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# VITAMIN A AND BONE GROWTH: THE REVERSIBILITY OF VITAMIN A-DEFICIENCY CHANGES

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In a series of publications (Mellanby, 1926, 1931, 1933, 1934, 1935) it has been shown that a deficiency of vitamin A in the diet of growing animals causes widespread nerve degeneration, both peripheral and central. Of the cranial nerves, the olfactory, the optic, the trigeminal (first and second divisions) and the auditory (both cochlear and vestibular divisions) are specially liable to suffer destructive changes. For the most part, under the conditions of these experiments, the cranial motor nerves escape. In the spinal cord, afferent nerves having their origin in the posterior root ganglia are often destroyed, together with their neurones passing into the cord. In addition, some of the endogenous ascending fibres are affected, especially the anterior and posterior cerebellar tracts.

A cause of this widespread degeneration was suggested when the examination of serial sections of the labyrinth and its capsule revealed that vitamin A deficiency results in a large bone hypertrophy which injures or destroys the nerve fibres and the cells of the spiral ganglion by pressing on the VIII nerve (Mellanby, 1938). Other observations made it clear that in vitamin A-deficient animals the whole of the nervous system was under greatly increased pressure (Mellanby, 1939*a*); the brain and spinal cord were closely packed into the cranium and spinal canal, and it soon became evident that, because of their abnormal growth, the skull and vertebrae had lost their primary function of protecting the nervous system and had become destructive agents.

Later (Mellanby, 1939*b*, 1941, 1943) descriptions were given of the abnormal bone growth near the spinal and cranial nerve cells, and in the Croonian Lecture of the Royal Society in 1943 a more detailed analysis of the abnormal growth in two positions, namely, the basi-occipital bone and the vertebral column, was presented (Mellanby, 1944). It was shown that the bone dysplasia which resulted from vitamin A deficiency and had such grave effects on the nervous system could be largely, if not entirely, explained by altered osteoclastic and osteoblastic activity.

A question raised in the Croonian Lecture was—how would the osteoclasts and osteoblasts of vitamin A-deficient puppies react if the animals were later given the vitamin? Would the cell activity be again reversed, and, if so, would the normal shape of the bones be restored? In a paper read before the Physiological Society in December 1945, this was answered, in part at least. It was shown that, under the conditions tested, the bone changes resulting from vitamin A deficiency are reversible, and that soon after the addition of the vitamin the osteoclasts and osteoblasts again become active in their normal positions, the osteoclasts especially being very numerous and active, as if making a special effort to remove the superfluous bone laid down or not absorbed in the period of vitamin A deficiency. The bones tend to return to normal, though no definite decision can yet be given as to whether they ever do so completely. Whether the small bone abnormalities seen in adult A-deficient dogs can be corrected by the addition of vitamin A to the diet is not yet known.

The dysplasia due to vitamin A deficiency appears to involve all the bones of the body to a greater or less extent. In previous reports attention has been concentrated mainly on the bones near the nervous system because it is their abnormal growth which causes such devastating effects on the animal's life. In the present publication also the study is confined to bones of the skull and, even so, only a few examples are given to illustrate the different ways in which growth of bone takes place and how these various processes are affected by the absence and restoration of vitamin A. It may be added, however, that sufficient study has been made of the action of vitamin A in the growth of other bones, including the long bones and pelvic bones, to ascertain that the vitamin plays a controlling part in what might be called the moulding of all bones.

An investigation of the present kind cannot be made without revealing some of the complexities of normal bone growth. The chief point of interest in this publication is in the study of the reparative or recovery stage of bone growth, especially of the cranial bones, when vitamin A is restored to an animal after a period of deficiency. The three bones discussed are (*a*) the basi-occipital, (*b*) the labyrinthine capsule and (*c*) the sphenoid bone. They were chosen because they represent three different types of bone growth, and it was thought desirable to describe what happens in each when vitamin A is largely removed from the diet and so ultimately from the animal's body and, secondly, when vitamin A is restored. It was hoped that by studying bones with different responses to the presence and absence of vitamin A it might be possible to get some insight into its mode of controlling bone growth. All that can be said at present is that vitamin A, when given in proper amounts under the experimental conditions adopted, ensures that bone growth or bone moulding proceeds along correct, predestined and safe lines and that, when the vitamin is deficient, growth continues in an orderly but wrong way, which leads to

injury or even destruction of the nervous tissue and ultimately to the death of the animal. The restoration of vitamin A to the diet of the deficient animal quickly brings about a response in the bone cells directed to correct the dysplasia resulting from the absence of the vitamin.

## EXPERIMENTAL METHODS

A full account has been previously given (Mellanby, 1931) of the symptoms which develop in puppies on diets similar to the one used in the experiments now to be described, which is as follows: wheatmeal bread 30–280 g., salt 1–2 g., baker's yeast 5% of cereal, separated milk powder 20–25 g., lean meat 15 g., peanut oil 10 c.c., ascorbic acid 5 mg., vitamin $D_2$ 200–2000 i.u.

The wheatmeal flour from which the bread was made varied between 80 and 85% extraction at different times during the period of the investigation and contained a trace of carotene. Similarly, the meat part of the ration no doubt included a small quantity of vitamin A. The diet was therefore not totally devoid of the vitamin or its precursor.

For the sake of brevity the term +A dog is used to denote one having an abundant supply of vitamin A, and −A dog for one receiving only the basal diet, and recovery dog or −A+A dog for one having at first the basal diet only, and later the same diet supplemented by abundant vitamin A.

The bones of −A dogs become thickened and cancellous as compared with those of +A littermates. To produce compact bones more calcium is needed. For instance, if extra calcium as calcium carbonate (1 g. daily) is added to the above basal diet, the bones of both +A and −A animals are less cancellous than without such addition, but the −A bone is still much thicker than the +A. Calcium is not, therefore, the controlling factor as regards bone hypertrophy (Mellanby, 1944).

In the case of +A diets, it has been usual to add to the basal diet vitamin A (2000–5000 i.u. daily) in the form of a proprietary preparation of mammalian liver oil or as pure vitamin A acetate. This applies both to the control +A dogs and to the recovery animals.

The histological side of this investigation, involving serial sections of large blocks of decalcified bone cut at carefully chosen angles in relation to the nervous tissue, was long and tedious. The blocks were usually embedded in celloidin, one in every ten sections being stained and mounted. One of the difficulties in this work was that bone overgrowth, especially in complicated structures, often made histological examination of identical positions in +A and −A dogs impossible. The difficulty was partly overcome by examining serial sections; for instance, Pl. 6, Fig. 1*a* (+A) shows the internal auditory meatus and the helix of the cochlea, but although Pl. 6, Fig. 1*b* (−A) shows a similar portion of the helix, it is necessary to examine other sections to trace the tortuous course of the internal auditory meatus of this animal.

Most of the results described in this paper refer to three litter-mate puppies, the experimental feeding of which started at the age of 7 weeks and continued in the case of the +A and −A dogs for 26 weeks. In the case of the third dog (referred to as the recovery or −A+A dog) the feeding of the −A diet lasted for 24 weeks and of the +A recovery diet lasted for 23 days, making the experimental feeding period 27 weeks in all.

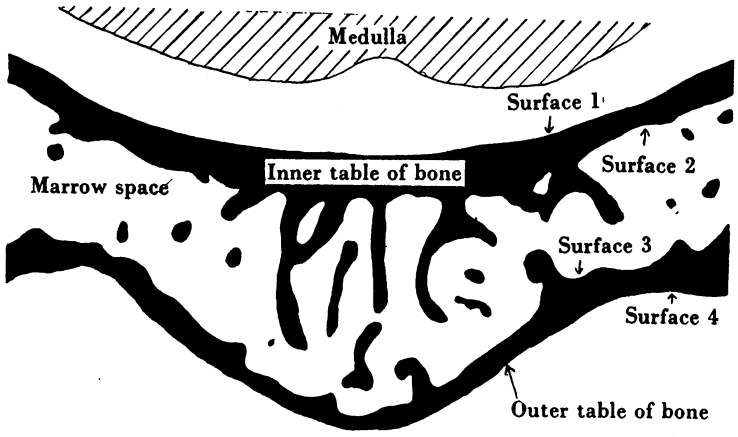
Less complete examinations were made on another three animals. The feeding period of the +A and −A dogs lasted from the age of 6 to 20 weeks, i.e. for 14 weeks, and the third (recovery) animal then had vitamin A added to the diet for 13 days. At the end of the experiment these animals were only 20–22 weeks old, as compared with the first set of three, which were about 33 weeks old at death.

For special points, bone tissues were examined from a number of other experimentally fed dogs, usually in order to see what happened to the bones of dogs of different ages, and in such cases time was saved by embedding decalcified bones in paraffin wax instead of celloidin. Serial sections were not prepared.

Although bone changes in both the recovery animals (−A+A) give similar pictures as regards the reaction of osteoclasts and osteoblasts, the age of the animals greatly affects the cell activity

observed in +A and −A dogs. It is probably not remarkable that this should be so, but the rapidity with which the points of maximum growth vary in bones at different ages was rather perplexing and added greatly both to the difficulty of understanding and of describing the detailed incidents of bone growth.

Reference will often be made to the osteoclasts and osteoblasts. In the case of the osteoclasts it is usually easy to count them in sections of bone when they are active and to judge of their relative activity by their size and shape and by the bone lacunae they form when engaged in removing bone. Early in the work it seemed difficult to understand how bone could have osteoblasts and osteoclasts respectively on opposite surfaces and at a later stage could have these same surfaces covered by the other type of cell. It is now evident that there are either active or inactive bone cells of both types present on most bone surfaces in the period of rapid growth, and that different circumstances, for example, the presence or absence of vitamin A, make a different type of cell active. Flattened inactive (Pl. 1*a*) and enlarged active osteoblasts (Pl. 1*b*) can be seen on many bone particles. Inactive flattened osteoclasts also can be seen on surfaces where, under other circumstances, they are apparently roused to activity and become rounder and more clearly multinuclear in the process. Osteoclasts of both forms can be seen in Pl. 1*c–f*. Occasionally active osteoclasts appear in great numbers on some bone surfaces where there was previously no evidence of inactive osteoclasts. In such a case it might be that these active osteoclasts were either transferred to the surface or possibly formed there from other precursors, i.e. possibly by a fusion of osteoblasts. This latter suggestion is not based on any definite observation.




Text-fig. 1. Diagram of general structure of basi-occipital bone. Surfaces have been numbered to simplify description in text. Surface 1 is the bone surface adjacent to the brain stem; it is separated from the nervous tissue by the closely attached periosteum (dura mater) and other membranes. Surface 2 is the marrow surface of the same inner table of bone. Surface 3 is the marrow surface of the outer table of bone. Surface 4 is the outer surface of the outer table of bone.

## RESULTS

### *Basi-occipital bone*

To simplify the description of this bone, reference will be made where possible to surfaces 1–4. Surfaces 1 and 2 limit the inner table of bone, 3 and 4 the outer table. Surface 1 is adjacent to the central nervous system, 2 and 3 are adjacent to marrow, and 4 is the external surface of the bone (see Text-fig. 1).

In the Croonian Lecture of 1943 it was shown that a −A diet produced a thick cancellous basi-occipital bone, the abnormal thickness of which was caused largely by lack of absorption of surface 1; indeed, actual deposition of additional bone on this surface (Text-fig. 1) could be seen at certain early stages of growth on these deficient diets. It appeared that absorption was greatly reduced or had even ceased because the osteoclasts, normally active and abundant in this position during growth, gave way to active osteoblasts. Conversely, on surface 2 osteoblastic activity had ceased and been succeeded by osteoclastic activity. This is shown in Pl. 2*a*, *b*, photomicrographs of mesial sagittal sections of the basi-occipital bone. Vitamin A deficiency caused but little change on surfaces 3 and 4, so that the outer table of bone continued to grow normally and therefore to withdraw from the inner table, with the consequent enlargement of the marrow cavity and thickening of the bone.

The above facts apply to dogs killed when 20–30 weeks old, which, judged by their behaviour during life, had been depleted of vitamin A stores for 8 or more weeks. In older animals the rate of growth of the basi-occipital bone diminishes and the differences between the number and activity of osteoclasts and osteoblasts on surfaces 1 and 2 in +A and −A dogs, although still evident, are not as obvious as in the younger animals (see Pl. 3*a*, *b*, compared with Pl. 2*a*, *b*).

Drawings of coronal sections of the central portion of the basi-occipital bone of older +A and −A litter-mates, animals which were 33 weeks old at death, can be seen in Pl. 4. In the +A dog (Pl. 4*a*), which had 5000 i.u. vitamin A daily, the inner and outer tables of bone have fused to form a compact structure and the marrow cavity has been eliminated. In the −A dog (Pl. 4*b*) the bone is cancellous, with a large marrow space, and surface 1 is almost flat as compared with that of the +A animal, which is modelled to the natural shape of the brain stem. It will be seen that on this surface in the +A dog there are still abundant osteoclasts which are more numerous in the centre than at the sides, a fact which may account for the hollowing near the midline. In the −A dog, on the other hand, there are osteoblasts at work, but no active osteoclasts. Thus, so far as surface 1 is concerned, the absence of vitamin A, as in the younger animal previously described, has reversed the type of bone cell at work. Surface 2, however, of the older −A dog (Pl. 4*b*) is different from that of the younger animal (Pl. 2*b*) in that there are no active osteoclasts. It is indeed interesting that the fusion of the inner and outer tables of bone in the +A dog appears to be accompanied in the corresponding −A animal with the disappearance of all active bone cells, both osteoclasts and osteoblasts, from surfaces 2 and 3 (Pl. 4*b*). In the case of surface 4, there are only a few scattered active osteoclasts left in the +A animal (Pl. 4*a*), whereas in the −A there are no osteoclasts and only a few active osteoblasts laterally (Pl. 4*b*).

The basi-occipital bone of a litter-mate which was killed at about the same age, having received an A-deficient diet for 24 weeks of the experiment and,

during the last 23 days of its life, vitamin A (5000 i.u. daily), is shown in the photomicrograph, Pl. 3*c*, and in the drawing, Pl. 4*c*. Though this dog is referred to as a recovery animal, it will be seen that the shape (Plate 4*c*) and texture (Pl. 3*c*) of the bone have not become normal; surface 1 is still flat and the bone cancellous. On the other hand, examination of the type and number of active bone cells on the different surfaces shows the great effort being made to bring about restoration of the bone to the normal shape. On surface 1 numerous active osteoclasts are apparently removing bone, while it is being laid down by the very active osteoblasts on surface 2, so that the inner table of bone is retreating to allow greater space for the brain stem (Pl. 4*c* and Pl. 3*c*). Surfaces 3 and 4 are also affected. Surface 3 shows osteoblastic activity, and on surface 4 active osteoclasts are seen, although there are many less than on surface 1.

It will be asked how soon after the addition of vitamin A to the diet the osteoclastic and osteoblastic activity returns to that seen in the young +A animal and whether the shape of the bones can ever be restored to normal. Insufficient evidence is as yet available to answer either of these queries fully, but it is known that 3 days after the addition of vitamin A to the diet cellular changes are negligible, but that after 13 days the osteoclasts and osteoblasts are rapidly correcting the abnormal growth of the period of vitamin A deficiency. This can be seen by comparing Pl. 5*a*, *b* and *c*. The three animals concerned were litter-mates. The +A and −A dogs were killed when 20 weeks old and from this time for 13 days the recovery animal was given 2000 i.u. vitamin A acetate daily. Pl. 5*a* is a photomicrograph of the basi-occipital bone (inner table) in the +A, Pl. 5*b*, in the −A, and Pl. 5*c* in the recovery animal. What the ultimate outcome of these changes in recovery animals will be, it is not yet possible to say. It would seem, however, that a continuation of the activity of the bone cells seen in Pl. 5*c* is calculated to bring about a return to the normal state both as regards the shape of the bone and the expansion of that part of the brain cavity bounded by the basi-occipital bone.

### *Labyrinthine capsule and VIII nerve*

The bony labyrinth is probably fully formed in the young animal before the time of special feeding in these experiments begins (6–7 weeks). At this age also the periosteal bone covering the labyrinth on the side adjacent to the brain is nearly fully formed, as is indicated by the few and inactive bone cells on this surface. On the same surface of the periosteal bone of the labyrinth there are in +A animals when about 20 weeks old a few inactive osteoclasts and inactive osteoblasts, in contrast to the active osteoclasts found on surface 1 of the basi-occipital bone.

In an A-deficient animal of this age these osteoblasts become very active, so that, whereas in the case of the basi-occipital bone the change produced by A deficiency is mainly, although not entirely, due to a failure to absorb bone,

the outstanding character of the same deficiency on the labyrinthine capsule is to produce excessive laying down of bone. As previously shown (Mellanby, 1938), parts of this capsule may in A-deficient animals become so thickened that it largely blocks the internal auditory meatus (Pl. 6, Fig. 1 *a*, *b*). The extra bone formed is, in the younger animals (Pl. 6, Fig. 2*a*, *b*), cancellous with large fatty marrow spaces. Fewer spaces are seen, however, as the animals grow older, until at 33 weeks of age (Pl. 7) the bone is relatively compact even after 26 or so weeks of the A-deficient diet. The marrow spaces of the younger, but rarely of the older, animals sometimes contain a few active osteoclasts.

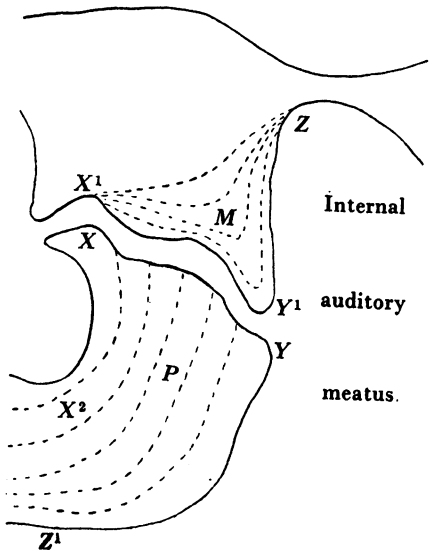
In the −A animals the enlarged periosteal bone adjacent to the brain increases the distance from the helix to the medulla, lengthens the internal auditory meatus and causes stretching of the VIII nerve. Within the internal auditory meatus there is a change in the position of bone cells reminiscent of that of the basi-occipital bone (Pl. 6, Fig. 2*a*, *b*), slight osteoclastic activity on the surface adjacent to the VIII nerve in the +A dog becoming in the −A animal osteoblastic activity with active osteoclasts on the marrow surface (equivalent to surface 2 of the basi-occipital bone). There is a definite deposition of bone within the meatus, which causes compression and, as it is not laid down equally but is found rather in nodules, tortuosity of the nerve.

Pl. 7*a*, *b* are drawings of the labyrinthine capsules of the +A and −A littermates which were 33 weeks old when killed. The increased thickness of the bone in the −A dog (Pl. 7*b*) is clear. There are practically no active osteoblasts or osteoclasts in either series of sections, thus indicating that both normal growth (Pl. 7*a*) and abnormal growth (Pl. 7*b*) have ceased. In Pl. 7*a* the few active osteoclasts seen are on the surface of the internal auditory meatus, which suggests that it is still necessary at this age to have a mechanism at work capable of modifying its size and shape.

If the absence of active osteoblasts and osteoclasts from the labyrinthine periosteal bone in the +A and −A animals of this experiment suggests absence of control by vitamin A, it is only an illusion. Proof of this can be seen in the labyrinthine capsule of the third litter-mate of the same age (Pl. 7*c* and Pl. 6, Fig. 2*c*), which was on a diet deficient in vitamin A for 24 weeks and then received for the last 3 weeks (23 days) of its life 5000 i.u. of vitamin A daily. The reaction on the addition of vitamin A is clearly tremendous, the surface of the periosteal bone being covered with active osteoclasts (Pl. 6, Fig. 2*c*) which are obviously there in an attempt to restore the bone to its normal (+A) shape (Pl. 7*a*).

There appears to be a definite plan in this absorption of excess bone, since great osteoclastic activity is found wherever osteoblasts have been active during the period of A deficiency. Thus the whole surface of the periosteal bone adjacent to the brain and within the internal auditory meatus is covered with active osteoclasts in the recovery animal (Pl. 7*c*). Since all this periosteal bone

overgrowth is due to the excessive activity of osteoblasts during the −A period, this result is in keeping with the general recovery reaction. One point of special interest is seen in the recovery animal, namely, the presence of abundant osteoclasts on a surface marked $X^1$–$Y^1$ in the sketch (Text-fig. 2), and the almost complete absence of these cells from the adjoining surface $X$–$Y$. Although the portion of bone marked $M$ is of periosteal origin, like that marked $P$, it seems probable that the mode of growth of $M$ is rather different from that of $P$. A possible difference is suggested in the sketch, and it will be seen that $M$ may grow in two directions, $Z$–$Y^1$ and $X^1$–$Y^1$, whereas $P$ probably grows in one direction as bony layers deposited on surface $X$–$X^2$. This explanation of the method of bone growth during the −A period (Pl. 7*b*) would allow the appearance of osteoblasts on surface $X^1$–$Y^1$ of the recovery animal (Pl. 7*c*) to be regarded as in line with the usual recovery reaction in regard to osteoclasts.




Text-fig. 2. Sketch of internal auditory meatus and overgrown periosteal bone due to vitamin A deficiency, to illustrate the way in which bone is apparently laid down in $M$ and $P$ (see Pl. 7). It will be seen that in $M$ the bone is added on both surfaces, $Z$–$Y^1$ and $Y^1$–$X^1$. Bone $P$, however, is laid down in the lines indicated, and the surface of growing bone faces in one direction only. It is usual in −A+A animals to see osteoclastic action where, in the −A, osteoblastic action has been predominant. Thus surfaces $Z$–$Y^1$, $Y^1$–$X^1$ and $Y$–$Z^1$ would be expected to show much osteoclastic activity and surface $Y$–$X$ to show little or none.

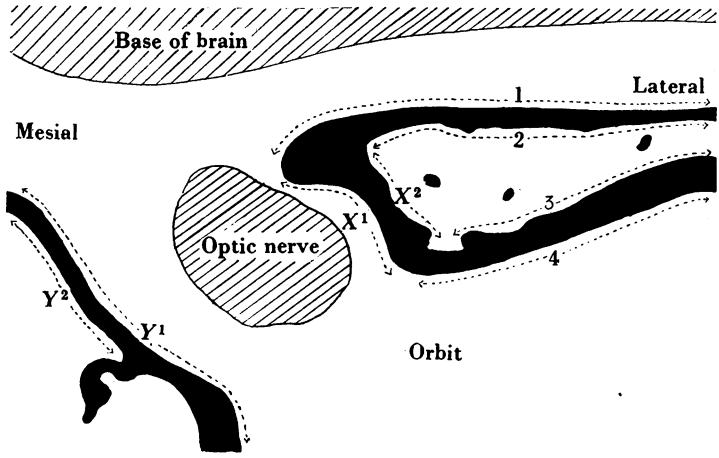
It seems legitimate to assume from this experiment that the apparent absence of active osteoclasts and osteoblasts on the normal labyrinthine capsule at this age does not mean that vitamin A has ceased to control, directly or indirectly, either the bones or the bone cells, but that it holds a guiding hand on these cells so that they produce and mould periosteal bone covering the labyrinth only in amounts and shape compatible with the easy passage of the VIII nerve from the labyrinth to the central nervous system. If, during the absence of the vitamin, the periosteal bone grows so that excessive bone is laid down, then the return of the vitamin to the body not only reverses the process but causes an exaggerated response of bone cells calculated to clear away the excessive bone formation and bring its shape quickly back as near to the normal as possible.

Comparing the reactions of the labyrinthine capsule and the basi-occipital bone in A deficiency, it is seen that the first is an example of excessive bone

formation following the loss of vitamin A, the return of the vitamin causing removal of the hyperplastic new bone. In the second, loss of vitamin A is followed primarily by cessation of removal of bone already laid down, although in the earlier weeks of the experiment there may be in addition a small deposition of new bone; on the return of the vitamin the bone removed by the hosts of active osteoclasts is for the most part bone which, under normal conditions, would have been removed earlier.

*Sphenoid bone and II nerve*

The general structure of this part of the sphenoid is similar to that of the basi-occipital bone. There is an inner bony plate (surfaces 1 and 2) adjacent to the brain and an outer bony plate (surfaces 3 and 4) remote from the brain, but in this case forming part of the orbit of the eye. Text-fig. 3 shows this formation.




Text-fig. 3. Diagram of general structure of the sphenoid bone at the level of the II nerve foramen. Surfaces have again been numbered to simplify description in text. Surface 1 is the bone surface adjacent to the brain stem. Surface 2 is the marrow surface of the same inner table of bone. Surface 3 is the marrow surface of the outer table of bone. Surface 4 is the outer surface of the outer table of bone. Surface $X^1$ is the lateral wall of the II nerve foramen and joins surfaces 1 and 4. Similarly, $X^2$ is the marrow surface joining surfaces 2 and 3. Surface $Y^1$ is the mesial wall of the II nerve foramen and $Y^2$ the marrow surface of the same plate of bone.

The sections to be described are coronal through the centre of the optic foramen, at right angles to the long axis of the head. They therefore show the mesial and lateral bone of the foramen wall, but not the anterior or posterior parts. For ease in description, the bone of the lateral wall of the optic foramen has been divided into surfaces $X^1$ (adjacent to the second nerve and connecting surfaces 1 and 4) and $X^2$ (the marrow surface connecting surfaces 2 and 3). The mesial

wall has been similarly divided into surfaces $Y^1$ and $Y^2$. Pl. 8*a* is a drawing of the optic foramen and sphenoid bone in a + A dog aged 33 weeks. It will be seen that there is no osteoclastic activity on surfaces 2, 3, $Y^1$ and $X^2$, a little on surfaces 1, 4 and $Y^2$, with rather more on surface $X^1$. Active osteoblasts are confined to surfaces $Y^1$ and $X^2$. This would suggest that there is very little growth and moulding occurring at this age, with the possible exception of a little on the walls of the foramen. From the positions of the osteoblasts on surfaces $Y^1$ and $X^2$ and the osteoclasts on $Y^2$ and $X^1$, it is reasonable to suppose that there is still a small lateral movement of the foramen taking place at this age.

Pl. 8*b* is a drawing of a litter-mate of the same age, which had received an A-deficient diet for 26 weeks. It will be seen that the osteoclastic activity has ceased on surfaces 1 and 4 and that osteoblastic activity (deposition) has taken its place on surface 4. Osteoclastic activity is seen on surfaces 2 and 3, so enlarging the marrow space. The absence of vitamin A has therefore brought about a thickening of the bone by reversing the osteoclastic and osteoblastic activity, as has been seen to happen in the case of the basi-occipital bone. The actual mechanism of bone thickening in the present case is different from that described above in the basi-occipital bone. In the latter case it was surfaces 1 and 2, forming the bony plate adjacent to the brain, which were principally affected by the absence of vitamin A. In the thickening of the bone surrounding the optic foramen it is the bony plate remote from the brain (surfaces 3 and 4) which is affected. This thickening of the bone has lengthened the optic foramen. What, meanwhile, has been happening to its wall? All changes of growth, both absorption and deposition, seem to have ceased on surfaces $X^1$ and $X^2$ in the −A animal, while growth processes have continued in a normal way on the lengthened surfaces $Y^1$ and $Y^2$. This would have the effect of narrowing the foramen, for, while the mesial surface would move laterally, the lateral surface would remain stationary, thereby constricting the nerve and vessels passing through the foramen.

If we now examine the recovery animal (Pl. 8*c*), a third litter-mate which was maintained on an A-deficient diet from the age of 7 to 31 weeks and then received 5000 i.u. of vitamin A daily for 3 weeks (23 days), we see that there is a return to the normal as regards position of active cells. Surfaces $Y^1$ and $Y^2$ again show no variation in type of cell, although there may be some increase in intensity of growth. Surfaces 1 and 2, which showed comparatively little change due to A deficiency, also show but little change on the return of the vitamin. The greatest response to the addition of the vitamin is seen on surfaces 3, 4, $X^1$ and $X^2$. There is obviously a great eating away of bone from surfaces 4 and $X^1$, with a correspondingly great deposition on surfaces 3 and $X^2$. It seems obvious that continuation of these processes will restore the foramen to the normal (+A) diameter, reduce its length by reducing the thickness of the sphenoid bone, and correct its position by moving it laterally.

*Notes on other bones*

Of the bones examined, most appear to react in the same way as the basi-occipital, but there are several special regions in which strong osteoclastic activity in the +A becomes reduced osteoclastic activity in the −A, complete cessation of absorption and deposition of bone being uncommon. One of these positions is part of the surface of the vertebral column adjacent to the lateral side of the posterior root ganglion. It was shown in the Croonian Lecture that osteoclasts may remain in this position in the −A animal, and further examinations have confirmed that these cells persist here in a comparatively inactive state when they have completely disappeared from the rest of the surface adjacent to the spinal cord. Reversal from osteoclastic to osteoblastic activity does, however, occur in very severe A deficiency. Recovery animals show strong osteoclastic activity over the whole of the surface of the vertebrae adjacent to the nervous system, including this special region, irrespective of whether the deficiency has been severe or only of a degree to produce reduced absorption.

Bones in which efforts are made by bone cells to correct the abnormal shape, when vitamin A is added to the diet of an animal showing signs of A deficiency, include the long bones (femur and radius), the pelvis, the petrous ridge and the walls of the V nerve foramen. Osteoclastic action to reduce the enlarged petrous ridge and to widen the V nerve foramen is particularly intense.

## DISCUSSION

The foregoing account of the effect on bone of vitamin A, its absence and its replacement in the diet of an A-deficient animal, shows clearly that it plays an important part in controlling bone growth and shape, its special function being to model bones. In the case of bones adjacent to the growing nervous system, even the finer degrees of moulding have to be specially adjusted. Thus, the precise chemical control of bone development in this position is of the utmost importance to the animal's well-being. As the nervous system and its branches grow, the brain and cord cavities and the foramina which allow the passage of nerves to and from the central nervous system must enlarge to accommodate these tissues. If vitamin A is deficient in the growing puppy, bone growth is so changed that the skull cavity and the spinal canal enlarge inadequately and irregularly, and the foramina may even become smaller, with disastrous results, which may include destruction of the olfactory, optic, trigeminal and auditory nerves and the posterior roots of the spinal cord.

If, after a period of some weeks, the vitamin is restored to the defective diet, there is a vigorous attempt to correct the wrong growth of the deficient period. This fact strengthens the evidence that vitamin A exerts a powerful controlling influence on the elements responsible for co-ordinated bone growth and thereby brings about the necessary adjustment between the growth of bone and nervous

tissue. Whether this effect of the vitamin on bone is direct or indirect is not known, nor is it known to what extent the effect extends beyond the period of active growth in early life. Evidence has been given to show, however, that the vitamin's influence on a bone may be present when that particular bone has apparently ceased to grow, e.g. the labyrinthine periosteal bone, although other bones are growing vigorously at the time. Also it is known that in adult dogs the removal of vitamin A from the body causes a very slow overgrowth of the periosteal bone of the labyrinth.

It may be added that bones remote from central nervous tissue, such as the long bones and pelvic bones, are also affected by vitamin A, the effect of its absence being loss of the finer moulding and the production of a coarser general appearance. What such a change in shape means in terms of the physiology of the long bones and their adjoining tissues cannot be discussed here, but it is certainly not so serious as the destruction or other pathological change in nervous tissue that follows the dysplasia of skull and vertebral bones. The pelvis of reduced internal dimensions produced by vitamin A deficiency, although unlikely to prove as important in parturition as that produced by vitamin D deficiency, may also be of some practical significance, but this is also a subject which, it is hoped, will receive further attention later.

Although the general result of these changes on bone growth and shape is obvious, the problem becomes more difficult when an attempt is made to generalize in terms of the reactions of osteoclasts and osteoblasts to vitamin A. One of the difficulties is the variation in intensity and manner of bone growth at different ages. In, for instance, the basi-occipital bone of a +A puppy aged 20 weeks, bone cell activity is greater than in an animal on the same diet aged 30 weeks. Correspondingly the abnormal cell activity in −A animals will be greater at 20 than at 30 weeks of age. If, however, vitamin A is added to the diet of a deficient animal when 30 weeks old, the bone cell activity returns to the normal type, but the intensity is greater than that seen in the normal 20-week +A animal, and therefore much greater than in the normal +A animal of 30 weeks. Then there is the further difficulty that the mode of bone growth in different parts of the skeleton varies. For instance, in the basi-occipital bone there is normally absorption of bone on surface 1 and deposition on the marrow surface (surface 2) whilst at the same time there is in the femur deposition on the periosteal or outer surface and absorption on the marrow surface. This is an extreme example, but these differences and others of a minor degree in osteoclastic and osteoblastic activity frequently occur, especially in bones of complicated structure. By examining this abnormal bone growth and perhaps more easily by noting the efforts to correct it that follow the addition of vitamin A to the deficient diet, the mechanism of normal growth and the answer to the question as to how vitamin A controls bone shape and growth at any point will become clear.

It might be asked why it should be assumed that the reactions of bones to vitamin A deficiency and to the return of this vitamin indicate the normal way in which a particular bone grows. The answer is that in the case of bones adjacent to nervous tissue and especially near the central nervous system, the absence of vitamin A in most cases results in bone dysplasia, which either destroys or threatens to destroy local nervous tissue. The bone change which follows the restoration of vitamin A to the body is in the direction of a return to normal shape or at least to a shape which brings relief of pressure to the nervous tissue. In such cases, therefore, it seems legitimate to assume that the recovery reaction of bone indicates an exaggerated form of the normal growth process. So far as is known, the only cells controlling bone growth are osteoblasts and osteoclasts, but it has been seen above that nature uses these two types of cells as regards number and degree of activity in different ways at different points of growth. In the examples given above, it is clear that the normal manner of growth, that is to say the growth which takes place in the presence of vitamin A, varies in each case. The salient facts as regards bone cell activity during growth in these positions are as follows:

*Basi-occipital bone*

Normally, there is removal of bone from the surface 1 adjacent to the brain stem by osteoclasts and a laying down of bone on the corresponding marrow surface 2. Deficiency of vitamin A reverses these processes and the growth in depth of the posterior fossa is reduced. Recovery changes produced by the addition of vitamin A to the diet again reverse the type of cell activity.

*Labyrinthine capsule*

Normally, after the early laying down of the periosteal bone covering the labyrinth, the osteoblasts are inactive and the few osteoclasts remaining active are found on the surface of the internal auditory meatus, possibly to enlarge the meatus to allow easy passage of the VIII nerve. Deficiency of vitamin A causes a great laying down of bone, which partially blocks the meatus and injures or even kills the nerve. Recovery changes which follow the addition of vitamin A are directed to the removal by osteoclasts of this superfluous bone.

*Optic foramen and the II nerve*

In normal growth the foramina move laterally away from the middle line and each other. In this movement the osteoblasts and osteoclasts work in a co-ordinated way so as to keep each foramen open and possibly to enlarge it for the safe passage of the growing optic nerve. When vitamin A is absent from the body, this lateral movement stops or is reduced, and the foramina are smaller in diameter and the optic nerves are squeezed. Addition of the vitamin to the diet of a deficient animal brings the osteoclasts and osteoblasts into a

state of hyperactivity, so as to correct the damage done during the period of deficiency.

Here then we have three instances of growth of bone adjacent to the nervous system where, although vitamin A deficiency and recovery therefrom have the same general results, disposition of the bone cells is different in each case.

The following summary of changes due to the presence and absence of vitamin A probably applies to all the growing bones described above.

(1) Neither in the presence nor in the absence of vitamin A are active osteoblasts and active osteoclasts intimately mixed up at any one part of a surface of a table of bone, although the activity of each may be much greater at one part of a surface than at another. For instance, the place where a muscle is inserted into a bone surface is more likely to have a greater number of osteoclasts than another point of the same surface where no muscle is inserted. There are rare occasions also when one portion of a surface shows the laying down of bone and another part of the same surface shows bone removal. When this happens the parts affected are distinct (see Pl. 8*b*, surface 3).

(2) When osteoclastic activity is evident on one surface, the opposing surface of the same piece of bone is usually covered with active osteoblasts. This type of co-ordination is clearly necessary, since otherwise the bone would disappear and the marrow would be exposed. This does happen occasionally in recovery cases over very small areas, but they are not sufficiently large to weaken the bone structure. By this means the basi-occipital bone is moved in the direction away from the base of the brain, thereby allowing expansion of the posterior fossa. Similarly, it is the method by which the optic foramina are moved away from the middle line during normal growth or during recovery from vitamin A deficiency.

(3) The effect of removing vitamin A from the animal body is usually to stop osteoclastic activity on the most effective surface, for example, in the basi-occipital bone, the surface near the nervous tissue (Text-fig. 1, surface 1). If the osteoclastic action has been great in the presence of vitamin A, then the removal of the latter usually results in cessation of activity at that surface or a weak osteoblastic action. If the osteoclastic action at the time the deficiency is established is slight, then the removal of vitamin A is more likely to be replaced by osteoblastic activity and a vigorous laying down of bone on that surface.

(4) The recovery changes in dysplastic bone following the restoration of vitamin A to a diet deficient in this substance are always vigorous, much more so in fact than the original growth processes in the presence of vitamin A. The cells—osteoclasts and osteoblasts—are restored to their normal positions but are greatly increased in number and activity.

(5) Although the absence of vitamin A during bone growth may cause devastating effects on the nervous system, its removal from the diet is not synonymous either with cessation of bone cell activity or with anarchy of

these cells. The natural process of osteoclastic and osteoblastic activity at each place seems to be pre-determined and, the orders having been given, vitamin A sees that they are carried out. If the vitamin is absent, growth goes awry, since the orders are not only disobeyed but reversed or changed in a regular way. Similarly, the recovery process which follows the restoration of vitamin A involves a return of cell activity to the normal positions but with increased activity, although, as we have seen above, the normal cell type varies from place to place.

Wolbach & Bessey (1941) confirmed in rats the destruction of nervous tissue by bone pressure produced by vitamin A-deficient diets (Mellanby, 1938, 1941), but their interpretation of the cause of the bone pressure was different. On their deficient diets they found that bone growth was retarded or stopped, but that the nervous system continued to grow at a normal rate and so became compressed. The explanation is not so simple, although recent evidence (Mellanby, 1944) showing the relative importance of osteoclastic activity in normal growth makes it easy to understand how cessation or retardation of bone growth might appear to be a satisfactory explanation, especially in short-term experiments. The prime factor in bone growth, however, must always be a laying down of bony tissue by osteoblasts, and this activity is not stopped by vitamin A deficiency although its place of action may be altered.

In the experience of this laboratory the particular vitamin A-deficient diets used in rat experiments have allowed good growth of bone and the rats have developed a bone dysplasia (Mellanby, 1938) more slowly than the animals described by Wolbach & Bessey. As in the puppies, this bone dysplasia, which causes compression of the nervous system, is the result of bone growth lacking the co-ordination found in normal growing bone. It is possible that variations in the basal diet, and thereby in the rate of depletion of vitamin A reserves, may account for the different results and interpretations in rat experiments. Clearly there is still a missing factor between the two investigations, and it is hoped that further experiments will bring them into agreement.

SUMMARY

1. The foregoing experimental evidence shows that the general function of vitamin A as regards growing bone is to control its shape and specially its fine moulding by influencing the position and the activity of osteoclasts and osteoblasts.

2. The addition of vitamin A to the diet of an A-deficient animal during growth brings about a return of osteoclastic and osteoblastic activity to the surfaces where it is normally found. This reaction is often of a very intense nature, and its object is clearly to restore the normal shape of the dysplastic bones formed during the deficient period. The present experiments did not continue long enough to establish whether this object is ever fully attained.

3. The change in position of activity and in number of osteoclasts and osteoblasts resulting from vitamin A deficiency is orderly, not anarchic, and is usually a modification or even a reversal at the effective surfaces, deposition of bone taking place where there was previously either absorption or no bone cell activity.

4. Although vitamin A deficiency causes a general thickening and dysplasia of bone by its effect on osteoclasts and osteoblasts, the method of producing this abnormal state varies, as is shown in the three regions discussed, namely the basi-occipital, the periosteal bone covering the labyrinth, and the sphenoid bone in relation to the optic foramen. The recovery changes on adding vitamin A are also different in each case.

5. The altered shape of bones adjacent to the nervous system may destroy cranial and spinal nerves and exert other harmful effects, but the recovery changes in dysplastic bone on the addition of vitamin A to the diet take place independently of the condition of the adjacent nerve or nervous tissue.

I wish to thank the staff of this laboratory for their important part in this investigation and especially to express my appreciation of the skilful help given by Mr R. J. C. Stewart.

## REFERENCES

Mellanby, E. (1926). *J. Physiol.* **61**, 24 *P.*
Mellanby, E. (1931). *Brain*, **54**, 247.
Mellanby, E. (1933). *Edinb. med. J.* **40**, 197.
Mellanby, E. (1934). *Nutrition and Disease*, p. 98. Edinburgh & London: Oliver and Boyd.
Mellanby, E. (1935). *Brain*, **58**, 141.
Mellanby, E. (1938). *J. Physiol.* **94**, 380.
Mellanby, E. (1939*a*). *IIIe Congrès Neurol. Internat., Copenhagen, Comptes rendus*, p. 797.
Mellanby, E. (1939*b*). *J. Physiol.* **96**, 36 *P.*
Mellanby, E. (1941). *J. Physiol.* **97**, 467.
Mellanby, E. (1943). *J. Physiol.* **101**, 408.
Mellanby, E. (1944). *Proc. Roy. Soc.* B, **132**, 28.
Wolbach, S. B. & Bessey, O. A. (1941). *Arch. Path.* **32**, 689.

## EXPLANATION OF PLATES

In the drawings of Pls. 4, 7 and 8 active osteoclasts are indicated by black dots and active osteoblasts by black lines; inactive cells of either type are not marked.

### PLATE 1

Photomicrographs of sections through basi-occipital bones to show inactive and active osteoblasts ( × 437) and osteoclasts ( × 328), as referred to in text. (*a*) Inactive osteoblasts—cells flattened on bone surface. (*b*) Active osteoblasts—cells cubical on bone surface. (*c*) Inactive osteoclasts —cells lying flattened between bone surface and periosteum; very little evidence of bone absorption. (*d*) Active osteoclasts—multinucleated cells prominent between bone surface and periosteum; note lacunae due to absorption. (*e*) One inactive osteoclast ( × 875). (*f*) One active osteoclast ( × 875).

PLATE 2

Photomicrographs of sagittal sections ( × 130) of the inner table of the basi-occipital bones of + A (*a*) and − A (*b*) litter-mate dogs to show reversal of osteoclastic and osteoblastic activity on surfaces 1 and 2. Duration of experiment 19 weeks and final age 25 weeks. (*a*) *+A diet*: Surface 1, many active osteoclasts, no active osteoblasts. Surface 2, many active osteoblasts, no active osteoclasts. (*b*) *−A diet*: Surface 1, many active osteoblasts, no active osteoclasts. Surface 2, many active osteoclasts, no active osteoblasts.

PLATE 3

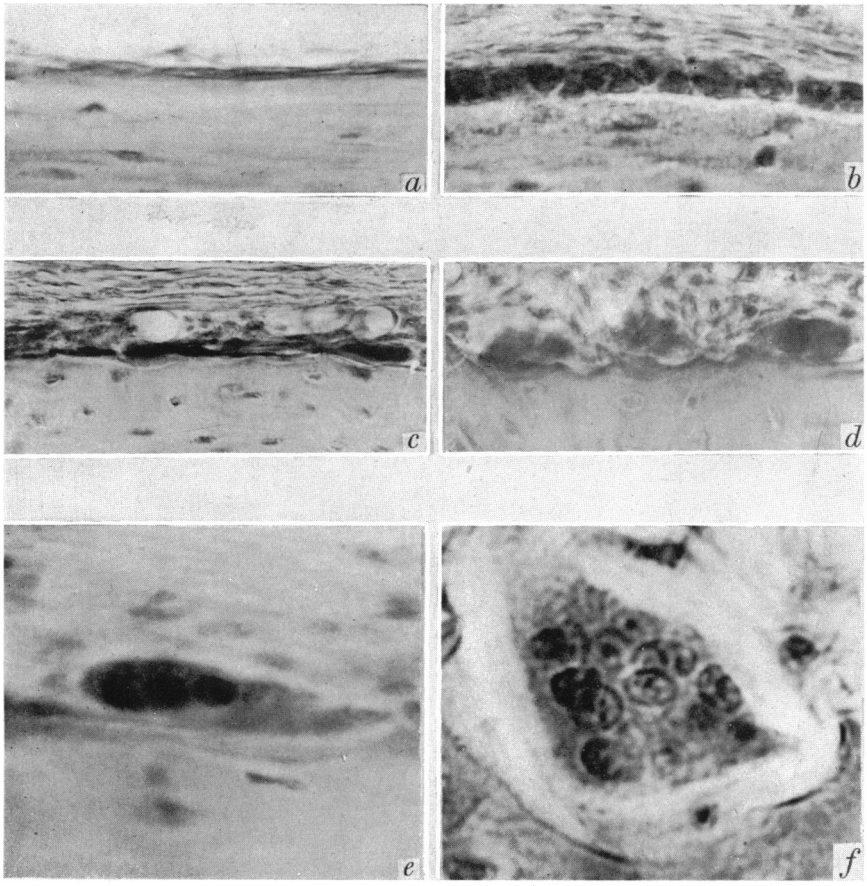
Photomicrographs of coronal sections ( × 114) of the inner table of basi-occipital bones of three litter-mate dogs, + A (*a*), − A (*b*) and recovery or − A followed by + A (*c*). Duration of experiment 26–27 weeks and final age 33–34 weeks. The effect of removing vitamin A from the diet is seen by comparing (*a*) with (*b*) and of adding vitamin A to an A-deficient diet by comparing (*c*) with (*b*). (*a*) *+A diet*: Surface 1, some slight osteoclastic activity. Surface 2, inactive osteoblasts, (*b*) *−A diet*: Surface 1, some slight osteoblastic activity. Surface 2, inactive osteoblasts. (*c*) *Recovery or −A followed by +A diet*: Surface 1, great osteoclastic activity. Surface 2, very strong osteoblastic activity. *Note.* The osteoclasts and osteoblasts have again become active in their normal positions.

PLATE 4

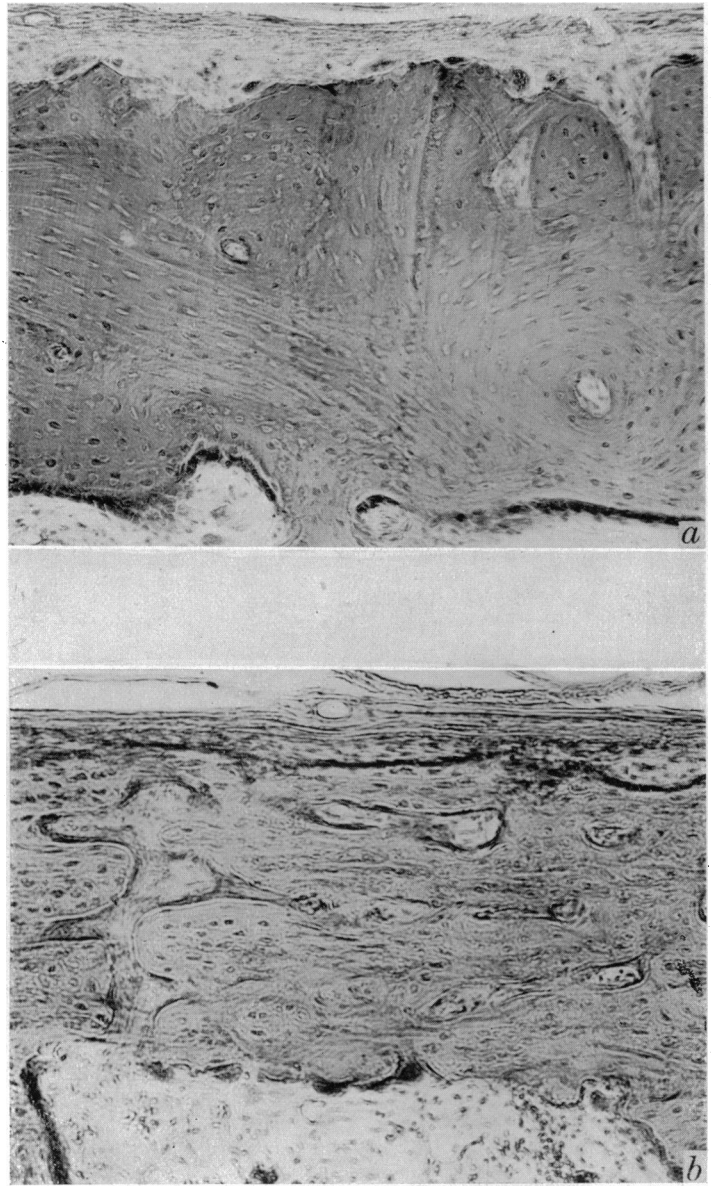
Drawings of coronal sections through basi-occipital bones of three litter-mate dogs, + A (*a*), − A (*b*) and − A followed by + A (*c*). Duration of experiment 26–27 weeks and final age 33–34 weeks. (*a*) *+A diet*: Inner and outer bone tables have fused, with elimination of surfaces 2 and 3. Surface 1, abundant active osteoclasts, especially towards middle line. Surface 4, a few scattered osteoclasts. (*b*) *−A diet*: Bone still cancellous, with large marrow cavity. Surface 1, some osteoblasts (reversal from same surface in (*a*)). Surface 2, no cell activity. Surface 3, no cell activity. Surface 4, a few active osteoblasts but otherwise no activity. (*c*) *Recovery or −A followed by +A diet* ( + A diet for last 3 weeks (23 days)): Surface 1, osteoclastic activity like (*a*) but much greater. Surface 2, much osteoblastic activity. Surface 3, some osteoblastic activity. Surface 4, a little osteoclastic activity, especially at periphery. *Note.* Reversal of action in (*c*) brought about by added vitamin A, especially on surfaces 1 and 2.

PLATE 5

Photomicrographs of sagittal sections ( × 114) of inner tables of basi-occipital bones of three litter-mate dogs, + A (*a*), − A (*b*) and − A followed by + A (*c*). Duration of experiment 14–16 weeks and final age 20–22 weeks. Dog (*c*) was on − A diet for 14 weeks and + A diet for 2 weeks (i.e. 13 days' recovery period). (*a*) *+A diet*: Surface 1, a few osteoclasts. Surface 2, many active osteoblasts. (*b*) *−A diet*: Surface 1, many active osteoblasts. Surface 2, some osteoclasts. (*c*) *−A followed by +A diet*: Surface 1, many active osteoclasts. Surface 2, many active osteoblasts. *Note.* There has been a reversal of cell activity at surfaces 1 and 2 when vitamin A was removed from the diet (compare (*b*) with (*a*)). On adding vitamin A to a deficient animal (*c*), the cells have again reverted to their normal position (*a*), but are much more numerous and very active.




*To face p.* 398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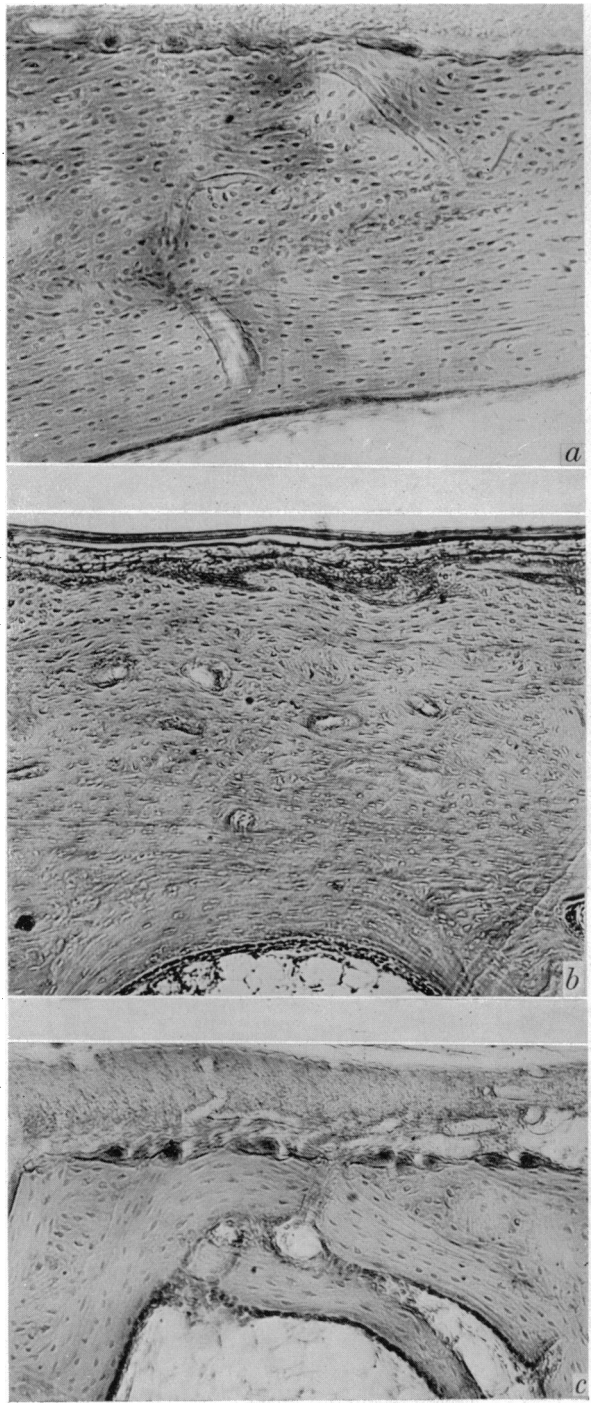
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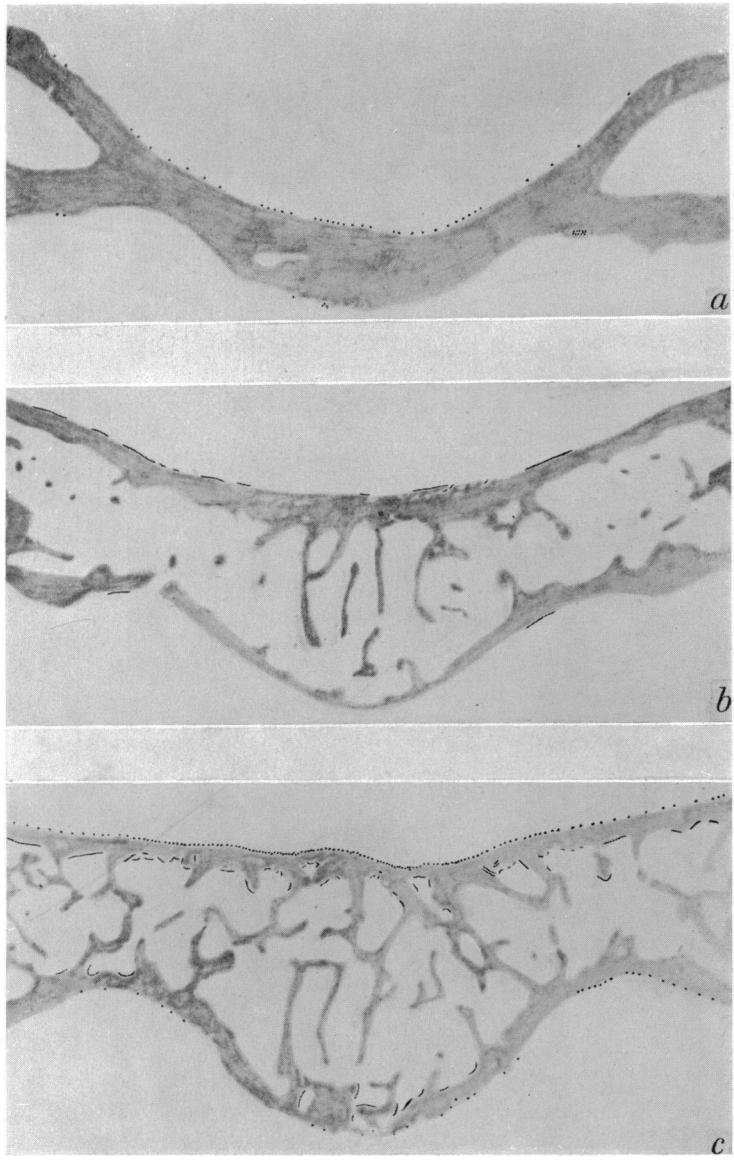
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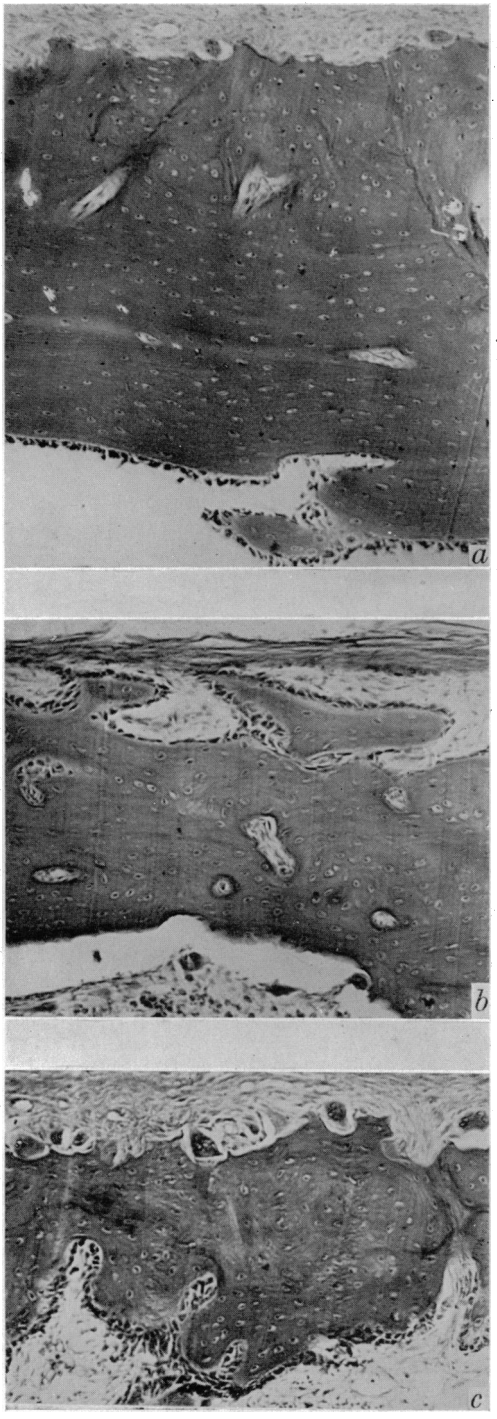
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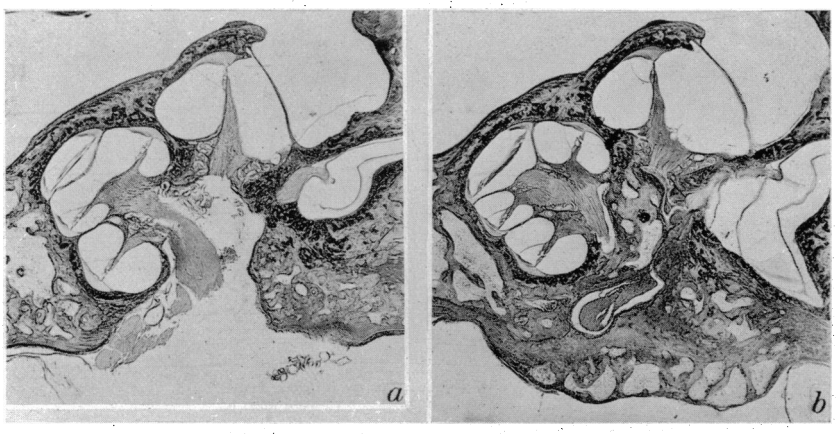



Fig. 1.

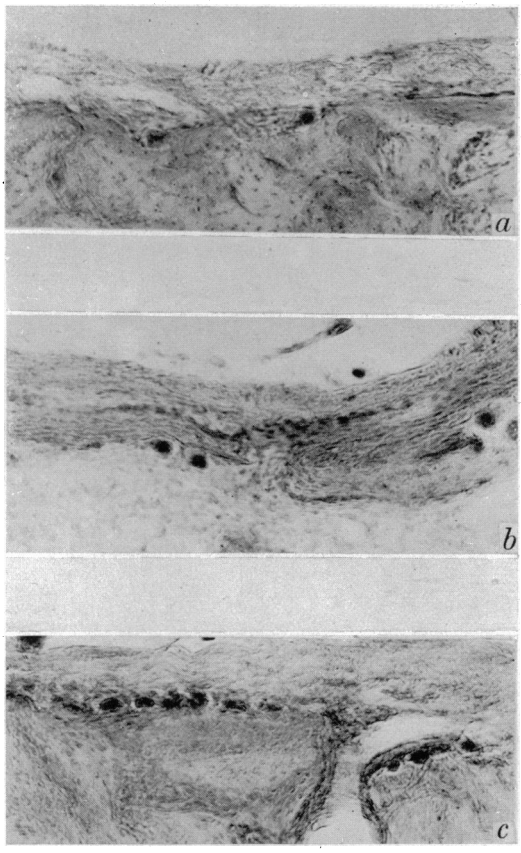
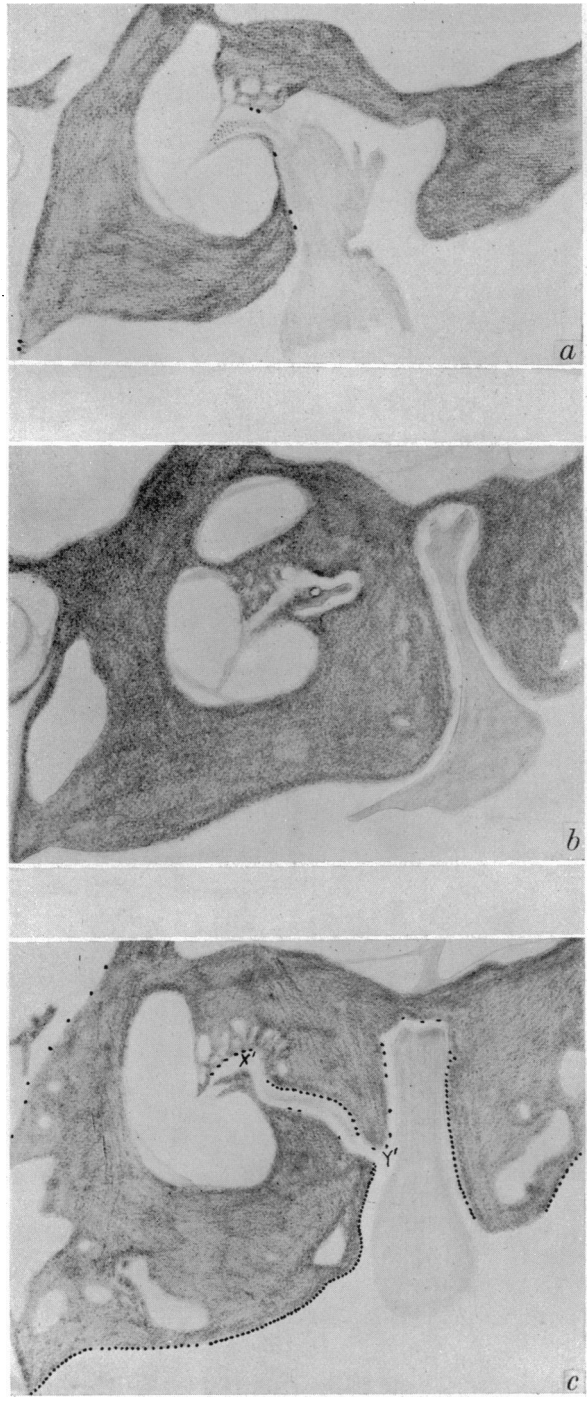



Fig. 2.


a
b
X'
Y'
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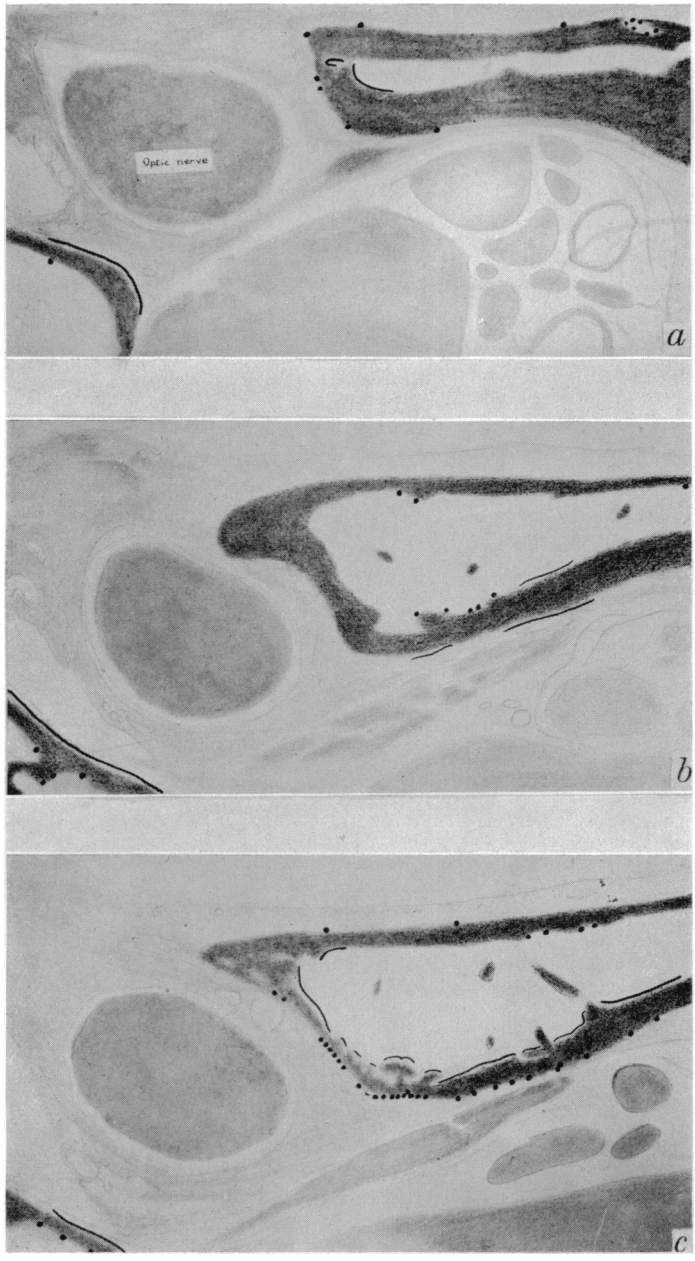
Optic nerve
a
b
c

PLATE 6

Fig. 1. Photomicrographs ( $\times 6{\cdot}1$) of the labyrinths of two litter-mate dogs, +A (*a*) and −A (*b*). Duration of experiment 22 weeks and final age 29 weeks. *Note* the laying down of much excess periosteal bone in (*b*). The internal auditory meatus appears to be occluded, but this is due to its tortuous course, and although it is much constricted the meatus is patent, as can be seen by examining serial sections. The VIII nerve is twisted, compressed and lengthened.

Fig. 2. Photomicrographs ( $\times 114$) of the bone adjacent to the internal auditory meatus in three dogs, +A (*a*), −A (*b*) and −A followed by +A (*c*). (*a*) *+A diet*: Duration of experiment 18 weeks and final age 27 weeks. Some osteoclastic activity on the bone surface adjacent to the VIII nerve. (*b*) *−A diet*: Duration of experiment 18 weeks and final age 27 weeks. Osteoblastic activity on the surface adjacent to the VIII nerve and osteoclasts now seen on the marrow surface. *Note.* Reversal of cell activity similar to that seen in the basi-occipital bones. (*c*) *−A followed by +A diet*: 24 weeks on −A diet followed by 3 weeks (23 days) on +A diet, and final age 34 weeks. Large number of active osteoclasts on the surface adjacent to the VIII nerve. *Note.* Cell activity has returned to the normal position, but is increased when compared with (*a*).

PLATE 7

Drawings of coronal sections of labyrinths of three litter-mate dogs, +A (*a*), −A (*b*) and −A followed by +A (*c*). Duration of experiment 26–27 weeks and final age 33–34 weeks. During the last 3 weeks of the experiment (23 days), (*c*) received 5000 i.u. of vitamin A daily. (*a*) *+A diet*: Practically no active bone cells visible except a few osteoclasts on inner surface of internal auditory meatus. (*b*) *−A diet*: No active cells visible. In the internal auditory meatus there has been a great formation of new periosteal bone, which has squeezed the VIII nerve: the increased activity of osteoblasts resulting in the overgrowth has ceased. (*c*) *−A followed by +A diet*: The addition of vitamin A has brought about a tremendous reaction and active osteoclasts in large numbers have returned to surface 1 with the object of removing the superfluous bone. *Note* the layer of osteoclasts on the surface $X^1$–$Y^1$.

PLATE 8

Drawings of coronal sections through the sphenoid bones of three litter-mate dogs, +A (*a*), −A (*b*), and −A followed by +A (*c*). Duration of experiment 26–27 weeks and final age 33–34 weeks. (See Text-fig. 3 for numbering of surfaces.) (*a*) *+A diet*: Very little cell activity on surfaces 1, 2, 3 and 4. Osteoclasts are seen on surfaces $Y^2$ and $X^1$, osteoblasts on surfaces $Y^1$ and $X^2$. (*b*) *−A diet*: No changes in cell activity on surfaces $Y^1$ and $Y^2$. Cessation of activity on $X^1$ and $X^2$. Surface 3 shows both osteoclastic and osteoblastic activity, and surfaces 1, 2 and 4 show reversal of cell activity. (*c*) *−A followed by +A diet*: No changes on surfaces $Y^1$ and $Y^2$. Activity on surfaces $X^1$ and $X^2$ has returned to the normal type, with an increase in intensity. Surfaces 3 and 4 show a great activity of bone cells and a reversal to the normal type; surfaces 1 and 2 show little change.