Terminations of Nerves in the Nuclei of the Epithelial Cells of Tortoise-shell.

By

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With Plate XLIII.

THE land tortoise (Testudo græca), so commonly imported into England from the south of Europe, appears to be a very sluggish animal. This is not really the case, and its movements on a hot summer day are the reverse of phlegmatic. In this condition its carapace is sensitive to the slightest impact. If the carapace or plastron be very gently tapped, the nearest leg is alone withdrawn, a heavier tap causing a withdrawal of its whole body. We have here, therefore, a structure which is a true sensitive surface, and like the soft skin of a frog or of a man, it is brought into relationship with the central nervous system. Like the soft skin of other animals it may be mapped out into areas, from which the nerve-fibres passing to the spinal cord are all especially connected with outgoing motor nerves, so that the definite reflex movements of limbs as already described may come about.

The above experiment naturally suggested that the sensory nerves passed right through the thick bone of the carapace and plastron, and ended near the outer surface, either in the epithelial tissue of the tortoise-shell itself or in the layer of connective tissue which unites it to the subjacent bone.

After removal of a scute of tortoise-shell the connective

tissue outside the bone was found, in confirmation of this surmise, to contain sensory nerve-fibres, for the application of acetic acid, or of an interrupted galvanic stimulus, caused definite reflex defensive movements, similar to those which follow the application of acid to the frog's skin.

Inasmuch, therefore, as sensory nerves evidently end quite superficially, it became an interesting question to determine their exact mode of termination in the curiously modified tissues of the carapace. Portions of the carapace, generally taken from the region of the costal plates, were softened in chromic acid and nitric acid fluid, frozen, and cut with a thick-bladed razor. In this way one can obtain fairly thin sections even of the tough tortoise-shell. The sections were treated in various ways, with a view of demonstrating nerves or nerve terminations, and in no case was I able to discover any nervous structure in the tortoise-shell itself.

In the subjacent connective-tissue layer, however, were bodies which I, at first sight, thought were end organs (Pl. XLIII, fig. 1). They turned out to be the transverse sections of curiously modified nerve-fibres. These nerve-fibres are easily distinguished from the blood-vessels (which in this situation are devoid of a muscular coat) by their solid appearance (fig. 2), strong connective-tissue covering, and by their occasional transverse section, which is very characteristic in appearance.

Fig. 1 represents, in transverse section, two of these fibres bound together by a common sheath of connective tissue (H). Each fibre consists of an external layer of concentrically arranged connective tissue, consisting of laminæ of colloid granular material with intervening connective-tissue corpuscles (M). Within this is a colloid-looking core (K), devoid of nuclei, and also staining pink with picro-carmine.

In the centre of the core is generally to be found a small spot, probably an axis-cylinder, somewhat differentiated from the rest of the core (G).

No trace of medullary matter is to be found in connection with any of these nerves, nor are ordinary medullated fibres

to be found in this region; and one is forced to conclude that owing to their peculiar situation—perhaps on account of the pressure of the hard scutes placed immediately above them—the medullated nerves are replaced by axis-cylinders, enclosed and protected by the sheaths of modified connective tissue just described.

These modified nerve-fibres can be traced back a little way into the bone, and no doubt ultimately pass into the ordinary medullated nerves found so plentifully on the inner surface of the carapace. Under the scutes they freely branch, becoming smaller and smaller, and ultimately terminate in the lower epithelial cells of the tortoise-shell. We have, therefore, medullated fibres passing from the central nervous system to the bone of the carapace and plastron, these then pass into the medullary nerve-fibres seen under the scutes, from which, as we shall presently see, fine terminal naked axis-cylinders run into the scutes.

The final intra-epidermic termination of the nerves was never seen in any of the sections, for the softening of the tissue previously to its cutting prevented their subsequent demonstration by staining agents. The nerve endings may, however, be demonstrated by another very simple method.

The scutes from a recently killed tortoise are removed in pieces with a sharp scalpel, care being taken to keep attached to their under surfaces as much as possible of the subjacent connective tissue; and it will be found advisable before doing so to remove as much as possible of the dense outer part of the scute. In this way one can obtain thin and fairly transparent pieces of tissue, consisting of the lower layers of the tortoise-shell and the tissue connected with it. These are placed in absolute alcohol 2 parts, and distilled water 1 part, and after twelve hours are thoroughly steeped in distilled water until every trace of alcohol is removed. The tissues are then placed in a solution of hæmatoxylin until they are sufficiently stained; they may then be mounted in balsam, the connective tissue or deeper layer being above the epithelium and next the cover-glass.

Hæmatoxylin Solution.

A. { Ammonia alum, 3 grammes; Distilled water, 100 c.c. B. { Pure hæmatoxylin, 3 grammes; Absolute alcohol, 16 c.c.

Mix A and B, keep in diffuse daylight for two weeks, and dilute with 20 volumes of distilled water.

On looking down into the connective tissue with a power of three or four hundred diameters, the modified nerve-fibres are seen branching in all directions. On deeper focussing the lower cells of the epithelium are seen from below. Their outlines are in most situations fairly well seen, and their nuclei should be stained with the hæmatoxylin.

In these preparations the nuclei frequently shrink within the nuclear cavities, appearing as dark blue granular masses (B, fig. 4); but in most cases they fill the nuclear cavity, and their chromatin filaments can clearly be made out. The greater number of cells are devoid of any nerves, but here and there nerve-fibres may be seen branching again and again in the connective tissue, and sending their finest ramifications to the nuclei of the epidermic cells. These are what appear to be definite sensitive spots where alone the nerves terminate. These spots are of variable size, so small as to correspond to a space occupied by only a dozen cells, or so large as to occupy two or three fields of the microscope. I should say that some twenty or thirty of these "spots" might be found on one square inch of a costal scute. Between these spots the epithelium presents, as already observed, nothing very remarkable, but within the spot the appearance is very striking.

The non-medullated fibres deeply stained with the logwood divide again and again, sending, in many cases, hundreds of fibres to the epithelial cells. Fig. 3 represents a sector of one of these spots carefully drawn from a specimen. At the circumference (B) the fibres terminate in only a few of the epithelial cells, but towards the centre all or nearly all of the cells receive fibres. The outlines of the cells are not well marked, the fibres at first sight appearing to terminate in little round blue masses, which are in reality the nuclei of the cells.

In fig. 4 a very small portion at the outer part of a sensitive spot is more highly amplified. At the upper part of the figure the epithelium outside the spot is seen. Below this the terminations of the nerves can readily be made out. They certainly pass into the nuclear cavity. Whether they end in little flat plates within the nuclear cavity and closely applied to the outside of the nucleus, or whether they are prolonged into the chromatin of the nucleus, I should not like dogmatically to state. I am inclined to believe in the latter view, and think it probable that they are continued into true nuclear substance. The appearances seen at E E E, fig. 4, are probably due to shrinkage as a result of treatment with alcohol; but in c c, fig. 4, the nuclear cavity is completely filled by the nucleus, all the chromatin substance having apparently gone to form the knob or cup at the end of the nerve, leaving the rest of the nucleus almost devoid of granular matter, and very faintly tinted by the hæmatoxylin.

The nerves end in the cells of the rete alone, for it is impossible to trace them beyond the deeper layer of the epithelium. This is what might be expected, for in the adult tortoise-shell the rete consists of one, two, or perhaps three layers of nucleated rounded cells, and above these, with hardly any transitional tissue, there are the dense laminæ of the horny layer, made up of flattened keratinised scales with unstainable nuclei.

It follows from the foregoing remarks that the scutes of the tortoise, in spite of their hard, dense nature, form a very typical epidermic sensory covering for the animal. As in the soft skin of mammals, the nerves end in localised sensitive spots in the epidermis, and before penetrating this tissue they form a horizontal plexus in the upper part of the connective tissue.

The final terminations of nerves in epithelium has received much attention from histologists, who have studied this subject perhaps most fully in the tadpole's tail.

In some situations the nerves appear to run entirely between the cells—indeed, this appears to be generally the case (Ranvier, 1; Klein, 2; Eberth, 3; Lebouc q, 4). They either end in a simple plexus, or terminate in very small knobs or plates, which,

judging from the drawings and preparations I have seen, are for the most part much smaller than the chromatin knobs of the tortoise.

But some authors have traced nerves into the epithelial cells themselves, where they appear to end in little knobs embedded in the cell protoplasm, *near* but never *in* the nucleus. Thus Pfitzner (5), working with the Amphibia, finds this to be the case; and more recently Macallum (6) describes nerves terminating both between and within the epidermic cells of the tadpole's tail.

In the tortoise-shell the nerves certainly pass right into the nuclear cavity, within which the only structures deeply stained by hæmatoxylin are the club- or cup-shaped masses into which the nerves pass. A very remarkable fact is the ease with which these preparations are obtained. I have made over twenty, and in all cases good demonstrations were obtained. I have tried several gold methods, but they were vastly inferior to the logwood, and, as usual, chiefly characterised by want of uniformity in the results obtained. In other situations the non-medullated nerves of the tortoise do not stain at all readily with logwood.

Papers quoted in Text.

1. Ranvier.—'Traité technique d'histologie,' p. 900.
2. Klein.—'Atlas of Histology.'
3. Eberth.—'Archiv f. mikr. Anat.,' Bd. ii, p. 490, 1866.
4. Leboucq.—'Bull. de l'Acad. Roy. de Belgique,' 1876.
5. Pfitzner.—'Morph. Jahrbuch,' Bd. vii, p. 726.
6. Macallum, A. B.—'Quart. Journ. Micr. Sci.,' vol. xvi.

DESCRIPTION OF PLATE XLIII,

Illustrating Dr. John Berry Haycraft's paper on "Terminations of Nerves in the Nuclei of the Epithelial Cells of Tortoise-shell."

FIG. 1.—Section through the lower part of the tortoise-shell, the subjacent connective tissue, and part of the bone of a costal plate. *A.* Lower laminæ of horny layer of tortoise-shell. *B.* Cells of rete, with big nuclei and deep ridges passing into subjacent connective tissue. (*c*) Process—profile view of a ridge—of connective tissue running into epithelial cell. *D.* Pigment-cell in tissue around nerve-fibres. *H.* Sheath common to two nerve-fibres. *m.* Outer covering of granular modified connective tissue. *K.* Inner part of nerve-sheath, consisting of granular non-nucleated connective tissue. *G.* Spot in centre, probably an axis-cylinder. *E.* Bone.

FIG. 2.—Longitudinal view of a nerve considerably smaller than the one represented in transverse section (Fig. 1).

FIG. 3.—Part of a sensitive spot; the tortoise-shell is viewed from below. × 250. *A.* Centre of spot. Branching nerve-fibres seen ending in nuclei of epithelium. Border of cells not seen because of low power used. *B.* At periphery of spot, where most of the epithelial cells are unconnected with nerve-fibres.

FIG. 4.—Part of the same highly magnified. × 800. *A.* Nucleus outside sensory spot. It contains chromatin filaments. *B.* Nucleus that has shrunk within nuclear cavity. *c c.* Nerve-fibre passes into nuclear cavity, and apparently ending in the chromatin of the cell, the rest of the nuclear cavity being filled with clear, almost colourless material. E E E. Nothing is to be seen inside the nuclear cavity except the knobs terminating the nerves. The rest of the nucleus has probably shrunk around this. *H.* Towards centre of spot the outlines of the epithelial cells are very indistinct.

Fig. 1.

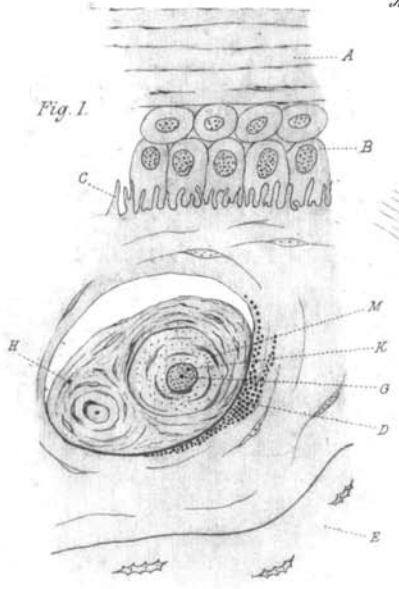
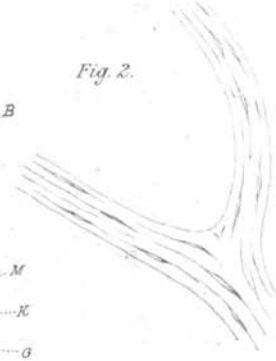


Fig. 2.



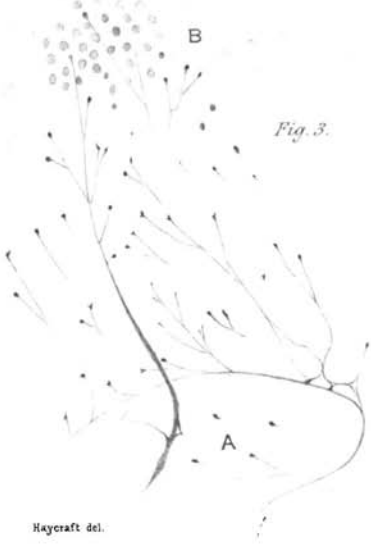


Fig. 3.

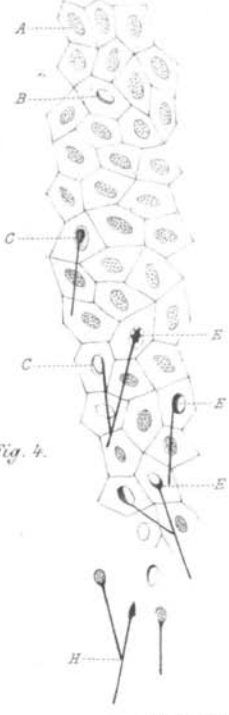


Fig. 4.