THE DOUBLE INNERVATION OF MUSCLES IN THE CLAM (*MYA ARENARIA*)

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(With One Plate and Two Text-figures)

IT is a matter of common observation that lamellibranch molluscs may close the valves of their shell by a fairly rapid twitch-like contraction of the adductor muscles, and may thereafter in certain circumstances relax the adductors, immediately allowing the valves to gape, while in other circumstances these muscles may be maintained for a considerable time in the shortened state even when large tensions are applied to the valves.

Bayliss *et al.* (1930) have shown that the two parts of the adductor muscle of scallops (*Pecten* sp.) have different physiological properties, the larger striated portion being responsible for the twitch-like contraction used in propulsion, while the small tough unstriated portion is very much slower both in contraction and relaxation and is responsible for the persistent closure of the valves. These authors seem to imply (*loc. cit.* p. 363) that a similar state of affairs may exist in the adductor muscles of other lamellibranchs. This is certainly not the case in the Dimyaria, where both the adductors and the other somatic muscles are homogeneous and consist of unstriated fibres, and it is therefore necessary to look for another explanation of their variable behaviour.

It is the purpose of this paper to show that certain of the muscles of the clam receive a double innervation by two groups of nerve fibres which can be recognized from the distinctive amplitudes of their action potentials and which produce different effects on the muscles. This double innervation may provide if not an explanation at least a clue to the equivocal behaviour of lamellibranch muscle.

The clam is in one respect particularly suitable for experiments on the neuromuscular system. In most lamellibranchs a peripheral "nerve net" is well developed, and reflexes can be elicited from small fragments of foot and mantle after all connexion with the central nervous system is severed. In the paper cited above an anomalous contracture of the "slow" muscle after removal of the visceral ganglion is described as occurring in many specimens, apparently in consequence of a local reflex of this kind (*loc. cit.* p. 369). In the clam, however, such peripheral ganglion cells seem only to occur in the syphon. The adductor muscles and the mantle muscles are completely inert once their central connexions are severed.

This paper is concerned particularly with the anterior adductor muscle and the muscle which withdraws the edge of the mantle in the anterior two-thirds of the animal and which I have called the retractor muscle of the mantle. Both of these muscles receive their motor nerve supply from the cerebral (cerebro-pleural) ganglia and the anatomy of the nervous system in the anterior region is shown diagrammatically in Text-fig. 1.

The anterior adductor muscle receives a motor branch from the anterior pallial nerve on each side. The retractor muscles of the right and left halves of the mantle are separately innervated by the right and left anterior pallial nerves respectively.
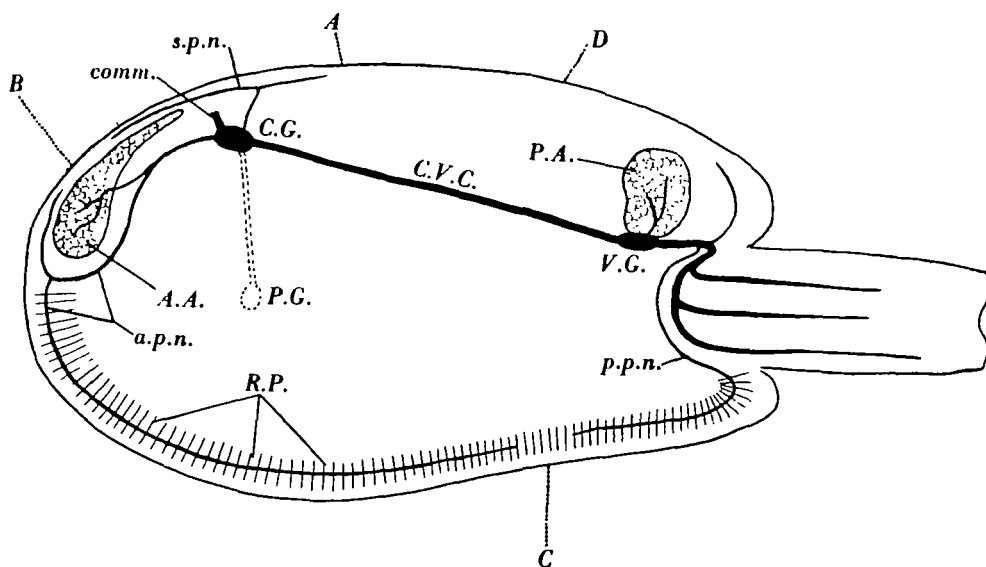
Text-fig. 1. Diagram of the relations of the nerves and muscles of the clam referred to here. *C.G.* cerebro-pleural ganglion; *V.G.* visceral ganglion; *P.G.* pedal ganglion; *comm.* cerebral commissure; *C.V.C.* cerebro-visceral connective; *A.A.* anterior adductor muscle; *a.p.n.* anterior pallial nerve; *s.p.n.* superior pallial nerve; *p.p.n.* posterior pallial nerve; *R.P.* retractor muscle of mantle; *P.A.* posterior adductor muscle; *A, B, C,* anterior sensory area; *C, D,* posterior sensory area.

Reflexly excited discharges in the motor fibres of the nerves may be elicited by tactile stimulation in two ways which give characteristically different motor effects. These effects are easily observed in an animal from which one valve of the shell has been carefully removed.

I. TACTILE STIMULATION OF THE ANTERIOR SENSORY AREA

The anterior sensory area (text-fig. 1, *A, B, C*) consists of the exposed edge of the mantle dorsally from the anterior adductor to the hinge and ventrally from the anterior adductor to within an inch of the origin of the syphon. The dorsal and ventral regions contribute afferent fibres to the superior and anterior pallial nerves respectively. In the ventral region at least there is no appreciable overlap of the fields of the right and left pallial nerves, though the edges of the two halves of the

mantle are fused everywhere except for a small aperture immediately ventral to the anterior adductor muscle through which the foot is protruded in digging.

Stimulation of any part of the anterior sensory area by touching it with a soft brush produces an efferent discharge in the anterior pallial nerve. The reflex centre is the cerebral ganglion, since the response is unaffected by the bilateral section of the visceral and pedal connectives. After section of the cerebral commissure the response is, as might be expected, confined to the side which is stimulated.

The character of the response varies with the vigour of the applied stimulus. This variation has been examined (a) by inspection and myographic recording of the activity of the muscles, and (b) by recording oscillographically the efferent discharge in the motor branches of the anterior pallial nerve. Three classes of response can be distinguished (by both methods of examination) which correspond to slight, moderate, and vigorous stimulation respectively.

Very gentle tactile stimulation results in a *slow* and incomplete shortening of the adductor and the mantle retractor muscles. The efferent discharge can be recorded oscillographically uncomplicated by the afferent discharge either by leading from the branch of the anterior pallial nerve which supplies the adductor or by leading from the mixed branches of the anterior pallial nerve which supply the retractor muscle of the mantle in the ventral region and stimulating the sensory area supplied by the superior pallial nerve. In both cases the response is seen to consist of an irregular discharge lasting a few seconds in fibres yielding very small action potentials [Pl. I, 4].

Moderate tactile stimulation (a single firm stroke of the brush) results after a perceptible reaction time in a relatively rapid contraction of adductor and retractor muscles followed by relaxation which is complete in 10–20 sec. Oscillograph records of the efferent discharge with moderate stimulation show an initial burst of activity in fibres giving action potentials of considerable amplitude (hereafter called large fibres). The discharge of the large fibres is complete in about $\frac{1}{4}$–1 sec. The discharge in the small fibres is more persistent, but has practically disappeared in 30 sec. (Pl. I, 1).

Vigorous tactile stimulation (by sweeping the brush over a large area or by pricking or crushing part of the mantle edge) also causes rapid shortening of both adductor and retractor muscles. It is sometimes observed that the shortening takes place in successive steps. Relaxation is much delayed and may not be complete in 3 or 4 min. Oscillograph records show an increased initial activity of the large fibres sometimes divided into successive short bursts. The activity of the large fibres save for a few isolated impulses is complete in 2 sec. or less. The discharge in the small fibres is much more prolonged and may continue with slowly diminishing intensity for 3–5 min. (Pl. I, 2).

These comparisons make it difficult to avoid the conclusion that the activity of the large fibres is necessary for complete and rapid contraction of the muscles, while the small fibres, though incapable of causing complete contraction, may by their activity greatly prolong a contraction initiated by the activity of the large fibres.

II. STIMULATION BY WAY OF THE CEREBRO-VISCERAL CONNECTIVE

Confirmatory evidence can be obtained by observing the reflex motor discharge to these muscles elicited by tactile stimulation of the syphon and of the posterior sensory region from both of which areas afferent fibres run to the visceral ganglion only. Such stimulation, however vigorous, fails to elicit more than a feeble contraction from the anterior adductor and the mantle retractor in the anterior region, and oscillographic records show that only the small fibre group can be reflexly excited in this way (Pl. I, 5). A more vigorous discharge, still limited to the small fibre group, can be elicited in the motor nerves by direct electrical stimulation of the cerebro-visceral connective, provided that the stimulus intensity is not too high.[1] If this procedure is followed the motor discharge is still inadequate to produce complete or rapid contraction (Text-fig. 2 (c)), but if the stimulation is maintained and at the same time moderate tactile stimulation is applied to the anterior sensory

(a)   (b)   (c)   (d)
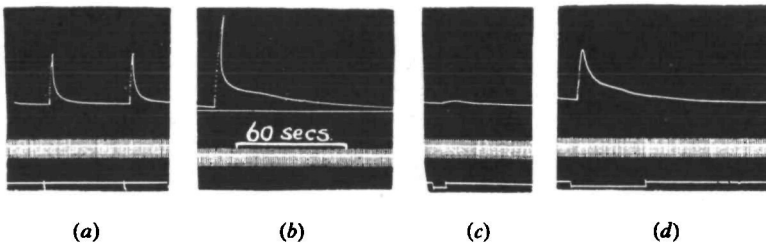
Text-fig. 2. Retractor muscle of the mantle, isometric recording, time signal: 1 sec. Lower signal: electrical stimulation. (a) Direct electrical stimulation of anterior pallial nerve, cut centrally (10 stimuli at 10 per sec.). Relaxation rapid; (b) reflex response to vigorous tactile stimulation anteriorly. Relaxation incomplete after 60 sec.; (c) reflex response to repetitive electrical stimulation (10 per sec.) of the cerebro-visceral commissure. Response slight and slow; (d) reflex response to slight tactile stimulation with concurrent electrical stimulation of the cerebro-visceral commissure. Relaxation delayed, incomplete after 30 sec.

area a normal reflex contraction of the muscle is elicited but relaxation is considerably delayed (Text-fig. 2 (b)).

Finally, if one of the motor nerves be cut and the peripheral end stimulated electrically, sufficiently to produce nearly maximal contraction of the muscle (about 10 maximal stimuli at the rate of 10 per sec.), it is found that relaxation occurs more rapidly than after the reflex contraction elicited by moderate stimulation of the anterior sensory area (Text-fig. 2 (a)).

Both groups of fibres are presumably excited by direct stimulation of the motor nerve, but the rapid relaxation may reasonably be associated with the absence of the prolonged after-discharge in the small fibre group, which is concomitant with the reflexly excited contraction.

There can therefore be little doubt that the activity of the large fibre group is necessary for full and rapid contraction, while the principal function of the small

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[1] Higher intensities of electrical stimulation applied to the cerebro-visceral connective set up an inhibitory state in the cerebral ganglion. This phenomenon will be described in another paper.

fibre group is to delay relaxation; and if this view is correct both groups must exercise their functions on the same individual muscle fibres.[1] No histological evidence can be adduced in favour of this conclusion. In fact the existence of "large" and "small" fibre groups can so far only be inferred from the magnitude and speed of conduction of their action potentials in the mixed nerve. The histology of the lamellibranch nervous system is technically difficult and no sections of nerve have yet been prepared sufficiently satisfactory for an estimate of fibre diameters to be made. But the physiological evidence is adequate to justify the main conclusions in default of histological evidence.

The concept of a double motor innervation is not new. Harreveld & Wiersma (1936) have demonstrated its existence in the muscles of certain crustacea by both histological and physiological methods, and the experiments of Young (1938) suggest a comparable condition in the muscle of the mantle of decapod molluscs. But in neither case do the functions of the two groups of nerve fibres seem to be closely analogous to the functions of the two groups described in this paper for *Mya*.

The function of the small fibre group of the clam is perhaps more comparable with that of the sympathetic innervation of vertebrate striated muscle first described by Ginetzinski (1924) and by Orbeli (1925), and confirmed by Maibach (1928).[2] Here stimulation of the sympathetic supply produces no contraction by itself but augments the contraction of fatigued muscle, elicited by stimulation of the fibres of the ventral roots and, moreover, prolongs the contraction.

SUMMARY

Two of the somatic muscles of the clam (*Mya arenaria*) are shown to receive a double motor innervation. The activity of one group of motor fibres, which yield action potentials of relatively great magnitude, is necessary to excite the muscle to complete and rapid contraction. The principal function of the second group which yield relatively small action potentials is to delay the relaxation of the muscle.

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[1] In this connexion it is worthy of note that Winton (1934) and later Fletcher (1937) have demonstrated that the smooth muscle of the lamellibranch *Mytilus* can exhibit two modes of contraction, namely, the normal summated response to repetitive stimulation accompanied by propagated action potentials, and a local unpropagated persistent contracture caused by stimulation by a constant current.
[2] For a general discussion see Kuntz (1929).
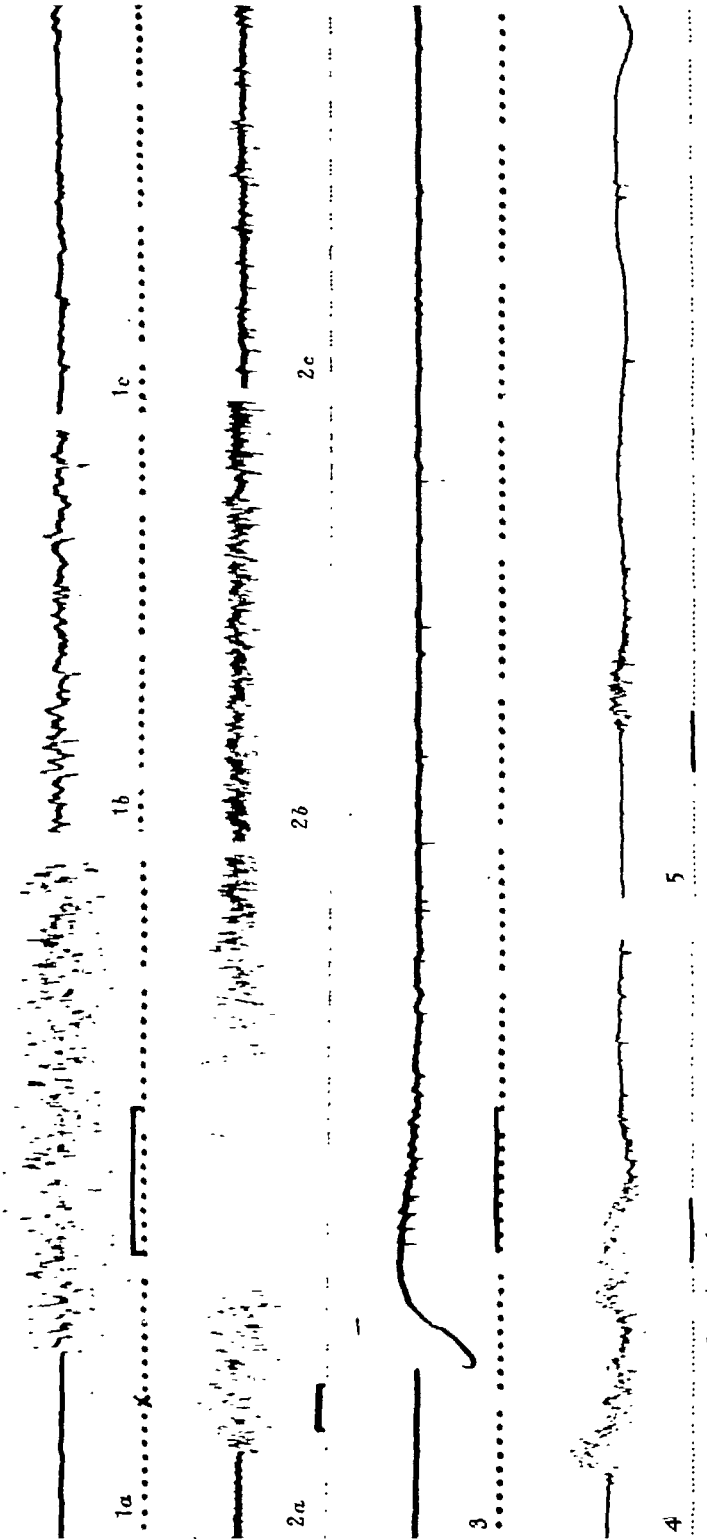
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(MYA ARENARIA) (pp. 500–505).

EXPLANATION OF PLATE I

All records from left motor nerve to anterior adductor muscle. In all records the heavy black line marks $\frac{1}{10}$ sec.

Fig. 1. Reflex response of whole nerve to moderate tactile stimulation of anterior sensory area. *a*, initial response; *b*, after 1 sec.; *c*, after 5 sec.

Fig. 2. Reflex response to vigorous tactile stimulation of the same. *a*, initial response; *b*, after 5 sec.; *c*, after 20 sec.

Fig. 3. Reflex response of subdivided twig of same nerve containing only one active "large" fibre and a number of "small" fibres. The stimulus in this case was a single submaximal shock applied to the peripheral part of the left anterior pallial nerve.

Fig. 4. Reflex response of whole nerve to very gentle tactile stimulation of anterior sensory area.

Fig. 5. Response of whole nerve to vigorous stimulation of posterior sensory area.