Lesions of the central and peripheral nervous systems produced in young rabbits by vitamin A deficiency and a high cereal intake / by Edward Mellanby.

LESIONS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEMS PRODUCED IN YOUNG RABBITS BY VITAMIN A DEFICIENCY AND A HIGH CEREAL INTAKE.

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In previous publications [1 (*a*), (*b*), (*c*), (*d*), (*e*), (*f*)] accounts have been given of the dietetic methods whereby degenerative changes in the central and peripheral nervous systems of young dogs and rabbits can be produced and prevented. The two outstanding factors which, when associated together, produce these changes are: (1) A deficiency of vitamin A and carotene in the diet; and (2) a high cereal intake. Of these, the absence of vitamin A and carotene is probably the more important in young animals, for if either is added to the basal diets so far tested the degenerative changes do not occur. On the other hand, when vitamin A and carotene are deficient, the nerve degeneration can be made worse by altering the amount and type of cereal eaten, or in the dog by adding certain cereal products such as the embryo on ergot.

Except for one publication [1 (*e*)] in which the effects of vitamin A and carotene deficiency in producing xerophthalmia in rabbits were related to degenerative changes in the ophthalmic division of the trigeminal nerve, most of the published results referred to experimental work on dogs. It seemed desirable, therefore, that a fuller account should be given of the complex changes in nerve-cells and nerve-fibres which can be readily produced in young rabbits by similar dietetic means. Clearly, for a herbivorous animal like the rabbit, the basal diet must be of a different nature from that used in the dog experiments, and yet the same specific dietetic defects must be present in both.

Experiments were originally begun on rabbits with the object of discovering if possible the chemical nature of the substances in ergot and in cereals which act as neurotoxins in dogs and probably in man in

the absence of vitamin A and carotene. It was hoped that it would be easier to continue this part of the work on smaller and cheaper animals. Thus, it happened that the diet of many experimental rabbits in this series contained either ergot, or some extract or residue of this substance. As the work progressed, however, it became more and more questionable whether ergot affected the nervous system of rabbits even in the absence of the protective substances. In any case its neurotoxic action did not seem as potent in the rabbit as in the dog under the experimental conditions so far tested. It is certain that most, if not all, of the specific nervous lesions to be described can be readily produced in rabbits by withholding carotene and vitamin A and without giving ergot or ergot derivatives.

Although the present investigation has been fairly extensive and includes the examination of central and peripheral nerves as well as many groups of cells and ganglia in a large number of rabbits it is by no means complete. A neurological expert would undoubtedly find many other points of interest both in the behaviour of the animals and in the lesions of the nervous system produced by these specific nutritional conditions. This work, however, may lead others to extend the inquiry.

Experimental Conditions.

Litters of rabbits of eight to ten weeks old were used. The basal diets had to be such that they were compatible with good health and growth during the period in which the specific lesions developed. The following diet deficient in carotene and vitamin A was found to fulfil this object.

The main ingredients were 4 parts of oats and 1 part of bran to which calcium carbonate was added to form 1·5 per cent. of the mixture; lemon-juice was given in some of the experiments as a source of vitamin C. According to the age of the rabbit, 40 up to 70 grm. of the oats-bran mixture, together with a constant amount of 10 grm. of dried alfalfa which had been heated at 120° C. for thirty-six hours, were given daily. The alfalfa was ground to a fine powder and then heated in thin layers on trays in an electric oven and stirred frequently during the heating process in order to allow full exposure to the air. By this means its carotene content could be entirely destroyed. A point of importance for the production of severe lesions is the degree of destruction of the carotene in the alfalfa. If it be only partially destroyed, there may be little or no degeneration, or it may develop slowly and not be severe. For intense lesions to develop rapidly the heating and

stirring of the alfalfa must be sufficient to ensure the complete destruction of the carotene. If unheated instead of heated alfalfa be given, the nervous lesions do not appear. Similarly, if cabbage or carrots or any other source of carotene or vitamin A be added to the standard basal diet, the nervous system of the rabbit is protected. Pure carotene, in amounts of 1 to 3 mgm. daily, was sometimes added to the basal diet to prevent the nerve changes. In view of Stockman's results [2] in which he has produced derangement of the nervous system by diets rich in cereals but containing very little available calcium, it is necessary to point out that in the present work there is no deficiency of available calcium in the diet. It may also be well to add that in each series of experiments a rabbit with a normal nervous system has been produced by adding a source of the protective factor to the same basal diet as that given to the affected animal.

Behaviour of rabbits on the carotene-deficient diets.—The young rabbits grow and remain well on the basal diet for some months. The order of the appearance of signs of nerve defect varies, but slight stiffness of the legs, and especially of the hind legs, is generally the first abnormality noticed; this may be seen after two to three months of the experimental diets. Xerophthalmia appears next, three to six months after the beginning of the experiment; a dull patch or band appears on the cornea, usually in the centre, running midway between and parallel to the eyelids, i.e. in that part most exposed [1 (*e*)]. At this stage examination will show that the pupillary reactions are sluggish. Head movements usually develop quickly after this, although in some cases they are noticed before the changes in the eyes. The animal moves its head in a curious and characteristic way, as if it did not know its position in relation to the rest of the body; sometimes it is moved up and down and sometimes from side to side, and when at rest is often askew with ears awry. In the later stages of xerophthalmia some of the head movements may be due to ocular defects and obscured vision. The rabbit often moves about as if suffering from alcoholic intoxication. It may go round in circles, turn its head permanently towards one side, wobble from side to side, and in extreme cases even fall over either to the side or backwards, sometimes being unable to regain the normal position.

When examined in the cage the early signs are not so obvious; the animal has a sleepy look, the eyes often being half closed; it sits quietly huddled in the corner of its cage.

At no time is there any definite paralysis, but the animals appear

heavy on their legs and show less spontaneous movement both in the cage and outside, and in advanced cases often refuse to move at all.

It will be seen below that the lesions of the central and peripheral nervous systems are often very extensive, and no doubt a closer examination, especially tests on the sensory side, would reveal many other functional defects. A casual examination, however, makes it clear that the co-ordinating mechanism both of the head and body generally is defective, and in some cases it is obvious that the animals are blind and probably deaf.

Histological methods for demonstrating changes in nerve fibres.—Post mortem changes due to a lapse of time between death of the animal and fixation of its tissues were avoided. In some cases it was found better to fix the tissues *in situ*, but large organs and those with resistant capsules were dissected out and the tissues placed in the fixative.

A series of experiments on peripheral nerves was undertaken in order to compare the appearances produced by rough handling in post mortem dissection and those found in experimental animals. Nerves which were crushed with forceps, stretched or left for periods up to forty-eight hours after death of the animal gave positive Marchi reactions, but in these cases the appearances were not the same as those produced by lack of carotene or vitamin A. Needless to say, changes produced by trauma were avoided as much as possible.

The tissues were usually fixed in formalin (10 per cent. tap water neutralized formol saline).

Although methods have been used for observing changes in the axis cylinders, those for demonstrating degeneration of the myelin in the fatty sheaths have received most attention. These include: (1) Osmic staining by Marchi's method (with modifications); (2) Nile blue and sulphurous acid (Lorrain Smith [3]); (3) Scharlach R.

For peripheral nerves, on the advice of Sir Charles Sherrington, a method described by Ramon-y-Cajal [4] was also tested in the later experiments. Fresh nerves were stained for ten to sixteen hours in 0·5 per cent. osmic acid, washed in distilled water, treated with alcohol and glycerin, and then teased before microscopic examination. This method is only useful in the case of peripheral nerves, in which it shows up the changes very beautifully (Plate VI, figs. 5 and 6).

For the staining of axis cylinders several silver methods were tried, but sufficient experience of this technique was not obtained to warrant description of the results, especially of the earlier stages of axis cylinder degeneration, in the present publication. Changes certainly

are seen in the axis cylinders of the nerves of rabbits fed on carotene-deficient diets before advanced myelin degeneration occurs.

In order to determine the position of complete disappearance of groups of nerve fibres, Kulchitsky's modification of the Weigert-Pal method was adopted.

Observations on the Histological Results obtained by the above Methods.

When examining nerve fibres most attention was fixed on the myelin sheath and the changes it was liable to suffer under the prescribed experimental conditions. It is well known that histological methods for demonstrating demyelination changes, especially those involving the use of osmic acid, are apt to give unreliable results unless examined very critically. A great effort was made to avoid wrong interpretations of the experiments owing to misleading pictures; most of the animal experiments were repeated many times: the tissues from each animal were in many cases stained by several methods, and, when adopting any given technique for unknown tissues, sections of material known to be normal were often carried through simultaneously.

When the animals are severely affected there is no difficulty in demonstrating degenerative changes in the nervous tissues; when, however, the effects are slight it is by no means easy with the methods at present available. In early cases only two or three scattered fibres in a tract may be grossly changed; sometimes, for instance in the posterior roots, none show typical Wallerian degeneration, that found being rather of the annular type (see Plate VI, fig. 3). In this form the fibres are swollen, the myelin is altered in chemical composition but is not yet disintegrated, so that in cross section stained by Marchi's technique axis cylinders are seen surrounded by a dark ring of altered myelin stained with osmic acid. This annular type of degeneration is commonly found in the peripheral nerves of rabbits fed on carotene-deficient diets, but more rarely in the central nervous system. There is evidence that nerves so affected can be brought back to normal fairly rapidly when the deficient nutritive agent, in this case vitamin A or carotene, is supplied to the animal. This point, which I have discussed elsewhere [1 (*e*) (*f*)] in relation especially to degenerative changes in the ophthalmic division of the fifth nerve in cases of xerophthalmia, is one demanding much closer study both because of its scientific and its practical interest. When the degeneration is more advanced and the myelin sheath has disintegrated, structural recovery takes a much longer time.

The probable interpretation of the differences between the changes seen in the nerves of the carotene-deficient animals and those which are more familiar, namely, so-called Wallerian degeneration following injury when the axon is actually or virtually separated from its cell of origin, is that in the former the changes come on more gradually and are much more prolonged. The structural changes following nerve section are well known and the appearance of a fibre at different times after severance from its cell of origin can be more or less accurately foretold. For instance, fourteen to sixteen hours after section, Marchi's staining method will usually show the myelin sheath of the distal part of the nerve to be intact although stained with osmic acid ; this, presumably the annular stage, is rapidly passed, however, and in the course of another eight hours the myelin sheath is breaking down and droplets are seen invading the axis cylinder. It seems probable, therefore, that the changes following injury and those caused by altered metabolism (toxic) differ only in degree and not in kind.

Toxic degenerative changes are much less understood, and, as regards the particular problems under discussion in this paper, have not previously been the subject of study. As might be expected from allied work on other animals, the time of onset and the degree of severity of the nerve lesions in rabbits fed on carotene-deficient diets varies. The age of the animal at the beginning of the experiment, the prenatal and postnatal feeding and probably the rate of growth and breed, all affect the results. The reason for these influences can in some cases be understood. For instance, it is obvious that a rabbit with large stores of vitamin A in the liver at the time of the initiation of the experimental feeding will resist longer the influence of a carotene-deficient diet than one with only small stores of this substance. Similarly, a rapidly-growing animal might be expected to use up its stores more quickly. But some of the factors influencing the rate and severity of the onset, especially the question of age, are more difficult to understand. Age seems to bring about a greatly increased stability of the nervous system, and an adult animal is very resistant to the dietetic influences discussed here which in younger animals rapidly result in extensive degenerative changes in parts of the nervous system.

In some experiments in the series the animals were killed soon after the general signs of abnormality were first observed ; in others a longer interval was allowed so that degeneration was more extensive. Sometimes, after the morbid condition had been produced, a source of carotene or vitamin A was added to the diet, with the result that further

degeneration was prevented and curative changes were observed. This part of the problem will be discussed in a later paper.

Distribution of nerve degeneration in carotene deficient rabbits.—An account will now be given of the more common positions of the different levels of the brain and cord in which lesions are found in rabbits brought up on diets previously described where vitamin A and carotene are deficient. As it is not easy to take low-power photomicrographs of Marchi preparations which show up the degenerated fibres, it was decided to make drawings showing the position and relative amount of degeneration at representative levels. At first it was thought that this would give as accurate a record as the work justified. On further consideration, since it seemed certain that some at least of the fibres involved in more obvious lesions could be identified, as those of the dorsal columns and of the spino-cerebellar tracts, attempts were made to associate more and more of the degenerated fibres with well recognized tracts. This procedure became more necessary when the medulla, pons and mid-brain were examined, as it was desirable if possible to make some kind of a unified story out of what at first sight seemed a mass of disconnected facts. The distribution of the degenerated fibres in the medulla and pons has helped considerably in the identification of degenerated fibres in the cord, for many tracts which are in close proximity in the latter separate out in the higher regions and therefore can be more readily differentiated. I have therefore attempted to describe the actual degenerative changes in terms of well-recognized tracts, in spite of the fact that in so doing some errors may have been made.

Although the brain-stem of the rabbit is built up on the same plan as that of other mammals, variations in the size and position of some of its component parts are apt to lead to difficulty in recognizing certain groups of nerve fibres and nerve cells (nuclei). For instance, the inferior olivary proturbances in the rabbit are small as compared with those of man. Again, in the human brain a large band of transverse pontine fibres are seen superficially, while in the rabbit the pons is a relatively small structure (see fig. 1) so that these fibres form a comparatively narrow band. Before attempting to identify the lesions in the affected animals, it was thought desirable to fix the "land marks" of the brain of the rabbit as compared with other mammals and for this purpose serial sections (30μ thick) were made right through the brain of a normal animal. Every tenth section was stained with hæmotoxylin after mordanting, so that a general view of the main anatomical and

histological arrangements in the rabbit's brain was obtained (fig. 1) and compared with similar levels in man and dog.

Even when the general relationship of the larger features of the brain are grasped, differences in the relative number and position of fibres forming established tracts in different species also bring difficulties in describing lesions in detail. The rabbit, in common with many other mammals, has no direct pyramidal tract in the ventral column so that the intense degeneration in this column, and especially in the fibres adjacent to the ventral fissure so commonly seen in the affected rabbits in this work, cannot belong to the direct pyramidal tract. Corroboration of this deduction is obtained by the fact that even in fairly severely

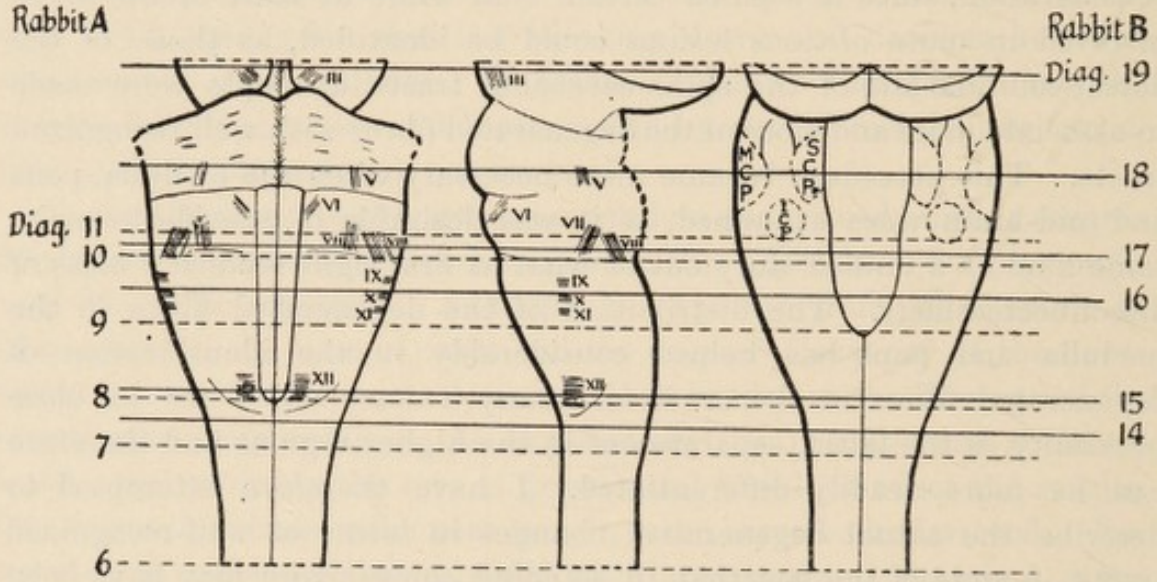


FIG. 1.—Diagrams of dorsal, lateral and ventral aspects of a rabbit's brain-stem to show the relative positions of diagrams 6-11 (rabbit A) and 14-19 (rabbit B).

affected animals it is rare to find degenerated fibres in the pyramidal tract of the pons and medulla, or in the crossed pyramidal tract of the cord.

A still further difficulty in interpreting lesions of the brain-stem is that, although the main tracts in the central nervous system of man and some animals have long been established, there are still many groups of neurons of whose origin, distribution and termination little is known, and this seems to be especially true in the case of some common laboratory animals like the rabbit and rat.

In face of these difficulties it may be necessary to amend the names given here to some of the degenerating tracts ; the positions indicated in the figs. 3 to 19, however, represent the lesions as found in the majority of the experimental animals fed on carotene-deficient diets.

In view of the varied nomenclature adopted by different workers to distinguish any one tract, it may be well to add that in this publication: (1) The terms lumbar, thoracic and cervical have been used for the three main regions of the cord; (2) that in cross-sections of the cord the three main divisions into which the long fibres are broken by the gray matter are referred to as the dorsal, lateral and ventral columns; (3) as far as possible the names used for the tracts indicate the origin and destination of the fibres. When two groups of fibres are found in different positions and yet have the same endpoint and the same origin they are referred to as ventral, dorsal or lateral in relation to each other, e.g. ventral spino-cerebellar and dorsal spino-cerebellar (both in lateral columns), also ventral spino-reticulo-thalamic (ventral column) and lateral spino-reticulo-thalamic (lateral column) (see fig. 2).

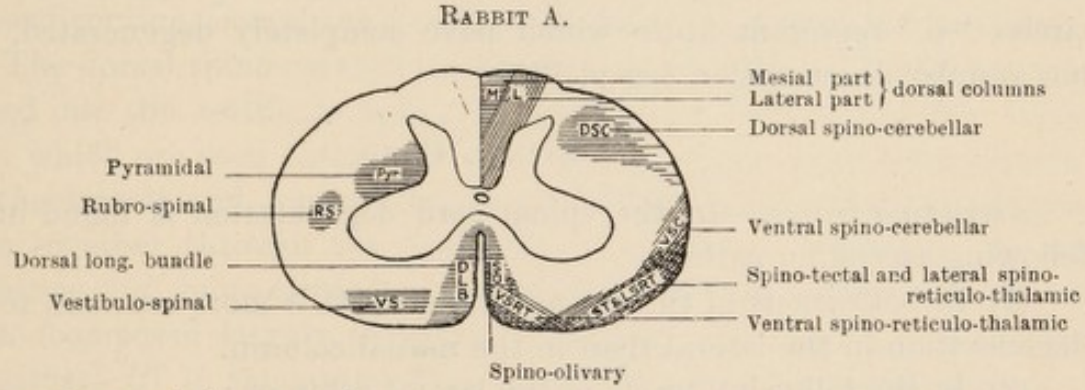


Fig. 2.—Rough diagrammatic representation of the position of the main tracts in a rabbit's spinal cord.

The drawings seen in figs. 3 to 19 were made from representative sections (stained by a modified Marchi method) of the cord and brain-stem of two rabbits (A and B) in which severe signs developed. Sometimes a section of one of these rabbits reveals the points it is desired to emphasize better than those of the other. The first animal A, shows much degeneration of the cerebellar tracts, whilst in the second animal B, most degeneration was found in the fillet. The diagrams do not represent the distribution of degenerated fibres in all the animals of this large series of experiments; they demonstrate, however, the positions in which degeneration is more commonly found.

Both rabbits had the same carotene-deficient diet (one had a daily addition of untreated and the other of treated ergot). In A the earliest symptoms appeared about the twelfth week and by the twenty-second week there was definite though slight inco-ordination. Xerophthalmia and head movements were seen about the twenty-third week, and by the

twenty-fifth the animal was very inco-ordinate and had difficulty in regaining its balance when put on one side. It was killed on the twenty-eighth week, sixteen weeks after the first signs of nervous abnormality. The second animal B was on diet only eight weeks when it showed the first signs of abnormality, especially slight stiffness and head movements. These gradually increased although there was slight improvement over one period. After the twenty-first week its head was held permanently to the left and there was definite xerophthalmia. It was killed after twenty-five weeks (seventeen weeks after appearance of the first signs), when the inco-ordination was great and the head-jerks such that the animal tended to fall over backwards.

An examination of figs. 3 to 19 shows the distribution of degenerated fibres and tracts in the spinal cord and brain-stem. In these drawings, black dots represent degenerating fibres as revealed by Marchi method, circles "o" represent fibres which have completely degenerated, and not annular degeneration (see p. 145).

I.—Spinal Cord.

Ascending tracts.—In the spinal cord degeneration is found in the following ascending paths:—

(1) In both tracts of the dorsal columns. It is more common to find degeneration in the lateral than in the mesial column.

(2) In the following tracts of the lateral columns:—

(*a*) dorsal spino-cerebellar;

(*b*) lateral spino-reticulo-thalamic;

[There is reason to believe that the ventral and lateral spino-thalamic tracts consist in part of fibres with synaptic interruptions, especially in the reticular formation of the brain-stem, and the two tracts are sometimes designated as the spino-reticulo-thalamic paths (Page May [5].]

(*c*) ventral spino-cerebellar; and

(*d*) in the spino-tectal;

[The fibres of this tract are in the cord associated with the lateral spino-reticular-thalamic tract, but it forms a separate tract in the mid-brain where degenerated fibres are seen (figs. 12 and 19).]

(3) In the ventral columns the most prominent ascending degenerated fibres belong to: (*a*) ventral spino-reticular thalamic (see note 2*b* above); and (*b*) possibly spino-olivary tracts.

Descending tracts.—There are degenerated fibres in the following descending tracts of the spinal cord: (*a*) the rubro-spinal; (*b*) vestibulo-

spinal; (*c*) the dorsal longitudinal bundle. The extent of the dorsal longitudinal bundle in the rabbit is not definitely established.

The main descending tract, namely the crossed pyramidal, is usually quite free from degenerated fibres, although in some of the carotene-deficient rabbits a few degenerated fibres are occasionally found in what is apparently the crossed pyramidal tract.

It is interesting to note in connection with the obvious impairment of equilibrium in these rabbits that all the degenerated descending fibres so far identified are connected with the vestibular and cerebellar systems.

II.—Brain-stem.

Ascending tracts.—In the brain-stem degeneration is found in the upward continuation of the ascending tracts of the spinal cord, including: (*a*) The dorsal spino-cerebellar, the degenerated fibres of which can be traced into the restiform body; (*b*) the ventral spino-cerebellar, fibres from which are seen entering the cerebellum via the superior peduncle; (*c*) the lateral and ventral spino-reticulo-thalamic tracts which pass close together through the brain-stem directly, or indirectly, to the thalamus; (*d*) spino-olivary; (*e*) degeneration is also found in the mesial fillet (composed largely of axons of cells in the nucleus gracilis and cuneatus); (*f*) in the lateral fillet which includes the second neurons of the auditory path; (*g*) the ascending fibres of the dorsal longitudinal bundle which connects the vestibular nuclei with the 3rd, 4th and 6th nuclei.

Descending tracts.—The descending tracts of the brain-stem showing degeneration include: (*a*) Rubro-spinal. Some degeneration is seen in the mid-brain where the fibres decussate (figs. 12 and 19) and in the medulla; in the pons and cord they are difficult to distinguish from degenerated fibres of other tracts; (*b*) vestibulo-spinal; (*c*) dorsal longitudinal bundle (below the vestibular nuclei).

Degenerated fibres are also seen in the intra-medullary portion of some of the sensory cranial nerves, especially the 8th and 5th.

It is interesting to compare and to contrast the behaviour during life with the amount of degeneration in the dorsal longitudinal bundles (vestibular connections) in these two animals: the head movements came on earlier and were much more pronounced in rabbit B than in rabbit A. Corresponding with this there was more degeneration in the dorsal longitudinal bundle of rabbit B. There was in rabbit A an access

of degenerated fibres in the dorsal columns at the lowest medulla level (fig. 7) (compare with the dorsal column of Cervical II (fig. 6)).

In examining the individual drawings and comparing them with one another, it will be seen that the actual and relative number of degenerated fibres in many tracts vary in the different segments.

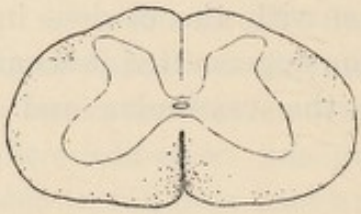


FIG. 3.—Lumbar, Segment IV.

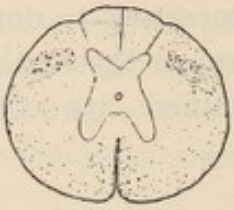


FIG. 4.—Thoracic, Segment VII.

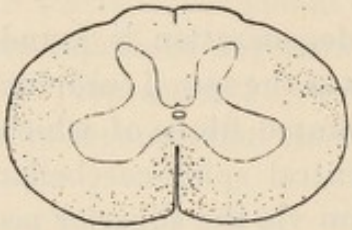


FIG. 5.—Cervical, Segment VI.

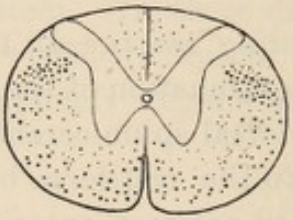


FIG. 6.—Cervical, Segment II.

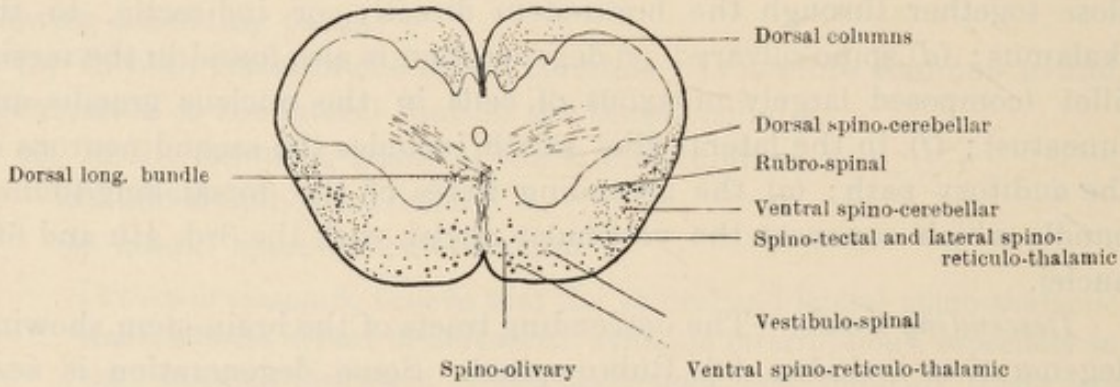


FIG. 7.—Medulla, pyramidal decussation.
Drawings illustrating degeneration in brain-stem and spinal cord of a rabbit on a carotene-deficient diet.

The following lesions were seen in the brain and cord of rabbits A and B.

Lumbar Segment IV (rabbit A) (fig. 3).—There are very few degenerated fibres in the dorsal columns; more are found in the cerebellar tracts and in the ventral columns adjacent to the ventral fissure. There is less degeneration in the lumbar and sacral regions than in any other levels examined. This is in general true for all rabbits fed on these carotene-deficient diets.

Thoracic Segment VII (rabbit A) (fig. 4).—There are many more degenerated fibres in this level than in lumbar IV. Dogs brought up on diets

deficient in vitamin A and carotene often had comparatively few degenerated fibres in the thoracic cord [1 (*c*)].

Cervical Segment VI (rabbit A) (fig. 5).—In this region the degeneration seems to have reached its maximum in the cerebellar and ventral column tracts, while the dorsal columns appear to contain fewer degenerated fibres than in the thoracic levels.

Cervical Segment II (rabbit A) (fig. 6).—The main change usually seen in this region is the scattered appearance of the degeneration in contrast to that in the lower levels of the cord; this is partly because the ascending tracts are separating out in preparation for redistribution in the brain-stem. At this level the degeneration in rabbit A is more evenly distributed between the lateral and mesial parts of the dorsal columns than is generally seen.

Medulla, pyramidal decussation (rabbit A) (fig. 7).—At the level of the motor decussation many degenerating fibres are seen in the dorsal columns near the nuclei cuneatus and gracilis, and also in the dorsal and ventral spino-cerebellar and lateral spino-reticulo-thalamic tracts. Rather less degeneration is seen in the white matter which includes the ventral spino-reticulo-thalamic, spino-tectal and spino-olivary tracts.

The fibres of the ventral columns naturally assume a more lateral position owing to the decussation of the pyramids, none of the fibres of which are affected, although some transversely cut fibres between the decussating ones are degenerated. These probably belong to the dorsal longitudinal bundle; there is some degeneration in what appear to be the vestibulo-spinal and the rubro-spinal tracts.

For the sake of clarity reference will first be made to some of the higher levels of rabbit A, then a few sections of rabbit B will be described.

Medulla, higher part of sensory decussation (rabbit A) (fig. 8). The inferior olivary and lateral reticular nuclei are just appearing at this level. Degenerated fibres are present in the remains of the dorsal columns, although many fibres have entered the nuclei gracilis and cuneatus. The axons of some of these cells can be seen sweeping through the reticular formation as internal arcuate fibres. There is much degeneration in the dorsal and ventral spino-cerebellar tracts but little in the mesial fillet (cf. fig. 15). The spino-reticulo-thalamic (above the level of the pyramidal decussation the lateral and ventral spino-reticulo-thalamic appear to run together), spino-tectal and possibly also the spino-olivary tracts contain some degenerated fibres and remain in approximately the same positions as in fig. 7. Of the descending fibres the dorsal longitudinal bundle and rubro-spinal tract show some degeneration; the vestibulo-spinal, still obscured by other tracts, is probably degenerated whilst the pyramidal is unaffected. The 12th nerve containing no degenerated fibres is seen passing ventralwards through the section.

Medulla, level of inferior olivary nucleus and the 4th ventricle (rabbit A) (fig. 9).—At this level in rabbit A there is not much degeneration in the fillet,

but there is still heavy degeneration in the dorsal spino-cerebellar tract which is moving towards and entering the restiform body, the ventral spino-cerebellar, the spino-reticulo-thalamic and the spino-tectal tracts. There is some, but not

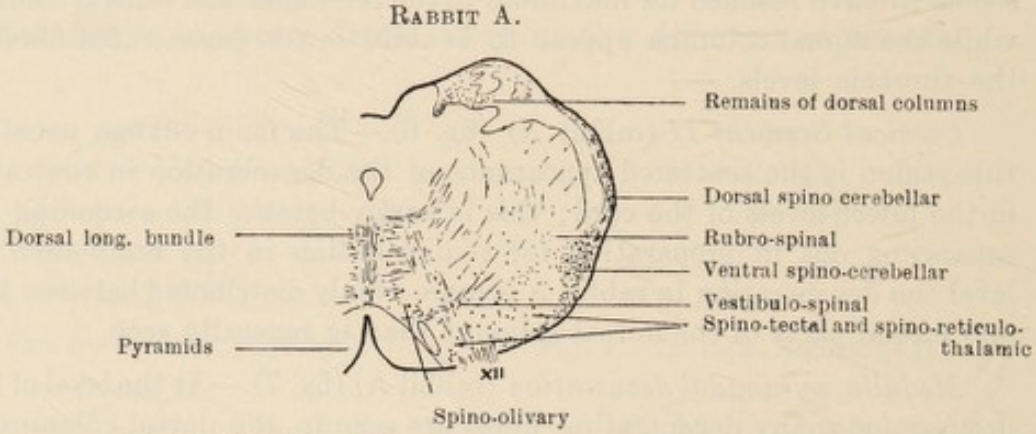


Fig. 8.—Medulla, higher part of sensory decussation.

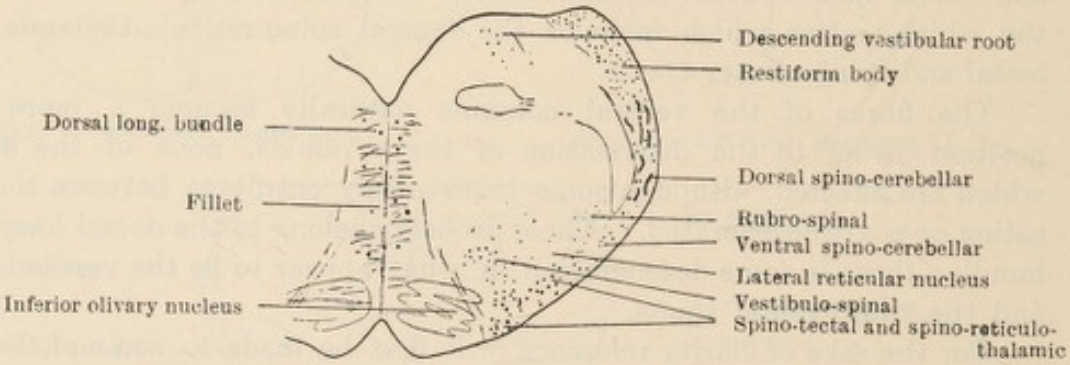


Fig. 9.—Medulla, level of inferior olivary nucleus and 4th ventricle.

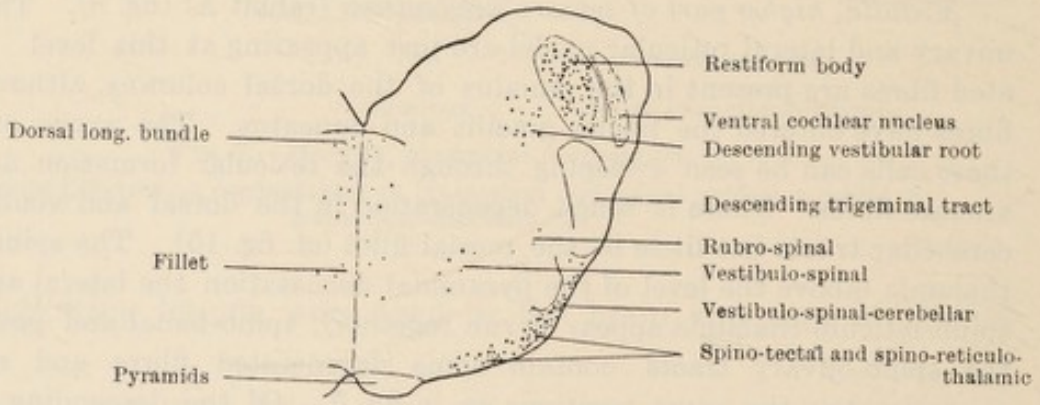


Fig. 10.—Section between medulla and pons.

Drawings illustrating degeneration in brain-stem and spinal cord of a rabbit on a carotene-deficient diet.

much, degeneration in the dorsal longitudinal bundle; of the other descending tracts, the vestibulo-spinal and what appears to be the descending vestibular root of the 8th nerve contain more degenerated fibres. The latter has only been

traced as low as the higher levels of the nuclei gracilis and cuneatus. Some small degenerated fibres are present in the position of the rubro-spinal tract. It is interesting to note that in this animal there are degenerated fibres among the cells of the lateral reticular nucleus. These are only found in animals having much degeneration in the lateral cord columns and the restiform body. Rabbit B, which has less degeneration in the lateral tracts, does not show any degenerating fibres among these cells. The lateral reticular nucleus is said to relay impulses from the lateral tracts of the cord to the restiform body.

Section taken between medulla and pons (rabbit A) (fig. 10). The transverse pontine fibres are not seen although this section is taken above the level of the inferior olivary nuclei. A few fibres of the mesial fillet are affected. The large group of degenerated fibres on the latero-ventral aspect belong to the ventral spino-cerebellar, the spino-reticulo-thalamic and spino-tectal tracts. There is much degeneration in the restiform body, mainly dorsal spino-cerebellar fibres. Degeneration is seen in the dorsal longitudinal bundle, vestibulo-spinal tract, the descending vestibular root and also in a few fibres of the rubro-spinal tract. The pyramids are free from degeneration, as also is the descending branch of the 5th nerve. (In one or two experiments this branch of the 5th nerve contained degenerated fibres.) Degenerated fibres are also found among the cells of the ventral cochlear nucleus.

Level of the 8th and 7th nerves (rabbit A) (fig. 11).—In this section the transverse fibres of the trapezoid body can be seen. These, some of which are degenerated in rabbit A, arise in cells of the ventral cochlear nucleus and form the greater part of the lateral fillet of the opposite side (second neurons of the auditory path). The pyramidal tract contains no degenerated fibres and remains at the ventral surface of the brain-stem. The degenerating fibres of the ventral spino-cerebellar tract can be seen between the 7th and 8th nerves, bearing dorsalwards to enter the superior cerebellar peduncle. The restiform body has already entered the cerebellum in spite of the fact that the pons is not yet established. There is now much less degeneration in the ventro-lateral region since the ventral spino-cerebellar tract has moved dorsalwards and some of the spino-reticulo-thalamic fibres have probably entered relay stations in the reticular formation of the medulla. The only descending tracts in which degenerated fibres appear are the dorsal longitudinal bundle and the rubro-spinal. This section, being at the level of the 8th nerve, shows no descending vestibular root or vestibulo-spinal tract. Heavy degeneration is seen in the 8th nerve, while the 7th and 6th are unaffected.

Superior corpora quadrigemina (rabbit A) (fig. 12).—The only ascending tract containing degenerated fibres at this level is the spino-tectal. The others which showed degeneration in fig. 11 have probably entered relay stations; for instance, the spino-reticulo-thalamic fibres of the lower levels may have made synapses with cells in the reticular formation of the medulla and pons, some fibres of the lateral fillet have probably entered the nucleus of this fillet, and the ventral spino-cerebellar tract has entered the cerebellum via the superior cerebellar peduncle. Degeneration is, however, seen in the dorsal

longitudinal bundle and in the rubro-spinal decussation. A few fibres of the 3rd nerve also take up Marchi stain. Although there is no degeneration in the internal capsule or the pyramidal tract of the lower brain stem, there are a few degenerated fibres in the crusta of this level.

Cervical, Segment II (rabbit B) (fig. 13).—It has previously been noted

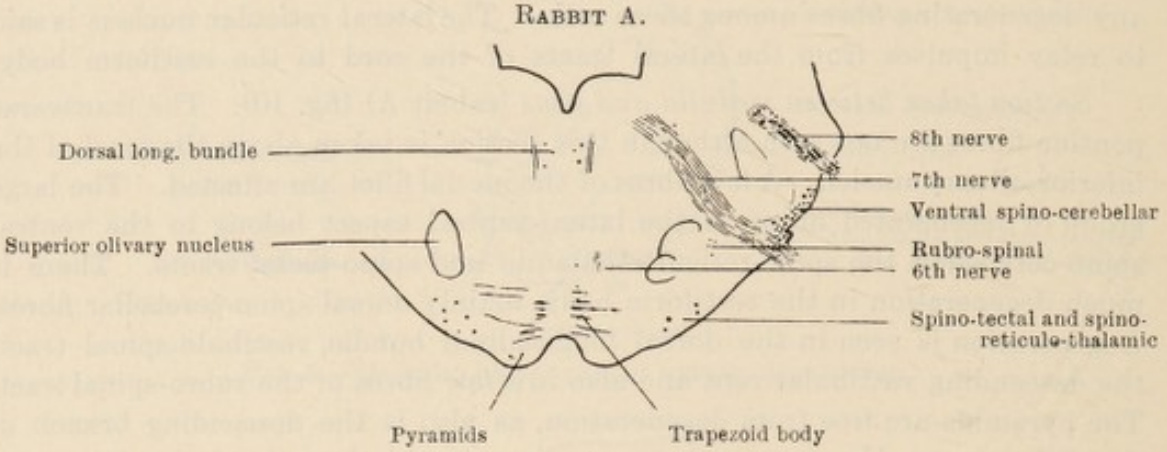


Fig. 11.—Level of 8th and 7th nerves.

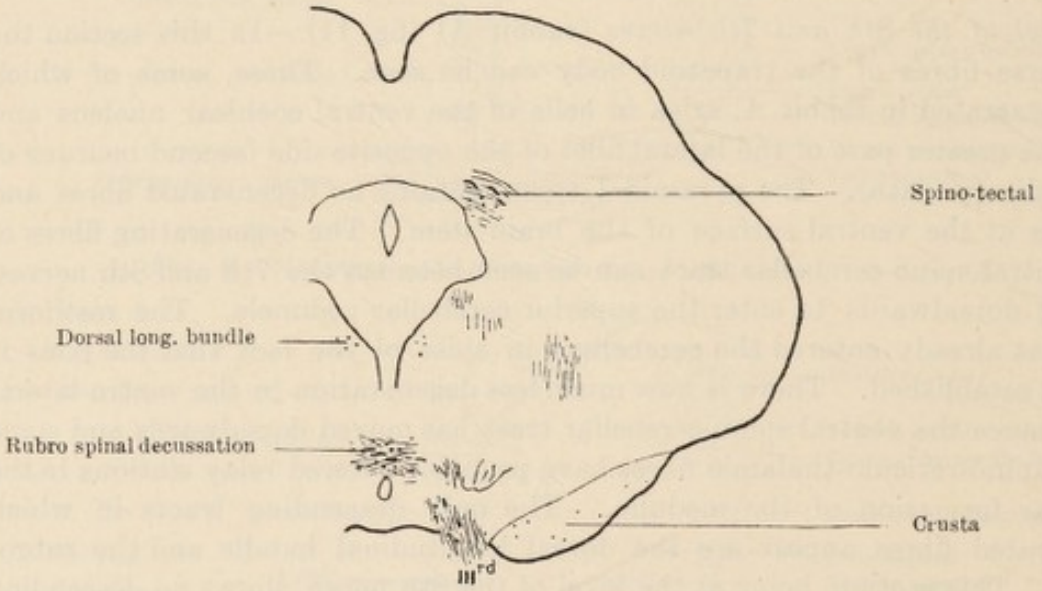


Fig. 12.—Superior corpora quadrigemina.

Drawings illustrating degeneration in brain-stem and spinal cord of a rabbit on a carotene-deficient diet.

that in rabbit B the degeneration is most prominent in the fillet and dorsal longitudinal bundle, whereas in rabbit A the heaviest degeneration is in the cerebellar tracts. This can be seen by comparing figs. 6 and 13. In rabbit B many fibres of the dorsal columns show very advanced degeneration with absorption of the myelin, and, as might be expected, there is also advanced degeneration of the second neurons of this path in the mesial fillet but no

RABBIT B.

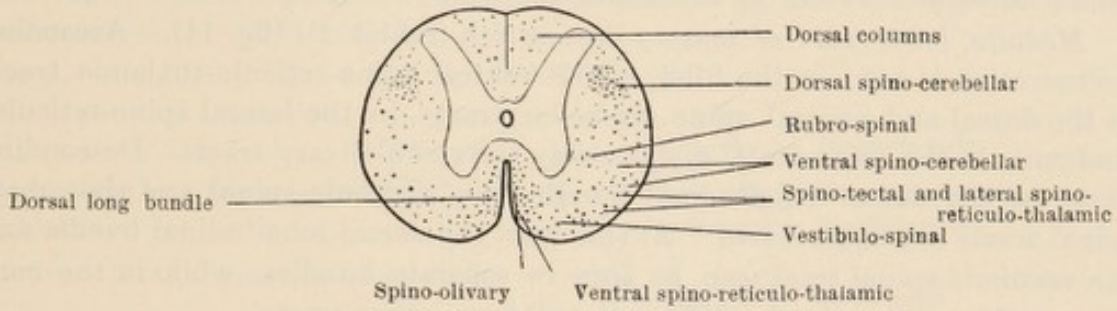


FIG. 13.—Cervical, Segment II.

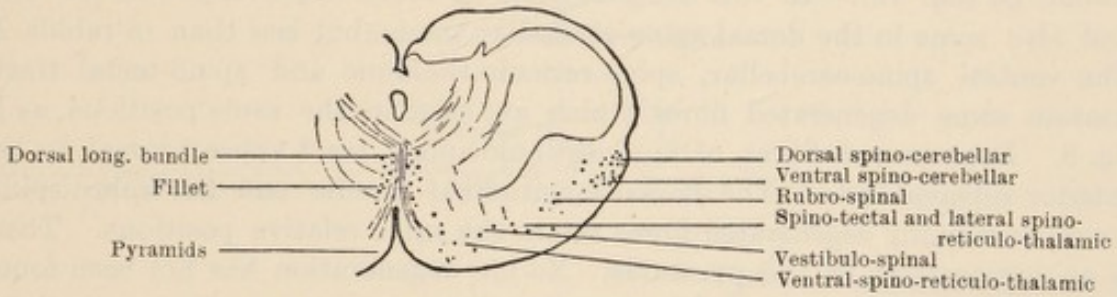


FIG. 14.—Medulla, lower part of sensory decussation.

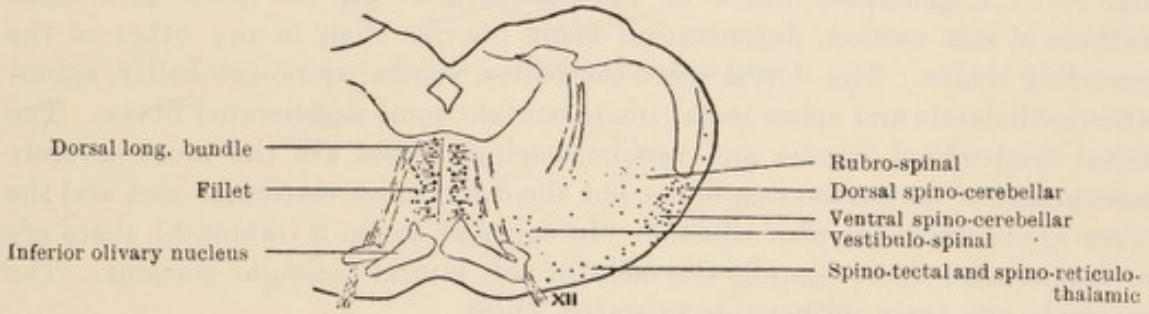


FIG. 15.—Medulla, level of inferior olivary nucleus.

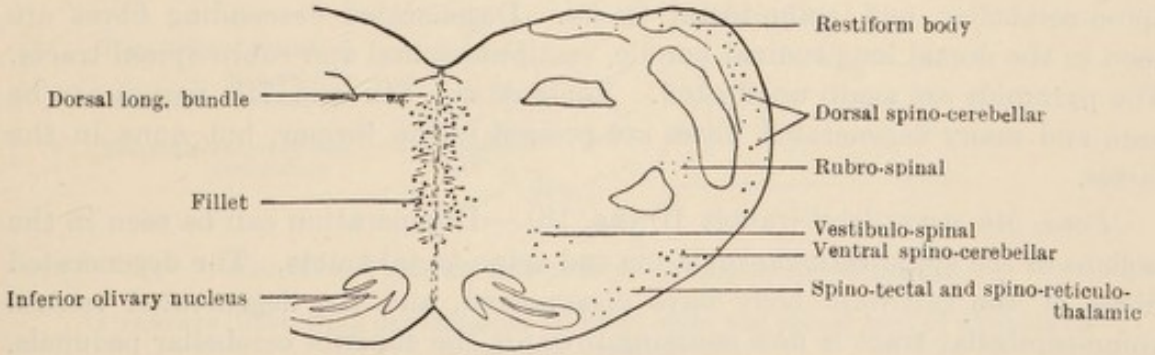


FIG. 16.—Medulla, level of inferior olivary nucleus and 4th ventricle.

Drawings illustrating degeneration in brain-stem and spinal cord of a rabbit on a carotene-deficient diet.

evidence of degeneration in a third neuron has been found either in this rabbit or in any others of the series so far examined.

Medulla, lower part of sensory decussation (rabbit B) (fig. 14).—Ascending degeneration is seen in the fillet, in the ventral spino-reticulo-thalamic tract, in the dorsal and ventral spino-cerebellar tracts, in the lateral spino-reticulo-thalamic, in the spino-tectal and possibly the spino-olivary tracts. Descending fibres in the dorsal longitudinal bundle, the vestibulo-spinal and the rubro-spinal tracts are degenerated. At this level the dorsal longitudinal bundle and the vestibulo-spinal tract can be seen in separate bundles, while in the cord they could not be clearly differentiated from other tracts.

Medulla, level of the inferior olivary nucleus (below 4th ventricle) (rabbit B) (fig. 15).—In this section there is much degeneration in the fillet and also some in the dorsal spino-cerebellar tracts, but less than in rabbit A. The ventral spino-cerebellar, spino-reticulo-thalamic and spino-tectal tracts contain some degenerated fibres which are seen in the same positions as in fig. 8. Degenerated fibres of the vestibulo-spinal tract appear dorsal to the inferior olivary body. The dorsal longitudinal bundle and the rubro-spinal tracts containing degenerated fibres retain the same relative positions. There is no degeneration in the pyramids. So far, degeneration has not been found in the 12th, 11th, 10th or 9th nerves.

Medulla, level of inferior olivary nuclei and 4th ventricle (rabbit B) (fig. 16). Degenerated fibres of the mesial fillet are the most prominent features of this section, degeneration being heavier than in any other of the ascending tracts. The dorsal spino-cerebellar, ventral spino-cerebellar, spino-reticulo-thalamic and spino-tectal tracts contain some degenerated fibres. The dorsal longitudinal bundle and vestibulo-spinal tracts are the most severely degenerated of the descending fibres, but the descending vestibular root and the rubro-spinal tracts are also affected. In contrast to fig. 9 (rabbit A) there are no degenerated fibres among the cells of the lateral reticular nucleus. The pyramids are again without degenerated fibres.

Level of 8th nerve (rabbit B) (fig. 17).—This section shows degenerated ascending fibres in the fillet, restiform body, spino-reticulo-thalamic, ventral spino-cerebellar and spino-tectal tracts. Degenerated descending fibres are seen in the dorsal longitudinal bundle, vestibulo-spinal and rubro-spinal tracts. The pyramids are again unaffected. Roots of the 8th and 7th nerves can be seen and many degenerated fibres are present in the former, but none in the latter.

Pons, 5th nerve level (rabbit B) (fig. 18).—Degeneration can be seen in the regions of the spino-reticulo-thalamic and spino-tectal tracts. The degenerated fibres of the restiform body have disappeared, and the degenerated ventral spino-cerebellar tract is now coursing towards the superior cerebellar peduncle. Degenerated fibres can be seen in the dorsal longitudinal bundle and what are presumably the rubro-spinal and vestibulo-mesencephalic tracts. The pyramids are again free from degeneration. Degeneration can be clearly seen in the fibres of the 5th nerve.

Posterior part of superior corpora quadrigemina (rabbit B) (fig. 19).—Much less degeneration is present in this section than in fig. 18. No degeneration is

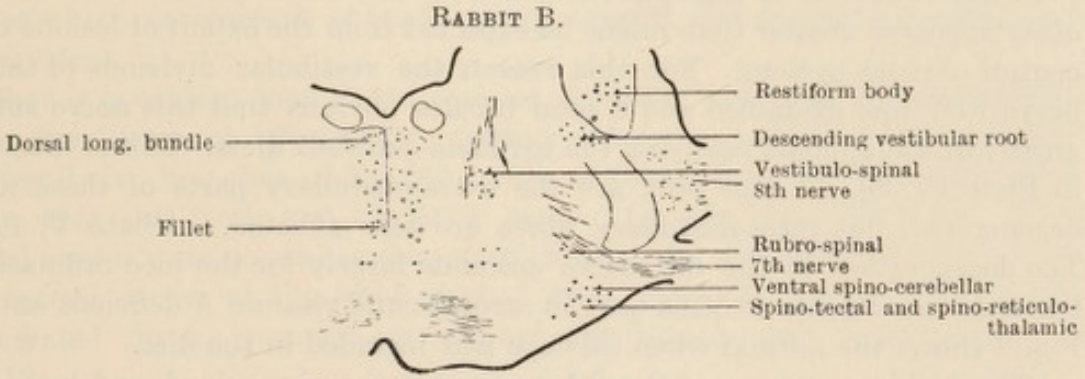


Fig. 17.—Level of 8th nerve.

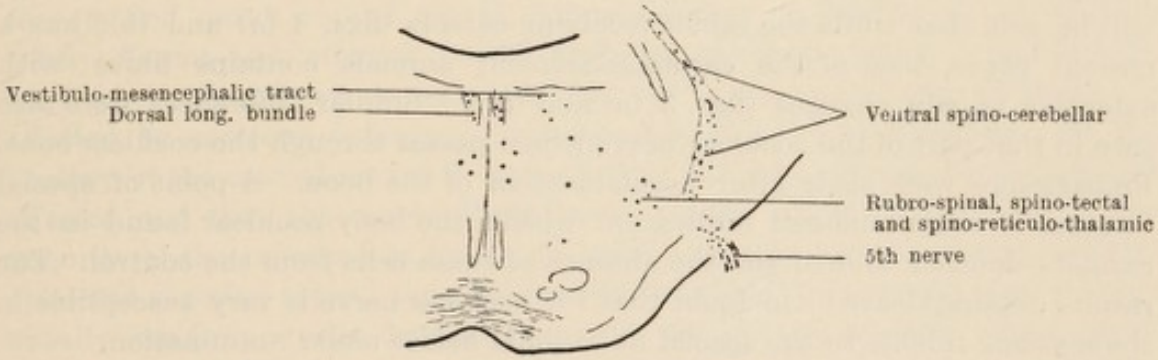


Fig. 18.—Pons, 5th nerve level.

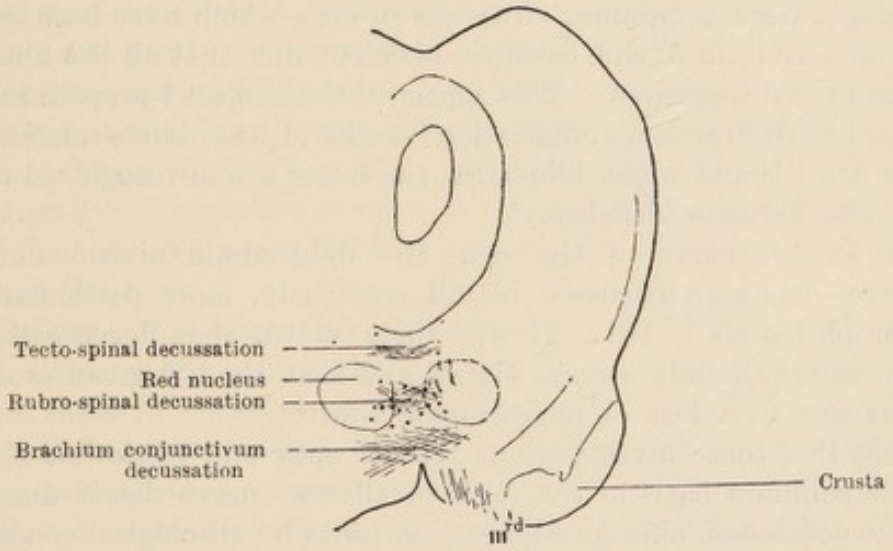


Fig. 19.—Posterior part of superior corpora quadrigemina.

Drawings illustrating degeneration in brain-stem and spinal cord of a rabbit on a carotene-deficient diet.

seen in the ascending tracts of this region, as they have probably entered relay stations. Degenerated fibres are present in the dorsal longitudinal bundle and in the decussation of the rubro-spinal tract.

Degenerative Changes in Peripheral Nerves.

Extension of the investigation to the peripheral nerves was begun because the inco-ordination of the animals, and especially the movements of the head, often appeared greater than might be expected from the extent of lesions of the central nervous system. For this reason the vestibular divisions of the 8th nerve were first examined and it soon became obvious that this nerve suffered greatly in the animals receiving the carotene-deficient diets. This is illustrated in Plate IV, fig. 1. Not only are the extra-medullary parts of these nerves degenerated, but intra-medullary fibres are also affected. (Plate V, fig. 1.) The degeneration in the 8th nerve accounts largely for the inco-ordination of the head movements so often seen in carotene and vitamin A deficient animals. Fig. 2 shows the normal when cabbage was included in the diet.

The cochlear division of the 8th nerve was then examined, and here again changes were revealed, which are illustrated in Plate IV, figs. 3 (*a*) and (*b*). It will be seen that while the rabbit receiving carrots (figs. 4 (*a*) and (*b*)) has a normal nerve, that of the carotene-deficient animals contains fibres with extensive myelin changes (figs. 3 (*a*) and (*b*)). Similar differences were also seen in that part of the cochlear nerve which passes through the cochlear bone. Preparations were made after decalcification of the bone. A point of special interest is the round-cell infiltration within the bony cochlear found in the carotene-deficient animal and the absence of these cells from the control. The results obtained leave little doubt that the cochlear nerve is very susceptible in these young rabbits to the special nutritional defect under examination.

Degenerating fibres were also found in the optic nerves of the carotene-deficient animals (Plate V, figs. 3 and 4). I have shown elsewhere [1 (*b*)] that these changes were sometimes so severe in dogs which have been fed for several years on a vitamin A and carotene-deficient diet that all the fibres of the optic nerve have disappeared. This aspect of the subject I propose to deal with at greater length in another publication because of its possible relationship to retrobulbar neuritis and night blindness, the latter a well-recognized result of vitamin A and carotene deficiency.

The other sensory nerve of the eye—the ophthalmic division of the trigeminal nerve—has been discussed in full previously, more particularly in relation to xerophthalmia [1 (*e*)]. It was there shown that degeneration of this nerve and xerophthalmia are associated, and that the latter can probably be regarded as due to a loss of neurotrophic control.

It is evident that these investigations on the optic and trigeminal nerves, by supplying a common basis in the form of afferent nerve defect due to a specific dietetic deficiency, offer an explanation for two pathological conditions affecting the eye, night blindness and xerophthalmia, two conditions which at first sight seem to have no relation to one another.

One other sensory nerve of the head, namely, the inferior dental nerve, has also been the subject of inquiry by M. Mellanby and J. D. King [6], who have shown that vitamin A and carotene deficiency bring about, in young rats, dogs and rabbits, degenerative changes in it. They also found evidence that these

demyelination changes were related to hyperplasia of the epithelium of the gum margin, especially of the part adjacent to the teeth, and the subsequent invasion of the epithelial cells by micro-organisms resulting in pyorrhœa, a disease which develops in animals eating a cereal diet deficient in vitamin A and carotene [7].

Thus it is evident that all the sensory nerves of the head so far examined in young rabbits are affected by carotene deficiency, including both auditory and vestibular branches of the 8th nerve, sensory branches of the 5th nerve to the eyes and teeth and the optic nerve. On the other hand the motor cranial nerves were normal in the experiments so far completed at a time when the sensory nerves of the head showed great demyelination changes; a few degenerated fibres, however, have been occasionally seen in the 3rd nerve (fig. 12).

In view of the almost clear-cut division between the susceptibility of the sensory and motor cranial nerves, it was a matter of interest to see whether other afferent and efferent nerves behaved similarly under these dietetic conditions. Sciatic nerves which contain both efferent and afferent fibres are affected by carotene deficiency (compare figs. 5 and 6 of Plate V). On the analogy of the cranial nerves it seemed likely that the afferent fibres would be affected and that the motor fibres would escape. This is the case in the majority of the experiments for, in the rabbits brought up on carotene-deficient diets and showing typical signs of the deficiency, the ventral roots (motor) were usually free from myelin degeneration, whereas the dorsal roots were always affected (Plate VI, figs. 1 to 4). Both roots of the animal fed on cabbage are devoid of degeneration, whereas in the animal not receiving carotene the dorsal root shows much degeneration, whilst the ventral root is normal. This is the usual result, but in animals where the dietetic defect has continued for a long time some of the ventral root fibres also may be degenerated. The affection of the dorsal roots can be clearly demonstrated by Cajal's osmic method (Plate VI, figs. 5 and 6). When the rabbit had the basal diet only, the degenerative changes are obvious (fig. 5), but when a few drops of concentrated vitamin A oil (0·1 c.c. = 100 blue values), were added to the diet the fibres are normal (fig. 6).

Reference has been made above to the vagus nerve. It might be expected that, although the motor fibres in this nerve would escape, its afferent fibres would suffer in the same way as most of the other afferent nerves. This has not been found to be true; in spite of many examinations of these nerves, even in severely affected animals, no degenerative changes have so far been seen in them. In rats brought up on a vitamin A deficient diet, Zimmerman [8] found some degeneration in the vagus nerves in only four out of eight animals examined, but in all there was extensive degeneration of the medullary sheaths of the brachial plexuses and sciatic nerves.

There is still a wide field left for future observation as regards these nerve changes. It is known that both the skin and epithelial surfaces are particularly susceptible to infection in vitamin A deficiency, and epithelial surfaces also

develop characteristic changes as hyperplasia and keratinization of their cells. On the analogy of the eye (xerophthalmia) and the gums (periodontal disease) it is probable that these epithelial changes are related to degeneration of the corresponding afferent nerves. It would be of interest to know whether the afferent nerves from joints and muscles are also affected by the dietetic deficiency under examination, and, if they are so affected, whether specific lesions are produced in these end organs which correspond to the pathological changes developing in the skin and epithelial surfaces.

Nerve-Cells.

In view of the great degenerative changes in the conducting fibres of the cord resulting from these specific nutritional defects, it is not surprising that many cells are also affected. Indeed, it is not improbable, considering the differentiation between the widespread degeneration of the intermedullary ascending fibres and the afferent nerves and the comparative immunity of the descending tracts and the efferent nerves, that many of the primary effects of the abnormal nutrition under consideration are on the cells and that the changes in the nerve fibres are secondary.

In spite of the large amount of work done by many investigators on the morbid anatomical changes of nerve-cells, there still remains a general lack of systematized knowledge as to the relation of the structures seen under the microscope after fixation and other treatment and their functional state during life. Nissl's bodies found in suitably stained sections vary in size, shape and arrangement in the different nuclei of any one species, and in different species cells having the same functions often vary greatly in appearance. This introduces a difficulty into the interpretation of experimental results even when care is taken to use controlled and efficient methods of fixation and to avoid post mortem and manipulative changes. However, when all technical errors in preparation have been avoided and all the processes are carried through as far as possible under identical conditions, there is no doubt at all that there are greater differences in the microscopical appearances of the nerve cells of the carotene-fed and carotene-free animals than can be accounted for by normal variations.

When nerve-cells undergo degenerative changes as the result of lesions of their axons, the process is usually called secondary degeneration. Cell changes brought about by toxic agents are known as primary degenerations. This phraseology was introduced by Marinesco.

Secondary degeneration of the nerve-cell due to injury of its axon, and particularly the changes in the chromophile elements, was discovered by Nissl [9]. It consists of a breaking up of the chromophile elements

first beginning round the nucleus, a swelling of the whole cell and a movement of the nucleus towards the periphery. All nerve-cells whose conducting fibres are cut do not react in this way. The best reactions are seen in motor cells, as those of the ventral horns or in the cortical motor cells, when their respective conducting fibres suffer transection. Peripheral afferent nerve-cells, as those of the dorsal root ganglion, undergo secondary degeneration when the peripheral portions of the fibres are divided, but not when their central processes, i.e. the dorsal roots, are injured.

In the central nervous system itself, apart from the chromolysis and other changes seen in the cells of Clarke's column when the dorsal spino-cerebellar tract is destroyed, but little is known of secondary cell changes in ascending systems. It is certain, however, that afferent cells in the central nervous system are by no means as susceptible to this type of change as the motor cells.

The question of primary or toxic reactions of nerve-cells, the so-called primary degenerations, is even more complex. Since the introduction of the Nissl technique, many investigations have been made to determine the changes produced in nerve-cells by known toxic substances. Nissl [11] himself investigated the action of many such substances including lead, arsenic, phosphorus, mercury, silver, alcohol, morphine, bromide, cocaine, trional, veratrine, strychnine, carbon disulphide, and tetanus toxin. Goldscheider and Flatau [12] also made many investigations into the effect of pathological conditions produced experimentally on nerve-cells. Russell [13] has studied the effect of parathyroidectomy on cells of the nervous system, and in more recent times Dye [14] has compared the effect of parathyroidectomy and cretinism on the nerve-cells of dogs. Other harmful procedures whose effects on nerve-cells have been studied include ischæmia, inanition, hyperpyrexia, uræmia, extirpation of the suprarenal bodies, and experimental and natural infection.

Although it is undoubted that nerve-cells undergo changes under many of the above conditions, the subject of primary degeneration has remained generally unsatisfactory aud undeveloped. One of the difficulties in work of this nature is, as Nissl himself recognised, that under conditions which produce primary degeneration of the nerve-cells even one group may show the widest variations, some appearing nearly normal and others having great degenerative changes. Even in normal animals the cells in one group may vary in their appearance. In his studies of the effect of parathyroidectomy and cretinism on the nerve-cells of dogs,

Dye [14] met the same difficulties. He found that chromolytic reaction in nerve-cells follows the same general course in conditions of such diverse symptomatology as parathyroid tetany and cretinism, and decided that chromolysis is a reaction of nerve-cells initiated by any factor which opposes the normal physiological equilibrium of these units, a conclusion similar to that reached years ago by Van Gehuchten.[1]

In primary degenerative changes, as pointed out by Van Gehuchten [15], the Nissl bodies or chromophile particles do not, as a rule, undergo real chromolysis. When altered in this direction there is no real dissolution of the chromophile substance, but rather an achromatosis or partial disappearance or change of chromophile elements in some part of the cell. Marinesco states that, when the lesions are primary, chromolysis commences in the periphery of the nerve-cells and spreads towards the centre, whereas in secondary or traumatic degeneration the chromolysis begins centrally and spreads towards the periphery.

In the present investigations the changes in the nerve-cells can be classified among the primary or toxic group, although produced by ordinary foodstuffs without the addition of any known toxic agent. On the whole, but not absolutely, Marinesco's generalisation as to the peripheral location of changes in the Nissl bodies in primary degeneration applies to the present results, although there are some exceptions. Real chromolysis (in the sense used by Nissl) is not very common, and when present generally affects a small portion of the cell either at the periphery or near the nucleus. A few instances of chromolysis comparable to that seen in secondary degeneration were found, more especially in certain cells of the medulla. More often the granules lose their discrete form and are in a powdery or lightly staining condition, the parapyknomorphic change of Nissl. At times the Nissl bodies may be aggregated into clumps, and in other cases they may be so changed that the cell takes on a more intense, even stain and is described as chromophile.

In addition to degenerative changes in the cytoplasm, the nucleus and nucleolus are often affected. They may be swollen or shrunken. Sometimes the nucleus leaves the centre of the cell and becomes eccentrically placed. Occasionally the nucleus seems to have disappeared or its space to be invaded by the cytoplasm with Nissl bodies. At times the nucleus may appear finely granular instead of clear as it is normally.

[1] For discussions of the various hypotheses concerning chromolysis in damaged nerve cells *see* Van Gehuchten, *Anatomie du Système Nerveux, de l'homme*, 1900, Vol. 1, pp. 313-339; also S. Ramon-y-Cajal, *Histologie du Système Nerveux*, 1909, Vol. 1, pp. 214-222.

If any one of these abnormalities appeared in a group of nerve-cells the problem of description would be relatively simple, but the fact is that in any group of cells in a single animal many of these appearances can be detected in different cells. Even in the control animals, where the diet contains abundant carotene and the condition of the animals is excellent, a few of the cells will often appear abnormal. Thus it often happens that, although the observer feels fully assured that the experimental condition under investigation has produced degenerative changes in particular groups of nerve-cells, when he comes to describe these it is a matter of great difficulty.

The following brief description of changes in nerve-cells that are produced by diets high in cereals and deficient in carotene is given not so much because of any claim to finality, but rather to introduce an aspect of nerve-cell pathology which is easily producible experimentally and which, because of its probable significance in man, is worth more detailed study, for it will be generally agreed that a pathological condition of the nervous system which can be produced by ordinary foodstuffs is an important field of investigation.

The chief fixatives used for nerve-cells were 10 per cent. neutralized formol saline and Carnoy's solution (Perdrau's modification). Of these Carnoy's gave the better results owing to more rapid penetration due to the presence of acetic acid, the subsequent staining being deeper and sharper as well as more lasting.

The stains employed for demonstrating the changes seen in nerve-cells include: methylene blue (polychrome), toluidene blue and the Unna-Pappenheim stain (pyronin and methyl green). Silver impregnation methods have so far been little used in these experiments although there are indications that such methods would give interesting results.

Changes in Nerve-cells.

Dorsal root ganglia (Plate VII, figs. 1 to 7).—In the dorsal root ganglia there are normally two types of cells, the larger staining rather faintly and the smaller more strongly. The most representative change in these ganglia in carotene-deficient animals is the powdering and loss of defined granules in the main body of the cell and the apparent concentration of granules around either the nucleus or the outer edge. The only obvious differences between degenerated large and small cells is that the small cells seem to be more affected by the "piling" reaction, but this may be more apparent than real owing to their deeper staining. This "piling" of the granules around both the nucleus and the outer edge has been seen in the same cell, but it is most common to find the chromophile substance concentrated around the

nucleus. Eccentric nuclei have also been found. The nuclei of the capsule or, according to Cajal, the sub-capsule, have sometimes proliferated around degenerate cells, giving by Nissl's method a picture akin to what has been called " neuronophagia " in the cells of the central nervous system.

Gasserian ganglia.—Changes in the cells of the Gasserian ganglion are also seen in the carotene-deficient animals. These include eccentric nuclei, proliferation of the nuclei of the cell capsule, powdery granules and " piling " of the granules around the nucleus and at the outer edges of the cell, the latter being very distinct. (" Piling " appears to be less obvious in cells of the central nervous system than in those of the dorsal root and the Gasserian ganglia, which are the only cells of the peripheral nervous system so far examined).

Clarke's column and other dorsal horn cells (Plate VIII, figs. 1 and 2.)—These cells are rather small, and even in the normal the granules are not clearly defined. There are, however, undoubted differences between the animals with and without carotene. In advanced cases of carotene-deficient diets some cells seem to have disappeared, leaving only a skeleton behind, but loss of granules without other apparent change is common. In less severe cases neuronophagia, loss of definition of the granules and eccentric and slightly granular nuclei are the usual changes.

Lateral horn cells.—No striking changes have so far been noted in these cells.

Ventral horn cells (Plate VIII, figs. 3 and 4).—The large motor cells are usually entirely free from degeneration ; occasionally, however, in advanced cases of vitamin A deficiency, clumping of the granules and even slight chromolysis, is found.

Nuclei gracilis and cuneatus.—The cells of these nuclei fall into line with the other cells of the afferent paths. Eccentric nuclei, powdery granules and loss of granules being seen in carotene-deficient animals, whilst in those on a carotene-containing diet they remain normal.

Lateral reticular nucleus (Plate VIII, figs. 5 and 6).—Powdering and loss of granules, clouding of the nucleus, eccentric nuclei and neuronophagia are the usual changes in the carotene-deficient animals. These groups of cells are usually only badly affected when there is much degeneration in the lateral columns of the cord and in the restiform body (see also p. 164, R. Cajal and and V. Gehuchten).

Olivary nucleus.—This nucleus receives sensory fibres from the ventral columns. Even in animals on the carotene-containing diets cells of the olivary nucleus with clearly defined granules seem to be rare. Eccentric nuclei have been found in cells of animals on a carotene-deficient diet and this seems to be the main difference from control animals.

Hypoglossal nucleus.—There are no definite differences in these nuclei between animals on carotene and carotene-deficient diets.

Nucleus solitarius (position rather more dorsal than in man).—This nucleus, which receives afferent branches from the facial, glossopharyngeal and vagus

nerves, is often severely affected. The cell changes are powdering and loss of granules, frequently with eccentric nuclei. Some of the cells in this nucleus in two or three carotene-receiving animals appear to be slightly degenerated compared with those considered as normal.

Nucleus ambiguus.—In this nucleus changes have been noted in the experimental animals. Powdering of the granules is by far the most common change, but eccentric nuclei have been seen; in a few carotene-deficient animals showing definite changes in many other nuclei no abnormalities have been seen in this group of cells. In the animals receiving carotene no degenerated cells have been found.

Vestibular nuclei (Plate VIII, figs. 7 and 8).—In carotene-deficient animals changes in the lateral and medial vestibular nuclei are seen regularly, the cells of the latter appearing most severely affected. Powdering of granules, eccentric nuclei and neuronophagia are the common changes, but in advanced cases many remnants of degenerated cells are also found. Cells of the control animals are normal.

Facial nerve nucleus (*7th*).—These cells are large and usually have well-defined nuclei and Nissl granules in both carotene and carotene-deficient animals. Degenerative changes have been found in some cells in two out of the ten facial nuclei of the deficient animals examined, but none in the carotene-fed rabbits.

Abducens and accessory 6th nuclei.—Few cells of these nuclei have been examined, but no differences between those in the animals with and without carotene have been observed. The slight changes seen in one case were within what are probably the normal limits.

Descending 5th nucleus.—Changes are usually seen in the small cells of this nucleus in the affected animals. In some advanced cases they appear to have been entirely destroyed, but powdery granules and eccentric nuclei can be found in most experiments in which the animal's diet did not contain carotene.

Oculomotor nucleus (Plate IX, figs. 1 and 2).—This nucleus, even in the control animals, contain cells which appear to be abnormal; more of these are seen in the carotene-deficient animals, and in one or two cases extensive degeneration was present.

Red nucleus (Plate IX, figs. 3 and 4).—Clumping of the granules is the most obvious change in the cells of this nucleus in the carotene-deficient animal. The granules appear larger and more widely scattered and have no definite arrangement as in the normal. Powdery granules have also been found fairly regularly, but on the other hand eccentric nuclei and neuronophagia have been so far rarely seen.

Purkinje cells (Plate VII, figs. 5 and 6).—Purkinje cells of the cerebellar cortex in rabbits receiving carotene are flask-shaped and have a large nucleus with a nucleolus situated towards the base of the flask. The protoplasm is normally granular and around the nucleus has a deeply staining demilune appearance. Of all the groups of cells examined in this work these show

perhaps the most consistent changes. In the well-established cases of carotene deficiency it is rare to see many Purkinje cells in which some change is not apparent. The nucleus becomes cloudy and can often only be recognized by means of the nucleolus, the demilune merges into the rest of the protoplasm and cannot be differentiated, the cell edges become frayed and sometimes, although the whole cell stains deeply, granules cannot be distinguished.

Dentate nucleus.—These cells in the carotene-deficient animals do not as a rule show such severe changes as the Purkinje cells, but loss of definition of the nucleus and powdery granules are often found.

Other groups of cells (Plate IX, figs. 7 and 8).—Here are included the medial accessory olivary nucleus and cells scattered in the median raphe, especially in the medulla. Many cells showing the changes described above are seen, but intense chromolytic changes such as are found when the axon of a motor cell is cut were obvious in some cells. These are the cells referred to on page 164 as exceptional in their reactions. Their appearance, in contrast with most other cells examined in this investigation, would suggest trauma of their conducting axon.

Discussion.

The foregoing account describes some of the changes in the central and peripheral nerve-fibres, and in the central and peripheral nerve-cells which can be readily produced in young rabbits by diets rich in cereals and deficient in carotene and vitamin A. Only the anatomical changes in relation to the general behaviour of the affected animals have been dealt with here. The experimental development of the study and its clinical significance I have discussed elsewhere [1 (*a*) (*b*) (*c*) (*d*) (*e*) (*f*)]. There [1 (*c*) (*d*) (*f*)] I have indicated a probable relationship between various diseases of the nervous system and diet which further work will no doubt expand. There is, for instance, good evidence that in some of the so-called toxic degenerations, especially those associated with restricted diets as convulsive ergotism, pellagra and lathyrism, a deficient intake of vitamin A and carotene is of ætiological significance. In sub-acute combined degeneration of the cord, the evidence is not so good, but it is difficult to escape the conclusion, not only that this disease is nutritional in origin, but that the responsible defect involves a biochemical mechanism in which vitamin A is concerned. Even in disseminated sclerosis there is suggestive evidence that a diet rich in vitamin A and other protective food factors increases the resistance of the nervous system to the toxic agent at fault, and that such a diet can improve the clinical condition of early acute attacks of this disease and possibly check the rate of advance in more established chronic cases.

It is necessary to emphasize that the investigations here described

and the interpretation of the results must only be regarded as of a preliminary nature ; it is for neurological experts to develop the subject which is essentially one of toxic degeneration of the nervous system produced by ordinary foodstuffs and the protective effect of other specific substances. The fact that the same kind of degenerative changes can be experimentally produced by dietetic means in dogs and rats, together with the fact that morbid conditions of a comparable nature are found in man, show the subject to be one of both scientific and practical interest. The work emphasizes the importance of the specific relation of nutritional substances to the structure and proper action of the whole nervous system. Possibly the results may prove of even greater significance ; the fact that degenerative changes are more easily produced in young than in old animals suggests that these same nutritional factors play a large part in determining the structural development of the nervous system, either as a whole or in part, in early life and even in the fœtus. If this is so, it is another instance of the supreme importance of the proper nutrition of the mother during pregnancy and lactation, and of the young child during the development of the nervous system.

I have already mentioned the difficulty of producing lesions in the adult animal by diet. Until recently I had not succeeded in either rabbits or dogs. Lately, however, some success in this direction has been obtained, but an account of this work I shall leave to a later publication. Suffice it to say that the intensity of the degenerative changes has not approached that described in this paper when young animals were investigated. In adult animals the amount of demyelination of fibres and the lesions in the nerve cells of the central nervous system are relatively small. There is some evidence that, apart from a deficiency of vitamin A and carotene and excess of cereal, some other nutritional defects aid in the production of degenerative lesions in the nervous system of adult animals. It would in fact appear that vitamin A bears the same kind of relation to the nervous system as vitamin D bears to bone and teeth structure. Had the experiments which led to the discovery of vitamin D been begun on adult animals, the successful issue would have been greatly delayed ; for although osteomalacia in adult animals or man is clearly comparable to rickets in early life, this disease does not develop when vitamin D insufficiency is the only dietetic defect as rickets does in infants. It is possible that the difficulty of producing nerve lesions in adult animals by vitamin A or carotene deficiency is due to the fact that vitamin A controls some form of metabolism and works in conjunction with other dietetic elements in a

way comparable to the interaction of vitamin D with calcium and phosphorus. Further work will have to be done before this question is settled. It can be said with assurance, however, that the adult nervous system is much more stable under the experimental conditions employed than that of young animals and that it is more difficult to produce nervous degeneration by defective diets.

It may be asked, does this work provide any solution to the question as to whether the nerve-cell or its conducting fibre is first influenced by the special dietetic conditions used? No definite answer can be given. It was hoped that the results of experimental studies of secondary (traumatic) degeneration might throw some light on this problem. Conducting fibres when cut off from their nerve-cells degenerate, but only in certain nerve-cells does chromolysis appear. For instance, motor-cells of cerebral nerves always react in this way, while spinal anterior horn cells sometimes show chromolysis after simple section of their axis cylinders, while in other cases severer injury of their conducting fibres is necessary to produce the typical change. Again, many of the afferent nerve-cells seem to remain normal, or at least escape sufficiently great change to be demonstrated by histological methods in general use, after injury of their conducting fibres, e.g. the posterior root ganglion cells after section of the posterior roots, also the ganglion of the vagus after section of the nerve central to the ganglion (van Gehuchten [16]). Thus it would seem that nerve-fibres can in some cases be damaged without the nerve-cells undergoing obvious degenerative changes, and that this applies specially to nerve-cells and fibres of the afferent system.

Recent work of Sherrington [17] has shown on the other hand that motor nerve-cells can show changes of degeneration without their conducting fibres being structurally damaged. In his recent experiments total spinal transection in the dorsal region of the cord of monkeys produced chromolysis in a number of motor horn cells in the hind-limb region, e.g. in the 5th lumbar segment. However, the motor fibres arising from chromolysed cells which supply the tibialis anticus, the anterior longus digitans and gastrocnemius failed to show any degeneration even seventeen days after spinal transection. It is clear that nerve-cells can show chromolysis and yet their conducting fibres escape obvious myelin degeneration. It is interesting to note that, although these fibres appeared normal in structure, evidence was obtained which indicated that they were not normal in function (Sherrington [17], and Fulton and Sherrington [18]. Thus the contraction of the innervated muscle in response to a single shock stimulus was of subnormal tension value

and very sluggish as compared with the response of the same muscle in a normal animal. Sherrington calls this type of reaction " transneuronal degeneration."

So far, then, the evidence of traumatic degeneration indicates that spinal motor nerve-cells may be either normal or abnormal when the conducting fibres are degenerated, that the nerve-fibres may appear normal when their corresponding cells show chromolysis and that, in the case of some afferent nerve units, the cells may escape chromolysis when the conducting fibres are degenerating. It is evident that these results of trauma offer no aid to the solution of the problem as to whether the primary lesion in experiments described in this paper is in the nerve-cell or its conducting fibre.

At one period of the work, when the demyelination changes in nerve-fibres seemed to be the outstanding effect of vitamin-A deficiency, H. N. Green [19], working in my laboratory, endeavoured to find out whether vitamin A had some special relationship to fat metabolism. If this had been established, it might have afforded some support for the view that the nerve-fibre, and especially its myelin covering, was primarily involved in vitamin A deficiency. However, there was no evidence of any specific relationship between vitamin A and fat metabolism.

There is, as indicated above, a good deal of specificity in the nerve cells and fibres affected, the afferent system being on the whole much more liable to change than the motor system. This might be regarded as evidence that the nerve-cell lesion is primary because it seems more likely that specificity of reaction would be shown by nerve-cells than nerve-fibres. It may be possible to settle this question in the adult animal, for early observations indicate abnormality of the cell without any obvious change in its conducting fibres. Thus in one adult animal the ganglion cells of the retina are degenerating and show chromolysis while there is no obvious myelin degeneration in the optic nerve.

In their biochemical aspect these experiments have a particular interest, especially in view of earlier and contemporary work showing the close interaction between specific chemical substances and the nervous system. I refer particularly to the recent work by Dale and his colleagues [20 and 21] on acetyl choline in relation to nerve-endings and nerve-cell activity, and to the earlier investigations which established the close relationship between adrenaline and most sympathetic nerve-endings (Elliott [22]). It would appear indeed that a new stage of biochemical knowledge regarding nervous tissue is opening up, and that the time is not far distant when there will be much more information available about

the physiological action of specific chemical substances in controlling the normal structure and function of nervous tissue. It has long been known that the nervous system is specially susceptible, and gives specific responses, to many drugs of plant origin, and it might, therefore, be expected that this system would also be controlled by chemical agents with a drug-like action which are, however, physiological and present in the body. Modern investigations, including the present work, show that this is certainly the case and that the field is one of great promise.

Summary.

(1) A diet is described deficient in carotene and vitamin A which allows young rabbits to grow until abnormal movements and general behaviour indicate widespread pathological changes in the nervous system which can be demonstrated post mortem.

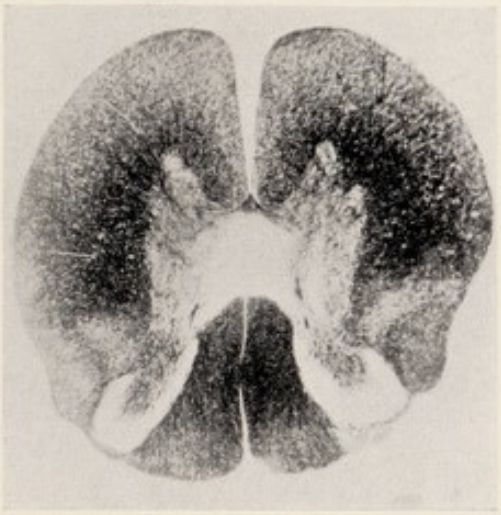
(2) The daily addition of 1 to 3 mgm. of pure carotene, or of some source of carotene (carrots, cabbage, &c.), to this basal diet prevents the development of these lesions of the nervous system. Vitamin A has the same protective effect.

(3) Generally speaking, it is the afferent side of the nervous system which is specially affected, but so far degenerative changes have only been found in the first and second neurons. All the afferent nerves of the head examined, including the optic nerve, the sensory fibres of the 5th, and the auditory and vestibular fibres of the 8th, suffer greatly. The motor cranial nerves usually escape, but some degeneration has occasionally been found in the 3rd nerve. In the body the same rule holds, the degeneration affecting primarily the dorsal root-fibres, those of the anterior roots remaining normal except when the nutritional deficiency has been very prolonged. No degeneration has been seen so far in the vagus nerve, even in its afferent fibres.

(4) A description of the distribution of myelin degeneration in the nerve-fibres and tracts inside the nervous system at different levels is given. Here, again, the fibres affected are mostly ascending and include the dorsal and ventral spino-cerebellar, the dorsal and ventral spino-reticulo-thalamic tracts, and the tracts of the dorsal columns. Some descending tracts suffer change, including the rubro-spinal, the dorsal longitudinal bundle and the vestibulo-spinal, all of which are associated with the mid-brain or medulla. The pyramidal tract generally escapes in the rabbit under these nutritional conditions.

(5) Degenerative changes of different degrees are seen in nerve-cells as well as in their conducting neurons; a description is given of the histological appearance of many such cells both in the central and

PLATE III.



Photomicrograph of section of high cervical cord, rabbit B, stained by hæmatoxylin after mordanting. Pale areas show complete loss of many fibres.

To illustrate paper by Edward Mellanby.

PLATE IV.

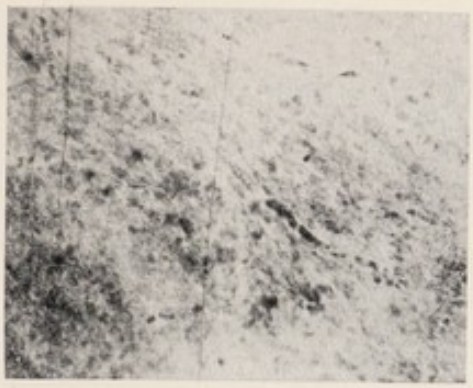


FIG. 1.

FIG. 2.

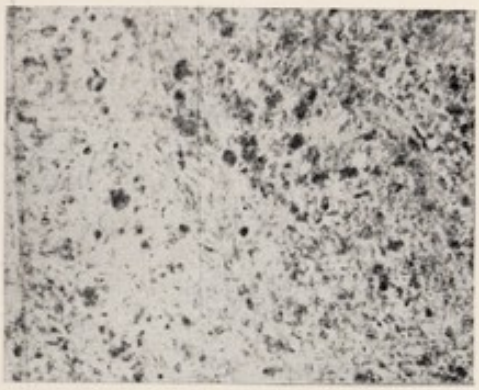


FIG. 3*a*.

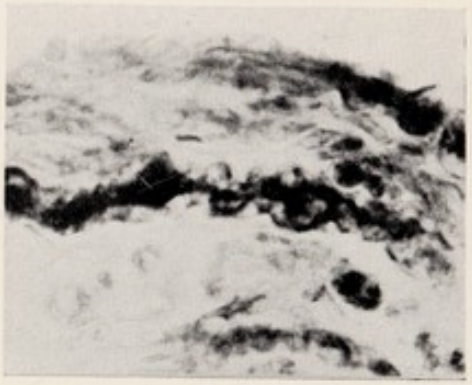


FIG. 3*b*.

FIG. 4*a*.

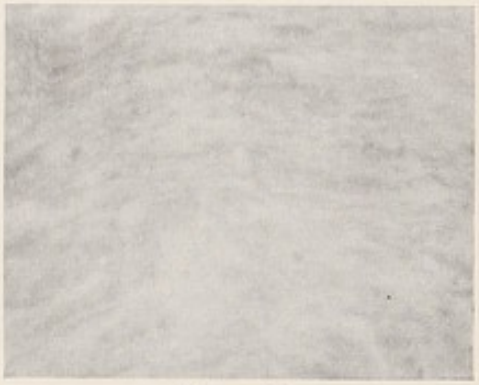


FIG. 4*b*.

FIG. 1.—Vestibular division of 8th nerve, diet deficient in carotene and vitamin A. Marchi stain × 280.

FIG. 2.—Vestibular division of 8th nerve, diet contained cabbage. Marchi stain × 280.

FIG. 3*a*.—Cochlear division of 8th nerve, diet deficient in carotene and vitamin A. Marchi stain × 280.

FIG. 3*b*.—Cochlear division of 8th nerve, diet deficient in carotene and vitamin A. Marchi stain × 750.

FIG. 4*a*.—Cochlear division of 8th nerve, diet contained carrot. Marchi stain × 280.

FIG. 4*b*.—Cochlear division of 8th nerve, diet contained cabbage. Marchi stain × 750.

(In this and the following plates the photomicrographs were reduced by one quarter in reproduction.)

Photomicrographs illustrating 8th nerves (vestibular and cochlear divisions) in rabbits, with and without carotene and vitamin A.

To illustrate paper by Edward Mellanby.

PLATE V.

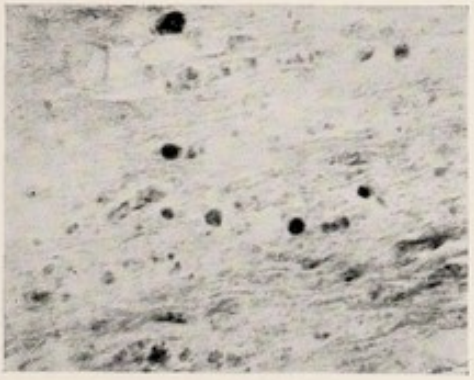


FIG. 1.

FIG. 2.

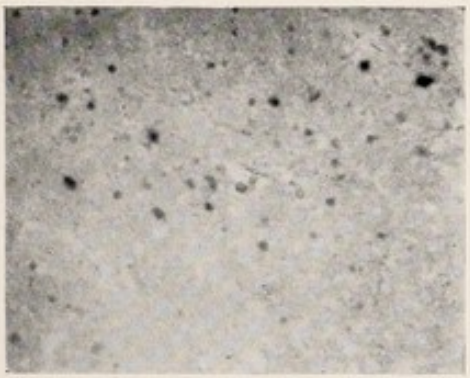


FIG. 3.

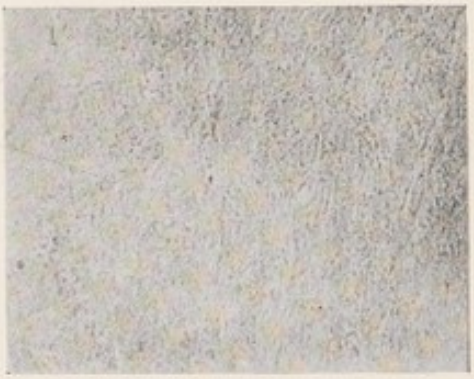


FIG. 4.

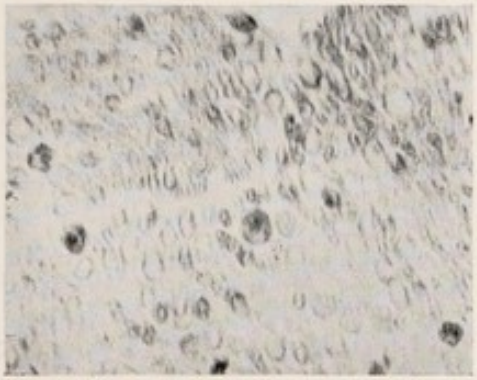


FIG. 5.

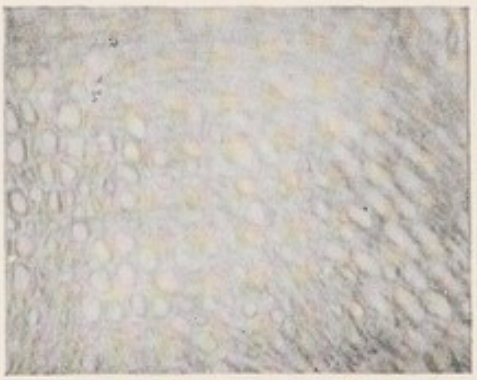


FIG. 6.

FIG. 1.—Intramedullary 8th nerve, diet deficient in carotene and vitamin A. Marchi stain × 280.

FIG. 2.—Intramedullary 8th nerve, diet contained pure carotene. Marchi stain × 280.

FIG. 3.—Optic nerve, diet deficient in carotene and vitamin A. Marchi stain × 280.

FIG. 4.—Optic nerve, diet contained carrot. Marchi stain × 280.

FIG. 5.—Sciatic nerve, diet deficient in carotene and vitamin A. Marchi stain × 280.

FIG. 6.—Sciatic nerve, diet contained cabbage. Marchi stain × 280.

Photomicrographs illustrating intramedullary 8th nerve, optic and sciatic nerves in rabbits, with and without carotene and vitamin A.

To illustrate paper by Edward Mellanby.

PLATE VI.

FIG. 1.

FIG. 2.

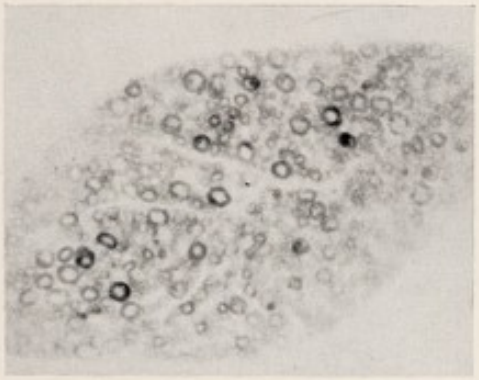


FIG. 3.

FIG. 4.

FIG. 5.

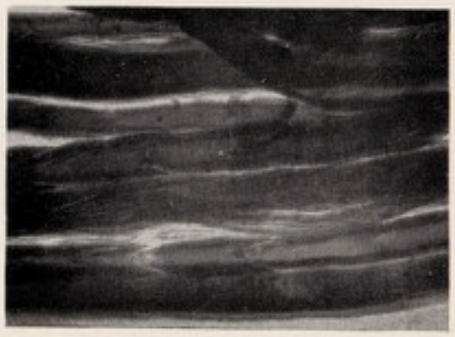


FIG. 6.

FIG. 1.—Ventral root, diet deficient in carotene and vitamin A. Normal. Marchi stain × 280.

FIG. 2. –Ventral root, diet contained cabbage and carrot. Normal. Marchi stain × 280.

FIG. 3.—Dorsal root, diet deficient in carotene and vitamin A. Annular degeneration. Marchi stain × 280.

FIG. 4.—Dorsal root, diet contained cabbage and carrot. Normal. Marchi stain × 280.

FIG. 5.—Dorsal root, diet deficient in carotene and vitamin A. Osmic method described by Cajal. × 420.

FIG. 6.—Dorsal root, diet contained vitamin A oil. Normal. Osmic method described by Cajal. × 420.

Photomicrographs illustrating ventral and dorsal roots of rabbits with and without carotene and vitamin A.

To illustrate paper by Edward Mellanby.

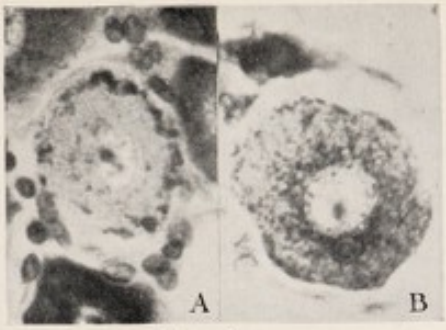


FIG. 1.

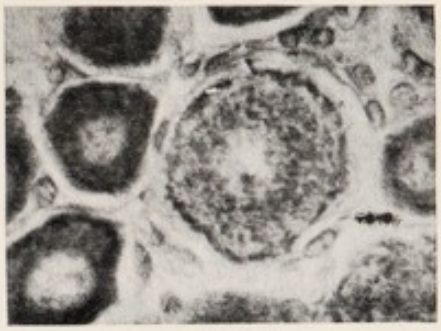


FIG. 2.

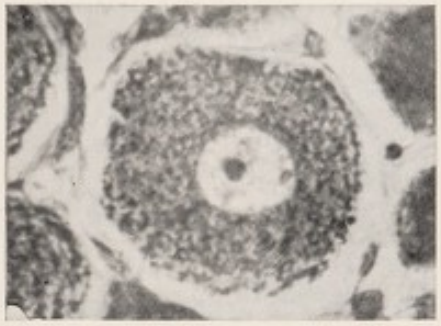


FIG. 3.

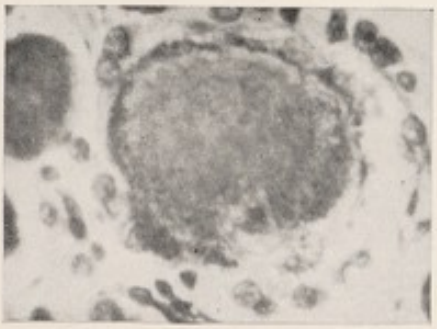


FIG. 4.

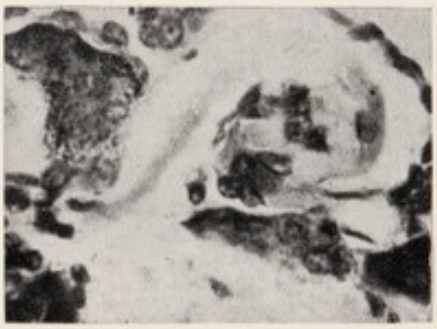


FIG. 5.

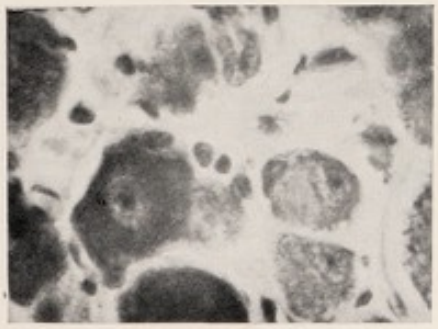


FIG. 6.

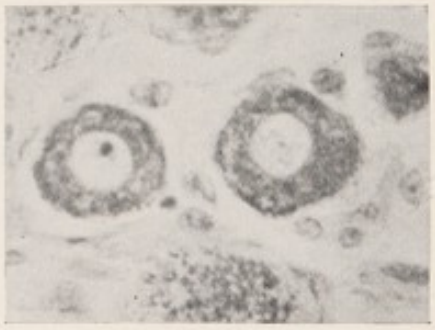


FIG. 7.

FIG. 1.—(*a*) Dorsal root ganglia × 750, large cell showing large granules around outer edge and rest of cell clear. (*b*) Dorsal root ganglia × 750, large cell showing concentration of granules around nucleus.

FIG. 2.—Dorsal root ganglia × 750, large cell showing large granules around outer edge, medium granules throughout.

FIG. 3.—Dorsal root ganglia × 750, large cell. Normal.

FIG. 4.—Dorsal root ganglia × 750, large cell with eccentric nucleus and concentration of granules around outer edge.

FIG. 5.—Dorsal root ganglia × 750, corroded cell of Cajal.

FIG. 6.—Dorsal root ganglia × 750, small cells showing eccentric nuclei.

FIG. 7.—Dorsal root ganglia × 750, small cells. Normal.

Photomicrographs illustrating dorsal root ganglia in rabbits with (figs. 3 and 7) and without carotene and vitamin A (figs. 1, 2, 4, 5 and 6).

To illustrate paper by Edward Mellanby.

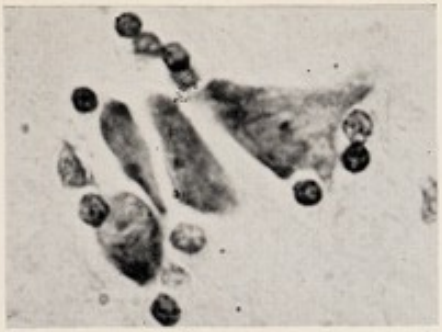


FIG. 1.

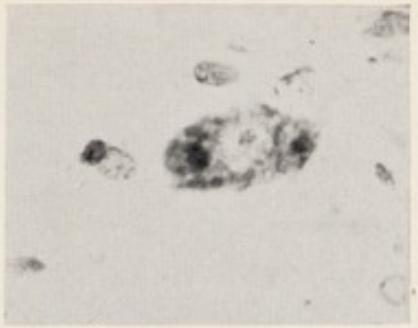
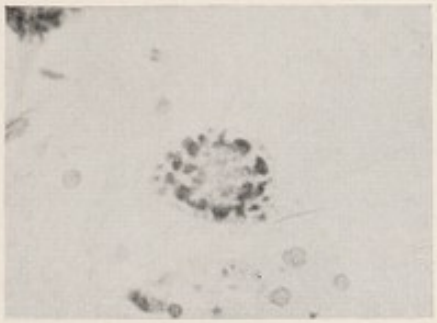


FIG. 2.



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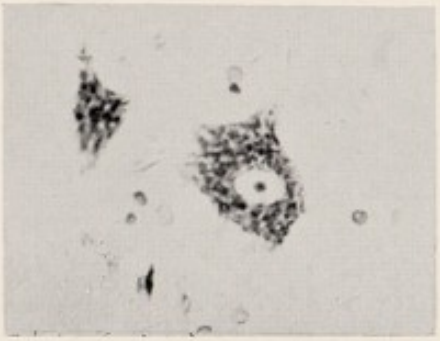


FIG. 4.

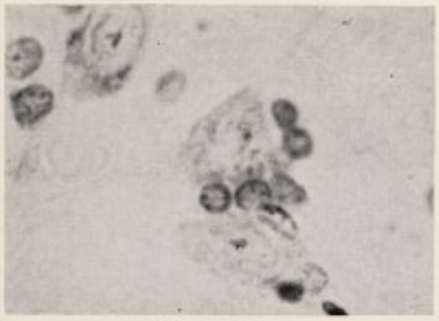


FIG. 5.

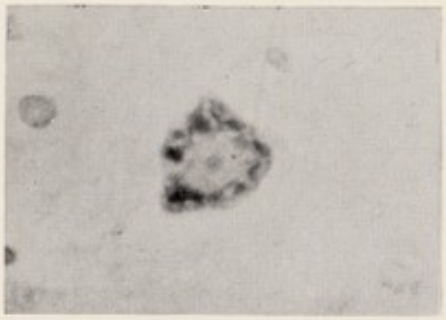


FIG. 6.

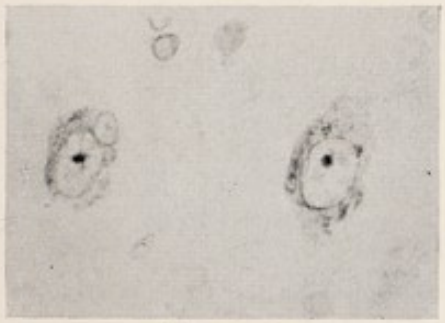


FIG. 7.

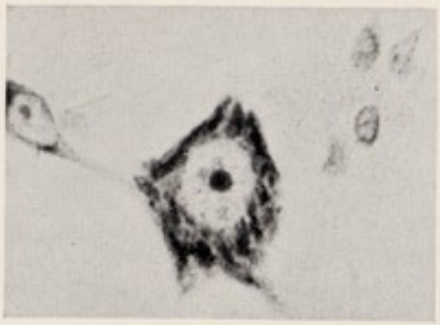


FIG. 8.

FIG. 1.—Clarke's column cells × 750, diet deficient in carotene and vitamin A. Eccentric nuclei, loss of granules and neuronophagia.

FIG. 2.—Clarke's column cells × 750, diet contained cabbage. Probably normal.

FIG. 3.—Ventral horn cells × 420, diet deficient in carotene and vitamin A. Clumping of granules.

FIG. 4.—Ventral horn cells × 420, diet contained yellow maize. Normal.

FIG. 5.—Cells of lateral reticular nucleus × 750, diet deficient in carotene and vitamin A. Loss of granules. Neuronophagia.

FIG. 6.—Cells of lateral reticular nucleus × 750, diet contained carotene. Probably normal.

FIG. 7.—Cells of mesial vestibular nucleus × 750, diet deficient in carotene and vitamin A. Loss of granules. Neuronophagia.

FIG. 8.—Cells of mesial vestibular nucleus × 750, diet contained yellow maize. Normal.

Photomicrographs illustrating cells in the central nervous system of rabbits with and without carotene and vitamin A.

To illustrate paper by Edward Mellanby.

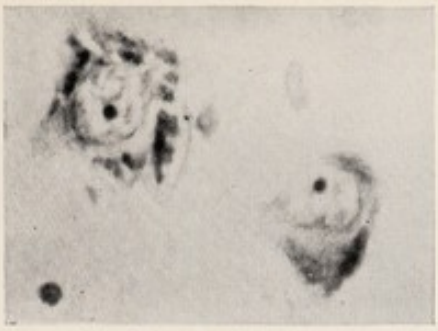


FIG. 1.

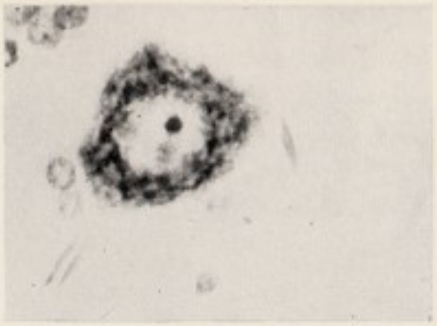


FIG. 2.

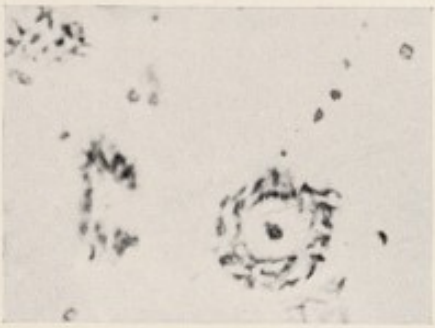


FIG. 3.

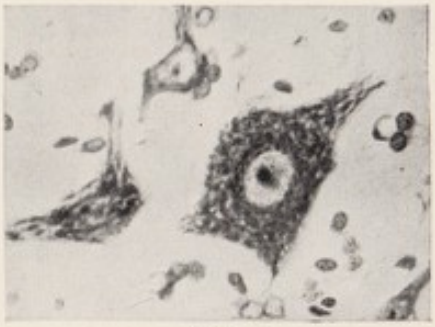


FIG. 4.

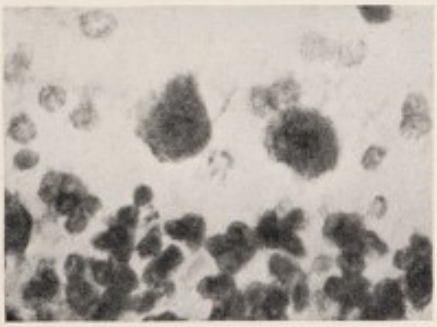
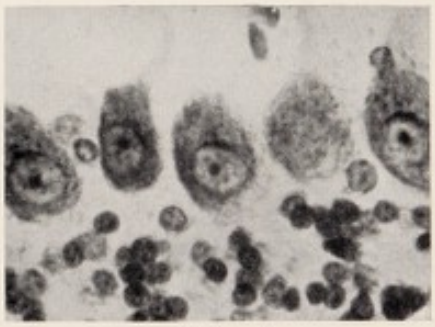


FIG. 5.



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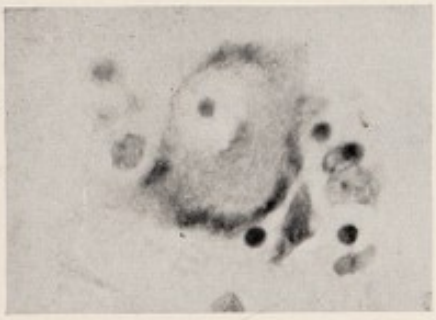

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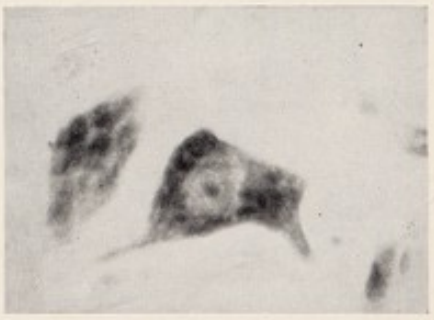

FIG. 8.

FIG. 1.—Cells of oculomotor nucleus × 750, diet deficient in carotene and vitamin A. Chromolysis and eccentric nuclei.

FIG. 2.—Cell of oculomotor nucleus × 750, diet contained carrot. Probably normal.

FIG. 3.—Cells of red nucleus × 420, diet deficient in carotene and vitamin A. Clumping of granules.

FIG. 4.—Cells of red nucleus × 420, diet contained vitamin A oil. Normal.

FIG. 5.—Purkinje cells × 750, diet deficient in carotene and vitamin A. Indefinite granules, loss of demilune and nucleus.

FIG. 6.—Purkinje cells × 750, diet contained cabbage. Normal.

FIG. 7.—Cell of medial accessory olivary nucleus × 750, diet deficient in carotene and vitamin A. Chromolysis and eccentric nuclei.

FIG. 8.—Cell of medial accessory olivary nucleus × 750, diet contained pure carotene. Normal.

Photomicrographs illustrating cells of the mid-brain and cerebellum of rabbits with and without carotene and vitamin A.

To illustrate paper by Edward Mellanby.

peripheral systems of animals receiving the carotene-deficient diet described. The groups of nerve-cells examined include those of the dorsal root and the Gasserian ganglia of the peripheral system, and the cells of Clarke's column, the Purkinje cells, those of the vestibular nucleus and many others in the central nervous system. In this case also, especially in the spinal cord, it is the afferent side which suffers most. In the mid-brain, however, the red nucleus and the dentate nucleus, and sometimes the 3rd nucleus, occasionally show some abnormalities.

I wish to express my indebtedness to my technical assistant, Mr. R. C. Stewart, for the great help he has given in this investigation, especially by improving histological methods so as to allow a large mass of material to be examined. The expenses of the work were paid by the Medical Research Council, to whom my thanks are due.

REFERENCES.

[1] Mellanby, E. (*a*) *Journ. Physiol.*, 1926, **61**, xxiv.
(*b*) *Brit. Med. Journ.*, 1930, **i**, 677.
(*c*) *Brain*, 1931, **54**, 247.
(*d*) *Edinb. Med. Journ.*, 1933, **40**, 197.
(*e*) *Journ. Path. Bact.*, 1934, **38**, 391.
(*f*) "Nutrition and Disease," 1934, Edinburgh, Oliver and Boyd (Croonian Lectures, 1933).

[2] Stockman, R., and Johnston, J. M. (*a*) *Journ. Hyg.*, 1933, **33**, 204.
Stockman, R. (*b*) *Ibid.*, 1934, **34**, 235.

[3] Lorrain Smith, J. *Journ. Path. Bact.*, 1906, **11**, 415.

[4] Ramon-y Cajal. "Degeneration and Regeneration of the Nervous System," translated R. M. May (Oxford University Press, 1928).

[5] May, W. Page. *Brain*, 1906, **29**, 742.

[6] Mellanby, May and King, J. D. *Brit. Dent. Journ.*, 1934, **56**, 2 Binding.

[7] Mellanby, May. *Sp. Rep. Ser. Med. Res. Coun.*, Lond., No. 153, 1930.

[8] Zimmerman, H. M. *Journ Exp. Med.*, 1933, **57**, 215.

[9] Nissl, F. *Z. f. Psychiatr.*, 1892, **48**, 197.

[10] *Idem.* *Zbl. f. Nervenheilk. u. Psychiatr.*, 1894, n.f. **5**, 337.

[11] *Idem.* *Z. f. Psychiatr.*, 1892, **48**, 675, and *Neurol. Zbl.*, 1896, **15**, 947.

[12] Goldscheider, A., and Flatau, E. *Fortschr. der Medizin*, 1897, **15**, 241, 609 and 793.
Also Flatau, E. *Ibid.*, 1897, **15**, 281.

[13] Russell, C. K. *Johns Hopkins Hosp. Bull.*, 1904, **15**, 196.

[14] Dye, J. A. *Quart. Journ. Exp. Physiol.*, 1927, **17**, 71.

[15] van Gehuchten, A. *Bibliographie anatomique*, 1896, referred to in *Anatomie du Système Nerveux de l'homme*, 1900, **i**, 338, by same author.

[16] *Idem.* *Anatomie du Système Nerveux de l'homme*, Louvain, 1900, pp. 335-337.

[17] Sherrington, C. S. *Journ. Physiol.*, 1932, **75**, 11 P.

[18] Fulton, J. F., and Sherrington, C. S. *Ibid.*, 1932, **75**, 17.

[19] Green, H. N. *Biochem. Journ.*, 1934, **28**, 25.

[20] Dale, H. H., and Gaddum, J. H. *Journ. Physiol.*, 1930, **70**, 109.

[21] Dale, H. H. Linacre Lecture, *Brit. Med. Journ.*, 1934, **i**, 335.

[22] Elliott, T. R. *Journ. Physiol.*, 1905, **32**, 401.