A Comparison of the Nervous Systems of two Sea-Anemones, *Calliactis parasitica* and *Metridium senile*

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With two plates (figs. 1 and 2)

SUMMARY

The properties of the actinian nervous system are known mainly from physiological experiments on *Calliactis parasitica* (Couch), and from histological work on *Metridium senile* (L.). The structure of the nerve-net in the mesenteries of *Calliactis* is now shown to resemble in general that in *Metridium*. Methylene blue stains a network of bipolar cells over the retractor muscle, together with sense-cells, and unlike *Metridium*, multipolar nerve-cells. The nerve-net over the radial surface of the mesentery is similarly much sparser. The distribution of nerve-cells and sense-cells in the column also resembles that in *Metridium*.

Experiments on *Metridium* show that as in *Calliactis*, the rate of conduction in the mesenteries is greater than in other parts of the anemone. The column, including the sphincter region, conducts more slowly. It is thus shown that the presence of a well-developed nerve-net over the retractors is associated with the development of fast tracts in the through-conduction system, and of rapid, facilitated contractions of the retractor muscles, in both species of anemone.

THE nervous system of sea-anemones has been studied from two points of view. Its physiological properties have been shown by experiment to resemble in many respects those of vertebrate nervous systems (Pantin, 1935), and histological work demonstrates a nerve-net whose arrangement agrees with these properties (Hertwigs, 1879; Pantin, 1952; Batham, Pantin, and Robson, 1960). This paper is about the correlation of the two sources of evidence in the analysis of the rapid responses of *Calliactis parasitica* (Couch) and *Metridium senile* (L.).

THE CLOSURE REFLEX

In the rapid closure reflex of *Calliactis* a specialized part of the conducting system is concerned with the response of sphincter and retractor muscles. Histological evidence that this system is nervous, while supporting the physiological work on *Calliactis*, has been derived largely from a different anemone, *Metridium*. It is thus desirable to assess any differences between the two species in relation to general conclusions about the actinian nervous system. A few observations on the structure of the nerve-net in *Calliactis* and on its rate of conduction in *Metridium* are now reported.

A diffuse, rapidly conducting system innervates the sphincter and retractor muscles in *Calliactis* (Pantin, 1935). It shows absolute and relative refractory periods and chronaxie similar to those of vertebrate nerves. Above threshold,

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frequency of electrical stimulation markedly affects the muscular response, and at shock intervals of less than 3 sec neuromuscular facilitation produces separate, graded contractions. Although 'interneural' facilitation may be demonstrated in certain regions of the anemone, the part of the conducting system which supplies the muscles of the column, the sphincter, and the retractor muscles is continuous physiologically. It has therefore been termed the 'through-conduction system'. In *Calliactis*, physiological experiments show that tracts which run vertically in the mesenteries and join a ring in the sphincter region conduct much more rapidly than the rest of the nervous system (fig. 5, F, p. 324). The through-conduction system may be excited by mechanical stimulation of the column (Passano and Pantin, 1955). A similar through-conduction system exists in *Metridium* (Hall and Pantin, 1937), although it has not yet been shown that fast tracts are present in the mesenteries.

Histological evidence from this anemone (Pantin, 1952; Batham, Pantin, and Robson, 1960) shows that corresponding to the position of rapidly conducting tracts in *Calliactis* a well-developed network of bipolar nerve-cells runs over the retractor surface of the larger mesenteries. Interneural and neuromuscular junctions have been demonstrated, and the processes of abundant sense cells along the junction of mesenteries and column are in contact with the mesenteric nerve-net. Similar but sparser nerve-nets are seen on the radial surface of the mesenteries and in the column.

These two lines of investigation suggest that the development of a rich network of bipolar nerve-cells is associated with the development of fast conduction and of rapid, facilitated contractions of muscles concerned in the closure reflex. In order to confirm this correlation, histological evidence of nerve-cells in the mesenteries of *Calliactis*, and physiological evidence for rapid through-conduction in the mesenteries of *Metridium* are required.

THE NERVE-NET IN *CALLIACTIS PARASITICA*

The nerve-net of *Calliactis* has been demonstrated by vital staining with methylene blue. Large anemones, allowed to expand in the dark for 2 or 3 h, were anaesthetized by transferring to approximately 0·15% propylene phenoxetol (Owen, 1955; Owen and Steedman, 1958). This was prepared by shaking the liquid with seawater in a test-tube, and stirring the emulsion into the main volume of sea-water. The medium was pipetted into the coelenteron at intervals, and large specimens were fully anaesthetized after 3 or 4 h. Staining was carried out at the same time by injecting reduced methylene blue into the

FIG. 1 (plate). Preparations of *Calliactis* mesenteries stained with methylene blue and fixed in Susa / phosphomolybdic acid.

A, part of the nerve-net over the retractor muscle, whose fibres run from left to right in the background.

B, a bipolar nerve-cell from the retractor nerve-net, with which the fine neurite of another cell makes several lateral contacts.

C, part of the retractor nerve-net, showing a neurite crossing numerous others. Underlying retractor muscle fibres run from left to right.

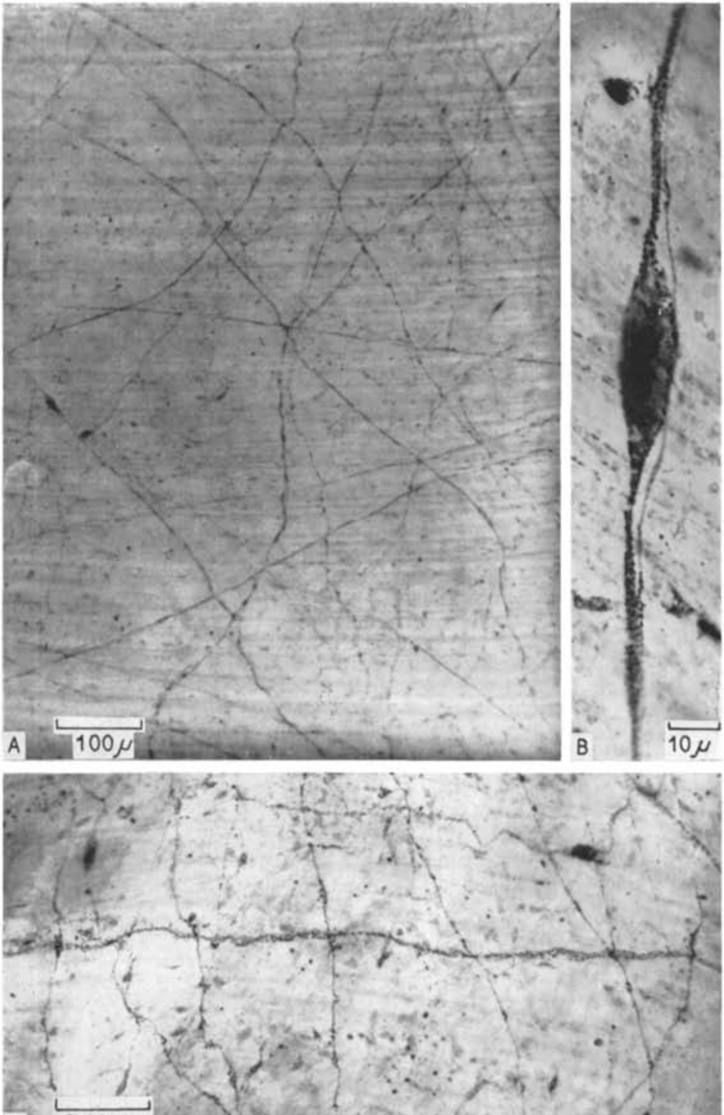

FIG. 1

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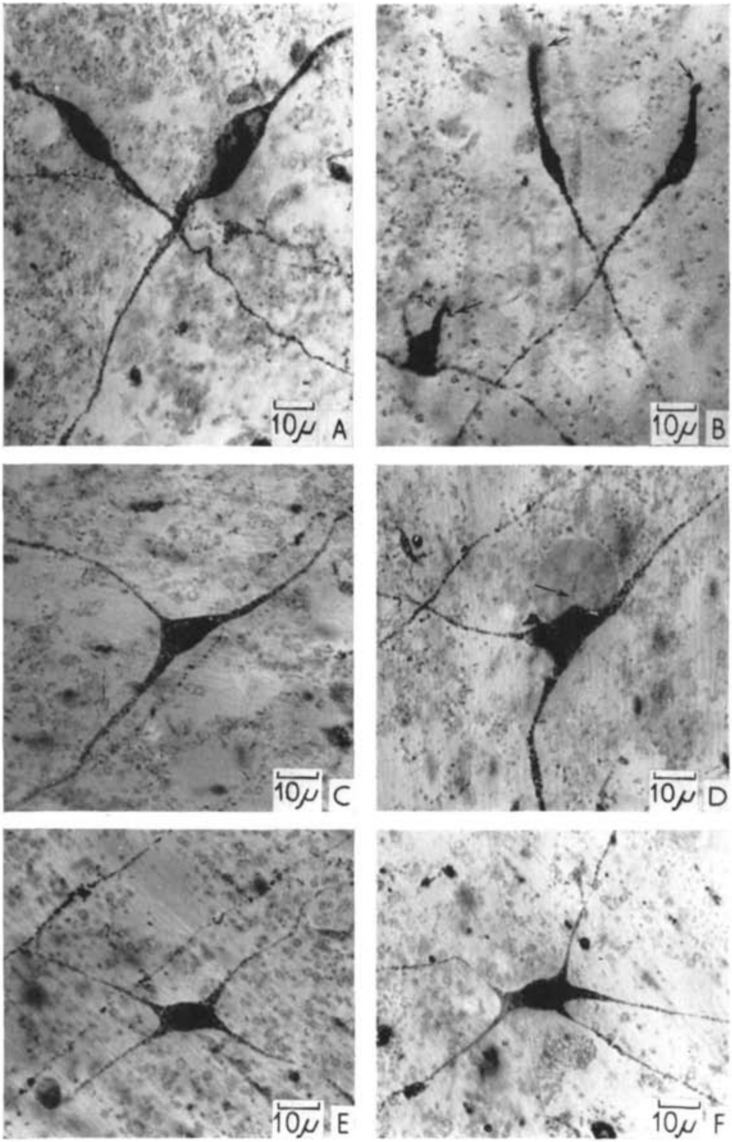

FIG. 2

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coelenteron an hour or two after the anemone was transferred to the anaesthetic. The most successful results were obtained after the dye had been diluted about 5 times with sea-water beforehand. Stained mesenteries and other parts of the anemone were then dissected out, pinned on to wax strips, fixed, and mounted in depex. Mesenteries were fixed in Susa/phosphomolybdate (Batham, Pantin, and Robson, 1960), which precipitates the dye completely although rather coarsely, and thicker preparations with ammonium molybdate.

The retractor surface of the mesenteries has a well-developed nerve-net (fig. 1, A) which is clearly similar to that of *Metridium*. It is composed mainly of bipolar nerve-cells (fig. 1, B), which cross one another at frequent intervals (fig. 1, C), and sometimes tend to run in the direction of the muscle-fibres. The nerve-net does not follow the pleated arrangement of the retractor muscle (Batham and Pantin, 1951; Batham, Pantin, and Robson, 1960) but is situated in one plane below the epithelium. Individual neurites may, however, sink down towards the muscle as in fig. 3. The overall length of bipolar nerve-cells in moderately extended mesenteries is difficult to determine in these preparations, but usually appears to be about 4 or 5 mm. Although they seem to be shorter than the bipolars in silver preparations of *Metridium*, which average 4 to 8 mm (Batham, Pantin, and Robson, 1960), a few cells 7 or 8 mm long suggest that the size ranges are probably similar. The shorter neurites may be incompletely stained in these preparations.

Relatively few nerve-cells are present on the radial surface of the mesentery, but as in *Metridium* their size range corresponds to that of the retractor nerve-cells. The numerical ratio of cells on the two surfaces cannot be measured in these preparations, but as in *Metridium* it is probably less than 10 to one.

Junctions between nerve-cells are numerous (fig. 2, A), although the region of synaptic contact appears in most cases to be more restricted than in *Metridium*. This might be due to the use of a different anaesthetic, since magnesium chloride, which was not used for *Calliactis*, might well affect the cytoplasm and cell membranes in such a way as to increase the area of adhesion. The main observation is, however, that in neither anemone is the nerve-net syncytial. *Calliactis* preparations show lateral contacts between adjacent neurites as in *Metridium* (fig. 1, B) (Batham, Pantin, and Robson, 1960). No new structural features have been observed.

The endings of neurites are difficult to trace in methylene blue preparations,

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FIG. 2 (plate). Preparations of *Calliactis* mesenteries stained with methylene blue and fixed in Susa / phosphomolybdic acid. All from the retractor surface, and at the same magnification as fig. 1, B.

A, cross-over junction between two bipolar nerve-cells.

B, three sense-cells, 2 with one neurite and one (lower left) with 3, to show junctions between these processes. Sensory cilia are not in focus but their position is indicated by arrows.

C, tripolar nerve-cell.

D, tripolar sense-cell, with the base of the faintly stained cilium indicated by an arrow.

E, multipolar nerve-cell, one of whose 4 neurites makes contact with at least one of the 2 others crossing the photograph.

F, multipolar nerve-cell with 5 neurites.

and have not yet been seen in *Calliactis*. In *Metridium*, their termination over muscle-fibres is best studied in silver preparations of fixed mesenteries.

As described by the Hertwigs (1879), *Calliactis* differs from *Metridium* in possessing multipolar nerve-cells with 3, 4, or 5 neurites, which make contact with the bipolar network. Examples from the retractor nerve-net are shown in figs. 2, C, E, F. Only occasional tripolar nerve-cells are found in the mesenteries

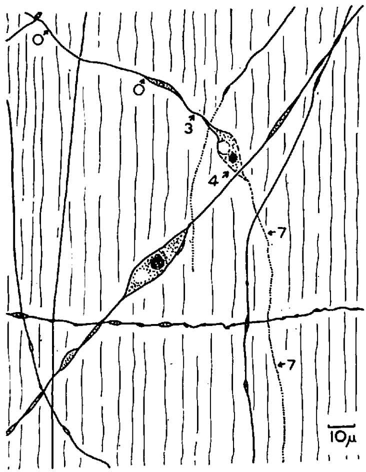

FIG. 3. Drawing to show how the neurite of a bipolar nerve-cell may drop down from the general level of the nerve-net to the folds of the retractor muscle. Numbers indicate distance below general level of focus in μ. (Camera lucida from fig. 1, A.)

of *Metridium* (Pantin, 1952). Sense-cells, differentiated by a short cilium (fig. 2, D), are present in the same preparations. Their processes make contacts with other sensory neurites and with the nerve-net (fig. 2, B, D). The sense-cells may possess 1, 2, or 3 neurites. These may exceed 500 μ in length, but they taper away to fine endings which are no easier to trace in *Calliactis* than in *Metridium*. Sense-cells occur on both surfaces of the mesenteries and may equal a nerve-cell body in size.

A few observations on the column and pedal disk show that these regions are similar to their counterparts in *Metridium* (Batham, Pantin, and Robson, 1960). Numerous sense-cells line the angles where mesenteries join the column and pedal-disk, and their neurites connect up with the retractor nerve-net. The presence of this system in *Calliactis*, inferred from mechanical stimulation experiments, is thus confirmed.

The distribution of the relatively few bipolar nerve-cells in the column is summarized in fig. 4. They often pass beneath the attachment of a mesentery within bundles of circular muscle-fibres, or else turn through 90° on reaching its insertion and either run along the junction or on to the surface of the mesentery. A few run wholly along the base of the mesentery. Sensory neurites may follow these pathways as well.

Multipolar nerve-cells, apparently absent from this region in *Metridium*, have also been seen in the column.

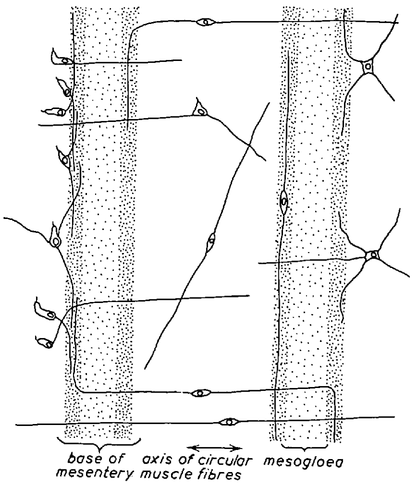

FIG. 4. Diagram to show distribution of nerve-cells and sense-cells in methylene blue preparations of the column of *Calliactis*. The bases of two mesenteries, shown by stippling, are traversed by neurites which accompany bundles of circular muscle-fibres. Only a few of the sense-cells in the angle of the mesentery are shown.

THE THROUGH-CONDUCTION SYSTEM

In *Calliactis* two muscles, the sphincter and mesenteric retractors, are able to give quick, facilitated contractions. The associated fast tracts of the through-conduction system have been shown experimentally to run in the mesenteries and in the sphincter region. The corresponding histological picture in this anemone now shows an extensive nerve-net over the retractor muscle and a similar but much sparser one on the radial surface of the mesentery, resembling the situation in *Metridium*. The innervation of the sphincter is unfortunately not yet known in either anemone.

In *Metridium*, in contrast to *Calliactis*, the retractor muscles withdraw the oral disk before the sphincter contracts (Pantin, 1935 *b*). Both give facilitated contractions, but the retractor muscle is in this case the more rapid of the two (Batham and Pantin, 1954). This agrees with the degree to which the nerve-net is developed over the retractors compared to the radial muscles and column (Batham, Pantin, and Robson, 1960). A few measurements now show that the rate of vertical conduction in the mesenteries exceeds that of any other region, confirming what has been found in *Calliactis*.

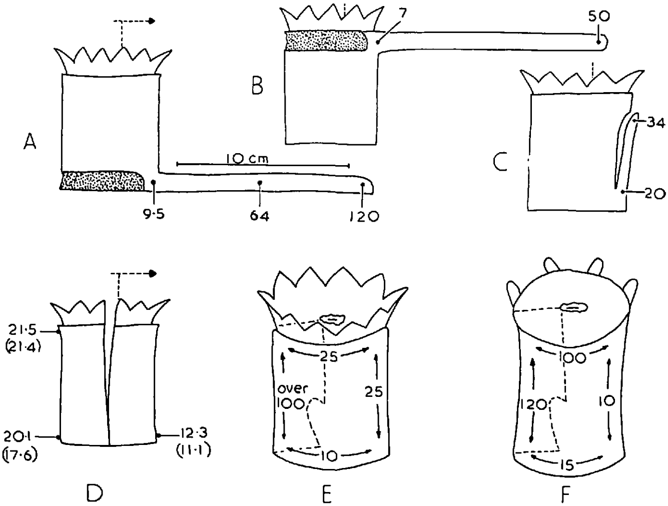

FIG. 5. A–D, diagrams of a few experiments on *Metridium*, showing latent periods in hundredths of a sec. The numbers represent time between the second of two electric shocks and contraction of retractor muscles recorded from a thread in the siphonoglyph. In D measurements from a different specimen are given in brackets. E, diagram showing approximate speeds of conduction in *Metridium* based on present results. F, diagram showing the comparable values in *Calliactis*, after Pantin (1935*b*).

Speeds of conduction were estimated from the latency of response to stimuli from silver / silver chloride electrodes in various positions (Parker, 1918; Pantin, 1935 *b*). Large *Metridium* (4 to 6 cm in diameter) were allowed to settle on glass plates, and any necessary incisions made after anaesthetizing with magnesium chloride (Batham, Pantin, and Robson, 1960). After the preparation had recovered in running sea-water, contractions of the retractor muscles were recorded by a thread through the top of the siphonoglyph. Condenser shocks were given by a slightly modified version of the stimulator used by Hall and Pantin (1937).

Two shocks, 1 sec apart, were given with the cathode in different positions. The latent period between the second shock and contraction of the retractor muscle was then estimated from kymograph records. Although a light spring lever was employed, at the drum speed used (22 cm/sec) the beginning of a contraction was not always clearly defined on the record. Errors due to this factor and to other inadequacies of recording technique are nevertheless small. Variation between different anemones, or in the same individual at different times, may also affect measurements of latent periods, but this similarly does not affect the order of general conclusions.

Fig. 5 summarizes the results. Latent period measurements were obtained in the column and sphincter from attached strip preparations. From these, speeds of conduction were calculated. Owing to the way in which the tissues of *Metridium* contract, the position of the cathode could only be measured to the nearest cm or so, and conduction velocities given in fig. 5, E are approximate. The results obtained nevertheless enable speeds of conduction in different parts of the anemone to be compared.

Speeds of vertical conduction in the mesenteries were obtained from animals divided vertically to the base, by stimulating at the top and bottom of the column on the side away from the recording thread (fig. 5, D). It is certainly higher than anywhere else in the anemone, and may perhaps exceed that of the fast tracts in *Calliactis*. The fact that the sphincter conducts no more rapidly than a vertical strip of column is unexpected by comparison with *Calliactis*, but agrees with the observed slower speed of the sphincter response in *Metridium* (Pantin, 1935*b*). It may thus be concluded that in both the anemones considered here, rapid vertical conduction in the mesenteries is associated with a well-developed retractor nerve-set, as compared to slower conduction on a sparser nerve-net in, for example, the column.

It may finally be noted that few properties of the actinian nerve-net have so far been correlated with its structure (but see Batham, 1956). It is not known, for example, how synaptic junctions between nerve-cells in a through-conduction system differ from those showing 'interneural' facilitation, although preparations such as that shown in fig. 1, C suggest that a retractor neurite may cross 50 others in the course of 1 mm; or whether nerve-endings differ in the sphincter and retractor from those in muscles which cannot give quick, facilitated contractions, or whether sensory neurites may innervate muscle-fibres directly as suggested by Parker (1919). The function of multipolar nerve-cells, if different from that of bipolars, is not known, and nothing is known of the histology of the sphincter region. It is therefore encouraging that in at least two different species of anemone, existing evidence supports the same conclusions.

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REFERENCES

BATHAM, E. J., 1956. 'A note on the mesenteric nerve net of the anemone *Metridium canum* (Stuckey).' Trans. roy. Soc. N.Z., **84**, 91.

BATHAM, E. J., and PANTIN, C. F. A., 1951. 'The organization of the muscular system of *Metridium senile* (L.).' Quart J. micr. Sci., **92**, 27.

—— —— 1954. 'Slow contraction and its relation to spontaneous activity in the sea-anemone *Metridium senile* (L.).' J. exp. Biol., **31**, 84.

—— —— and ROBSON, E. A., 1960. 'The nerve-net of the sea-anemone *Metridium senile* (L.): the mesenteries and column.' Quart. J. micr. Sci. **101**, 487.

HALL, D. M., and PANTIN, C. F. A., 1937. 'The nerve-net of the Actinozoa. V. Temperature and facilitation in *Metridium senile*.' J. exp. Biol., **14**, 71.

HERTWIG, O., and HERTWIG, R., 1879. *Studien zur Blättertheorie. Heft. I; Die Actinien.* Jena (Fischer).

OWEN, G., 1955. 'The use of propylene phenoxetol as a relaxing agent.' Nature, **175**, 434.

—— and STEEDMAN, H. F., 1958. 'Preservation of molluscs.' Proc. malacol. Soc. Lond., **33**, 101.

PANTIN, C. F. A., 1935*a*. 'The nerve net of the Actinozoa. I. Facilitation.' J. exp. Biol., **12**, 119.

—— 1935*b*. 'The nerve net of the Actinozoa. II. Plan of the nerve net.' Ibid., **12**, 139.

—— 1935*c*. 'The nerve net of the Actinozoa. III. Polarity and after-discharge.' Ibid., **12**, 156.

—— 1935*d*. 'The nerve net of the Actinozoa. IV. Facilitation and the "staircase".' Ibid., **12**, 389.

—— 1952. 'The elementary nervous system.' Proc. roy. Soc. B., **140**, 147.

PARKER, G. H., 1918. 'The rate of transmission in the nerve-net of the coelenterates.' J. gen. Physiol., **1**, 231.

—— 1919. *The elementary nervous system.* Philadelphia (Lippincott).

PASSANO, L. M., and PANTIN, C. F. A., 1955. 'Mechanical stimulation in the sea-anemone *Calliactis parasitica*.' Proc. roy. Soc. B, **143**, 226.