Observations on the Nerve Fibres of *Aurellia aurita*

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With two plates (figs. 3 and 4)

SUMMARY

The nerve fibres of *Aurellia aurita* were originally described by E. A. Shäfer from gold preparations. This work has been repeated with Holmes's method of silver staining on the slide. Results have been obtained substantially in agreement with those of Shäfer.

The same nerves can be seen in the living animal when a phase-contrast microscope or oblique illumination is used. By the use of these techniques, a study has been made of the plan of the nerve net over the subumbrellar surface of the bell.

This work is intended as an anatomical foundation for physiological studies to be described elsewhere.

ROMANES (1878) mentions that he saw fibres in the subumbrellar epithelium of living specimens of *Aurellia*, similar to the nerve fibres demonstrated by his friend E. A. Shäfer (1878). The fibres to be described in this paper are of exactly the same kind as the ones that Shäfer described from gold preparations of fixed material. The general appearance, the shape of the cell-body, and the position in the epithelium are characteristic, and there are no other structures with which they can be confused.

MATERIAL AND METHODS

At the beginning of this study a phase-contrast microscope was used for the observation of the living nerves of *Aurellia aurita* Lamarck. The mesogloeal jelly of the exumbrellar surface was cut away to make a flat preparation a few millimetres thick on which the muscular sheet of the subumbrella was displayed. A 16-mm. objective was sufficient to find the nerves which appeared to be right in the subumbrellar epithelium. Later it was found that the nerve fibres scatter light considerably, so that they are easily visible by oblique or dark ground illumination. This allowed the use of an ordinary binocular microscope, as used for dissection, which was very convenient when handling the fibres experimentally.

For the silver-stained preparations whole animals were fixed overnight in a mixture of 3 volumes of saturated picric acid solution in sea-water with 1 volume of 40 per cent. formalin. Strips of epithelium were peeled off from the jelly and fastened to microscope slides with cotton or with albumen. Staining was by the method of Holmes (1947) with slight modifications. Well-washed

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slides were soaked in 20 per cent. silver nitrate solution for an hour, then washed again before they were put in the impregnating solution. Impregnation was for 2–3 days in dilute silver nitrate solution 1 in 10,000, buffered to about pH 8 with boric buffer, and with pyridine added to a strength of 1 in 10,000. I have made use of Dr. Batham's experience with the nerves of *Metridium*, but my results have not been so successful as her beautiful preparations (see the illustrations in Pantin, 1952).

Living Preparations

With a phase-contrast microscope, fibres similar to those described by Shäfer are easily discovered in the epithelium of the concave surface of the bell. Lower down, in the mesogloea, many supporting fibres are found, but these look quite different, being thin, sharp, straight, and non-cellular. There is no doubt that the fibres seen in the epithelium of the living material correspond to the cellular axons which can be demonstrated by staining. Experimental work (Horridge, 1953) has shown that these living fibres are indeed nerves.

When oblique illumination is used, the great scattering of light makes the nerve fibres appear very wide. Even when allowance is made for this, the axons appear to be full of fluid; some are 12 μ thick, others only 6 μ, most have a diameter between these two values. In fixed preparations they are collapsed and even ribbon-shaped. The smaller ramifications of these axons have not been seen in studies of living epithelium.

Along the axons are swellings at irregular intervals and although the nature of these is not clear, their appearance suggests that they may be due to damage to the wall of the axon. These swellings increase in frequency under adverse conditions. They recall the beading found when nerve axons are vitally stained with methylene blue. Despite the presence of these swellings the single axon in a bridge of tissue between two pieces of *Aurellia* would still conduct the excitation leading to a contraction wave.

Under an ordinary binocular a large living axon can usually be traced for about 5 mm., sometimes much less. Exceptionally I have been able to follow one for as much as a centimetre. These figures give no idea of the extent of muscle supplied by all the branches of a single neurone, since silver preparations show thin ramifications never visible during life.

In the examination of the living animal with oblique illumination it is very difficult to make out the cell-bodies belonging to the axons. Fixed preparations show bipolar cell-bodies about midway along the axons; in the living tissue the observed axons are interrupted somewhere along their length. At this interruption there is an apparent break 10–15 μ long in the course of the fibre and careful examination reveals a large nucleus, a highly refractile nucleolus, and a faint surrounding cell-membrane. It happens that under the conditions of illumination used the cell-body does not scatter light to the same extent as the axon, but a phase-contrast microscope shows the cell more clearly.

At the frequent places where axons cross each other, they appear to do no more than pass one over the other. Since the specimen is viewed from above

with optical conditions that are necessarily inadequate for demonstrating the detailed structure, it is impossible to claim that there is either contact or syncytial continuity at these points. However, besides these simple crossings, all kinds of more intimate contact, in which the axons twist about each other or run alongside, can be found in an exhaustive search; but it is quickly noticeable that one sort of relation is the most frequent. Two fibres cross or come together at an acute angle, run side by side so closely that they are not resolved as two fibres in the halo of scattered light, and then they separate and

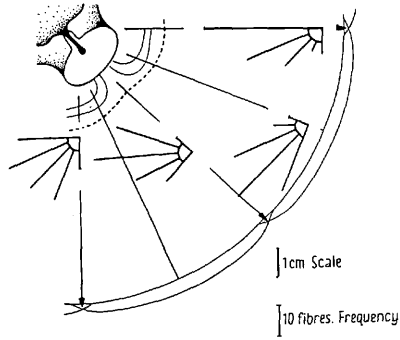

FIG. 1. The angular distribution of large nerve fibres at four places on the concave surface of the bell of *Aurellia*. At each place 100 fibres were examined by using an ordinary binocular microscope and oblique illumination. A histogram with one angular co-ordinate has been drawn at each place; the scale giving the frequencies is shown.

usually resume their former courses. These points where the axons run together correspond to the contacts between neurones seen by other workers (Bozler, 1927), and have been referred to as synapses.

Branching of the observed living axons is not unusual and side branches are noticed occasionally, but in general all that are seen are surprisingly straight fibres, which fade out at their ends without giving an impression that they stop at once.

Taking advantage of this straightness, it is not difficult to plot the angular distribution of the nerve fibres (fig. 1). The principal directions of the observed nerve fibres were recorded on a piece of squared paper by the use of a squared eyepiece. A small area about 1 centimetre square was scanned systematically, by using a mechanical stage, until 100 fibres had been recorded in the area at each of four positions on the under surface of the bell, as shown in fig. 1. As far as possible no fibre was counted twice. The axons run in all directions, but most of them are sufficiently straight to justify an estimate of the angular distribution. There is no preferred orientation of the nerve fibres except in two regions of the bell. Along the edge of the bell few of the axons run radially and in the region just central to each of the marginal bodies the axons appear

to radiate from the stalk of the marginal body and spread over the neighbouring epithelium. There is no physiological evidence to suggest that elsewhere in the net there is any orientation of conducting pathways. In *Cyanea*, however, there is quite a different organization of the nerve net (fig. 2); the muscle fibres are concentrated into distinct blocks. Although the orientation of the nerve cells and processes is quite different, the individual axons are similar to those of *Aurellia*.

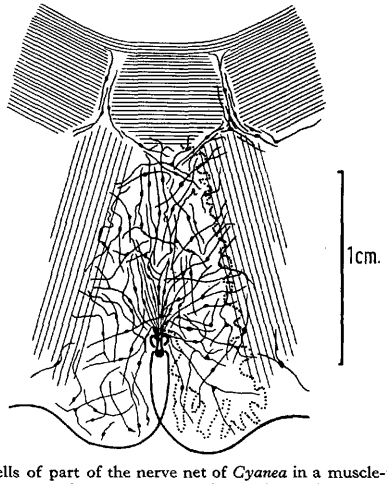

FIG. 2. The large cells of part of the nerve net of *Cyanea* in a muscle-free region. Two of the radial muscles and part of the circular muscle are shown. In a small specimen 5 cm. in diameter the number of large nerve cells visible in the most favourable preparations would be approximately as shown.

In *Aurellia* the abundance of the nerve axons seen under an ordinary binocular varies enormously from place to place and between individuals. Coming from each marginal ganglion is a concentration of axons. Near the marginal ganglia they are often more numerous than elsewhere but not particularly so, and this is not true for some specimens. The photograph (fig. 3, A) is of an area chosen because of the great abundance of axons. In a field of view 2 mm. in diameter there are usually three or four axons visible and sometimes as many as ten, but the number noticed depends upon the conditions of illumination, which vary with the thickness of the jelly.

FIXED PREPARATIONS

A few successful preparations were made by Holmes's method of silver staining. The photographs (fig. 4, B and D) show some of the qualities of these nerve fibres in *Aurellia*.

The larger cells, 10–20 μ wide, are not usually stained at all with the silver. Their large nuclei can be picked out among the much smaller nuclei of the muscle fibres in preparations stained with Heidenhain's haematoxylin. The most conspicuous feature of the nerve cell is the single, heavily staining, round nucleolus in the comparatively large nucleus. A few of the large axons are more than 8 μ wide. Most are more than 5 μ wide, but towards their ends they get thinner and branch to fine ramifications. The thinner branches stain more readily with the silver.

These nerve fibres in the fixed preparations show the same relations to each other as the nerve fibres seen in the living preparations; sometimes two run alongside each other in contact for as much as 50 μ, sometimes they twist together but always separate again. It seems characteristic of coelenterate nerves that they have synapses along the axon lengths, as Pantin (1952) has shown. Bozler (1927) depicts, in addition, a different kind of synapse in which a very fine axon termination makes a synaptic connexion with the middle of another axon in *Rhizostoma*, but in *Aurellia* I have not seen junctions of this kind. In *Aurellia* the ends get finer and finer after branching and eventually cannot be traced because they become so very thin. In fact they pass beyond the resolving power of the microscope. The fineness of the detail excludes any conclusion about the question of syncytial fusion with other thin endings of the nerve net. However, in the case of the relations between the larger fibres of the through-conducting system of *Metridium* (Actinozoa), Pantin (1952) has shown the discontinuity at the synapses between the axons. All the work on *Aurellia* (Shäfer; Woollard and Harpman) shows a similar picture. The principal dissenter from the view that axons are only in contact is Bethe (1903), and his figures show fibres continuous from cell to cell, a condition which other authors deny. It seems likely that Bethe's method of fixation with nitric acid followed by staining with toluidine blue led to artifacts which happened to be similar to fibres. Impregnation with heavy metals can produce similar impression of continuity.

The relations, called synapses, between the larger fibres in the nervous system of medusae are not as clear as the definite pictures of those in *Metridium*. The axons in *Aurellia* and in *Rhizostoma* come together at some points and run in contiguity for a distance of up to 50 μ, many times their width. The same can be seen in the living nerve net (fig. 4, A). This is similar to the succession of small contiguities between parallel fibres described by Pantin. In *Metridium* these large concurrences are found and it is also clear in the actinian that at the crossing of one fibre over another there is a structure which suggests that excitation can pass from one axon to the other. In medusae these simple crossings have not been demonstrated to be points of contact, although there are indications that such contact exists in my own preparations.

Discussion

The results here described were obtained in a histological study that accompanied experimental work on the nerve net of *Aurellia* and other Scyphozoa.

By making cuts in different directions, Romanes (1878) showed that the contraction wave is transmitted in all directions across the under surface of the bell. He had evidence that the transmission was through nerve fibres; for example, in narrow bridges transmission always broke down at a definite point that could be located with a fine electrode. At Romanes's suggestion, Shäfer (1878) showed that there was in fact a network of nerve fibres which accounted for the physiological results.

Shäfer described a network of bipolar and tripolar cells with processes running in all directions in the epithelium of the lower surface of the bell, over the circular muscle. He emphasized that, although the fibres run alongside and twist about one another, in no case did he find continuity between the fibres of two cell bodies. In fact he was rather at a loss to explain how the continuity of the contraction wave could be maintained over the whole bell.

The identification of nerve cells in coelenterates has depended upon circumstantial evidence of two kinds (Pantin, 1952): the histological appearance, and the agreement of this appearance with the results of physiological experiments. The present paper shows some of the characteristics of the living nerve fibres. The re-discovery that these could be examined has made it possible to show the truth of the inference that these nerve fibres are responsible for the propagation of the contraction wave (Horridge, 1953). The observations described here give an anatomical background which corresponds with physiological results to be presented in another paper.

A number of authors, particularly Bethe (1903), concluded that the nerve net in several species of Scyphozoa consists of anastomosing fibres which are continuous from cell to cell. This is contrary to the clear evidence of Bozler (1927) and Pantin (1952), and it seems likely that an appearance of continuity was brought about by artifacts in the preparations.

Bozler (1927) has used vital staining with methylene blue to investigate the structure of the nerve net of *Rhizostoma*. This careful and detailed work has been largely responsible for the downfall of the belief that continuous fibres run from cell to cell of the coelenterate nervous system. Both bipolar and multipolar cells were described; the former closely resemble the large bipolar nerve cells of *Aurellia* and can be seen without staining. Bozler described two types of synapse between the bipolar cells. There were places where two nerve cells ran close together and then separated again; and there were contacts between the end of one axon and the thicker portion of another. In *Aurellia* only the first of these has been discovered, but Bozler studied a muscle-free area where alternative connexions on to the muscle fibres were not possible.

The results here described for *Aurellia* and *Cyanea* are concerned with the

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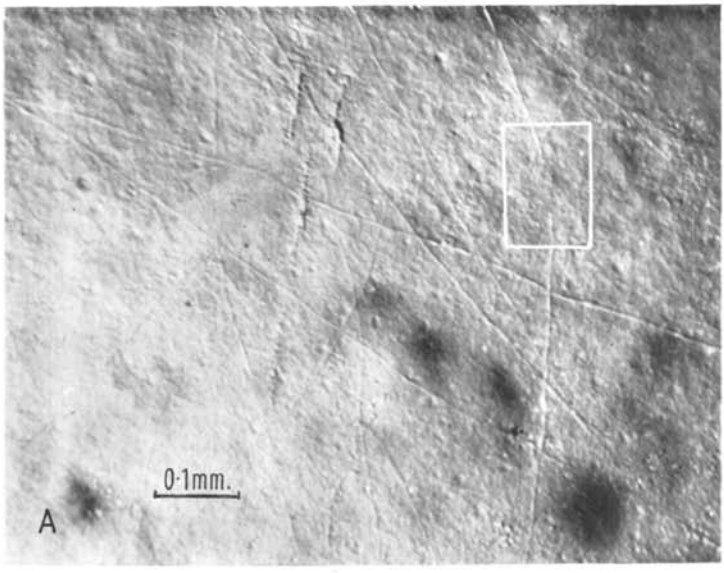
FIG. 3. A, the living nerve fibres of *Aurellia* running over the sheet of circular muscle. The photograph was taken through an ordinary binocular microscope with oblique lighting. Within the rectangle marked, the apparent gap in the course of the fibre shows the position of the cell-body.

B, the striated fibres of the circular muscle of *Aurellia*, impregnated with silver by Holmes's method.



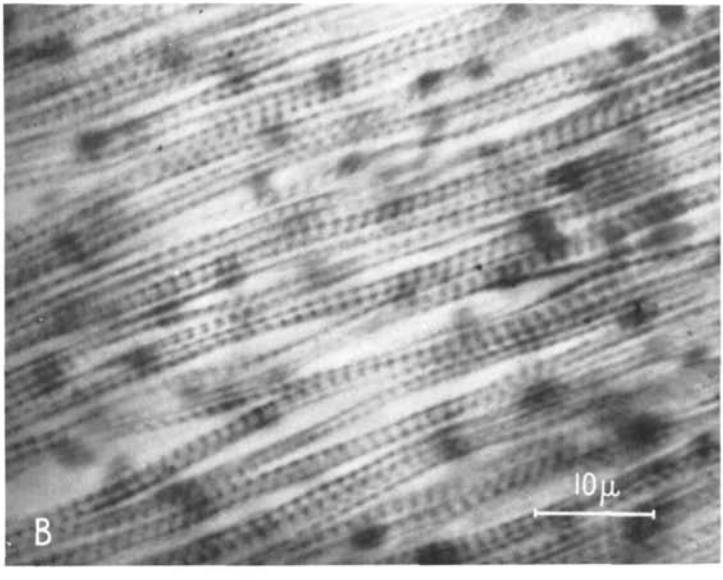


FIG. 3.

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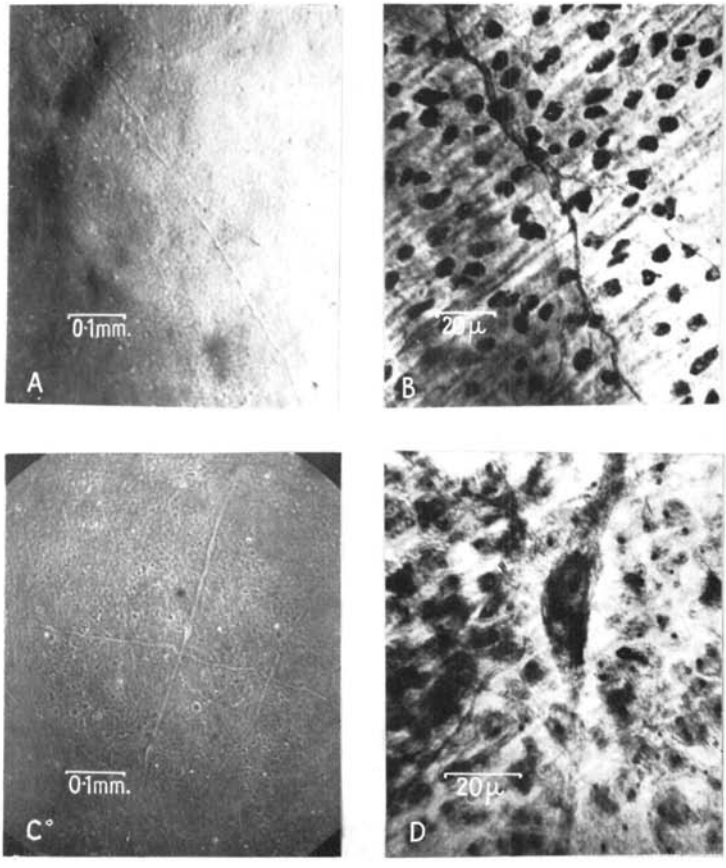

FIG. 4

A. HORRIDGE

large bipolar cells and their processes which propagate the contraction wave. The structure of this nerve net agrees with the fibres Shäfer described and the details agree with those of the large bipolar cells which Bozler described. There is general agreement that this network is make up of separate cells which touch at well-defined points which can be named synapses. This is a histological definition independent of the results of physiological experiments. The system is normally through-conducting but the existence of synapses may become physiologically apparent under the influence of anaesthetics, for example, magnesium chloride solutions.

Apart from the fact that they are normally through-conducting, these synapses differ in certain respects from most synapses in the higher animals since they appear to occur indiscriminately between any axon of this system and any other. Bozler (1927) suggested that there are several distinct systems of large bipolar nerve cells in the Scyphozoa but came to the conclusion that fixed preparations would be necessary to be certain of this. The observations on *Aurellia* indicate that only one system of large bipolar cells is present, and contacts occur indiscriminately between the fibres of this system. Other nerve cells occur besides those of the through-conducting system and it remains to be seen whether the relations between these cells are synaptic contacts.

There is much less agreement about the smaller multipolar cells. Bozler considers that axons of all the nervous systems of coelenterates studied are never continuous from cell to cell and he also draws connexions of a contact nature between multipolar cells and the larger axons of bipolar cells. In *Aurellia* these multipolar cells were never well stained with silver, and methylene blue produced no results at all. In *Cyanea* there is also a network made up of these small nerve cells in places where the large cells of the through-conducting system are absent both histologically and physiologically. In *Cyanea* and *Aurellia* the thinnest fibres of the nerve net are at the optical limit of resolution. There is no evidence that the concurrence of a large and a small axon, such as Bozler saw, is in fact the site of a connexion, where excitation may pass from one axon to the other. Where a thinner axon runs alongside a larger the normal forces during growth may be responsible for a misleading appearance. Much of the difficulty of interpretation has arisen because the finest structures observed in methylene blue preparations of *Cyanea* and silver preparations of *Aurellia* are near the limit of resolution of the microscope.

Nerve fibres have been described by Woollard and Harpman (1939). I quote part of their summary; '2. It is concluded that the nervous system in

FIG. 4. A, 'synapse' between two living nerve fibres. (At the bottom right hand-side there is a simple crossing of one fibre over another.)

B, silver-stained nerve fibres running over the muscle fibres among the nuclei of the subumbrellar epithelium of *Aurellia*.

C, *Aurellia*, photograph of the living epithelium taken with a phase-contrast microscope to show a number of finer nerve fibres besides the two larger nerve fibres.

D, bipolar cell-body in the nerve net of *Aurellia*, silver-stained. The darkly stained nucleolus appears in the centre of the oval nucleus.

these Coelenterates consists of nerve cells the processes of which are discontinuous. 3. The nerve fibres frequently intertwine but never fuse. 4. Nerve fibres terminate on muscle cells by small expansions.' They studied tentacles mainly and were dealing with small fibres of the primary sensory nerves and the connexions of small fibres with the locally controlled muscle fibres. They decided that end to end fusion of axons does not occur but that the smallest axons disappear beyond the limit of resolution.

In his Croonian Lecture, Pantin (1952) has described and illustrated the nerve fibres of the through-conducting system of *Metridium senile*. Both functionally and histologically these are similar to the fibres of the nerve net of *Aurellia* and it seems likely that a study of one leads to conclusions valid for the other. In the present description of the axons found in *Aurellia*, comparisons are made with the structures found in the anemone.

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