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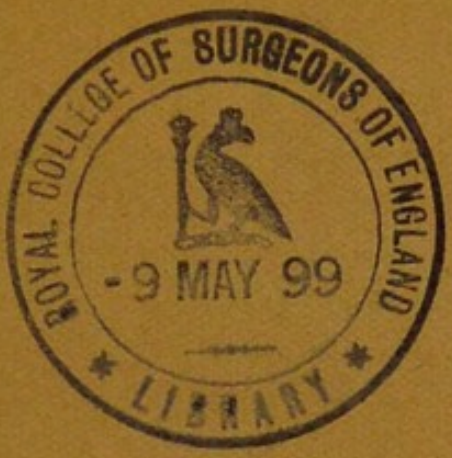

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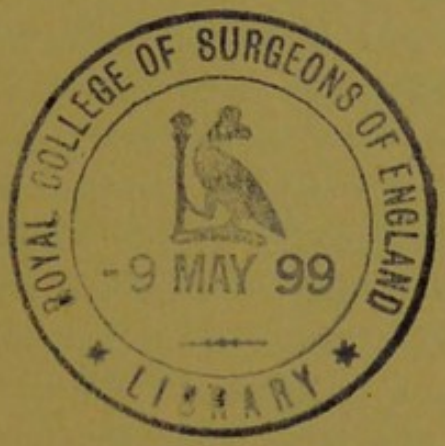

ON THE NERVE TERMINATIONS IN THE SELACHIAN CORNEA.

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Throughout the vertebrate body there is hardly an organ whose innervation has received so much attention as that of the cornea.

Time and again as new neurological methods have been discovered they have been applied to the study and re-study of the corneal nerve endings. Ever since 1866 when Cohenheim (1) published his celebrated paper on the termination of sensory nerves in the cornea, as demonstrated by him with his gold chloride method, down to the more recent researches of Dogiel (2 and 3) with methylen blue, an innumerable number of papers have appeared.

Despite the fact, however, that so much has been done and said on this subject, little is really known about these nerve terminations in the cornea of vertebrates lower than amphibians,

and it is surprising to find on what few types our knowledge rests, the frog, rabbit and human subject being the regular stand-bys. As nothing to my knowledge had been done on selachians I thought it might be worth while studying the conditions there presented. My results soon gave me reason to believe that this hope had not been misplaced and that the termination of the sensory nerves in the selachian cornea was evidently different from that of amphibians and mammals according to the classical researches of Hoyer, Arnold, Izquierdo, Klein, Kölliker, Dogiel, and so well worthy of further study. The following remarks apply to some short and very imperfect work which I have done on the subject within the last few months. The material I have used mostly was from the ordinary form of "Smooth dog fish" (Mustelus canis) so abundant here, although I have secured the corneas from the following sharks occasionally: Galeocerdo tigrinus, Carcharhinus obscurus, Sphyrna zygaena, Carcharias littoralis, for all of which the following results also hold.

The methylen blue method of staining was adopted and so far I have used it only. The particular modification of the blue method used was that recommended by Dogiel (3). Apáthy's (4) fixation also gave good results, but for thorough action Dogiel's fixation is more to be depended upon. Bethe fixation (5) has been used for sections, but on account of the trouble experienced in cutting I have had few results with it, for the cornea tissue proper so hardens in the usual processes of embedding that it is nearly impossible to cut it. At first I had trouble with my fixing fluids in that they caused too much maceration. This was stopped by adding a few drops of 1-10 per cent. solution osmic acid, which was not enough to blacken the tissues, and so render them obscure.

A few words as to the general histological structure of the cornea in Selachians.

The anterior corneal epithelium is somewhat thicker altogether than that of the cornea stroma proper, composed of large cells, having centrally placed rounded nuclei. The epithelium is on an average 12 to 18 layers of cells deep, the su-

perficial cells having the usual flattened scale-like appearance, the layer next the corneal substance proper tall cubical columnar, and the cells of the middle layers present the well known "prickle" appearance. There appears to be no membrane of Bowman or of Descemet; the posterior epithelium consists of a single layer of cells. The cornea substance proper presents the usual clear laminated appearance composed of about 12-14 sheets with corneal cells and lymph canals.

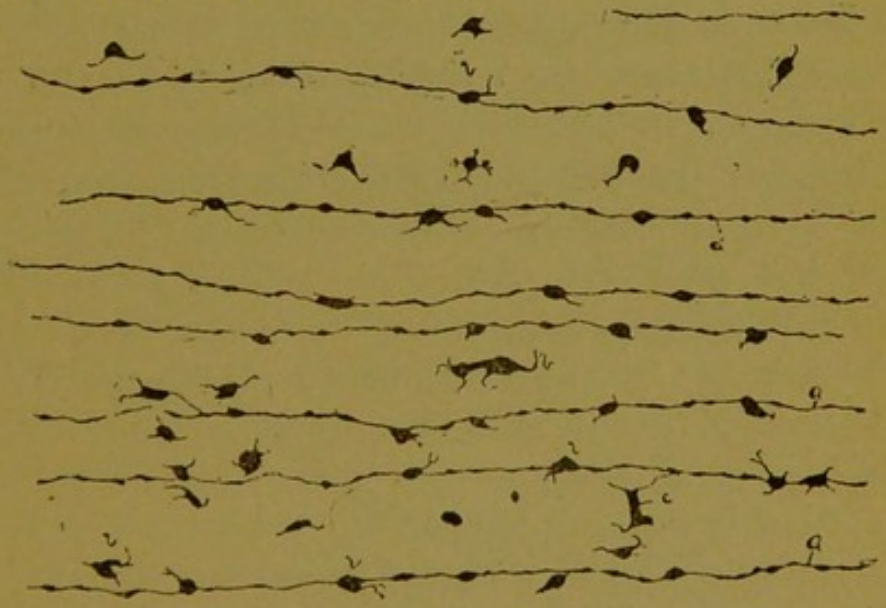

Fig. 1. Methylen blue preparation from the dog-fish cornea showing straight unbranching inter-epithelial fibers with dark bodies *b*. 8 mm. obj. comp. oc. 4, Zeiss, Camera.

Examining one of these corneas properly stained and fixed one is struck with the great number of nerves present, their relatively straight course from the border of the cornea inwards towards the center, and their unbranching course. It is surprising to find how long some of the fibers are, going apparently in some cases right across the whole cornea. Again one notices the very regular distances they keep from one another, always more or less parallel, looking under a low power of the microscope like a series of ruled lines. This condition is very different from what Klein describes in the frog where the nerves run very irregularly, crossing one another sometimes nearly at right angles. Some of these fibers give off lateral branches which cannot be followed for any distance

and soon become lost. These lateral branches do not anastomose with one another, as can be seen from the magnified camera drawing of Fig. 3, *b*. They suggest small fibrils coming off to form a network, but the closest examination does not show this to be the case, and I am pretty certain that a true inter-epithelial plexus is wanting in Selachians. The unbranched condition of these nerve fibrils is perhaps made more striking by comparing Fig. 2 with Fig. 1, which represents a similar preparation from the cornea of one of the osseus fishes (Prionotus carolinus) where the irregular joining and course of the fibers is apparent, besides in the cornea of the dog-fish the fibers are much larger and thicker. These nerves are covered with a

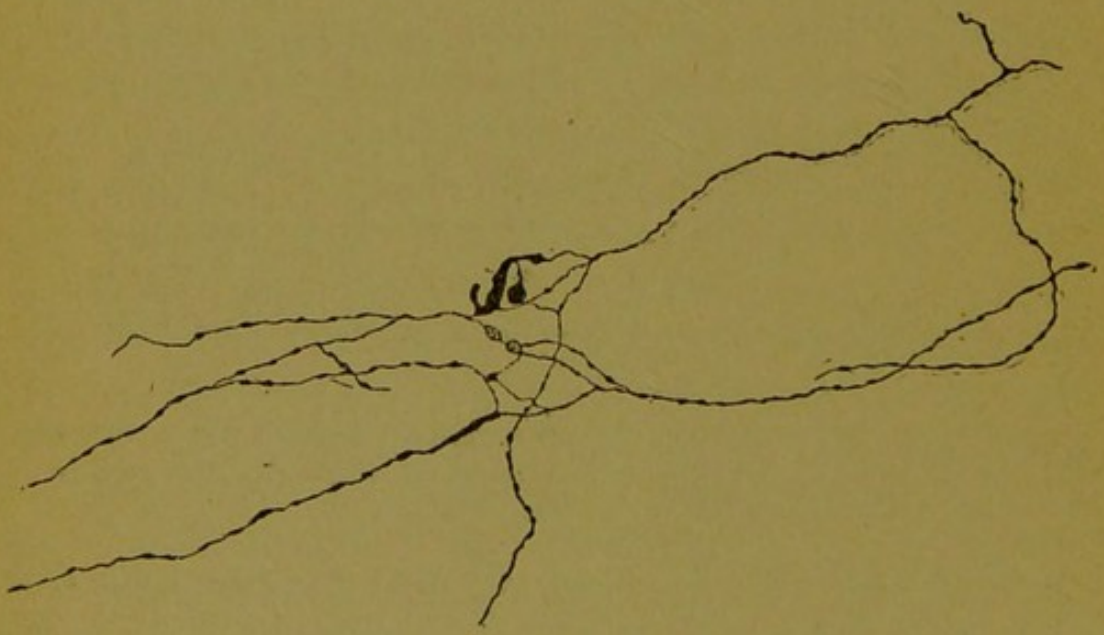

Fig. 2. Methylen blue preparation from cornea Sea Robin (Prionotus carolinus) Zeiss 8 mm. obj. Comp. oc. 4. Camera.

sheath which in some places pulls away from the fiber axis proper, leaving a clear space, at the other points swelling up. This sheath however does not in any way resemble the half medullated sheath which Dogiel (*l. c.*) has described as occurring on the corresponding nerves of the human cornea. But is most probably a result of the changes caused in the nerve fibre by the staining process, and which is always characteristically obtained when the blue method is used.

Where these nerves enter the epithelium around the corneal border small fibrils are often given off which may be traced for some distance winding in and out among pigment cells which are always collected there in considerable numbers. Many of these fibrils enter into close relation with these cells, in every case they can be shown not to end on them although sometimes forming loops around them. Some of these pigment cells presented the appearances of contraction and expansion figured by Ballowitz, but no nerve endings as he describes in relation with them.

Along the course of the nerves Fig. 1, *b*, *b*, Fig. 3, *c*, and scattered throughout the field are seen dark staining bodies bearing processes looking like delicate nerves. These bodies will be seen to be of varying size and shape, in some places gathered together in clusters, in other places scattered and irregularly disposed. In some cases a nerve will send off a delicate fibril to one of these cells lying near to its course, in others to terminate directly in it, but generally passing on to another body further on. The processes coming off from these bodies which are shown in Fig. 3, *d*, *d*, wind in and out among the cells for a short distance and then become lost from view and indistinct; apparently not joining with one another. I cannot help thinking these bodies are similar to the bodies which Dogiel (2) describes as ending bodies in man. The fact that they have these processes however seems to be against this and one brings to mind the assertions of Inzani about special terminal ends situated amongst epithelial cells and which Klein (7) lays to imperfect specimens and bad technique. No bodies similar to Dogiel's complicated "knäulchen" were met with.

That these nerves and bodies are within and limited to the epithelium is easily demonstrated by transverse sections, and by macerating the epithelium off from the cornea substance proper. This method of maceration by over fixing is perhaps the best way to obtain good preparations of these nerves and bodies; in some maceration preparations the epithelium which comes off in one piece becomes broken into several pieces by the

pressure of the cover glass; these pieces will separate a little bit leaving a clear space between them. Across these spaces the nerves will be seen running from one piece to the other unbroken, showing their strength and elasticity.

Some observers have described the nerves of the epithelium as giving off short hook-like branches which bend back

Fig. 3. Methylen blue preparation of the inter-epithelial nerves of the Dog-fish under high magnification, showing dark bodies *c*, lateral fibers *b*; *d*, processes from the dark bodies. Zeiss homog. immers. Comp. oc. 4. Camera.

and enter into relation with stromal plexus of the cornea tissue proper and it occurred to me that these short lateral branches (Fig. 3, *b*) were of this nature; but after repeated examination I could not determine whether they did or not, but from the fact that I could not find branches running down between the deepest layer of cells towards the cornea tissue proper, I do not believe this to be the case. I have already stated I have been unable to find any true plexus or nervous network within

the epithelium which could in any way answer to the various networks described by Klein (7).

Klein states that the sub-epithelial network is situated beneath Bowman's membrane and that he was able to remove the entire epithelium without disturbing this net-work. On examining a similar preparation from the dog-fish with the epithelium so removed no trace of this plexus is found, but instead we get a view of regular plexus of the corneal tissue proper (Fig. 4) so very different in appearance from the much larger nerves of the epithelium.

This network, of which Fig. 4 is but a very poor representation, is of the very finest texture, the fibers forming it being of the very finest in size, perfectly uniform throughout their course and at once to be distinguished from the nerves of the epithelium by the way in which they branch at right angles and their irregular course and all pretty much within the same plane. Every sheet of the corneal substance proper seems to have a special network of these fibers over it. When one network is within focus the the networks of the layers deeper can be faintly made out, and by properly adjusting up and down one can bring nearly all the networks into view one after the other. One peculiarity of these nerve fibers is their sharply granular appearance as if made up of a series of closely arranged dots one after the other in a delicate strand. These fibers in branching and winding about amongst the corneal cells do not keep any definite relation with them, and it is needless to say no anastomoses with them or their processes was to be distinguished. As Dogiel has found in man, the nerve fiber never comes into real relation with the cell but simply passes over it or along one border.

On comparing Fig. 4, with Fig. 7 of Dogiel's (2) paper the general resemblances of this network in selachians and man is very apparent. There is a tendency in selachians to greater regularity of branching of the fibers at right angles, they run in one direction for a certain distance then abruptly turn and run at right angles to their former course, while in man the change of direction is less sharp and sudden.

As to the distribution of this network it seems to be uniformly all over the surface of each lamella. No large trunks are ever seen to join it from the border and going to form it. The nerves composing it appear to be very continuous, where an apparent ending takes place the fiber seems to fade out in a manner which renders it impossible to tell whether it is a free ending or not.

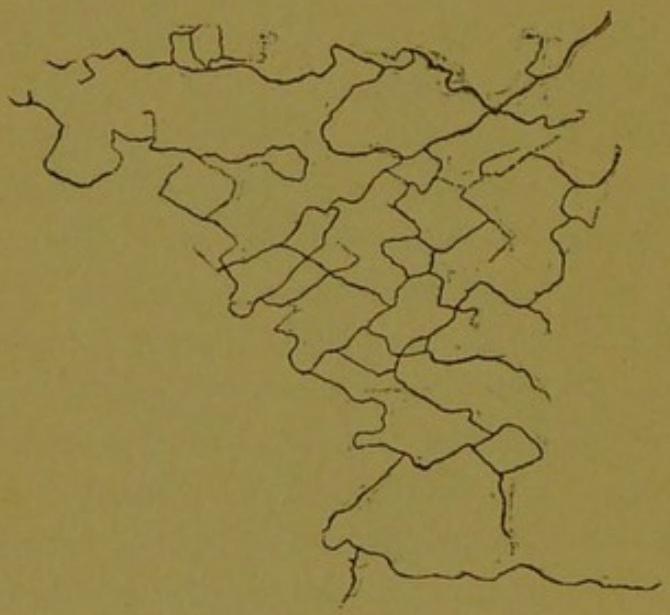

Fig. 4. Fine plexus of the cornea substance proper, methylene blue, showing the right angled courses of the fibers. Zeiss homog. immers. oc. 6. Camera.

To sum up. The chief peculiarities presented by the nerves of the selachian cornea are:

1. The relatively straight, thick, nerve trunks which run in the anterior epithelium and their parallel courses with relation to one another.

2. The dark bodies into which these nerves run and sometimes terminate.

3. The unbranching condition of these nerve fibres.

4. The lack of apparent relation between the nerves of the epithelium with those of the cornea substance proper and the lack of all nerve fibers in the cornea stroma proper similar to these nerves of the epitehlium.

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