 3: 679, November, 1933.

[6] S. B. Wolbach and P. R. Howe, *Jour. Exper. Med.*, 57: 511, March, 1933.

deficiency metaplasia, while the sequences of the reverse changes are very similar to recovery changes in vitamin A deficiency, including vacuolar degeneration, leucocytic infiltration and desquamation. As the metaplasia of vitamin A deficiency and its recovery is a cycle that probably does not occur in animals in natural habitats, it is of interest to find that it can be correlated with normal processes. Comparison of the A deficiency phenomena with the histological sequences in the estrous cycle makes us note that the greatest similarity exists between vitamin A reparative sequences and the sequences in the di-estrous. Hence, the addition of vitamin A in the deficiency produces results like those presumably due to the wane of an hormonal effect.

Vitamin A, in its effect upon epithelium and in its repair, offers two opportunities to identify physiological shifts with structural detail.

The biochemistry of vitamin A is practically unknown except in its rôle as a constituent of visual purple. Here it is a necessary material for the making of a photosensitive compound, a conjugated protein with vitamin A in the prosthetic group which, in undergoing changes due to light, initiates nerve impulses. A supply of vitamin A is necessary for the resynthesis of visual purple. Important facts to consider are that visual purple is a vitamin-protein compound and that in this instance vitamin A is a structural material and that profound cytological changes are wide-spread in many organs in the deficiency. These facts suggest that vitamin A may be solely concerned in maintaining an apparatus within cells and not in the chemical processes for which the apparatus is necessary.

The most interesting consequences of vitamin A deficiency are found in the incisor teeth of rats and guinea pigs because these structures grow at a fairly rapid rate throughout the life of the animal and because the leading rôle in the organization of the tooth at the formative end is played by the enamel organ, an epithelial structure which in vitamin A deficiency atrophies and undergoes keratinizing metaplasia.[7] The enamel organ atrophy is followed by atrophy and loss of polar deposition of dentine matrix on the part of the odontoblasts—cells of mesenchymal origin. The odontoblasts remain morphologically normal and functionally active on the labial side of the tooth in apposition to the enamel organ long after complete disappearance upon other surfaces. (The enamel organ is found on the labial side of the tooth only, except at the formative end of the tooth, where it exists as a sheath extending about 1 mm from the base.) With complete enamel organ atrophy in the rat, the odontoblasts disappear also on the labial side. For a considerable time, however, the odontoblasts survive. They lose their columnar shape but continue to deposit dentin, no longer restricted to the outer pole, but in centrifugal fashion like osteoblasts. Therefore, we have characterized the odontoblast as a polarized osteoblast and regard the enamel organ as the polarizing agent. Ultimately in complete A deficiency, all activities of the odontoblasts cease, formation of dentin stops, and inclusions of enamel epithelium occur through plication occasioned by stress of the imperfect dentine. The inclusions are carried forward as the tooth grows.

In repair following vitamin A administration, the organizing influence of these enamel epithelium inclusions is shown by the formation of odontoblasts from adjacent connective tissue cells of the pulp. The recent production, by means of long-continued vitamin A deficiency by Burn, Orten and Smith, of Yale, of tumor-like formations and tooth duplications at the formative end of rat incisors reveals much more strikingly the possibilities of this technique for the study of sequences of cell differentiations and organogenesis.[8]

Whereas vitamin A deficiency shows the dependence of odontoblasts upon the enamel epithelium, complete atrophy of the odontoblasts in vitamin C deficiency has no effect upon the enamel organ. Enamel organ changes, attributed to scurvy, are caused by trauma due to the loosening of the teeth consequent to resorption of anchoring structures, fibrous and bony.

In guinea pigs in vitamin A deficiency, the rapidity and completeness of enamel epithelium atrophy are greater than in white rats. Before the atrophy is complete, globules of calcified material are deposited between the enamel organ papillae and upon the tooth surface of the organ. This occurs less strikingly in rats. In guinea pigs, such deposits may pile up until broad sheets of cementum-like material are formed. I am certain that this deposit does not begin in dead cells. It is found in the region of greatest vascularity of the enamel organ. The cells directly responsible for the elaboration of a specific calcified structure enamel are partly or completely inactive. A reasonable explanation of the deposit of calcified material is that capillaries continue to deliver the inorganic ingredients of enamel to the tissues and thus give evidence for a selective permeability of the capillary walls or of functional activity on the part of the endothelium. The high mineral content of enamel and its rapid growth rate present problems concerning concentration that make such speculations attractive.

Another consequence of vitamin A deficiency, but common to any athrepsia which may prove useful in

[7] S. B. Wolbach and P. R. Howe, *Am. Jour. Path.*, 9: 275, May, 1933.

[8] A. N. Orten, C. G. Burn and A. H. Smith, *Proc. Soc. Exper. Biol. and Med.*, 36: 82, February, 1937.

studies of endochondral bone growth is that growth of bone ceases because of cessation of proliferative activity of the epiphyseal cartilage. A narrow band of atrophic cartilage is the result which becomes bounded by a thin plate of bone on the diaphyseal side duplicating conditions also normally found in adult rats. In recovery from vitamin A deficiency, the cartilage regenerates, blood vessels from the diaphyseal marrow penetrate the limiting bony plate, and normal endochondral bone formation is resumed. Resumption of bone growth may be induced in vitamin A deficient rats who have become chronologically adult while under the deficient regimen. Of course, the effect may be due to release of hormones as a part of the secondary recovery phenomena.

Finally, in the consideration of vitamin A deficiency, I mention that, associated with the anemia, there is in comparison with other vitamin deficiencies a heavy deposition of hemosiderin in the liver and particularly in the spleen. In recovery, following an outburst of erythroblastic activity in spleen and bone marrow, the hemosiderin rapidly disappears from the organs, which may be regarded as presumptive evidence that the stored iron is utilized.

VITAMIN C DEFICIENCY

In 1926, Howe and I characterized the condition of scorbutus as the inability of the supporting tissues to produce and to maintain intercellular substances.[9] These conclusions were reached through histological studies of human infantile scurvy and largely through studies of the histological sequences of progressing scurvy in growing guinea pigs and of the repair following administration of vitamin C in natural forms. Subsequently, further verification that vitamin C was the only missing factor in scorbutus, concerned in the inability of the tissues to produce intercellular material, was obtained through the study of reparative processes following the oral and parenteral administration of crystalline ascorbic or cevitamic acid.[10] The intercellular substances requiring ascorbic acid for their formation and maintenance are the collagen of all fibrous tissue structures, the matrices of bone, dentine and cartilage, and probably all non-epithelial cement substance, including that of the vascular endothelium. The relation of ascorbic acid to elastic tissue has not been studied. The reparative proliferative powers of epithelial cells, endothelium, fibroblasts and osteoblasts are not impaired. The mechanism of calcification is not interrupted.

Cells which produce intercellular substances may undergo striking morphologic changes. Such cells, notably the freely dividing osteoblasts in periosteum and at sites of endochondral ossification, migrate away from recently formed bone and assume the appearances of young fibroblasts. In long-continued partial vitamin C deficiency in animals receiving inadequate rations of ascorbic acid, striking accumulations of connective tissue cells may build up, notably at attachments of muscles to bones and fasciae. This I interpret as a compensatory hyperplasia, occasioned by mechanical weakness due to diminished collagen production.[11]

As a tool of investigation, I have used C deficiency and repair to study the manner and source of collagen formation, taking advantage of the fact that the repair of blood clots in guinea pigs in absolute scorbutus is by avascular organization and that fibroblasts wander far from their sources and remain isolated in the blood clot.[12] No collagen is formed until ascorbic acid is administered. This affords means to study the appearances and staining behavior of collagen at various periods following the initial deposit. It appears first as a homogeneous material in which argyrophile or reticulum fibrils promptly follow. Coincidentally, with the appearance of the argyrophile fibrils, the stains in common usage for demonstrating collagen show the presence of collagen fibril bundles. The distribution of the collagen is dependent upon the form of the cell and with isolated cells is confined to zones immediately adjacent to the cell body and its processes, including the entire length of fibroglia fibrils.

The course or direction of the collagen and argyrophile fibrils is parallel to surfaces of the fibroblast and its processes. Because of this arrangement and the absence of a radiating pattern, collagen fibril formation must be influenced by factors unlike those operative in the formation of fibrin strands. The pattern of fibrils formed rapidly in groups of cells without processes suggests that it is determined by the resultant of forces acting in the homogeneous or amorphous stage of collagen formation, presumably influencing the alignment of the elongated collagen molecules. The sequences of collagen formation and the appearance of the fibroblasts, before and after the effect of ascorbic acid is apparent, indicate that collagen is a secretory product of the cell and is laid down extracellularly. Neither fibrin nor any other surrounding material enters into its composition, as has been claimed by various investigators.[13]

In the repair, or rather the resumption of normal growth of bone, the sequences of bone matrix or osteoid formation can be followed. The first appear-

[9] S. B. Wolbach and P. R. Howe, *Arch. Path. and Lab. Med.*, 1: 1, January, 1926.

[10] V. Menkin, S. B. Wolbach and M. F. Menkin, *Am. Jour. Path.*, 10: 569, September, 1934.

[11] Unpublished.

[12] S. B. Wolbach, *Am. Jour. Path.*, 9: Supplement 689, 1933.

[13] J. Nageotte, "L'organisation de la matière dans ses rapports avec la vie." Felix Alcan, Paris, 1922.

ance of the matrix are identical with those of collagen formation, including the formation of fibrils. Subsequently, the material characterizing osteoid is added. By observation of the character of intercellular material deposited about the cells in the "gerüst mark" zone of scorbutic bones, it has been possible to show that these cells having the appearance of immature fibroblasts are in reality osteoblasts, in corroboration of my observation that, in the development of scurvy, osteoblasts applied to newly formed bone trabeculae of the primary spongiosa of endochondral bone formation assume the morphology of fibroblasts and migrate toward the marrow.

The most striking and immediate effect of C deficiency in incisor teeth of guinea pigs is upon the odontoblasts. Dentine matrix formation becomes atypical. An excessive amount of material is produced. This material for a time is probably a rarefied matrix; later the appearances indicate that it is a liquid. The pulp becomes shrunken and the layer of atrophic odontoblasts becomes much folded and widely separated from the old dentine. Following the administration of vitamin C, the gap between the wall of dentine and the shrunken pulp rapidly becomes filled with dentine matrix. The layer of new and normal-appearing dentine matrix formed within a few days may exceed in thickness the original tooth wall.

In bone as well as in incisor teeth we see evidence that in complete vitamin C deficiency, cells concerned in the formation of matrices continue to secrete a material which is liquid in character. This is our interpretation of the edematous appearance of the "gerüst mark" zone in experimental scurvy. The volume and rapidity of osteoid formation (about osteoblasts which have masqueraded as fibroblasts) during repair in this zone are as impressive as are the reparative sequences in teeth. Further support to this interpretation of observed sequences is that the fibroblast in the avascular organization of lesions in absolute scurvy are vacuolated with a material faintly stainable by the anilin blue method. Many observations lead to the conclusion that the rôle of ascorbic acid in synthesis of intercellular materials concerns the gelling quality of a product which is liquid up to its passage through the cell wall. In absolute scurvy this product remains liquid and may gel promptly when recovery is instituted. It should not be beyond ingenuity to put this hypothesis to direct test. As far as can be judged from microscopic appearances, the amount of intercellular substances formed suddenly in repair of absolute scurvy and in long-continued incomplete C deficiency bears a quantitative relationship to the amount of ascorbic acid administered.

That vitamin C is concerned in the maintenance of intercellular materials may be inferred by the lesions of scurvy in consequence of weakness of fibrous tissue structures and the occurrence of osteoporosis independently of osteoclasis and in the presence of undisturbed Ca : P metabolism. Study of bones undergoing osteoporosis in absolute scurvy has offered evidence that some liberated bone cells survive and increase in size and acquire appearances usual to fibroblasts. Fibroglia fibrils can be seen in enlarged bone canaliculae and to extend into the enlarged marrow cavity. Traces of matrix extending inward from the cortical bone lie parallel to fibroglia fibrils. Thus we can see in reverse order what takes place in the formation of bone.

Without intention, the study of vitamin C deficiency and its recovery phenomena in bone provided almost ideal conditions for testing some of the most important hypotheses of Leriche and Policard.[14] These authors believe that the osteoblast is a degenerated connective tissue cell. They deny that the osteoblast contributes to the formation of bone matrix. Bone matrix, their pre-osseous substance, is collagen ("connective tissue substance") modified by union with a material ("interstitial lymph") of humoral origin. Osteoblasts, wherever situated, including the periosteum, serve to limit or confine bone formation. No secretory product of cells contributes to the formation of bone matrix. They admit release of enzymes from degenerating (osteoblasts) cells, as a possible factor in matrix formation from collagen and humoral substances. Our observations, as outlined above, are in complete disagreement regarding the functions of osteoblasts and bone cells, periosteal bone formation and source of bone matrix.

While cartilage matrix formation ceases in absolute scorbutus and the density of epiphyseal cartilage undergoes marked changes, the responses to the deficiency and to repair are less prompt than with other matrices. Argyrophile fibrils do appear in the first matrix formed during repair. As far as I can determine, no appreciable amount of fibrillary collagen is laid down as in bone formation. Fibroglia fibrils do not persist and I doubt if they occur at any stage in the development of the cartilage cell from the perichondrium or from cells of an early callus *following* fracture.

Extramedullary hematopoiesis is not an uncommon finding in the experiences of pathologists. In the scorbutic guinea pig it occurs in the neighborhood of extensive hemorrhages of spontaneous origin, notably in the wall of the urinary bladder beneath the mucosa. In tissues adjacent to blood clots formed after excision of skeletal muscle, islands of erythroblastic cells are found about capillaries and apparently are derived

[14] R. Leriche and A. Policard, "Les Problems de la physiologie normale et pathologique de l'os." Masson et Cie., Paris, 1926.

from vascular endothelial cells which accumulate in consequence of failure of capillary formation. The erythroblastic activity is increased after administration of vitamin C. More intensive studies than I have made are necessary to establish beyond doubt that the capillary endothelium has embryologic potentialities and is the source of blood-forming cells.

VITAMIN D

The rôle of vitamin D, whether or not identical with the pure substance, calciferol, or viosterol, in the processes concerned in the deposition of calcium phosphate in bone matrix 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 phosphorus in the diet prevent rickets, yet vitamin D cures rickets produced in the rat by dietary methods. Likewise, restoration of a proper calcium and phosphorus intake is curative. According to Shohl, "The essentials for the production of rickets are an inadequacy of vitamin D accompanied by a relative deficiency of calcium or phosphorus or an absolute deficiency of either or of both."[15]

Rickets is exhibited in two striking ways. First in importance is the chemistry involved in the calcification of bone in which vitamin D is operative in maintaining normal concentrations of calcium and phosphorus in the blood. The second is the interruption of cartilage cell sequences necessary to the endochondral growth of bone and logically would seem to be a consequence of the first. Rickets also illustrates beautifully a deficiency condition in which the retardation or suppression of normal processes is expressed by strikingly obvious morphological changes. Also, as in scurvy, there are quantitative relations between morphological effects and intake of the essential dietary factors, by which the degree of the deficiency can be estimated or gauged. In each of these deficiencies, the characterization in terms of interrupted sequences explains completely the gross and microscopic pathology. I shall refer only to the morphological aspects of rickets.

The sequences in endochondral bone formation which are disturbed in rickets may be stated briefly as follows. The epiphyseal cartilage, at the upper end of the tibia for example, in normally growing white rats consists of a narrow plate of cartilage firmly supported by bone on the epiphyseal side and uniformly penetrated by blood vessels of capillary dimensions on the diaphyseal side. Very little evidence of growth is present at any one time 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 at a corresponding rate on the diaphyseal side. The cavities occasioned by the degeneration and disappearance of the cartilage cells at the diaphyseal end of the columns are invaded by capillaries accompanied by cells (osteoblasts) which are responsible for the deposition of bone matrix upon the exposed cartilage matrix. Endochondral growth of bone is thus achieved by a continuously retreating gap in the continuity of tissues, a gap maintained on the epiphyseal side by a continuous renewal of cartilage cells and repaired on the diaphyseal side by vascular ingrowth comparable to repair of a defect of tissue by the process of organization or granulation tissue formation. Since the ingrowth of capillaries is secondary to or dependent upon the degeneration of cartilage cells, the advantage of the columnar arrangement of the latter is apparent as a factor in securing orderly growth of bone. In normal growth there presents, on the diaphyseal side of the marrow epiphyseal cartilage, a continuous layer of clear or empty cartilage cells forming an almost straight line.

The first histological evidence of rickets is the absence in whole or in part of the layer of clear cells and the consequent absence of ingrowth of capillaries. The thickness of the epiphyseal cartilages increases because the normal cycle of proliferation and degeneration of the cartilage cells has been interrupted at the end stage. Proliferation and differentiation continue to maturity, but there is no degeneration and hence no opportunity for capillaries to enter. Death of the cartilage cells is an essential to growth of bone, yet this complete degeneration is differentiation carried to an extreme, for it is of indispensable service. Not only does it permit the ingrowth of capillaries, but it is attended by the deposition of calcium salts in the surrounding matrix. Studies of calcification of epiphyseal cartilage in normal growth and in the repair of rickets shows that the calcification of the "provisional zone" takes place coincidentally with the degeneration of the cartilage cells and only in the matrix surrounding cells which show very evident morphological aspects of degeneration.

The dependence of endochondral bone formation upon proliferation, differentiation, and degeneration ending in death and disappearance of the cartilage cells illustrates and epitomizes *cytomorphosis* in its four essential stages as defined by Minot.[16]

We may then contemplate endochondral bone for-

[15] A. T. Shohl and S. B. Wolbach, *Jour. Nutrition*, 11: No. 3, 1936.

[16] C. S. Minot, *The Popular Science Monthly*, August, 1907.

mation as expressive of the general cytomorphic law operative throughout embryonic development and the whole life of the organism. To quote Minot: "Cytomorphosis, the succession of cellular changes which goes on in the body, is always progressive. It begins with the earliest development, continues through youth, is still perpetually occurring at maturity, and in old age. The rôle of the last stage of cytomorphosis, that is of death in life, is very important, and its importance has only lately become clear to us."

Other than rickets and its repair, I know of no clean-cut method of manipulating cytomorphic sequences.

In vitamin A deficiency, in the keratinizing metaplasia, we see the substitution of one expression of this law for the usual. In rickets, apparently through changes in the calcium and phosphorus concentration in the blood, we suspend cytomorphosis and no mechanism appears for the disposal of superfluous cartilage cells. Perhaps the accumulation of osteoid (bone matrix) which occurs around capillaries in the diaphysis adjacent to the cartilage is another expression of suspended cytomorphosis for normally only a small part of the primary spongiosa of bone survives. One may speculate that non-calcified bone matrix has, in an ontogenetical sense, never given occasion for the development of a process of removal and hence in rickets it remains until it has fulfilled its destiny of calcification, then removal of the excess is initiated.

Resumption of cytomorphosis is the first visible effect in the repair of rickets and is shown by the degeneration of the cartilage cells on the diaphyseal border. These probably are the oldest cells, they are cells in closest proximity to blood vessels, and therefore the first to be influenced by a change in chemical environment. This reparative effect takes place promptly and is clearly evident within twenty-four hours. Also, within twenty-four hours there is calcification of the matrix lateral to these cells and ingrowth of blood vessels into the empty cartilage spaces has begun.

The first osteoid to become calcified in repair is that laid down in the resumption of normal sequences. The osteoid that has accumulated in the diaphysis during the deficiency later becomes calcified and then only is mostly removed by osteoclasis.

It would be interesting to follow the effects of mechanical stresses upon the pattern produced by osteoclasis in the removal of the calcified excess osteoid in repair of advanced rickets. The rapid rate of the renewed growth of bone makes it seem possible that we have at hand a convenient tool for the study of effects of stresses in the architecture of cancellous bone.

The degree or severity of rickets may be estimated and recorded on the basis of the prominence of anatomical changes demonstrable by roentgenograms or histological study. Obviously, two factors enter into the production of the pathological picture, the duration of the deficient diet and the degree of the deficiency as measured by calcium and phosphorus blood concentrations and ratios. Either factor can be made the variable and thus the time factor can be calibrated against the chemical factor, in so far as increased width of epiphyseal cartilage and accumulation of osteoid are concerned.

DISCUSSION

I have sketched the important morphological consequences of the vitamin deficiencies I know best. I have tried to learn about others and have refrained from presenting bits of information about them which, though interesting, are not now amenable to correlation with known normal sequences.

Vitamins must have other activities than those expressed by morphological changes, but those I have considered are undoubtedly concerned with the maintenance of structure. Biochemistry and morphology meet in the field of vitamin research, and together promise new progress in the understanding of the organization of the cell and of tissues.

Of value in using vitamin deficiencies as a method of investigation is the fact that in repair the released activities for a time proceed at a rate greater than the normal. This is proved by the measured rate of increase in size and weight in recovery from A, B_6, and lactoflavin deficiencies and is very evident in the histological sequences in recovery from C and D deficiencies. Details of growth of bone, for example, are easier to follow than in the normal because of the rapidity of processes and the accurate correlation in time that is possible.

Thus far only the obvious morphological consequences of the vitamin deficiencies have been studied by simple techniques. Minute cytological studies will probably bring to light much new information of value. Experimental scurvy and rickets, I believe, offer to the combined attack of biochemist and pathologist the best approach to urgent problems in the physiology and pathology of intercellular substances and in particular, collagen.

Modification of form and function of cells in vitamin deficiency may be of value as premises in problems, hitherto approached only by the methods of experimental embryology, concerning the differentiation of tissues and perhaps the ideas expressed in the term "dependent differentiation" as contrasted to "self-differentiation."[17] Hormones have forced their way into the horizon of embryologists and vitamins will follow, for they too are agents having profound effects upon cells designed to respond. Vitamins are essential for the conversion of metabolites into energy and

[17] P. Weiss, *Physiol. Rev.*, 15: 639, October, 1935.

secretory products, and hence indirectly as well as directly are concerned in maintenance of normal structure. Conceivably the "fields" under vitamin influence include cells other than those in which vitamins operate.

I acknowledge a hazy embryological perspective and admit my vagueness of ideas, yet it seems to me that the morphological responses to vitamin deficiencies should in a very limited way influence thought in research upon embryonic organization and, in particular, postnatal differentiation of tissues. More limited perhaps in possibilities for such purposes than the hormones, the vitamins offer more direct attack or easier isolation of phenomena in forms amenable to research.

A number of problems involving cells and tissues primarily affected by vitamin deficiencies can be made easier of approach by making use of the facts that we can retard, suppress and release at will some tissue activities, including growth of cells, source, maintenance and formation of intercellular materials, calcification of bone and cartilage, compensatory hyperplasias, and other applications, all of which have been mentioned in their appropriate setting.

The observations submitted may seem trivial in relation to the broad front of attack upon the problems indicated, but none the less they are interesting and thought-provoking. For the *how* of vitamin deficiency consequences, demonstrated morphological sequences only can be offered; for the *why,* retreat to two refuges or expedients I have long employed in teaching—certain invariable histological sequences in pathology and the conviction that all pathological processes subsequent to injury recapitulate normal events of growth.

AWARD OF THE MEDALS OF THE ROYAL SOCIETY[1]

By Sir WILLIAM BRAGG
PRESIDENT OF THE ROYAL SOCIETY

SIR HENRY DALE is awarded the Copley Medal. His most important contributions to physiology and pharmacology lie in two different but closely related fields: (1) the isolation of certain chemical substances, notably histamine and acetylcholine, from animal tissues, and (2) the discovery of the part played by these in a large number of important physiological and pathological processes.

His earlier work (1905–11) on the active principles of ergot led to progress in many allied subjects. The study of histamine, isolated from ergot extract and later found as a normal constituent of certain tissues, has modified profoundly our views of the capillary circulation and of the conditions known as "wound shock" and "anaphylactic shock." In 1914 he became interested in the choline esters, and with extraordinary prescience singled out acetylcholine as the most interesting member of the series and pointed out the extreme likeness of its action to that of stimulating the parasympathetic.

In 1924, Loewi demonstrated that a substance indistinguishable from acetylcholine is liberated by the heart when the vagus nerve is stimulated. The researches of others, prominently among them Dale himself and his colleagues, have since shown that acetylcholine is liberated at many other junctions between conducting tissues, and the results with acetylcholine and adrenaline are embodied in the description of nerves as "adrenergic" and "cholinergic." Recently convincing evidence has been given by Dale and his collaborators that acetylcholine plays an important, possibly an essential, part in the transmission of impulse from nerve to voluntary muscle: a discovery which has direct practical bearings on muscular fatigue and in various pathological conditions, and also is of the greatest interest [illegible]he theory of the mechanism of the nervous and n[illegible]omuscular systems.

As director of [illegible]e National Institute for Medical Research, Dale h[illegible]s inspired and directed a wide variety of investi[illegible]tions outside his special field, and numerous invest[illegible]tors from many countries have worked under his guidance.

A Royal Medal is awarded to Professor Nevil Vincent Sidgwick. He has always been primarily interested in the causes which determine molecular structure, and his earlier experimental work chiefly dealt with such subjects as tautomerism, and the vapor pressures, boiling-points and solubilities of isomerides. The development of the conception of the nuclear atom, more particularly by Bohr and Moseley, made possible for the first time a quantitative treatment of chemical valency other than purely formal, and the first steps in this direction were taken by Langmuir, G. N. Lewis and Kossel during, or just after, the war. Others followed with theoretical or physical extensions.

Sidgwick's post-war experimental work has all been concerned with particular problems of structure, utilizing to the full available physical methods of attack. To take a few examples, he has shown the existence

[1] Made at the anniversary meeting, Burlington House, London, November 30, 1937.