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THE EFFECT OF BONE DYSPLASIA (OVERGROWTH) ON CRANIAL NERVES IN VITAMIN A-DEFICIENT ANIMALS

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This report is a continuation of one dealing with the wider problem of skeletal changes produced in young animals by vitamin A-deficient diets and their effect on the central nervous system [E. Mellanby, 1941]. An account is given here of overgrowth of bone in the region of the cranial nerves and its effect upon them, with the exception of the VIIIth nerve, which was dealt with in detail in a previous paper [E. Mellanby, 1938].

Degeneration of the optic nerve, the trigeminal and the auditory and vestibular divisions of the VIIIth nerve resulting from vitamin A and carotene deficiency in the diet were described in 1933 and 1934*a* [E. Mellanby]. It was thought at that time that these degenerative changes, together with others in the brain stem and spinal cord, were due mainly to the abnormal metabolism associated with vitamin A deficiency having a direct, destructive effect on certain nerve cells. A detailed study of the cochlea and vestibule made it clear, however, that the destruction of the VIIIth nerve was secondary to bone overgrowth. It was soon obvious that the degenerative changes in other cranial nerves and indeed of some peripheral nerves were associated with pressure resulting from abnormal bone growth [Mellanby, 1938, 1939*a*, *b*].

Methods

Litters of puppies from 6 to 10 weeks old were fed on basal diets of the following type: separated milk powder 20 g., cereal (usually white bread) 100–300 g., lean meat 15–20 g., yeast 3–12 g., peanut or olive oil 10 ml., sodium chloride 1–2 g., lemon or orange juice 5 ml., irradiated ergosterol (vitamin D_2) 1000–2000 i.u.

There is a small amount of vitamin A in this diet, but not sufficient to prevent depletion of the body. Under the conditions of these experiments the calcium in the diet is not high enough for optimal bone formation when growth is rapid, but increasing the calcium modifies, though it does not prevent, the effects

described in −A animals.[1] In each litter one or more animals were given supplementary vitamin A or carotene, sometimes as mammalian liver fat or cod-liver oil or as cabbage. In such cases the bones developed normally and no pathological changes in the nerves resulted. The nervous signs characteristic of vitamin A deficiency develop within varying times: the younger the dogs and the more rapid the depletion the quicker do the abnormalities appear. This period may be as short as 2 months and as long as 4–6 months; in adults it is a year or more.

At the end of the experiment many of the animals were injected intra-arterially with Wittmaack's solution so as to fix the tissues in situ. It was usual, however, in such cases to ligature the femoral artery to one leg before injecting the Wittmaack's solution in order to allow estimation of calcium in the femur of that leg. After fixation, blocks of tissue containing the different nerves together with the adjacent bone were decalcified by nitric acid and, after embedding in celloidin, serial sections were cut and stained with Ehrlich's haematoxylin, Biebrich's scarlet and eosin. By this means it was possible to follow the relation of the bone to the nerve at each point of its course. For myelin degeneration, histological examination was made by a modified Marchi method [Stewart, 1936].

Results

Ist nerve (olfactory)

In a −A animal, examination of the olfactory nerve in its passage from the mucous membrane of the nose to the brain shows that it is liable to experience abnormal conditions, owing to bone overgrowth, but it is difficult to say to what extent the function of the nerve is affected.

The most obvious change produced by A deficiency is the thickening and enlargement of the cribriform plate (Figs. 2 and 3 *a*, *b*). This bony structure, through which fibres of the olfactory nerve pass, can be seen to be swollen in the −A dog (*b*), because of the enlargement of the marrow spaces between the limiting plates which, however, are not themselves increased in thickness. Not only are the marrow spaces increased in that part of the plate which is parallel to the brain tissue, but they are also enlarged in the projections of the plates into the folds of the olfactory mucous membrane, so that, whereas in the +A animal the bony trabeculae entering these projections may only appear as spicules of bone with small marrow spaces (Figs. 2*a*, 3*a*), in the −A animal a shell of bone with a large marrow space is often seen (Figs. 2*b*, 3*b*).

Figs. 2 *a*, *b* and 3 *a*, *b* also show that the enlargement of the cribriform plate does not much increase the average distance to be traversed by the olfactory nerve, but it reduces the size of the nerve bundles which pierce the plate at

[1] For the sake of brevity the terms +A and −A dog (or animal) will be used to denote dogs brought up on diets containing, and those deficient in, but not devoid of, vitamin A respectively.

any one point and subjects such nerve fibres to mechanical pressure. In the +A animal a number of bundles of nerve fibres traverse a single passage in the cribriform plate, but in the −A animal they seem to be broken up into individual bundles by the encroaching and irregular bone overgrowth; some of the bony passages in the plate are so narrow that even these smaller nerve bundles passing through them are pinched, as can be seen in Figs. 2*b* and 3*b* (see *N*). The compressed nerve can be seen in Fig. 1, which shows high-power photomicrographs of nerve sections in +A and −A animals respectively just before passing, during passage, and after passing through the cribriform plate towards the brain. The sections are stained to accentuate the appearance of the nuclei of the sheaths of these non-medullated nerve fibres. Fig. 1*b* (ii) shows that in the −A animal the nuclei are much more crowded during the passage of the nerves through the plate, indicating squeezing of the nerve at this place, whereas both before and after passage the nuclei are more dispersed and similar to the unsqueezed appearance of comparable sections in the +A animal (Fig. 1*a*). In spite of the pressure suffered, these particular nerves have not been completely destroyed, although it may be that other bundles of nerves or some nerves in any bundle have degenerated and disappeared. No histological preparations have been made which show whether changes are present in the axis cylinders of these non-medullated nerves.

In this, as in other situations, changes are sometimes observed in the dura mater and subarachnoid space in −A animals. The dura mater, which accompanies the nerve bundles in part of their passage through the cribriform plate, is thickened and may add to the pressure effect on the nerve bundles referred to above. The subarachnoid space of the +A animal contains much nerve tissue and some other connective tissue elements, whereas in the −A animal it seems relatively free from such tissues. The olfactory nerve fibres which pass across it to the olfactory lobe of the brain are broken up into much smaller bundles and appear to be greatly reduced in number, an indication that many fibres have been killed by the overgrown cribriform plate.

There is, however, one other factor to be considered in regard to the reduction in olfactory nerve fibres crossing the subarachnoid space towards the olfactory lobe of the brain. This is the raised intracranial pressure in some of these −A animals [E. Mellanby, 1939*a*, 1941]. Increased pressure in the subarachnoid space in this area may affect both the olfactory fibres crossing it and the surface layers of the olfactory lobe itself. That destruction of nerve fibres on the surface of the olfactory lobe does occur is indeed evident from the fact that in some of the experiments myelin degeneration has been observed in this position. The superficial layer of the olfactory lobe of the brain is often thinner in −A animals, so that the glomerular layer of cells is nearer the brain surface (Figs. 2*b*, 3*b*). This again may be partly due to compression associated with increased intracranial pressure, but the more important factor is doubtless

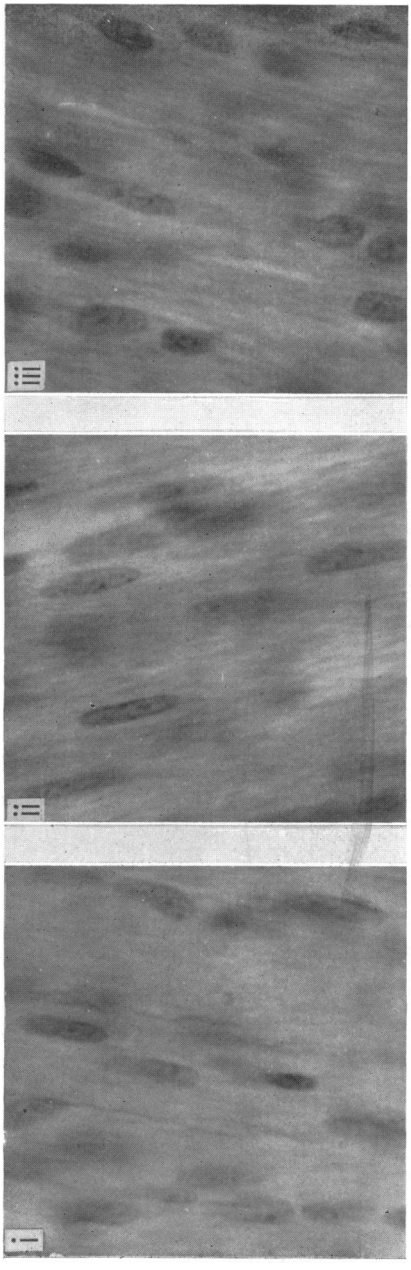

Fig. 1*a*.

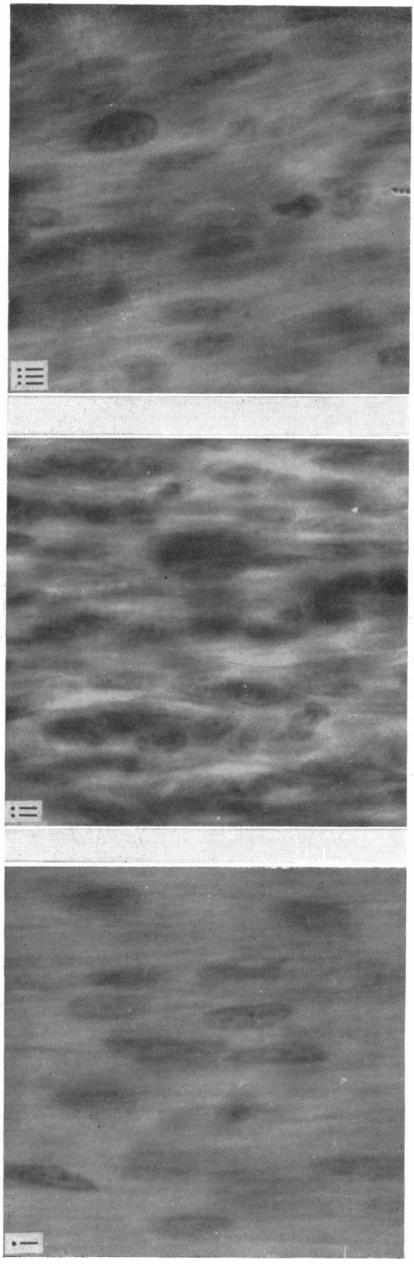

Fig. 1*b*.

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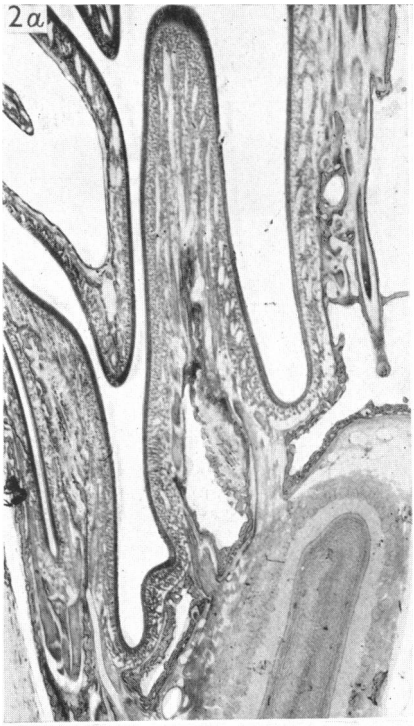


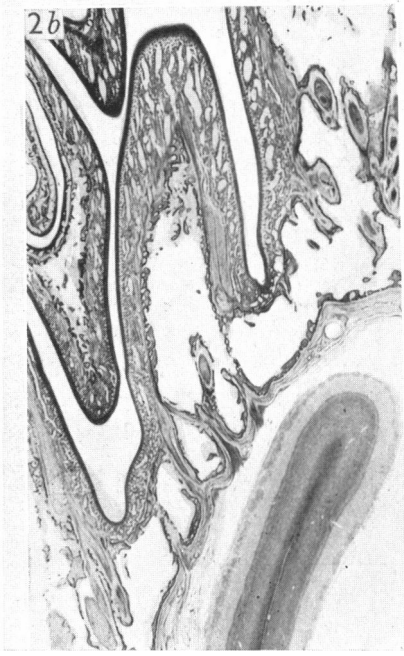
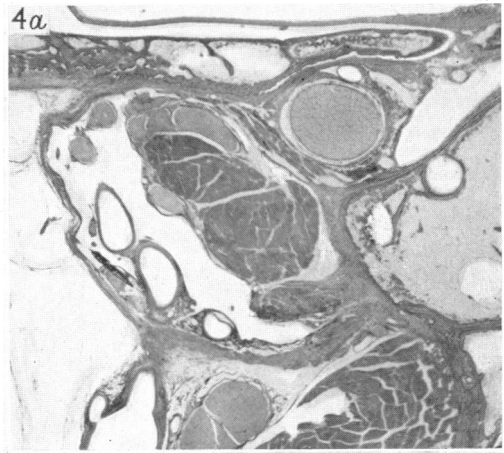


Fig. 2.



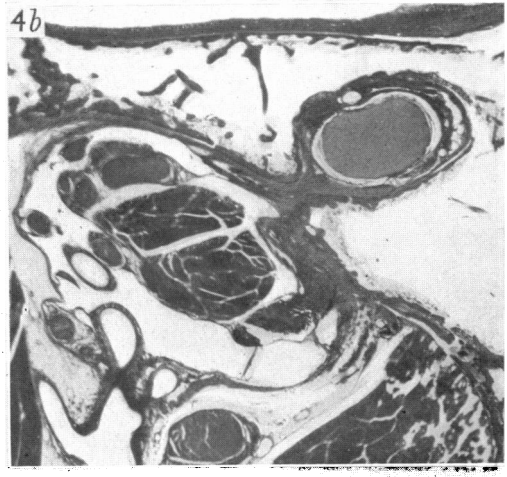
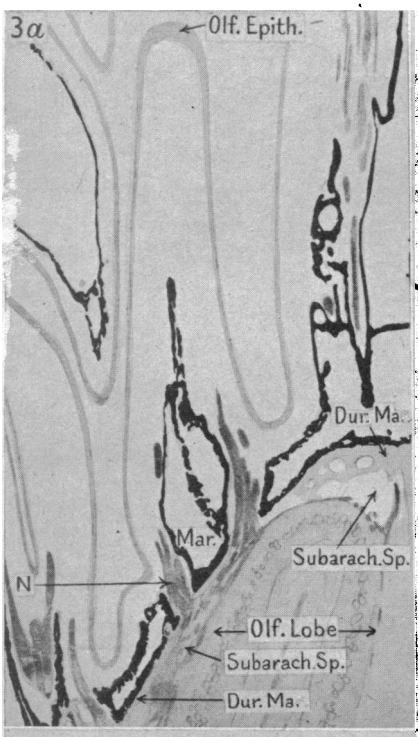


Fig. 4.



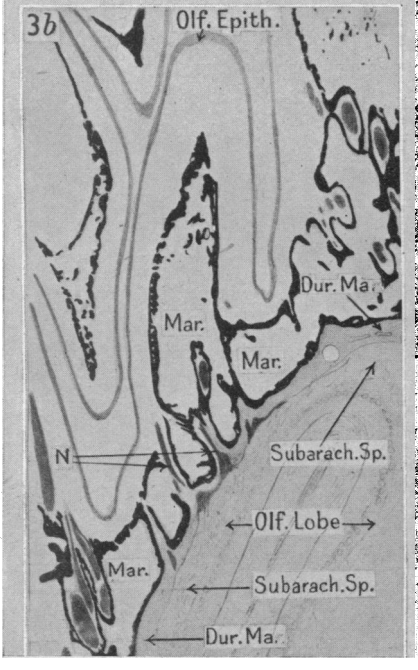


Fig. 3.

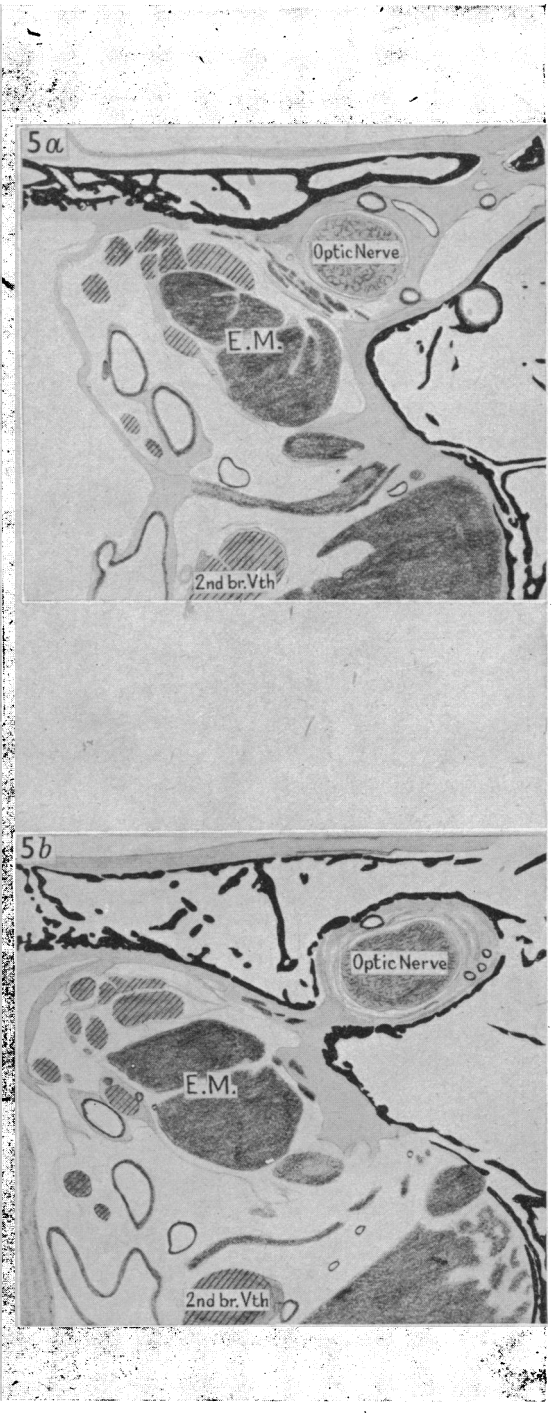


Fig. 5.

For description of Figs. 3 and 5 see bottom of p. 412.

the disappearance of many olfactory nerve fibres running into the brain in this position.

Histological examinations so far made have not indicated to what extent the normal function of the olfactory nerves in −A animals is affected. Individual nerve bundles are certainly seen to be compressed in their passage through the cribriform plate, and nearer the brain the number of actual nerve fibres passing across the subarachnoid space seems to be greatly reduced. On the other hand, those fibres that can be seen appear to be normal, except for some degeneration on the surface of the olfactory lobe.

The epithelial or sustenticular cells of the olfactory mucous membrane do not show any great changes in −A animals, although the cell layers may become irregular in appearance and the nuclei are sometimes found nearer the surface than in +A animals. The cells remain columnar in shape and do not become squamous or keratinized. The bipolar olfactory nerve cells in the mucous membrane with their hairlets may be reduced in number, but as yet no definite change in individual cells has been observed. As the relative number of receptor cells is known to vary in different parts of the olfactory mucous membrane, even the question of reduced numbers is not certain.

The behaviour of the animals and the histological appearance of the olfactory apparatus above described suggest that the effect of feeding on −A diets is to reduce both the sense of smell and the number of olfactory nerve fibres. Animals so fed are apt to run about with their noses near the ground sniffing vigorously, as if they were attempting to make up for their deficient smelling power by excessive sniffing. This is the behaviour that might be expected in dogs whose sense of smell, normally well developed, is impaired.

Fig. 3 (*a* and *b*). Drawings to explain Fig. 2 (*a* and *b*), showing the passage of the olfactory nerve through the cribriform plate. (See also p. 409). (*a*) Dog whose diet contained vitamin A. (*b*) Dog whose diet was deficient in vitamin A.

Note: (1) swelling of bones in (*b*) with enlargement of marrow cavities (Mar.); (2) constriction of foramina of cribriform plate causing compression of olfactory nerve bundles (*N*) in (*b*) (− A dog) as compared with (*a*) (+ A dog); (3) dura mater thickened and subarachnoid space larger in (*b*) than in (*a*); fewer nerve fibres cross the subarachnoid space in the vitamin A-deficient animal (*b*); (4) outer layers of olfactory lobe thinner in (*b*) than in (*a*).

Black represents calcified areas of bone.

Fig. 5 (*a* and *b*). Drawings to explain Fig. 4 (*a* and *b*), optic nerve near orbit. (See also p. 413). (*a*) Dog whose diet contained vitamin A. (*b*) Dog whose diet was deficient in vitamin A.

Note: In (*a*) both openings of the bony canal through which the optic nerve passes from the orbit to the chiasma can be seen; in (*b*) the nerve is almost surrounded by bone and, owing to the lengthening and twisting of the canal by the bone overgrowth, only one opening of it is evident.

Black represents calcified areas of bone. Diagonally shaded areas = various portions of IIIrd, IVth, Vth and VIth nerves. E.M. and similarly shaded areas = eye muscles.

IInd nerve (optic)

Reference has previously been made to the degenerative changes in the optic nerve and retina in −A puppies [Mellanby, 1934*a*] and to the pressure effect of the bone overgrowth surrounding this nerve [Mellanby, 1938]. Only in one of the animals has the optic nerve been completely destroyed, and that animal was on an A-deficient diet for several years [Mellanby, 1934*a*], a much longer time than the experimental period of the animals examined in the present work, which was usually not over 6 months. Moore, Huffman & Duncan [1935*a*, *b*] described in calves blindness of a nutritional type resulting from atrophy of the optic nerve where it passes through the optic foramen. This atrophy, they suggested, was caused by improper development of the foramen, and was probably due to bone pressure. They thought that vitamin A deficiency might or might not be the nutritional factor concerned and noted that, while corn silage, timothy hay and cod-liver oil prevented the blindness, 10,000 units of vitamin A ('caritol') did not prevent it. The authors did not notice any bone exostosis but said that 'the bony canal gave more the appearance of having had pressure applied from above, which caused it to become smaller as growth proceeded'. Moore [1939] later, however, came to the conclusion that carotene deficiency was the cause of nyctalopia, papillary oedema and permanent blindness in calves and, although he again ascribed the nerve degeneration to bone pressure due to stenosis of the bony canal, he still thought it was 'difficult to associate vitamin A deficiency with any bony malformation', and that increased intracranial pressure probably accounted for the abnormal bone development.

Evidence will now be given of the manner in which the optic nerve may suffer compression and stretching by bone overgrowth when animals are brought up on diets deficient in vitamin A and carotene. In advanced cases of A deficiency in dogs, the bone surrounding the optic foramen can be seen from the inside of the skull to be gripping the nerve more closely than usual. Whereas in a normal (+A) dog the optic nerve, as it passes through the foramen, lies in loose connective tissue, in a −A animal it is closely surrounded by bone with but little intervening connective tissue, and in some cases the nerve is gripped tightly and compressed by these tissues. Another possible cause of deformity in the case of the optic nerve, as it passes from the optic foramen into the skull cavity, is pressure from the dura mater which closely surrounds it at this spot; the dura mater in these −A animals is usually thickened and, possibly because it does not easily adapt itself to the shape of the locally hypertrophied bone, it may be in a state of increased tension at points where nerves pass through it.

Bone overgrowth around the optic nerve in a −A dog can be clearly seen by comparing serial sections of the nerve in its course from the orbit to the chiasma in +A and −A dogs. Fig. 4 *a*, *b* are photomicrographs and Fig. 5 *a*, *b* drawings of sections through the optic nerves of two dogs in the region of the

canal formed by the sphenoid bone just as the nerve leaves the orbit on its way to the brain. In the −A dog (Figs. 4 *b*, 5 *b*) the fatty marrow spaces are enlarged, thus increasing the bulk of the bone, which almost completely surrounds the nerve in this position, leaving only a small opening into the orbital cavity and none into the cranial cavity. In the corresponding section of the +A animal (Figs. 4 *a*, 5 *a*) the opening into the orbital cavity is much wider and the optic foramen into the skull cavity is seen. The connective tissue surrounding the nerve is loose in the +A animal, whereas in the −A animal it is tightly packed. That there is some compression of the nerve in the −A animal (Figs. 4 *b*, 5 *b*) at this point is indicated by the fact that: (1) the cross-section of the nerve is oval and not round, as in the +A animal (Figs. 4 *a*, 5 *a*); and (2) the blood vessels in the connective tissue are smaller than in the +A animal. It may be added that, owing to the bone overgrowth described, the optic foramen in the −A animal is longer and more tortuous than that in the +A animal and there is a corresponding increase in length of the nerve.

The appearance of the optic nerve as it emerges from the optic foramen and passes over the surface of the base of the skull towards the optic chiasma can be seen in drawings of serial sections (Fig. 6 *a*, *b*). The bone enlargement in the −A animal (Fig. 6 *b*) again stands out prominently and is seen to be due to the increase in cancellous tissue. The compact bone is not as abundant as in the normal animal (Fig. 6 *a*), so that the total calcified bone of the −A animal is not nearly as great as its increased mass would suggest. It will be noticed that, whereas the optic nerve in the normal animal runs along a gentle depression in the sphenoid bone towards the optic chiasma and is round in cross-section (Fig. 6 *a* (ii, iii)), the depression is much deeper in the −A animal and the nerve is compressed, as can be seen by the distorted shape of its cross section (Fig. 6 *b* (ii, iii)). These are the main bone changes which appear directly to affect the optic nerve. Although there are changes in the anterior clinoid processes of the sphenoid bone, they probably have a greater compressor effect on the pituitary body than on the optic chiasma.

Besides the direct mechanical squeezing of the optic nerve by the overgrown bone, it is probable that the raised intracranial pressure [Mellanby, 1939*a*; Moore & Sykes, 1940] which occurs when there is great bone overgrowth, especially round the posterior fossa, must also be a factor of destruction.

Clinical experience in man teaches that the optic nerve is particularly susceptible to mechanical pressure in any part of its course, and when a nerve fibre is thus injured degenerative changes do not follow the Wallerian law, but the whole neuron may be destroyed. It would be expected, therefore, that in these −A dogs the bone overgrowth which has been shown to exert undue pressure on the optic nerve during its bony passage, as well as the raised intracranial pressure acting on the nerve within the cranial cavity, would have a destructive effect on the optic nerve.

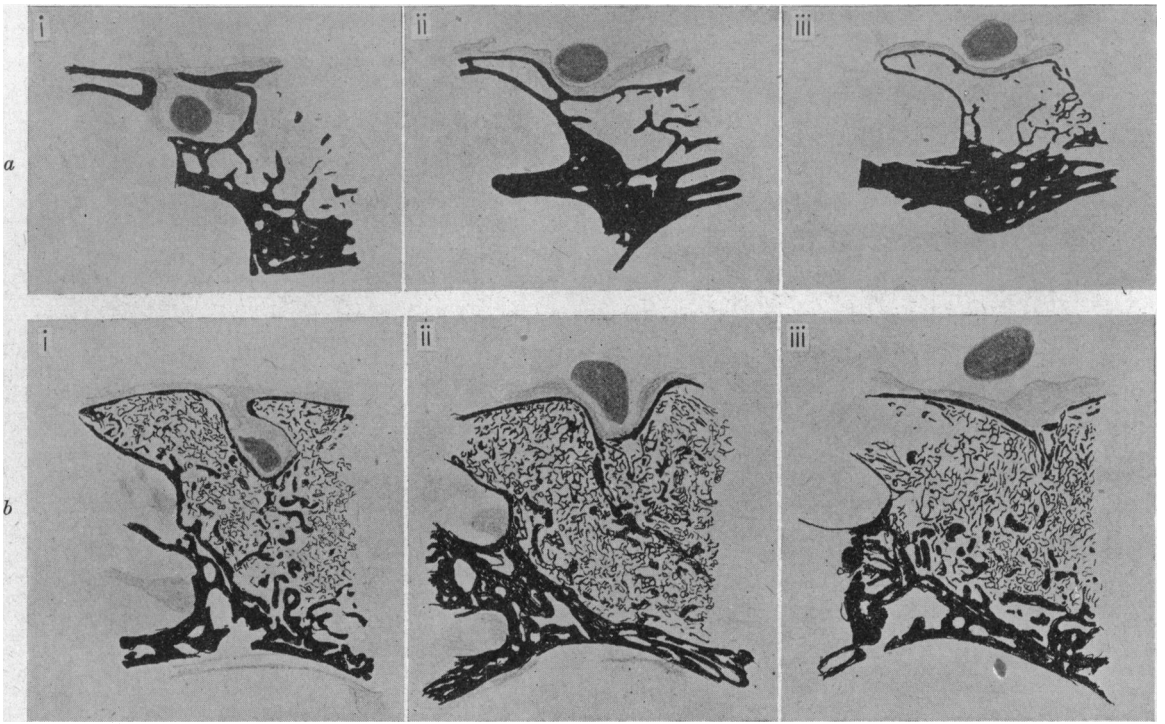


Fig. 6 (*a* and *b*). Drawings of sections of three regions of the optic nerve between the orbit and the optic chiasma in + vitamin A and − vitamin A dogs. (See p. 414.) (*a*) Dog whose diet contained vitamin A. (*b*) Dog whose diet was deficient in vitamin A.

Note: In (*b*) greatly enlarged cancellous bone as compared with (*a*) and alteration in shape of the nerve due to compression. Sections (*a* (i, ii and iii)) are as nearly comparable in position as possible to (*b* (i, ii and iii)).

Black represents calcified areas of bone.

Are the pressure effects sufficient to account for the abnormal and degenerative changes that can be observed in −A dogs? The probability is that they are not and that another factor must be taken into consideration, namely, a degenerative change which begins in the retina itself. It is already established that vitamin A deficiency impairs retinal function, as is evident in loss of night vision in man and animals. This loss of function is associated with an upset of the mechanism responsible for the production of visual purple. Since this mechanism begins to fail even in adults after only a few weeks' deprival of vitamin A, it cannot be associated with any bone hyperplasia or pressure on the nerve, but must be a direct effect of abnormal metabolism on the retinal cells. In dogs other retinal changes develop later, such as degeneration of the ganglion cells which show eccentricity of the nuclei, powdery Nissl granules and reduction in number. The bipolar cells (inner nuclear layer) are also reduced in number and lose some of their affinity for basic dyes [Mellanby, 1934*a*]. Ultimately, the whole of the ganglion cells of the retina and the neurons of the optic nerve may disappear, but this degree of degeneration is only reached after many months of A deficiency. It is, of course, probable that many of the retinal and nerve changes, especially the later changes, may be produced by pressure of overgrown bone on the optic nerve. On the other hand, some of the degeneration may be an extension of the retinal defect of night blindness produced directly by vitamin A deficiency. Is there any evidence of this?

In 1939 Moore pointed out that papilloedema and bleaching of the tapetum could be observed in vitamin A-deficient calves by ophthalmoscopic examination. Similar changes are found in −A dogs, but there is reason to believe that the condition of the optic disk is not simply one of papilloedema. The earliest retinal change observed in these animals by ophthalmoscope is an alteration in the colour of the tapetum lucidum, which loses its blue component. The blue band contiguous with the tapetum nigrum becomes green and the green coloration gradually changes to yellow from its upper boundary downwards to the tapetum nigrum. Ultimately, the whole of the tapetum lucidum is yellow and the tapetum nigrum in some of the −A dogs' eyes seems rather a darker brown than in the normal dogs.

The early changes in the tapetum are soon followed by pallor of the disk, which may be associated with protrusion of about half a diopter. Swelling of the optic disk to a degree which can be described as definite papilloedema takes place slowly after these initial changes, and in some of the dogs reaches as much as 8 diopters. In the early stages the pale disk, when viewed with the ophthalmoscope, has a sharp outline. Later the edge is blurred and irregular, in some cases with apparent extensions of the disk along the vessels, especially on the nasal side.

An attempt was made in the course of the work to see whether there was any

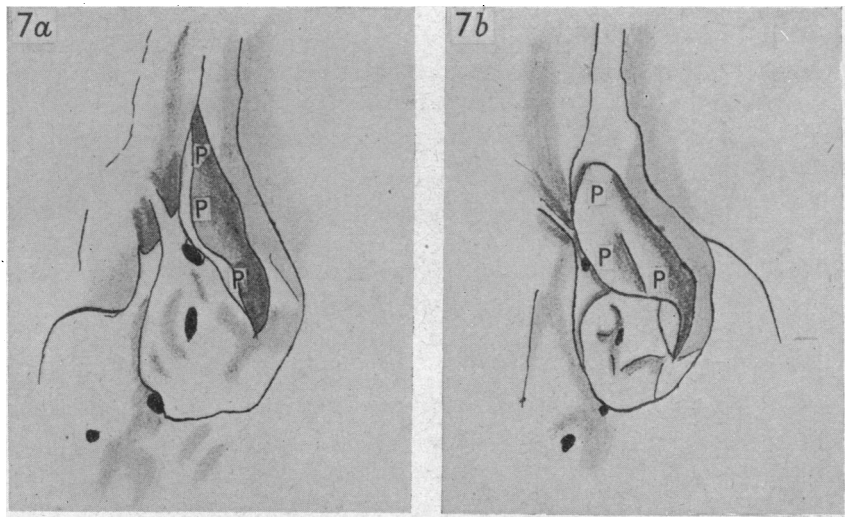
relationship between the degree of abnormality of the optic disk and the degree to which overgrown bone compressed the nerve as it entered the cranial cavity from the optic canal, but the evidence was inconclusive. This may possibly be regarded as supporting the view that a factor other than pressure of bone and raised intracranial pressure is involved. This other factor is probably the direct action of vitamin A deficiency on the retinal cells which begins to manifest itself early in the experimental period by bleaching of the tapetum lucidum and pallor of the disk. At a later stage bone pressure on the optic nerve and the increased intracranial pressure become effective and produce a condition of papilloedema. While, therefore, it cannot be said that the position is yet clarified, ophthalmoscopic and histological evidence suggest that the optic nerve degeneration in −A animals is really a double mechanism, one a condition of optic atrophy starting in the retina itself, and the second a condition of papilloedema superimposed on the atrophy by the pressure of bone on the optic nerve and by the increased intracranial pressure.

IIIrd, IVth and VIth nerves (*oculomotor, trochlear and abducens*)

These nerves, together with the first branch of the Vth, pass through the superior orbital fissure. There appears to be but little bone change in this region; at least, there is not sufficient change to compress the nerves to any great extent; and the only nerve passing through this fissure which is regularly found to be degenerated is the first branch of the Vth, which will be considered later with the trigeminal system. Allusion has been made above to the distorting effect of the dura mater on the optic nerve. A similar effect can sometimes be seen on the IIIrd nerve of −A dogs. This nerve is normally slightly bent at the point where it passes through the dura mater into the cavernous sinus, but in −A dogs the distortion is exaggerated. It may be noted that in the one case in which the region of this distortion was examined histologically, a few degenerated fibres were found. Since, however, there is seldom much degeneration in the IIIrd nerve in −A animals, and indeed this applies to all the motor nerves of the eye, the mechanical distortion produced in this region by the dura mater alone does not apparently interfere to any great extent with the structure of the nerve fibres or their function.

Vth nerve (*trigeminal*)

Degenerative changes in the Vth nerve and in the Gasserian ganglion in −A animals have been previously described [Mellanby, 1934*b*]. It was shown that there was often an association between degeneration of the fibres of the first division of the Vth nerve and xerophthalmia, and it was suggested that this latter condition might be a manifestation of loss of neurotrophic control of the nerve over the conjunctiva and cornea. Whether this is so or not, the two conditions, xerophthalmia and degeneration of the sensory branches of the Vth



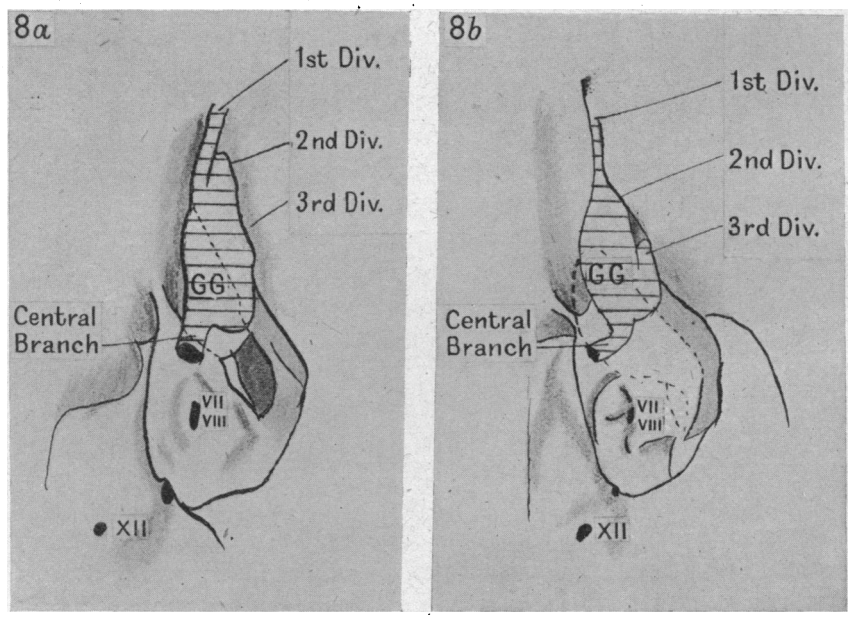


Fig. 7 (*a* and *b*). Drawings of the petrous ridge (*P*) of temporal bone of +vitamin A and −vitamin A dogs. (See p. 419.) (*a*) Dog whose diet contained vitamin A. (*b*) Dog whose diet was deficient in vitamin A.

Note: Thickened bulbous ridge in (*b*) as compared with (*a*).

Fig. 8 (*a* and b). Drawings of the same specimens as Fig. 7 (*a* and *b*) after removal of the petrous ridge to expose the Vth nerve and Gasserian ganglion (*G.G.*). (*a*) Dog whose diet contained vitamin A. (*b*) Dog whose diet was deficient in vitamin A.

Note: Bone overgrowth in (*b*) has twisted the central branch of the Vth nerve; in (*a*) this branch is straight and normal. Note also the reduced size and folded wall of the VIIIth nerve foramen in (*b*).

nerve, are generally found together in dogs, rabbits and rats. M. Mellanby & King have shown in −A animals degeneration of Vth nerve fibres supplying the gums and teeth, together with hyperplasia of the gum epithelium [Mellanby & King, 1934] and changes in the dental roots [King, 1936]. There is therefore no doubt about the destruction of sensory fibres of the Vth nerve in −A animals, and it now remains to see whether the bone changes at the base of the skull in the neighbourhood of the nerve are sufficient to account for this degeneration.

Macroscopic examination of the skulls of −A dogs reveals at once large overgrowth of the bone surrounding the Vth nerve system as it passes under the petrous ridge of the temporal bone. The petrous ridge is swollen and blunted, the increase in size being in depth and width rather than in length, as can be seen in Fig. 7*b* (cf. Fig. 7*a*, the normal). When the bone of the petrous ridge is removed (Fig. 8 *a*, *b*), it will be seen that the bone overgrowth has greatly affected the course of the Vth nerve. In the −A animal (Fig. 8*b*) the central branch of the nerve is bent and it passes from the bony foramen towards the pons in a more mesial position. In the normal animal (Fig. 8*a*) the central branch of the nerve runs straight from the Gasserian ganglion to the pons without any bending.

Fig. 9 *a*, *b* shows drawings of sections, as far as possible comparable, through the trigeminal nerve and petrous bone of a +A and a −A dog respectively. Whereas in the +A animal (Fig. 9*a* (i, ii)) the nerve is cut transversely to its length and occupies a relatively wide space in the bone, the twisting of the nerve in the −A animal is such that it is seen to be cut parallel to the plane of the section (Fig. 9*b* (i, ii)). The overgrowth of the petrous ridge has also encroached on the space occupied by the nerve and has compressed it. A small portion of bone (*X*) can be seen immediately beneath the nerve in Fig. 9*a* (i, ii), but in the −A animal (Fig. 9*b* (i, ii and iii)) this piece of bone (*X'*) is much greater in size and, together with the overgrowth of the petrous ridge, has caused considerable compression of the Vth nerve. Fig. 9*a* (vi) shows the normal appearance of the Gasserian ganglion in relation to the bone surrounding it. In Fig. 9*b* (vi), a drawing of a section through the same region in a −A animal, the Gasserian ganglion is compressed and elongated by the great overgrowth of the petrous portion of the temporal bone. It will also be seen that the depression of the internal surface of the petrous ridge into which the ganglion fits (Fig. 9*a* (vi)) has become flat in the −A animal (Fig. 9*b* (vi)). Although the Gasserian ganglion is squeezed between the apex of the petrosal portion of the temporal bone and its petrous ridge, the nerve cells do not show the elongation that might be expected; they do, however, undergo chromolytic and other degenerative changes [E. Mellanby, 1934*b*], which may be due to the mechanical pressure of the abnormal bone.

In cases where the effects of the A-deficient diet are slighter, the overgrowth of the petrous portion of the temporal bone may not be so obvious and twisting

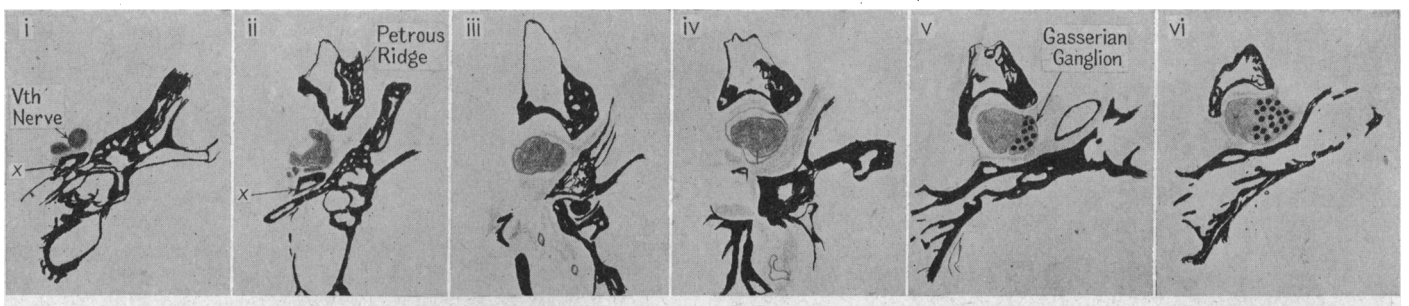


Fig. 9 *a*.

Fig. 9 *b*.

Fig. 9 (*a* and *b*). Drawings of serial sections through the Vth nerve, Gasserian ganglion and surrounding bone in + vitamin A and − vitamin A dogs. **(See p. 419.)** **(*a*) Dog whose diet contained vitamin A. (*a*) Dog whose diet was deficient in vitamin A.**

Note: **(1) overgrown petrous ridge in (*b*) as compared with (*a*); (2) in (*b* (i, ii and iii)) the nerve is cut longitudinally, whereas in (*a* (i, ii and iii)) it is cut transversely; this is due to twisting of the nerve in (*b*) similar to that shown in Fig. 8 (*b*); (3) in (*b* (v)) the ganglion can be seen to be compressed between the petrous ridge and the apex of the petrous bone, as compared with the ganglion in (*a* (v)).**

Black represents calcified areas of bone.

***X* and *X'*. For explanation see text.**

of the central branch of the Vth nerve may not occur. Fig. 10*b* is a section through the foramen lacerum of such a dog and compares with a section from a control dog of the same age (Fig. 10*a*). The overgrowth of bone in the −A animal is again obvious, the additional bulk being made up of loose cancellous bone at the expense of the compact bone seen in the control animal (Fig. 10*a*). It will be seen also in Fig. 10*b* how deeply placed the nerve is, due to the bone hypertrophy. The deformity of the nerve in Fig. 10*b* is obvious; it is now

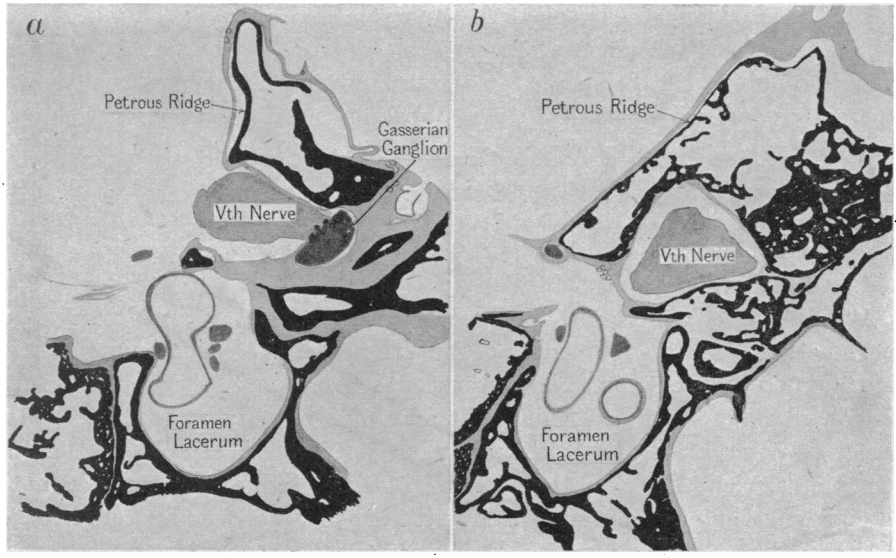


Fig. 10 (*a* and *b*). Drawings of sections through the Vth nerve and surrounding bone at the level of the foramen lacerum in +vitamin A and −vitamin A dogs. (*a*) Dog whose diet contained vitamin A. (*b*) Dog whose diet was deficient in vitamin A.

Note: In (*a*) the nerve and part of the ganglion are surrounded by loose tissues, whereas in (*b*) no ganglion cells are seen and the nerve is almost surrounded by overgrown and encroaching bone which has altered its shape.

Black represents calcified areas of bone.

triangular in cross-section to conform to the shape of the bony canal (cf. Fig. 10*a*), and this change of shape again indicates a mechanical effect of the bone on the nerve during its passage to the brain from the tissues outside the skull.

When the Vth nerve has emerged from under the petrous ridge in its passage towards the periphery, it assumes a more normal appearance, since the bone overgrowth produced by the vitamin A-deficient diets is less prominent, although still definite. For instance, if one examines sections passing through the foramen ovale, the sphenoid bone is seen to be thickened and the foramen is narrowed, but not sufficiently to press on the third (mandibular) branch of

the Vth nerve and distort its shape. The sphenoid bone surrounding the foramen rotundum, through which the second (maxillary) branch of the Vth nerve passes, is also slightly thickened and the foramen narrowed, but again there is no evidence of bone pressure on the nerve.

The first (ophthalmic) branch of the Vth nerve, together with the IIIrd, IVth and VIth nerves, passes through the superior orbital fissure towards the orbit. The bone is thickened in this position, but not so as to reduce the fissure, and there is no evidence of bone pressure on the nerves traversing it. It is possible that the nerves in this position are affected by increased intracranial pressure, suggestive evidence of which is obtained by examining the blood vessels running with the nerves. In the cases examined they are smaller than the corresponding vessels in the same position in normal animals. The connective tissue surrounding the nerves and vessels seems also to be compressed and not only to occupy a smaller space but to be denser in the vitamin A-deficient dogs than in the control animals. This possible effect of increased pressure on blood vessels in the brain may be of importance and requires more detailed study.

One other mechanical effect of bone overgrowth which may be of some importance, is the increase in length of the Vth nerve. This lengthening becomes more apparent on cutting serial sections of the nerve and its surrounding bone in comparable +A and −A dogs. It may involve actual stretching, or the nerve may simply lengthen without any increase in tension. It was thought at first that the lengthening of the nerve might itself produce degeneration, but a similar lengthening produced by thickening of bone was found to occur in other cranial nerves, without comparable degenerative changes. This applies especially to the IXth, Xth, XIth and XIIth and, since little or no degeneration is found in these nerves in −A animals, it is probable that the degeneration suffered by Vth nerve is not due to lengthening.

It seems from the foregoing account that the great susceptibility to destructive change of the more purely sensory divisions of the Vth nerve in −A dogs could be accounted for by bone overgrowth pressing on the Gasserian ganglion.

VIIth nerve (facial)

It has been shown in earlier publications that the motor cranial nerves are relatively little damaged in −A animals in which the sensory cranial nerves are severely affected [Mellanby, 1935]. Of the motor cranial nerves, probably the VIIth is more often affected by degenerative changes whilst, among the sensory cranial nerves, the adjacent VIIIth is the most often destroyed. On the other hand, there is a great difference between the liability of neurons of the VIIth and VIIIth nerves to destructive changes, due probably in large measure to the different positions of their cells of origin.

In its passage from the central nervous system to its exit from the skull, the VIIth nerve may be roughly divided into five parts: (1) the portion from the point of issue from the brain stem to the internal auditory meatus; (2) the portion running close to the VIIIth nerve within the internal auditory meatus; (3) a short portion which leaves the internal auditory meatus near the modiolus and connects with the geniculate ganglion; the nerve here is within the facial canal which passes in an antero-lateral direction; (4) a part which, beginning at the geniculate ganglion, bends through an angle of practically 120° and then passes posteriorly through the facial canal in the upper wall of the tympanic cavity; and (5) the portion which passes through the stylomastoid foramen.

Taking these parts separately, it is obvious that the first part of the VIIth nerve running from the brain stem to the internal auditory meatus will not be affected directly by bone overgrowth and, in fact, it will only be subjected to the same increase of intracranial pressure as may influence any other part of the central nervous system in these −A animals. The second part of the nerve will be subjected to the same conditions as the VIIIth nerve in this position. Bone overgrowth in the neighbourhood of this part of the course of the VIIIth nerve in −A animals has been described in an earlier publication [E. Mellanby, 1938]. The internal auditory meatus is often greatly lengthened and twisted by overgrown periosteal bone of the labyrinthine capsule, so that the passage may in individual sections seem to be occluded, but if a series of sections is examined it is found that the meatus is constricted but patent. If there is ever complete destruction of the VIIth nerve due to bone overgrowth at this part of its passage, it must be very rare. Nor is it this particular bone overgrowth around the internal auditory meatus which is responsible for the severe degeneration of the cochlear division of the VIIIth nerve. The new bone mainly responsible for this change is found at the modiolus end of the internal auditory meatus adjacent to the helix which contains the ganglia of the auditory division. These ganglion cells, as previously described [Mellanby, 1938], are often destroyed and the whole neuron may be killed by bone pressure. The VIIth nerve, however, does not reach the modiolus, but turns away from the VIIIth nerve to enter the facial canal some distance before the helix is reached. It would appear, therefore, that although the VIIth nerve, when passing through the internal auditory meatus, is liable to suffer some stretching and compression by overgrowth of periosteal bone, there is no evidence that it suffers severe destructive changes in this position.

In the third part of its course through the facial canal, the VIIth nerve is again liable to be compressed by the partial closure of the canal. Layers of newformed bone can be seen lining the canal in −A animals. The resultant narrowing of the canal does not proceed to complete occlusion, but it may be sufficiently great to press on the geniculate ganglion and elongate the cells in a way similar to, but to a less degree than, that seen in the posterior root ganglion

and in Scarpa's ganglion in −A dogs [Mellanby, 1938]. In spite of the elongation by pressure, the cells of the geniculate ganglion, from which the sensory fibres of the VIIth originate, suffer but little destructive change, and their Nissl's granules and nuclei generally seem normal or nearly so. In severe cases, however, there is definite destruction of some of the cells. It is obvious from the appearance of the cells and the canal in such cases that the cells can withstand a good deal of pressure and distortion without degenerating. It may be that, when degenerating fibres have been found in the VIIth nerve, they are fibres having their cells of origin in the geniculate ganglion.

In the fourth part of its course, where the VIIth nerve runs through the facial canal in the upper wall of the tympanic cavity, the compression due to bone overgrowth may be severe. In several cases the nerve has been seen to be compressed to a thin ribbon, but complete occlusion of the canal has never been observed even in the most severely affected cases; nor, indeed, have the blood vessels which pass with this part of the nerve appeared unduly narrowed.

The fifth part of the nerve now issuing from the skull wall through the stylomastoid foramen is unrestricted by bone overgrowth and the passage usually appears to be quite normal.

It seems, therefore, that the VIIth nerve is liable to be affected by bone overgrowth in the second, third and fourth parts of its course, and it is in these positions that degenerating nerve fibres may sometimes be found. Even in severe cases of A deficiency, however, the number of such fibres is relatively small.

Reference may be made here to two nerves which pass into the VIIth nerve, namely, the greater superficial petrosal nerve going from the spheno-palatine ganglion on the second division of the Vth nerve to the geniculate ganglion on the VIIth nerve and the auricular nerve which passes from the jugular ganglion of the Xth nerve to join the VIIth nerve near the stylomastoid foramen. Both these nerves may be compressed in −A animals, but especially the greater superficial petrosal nerve which, in advanced cases, may be pressed to a ribbon shape by the encroaching bone. In spite of this distortion, however, there may be only a few fibres in the nerve showing degenerative changes. The compression of the auricular branch of the Xth nerve is not so great, and here again the nerve usually shows only a few degenerated fibres.

Facial paralysis has not been recognized in the animals used in these experiments and, in spite of the severe constriction of the nerve, sufficient degeneration to justify paralysis has not been found. In human beings also the VIIth nerve is known to be very resistant to pressure. The absence of facial paralysis in dogs and man in spite of compressed VIIth nerves, emphasizes the fact that some nerve fibres can withstand a surprising amount of mechanical pressure and distortion without loss of function.

IXth, Xth and XIth nerves (glosso-pharyngeal, vagus and accessory)

These nerves pass together from the cranial cavity through the jugular canal, the walls of which are formed by portions of the temporal and occipital bones. Both these bones are thickened in −A dogs, but in spite of this, the jugular canal is only slightly narrowed, the main effect being the lengthening of the canal from the base of the brain to the wall of the tympanic cavity. At this latter point the jugular canal turns slightly and passes over (round) the wall of the tympanic cavity. It might be expected that, in passing over the tympanic cavity and under the thickened basi-occipital bone, the nerves would be subjected to increased pressure. This, however, does not appear to be the case. It seems probable that constriction of the canal is avoided at this point because the space occupied by the thickened occipital bone is obtained at the expense of the tympanic cavity which is smaller and surrounded by thicker walls than in the +A animals.

The only change in diameter of the canal appears to be near the internal end. Here the bone is sometimes seen to be folded and the ganglia on the nerves seem to fit more tightly in the canal. Changes in these ganglia when examined either by naked eye or histologically are very slight, even in severe vitamin A deficiency. Generally speaking, the only mechanical change observable in these animals is the lengthening of the nerves owing to bone hypertrophy, and there is no conspicuous pressure of bone on either the nerves or their ganglia. Examination of the nerves by Marchi's method shows that very few degenerated fibres are present in those parts which are within the jugular canal. After the nerves have emerged from the canal a few more degenerated fibrés can be seen.

XIIth nerve (hypoglossal)

In −A animals the hypoglossal canal, which passes through the basi-occipital bone, is lengthened because of the thickening of the bone, but apart from this lengthening and an occasional fold of bone, which occurs in severe −A cases, the changes are slight. The hypoglossal nerve shows a similar lengthening, but no other mechanical interference arises, and when examined by a modified Marchi technique, the nerve shows no significant degeneration even in cases of severe A deficiency.

Discussion

The main facts of the nature and degree of bone overgrowth in relation to the cranial nerves and their degenerative changes observed in vitamin A-deficient dogs have been described. It is difficult to correlate the facts into any simple statement, for there appear to be unknown or only partially known factors involved other than those of simple mechanical pressure of bone on the nerves and nerve cells.

One outstanding fact is that under the conditions of these experiments the sensory nerves are largely damaged, while the motor nerves generally escape destructive changes. It has been found that the VIIIth nerve (both divisions, but especially the cochlear division), the sensory fibres of the Vth (all divisions), the optic nerve and the olfactory nerve are partially destroyed. The IIIrd, IVth, third branch of the Vth, VIth, VIIth, IXth, Xth, XIth and XIIth tend to escape destructive changes.

In considering this problem the following factors must be taken into account: (*a*) direct pressure of overgrown bone on nerve cells and fibres, (*b*) the restriction of blood supply to nerve cells and fibres by the overgrowth of bone, (*c*) an increased intracranial pressure, again due to overgrowth of bone, (*d*) a direct effect of vitamin A deficiency on certain nerve cells. There is little doubt that the direct pressure effect on nerve cells and fibres is the main cause of nerve degeneration in these animals, but it is still far from clear what part the other factors may play.

It is probable that the chief reason for the difference between sensory and motor nerve susceptibility to damage is the presence in the course of the sensory nerves of ganglia outside the central nervous system and the pressure to which they are subjected by overgrown bone. Motor nerves, having their ganglionic origin within the central nervous system, escape this form of pressure, and only the nerve axons can be squeezed by direct bone overgrowth. Of the sensory nerve systems, the Gasserian ganglion of the trigeminal nerve is liable to be compressed by the overgrowth of the petrous bone, so that sensory fibres of all three divisions show large degenerative changes. The third (mandibular) division contains a much smaller proportion of degenerated fibres than the second (maxillary) or the first (ophthalmic) division. The motor fibres of the third division apparently escape destructive changes, for, although their axons run with the Gasserian ganglion through the petrous bone and suffer the same pressure changes, their cells of origin are inside the central nervous system.

In the case of the optic nerve, a further instance must be considered of the explanation offered in 1938 [Mellanby] to account for the differences in susceptibility of the cochlear and vestibular divisions of the VIIIth nerve to destructive changes in vitamin A-deficient animals. In that publication the evidence was given to show that, whereas pressure on any part of the neuron of the cochlear division caused complete degeneration, similar pressure on the vestibular neuron only caused degeneration expected by the Wallerian law, namely of that part of the neuron peripheral to the injury. Clinical experience in man shows that pressure on any part of the optic nerve causes degeneration both of the nerve and of its ganglion cells in the retina.

It seems established, therefore, that, apart from the degree of pressure produced on nerves by local bone overgrowth and apart from the exposure of the ganglia of certain cranial nerves to such pressure, the greater degree of

susceptibility of some cranial nerves than of others to injury must also be a factor in determining the amount of degeneration produced in animals having vitamin A-deficient diets. This variation in susceptibility to injury, while clearly of importance in determining the degree of degeneration in the sensory nerves of the brain, may well also partly account for the relative immunity of the motor nerves to degeneration. Thus, whereas many fibres of the olfactory nerve are killed by bone pressure in their passage through the overgrown cribriform plate in vitamin A-deficient animals, the facial nerve often suffers great deformity by pressure in the same animals without showing any destruction. The same resistance to destruction shown by the facial nerve has been observed clinically in man, and Perlman & Willard [1941] have pointed out that this nerve 'may be compressed to a microscopically thin ribbon on the capsule of the tumour and yet no facial paralysis is seen'. It may be therefore that, even excluding the auditory division of the VIIIth nerve and the optic nerve because of their exceptional susceptibility to injury, motor nerve axons escape destructive changes when exposed to those pressure effects which would destroy the axons of sensory nerves. On the whole, however, in the present experiments the motor nerves, except the motor fibres of the Vth and the facial nerve, escape pressure changes due to bone hypertrophy. Some of them, especially the IXth, Xth, XIth and XIIth, may be lengthened owing to the increased thickness of the temporal and occipital bones in vitamin A-deficient dogs, but this change does not cause degeneration. It seems probable that these nerves are never seriously stretched but adapt themselves by growing in length as the bone increases in thickness.

Attention has already been called to the increased susceptibility of the optic nerve to injury, and at one time it was thought that this susceptibility accounted for the fact that the optic nerve showed more degeneration than would be expected from the degree of bone pressure it experienced in these animals. Some doubt has arisen about this recently because of the early retinal changes that develop in −A animals, and it is possible that a deficiency of this vitamin has a degenerative effect on the ganglion cells of the retina which is independent of pressure on its nerve fibres. The point has been discussed above, and evidence was given which suggested that the optic nerve in −A animals suffered two kinds of destructive change: (1) an early condition of optic atrophy probably beginning in the retina itself, and (2) papilloedema superimposed on the optic atrophy and due to pressure on the nerve by the neighbouring overgrown bone together with the increased intracranial pressure. If it should prove that the retinal cells are directly influenced by vitamin A deficiency and that optic atrophy is produced independently of bone overgrowth and pressure, the problem reverts partially at least to its earlier position where it was considered that the abnormal metabolism associated with vitamin A deficiency might itself cause degeneration of certain nerve ganglia [Mellanby, 1934*b*].

It raises the possibility once more that, in addition to the destructive nerve changes due to bone and intracranial pressure, there may be other instances of nerve degeneration in the central nervous system which depend directly upon the normal metabolic changes associated with A deficiency.

One factor which has not been considered in this publication is the effect of the bone overgrowth on the blood supply. If, as seems possible in areas of great malformation, the blood supply is interrupted or reduced, the effect on the nutrition of the nerves, and more especially the nerve cells, would be considerable. No instance has been seen of a foramen being so occluded as to obliterate the blood vessels, and it may be that the blood supply remains adequate.

Wolbach & Bessey [1941] have recently given their authority to the view that degenerative changes in nervous tissue in −A animals are easily explained by cessation of bone growth at a time when the nervous system is growing normally. This, they claim, causes compression of the nervous system and subsequent degeneration. They refer to the older observations of Hess, McCann & Pappenheimer [1921] and those of Wolbach & Howe [1925] on cessation of bone growth in −A rats and suggest that this older work suffices to explain the recent results. It may be permissible to draw attention to the fact that the diets used in these older experiments were deficient both in vitamin A and vitamin D. It would be unfortunate if this view of Wolbach & Bessey were accepted, for the evidence indicates that, in vitamin A deficiency, growth of bone does not cease in dogs, rabbits or, in my experience, even in rats.

Wolbach & Bessey [1941] confirm the observations of Mellanby [1938] and of Loch [1939] on the increase in the periosteal bone of the labyrinthine capsule and on the formation of exostosis in the internal auditory meatus in vitamin A-deficient rats, while Perlman & Willard [1941] have reported similar excessive bone growth in rabbits. The same intrusion of cancellous bone into the canal surrounding the optic nerve of calves is shown in a photomicrograph published by Moore, Huffman & Duncan [1935*a*], although they do not comment in this paper on the cancellous bone formation nor relate it to vitamin A or carotene deficiency. There is therefore good evidence that a deficiency of vitamin A and carotene in all young experimental animals tested produces bone overgrowth of a specific kind. There may be some special reason for the experimental results obtained by Wolbach & Bessey in rats, and the conclusion of these authors that bone growth ceases in vitamin A deficiency.

In the investigations described here and elsewhere certain bones of vitamin A-deficient dogs are larger than those of normal animals and they grow in an abnormal way. Some bones grow more than others and some parts of a bone grow more than other parts. Their general normal outline is changed and they become coarser in appearance and lose their fine moulding. In the dog the greater thickness of the malar bone and the zygomatic portion of the temporal

bone can be seen on examining the skull. Similarly, the lower jaw is often greatly thickened. Other instances, e.g. supra- and basi-occipital, and the sphenoid and temporal bones, have been given of excessive bone growth in the sense described.

It is true that some of the internal openings of the cranial foramina in −A dogs are smaller than usual and compress the nerves, and this may at first glance suggest cessation of bone growth at a time when the nerve tissues continue to grow at a normal rate. Further examination of the bone at these points, however, shows that this interpretation is incorrect, for the openings are not circular or oval as in the normal animal, but the walls are folded because of bone overgrowth, thus making the foramina triangular or irregular in shape. In contrast to this, it can sometimes be observed that the external ends of these bony canals are larger than normal.

The evidence indicates that a certain amount of vitamin A is necessary for normal bone growth and that, when there is a deficiency, bone does not stop growing, as suggested by Wolbach and Bessey; but a controlling influence on its growth is lost. The size of the bones continues to increase but some are malformed and contain an excessive amount of cancellous tissue whose spaces are often full of fatty marrow. This overgrowth of cancellous bone may be accompanied by a reduction in compact bone, as is shown in the illustrations above (Fig. 6), but this is not always the case, and if the calcium, phosphorus and vitamin D of the diet are generous, the compact tissue may also be thicker, even if not normal in structure, in some vitamin A-deficient bones. In vitamin A deficiency there is a bone dysplasia. The function of this vitamin in young growing animals is not that of a stimulant to bone growth but rather that of a controller of certain growth elements, the co-ordinated activity of which is necessary for normal bone production. When vitamin A is deficient, the activity of these bone elements is unco-ordinated, the growth becomes excessive in places, and the nervous tissue is compressed and suffers destructive changes. In the adult animal vitamin A acts in a similar way, but since ordinary growth has ceased, the specific effects on bone are much slower in developing and are never as conspicuous as in young animals.

Summary

1. When young dogs are brought up on diets deficient in vitamin A and carotene, local overgrowth of certain skull bones causes compression, twisting and lengthening of most cranial nerves, some of which show large degenerative changes. These changes are intensified if, as happens in the VIIIth and Vth nerves, the ganglion cells are also affected by bone overgrowth.

2. Destructive changes are largely confined to the sensory nerves, the motor cranial nerves for the most part escaping.

3. Cranial nerves, especially those with motor function, such as the VIIth, can often suffer compression, lengthening and twisting as the result of bone overgrowth without degenerating.

4. In the experiments described the nerves most affected in diminishing order are somewhat as follows: (*a*) cochlear and vestibular divisions of the VIIIth nerve, especially the former, (*b*) Vth nerve (trigeminal) (first and second branches especially), (*c*) IInd nerve (optic), (*d*) Ist nerve (olfactory).

5. Degeneration in the optic nerve may be produced in −A animals, not only from direct pressure of overgrown bone and from increased intracranial pressure, but also from a primary degenerative change beginning in the retina itself. The early optic atrophy associated with bleaching of the tapetum is probably a direct effect of A deficiency on retinal cells, while papilloedema, due to bone overgrowth and increased intracranial pressure, is superimposed later.

6. Whereas the internal ends of foramina in the skulls of vitamin A-deficient animals are generally stenosed, with folded outlines due to bone overgrowth and not to cessation of growth, the external openings are not usually smaller than normal.

7. In these experiments, where the calcium intake was not high, the increased bulk of certain bones is due to the formation of an excess of cancellous tissue.

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EXPLANATION OF PLATES 1 AND 2

PLATE 1

Fig. 1 (*a* and *b*). Photomicrographs (× 500) of sections of branches of the olfactory nerves (non-medullated) of + vitamin A and − vitamin A dogs. (*a*) Dog whose diet contained vitamin A. (*b*) Dog whose diet was deficient in vitamin A. (*a* (i)) and (*b* (i).) Before entering the bony plate; *note* approximately same number of sheath nuclei in each. (*a* (ii) and *b* (ii).) Within the bony plate; *note* many more nuclei in the − A (*b*) than in the + A (*a*) owing to squeezing of the nerve by the bone. (*a* (iii) and *b* (iii).) After emerging from the bony plate; *note* nuclei rather more concentrated in (*b*) than in (*a*), but less concentrated than in *b* (ii). (See p. 410.)

The olfactory nerve of the − A dog has been squeezed in passing through the cribriform plate.

PLATE 2

Fig. 2 (*a* and *b*). Photomicrographs (× 6) of sections showing olfactory nerves passing through the cribriform plate of + vitamin A and − vitamin A dogs. (See Fig. 3, representing drawings of Fig. 2, for explanation.) (*a*) Dog whose diet contained vitamin A. (*b*) Dog whose diet was deficient in vitamin A.

Fig. 4 (*a* and *b*). Photomicrographs (× 6) of sections of the optic nerve (near orbit) and surrounding tissues of + vitamin A and − vitamin A dogs. (See Fig. 5, representing drawings of Fig. 4, for explanation.) (*a*) Dog whose diet contained vitamin A. (*b*) Dog whose diet was deficient in vitamin A.