COMMUNICATIONS

REACTION OF CORNEAL NERVE FIBRES TO INJURY*

BY

E. ZANDER† AND G. WEDDELL

From the Department of Anatomy, University of Oxford

IN a recent paper (Zander and Weddell, 1951) a description of the innervation of the normal cornea is given so far as it can be determined by direct observation. In the present paper the results are described of a number of experimental procedures designed to test the validity of observations made by the direct method and also to add facts not obtainable by that method.

Apart from information on the general pattern of corneal innervation, a study of degeneration and regeneration of nerve fibres in this tissue provides other data of considerable interest.

Corneas were examined after the following procedures had been carried out:

- (1) corneal autografting;
- (2) keratotomy;
- (3) section or avulsion of the infra-orbital nerve;
- (4) section of the ciliary nerves;
- (5) removal of the superior cervical sympathetic ganglion;
- (6) destruction of the Gasserian ganglion.

MATERIAL AND METHODS

The animals used were rabbits weighing between 2 and 3 kg. and two rhesus monkeys. For the operative procedures they were anaesthetized with nembutal given intravenously. At the end of the experimental period the animals were killed with an overdose of nembutal.

The corneal nerve fibres were either stained with methylene blue or impregnated with silver. Details of the methods used have already been published (Weddell and Zander, 1950). Briefly, staining is carried out by the introduction of a dilute solution of dye into the anterior chamber of the eye, where it is allowed to remain for a specified time. After staining, the cornea is very carefully removed with sharp scissors and prepared for microscopical examination after fixation of the dye with a mixture of ammonium molybdate and ammonium picrate. The details of the fixation process are complicated

* Received for publication October 18, 1950.

† In receipt of a grant from the Swiss "Stiftung für biologisch-medizinische Stipendien" and on leave from the Neurosurgical Clinic, University of Zürich.

and must be carried out with extreme care. The silver-impregnation method has its basis in the procedure described by Glees (1946), but a number of modifications have been introduced to make it suitable for the cornea.

EXPERIMENTAL PROCEDURES

(1) CORNEAL AUTOGRAFTING.

The primary purpose of these experiments was to study the process of degeneration of corneal nerve fibres.

It has been claimed by a number of authors that the process in the cornea is much slower than elsewhere in the body (e.g., Leoz Ortin, 1915, 1917; Escapini, 1948), and autografting some two-thirds of the cornea seemed the most suitable method of testing this statement. In addition, it was hoped that some information on the relation between opacity in grafts and their nerve supply might be obtained.

Little work seems to have been done on the behaviour of nerve fibres in corneal grafts. Franceschetti and Babel (1947) have described the nerve supply of a corneal heterograft in man 6 years after it had been transplanted. The graft had remained transparent since it was applied. Following the removal of the cornea they used silver-impregnation methods to demonstrate the nerve fibres. They found that no nerve *bundles* entered the graft from the host's cornea; the bundles either took a recurrent course or ran round the graft. A few *single* nerve fibres penetrated the scar and entered the graft, where they gave rise to a profuse plexiform network of nerve fibres situated just beneath the epithelium. The authors concluded that the presence of these nerve fibres was responsible for the transparency of the graft, for no nerve fibres were found in opaque grafts which they examined. Escapini (1948) studied the course of degeneration and regeneration of nerve fibres in relation to corneal transplants in rabbits and concluded that the processes of nerve degeneration and regeneration in the cornea resemble those in other tissue except that they are much slower. He finds, in contradistinction to Franceschetti and Babel, that opaque grafts appear to be as well innervated and as sensitive as clear ones.

Operation.—In all, fifteen rabbits were used. The animals were anaesthetized with nembutal, and sufficient eserine solution was placed in the conjunctival sac to obtain a constricted pupil. A small circumferential incision was made right through the cornea with a keratome, one-third of the distance from the limbus to the centre of the pupil, and this was immediately resutured with a No. 1 nylon thread. This procedure was continued until two-thirds of the central part of the cornea had been completely excised and resutured into its original position with about ten stitches. If there was an iris prolapse which could not be reduced, the protruding portion was excised. The lids were subsequently sewn together and remained closed for 14 days, after which the stitches were removed. After removal of the sutures sensory tests were commenced. They were carried out with a No. 1 nylon suture and cotton-wool, attempts being made to elicit a blink reflex.

Observations.—The results of the operations and the fate of the grafts are shown in Table I, further observations being grouped under five heads, as follows:

- (a) degeneration of nerve fibres ; (d) reaction of the corneal corpuscles;
 (b) return of sensibility ; (e) reaction of the Schwann cells.
 (c) regeneration of nerve fibres;

TABLE I
ANALYSIS OF CORNEAL AUTOGRAFT OPERATIONS

Rabbit No.	Time between operation and end of experimental period (days)	State of graft at end of experimental period	Blink reflex after grafting				Neurohistological method used
			1 week	2 weeks	3 weeks	at end of period	
1	2	whole cornea slightly opaque	—	—	—	0	silver impregnation
2	2	whole cornea slightly opaque	—	—	—	0	methylene blue
3	7	whole cornea slightly opaque	0	—	—	0	silver impregnation
4	7	whole cornea slightly opaque	0	—	—	0	methylene blue
5	14	clear	0	0	—	0	silver impregnation
6	14	whole cornea slightly opaque	0	0	—	0	methylene blue
7	21	clear	0	0	+	+	silver impregnation
8	21	upper half of cornea opaque	0	0	0	0	methylene blue
9	50	clear	0	0	+-	+	silver impregnation
10	50	centre opaque	0	0	+	+	methylene blue
11	80	centre opaque	0	0	—	++	silver impregnation
12	110	clear	0	0	0	++	silver impregnation
13	110	clear	0	0	0	—	methylene blue
14	220	upper half of cornea slightly opaque	0	0	+-	+++	silver impregnation
15	220	clear	0	0	—	+++	methylene blue

0 = no reflex

+- = doubtful response

+ = weak response

++ = brisk response

+++ = response equivalent to that obtained in unoperated eye

— = reflex not tested for

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

FIG. 7.

FIG. 8.

FIG. 9.

FIGS 1-9.—Condition of degenerating nerve fibres in rabbit corneal autografts at various periods after operation.

FIG. 1.—Silver-impregnated preparation 2 days after operation. It shows a nerve bundle in the substantia propria. Some of the nerve fibres are severely affected; even the smooth axis cylinder is very swollen. (x 463)

FIG. 2.—Methylene-blue stained preparation 2 days after operation. It shows a nerve bundle in the substantia propria in which all the axons are showing signs of degenerative change. (x 193)

FIG. 3.—Silver-impregnated preparation 2 days after operation. The axon in the substantia propria is swollen and "neurofibrillae" are developing. (x 463)

FIG. 4.—Silver-impregnated preparation 2 days after operation. The axons in the substantia propria show typical signs of degenerative change. (x 1130)

FIG. 5.—Silver-impregnated preparation 7 days after operation. The axons in the substantia propria are in an advanced state of degeneration. (x 595)

FIG. 6.—Silver-impregnated preparation 14 days after operation. It shows axon fragments lying on the surface of a Schwann cell in the substantia propria. (x 655)

FIG. 7.—Silver-impregnated preparation 21 days after operation. It shows Schwann-cell pathways in the substantia propria. The cytoplasm of the cells contains beaded threads. No axon fragments can be seen. (x 655)

FIG. 8.—Methylene-blue stained preparation 21 days after operation. It also shows Schwann-cell pathways in the substantia propria, devoid of axons, but containing beaded intra-cytoplasmic threads. (x 519)

FIG. 9.—Silver-impregnated preparation 2 days after operation. It shows the peculiar reaction of the corneal corpuscles. (x 575)

(a) DEGENERATION OF NERVE FIBRES

Two days after Grafting.—The process of degeneration was marked, and generally similar in type and degree to that seen among non-myelinated nerve fibres in the skin of the rabbit's ear by Weddell and Glees (1941), the signs of degeneration taking the form of a breaking up of the axis cylinder into "neurofibrillae" or of the formation of vacuoles within the axis cylinder (Figs 1, 2, 3, 4). At this early stage (as in skin) some fibres appear to suffer less than others although all nerve fibres are affected to some extent (Fig. 1) in some part of their course. Sections taken to include the incision and tissue on either side of it show that nerve fibres proximal to the incision were similarly affected from some 2 mm. More proximally, the fibres appeared to be quite normal.

Seven days after Grafting.—The degenerative processes are again similar to those seen in the skin of the rabbit's ear, all the axis cylinders being in a disintegrating condition (Fig. 5) except for a few single nerve fibres in the substantia propria at the centre of the cornea, which appeared to be more "resistant".

Fourteen days after Grafting.—The changes are still more severe. All nerve fibres are seen to exhibit extensive degenerative changes (Fig. 6). The rate and nature of the degenerative process of nerve fibres in the cornea are thus similar to that undergone by non-myelinated nerve fibres in skin from the rabbit's ear.

Twenty-one days after Grafting.—Only traces of the original nerve fibres can be seen as fragments lying along the original Schwann-cell pathways, the process of degeneration being virtually complete (Figs 7 and 8). However, regenerating nerve fibres can now be seen coursing along a certain number of Schwann-cell pathways. They arise from the severed axons proximal to the scar.

(b) RETURN OF SENSIBILITY (Table I)

This was tested as follows: either a No. 1 nylon suture or a wisp of cotton-wool was lightly applied at right angles to the surface of

FIG. 10.

FIG. 11.

FIG. 12.

FIG. 13.

FIG. 14.

FIG. 15.

FIG. 16.

FIG. 17.

Figs 10-17.—Condition of regenerating nerve fibres in rabbit corneal autografts at various periods after operation.

FIG. 10.—Silver-impregnated preparation 21 days after operation. It shows single regenerating axons in the substantia propria which have pierced the scar and entered the cornea. They are not related to Schwann-cell pathways. (x 670)

FIG. 11.—Methylene-blue stained preparation 50 days after operation. It shows a bundle of immature axons passing along a Schwann-cell pathway in the substantia propria. (x 390)

FIG. 12.—Silver-impregnated preparation 50 days after operation. It shows a regenerating axon making its way between the cells of the basal epithelial layer. (x 545)

FIG. 13.—Methylene-blue stained preparation 80 days after operation. It shows regenerating beaded axons lying among the epithelial cells. (x 465)

FIG. 14.—Silver-impregnated preparation 110 days after operation. It shows mature regenerated axons in the substantia propria. The number of branches to which one of the axons gives rise is greater than that seen in normal specimens. One daughter axon in particular has divided into a number of branches on its way round a cell, the nucleus of which can be seen. (x 254)

FIG. 15.—Silver-impregnated preparation 110 days after operation. It shows an axon taking a very complicated course in the substantia propria. (x 420)

FIG. 16.—Silver-impregnated preparation 220 days after operation. It shows a nerve bundle containing relatively fewer axons than normal, some of which are still immature, in the substantia propria. (x 673)

FIG. 17.—Methylene-blue stained preparation 220 days after operation. It shows immature axons ramifying among the epithelial cells. (x 635)

the graft, these procedures having been found to elicit a brisk corneal reflex from a normal cornea at each contact.

Fourteen days after Grafting.—No corneal reflex could be obtained, whether the graft was clear or opaque.

Twenty-one days after Grafting.—A feeble blink reflex could occasionally be obtained in the periphery of the graft, but it tired quickly. No response could be elicited with cotton-wool, however. The reflex was obtained from animals with opaque as well as clear grafts.

Seven weeks after Grafting.—A response could be obtained at every stimulation in the periphery of the graft, but the reflex was not a strong one. Only an occasional response was obtained from the centre of the graft. Cotton-wool still elicited no response.

Twelve weeks after Grafting.—A brisk and moderately strong response was obtained at each stimulus throughout the graft. Cotton-wool now elicited a feeble response on some occasions in the periphery of the graft only.

Sixteen weeks after Grafting.—The response to the nylon suture was stronger still and cotton-wool now elicited a moderate response each time it was applied to the centre of the graft.

Twenty-eight weeks after Grafting.—The response from the grafted side was indistinguishable from that obtained from the unoperated side.

In every case recovery of sensibility in opaque grafts followed a course similar to that in clear grafts.

The observations tabulated above broadly determined the times following operation at which the grafts were examined histologically.

(c) REGENERATION OF NERVE FIBRES

Twenty-one days after Grafting.—A few fine isolated nerve fibres can be seen coursing throughout the substantia propria. They can be recognized as regenerating nerve fibres by reason of their origin from nerve bundles proximal to the scar and by their morphological features (Fig. 10). The fibres are fine, but not of uniform diameter; they are made up of a series of interconnected elongated swellings joined by segments which are only half as broad. They are quite similar in appearance to those seen regenerating in the skin of the rabbit's ear in the early stages except that they seldom appear to terminate in growth cones; nor do they necessarily grow along Schwann-cell pathways: in fact many lie freely among the connective tissue elements of the substantia propria. Individual fibres can be traced for relatively long distances and many have covered a distance of not less than 0.5 cm. from one side of the graft to the other.

Fifty days after Grafting.—Many nerve fibres have now invaded the graft, but they only penetrate the scar singly or in pairs, not in the form of closely packed bundles. Those fibres which penetrate the scar in the region of a pre-existing Schwann-cell pathway follow the latter; but many fibres appear to have forged their own pathways and to have progressed as far as those associated with Schwann cells. Thus at this stage there are more nerve fibres unassociated with Schwann pathways than in the normal cornea. The bundles of nerve fibres situated just beneath the epithelium, which are so striking a feature in the normal cornea, are starting to re-form; but the course pursued by individual axons composing the bundles is far more complex than in the normal cornea. Some axons have reached the epithelium and are giving rise to finely beaded fibres (Figs 12 and 13). A proportion of the nerve fibres now appears smooth in outline, *i.e.*, they have presumably reached maturity. Many fibres are still in a state of immaturity, however; particularly those entering the plexiformly arranged bundles (Fig. 11).

Eighty days after Grafting.—The graft is full of mature nerve fibres, which, however, are all derived from relatively few axons that have penetrated the scar. There is still a large number of immature fibres passing towards the epithelium, and the number of small beaded fibres in the epithelium is far below that in a normal cornea. The peculiar paths taken by, and the complex twistings of, many mature axons are evidence of the fact that they are regenerated fibres. In some cases, just as in the skin of the rabbit's ear, fibres appear to have avoided cells in their pathway, splitting and rejoining around them (Fig. 14). In contrast with the ear skin, however, growth cones are seldom seen (except in the region of the scar); nor are there large swellings on the course of the regenerated nerve fibres, suggestive of obstruction. There is still a number of Schwann-cell pathways devoid of nerve fibres.

One hundred and ten days after Grafting.—Apart from the fact that the axons penetrating the scar are not grouped into bundles consisting of up to 30 axons, the mass of nerve fibres present in the graft makes it hardly possible to distinguish these specimens from normal corneas. Only examination of individual axons, by reason of their complex and bizarre course, shows the true state of affairs (Figs 14 and 15). The majority of nerve fibres appear to have matured.

Two hundred and twenty days after Grafting.—Many axons are seen in both the substantia propria and the epithelium. It is again difficult to distinguish these specimens from normal corneas except by examination of individual axons under high magnification. There are still immature axons present (Figs 16 and 17). In the region of the incision, more axons are now seen to have penetrated into the cornea, some in the form of bundles containing ten to fifteen nerve fibres.

(d) REACTION OF THE CORNEAL CORPUSCLES

Two days after Grafting.—Many corneal corpuscles have undergone some change which makes them visible when the cornea is stained or impregnated by methylene blue or silver, using the procedures that are optimal for revealing nerve fibres. (In the normal cornea, under these conditions, they remain invisible.). They have assumed a stellate form, and their processes have multiplied and become thicker, smoother and longer than normal (Fig. 9). These changes are most marked in the region of the scar, where every visible corneal corpuscle seems to have been affected in this manner. Towards the centre of the graft, whilst the majority of corpuscles are rendered visible by techniques optimal for nerve fibres, the morphological changes in many are of less degree than in the region of the scar and the shape of the cells resembles more nearly those of normal corneal corpuscles. In thin silver preparations it is on occasion almost impossible to distinguish processes of altered corneal corpuscles from normal nerve fibres. However, in thicker silver sections and methylene-blue stained specimens they can be traced from origin to destination, and may thus easily be distinguished from nerve fibres (which at this stage all show clear signs of degeneration).

Three weeks after Grafting.—The changes are similar to those seen two days after grafting.

Seven weeks after Grafting.—The corneal corpuscles have returned to their normal state except in the region of the scar. They are now only rendered visible in large numbers by unspecific neurohistological techniques (that is by methods which stain other tissue elements as well as nerve fibres), and under such conditions are seen morphologically to resemble normal corpuscles. In the region of the scar the cell processes lie parallel to the incision, and the cells and their numerous processes are still more easily stained or impregnated than usual.

Twelve, Eighteen, Twenty-four, and Thirty-one weeks after Grafting.—No further change in the state of the corneal corpuscles has occurred.

(e) REACTION OF THE SCHWANN CELLS

Two days after Grafting.—The Schwann cells are also rendered visible by techniques optimal for the demonstration of nerve fibres. They now appear as a series of solid, interconnected strands, containing elongated nuclei and beaded intra-cytoplasmic threads. Whereas in the normal state it is only possible to render visible the beaded intra-cytoplasmic threads by overstaining or impregnating, and no other portion of the cytoplasm, after denervation all the cytoplasmic content of the cells becomes visible.

Three weeks after Grafting.—The Schwann cells react in the same way as they do two days after the operation. In a few cases some of the cell columns are seen to be associated with regenerating nerve fibres, but this does not alter their staining or impregnation reaction (Figs 7 and 8).

Seven weeks after Grafting.—No significant change can be observed.

Twelve weeks after Grafting.—The Schwann-cell pathways associated with mature regenerated nerve fibres are now less readily stained or impregnated. The Schwann-cell pathways not associated with nerve fibres still show no significant change.

Sixteen weeks after Grafting.—It is now difficult to render visible any Schwann-cell pathways unless unspecific neurohistological methods are used. No Schwann-cell pathways not associated with nerve fibres can be seen, but throughout the graft it is occasionally possible to find short elongated columns of cells which are not seen in the normal cornea or in grafts before this length of time has elapsed since the operation.

Discussion.—It is clear from these observations that, after being interrupted, corneal nerve fibres undergo degenerative changes quite similar temporally and morphologically to those of unmyelinated nerve fibres in skin from the rabbit's ear (Weddell and Glees, 1941). It is possible that previous authors have mistaken the processes of the altered corneal corpuscles for nerve fibres, which would make it appear that the process of nerve degeneration in the cornea is a slow one. The rapidity with which regenerating nerve fibres enter the graft may also have caused confusion, particularly if small grafts were used or the cornea was not completely incised. A study of the reactions of corneal nerve fibres after keratotomy (*vide infra*) makes it clear that an uncomplicated study of nerve degeneration in the cornea is not possible unless a relatively large and *complete* graft is made.

The rate of regeneration of nerve fibres in the rabbit's cornea is comparable with that seen in the skin from the rabbit's ear (Weddell, 1942). There are, however, minor differences to be noted in the behaviour of the nerve fibres. In general, regenerating nerve fibres entering and ramifying in the cornea do so without necessarily becoming related to Schwann-cell pathways, a condition which does not occur in the skin. Moreover, regenerating corneal nerve fibres seldom terminate in growth cones as they do in the skin.

The changes in the Schwann cells after denervation resemble those seen in the skin, and it is interesting to note that, in Schwann-cell pathways which have been completely denervated, the cytoplasm of the cells contains the beaded threads which have in the past been confused with nerve fibres (Weddell and Zander, 1950). Indeed, it is probable that the presence of these threads led to the notion that regenerating nerve fibres advance within the cytoplasm of the Schwann cell, when in fact they run along their surface (Holmes and Young, 1942). It cannot at present be explained why the cytoplasm of Schwann cells as a whole becomes more easily demonstrated after denervation. Observations on autografts, like those on the normal cornea, have failed to demonstrate the presence of epineurial sheaths or endoneurial tubes.

The most striking change to follow denervation is seen in the corneal corpuscles; this has apparently not been noted previously. In view of the fact that the reaction of the corpuscles is both more severe and more permanent in the neighbourhood of the scar, and less severe following destruction of the Gasserian ganglion, it seems probable that it is in part related to inflammatory processes consequent on the operation.

Finally, the recovery of sensibility in autografts parallels the course of regeneration and is strikingly similar to that obtained in the skin of the rabbit's ear; the most recently regenerated nerve fibres apparently not conveying effective impulses when stimulated.

Maturation of some degree is a pre-requisite for this function. It is quite clear that both the regeneration of nerve fibres and sensory recovery take place equally well in transparent and opaque transplants.

(2) KERATOTOMY.

Few workers have reported observations on the neurohistological changes after keratotomy. However, the operation has been performed, in connexion with other studies, and much work has been done on the ensuing sensory changes (Schroeder, 1923).

In 1878 Tizzoni examined corneas from rabbits 4½ months after keratotomy, using a gold-chloride impregnation method. He gives a brief description of the process of degeneration and regeneration of nerve fibres, and remarks that degeneration is slow in the cornea in view of the presence of degenerating fibres at this long period following operation. The process of regeneration, he says, takes the form of a replacement of the old nerve fibres by new nerve fibres, the growing ends of which are not surmounted by growth cones. Schultz (1881), using frogs and a gold-chloride technique, states that 3 weeks after keratotomy he was unable to determine exactly what was happening, for both degeneration and regeneration processes appeared to be taking place simultaneously. Ranvier (1881) performed keratotomies in rabbits, only extending as deep as Descemet's membrane, and impregnated them 7 days later with gold-chloride. He states that after this period the sector of the cornea related to the lesion shows a complete absence of nerve fibres in the epithelium, and is sharply distinguished from the surrounding cornea in this respect. However, the nerve fibres in the "plexus fundamentalis" of the substantia propria are all intact. The most recent publication on the subject is by Jent (1948), who used rabbits, staining the corneal nerve fibres with methylene blue. He performed his keratotomies in the same way as Ranvier; that is, extending only as far as Descemet's membrane but completely encircling the cornea 1 mm. from the limbus. He correlated his histological observations with sensory tests, and observed that 24 to 48 hours after operation the nerve fibres did not stain as well as normally. This he attributed to degenerative changes. He believed degeneration to be complete after 5 days, for at later periods he could no longer demonstrate nerve fibres with methylene blue; moreover, he could obtain no blink reflex by stimulation of any part of the cornea. He could elicit a feeble, sluggish corneal reflex 28 days after operation and observed that nerve fibres could once more be seen after staining with methylene blue, thus indicating that regeneration was taking place.

It is thus not clear from the literature how much of the cornea is denervated as the result of keratotomy; nor is it clear how, or from where, the denervated portion is re-innervated. In view of this, and

in order to determine the complete area over which a single corneal nerve fibre is distributed—a fact which cannot readily be determined in the normal cornea—a series of keratotomies was carried out as detailed below.

Operation.—Only rabbits were used in these experiments. Thirty-eight keratotomies passing completely through the cornea were successfully performed, after eserization to obtain a constricted pupil. The length of the incisions varied from $\frac{1}{4}$ to $\frac{1}{2}$ of the circumference of the cornea, and they were placed about 1 mm. on the corneal side of the limbus. Any irreducible iris prolapse was excised. The incision was closed with one or two No. 1 nylon sutures after the operation. The corneal sutures were removed 3 weeks after incision in those animals which were not killed before this time. In some animals the lids were sutured together for 3 weeks, after which both the tarsal and corneal sutures were removed. The operated eyes were examined at intervals between 12 hours and 220 days after incision. Nineteen specimens were stained with methylene blue and examined as whole preparations; nineteen were sectioned and examined as a series of silver-impregnated preparations of varying thickness.

Observations.—Sensory tests and histological investigations were carried out at varying periods after operation.

(a) SENSORY

By using a No. 1 nylon suture and cotton-wool in the manner described in connexion with corneal autografts, the relation between the ability to arouse a blink reflex from the cornea and the time since keratotomy was examined. The results are illustrated in Diagram 1 (opposite).

Keratotomy results immediately in an area of sensory loss in which the stimulus employed does not arouse a blink reflex. This area does not take the form of a sector of the cornea subtending the incision, but is considerably less; on the other hand, the area of diminished sensibility is greater. The area of apparently complete loss, however, diminishes rapidly during the first 7 days after the operation, after which the changes are much slower. On the other hand, the area of diminished sensibility remains about the same until some 3 weeks after the operation, when it begins to diminish. By about the 90th day, and subsequently, sensory recovery (as judged by the tests employed) is complete. It is of some interest that about 28 days after operation the region of the scar is abnormally sensitive so that the lightest stimulus with cotton-wool arouses a vigorous blink reflex. As time goes on, this abnormal sensitivity recedes and by the 50th day it has disappeared.

(b) NEUROHISTOLOGICAL

Twelve hours after Operation.—In the area from which no blink reflex can be aroused, all the nerve fibres, particularly those in the epithelium, show signs of degenerative changes (Fig. 18). The changes seen are quite similar to those described 12 hours after nerve section among non-myelinated fibres in the skin from the rabbit's ear illustrated

DIAGRAM 1.—Results of sensory tests at intervals after operation.

Black area	complete sensory loss.
Hatched area	partial sensory loss.
Crosses	hypersensitivity.
Arrows	extent of incision.

by Weddell and Glees (1941). There are also some signs of early degenerative changes among nerve fibres beyond the sector subtending the incision.

Two days after Operation.—The condition in various parts of the cornea is illustrated in Diagram 2. There are apparently no normal nerve fibres in the region of the cornea more than halfway from the scar towards the centre of the cornea. There are, however, degenerating fibres to be seen more than halfway from the centre of the cornea to the limbus opposite to the site of the operation. The degenerative

DIAGRAM 2.—Zone of (a) epithelium (b) substantia propria over which corneal nerve fibres are affected 2 days after operation.

Black area	degenerating nerve fibres.
Hatched area	both degenerating and normal nerve fibres.
Unshaded area	occasional degenerating nerve fibres.
Arrows	extent of incision.

FIG. 18.

FIG. 19.

FIG. 20.

FIG. 21.

FIG. 22.

FIG. 23.

FIG. 24.

FIG. 25.

FIG. 26.

FIG. 27.

Figs 18–27.—Condition of nerve fibres in affected zones of rabbit corneas at various periods after keratotomy.

FIG. 18.—Methylene-blue stained preparation 12 hours after operation. It shows early degenerative changes affecting nerve fibres in the epithelium in an area of complete sensory loss. (x 220)

FIG. 19.—Silver-impregnated preparation 2 days after operation. It shows a bundle of degenerating nerve fibres in the substantia propria in the area of complete sensory loss. (x 560)

FIG. 20.—Silver-impregnated preparation 2 days after operation. It shows a degenerating axon in the substantia propria in the area of complete sensory loss. (x 1050)

FIG. 21.—Methylene-blue stained preparation 5 days after operation. It shows a degenerating nerve fibre lying beside a fine normal loaded nerve fibre in the substantia propria in the area of partial sensory loss. (x 420)

FIG. 22.—Silver-impregnated preparation 5 days after operation. It shows a small bundle of degenerating nerve fibres in the substantia propria in the area of complete sensory loss. (x 420)

FIG. 23.—Silver-impregnated preparation 7 days after operation. It shows a small degenerating nerve bundle and a regenerating nerve fibre unrelated to it in the superficial layers of the substantia propria. The fibre is derived from a normal axon in the periphery of the affected area, in which sensibility is "normal". (x 560)

FIG. 24.—Methylene-blue stained preparation 14 days after operation. It shows a single regenerating axon surmounted by a growth cone; it lies in the substantia propria on the conjunctival side of the incision. (x 220)

FIG. 25.—Methylene-blue stained preparation 28 days after operation. It shows regenerating axons in the substantia propria arising from a severed nerve bundle sweeping round the margin of the scar to reach the denervated zone. (x 250)

FIG. 26.—Methylene-blue stained preparation 28 days after operation. It shows an immature regenerating axon in the substantia propria in the area of complete sensory loss. (x 560)

FIG. 27.—Methylene-blue stained preparation 40 days after operation. It shows a normal axon and fragments of recently degenerated axons in the epithelium. This region was originally insensitive. (x 465)

changes are more advanced in axons in the epithelium than in those in the substantia propria (Figs 19 and 20).

Five, Seven, and Nine days after Operation.—A few apparently normal nerve fibres can be seen extending as far as the centre of the area previously occupied solely by nerve fibres showing early signs of degeneration. They enter the otherwise denervated area from the normal region of the cornea opposite to the keratotomy incision, and are surrounded only by degenerated fibres. A few regenerating axons can be seen advancing from normal nerve fibres in the periphery of the affected area (Figs 21, 22, and 23).

Fourteen days after Operation.—In cases where healing has proceeded in an uncomplicated way capillaries have invaded the scar and crossed it up to 1 mm. towards the centre of the cornea. In this position many polymorphonuclear leucocytes can be seen. Single regenerating nerve fibres derived from nerve bundles proximal to the incision have crossed the scar and are just entering the denervated area. Other regenerating nerve fibres from the same source, forming complicated loops and spirals, have not crossed the scar; the apex of some of them terminates in growth cones (Fig. 24).

At the lateral margins of the incision many nerve fibres derived from nerve bundles proximal to the incision are streaming towards the denervated area. They are pursuing their own way, unrelated in most cases to pre-existing Schwann-cell paths. Some of these fibres show swellings along their course, suggestive of recent obstruction; some twist and turn around cells; others terminate in growth cones. Unlike nerve fibres in the normal cornea, these single fibres pursue a course parallel to one another.

In the area of diminished sensibility the condition remains unchanged.

FIG. 28.

FIG. 29.

FIG. 30.

FIG. 31.

FIG. 32.

FIG. 33.

FIGS 28-33.—Affected zones in rabbit corneas at various intervals after keratotomy.

FIG. 28.—Silver-impregnated preparation from region of scar 50 days after operation. It shows single axons in the substantia propria passing towards the scar and then turning away again. Empty Schwann-cell pathways are seen close to these axons; they are on the left of the picture. (x 235)

FIG. 29.—Methylene-blue stained preparation 50 days after operation. It shows immature regenerating nerve fibres in the basal layer of epithelium in the area of complete sensory loss. (x 425)

FIG. 30.—Silver-impregnated preparation 110 days after operation. It shows mature regenerated nerve fibres in the substantia propria in a zone of normal sensibility. This region was previously at the centre of the zone of complete sensory loss. (x 164)

FIG. 31.—Methylene-blue stained preparation 110 days after operation. It shows immature regenerating axons in the substantia propria sweeping around the lateral margin of the scar. (x 215)

FIG. 32.—Silver-impregnated preparation 220 days after operation. It shows bundles of mature axons in the substantia propria in a zone of "normal" sensibility. This zone was originally completely insensitive. (x 510)

FIG. 33.—Silver-impregnated preparation 220 days after operation. It shows a bundle of immature axons coursing along a Schwann-cell pathway in the substantia propria, lying beside a mature axon which in unrelated to Schwann cells. This specimen came from a zone of "normal" sensibility which was originally completely insensitive. (x 1050)

Twenty-eight days after Operation.—In the region of the incision more nerve fibres have penetrated the scar, and many more fibres have entered the denervated area after passing around its lateral margins (Fig. 25).

In the area of diminished sensibility regenerating nerve fibres derived from normal and apparently pre-existing nerve bundles are advancing towards the denervated area from a position opposite to that of the incision (Fig. 26). About this time it is possible to trace almost the complete course of single nerve fibres entering the cornea lateral to the keratotomy and terminating in the region of apparently complete sensory loss. Diagrams 3(a) and 3(b) show the course of two such fibres which could be so analysed.

DIAGRAM 3(a) and (b).—Camera lucida drawings showing approximate distribution of single axons ending in substantia propria of cornea, made 28 and 100 days respectively after keratotomy.

Position and extent of incision indicated by arc.
Solid lines continuous axon.
Interrupted lines beaded terminals.

Forty days after Operation.—In fully-stained preparations at this time counts were made of the number of nerve fibres in larger nerve bundles passing from a position near the scar towards the opposite limbus through the centre of the cornea. The counts were made in two positions in each bundle. One position was approximately one-third of the way from the scar towards the centre of the cornea, and the other was at the centre of the cornea. Similar counts were made 80, 100, 110, and 115 days respectively after keratotomy. In each case the number of axons at the centre of the cornea is greater than that towards the scar. (See Table II).

At this time single regenerating nerve fibres are seen in the epithelium in the region from which it is still impossible to arouse a blink reflex by the methods employed (Fig. 27).

TABLE II
NUMBER OF AXONS IN NERVE BUNDLES

Days after operation	Bundle	Number of axons close to scar	Number of axons at centre of cornea
40	1	4	12
	2	6	15
80	3	11	17
	4	7	14
100	5	6	15
	6	8	17
110	7	9	13
	8	14	18
115	9	8	14
	10	5	11

Fifty days after Operation.—It is now apparent that the pattern assumed by the regenerating nerve fibres in the substantia propria of the previously insensitive area is superficially very similar to that seen in the normal cornea. The nerve fibres entering the previously insensitive area can be traced to their origin and are found to derive from:

- (i) nerve trunks severed at the time of the keratotomy, piercing the scar;
- (ii) nerve trunks severed at the time of the keratotomy, sweeping around the lateral margins of the scar (Fig. 28);
- (iii) normal nerve trunks opposite the incision.

It has not proved possible to determine quantitatively the number of fibres derived from each source, for two reasons. Firstly, the complex pathways pursued by the regenerating fibres make the task a very difficult one. Secondly, from the 28th to the 120th day, in all regions where regenerating nerve fibres are seen, a few nerve fibres in various stages of *degeneration* can also be recognized (Fig. 27).

The pattern assumed by the nerve fibres in the *epithelium* is different from that which obtains normally. Single axons penetrate Bowman's membrane and, instead of breaking up immediately into a large number of branches, at once proceed to ramify among the epithelial cells, giving rise at intervals to daughter axons which terminate among the cells (Fig. 29).

Eighty, One hundred, One hundred and ten, and One hundred and twenty days after Operation.—Re-innervation gradually becomes more complete during this time. Under low powers of the microscope (§ objective) it is difficult to differentiate the

affected region from the surrounding normal cornea. Under higher powers, however, regenerating nerve fibres can still be recognized, but they gradually become fewer in number. In addition, the nerve fibres in the affected regions are seen to take more complex pathways, and the pattern assumed by the epithelial nerve fibres is still abnormal although the number of fibres seen in any given area approaches normal (Figs 30 and 31). It is remarkable that a number of nerve fibres in all states of degeneration is still seen throughout the affected area.

Two hundred and twenty days after Operation.—More nerve fibres have penetrated the scar, and in one specimen only ten single nerve fibres were seen sweeping round its lateral margin. More nerve fibres penetrating the scar are grouped into bundles. The appearance and number of nerve fibres reaching the cornea through the scar are, in fact, almost comparable with that seen in an unoperated sector of the eye (Fig. 32). Owing to the normal variation in numbers, however, counts of nerve fibres were not made, for they would have been misleading. Despite the superficial appearance of normality at low magnification, a number of immature nerve fibres can still be seen under higher magnification, although no degenerating nerve fibres are evident (Fig. 33).

Discussion.—It is now clear that the changes following keratotomy are complex and cannot be clearly understood unless specimens are examined at successive stages following operation.

Fourteen hours after keratotomy early signs of nerve degeneration are apparent among all the nerve fibres in the area from which no blink reflex can be aroused. This is in accordance with the observations made concerning nerve degeneration after autoplasmic grafting, but the changes were seen at an earlier stage than in any specimen examined in that series.

The fact that *no* normal nerve fibres were seen among the degenerating fibres was surprising, for a few degenerating nerve fibres were seen among normal nerve fibres at a considerable distance from the lesion towards the opposite limbus. The appearance of an occasional normal nerve fibre 5 days and subsequently after operation in the insensitive area was also surprising. These findings are most easily explained on the basis that the few normal nerve fibres present amongst the many degenerating fibres had undergone a reversible degenerative change of a non-Wallerian type (Denny-Brown and Brenner, 1944a and b).

It is unfortunate that, owing to the mass of degenerating nerve fragments present, the few normal axons cannot be traced throughout their course until some 2 to 4 weeks after the lesion, for one object of these experiments is thereby defeated. It was hoped as the result of keratotomy (i.e. partial denervation) to obtain an accurate measure of the area over which single corneal nerve fibres are distributed; but, by the time their distribution can be mapped out, nerve fibres from surrounding normal nerves are invading the denervated area. The invading fibres, it is true, can be classed morphologically as regenerating fibres, whereas those present in the insensitive area appear quite normal. Thus the picture given of the distribution of a single corneal nerve fibre is probably very similar to

that which it occupies in the normal cornea, although it may be somewhat exaggerated by extension.

The denervated area is re-innervated by fibres from three sources:

- (i) nerve fibres arising from severed nerve bundles proximal to the incision, which pierce the scar;
- (ii) nerve fibres from the same source which pass round the lateral margins of the scar;
- (iii) nerve fibres which invade the denervated area and which are derived from the surrounding normal nerve bundles.

Unfortunately, the pattern formed by the nerve fibres entering the denervated area is so complex that it is not possible to determine the relative proportion of nerve fibres derived from each source at any particular stage after the operation. Nevertheless, it is clear from Table II that fewer nerve fibres enter the cornea by piercing the scar, until a very late stage after operation, than normally enter from the periphery. Such an assessment would, in any case, be of doubtful value, for the reparative process is a long and continuous one, lasting more than 120 days after the operation. Moreover, throughout this time nerve fibres in early stages of degeneration can be seen, a finding in marked contrast to that seen after autoplasmic grafting.

It is now clear why workers who examined corneas after keratotomy believed that nerve degeneration was such a slow process in this tissue.

The abnormal sensitivity of the scar about 28 days after operation is explained on the basis that at this time the number of axons surmounted by growth cones at this site reaches a maximum (Weddell, Sinclair, and Feindel, 1948).

The return of sensation as judged by the presence of a blink reflex shows that the last region to recover is that closest to the scar. These observations are in accordance with those reported by Schroeder (1923) in a careful clinical study after keratotomy. They suggest that the invading nerve fibres play a not unimportant role in the re-innervation process. However, in the specimens taken after 220 days the number of nerve fibres entering the originally denervated area by piercing the scar appears to be approaching that which would have entered the cornea in such a position under normal conditions. The nerve fibres have re-grouped themselves into bundles, and in one case only ten nerve fibres were seen sweeping round one lateral margin of the scar to reach the originally denervated area. Moreover, at this stage no nerve fibres in early stages of degeneration could be seen.

These observations are explained on the basis that the denervated area is at first re-innervated by invasion from surrounding normal nerves which advance to meet the nerve fibres regenerating through and around the scar. As those regenerating through the scar become more numerous, so those invading the territory from other regions degenerate, the process continuing until stabilization is reached.

The stage at which stabilization takes place clearly depends upon time, and presumably upon the nature of the scar. It is possible that in some cases the affected area may even become fully re-innervated by nerve fibres from severed nerve bundles piercing the scar. In the two cases examined this had all but occurred at the end of 220 days; however, despite the fact that no degenerating nerve fibres were seen, it may be presumed that stabilization had not been attained, for immature nerve fibres could still be seen. It is, therefore, possible that complete restitution might have occurred at a still later date.

Regenerating nerve fibres apparently grow into the denervated zone of the cornea between the layers of the stroma as easily as they do along Schwann-cell pathways. This is in marked contrast to what takes place in the skin. However, the mere presence of the requisite quota of nerve fibres in the affected zone does not constitute the end of the process. There are apparently some factors which influence the process sufficiently to determine that, by degrees, the original pattern of innervation is restored at the expense of the fibres which have taken new pathways to arrive at their destination.

The processes of nerve repair after keratotomy in mammals are of interest in that they are in broad agreement with the hypotheses concerning nerve patterns and the mechanics of nerve growth expressed by Weiss (1941) as the result of work on amphibians and the examination of the growth of neuroblasts in tissue culture.

Finally, keratotomy demonstrates clearly how labile the peripheral nervous system is, and illustrates the danger of assuming that partial denervation experiments will necessarily provide a picture which represents the pattern normally assumed by the unoperated nerve fibres.

(3) SECTION AND AVULSION OF THE INFRA-ORBITAL NERVE.

As Vonderahe (1928) suggested that the infra-orbital nerve supplies the lower half of the cornea in man, it was thought advisable to determine whether this was also the case in the rabbit. Although examination of normal material had excluded the possibility that many nerve fibres reach the cornea from the infra-orbital nerve, the possibility that some may do so cannot be excluded by this method.

Operation.—The infra-orbital nerve was approached by the method described by Krause (1884). After identification, it was divided and then avulsed from the infra-orbital canal on the left side in four rabbits. Periods of 7 days (two rabbits) and 20 days (two rabbits) respectively were left for nerve degeneration to occur. The corneas from two of the rabbits were examined after methylene-blue staining, and from two after silver impregnation.

Observations.—No differences were found in the blink reflex on stimulation of the cornea with a wisp of cotton-wool between the

normal and operated side or between the upper and lower half, periphery, and centre of the cornea on the operated side, although there was apparently complete anaesthesia of the skin supplied by the infra-orbital nerve. No "trophic" changes were observed in the corneas on the operated side and no changes in the conjunctival vessels. The corneas were well stained or impregnated throughout their extent. Careful examination failed to show any clear-cut differences in the appearance of the nerve fibres between the normal and operated sides.

Discussion.—Vonderahe has suggested that in man the infra-orbital nerve (and thus the second division of the fifth nerve) supplies the cornea, for he found in a case of traumatic injury involving the second division of the fifth nerve that sensibility in the lower half of the cornea of the affected side was impaired. In addition, Boucheron (1890, 1891) suggests that the periphery of the cornea may be supplied by nerve fibres which reach it from other than the ciliary nerves. This implies that the infra-orbital nerve may be implicated.

Vonderahe's observations and Boucheron's statements have given rise to the suggestion that in testing for corneal sensitivity in the clinic reactions from the upper and lower halves of the cornea respectively should be compared.

In the rabbit it seems improbable that the cornea receives sensory nerves from the infra-orbital nerve. In view of the fact that Vonderahe's case was not an uncomplicated section of the infra-orbital nerve, it is clear that observations of this kind in man should be re-examined, for it is possible that fibres from the maxillary division of the fifth nerve may reach the cornea by routes other than by way of the infra-orbital nerve, as suggested by Boucheron (1890, 1891).

(4) SECTION OF THE CILIARY NERVES.

The purpose of these experiments was to see whether any nerve fibres reach the cornea by routes other than the ciliary nerves. As already noted, Boucheron (1890, 1891) believed that the periphery of the cornea is innervated by nerve fibres which do not travel with the ciliary nerves, and Vonderahe (1928) has suggested that in man the lower half of the cornea is innervated by the fibres travelling in the infra-orbital nerve.

Operation.—The corneas from three rabbits were examined on the side on which all the ciliary nerves, together with the optic nerve, had been severed, close to their entry into the eyeball, 3 days previously. Care was taken to cause as little damage as possible to the ciliary vessels.

Observations.—Unfortunately, the operated eye in every case became so rapidly and so seriously affected that it was not considered useful to keep any animal longer than 3 days after operation. On the fourth day after the section of the nerves, the cornea in almost

every case becomes opaque and is grossly distorted; in addition, some degree of keratitis is present (a condition recalling early phthisisbulbi in man). In the corneas examined 3 days after operation only degenerating nerve fibres were seen. No normal nerve fibres whatever were encountered. The corneal corpuscles are affected in the same way as after autoplasmic grafting.

Discussion.—These experiments are inconclusive in view of the observations made after keratotomy, for there may have been a few nerve fibres in these corneas undergoing reversible degenerative changes. Nevertheless, the experiments do indicate that the majority of nerve fibres in the rabbit reach the cornea via the ciliary nerves: thus confirming what appears to be the case in normal material (Zander and Weddell, 1951).

(5) EXCISION OF THE SUPERIOR CERVICAL SYMPATHETIC GANGLION.

Although Boeke (1935) stated that few, if any, sympathetic nerve fibres supply the cornea, Rodger (1950) mentioned their presence. In view of this divergence of opinion, and of the fact that the method of direct observation in normal material cannot solve the problem, it was decided to determine experimentally whether post-ganglionic sympathetic fibres leave the superior cervical ganglion to supply the cornea.

Operation.—The superior cervical sympathetic ganglion was excised on the left side in each of five rabbits. The operation was carried out in accordance with the instructions of Krause (1884) and resulted in a constriction of the pupil, dilation of the conjunctival blood vessels, and an increase in temperature and flushing of the ear on the affected side. Periods of 7, 14, 21, and 50 days were allowed for degeneration to occur. The corneas from three rabbits were examined after methylene-blue staining, and from two after silver impregnation.

Observations.—After operation no differences in the corneal sensitivity of the two sides could be detected by the use of a wisp of cotton-wool to arouse a blink reflex. There were no obvious trophic changes or differences in transparency between the two sides. The staining and impregnation of the corneas was complete and uniform. No obvious differences in the nerve supply between the normal and operated corneas was observed. A count of the nerve fibres in the nerve bundles in the periphery of the corneas was carried out in the methylene-blue stained specimens of one of the animals, and no significant differences were noted between the normal and operated sides (see Table in Zander and Weddell, 1951, which indicates the normal variation in the numbers to be expected).

Discussion.—These observations suggest that few post-ganglionic nerve fibres, if any, pass directly from the superior cervical ganglion to the cornea. It is possible that fibres reach the cornea via ganglion-cell relays situated between the superior cervical ganglion and the cornea, but it appears unlikely, from these observations, that

FIG. 34.

FIG. 35.

FIG. 36.

FIG. 37.

FIG. 38.

FIG. 39.

FIG. 40.

FIGS 34-40.—Silver-impregnated preparations from normal and affected corneas 5 days and 21 days after destruction of Gasserian ganglion.

FIG. 34.—Completely denervated Schwann-cell pathway in substantia propria of monkey's cornea 5 days after operation. (x 775)

FIG. 35.—Normal nerve bundle in substantia propria of monkey's cornea on unaffected side. The specimens from which Figs 34 and 35 were taken were impregnated together. (x 775)

FIG. 36.—Empty Schwann-cell pathways in substantia propria of monkey's cornea 21 days after operation. (x 690)

FIG. 37.—Fragments of degenerating axon in substantia propria of rabbit's cornea 21 days after operation. (x 775)

FIG. 38.—Fine regenerating axon in substantia propria of monkey's cornea 21 days after operation. (x 605)

FIG. 39.—Fine regenerating axon surmounted by growth cone in substantia propria of monkey's cornea 21 days after operation. (x 605)

FIG. 40.—More mature regenerating axon in substantia propria of rabbit's cornea 21 days after operation. The axon apparently found the cell, whose nucleus is seen, obstructing its course, for it has by-passed it. This kind of picture is never seen in the normal cornea. (x 775)

such fibres, should they exist in any number, convey sensory impulses. The experiments of Asher and Chervet (1934) suggest that the cornea is more vulnerable to the effects of ultra-violet light after the removal of the superior cervical ganglion. The explanation may reside in the removal of pre-ganglionic nerve fibres the post-ganglionic fibres of which are destined for the cornea, or in the effect on the blood vessels in the orbit. That the latter rather than the former is correct is suggested by the work of Boeke, who found few, if any, sympathetic nerve fibres supplying the cornea.

In man, removal of the superior cervical ganglion causes no changes in corneal sensibility; this is in conformity with the observations in the rabbit.

(6) DESTRUCTION OF THE GASSERIAN GANGLION.

The purpose of these experiments was to determine whether any nerve fibres not derived from the fifth cranial nerve normally enter the cornea. They were intended to be complementary to the experiments which involved removal of the superior cervical ganglion of the sympathetic chain.

Operation.—Seven rabbits and two macaque monkeys were used. In the case of the rabbits, unilateral destruction of the Gasserian ganglion proved an extremely difficult procedure. In three rabbits the method used followed that described by Magendie (1824) and Ranvier (1881), but in the only rabbit to survive the operation the three divisions of the fifth nerve were not completely divided and a portion of the Gasserian ganglion appeared to be intact. In the remaining rabbits the left Gasserian ganglion was destroyed under vision after removal of a portion of the temporal lobe of the brain. Two animals survived the operation. In the case of the monkeys the operation was straightforward, following the lines adopted by neurosurgeons in the human subject. The left Gasserian ganglia were completely destroyed by coagulation after section of their central and peripheral roots. After operation, the lids of the affected eye in the rabbits and in the monkey which was allowed to survive for 3 weeks were sutured together. The other monkey was killed 5 days after operation. Both corneas in each animal were impregnated with silver and sections of varying thickness examined.

Observations.—Sensory tests and histological investigations were carried out with the following results.

(a) SENSORY

In every case the cornea was completely insensitive, no blink reflex being aroused by the application of cotton-wool or a No. 1 nylon suture.

(b) HISTOLOGICAL

The cornea from the monkey killed 5 days after operation showed no macroscopical evidence of keratitis, and few polymorphonuclear cells were seen; in addition, the corneal corpuscles appeared to be very little affected. No normal nerve fibres were seen, but the Schwann-cell pathways were clearly outlined and seen to have fragments of degenerating axons lying along their surface (Fig. 34). In the conjunctiva of the operated eye there were blood vessels with nerve fibres beside them at the margin of the cornea. The unoperated eye, which was also prepared for histological examination, appeared to be normally innervated (Fig. 35). The corneas from the animals killed 3 weeks after operation showed some macroscopic evidence of keratitis and the corneas were infiltrated by a number of polymorphonuclear cells. The corneal corpuscles showed a reaction similar to that seen 3 weeks after grafting. Numerous Schwann-cell pathways, having only an occasional axis-cylinder fragment lying on their surface, were seen (Figs 36 and 37). However, there were throughout the cornea a few isolated regenerating nerve fibres (Figs 38, 39, and 40). The ultimate source of these fibres was not determined, but they entered singly from the conjunctiva. The eyes from the unoperated side were also prepared for microscopical examination and appeared to be normally innervated.

Discussion.—These experiments show clearly that normally few nerve fibres reach the cornea other than by way of the fifth cranial nerve, and thus we are able to confirm Boeke's statement that if the cornea does contain any sympathetic nerve fibres at all, these must be very few. They also show once again how easily nerve fibres grow into the cornea, the source of the regenerating nerve fibres which were seen 3 weeks after operation almost certainly being from the vascular nerves in the conjunctiva by a process of extension. This observation suggests that great care needs to be exercised when interpreting results following experimental nerve section. It is clearly not safe to conclude that, following removal of one set of nerve fibres, the remaining axons will remain morphologically quiescent. Of particular interest in connexion with these experiments are those of Clark (1942, 1943) who showed that the work of Tello (1911) concerning the regeneration of nerve fibres in the central nervous system was explicable on the basis of vascular nerves invading the

region of the lesion, and that in fact no regeneration of central nervous system axons takes place after injury.

The observation that the corneal corpuscles show so few signs of reaction 5 days after destruction of the Gasserian ganglion in the monkey is interesting. It may be due to the fact that the lids were not sutured in this case, thereby preventing surface abrasion of the cornea.

Reiser (1936, 1937) stated that 2 days after destruction of the Gasserian ganglion, although degenerative changes affected all the nerve fibres of the main nerve bundles, the preterminal network showed only slight evidence of degenerative change and the terminal reticulum had not changed at all. His statement can be criticized on two accounts. Firstly, using *thin* silver sections and the Bielschowsky method, 2 days is too short a time to be certain of seeing degenerative changes in every isolated axon segment; this may account for his statement that the pre-terminal network is only slightly affected. Secondly, the so-called terminal reticulum has been shown to be an artefact (Weddell and Zander, 1951).

SUMMARY

(1) The rate and character of nerve degeneration in the rabbit's cornea are similar to that in the skin of the rabbit's ear.

(2) The rate of regeneration of nerve fibres in the cornea is comparable with that in the skin of the rabbit's ear.

(3) Regenerating nerve fibres within the cornea seldom terminate in growth cones; they apparently pursue their way as easily when unrelated to Schwann cells as when related to them.

(4) The area over which a single corneal nerve fibre is distributed is at least as large as a quadrant of the cornea.

(5) After denervation, both Schwann cells and corneal corpuscles undergo characteristic changes.

(6) After keratotomy, the denervated area is re-innervated from three sources:

- (i) regenerating nerve fibres which penetrate the scar,
- (ii) regenerating fibres which pass around the lateral margins of the scar,
- (iii) invading fibres which sprout from surrounding normal nerve fibres and invade the denervated area.

(7) From the 50th to the 120th day after keratotomy there are signs of active nerve degeneration as well as nerve regeneration.

(8) Recovery is histologically nearly complete 220 days after keratotomy. It appears that the nerve fibres which originally invade the denervated zone from normal surrounding nerves, and by sweeping around the incision, have retreated in favour of regenerated

nerve fibres originating from those severed at the time of keratotomy which traverse the scar to reach their destination.

(9) As the result of section or destruction of the following structures: the infra-orbital nerve, the superior cervical sympathetic ganglion, the ciliary nerves and the Gasserian ganglion, it is clear that the rabbit's cornea is probably supplied solely by axons from the fifth cranial nerve reaching the cornea by way of the ciliary nerves.

(10) Five days after extirpation of the Gasserian ganglion only degenerating nerve fibres can be seen throughout the cornea.

(11) Three weeks after extirpation of the Gasserian ganglion regenerating nerve fibres can be seen in the cornea, which is completely insensitive. The source of these fibres is unknown, but it is likely that they arise from vascular nerves in the conjunctiva by a process of extension.

This investigation was made possible by a grant from the Rockefeller Foundation for which we wish to express our gratitude. We should also like to thank Mr. Arthur Dent, Miss Jean Gurden, and Mr. Patrick Selwood for their skilful technical assistance. At the outset of these investigations we had the invaluable surgical assistance of Mr. J. P. F. Lloyd, F.R.C.S., to whom we offer our sincere thanks.

REFERENCES

- ASHER, L., and CHERVET, N. (1934). *Klin. Wschr.*, **13**, 1688.
 BOEKE, J. (1935). *Z. mikr.-anat. Forsch.*, **38**, 594.
 BOUCHERON, A. (1890). *C.R. Soc. Biol. Paris*, 9 sér., **2**, 71.
 ——— (1891). *Ibid.*, **3**, 59.
 CLARK, W. E. LE GROS (1942). *J. Anat., Lond.*, **77**, 20.
 ——— (1943). *Ibid.*, **77**, 251.
 DENNY-BROWN, D., and BRENNER, C. (1944a). *Arch. Neurol. Psychiat., Chicago*, **51**, 1.
 ——— (1944b). *Ibid.*, **52**, 1.
 ESCAPINI, H. (1948). *Arch. Ophthal., Chicago*, **39**, 135.
 FRANCESCHETTI, A., and BABEL, J. (1947). *Ann. Oculist., Paris*, **180**, 142.
 GLEES, P. (1946). *J. Neuropath. exp. Neurol.*, **5**, 54.
 HOLMES, W., and YOUNG, J. Z. (1942). *J. Anat., Lond.*, **77**, 63.
 JENT, M. (1945). *Helv. physiol. pharmacol. Acta*, **3**, 65.
 KRAUSE, W. (1884). "Die Anatomie des Kaninchens". 2nd ed. Engelmann, Leipzig.
 LEOZ ORTIN, G. (1915). *Arch. oftal. hisp.-amer.*, **15**, 225.
 ——— (1917). *Ibid.*, **17**, 615.
 MAGENDIE, F. (1824). *J. Physiol. exp. path., Paris*, **4**, 176.
 RANVIER, L. (1881). "Leçons d'anatomie générales faites au Collège de France par L. Ranvier; année 1878-1879". Baillière, Paris.
 REISER, K. A. (1937). *Arch. Augenheilk.*, **110**, 253.
 RODGER, F. C. (1950). *British Journal of Ophthalmology*, **34**, 107.
 SCHROEDER, E. (1923). *Graefes Arch. Ophthal.*, **111**, 17.
 SCHULTZ, F. (1881). "Experimentelle Studien über Degeneration und Regeneration der Cornealnerven". Laakman, Dorpat.
 TELLO, F. (1911). *Trab. Lab. Invest. biol. Univ. Madr.*, **9**, 123.
 TIZZONI, G. (1878). *Zbl. med. Wiss.*, **16**, 225.
 VONDERAHE, A. R. (1928). *Arch. Neurol. Psychiat., Chicago*, **20**, 836.
 WEDDELL, G. (1942). *J. Anat., Lond.*, **77**, 49.
 ——— and GLEES, P. (1941). *Ibid.*, **76**, 65.
 ——— and ZANDER, E. (1950). *Ibid.*, **84**, 168.
 ———, SINCLAIR, D. C., and FEINDEL, W. H. (1948). *J. Neurophysiol.*, **11**, 99.
 WEISS, P. (1941). *Growth*, **5**. 3rd Growth Symposium, p. 163.
 ZANDER, E., and WEDDELL, G. (1951). *J. Anat., Lond.*, **85**, 68.

CORRIGENDA

WILLIAM CHESELDEN (1688-1752)

It has just come to our notice that in Kirby's "Surgery of Cataract" (J. B. Lippincott Co., Philadelphia, 1950), p. 22, one of the greatest pioneers of English surgery is described as a "well-known charlatan". Cheselden was certainly well-known, not only to the staff of St. Thomas's Hospital, but throughout the world, and today his portrait occupies an honoured place among the great in the Royal College of Surgeons in England. Not only was he the first to practise the operation of iridotomy but his contributions to general surgery were immense and classical.

CORNEAL NERVE FIBRES

DRS ZANDER and WEDDELL regret that their paper "The Reaction of Corneal Nerve Fibres to Injury" (*Brit. J. Ophthalm.*, 35, 61) appears to suggest that Dr. F. C. Rodger had stated in his paper "The Pattern of the Corneal Innervation in Rabbits" (*Brit. J. Ophthalm.*, 34, 112) that the cornea contained sympathetic nerve fibres. Dr. Rodger described two anatomically distinct types of fibre in the rabbit cornea, and remarked on the interesting similarity of one of them to nerve fibres seen in the vicinity of blood vessels in the skin and iris, but he did not conclude that these fibres were sympathetic in origin.

BOOK REVIEW

Genetics in Ophthalmology. By ARNOLD SORSBY. 1951. Butterworth, London. Pp. 251, 233 figs (10 col.) (42s.).

In the preface the author places ophthalmological genetics in perspective by indicating that infective diseases have been replaced by cataract, glaucoma, myopia, senile macular degeneration, and a group of congenital, hereditary, and developmental defects as the main causes of blindness. None of these is explicable in terms of bacteriology and all may have a genetic basis. It has become apparent that, in medicine, the constitutional factor, as opposed to the extraneous factor, of disease has come to be the starting point for further advance.

The first section of the book proceeds from a description of the behaviour of the nucleus during division of the germ cell to build up, step by step, the theoretical knowledge which we have inherited from past workers on the modes of inheritance, the gene and chromosome types of inheritance (illustrated by human pedigrees), and clinical varieties of genetic disease. This section closes with a discussion on the prospects of the control of genetic disease, drawing from experiences with diabetes, Rh factor, acholuric jaundice, etc., a modest optimism. Experimental medical genetics is briefly touched upon, and sound advice is given regarding the responsibility of the physician in guiding patients about the possible transmission of their inherited defects.