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The effect of vitamin A on tissue structure

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In animals vitamin A exerts a powerful influence over the functional activities of a wide range of organs including the eye, the nervous system, the respiratory tract, certain glands, the reproductive organs and the skeleton; either its deficiency or its excess in the diet has severe and sometimes fatal effects (for a comprehensive account of the physiology and chemistry of vitamin A see Moore, 1957). I propose to deal with only one limited aspect of the subject, namely vitamin A as a morphogenic agent, and to indicate briefly how the structure of certain tissues depends on the amount of vitamin A available to them. The tissues that respond most dramatically to the influence of the vitamin are the cartilage and bone of young animals and various types of secretory epithelium.

Effects on cartilage and bone

In vivo. The young skeleton is a very plastic structure, and as the animal grows it is being continually remodelled by removal of bone from some regions and fresh deposition in others, according to a definite pattern. In the limb bones and some other parts of the skeleton, growth in length takes place by means of a delicately balanced mechanism, the epiphysial plate, which is a plate of cartilage interposed between each of the terminal ossification centres or epiphyses and the shaft or diaphysis. On the diaphysial surface of the plate the cartilage is constantly being excavated and replaced by bone, but this loss is made good by multiplication of the

cartilage cells near the epiphysial surface. In later life the epiphysial plate disappears and growth in length then ceases.

The equilibrium that is normally maintained between the deposition and resorption of bone during remodelling and between the proliferation and replacement of the cartilage of the epiphysial plate during growth in length can be profoundly disturbed by too little or too much vitamin A. Let us first consider the changes produced in the growing skeleton by vitamin A deficiency. In the skull and spinal column, the normal pattern of remodelling is drastically altered (Mellanby, 1944, 1947); the resorption of bone is diminished while its deposition continues, and in addition the normal sites of resorption and deposition may be completely reversed. Severe deformities (Pl. 1*a,b*) result which in turn cause degenerative changes in the brain, spinal cord and nerves. In the limb skeleton also (Wolbach, 1947) resorption of bone is reduced while the apposition of new bone goes on, and at the same time the epiphysial plate atrophies so that growth in length ceases; as a result of these changes, the limb bones become abnormally short and thick. If the effects of the deficiency have not progressed too far, they can be reversed by restoring vitamin A to the diet.

The administration of a large excess of vitamin A is equally injurious to growing bones (Wolbach, 1947) but in a different way. Resorption is greatly increased so that the bone becomes fragile and may fracture spontaneously; new bone is still formed, sometimes profusely, but, being mechanically much weaker than the mature bone that it replaces, it cannot prevent fractures. In the epiphysial plate excavation of the cartilage at the diaphysial surface and maturation and degeneration of the cartilage cells are accelerated without a corresponding increase in cell division, so that the plate becomes abnormally thin and growth in length of the bone is retarded.

Recently Lewis Thomas and his colleagues (personal communication) have demonstrated striking changes in the matrix or ground substance of the cartilage of young rabbits fed with excess vitamin A. One of the main components of the ground substance is chondroitin sulphate which in histological sections causes the matrix to stain a deep purplish red with such stains as toluidine blue, a staining reaction known as metachromasia. Under the influence of excess vitamin A this substance is rapidly lost, especially from the epiphysial plate and the tracheal rings; the ear cartilage also is affected so that the ends of the ears droop. In histological sections the matrix no longer gives the characteristic metachromatic staining.

All the changes I have described are quickly reversed when the animal is returned to a normal diet.

In vitro. At first it was not known whether excess vitamin A acted directly on the skeleton or whether its action was mediated through the endocrine glands. To answer this question, the limb bones of mouse foetuses at a late stage of development were isolated and grown in culture on a nutritive medium with and without the addition of excess vitamin A (Fell & Mellanby, 1952). For these experiments natural or synthetic vitamin A alcohol was used, dissolved in ethanol and added to the culture medium to give a concentration of 10–15 i.u./ml medium (+ A medium); controls received the same quantity of ethanol without the vitamin. These late foetal bones, though small, are well developed with large cartilaginous ends and a stout bony

shaft enclosing a marrow-cavity. After a few days in the +A medium, the explanted bones began to show changes similar to but more exaggerated than those seen in young hypervitaminotic animals (Pl. 1c,d). The terminal cartilage shrank, the matrix lost its characteristic metachromatic staining reaction, and the cartilage was rapidly excavated on its diaphysial surface by cells from the marrow-cavity. Meanwhile the bony shaft was resorbed with extraordinary speed. After about a week all that remained of the explant was a sheet of actively growing unorganized cells in which lay a few fragments of bone and cartilage. Control bones grown in normal medium showed none of these changes.

Attempts were made to shed some light on the biochemical effects produced in explanted cartilage by excess vitamin A. The chondroitin sulphate of normal cartilage matrix contains a considerable proportion of inorganic sulphate. The cartilaginous limb-bone rudiments of 6-day chick embryos were therefore grown in normal and in +A medium and treated with inorganic [³⁵S]sulphate (Fell, Mellanby & Pelc, 1956); the bones were then fixed and sectioned and autoradiographs were prepared by covering the slides with photographic film and leaving them in the dark for a few weeks. When such preparations are developed the distribution of radioactive material in the sections is shown by the blackening of the overlying film. The control rudiments in normal medium readily took up the sulphate, most of which was retained in the matrix when the explants were transferred to unlabelled medium. In the +A medium, on the other hand, the cartilage first failed to incorporate new sulphate and then, as the changes produced by the vitamin advanced, the sulphate already present in the matrix was lost; the loss of the sulphate went parallel with the disappearance of the metachromatic staining reaction of the ground substance.

Comparison of the effects of papain and of vitamin A in vivo and in vitro. Thus the removal of chondroitin sulphate from cartilage matrix is a characteristic effect of excess vitamin A both in the animal and in organ cultures. Recently Lewis Thomas and his co-workers (Thomas, 1956; McCluskey & Thomas, 1958) found that the proteolytic enzyme papain, when injected into young rabbits, also removed the chondroitin sulphate from all the cartilage of the body, causing the ears to droop like a spaniel's. The histological effects on the cartilage matrix of a small dose of papain were almost indistinguishable from those of excess vitamin A (Lewis Thomas, personal communication). Similar changes were produced (Fell & Thomas, unpublished) on the cartilage of late foetal mouse bones grown in medium containing 20 mg crystalline papain protease/ml. In this respect the effect of the protease resembled that of excess vitamin A, but there was one important difference: papain produced no obvious change in the bone, whereas the vitamin caused its rapid and almost complete resorption. When both agents were present in the culture medium, they had a drastic additive effect on the cartilage, but resorption of the bone was not accelerated by the protease.

Dr Lewis Thomas and I are now considering the possibility that vitamin A may enhance the hydrolytic activity of a number of cellular enzymes, one of which resembles papain protease in its action. I must emphasize that this idea is merely a working hypothesis.

Effects on epithelia

In vivo. We come now to the second morphogenic action of vitamin A: its influence on the structure of certain epithelia (Mori, 1922; Wolbach & Howe, 1925). One of the most obvious and disastrous effects of severe vitamin A deficiency is on the cornea; it becomes increasingly opaque and, when examined microscopically, the epithelium is seen to have developed a horny layer of keratin on the surface like that of the normal epidermis. An extreme example of this transformation has been described by Schmidt (1941) in a calf born of a vitamin A-deficient cow: in the centre of one cornea, a patch of thick hair had been formed. The mucous, ciliated epithelium of the trachea and bronchi undergoes a similar epidermoid change. The basal germinative cells multiply and form a stratified, keratinizing epithelium beneath the ciliated and mucous elements which degenerate and are shed (Pl. 1e,f). Similar effects are produced in the salivary glands, bladder, ureter, seminal vesicles, kidneys and some other organs. If animals are returned to a normal diet, the epithelial transformation is reversed.

Excess vitamin A produces the opposite effect in the vaginal epithelium. In the normal animal this epithelium keratinizes at a certain stage of the oestrous cycle, and at a later stage the keratinizing cells are shed and replaced by mucous elements. This keratinization, which is caused by the secretion of oestrogen, can be overcome by large doses of vitamin A both in the body (Hohlweg, 1951) and in organ cultures of the vagina (Kahn, 1954).

In vitro. A similar reversal of differentiation can be produced by growing embryonic chicken skin as organ cultures in a medium containing excess vitamin A (Fell & Mellanby, 1953; Weiss & James, 1955; Fell, 1957). In normal medium the epithelium develops into a squamous, keratinizing epidermis, but in the presence of excess vitamin A keratinization is prevented and a mucus-secreting epithelium, which may even contain ciliated cells, is formed (Pl. 1g,h). C. B. McLoughlin (unpublished) has shown that this mucous change is a direct effect of the vitamin on the epithelium, since it can be produced in the absence of the dermal connective tissue in a pure culture of embryonic epidermis.

Experiments have been made (Pelc & Fell, 1960) to try to identify by autoradiography some of the earliest metabolic changes that accompany this mucous metaplasia of the embryonic epidermis. Explants of skin from the legs and feet of 13-day chick embryos were used. Most of the observations were made during the first 2–3 days' growth by the autoradiographic method described for the bone rudiments. Of the amino acids investigated, leucine and methionine were taken up equally well by the epidermis in both normal and +A medium, but the vitamin reduced the incorporation of tyrosine in the superficial, though not in the deeper, layers and greatly diminished that of cystine throughout the epithelium. These effects of vitamin A are probably correlated with the inhibition of keratinization for which both tyrosine and cystine are extensively utilized.

Animals incorporate inorganic sulphate into sulphated mucopolysaccharides, but not into amino acids. Since the mucous transformation of the epidermis involves

changes in the production of mucopolysaccharides, the uptake of inorganic [³⁵S]sulphate was studied. In the deeper layers of the epithelium there was little difference in uptake between the +A and control cultures, but in the superficial layers incorporation was very slight in the keratinizing controls, whereas in the +A explants it was at first about the same as in the basal cells, and then, as secretory activity developed, steadily increased to a relatively high level. This observation suggests that in the normal epidermis the basal cells have a mechanism for synthesizing mucopolysaccharides which is blocked by keratinization but not when keratinization is stopped by excess vitamin A.

Although we now know a little about the biochemical changes that accompany some of the diverse morphological effects of vitamin A, we remain profoundly ignorant of the fundamental nature of its action. It seems probable that there must be a factor common to its effects on the structure of cartilage, bone and epithelium, but at present we can only speculate about what this factor may be.

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EXPLANATION OF PLATE

- a* and *b*. Axis and atlas vertebrae of (*a*) normal and (*b*) vitamin A-deficient litter-mate dogs of the same age. The vertebrae of the deficient animal are coarse and blunted and have lost their delicate outlines; the spinal canal is smaller than in the normal vertebrae (Mellanby (1944), by courtesy of the Royal Society).
- c* and *d*. Explanted radii from the same mouse foetus, grown for 4 days in (*c*) medium containing 10 i.u. synthetic vitamin A alcohol/ml and (*d*) normal medium. Both sections stained with toluidene blue. In (*c*) note the shrinkage of the cartilage, loss of metachromatic staining of the peripheral cartilage matrix, and partial resorption of the bony shaft. The control (*d*) has retained its normal appearance and staining properties.
- e* and *f*. Tracheal epithelium of (*e*) a vitamin A-deficient guinea-pig, from a preparation given to me by the late Professor S. B. Wolbach, and (*f*) a normal guinea-pig, showing columnar ciliated cells. In (*e*) the basal cells have formed a squamous, epidermoid epithelium beneath the ciliated mucous elements which are being shed. Haematoxylin and eosin.
- g* and *h*. Skin explants from the shank of a 13-day chick embryo: (*g*) grown for 12 days in medium containing 15 i.u. vitamin A/ml, followed by 2 days in normal medium; (*h*) grown for 14 days in normal medium. In (*g*) the epidermis has become transformed into a mucous membrane with typical goblet cells and is covered by a thick layer of mucin; in (*h*) a squamous, keratinizing epidermis has been formed. Periodic Acid Schiff, Mayer's acid haemalum.

