Xerophthalmia, trigeminal degeneration and vitamin A deficiency / E. Mellanby.

612.392.013A [617.713—005.8+616.833.15—003.8

XEROPHTHALMIA, TRIGEMINAL DEGENERATION AND VITAMIN A DEFICIENCY.

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(Plates LI.-LVI.)

It is disconcerting at first sight to be faced with the fact that two of the main lesions produced in young animals by diets deficient in vitamin A and carotene are so dissimilar, namely (1) hyperplasia and metaplasia of epithelium and mucous membranes, often accompanied by the invasion of these areas by micro-organisms and the production of local infective foci (Mori 1922, Wolbach and Howe 1925, Goldblatt and Benischek 1927, Green and Mellanby 1928, 1930), and (2) degenerative changes of medullated nerves both in the central and peripheral nervous systems (Mellanby 1926, 1930, 1931, 1933). Two such different morbid changes do not appear to have any relation to one another. On the other hand, since the chief causative agent or agents in both cases is the same, it is clearly necessary to make the attempt to correlate these phenomena.

The present publication, therefore, deals with an investigation whose object was to see whether there was a possible connecting link which could bring them into close association. It is true that the investigation deals with lesions of only one organ, the eye, but if evidence of a relationship can be produced in this case, it is probable that a similar mechanism holds in other organs where epithelial changes and infective foci are commonly produced by a deficiency of vitamin A in the diet.

In previously published accounts describing the effect of vitamin A deficiency on the nervous system of experimental animals, I have shown that the resulting nerve degeneration affected both the central and peripheral systems (Mellanby, *op. cit.*). In the former, ascending fibres, both exogenous and endogenous, seem to be principally affected; in the latter, the afferent rather than the efferent nerves. It was the demyelination seen in the medullated afferent nerves which suggested the clue for the association of nerve degeneration and peripheral infective lesions, for it seemed possible that xerophthalmia and the subsequent invasion of the cornea and eye generally by micro-organisms might be secondary to a loss of the trophic control normally exerted by the

sensory nerve of the conjunctiva and cornea. Such a loss of control would be expected if the first division of the fifth nerve showed degenerative change, since it is from this source that the cornea receives its sensory nerve supply (Mellanby 1933).

That the fifth nerve does exert a trophic control over the conjunctiva and cornea has been recognised ever since removal of the Gasserian ganglion for trigeminal neuralgia was introduced. One of the most feared complications of this operation is the development of keratitis neuro-paralytica. In an attempt to avoid this complication, ophthalmic surgeons usually try to protect the eyeball by sewing the eyelids together following this operation. The same phenomenon is sometimes seen when herpes zoster attacks the Gasserian ganglion, and here again resort is often made to the increased protection of the eye by the same means. It is clear, therefore, that conditions which interfere with the sensory nerve supply to the cornea are liable to be followed by infection. In consequence of this relationship, it is usually held that the afferent nerve supply to the cornea exerts some neurotrophic control.

The mechanism whereby fat-soluble vitamin* deficiency leads to the development of xerophthalmia has been investigated by several workers. Mori (1922) regarded the condition as due to desiccation following the loss of secretion of the lachrymal glands and the ulceration of the cornea as the result of secondary infection by micro-organisms. Examination of the lachrymal, Harderian and Meibomian glands supported this view. He found the secretory cells of the lachrymal glands shrunken, while those of the Harderian gland were either dilated or empty. In the Meibomian glands of fat-soluble vitamin-deficient animals the duct epithelium became hyperplastic and prevented the passage of secretion from the secretory cells. The main fact which has been advanced against the hypothesis of Mori is that lachrymation often persists after xerophthalmia has been established. Wolbach and Howe (1925) also pointed out that keratinisation of corneal and conjunctival epithelium may occur without appreciable atrophy of the paraocular glands. Findlay (1925) has suggested that the most important factor in the onset of xerophthalmia in fat-soluble vitamin deficiency is a loss or decrease in the lysozyme in the tears. He also emphasised the fact that the onset of the disease is associated with increased lachrymation lasting for several days, after which the secretion is reduced in amount and is sero-sanguineous in character. He noted that the conjunctival epithelium becomes keratinised during the period of increased lachrymation. Yudkin and Lambert (1923) have advanced the view that focal inflammatory lesions of the conjunctiva are primary, and keratinisation a secondary change. Wolbach and Howe, on the other hand, regard keratinisation of the conjunctival and corneal epithelium as the primary change and the inflammatory reaction as secondary and incidental in the sense that it need not occur in animals in which the vitamin deficiency had been carried to an end result. Collins (1930) thinks that the lachrymal secretion acts mainly as an irrigating and bactericidal fluid and that a mucous secretion preserves the corneal epithelium from keratinisation in those animals in which it is present. He suggests that whereas in most animals this mucous

* It must be remembered that the term vitamin A, according to present usage, has a narrower meaning than it originally had when it covered both the anti-infective growth factor (to which it is now limited) and the antirachitic or calcifying factor (now called vitamin D).

secretion comes from the Harderian gland, in primates the function is taken over by unicellular mucous glands in the retrotarsal folds and ocular conjunctiva. In vitamin A-deficient animals he found that the Harderian gland soon became atrophied and ceased to function.

With the nature of these mechanisms I am not concerned in the present paper. My object is primarily to show that xerophthalmia is usually accompanied by obvious derangement of the sensory neuromechanism of the eye and that the changes in the cornea and its sensory nerve supply are related.

Experimental methods.

A difficulty in work of this nature, especially in the case of rabbits, is to find a diet which is compatible with good health apart from the production of the specific lesions required. Anybody with experience of animal feeding knows that a small deviation from the standard condition described may result in a high mortality and make the work useless.

Rabbits, dogs, rats and chickens have been used in this research. Rabbits are for many reasons convenient for general purposes. In my experience, their eyes show xerophthalmia regularly on a vitamin A-deficient diet as given in these experiments.

A standard basal diet deficient in vitamin A and carotene, which will produce the lesions described below and keep rabbits in fair general health from the age of about 9 weeks, is as follows:—oats 4 parts, bran 1 part, $CaCO_3$ 1·5 per cent. 10 grms. of alfalfa heated for 36 hours at 120° C. to destroy the carotene are also given daily. The alfalfa is distributed in thin layers on the trays of an electric oven and turned frequently to allow full exposure to the air. As the rabbit grows, the oats-bran portion of the diet is gradually increased from 40 to 70 grms., but the alfalfa is not necessarily raised correspondingly. When sufficient unheated alfalfa is given instead of heated, neither xerophthalmia nor nerve degenerative changes develop, for as shown previously, carotene acts like vitamin A in preventing such degeneration (Mellanby 1931).

Experimental observations.

A series of rabbits brought up from the age of 8 to 10 weeks on diets deficient in vitamin A or carotene were killed at various periods after the development of xerophthalmia. Sections of the first division of the trigeminal nerve, taken peripherally and sometimes also centrally to the Gasserian ganglion, were found to contain degenerated fibres or fibres undergoing degenerative changes according to the severity of the condition.

The most prominent change was in the myelin sheath and so a modification of Marchi's method for degenerating myelin was used as

a routine, but sections were also stained with one or more of the following:—methylene blue-sulphurous acid mixture, Scharlach R, Sudan III, Weigert-Pal (before and after alcohol treatment of the tissue), and in some cases one of the silver nitrate axis cylinder methods. As a further control, sections of known normal nerves were usually taken through at the same time.

Control rabbits in each family on the same basal diet but with the addition of some food containing vitamin A or a vitamin A or carotene preparation showed no such lesions either in the cornea or in the sensory nerve provided sufficient of the supplement was given (figs. 1 to 4).

The following examples show the histological changes in the sensory nerve supply as well as the macroscopic and microscopic changes in the eye itself at varying periods after the onset of the disease. In most of the experiments the cornea only was studied, as it is the tissue most obviously affected in the early stages of the disease in rabbits.

Xerophthalmia without infective invasion of cornea.

Very early xerophthalmia (*less than* 1 *day's duration*). Serial sections were made of the corneas of two rabbits in which the xerophthalmia had not been present more than 24 hours as gauged by naked-eye examination; (the lesions were too small to be photographed with the methods available). Photomicrographs of sections of one of the corneas and of the first division of the corresponding trigeminal nerve are shown in figs. 5 and 6. In the one area in which changes were found in serial sections the corneal tissues were only slightly affected and there were a few degenerating and swollen fibres in the sensory nerve.

At the onset of the disease a cornea may sometimes appear to the naked eye to be affected one day but normal the next, and affected again the following day, perhaps then to remain so and gradually get worse. In the case illustrated in fig. 5, the results are of an initial attack of the disease which had not been obvious to the naked eye longer than 24 hours.

Xerophthalmia of 6 *days' duration.* The central portion of the right cornea in this animal was seen to be slightly clouded about 6 months after the beginning of the experimental diet. The xerophthalmia got gradually worse during the following 6 days (fig. 7); the animal was then killed and the tissues examined. Serial sections showed that most of the cornea was normal and unaffected, but in a central region there was some early keratinisation, whilst in an area close to the corneo-scleral junction a small patch of abnormal epithelium was noted. In the central area the epithelial cells were definitely abnormal; they were becoming flattened and keratinised but nuclei could still be seen (figs. 8 and 9). Some fibres of the trigeminal nerve were found to be degenerated, but the photograph (fig. 10) suggests rather more degeneration than was actually present,

NORMAL CORNEA AND SENSORY NERVE OF RABBIT WHOSE DIET CONTAINED VITAMIN A

FIG. 1.—Cornea showing normal stratified epithelium. × 100.

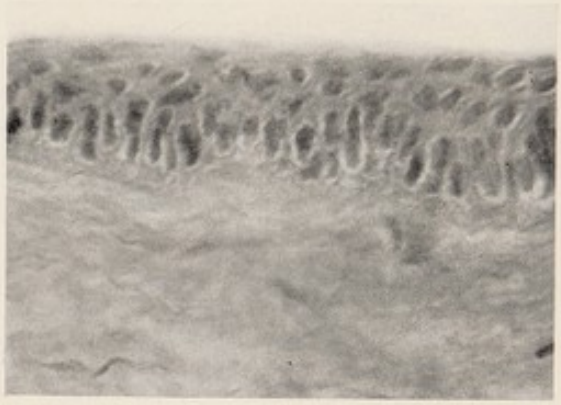


FIG. 2.—Cornea showing normal epithelium. × 450.

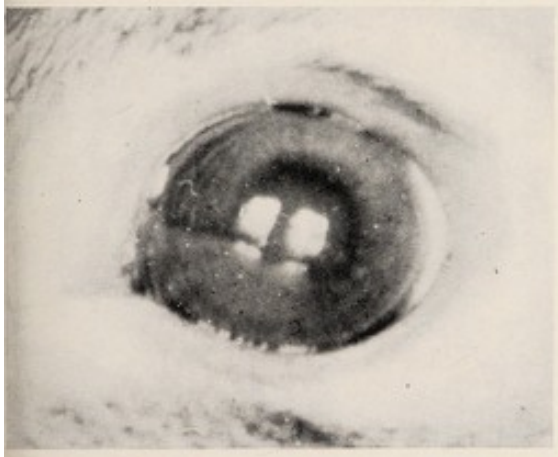


FIG. 3.—Photograph of normal eye showing clear reflected image of a window.

FIG. 4.—First branch of trigeminal nerve stained modified Marchi, showing no degenerated fibres. × 280.

VERY EARLY XEROPHTHALMIA OF RABBIT ON A-DEFICIENT DIET (LESS THAN 24 HOURS' DURATION)

FIG. 5.—Cornea, showing early keratinisation of surface epithelium. × 100.

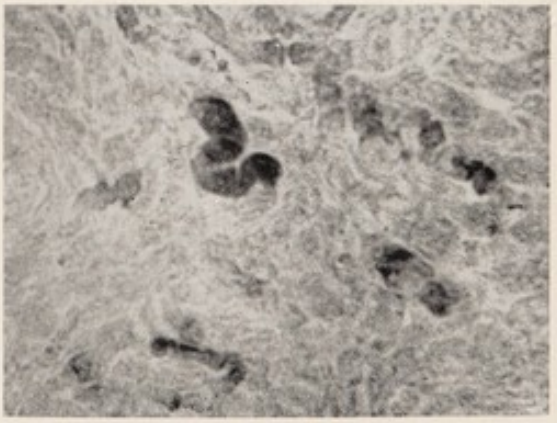


FIG. 6.—First branch of trigeminal nerve, showing a few degenerated fibres cut obliquely; Marchi's stain. × 280.

OPHTHALMIA OF 6 DAYS' DURATION OF RABBIT ON A-DEFICIENT DIET (figs. 7 to 10)

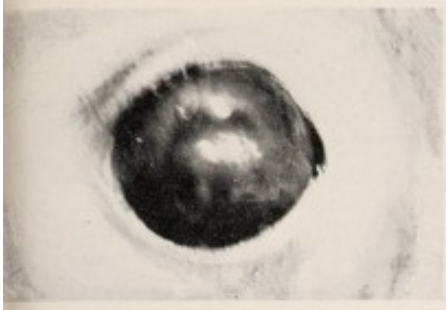
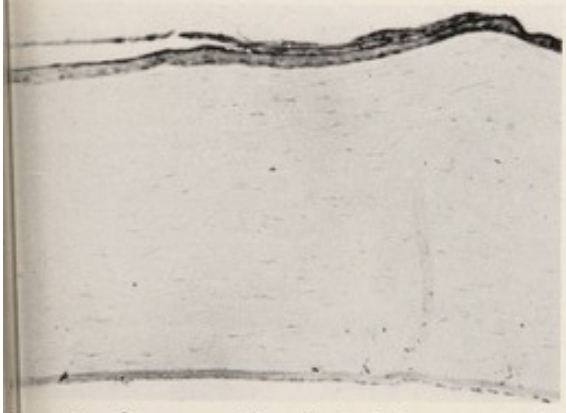
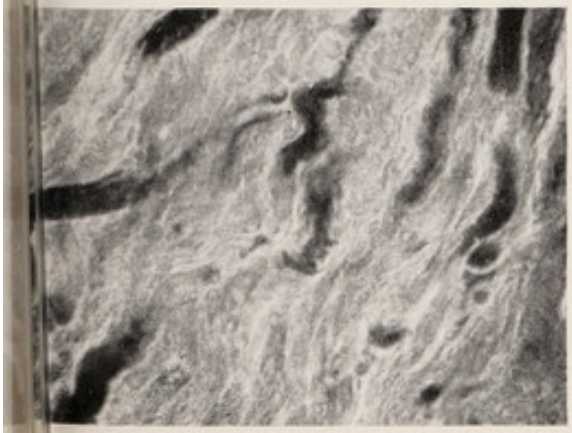


FIG. 7.—Photograph of eye with xerophthalmia showing white film on cornea.



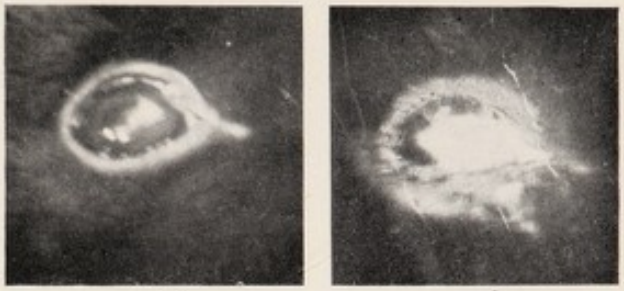
IG. 8.—Cornea. Keratinised layer can be seen splitting away from supporting epithelium. ×100.

. 9.—Cornea. Early epithelial changes of erophthalmia. Note all cells flattened; uclei of even surface cells still present. 450.



10.—First branch of trigeminal nerve. me degenerating fibres; modified Marchi. e degeneration is less than is suggested by photograph as all the fibres shown are longitudinally or obliquely. ×280.

XEROPHTHALMIA OF 3 WEEKS' DURATION OF RABBIT ON A-DEFICIENT DIET (figs. 11 to 14)



a *b*

FIG. 11.—Photographs of eyes showing films, (*a*) thin and localised, (*b*) thick and widespread, 2 days later.

FIG. 12.—Cornea showing thick layer of keratinised epithelium. ×100.

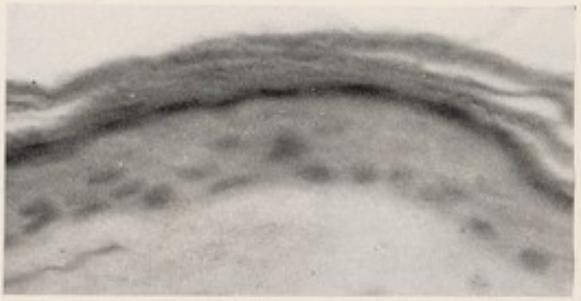


FIG. 13.—Cornea. No nuclei in most of epithelial cells (cf. fig. 9). ×450.

FIG. 14.—First branch of trigeminal nerve, showing much degeneration; modified Marchi. Weigert preparations also showed degenerated fibres.

partly because the fibres are cut obliquely and partly because in early or slight cases of xerophthalmia nerve fibres in certain bundles only of the whole nerve may be affected. Fig. 10 happens to represent one of these bundles. Another possible reason will be mentioned later.

A point of interest in this case is that whereas the right eye showed early but definite xerophthalmia no naked-eye abnormality could be seen in the left, nor could any microscopic changes be found in the serial sections of the cornea. The corresponding sensory nerve contained some degenerated fibres. Thus it appears that demyelination may be present in the sensory nerve supply to the cornea at a time when no xerophthalmia is present, suggesting that the corneal changes are secondary to the nerve degeneration. However, in three other rabbits in which one cornea became affected whilst the other remained apparently unaffected, both cornea and nerve of the unaffected eye were found to be normal on microscopic examination.

Severe xerophthalmia of 3 *weeks' duration.* The disease began in both eyes simultaneously. The lesions were severe and involved practically the whole of both corneas. On some days a thick mass covered each cornea and this disappeared periodically, leaving only a thin film which later thickened and was again rubbed off or fell off. Figs. 11 (*a*) and 11 (*b*) are photographs of a rabbit's eye taken on different days. In 11 (*a*) the film is thin, in 11 (*b*), two days later, it is much thicker. Sections of the cornea (figs. 12 and 13) show the epithelium to be very abnormal and covered by a keratinous layer. The first division of the trigeminal nerve (sensory) shows much degeneration (fig. 14). In the photomicrograph, there is not only more degeneration than in previous representations of this change (*i.e.* figs. 6 and 10) but the breakdown of the myelin sheaths is more complete, there being large numbers of degenerated myelin globules over the whole section. Staining by a modified Weigert hæmatoxylin method showed that many fibres had completely disappeared.

Severe xerophthalmia of 4 *weeks' duration.* An eye covered with a thick infected film is shown in fig. 15, taken 4 weeks after the first signs of the disease had been noticed. The eye is enophthalmic and the whole cornea is involved. Three days after the onset of the disease the eye seemed perfectly clear, but on the fifth day the changes were again obvious and finally developed rapidly into a very severe condition. Sections showed a heavy keratinised layer over the greater part of the cornea (fig. 16). Areas of infection were found scattered through this layer, some of the bacteria taking basic and others acid stains. The substantia propria appeared to be normal. Most of the sensory nerve fibres were affected (fig. 17), swollen fibres with annular or complete staining of the myelin sheath being almost general.

Slight xerophthalmia of prolonged duration (14 *weeks*). This animal never developed a severe xerophthalmia although it was affected for 14 weeks. Six times during this period the eye was seen to be covered

by films which disappeared leaving only dull areas on the cornea. Microscopically the cornea showed a crenated and slightly keratinised epithelium towards the periphery of the dull area (fig. 18). The latter had only a single layer of epithelial cells as a covering at this time, *i.e.* at the post-mortem examination. The first division of the trigeminal nerve showed some changes of the annular type, but these were not as advanced as in the majority of cases of severe xerophthalmia of 3 or 4 weeks' standing (fig. 19).

Xerophthalmia with infective invasion of cornea.

As is well known xerophthalmia is often accompanied by an invasion of the substantia propria of the cornea by micro-organisms. Instances of this will now be considered.

Xerophthalmia of 4 weeks' duration with infection of cornea. Fig. 20 shows the cornea of a rabbit in which not only the epithelium but also the substantia propria is infected; changes in the cornea had been noted for 4 weeks before death. Little epithelium is seen in the area photographed; there was no keratinisation in any of the sections but a large infiltration of polymorphonuclear leucocytes into the substantia propria was present in the central region. The trigeminal nerve central to the Gasserian ganglion, as well as the ophthalmic branch, was examined, and showed much degeneration (fig. 21).

Xerophthalmia of 9 weeks' duration with infection. Both eyes of this animal were severely affected, the corneas being covered by films which at various times fell or were rubbed off. Disintegrating cocci were present in smears taken from the surface of the corneas during life. When no film could be seen the eye had a very dull appearance and a white mass behind the cornea became visible. Fig. 22 shows the eye with a covering of film, fig. 23 without it, and in the latter the white mass within the anterior chamber can be seen. The corneal epithelium (fig. 24) showed no keratinisation, but an increase in the connective tissue of the substantia propria indicated a response to an infective invasion. The animal was killed during a period following the disappearance of the film, and the epithelium was only one or two cells deep; in some places it was missing. The ophthalmic branch of the trigeminal nerve showed much degeneration in some bundles (fig. 25).

Many other rabbits' eyes in which the disease was noted for different periods have been examined. As a general rule the more intense the disease the greater was the nerve degeneration.

Relative response of cornea and trigeminal nerve in curative experiments.

In the following experiments cabbage was added to the diet after xerophthalmia had developed in both eyes of two rabbits. In the first, as soon as the eye condition had cleared up the animal was killed and the trigeminal nerves were examined. In the second there was an

XEROPHTHALMIA OF 4 WEEKS' DURATION OF RABBIT ON A-DEFICIENT DIET (FIGS. 15-17)

FIG. 15.—Photograph of eye just before death showing severe xerophthalmia and enophthalmos.

FIG. 16.—Cornea. Extensive keratinisation with infected film, but substantia propria not infected. × 100.

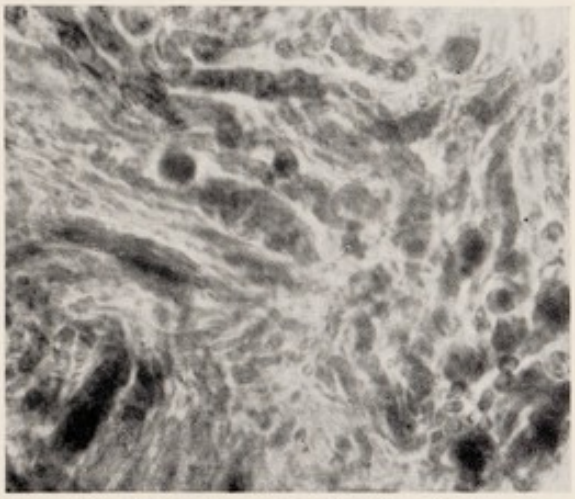


FIG. 17.—First branch of trigeminal nerve showing much degeneration; modified Marchi. × 280.

XEROPHTHALMIA OF 14 WEEKS' DURATION WITH PERIODS OF IMPROVEMENT OF RABBIT ON A-DEFICIENT DIET

FIG. 18.—Cornea. Worst part showing folded and keratinised epithelial layer. The changes were apparent on and off for 3½ months but were never severe. × 100.

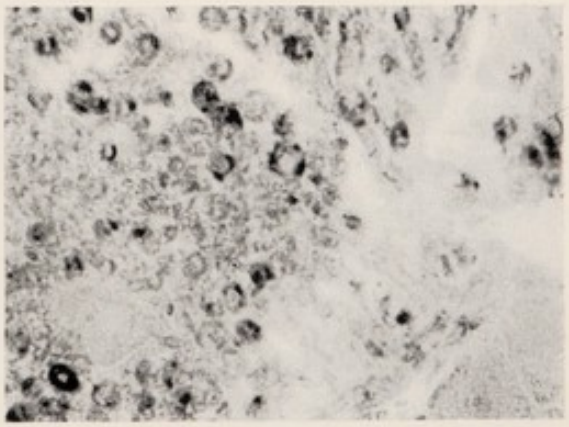


FIG. 19.—First branch of trigeminal nerve. Much "early" degeneration, with ringed effect; modified Marchi. × 280.

SUPERFICIAL INFECTION OF 4 WEEKS' DURATION OF RABBIT ON A-DEFICIENT DIET (FIGS. 20 AND 21)

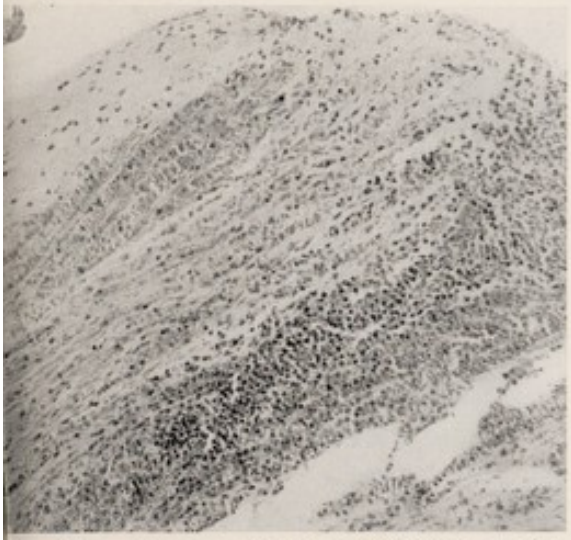


FIG. 20.—Cornea. No keratinisation seen in any sections made. Epithelium missing over infected area. Substantia propria infected. × 100.

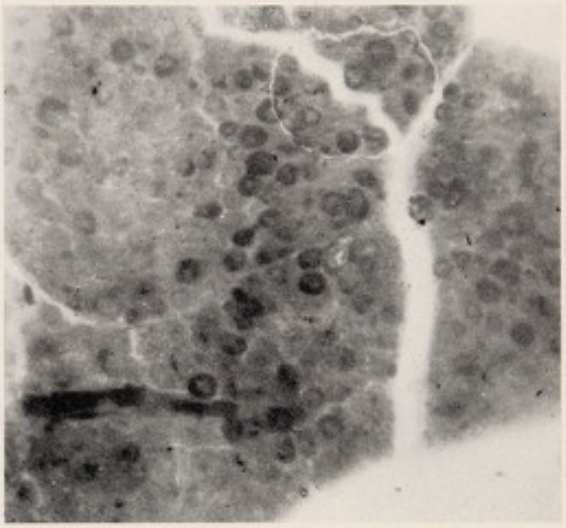


FIG. 21.—Fifth nerve, showing stages of early and later degeneration; modified Marchi. × 280.

XEROPHTHALMIA OF 9 WEEKS' DURATION ASSOCIATED WITH HYPOPYON IN RABBIT ON A-DEFICIENT DIET

FIG. 22.—Photograph of eye taken about two months before death, showing thick film over external corneal surface.

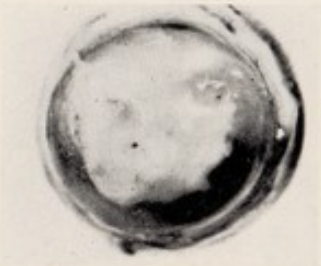


FIG. 23.—Photograph of eye at post-mortem. The white areas are within the anterior chamber and represent pus.

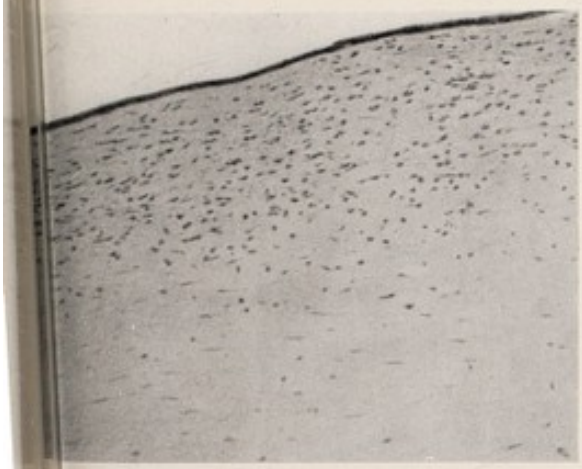


FIG. 24.—Cornea with only a single layer of epithelial cells (surface layers probably worn off) and a connective tissue cell infiltration of the substantia propria. × 100.

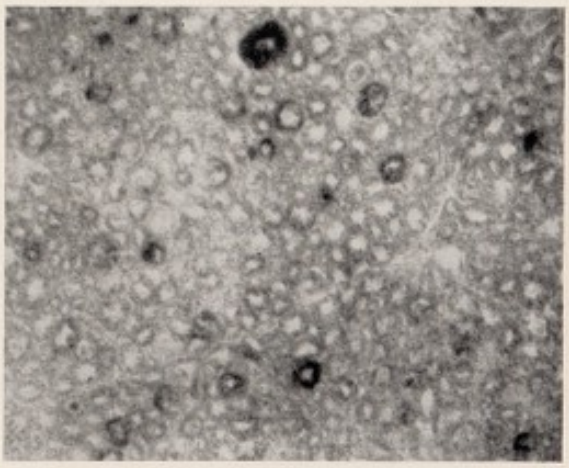


FIG. 25.—First branch of trigeminal nerve showing degeneration especially of the annular type. × 280.

interval of 12 days between the disappearance of the eye trouble and the end of the experiment. In neither animal did the trigeminal nerve show any abnormality. In other words, healing of the cornea was probably accompanied by recovery of the corresponding sensory nerve.

Recovery after xerophthalmia of 10 *days' duration* (*rabbit*). Both eyes became affected at the same time and 10 days later carotene (50 gr. cabbage daily) was added to the diet. The left eye was completely clear 6 days, the right 9 days after this addition to the diet; on the 10th day the animal was killed. The first division of both trigeminal nerves was examined and was apparently normal.

Recovery after 16 *days' xerophthalmia* (*rabbit*). The second animal developed xerophthalmia in both eyes at the same time and 16 days later 50 gr. cabbage daily was added to its diet. The right eye cleared in 3 days, the left in 5, and the animal was killed after 17 days of curative therapy. No degeneration could be found in the ophthalmic division of the trigeminal nerve. Other experiments of this nature but of longer duration are now in progress.

Experiments on other animals.

The experiments described above were made on rabbits. Dogs, rats and chickens have also been used, but much of this material has not yet been examined and is therefore not available for the purposes of the present paper. However, it may be stated that although there are differences in reaction to vitamin A deficiency in different animals the results are in general the same. In rabbits the first obvious change in the eye is usually in the central or exposed part of the cornea. In the dog the earliest signs of abnormality vary; sometimes the eyelids are first affected, sometimes the cornea appears to be slightly opaque, either in part, or more often over the whole area. It is not necessarily rough and dry as is common in the rabbit. In the rat, the earliest sign is usually in the eyelids which sometimes lose hair and become puffy. Corneal changes are only seen later and indeed if the vitamin A deficiency is great the animals often die before any macroscopic corneal changes are obvious.

Dogs. Puppies of 6 or 8 weeks old were given a diet of the following composition:—separated milk powder 15 to 25 grms., cereal such as bread 100 to 250 grms., lean meat 10 to 20 grms., yeast 5 to 10 grms., orange or lemon juice 5 c.c., olive or peanut oil 10 c.c., and sodium chloride 2 to 4 grms. Xerophthalmia often develops on a diet of this nature in 3 to 5 months but not with the same regularity as in rabbits on the basal diet previously described. Widespread degenerative changes take place in both central and peripheral nerves. When xerophthalmia appears, the ophthalmic division of the trigeminal nerve shows degenerative changes and sometimes this nerve is affected when there is no obvious eye trouble. In other words, the nerve degenerative changes are usually found but xerophthalmia is probably an incident

which may or may not develop during the feeding period. The longer the experiment the more likely are eye changes to occur.

Severe xerophthalmia in a dog on an A-deficient diet. The first signs of xerophthalmia appeared after 5 months of the diet. The disease became worse in both eyes and the animal was killed 2 months after the onset. Figs. 26 (*a*), 26 (*b*) and 27 show the appearance of the corneas at death. Sections showed no keratinisation, the surface epithelium being usually only one cell deep. The substantia propria was perforated in two places; one appeared to be an extrusion of the iris, the other a free mass containing many white and red blood corpuscles. The substantia propria showed infiltration of leucocytes (fig. 28). There was some degeneration in the ophthalmic branch of the trigeminal nerve (fig. 29), but not as much as might have been expected from the appearance of the cornea.

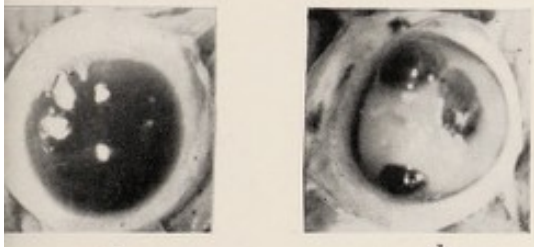
Rats. Several A-deficient basal diets have been given to young rats, in all of which xerophthalmia developed sooner or later. At varying times after beginning the diet, eye changes are seen and corresponding changes in the trigeminal nerve. When the eyelids are swollen and no xerophthalmia is present, the trigeminal nerve, as far as examined, always shows typical changes in the myelin sheaths. Since the first division supplies the eyelids as well as the cornea it is probable that this nerve is responsible for the maintenance of the normal structure and function of their epithelial cells also. In the rat, however, the corneal covering cells are generally more resistant to loss of the neurotrophic influence than the glandular cells of the eyelids and related tissues.

Disappearance of nerve fibres in a severe and long-standing case of xerophthalmia in a dog.

So far I have dealt with the actual degenerative changes which occur in the ophthalmic branch of the trigeminal nerve associated with xerophthalmia. It may be asked whether these nerves ultimately disintegrate and disappear if the process continues long enough and is of a sufficiently severe degree. The following example, one of several long-continued experiments on dogs, shows this to be the case.

The animal was on a vitamin A-deficient diet for 6 months. During this period it developed severe xerophthalmia, which was followed by ophthalmia and hypopyon. Cod-liver oil and other sources of vitamin A were then added to the diet, and the eye condition improved very much during the following four months until the end of the experiment. Figs. 30 and 31 represent sections of the trigeminal nerve stained respectively by methylene blue and Weigert-Pal. They show two things: (1) that the trigeminal nerve still contained much degenerating myelin and that the nerve degeneration in this case was not of the annular but of the Wallerian type which takes many months to regenerate; and (2) that many bundles of fibres have completely disappeared

CORNEA AND SENSORY NERVE OF DOG ON VITAMIN A-DEFICIENT DIET FOR 6 MONTHS. XEROPHTHALMIA FOLLOWED BY HYPOPYON (FIGS. 26-29)



a *b*

IG. 26 (*a*) and (*b*).—Photograph of cornea of both eyes.

FIG. 27.—Cross-section of anterior part of eye showing ulceration and rupture of cornea together with pus in anterior chamber. ×6.

IG. 28.—Cornea. The epithelium has disappeared and the substantia propria is invaded by leucocytes. ×100.

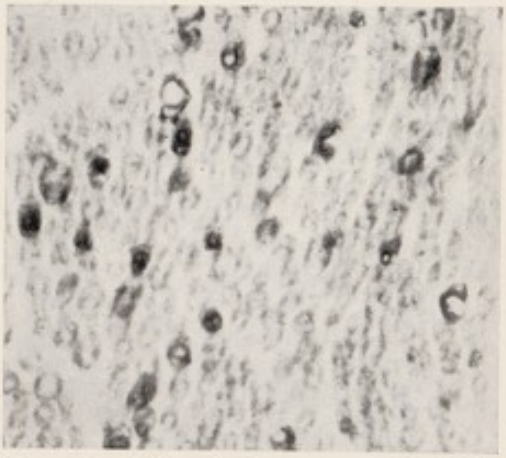
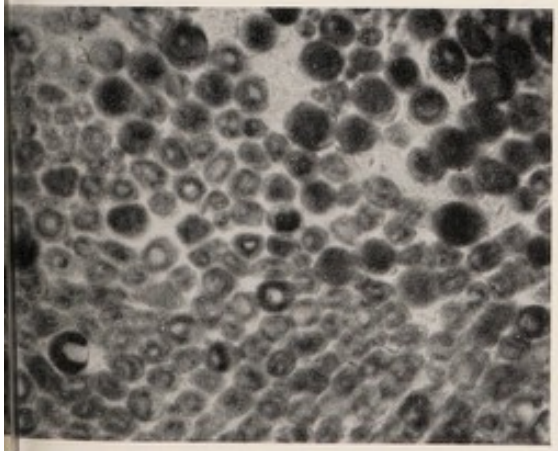


FIG. 29.—Trigeminal nerve stained by Marchi's method. Degeneration is present but not as much as might be expected considering the state of the eye. ×280.

ORY NERVE OF DOG ON A VITAMIN A-DEFICIENT DIET FOR 6 MONTHS FOLLOWED BY A DIET RICH VITAMIN A FOR 4 MONTHS. XEROPHTHALMIA AND INFECTIVE INVASION WERE GREATLY IMPROVED CHANGE OF DIET.



G. 30.—Trigeminal nerve stained by methylene blue. There is still much degeneration even after 4 months of vitamin A therapy. ×280.

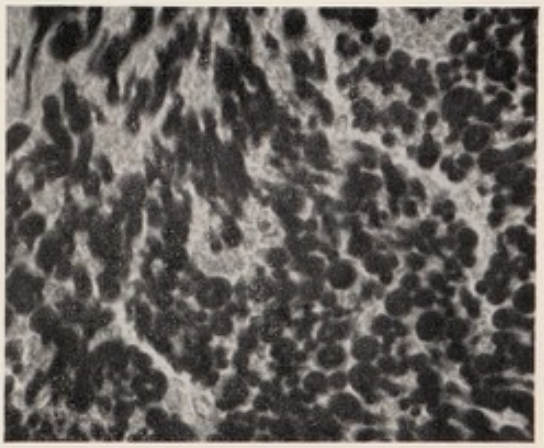
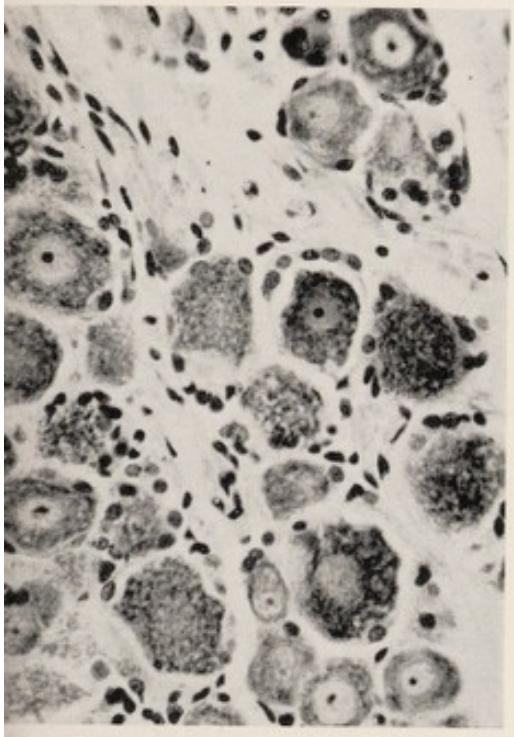


FIG. 31.—Trigeminal nerve stained by the Weigert-Pal method. The light areas are those where the nerve fibres have disappeared. ×280.

GASSERIAN GANGLION CELLS OF ANIMALS ON DIETS WITH AND WITHOUT VITAMIN A



32.—Gasserian ganglion cells from rabbit a diet containing carotene; cells nearly rmal. ×280.

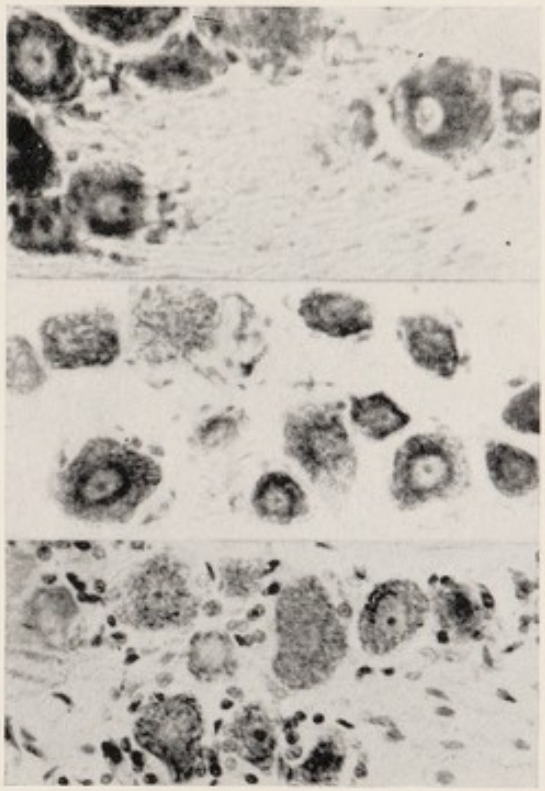
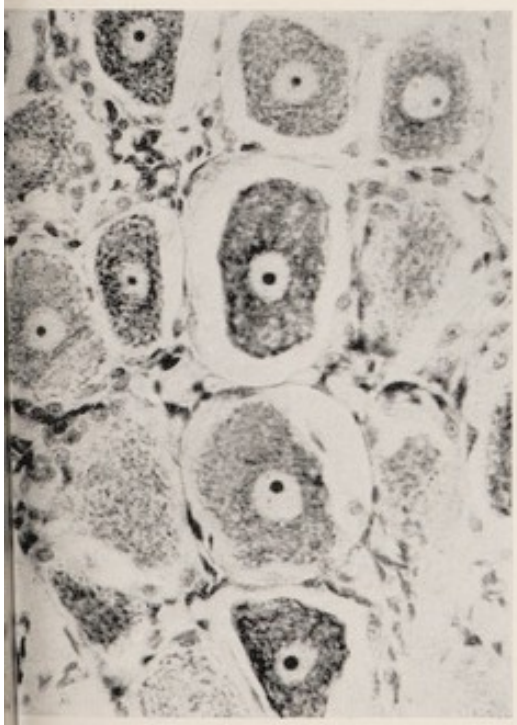


FIG. 33.—Gasserian ganglion cells from rabbit on carotene-deficient diet: many cells show degenerative changes. Note indistinct and powdery Nissl granules, deeply staining areas around nuclei, eccentric and granular nuclei and indistinct nucleoli. ×280.



—Gasserian ganglion of dog on vitamin . Note well-defined Nissl granules, well- d nuclei, and deeply staining nucleoli. have shrunk in formalin fixation.

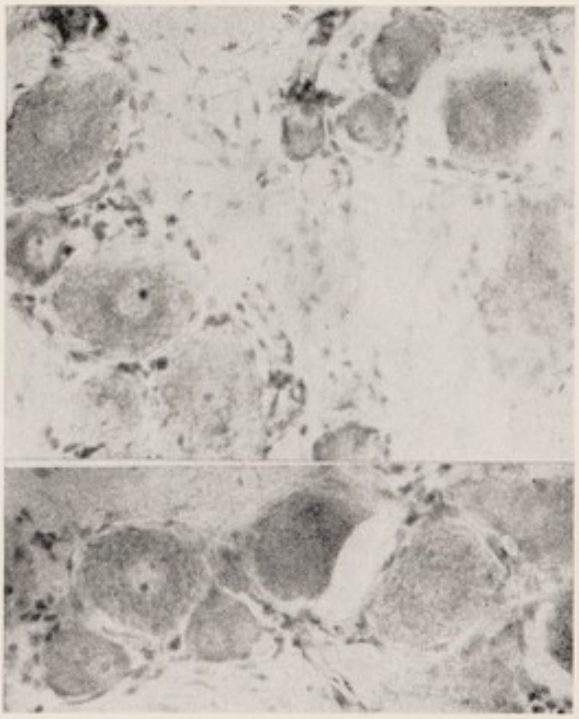


FIG. 35.—Gasserian ganglion of dog on A-deficient diet. Note loss of definition of Nissl granules and of nuclei. Nucleoli not prominent; nuclei eccentric.

(fig. 31, the Weigert-Pal section), indicating that the degenerative process had proceeded to the point of destruction of the nerve fibre. In this section the light areas represent places where the nerve fibres have disappeared and have been replaced by endoneurium.

It is evident that the degenerative changes in the nerves brought about by vitamin A deficiency may be so profound that even a source of vitamin A added to the diet, although it causes great improvement in the eye, cannot save some of the nerve fibres from complete destruction. In early and slight cases of xerophthalmia, however, this does not happen and the addition of vitamin A or carotene to the diet rapidly brings both the corneal epithelium and the sensory nerve back to normal.

Degenerative changes in the cells of the Gasserian ganglion.

In the foregoing, attention has been confined to the degenerative changes in the medullated nerve fibres and it has been pointed out that changes affecting the myelin sheath and probably also the axis-cylinders are found both peripherally and centrally to the Gasserian ganglion. It seemed probable that the nerve cells also would show degenerative changes, and even that these changes would prove to be the primary lesion. Whether this is so is not yet certain, but evidence will now be given to show that the nerve cells of the Gasserian ganglion are involved in this nutritional syndrome.

In fig. 32 are shown typical examples of Gasserian ganglion nerve cells of a rabbit on a diet containing carotene and in fig. 33 corresponding nerve cells of a rabbit on the same diet except that no source of vitamin A or carotene was added to the basal diet. It will be seen that the nuclei of the carotene-deficient animals instead of being centrally placed and clear, with distinct single or double nucleoli, tend to be eccentric in position and granular in appearance with indistinct nucleoli. Again the degenerating cells often have their Nissl's granules collected round the nucleus instead of being more or less evenly distributed throughout the cytoplasm, their outline is uneven and their protoplasm often appears lobulated. The general impression is that many of the cells in the carotene-deficient animals are disintegrating, and glial cells are often concentrated around them.

How early these changes appear and how far they proceed in severe and prolonged cases of xerophthalmia cannot yet be stated, but as whole nerve fibres disappear ultimately under these conditions (fig. 31) it is probable that the nerve cells also disintegrate entirely.

In figs. 34 and 35 similar differences in the cells of the Gasserian ganglion of the dog according to whether vitamin A was present in or absent from the diet are shown.

Discussion.

The object of this investigation was to see if there is any association between the two main morbid conditions produced in animals, especially young animals, by diets deficient in vitamin A and carotene, namely:—(1) epithelial and mucous membrane changes, including hyperplasia and metaplasia, often giving rise to local infective lesions, in the eye, genito-urinary tract, lungs, middle-ear and nasal sinuses, and alimentary tract; (2) degenerative changes in the nervous system as evidenced by demyelination and final disappearance of fibres in central and peripheral nerves.

In this paper only the eye, its related sensory nerve and the ganglion cells from which the latter arises have been considered. Of the ocular tissues supplied by this nerve, the cornea has been studied in more detail than the paraocular glands and eyelids. Evidence has been given to show that xerophthalmia is generally if not always associated with degenerative changes in the fibres of the first branch of the trigeminal nerve and its cells of origin. When the eye has been affected by xerophthalmia, even in the early stages, the corresponding sensory nerve has nearly always shown some abnormality of the myelin sheath, and in the animals so far examined, curative changes in the eye in early cases of the disease have been accompanied by a return to normal of the corresponding nerve.

The nerve changes that accompany xerophthalmia are not always of the typical Wallerian type. It is unusual in the earlier stages to find the myelin droplets invading the axis cylinder portion of the nerve. More commonly the nerve fibres are swollen, and the staining of the myelin with osmic acid (Marchi method) and other differential stains is confined to the myelin sheath itself, so that in cross sections thickened and stained rings of degenerated myelin are seen mingled with the normal fibres. In more advanced cases many fibres are found in which degenerated myelin occupies the position of the original axis cylinders. This annular form of nerve degeneration has not received much consideration by earlier workers, but occasional references to it have been made by neurologists. Thus Kinnier Wilson (1913-14) comments on the frequency of degenerated myelin sheaths which have not disintegrated or become discontinuous, but which stain with osmic acid, in the peripheral nerves of pellagrins.

It will be asked which change comes first, the corneal or the nervous. This question cannot be answered definitely at present, as the evidence indicates that they are closely related in point of time. In order to get more evidence as to which change is primary, both eyes and the corresponding nerves of 4 rabbits were examined in which one eye of each animal showed early xerophthalmia while the other eye was unaffected. In only one instance did the trigeminal nerve of the normal eye contain degenerating myelin; the remaining three unaffected

eyes had normal myelin sheaths as far as could be ascertained by the methods adopted. The affected eye in each animal had degenerating fibres in the corresponding trigeminal nerve. Since it is probable, on the basis of other experiments, that the normal eye would soon have developed xerophthalmia, it seems clear that in these animals the development of the two lesions must have been almost synchronous, but that the nerve changes may have slightly preceded the corneal. More recent results on rats not described in detail in this paper have, however, shown that the sensory nerve of the eye may be degenerate without any obvious xerophthalmia, although the paraocular glands were evidently abnormal.

The experiments of a curative nature gave results which also suggested the almost synchronous origin of the corneal and trigeminal changes. When the corneal condition was cured by adding cabbage to the diet, the trigeminal nerve no longer showed abnormality. This rapid recovery of the nerve on adding a source of vitamin A to the diet, in one experiment after only a few days' feeding, indicates that a nerve with early degeneration caused by vitamin A deficiency can resume its normal structure and probably its function much more quickly than a nerve which shows demyelination of the ordinary Wallerian type.

When nerve fibres have assumed a more or less typical Wallerian stage of degeneration, as is often found in some fibres of a nerve, especially in advanced cases of vitamin A deficiency, then a much longer period of correct feeding is necessary before function can again be resumed. One instance out of several of a curative experiment in a dog with a severely infected eye following xerophthalmia has been described above. Even after four months of good feeding, although the eye condition had greatly improved the nerve had some fibres in which the Wallerian type of degeneration could still be seen. Sections of the trigeminal nerve of this dog stained by Weigert's method showed that patches of nerve fibres had disappeared and been replaced by connective tissue (fig. 31), while others stained with osmic acid.

It has been shown above that not only is the myelin sheath of the nerve affected by the specific nutritional defect under consideration, but that many of the nerve cells in the Gasserian ganglion are changed. The cells are generally somewhat shrunken, Nissl's granules are altered in number and distribution and are powdery, the nuclei of the cells are less clear and seem to have a groundwork not obvious in the properly nourished cells and they may be moved to an eccentric position. These changes in the nerve cells support the view that the swelling of the nerve fibre and the alteration in appearance of the myelin sheath so often found in the trigeminal nerve in vitamin A deficiency are not artefacts but represent real degenerative effects. The question as to whether the nerve cell or the myelin sheath change is primary is a point which can only be settled by further work. It is probable, however, that the nerve cells are first affected by the metabolic defect

because, as previously indicated, there is a clear differentiation as regards medullary changes between the afferent and efferent nerves, the former being affected while the latter escape, at least in the early stages. There does not seem to be any reason why medullated nerves should be so differentiated unless the nerve cells of the afferent and efferent systems are susceptible in different degrees to the absence of vitamin A and carotene from the diet. Observations made up to the present time indicate that afferent nerve cells such as are found in the Gasserian and posterior root ganglia show more typical alterations with this diet deficiency than efferent nerve cells represented by those of the anterior horns of the spinal cord. On the whole, therefore, it seems probable that the degenerative changes in the nerve roots of the trigeminal nerve are secondary to changes in the Gasserian ganglion.

Another problem which awaits further investigation is the degree to which the axis cylinder is involved in these changes, especially in the early stages. It is clear that the axis cylinders of the afferent trigeminal nerve fibres degenerate completely in advanced cases. This can be seen in fig. 31, which represents a cross-section of this nerve stained by Weigert's method in a long-standing case in a dog. The light patches indicate complete disappearance of bundles of nerve fibres. This, however, does not settle the question of the condition of the axis cylinder in early cases where the medullary sheath although not normal is still intact and where the nerve can apparently be brought rapidly back to normal, both in appearance and function, by adding a source of vitamin A or carotene to the diet. We are at present investigating the state of the axis cylinder in these early cases by the use of silver stains and it appears that the axis cylinders are also altered even in fibres which show only annular myelin degeneration and perhaps even at a still earlier stage.

I am conscious that experimental work of this type, which depends so largely on histology and especially on microscopic changes in the myelin sheath of nerves, rests on an insecure foundation. Every effort has been made to overcome this difficulty. In most cases the Marchi method of demonstrating degenerating myelin has been supplemented by the use of other stains including methylene blue, Scharlach R and Sudan III, with in most cases comparable results. Again in the majority of cases a known normal nerve has been treated at the same time as the unknown for comparison.

Although this paper deals with the trigeminal nerve, the whole investigation, of which only a small part is reported here, has been extensive and has included the examination of the central nervous system and many peripheral nerves. Histological findings have been constantly supported by the clinical condition of the animals, which itself shows that nervous function in these animals is greatly changed. Indeed, the combined evidence seems to me so overwhelming that

I regard the histological results as definite indications of structural change. It is true that the annular form of degeneration involving the swelling of the nerve fibre and altered staining of the myelin sheath has been found so far most commonly in peripheral nerves. Nevertheless, annular changes seem to me to be a quite definite indication of degeneration and not artefact.

The fact of the matter is that practically all present knowledge of the degenerative changes in nerve fibres has been obtained by experiments involving the cutting of nerves, and it is evident that the changes which follow metabolic abnormalities in the body are, in the earlier stages at any rate, of a different nature and probably more dependent on primary alterations in nerve cell function and structure. So far as the present work is concerned it would appear that some changes in afferent nerve cells and fibres produced by specific nutritional influences can be quickly brought back to normal in the early stages but that later when the myelin sheaths are broken down and invade the axis cylinders, recovery may either not take place, or if it does, it is a slow prolonged process taking many months, just as does recovery of a cut peripheral nerve showing the typical Wallerian degeneration.

This subject of metabolic degeneration in nerve cells and fibres is by no means academic and of interest only so far as the present work is concerned. Although the evidence that cut or mechanically damaged fibres in the central nervous system do not regenerate is very strong, clinicians are generally agreed that nerve fibres in the central nervous system which have lost their function may regain it. This seems to be specially the case in subacute combined degeneration, where, although much recovery of function is undoubtedly due to peripheral nerve regeneration (Baker, Bordley and Longcope 1932, Carmichael 1932), part of the improvement is thought by some neurologists to follow central nervous system recovery. It may well be that a metabolic degenerative change in the fibres of the central nervous system, as found in subacute combined degeneration if it has not gone too far, can recover and the affected nerves assume their normal structure and function. It would be of interest to know exactly at what stage of alteration of histological structure as revealed by staining methods a nerve loses its function.

These results bring to the fore again the long-debated question of neurotrophic influence. Are the epithelial changes in the cornea the direct effect of an abnormality of the sensory nerve? Does the susceptibility to infection of an eye in this condition depend on loss of neurotrophic control? What really is the relationship between xerophthalmia and keratitis neuroparalytica as seen for instance in cases after removal of the Gasserian ganglion? In keratitis neuroparalytica the corneal epithelium is affected in the earliest stage and assumes the same dry appearance as in xerophthalmia, but whereas in

the latter the spread of the change over the whole epithelium is usually slow, in the former it is often extremely rapid. It may be that one explanation of this difference lies in the complete loss of sensory innervation in keratitis neuroparalytica, whereas the loss is often only partial in xerophthalmia. In the experimental animals some bundles of fibres may be affected, the rest of the trigeminal nerve remaining normal. Some of the difference may also possibly be explained by the complete loss of blinking reflex after removal of the Gasserian ganglion, whereas this reflex is not entirely lost in the experimental animals referred to above. It is, however, of interest to note that clinicians have long known that the eye in xerophthalmia is relatively insensitive, a condition which itself indicates an abnormality in the sensory nerve; the abnormality has usually been thought to be due to changes in the actual nerve-endings which accompany the changes in the corneal epithelium.

The other well-known disease in which both the cornea and the trigeminal nerve are often implicated is herpes zoster ophthalmicus. The changes in the cornea in this disease differ somewhat from those which develop in xerophthalmia and keratitis neuroparalytica. Instead of the dry appearance of the cornea found in the latter, vesicles often form in herpes zoster ophthalmicus, and these rupture on the surface, leaving superficial ulcers which remain discrete or become confluent according to the severity of the attack. Permanent opacity tends to be left by this disease, but the ulcers do not usually perforate. In xerophthalmia rupture of the cornea readily develops if the condition be not treated, but no corneal opacity is left if the case is treated in reasonable time by adding vitamin A or carotene to the diet. There are obviously some differences in the ætiological factors involved in the keratitis of herpes zoster ophthalmicus and in xerophthalmia. Possibly the presence of the herpes zoster virus alone will explain the differences in the lesions. At least it is clear that in both conditions a neurotrophic factor associated with the sensory nerve supply to the cornea is involved.

Any discussion of the nature of the neurotrophic influence of the trigeminal nerve on the cornea seems unprofitable at the present time. A difficulty which has always presented itself in discussions on this point is why only a proportion of the cases in which the Gasserian ganglion has been removed develop keratitis neuroparalytica. Parsons (1924) has suggested as the result of the examination of Victor Horsley's surgical cases that irritation of the cut surface was probably a determining factor. He also suggested that the neuropathic effect was bound up with the passage of anti-dromic impulses passing down the fifth nerve from the point of injury. This same difficulty, the limitation of corneal complications to a portion only of the cases, is seen in herpes zoster ophthalmicus. It is possible that one determining factor in the development of keratitis in both keratitis neuroparalytica

and herpes zoster ophthalmicus is nutritional, and by providing a high vitamin A intake both might be reduced or avoided. This, however, is purely surmise.

The evidence given strongly favours the view that xerophthalmia is bound up with degenerative changes in the sensory nerve of the cornea. Now, as stated above, this is only one of a large number of organs in which epithelial changes of a hyperplastic and keratinising type develop in young animals brought up on a vitamin A-deficient diet and in which there is a great tendency to the production of septic foci. Such changes are found all over the body, for instance in the respiratory, genito-urinary and alimentary tracts, and indeed in all mucous membranes and duct epithelia. Not only so but the involvement of afferent nerves throughout the body is equally extensive, the optic nerve, the cochlear and vestibular divisions of the eighth nerve, the sciatic nerve and all the posterior roots of the spinal cord usually show extensive degeneration. If xerophthalmia and subsequent inflammation of the cornea are closely associated with the removal of a neurotrophic influence accompanying degenerative changes in the trigeminal nerve, it is difficult to avoid the assumption that there is a similar ætiological relationship between the extensive afferent nerve degeneration and the hyperplastic, keratinising changes in other epithelia and mucous membranes and the development of abscesses in these places. If this is the case, and it seems most probable that such a relationship holds, it would be necessary to extend greatly the significance of neurotrophic relationships to inflammatory conditions. Up to the present time neurotrophic problems have been considered only in a narrow field which includes the eye, the skin and some joints, the so-called Charcot's joints of tabes dorsalis. The work described above suggests, however, that it may be necessary to extend this conception and to admit the possibility that such conditions as broncho-pneumonia, some forms of dysenteric and other alimentary infections, pyelitis, cystitis, septic nasal sinuses, middle ear disease, and other septic foci may on occasion have a neurotrophic basis. In such a clinical condition as pellagra, for instance, it would appear most probable that not only the skin but the alimentary tract and mouth changes have the loss of a neurotrophic control as an ætiological basis. Again, the state of the tongue in pernicious anæmia may be similarly related. At least it can be said that the present work affords a method whereby the problem of neurotrophic influences and their relation to susceptibility to infection can be experimentally studied in the laboratory.

Summary.

1. Evidence is given which suggests that xerophthalmia produced in animals by diets deficient in vitamin A and carotene may be secondary to a loss of the neurotrophic control normally exerted on the cornea by the ophthalmic division of the trigeminal nerve.

2. When xerophthalmia is present, the corresponding trigeminal nerve usually shows degenerative changes in the myelin sheaths, and in the rabbit their development is commonly almost synchronous.

3. In early and slight xerophthalmia when the corneal epithelium returns to normal as the result of adding carotene or vitamin A to the diet, the nerve also returns to normal. In such cases the myelin degeneration is of the annular type and although the fibres may be swollen the myelin does not invade the axis cylinder. This type of nerve degeneration seems capable of rapid recovery.

4. In more severe cases of xerophthalmia, in addition to degeneration of the annular type, many of the fibres show typical Wallerian degeneration, the axis cylinder being invaded by degenerated myelin while globules of the latter are scattered throughout the nerve. In cases of this type recovery of the nerve fibre either does not take place or does so only after months of vitamin A or carotene therapy.

5. The nerve cells of the Gasserian ganglion as well as their nerve fibres show degenerative changes. Indeed it is possible that the original lesion is in the cells and that the trigeminal changes, peripheral and central, are secondary thereto.

6. In the rat, where as the result of vitamin A deficiency, the eyelids become puffy, the cornea may escape xerophthalmic changes. In such cases also the trigeminal nerve shows demyelination.

7. Since degeneration of the afferent nerves is widespread in animals brought up under these experimental conditions, it is probable that hyperplasia and metaplasia of other epithelial and mucosal surfaces throughout the body, and the subsequent invasion of these tissues by micro-organisms, are also related to changes in their afferent nerve supply.

The expenses of this research were paid by the Medical Research Council, to whom my thanks are due.

REFERENCES.

BAKER, B. M. Jr., BORDLEY, J. III, AND LONGCOPE, W. T. — *Amer. J. Med. Sci.*, 1932, clxxxiv. 1.

CARMICHAEL, E. A. . . . . — Verbal communication to Medical Research Society, May 1932.

COLLINS, E. T. . . . . — *Trans. Ophthal. Soc.*, 1930, l. 201.

FINDLAY, G. M. . . . . — *Brit. J. Exper. Path.*, 1925, vi. 16.

GOLDBLATT, H., AND BENISCHEK, M. — *J. Exper. Med.*, 1927, xlvi. 699.

GREEN, H. N., AND MELLANBY, E. — *Brit. Med. J.*, 1928, ii. 691.

GREEN, H. N., AND MELLANBY, E. — *Brit. J. Exper. Path.*, 1930, xi. 81.

MELLANBY, E. . . . . — *J. of Physiol.*, 1926, lxi.; *Proc. Physiol. Soc.*, p. xxiv.

„ . . . . . — *Brit. Med. J.*, 1930, i. 677.

„ . . . . . — *Brain*, 1931, liv. 247.

„ . . . . . — *Edin. Med. J.*, 1933, xl. 197.

MORI, S. . . . . *Bull. Johns Hopkins Hosp.*, 1922, xxxiii. 357.

PARSONS, J. H. . . . . *Trans. Ophthal. Soc.*, 1924, xliv. 156 (Discussion on trigeminal system opened by Ivy Mackenzie.)

WILSON, S. A. K. . . . . *Proc. Roy. Soc. Med.* 1913-14, vii. (Neurological Section), 31.

WOLBACH, S. B., AND HOWE, P. R. *J. Exper. Med.*, 1925, xlii. 753.

YUDKIN, A. M., AND LAMBERT, R. A. *J. Exper. Med.*, 1923, xxxviii. 17.

PRINTED IN GREAT BRITAIN BY OLIVER AND BOYD LTD., EDINBURGH