A NERVE-MUSCLE PREPARATION FROM THE SNAIL

By J. A. RAMSAY

From the Zoological Laboratory, Cambridge

(Received 30 October 1939)

(With Twelve Text-figures)

I. INTRODUCTION

In contrast to the Vertebrata, the Invertebrata as a whole do not lend themselves to the study of nerve-muscle preparations which can be isolated from the animal. The essential properties of a good nerve-muscle preparation are:

1. That it should be possible to prepare the muscle free from other tissues and with its ends normally connected to relatively inextensible structures, e.g. bone or tendon, to which writing levers may be attached; and

2. That it should be possible to prepare the nerve free from other tissues and that the nerve fibres in the nerve trunk should run direct to the neuro-muscular junctions without any other synaptic junctions intervening.

A nerve muscle preparation can be obtained from the snail (*Helix pomatia*) which, it is believed, very largely satisfies these conditions.

The muscle in question is the retractor of the buccal mass, a slip of the columellar muscle running from the columella to the ventral surface of the buccal mass. It varies in length from about 0.5 to 5.0 cm. according to the state of contraction. This slip is really double and is supplied by two nerves arising from the suboesophageal ganglion and entering the substance of the muscle near the buccal mass end. The anatomical arrangement is indicated in Fig. 1, and in a reasonably large snail it is possible to obtain a nerve trunk of about 1.0 cm. in length without undue stretching. Attachment can be made to the muscle without injuring it by making use of the buccal mass at one end and a small piece of the columella at the other.

The mechanical properties and heat production of this muscle have been studied by Bozler (1930a, b; 1931) and a certain amount of histological work has been done upon it by Plenk (1924). No work appears to have been done upon the nerve trunks supplying it, but the intra-muscular nerves have been described by Röchling (1922). In anticipation it may be mentioned here that the muscle responds to acetylcholine, and that neuro-muscular conduction is modified by such drugs as atropine, eserine and veratrine. In view of the recent advances in the humoral theory of neuromuscular conduction which have been made in studies of Vertebrate physiology,
this nerve-muscle preparation from the snail appears to afford an opportunity of seeing how far the principles applicable to the vertebrate system apply also in the Mollusca.

**Fig. 1.** a, columella; b, buccal mass; c, cut end of main columellar muscle; d, central nervous system; e, e., nerves to retractor of buccal mass.

**II. METHOD OF PREPARATION**

As already stated, this muscle has been used isolated from the snail’s body by Bozler (1930a) and in that paper he has described a method of its preparation. Since, however, the nerves were not prepared by Bozler, it is thought advisable to give an account of the method used in the present work.

The snail is first bled by cutting through the first whorl of the shell just above...
its opening and by puncturing the wall of the haemocoele. After the blood has drained out—usually about 4-0 c.c. are obtainable—the rest of the shell is cut away, leaving only that portion of the columella to which the muscles are attached. The snail is then pinned out and the dorsal body wall is opened by a slit from the visceral mass to the head. The viscera are removed as far as possible. The nerve collar is slipped forwards over the buccal mass (if it is not already in this position) and the buccal mass is lifted with a pair of forceps: this serves to separate the retractor from the rest of the columellar muscle and the main part of the muscle is cut through as near to the columella as possible. The small piece of columella with the muscle attached is then turned forwards.

The nerves are now exposed, as shown in Fig. 1. They are very thin and difficult to see except where they deform the surface film of moisture. In order to obtain as great a length of nerve as possible, the nerve collar is cut through on each side just dorsal to the origins of the nerves: this enables ligatures to be tied nearer the origins than would otherwise be the case. As ligatures, fine strands of thread are used, prepared by unravelling ordinary cotton thread into six strands. Manipulating these strands with fine forceps, one ties the ligatures and cuts away the surplus thread leaving only a piece about 1 cm. in length attached to each nerve. Each of these threads is held in turn in the forceps and the nerves are cut between the ligatures and the nerve collar: they are then laid forwards over the muscle.

An ordinary thread ligature is tied round the buccal mass anterior to the attachment of the muscle and another is tied to the columella. The preparation can then be cut away from the rest of the body.

Bozler reported that he was unable to find an artificial medium in which the preparation would behave satisfactorily and he was driven to use snails' blood to moisten the preparation. The same procedure has been followed in the present experiments.

III. APPARATUS

Various methods of mounting this muscle have been used from time to time, but for the present purpose it will be sufficient to describe two of these. The first, A, was used when it was desired to record the mechanical response only: the second, B, was used when electrical records were also required.

A. The arrangement is indicated in Fig. 2. The actual muscle chamber, a, was a small ebonite trough lined with wax, 6 cm. long by 1 cm. wide and of depth varying between 0.5 and 1.0 cm. The muscle was set in this trough and attached to it by the thread of the buccal mass ligature which was waxed on to the end of the trough. The buccal mass was further secured by a pin passing through it and into the ebonite. The thread from the columella passed through a groove at the other end of the trough and was attached to the writing lever.

The "base", b, of the apparatus was a piece of \(\frac{1}{2}\) in. ebonite, with a slot into which the trough could be slipped and in which it was held by a clamping screw. Into the base was fitted a brass tube, c, and upon this tube was mounted the
A Nerve-muscle Preparation from the Snail

adjustable carriage, \( d \), of the writing lever \( e \). In Fig. 2 an isotonic lever is shown, but this could readily be replaced by one of the "isometric" type if required.

There were two pairs of silver wire electrodes, each pair mounted upon an adjustable arm, and they could be made to dip into the trough. Only one such pair, \( f \), is shown in Fig. 2, and the adjusting device is omitted for the sake of simplicity. The leads from the electrodes were brought to four terminals upon the ebonite base. About 2·5 c.c. of blood were required to cover the muscle, and the nerve, when laid over the electrodes was also completely immersed in blood.

The whole apparatus was mounted upon the vertical rod, \( g \), of an adjustable stand and held in position by a tightening screw. The main advantage of the whole arrangement lay in the fact that the writing lever was carried upon the same support as the muscle, so that the whole system could readily be moved from one stand to another or could be raised and lowered without the necessity of having to adjust the connexion between the muscle and the lever.

B. For purposes of recording electrical changes it is not possible to have the preparation completely immersed in blood: and attempts to find a satisfactory moist-chamber technique, under which the nerve would remain viable for long periods, were unsuccessful. Apparatus B (Fig. 3) was developed to meet this difficulty, and its main feature was that a bath of blood, \( a \), in which the preparation was normally immersed, could be lowered temporarily while a determination was being made. The buccal mass ligature was tied to a silver plate, \( b \), which served as one of the recording electrodes. The columnellar end of the muscle was impaled upon a silver hook attached to an isotonic lever, \( c \), and this served as a second electrode. Besides these two electrodes, a silver pin, \( d \), could be inserted into the muscle substance at any desired point; there were thus three possible leads from the muscle, \( b \), \( c \) and \( d \). The silver plate, and the isotonic lever were mounted upon a rod, \( e \), with ebonite insulation (shown shaded in Fig. 3). The nerve was laid upon two pairs of fine gold or platinum electrodes, \( f \) and \( g \), which could be used either for

![Fig. 2. For explanation see text.](image-url)
stimulating or for leading off. One of these pairs was carried upon the rod $e$; the other was carried upon an independent mounting.

The whole apparatus, excepting the upper part of the lever, was covered with an earthed gauze shield having a small slit sufficient to allow of the lever's movement.

The mechanical response could be recorded graphically or, alternatively, optically. For graphical recording a writing point was attached to the top end of the lever and allowed to write upon a smoked drum with axis horizontal. For optical recording the writing point was replaced by a small gold bead. This was illuminated by a horizontal beam of light in the plane of movement and it reflected a point of light whose movements were recorded by a film camera. By arranging matters so
that the gold bead traversed the screen of a cathode-ray oscillograph it was possible to obtain a record of mechanogram and electrogram simultaneously. Both muscle and nerve were led off to the oscillograph together and since the action current of the nerve was over before the action current of the muscle began, simultaneous records of the nerve action current, the muscle action current and the muscle contraction were obtained (see Fig. 9). This arrangement was not altogether satisfactory. The movement of the gold bead allowed for the complete contraction of the muscle to be recorded: but as the speed of the film was necessarily fairly rapid and as the mechanical responses to individual stimuli were a very small fraction of the total possible contraction, the mechanogram was not altogether easy to interpret.

The methods used for stimulating the preparation have been: alternating current (50 cyc./sec.): induction shocks: condenser discharges controlled by metronome, commutator or by automatic neon lamp or thyatron circuits. These methods are sufficiently well known to require no special description here, but an account is given below of two more special pieces of apparatus used in this work.

1. The commutator. This was simply a split ring upon which rested four phosphor bronze brushes (A, B, C, D, Fig. 4). One of these, D, was mounted upon an arm and could be rotated about the axis of the commutator so that the distance apart of C and D, measured over the circumference of the commutator, could be varied.
I am indebted to Dr W. A. H. Rushton for bringing to my notice the possibilities of this device, which can be put to various uses: in the present experiments it was used in connexion with the circuit shown in Fig. 4 to give two shocks at variable time intervals apart.

In order to prevent mains ripple being picked up by the stimulating circuit and carried to the oscillograph, a small transformer was interposed in the electrode leads after they passed within the shield of apparatus B.

2. The multiple rheotome. This instrument is shown in Fig. 5. It is built up upon a standard kymograph drum as supplied by Messrs Palmer. A $\frac{1}{2}$ in. steel rod, $a$, is screwed into the base and on this is carried a $\frac{3}{4}$ in. rod, $b$, free to rotate upon $a$. The rod $b$ carries six knock-down keys of the type fitted to the Keith Lucas spring rheotome (Cambridge Instrument Co.). On the spindle, $c$, are carried six strikers. The spindle also carries a $360^\circ$ protractor, $d$. A pointer, $e$, and a peep-sight, $f$, are carried on rod $a$. The pointer is sighted through the peep-sight and the zero of the protractor is brought into line with it: one of the strikers is then lined up with the pointer. The spindle is now turned until the required angle is indicated on the protractor and the next striker is lined up. In this way the strikers can be set at various angles and the time interval between the openings of any two keys will be proportional to the angle between the strikers and inversely to the speed of rotation of the spindle.

This instrument has been used in conjunction with induction coils, but more satisfactory results have been obtained by the use of condenser discharges. Each key is interposed in the grid circuit of a thyratron valve and when this circuit is broken a condenser is allowed to discharge through the valve with a potentiometer in series.
The accuracy of this instrument is probably not high but it has been found sufficient for the present work.

I was fortunate in being able to make use of an apparatus built by Dr R. J. Pumphrey. It is expected that a description of this apparatus will appear shortly (Pumphrey et al. 1939) and it would not be proper for me to say more here than to indicate its capabilities. It consisted of an amplifier and cathode-ray oscillograph, the initial stages of the amplifier being direct-coupled or condenser-coupled according to the requirements of the experiment. It was also provided with a condenser-thyratron variable frequency stimulator and the sweep of the oscillograph could be automatically synchronized with each stimulus at all frequencies, so that the stimulus artefact and subsequent electrical response of the tissue could be viewed on the screen as a "stationary wave".

It is appropriate at this point to record my indebtedness to Dr Pumphrey. Not only did he spend much time explaining to me the use of the apparatus, but he also assisted me at many of the experiments and I have had the benefit of his advice upon many of the electrical and electro-physiological problems which have arisen in the course of this work.

IV. REPORT OF INVESTIGATIONS

Most of the snails used were obtained from France through the agency of Messrs Gaudin. Others were obtained from a chalk pit near Cambridge into which they were known to have been introduced some years ago. The stock was kept in a cold room—at about 5° C.—until required. About 48 hr. before the experiment the chosen specimens were brought into the laboratory and supplied with cabbage and water. It has been shown (Wells & Howes, 1934) that the snail's normal waking life exhibits a well-marked periodicity, and it was hoped that if this routine were adhered to, the animals would come to the experiment in approximately the same physiological state.

The preparation is very viable and has been known to respond to stimulation 72 hr. after removal from the snail.

Only one nerve (and therefore only one half of the muscle) was studied at any one time. In the earlier experiments the other half of the muscle was cut through, but, as this had no obvious effect upon the results, the practice was abandoned.

A. The nerve

As already mentioned, histological information about the nerve trunks supplying the retractor of the buccal mass is lacking. Other nerves in Helix have been studied, notably by Baeccker (1932) who gives some account of their structure. It can readily be observed that a sheath consisting of fibrous tissue and "Blasenzellen" surrounds the nerve trunk proper, from which the latter can be separated. The nerve trunk is a very delicate structure, having the consistency of a jelly. It is impossible to tease it out and if compressed under a cover-slip it bursts, exuding a mass of apparently
homogeneous protoplasm. According to Baecker, the shape of the nerve trunk is maintained by neuroglia fibres, forming a superficial membrane from which septa extend radially into the nerve trunk separating the nerve fibres into bundles of varying size. Still less information is available regarding the nerve fibres. Röchling (1922), using methylene blue, has noted that within the substance of the retractor muscle it is possible to distinguish two types of nerve fibre, one thick and lightly staining, the other thinner and more deeply staining, and that when branching occurs both types of fibre divided together. He compares this with the condition already known in the Crustacea.

Fig. 6. a, camera lucida drawing of nerve fibres in nerve trunk; b, camera lucida drawing of intra-muscular nerve fibres.
A nerve-muscle preparation from the snail

A number of methylene blue preparations have been made of the nerve fibres, both in the nerve trunk and in the muscle. In the nerve trunk it appears that there are two types of nerve fibre present: the thicker, less deeply staining type is 6–10μ in diameter, and the thinner more deeply staining type is 1–3μ. The apparent diameter measured in optical section varies slightly from one part of the fibre to another. This is due partly to variation in the true diameter, and partly to variation in the disposition of fibres which are not truly circular in cross-section: partly also to the beaded appearance indicated in Fig. 6a which is almost certainly an artefact.

Branching of the thin fibres has been clearly observed in the nerve trunk and appearances highly suggestive of the origin of the thin fibres from the thick have been seen, but it has never been possible to follow fibres for a sufficient distance along the nerve to be absolutely certain that the thick fibres eventually break up into the thin ones. The greatest number of thick fibres observed in any nerve trunk was three: the number of thin fibres was often too great to count.

Within the muscle the two types of fibre have been identified and the method of branching commented upon by Röchling has been confirmed (Fig. 6b). It should be emphasized, however, that appearances of this type are by no means universal, and that when the fibres are followed through the muscle the distinction between the two types diminishes, and they no longer are distributed in parallel. The thick fibre becomes gradually thinner and indistinguishable from the thin one; eventually both types become vanishingly thin. No evidence has been found of differentiated motor nerve endings in these preparations, and no mention is made of them in the literature.

No evidence has been found of nerve cells in the course of the nerve fibres either in the nerve trunk or in the muscle.

The nerve action currents elicited by electrical stimulation present rather a complicated picture. Usually it is of the following type. Suppose that the nerve is subjected to a series of shocks at a frequency of 10 per sec. As the intensity of stimulation is raised to the threshold value a single impulse appears. When the intensity reaches about 1.5 threshold a second impulse appears, arriving at the electrodes just after the first (Fig. 7b) and with still further increase a third impulse may appear after, and in addition to, the first two. But in some cases, as the intensity is increased, an extremely ragged volley is produced in which four or five impulses are suggested (Fig. 7a). The appearance of more than one impulse in response to a single shock may be attributed either:

(1) to the existence of nerve fibres with widely different conducting rates; or
(2) to repetitive discharges in some or all of the fibres; or
(3) to a combination of (1) and (2).

If a sufficient length of nerve were available it should be possible to decide whether the impulses are conducted at different rates or at the same rate: but it has not yet been possible to obtain measurements of any great accuracy. In one case when as great a length as 1.2 cm. of nerve was obtained, three pairs of electrodes
were placed upon it. The pair nearest to the muscle were used as stimulating electrodes and the action current could be led off by either of the other two pairs. From the records it was quite clear that the further from the stimulating electrodes, the greater the distance between the first and second impulses on the record. This, however, might be expected with repetitive discharges also, if the second impulse was started during the relative refractory period following the first: but the following arguments can be put forward in addition to suggest that repetitive discharge is not the sole explanation:

(1) As the intensity of stimulation is raised beyond the threshold of the second impulse, the amplitude of this impulse may exceed the amplitude of the first (Fig. 7b). It is improbable that the amplitude of a repetitive discharge will exceed that of the initial discharge in response to the stimulus.

(2) Records have been obtained in which, as the intensity is raised, the second impulse to appear on the record does so between the stimulus artefact and the first impulse: that is to say, it arrives at the leading off electrodes before the first impulse and therefore it cannot be a repetitive discharge in the same fibre or group of fibres.

(3) Not infrequently, as the intensity is raised to the threshold, two impulses appear ab initio (Fig. 7a).
In view of the differences in the diameters of the fibres in the nerve trunk, differences in conduction rate are to be expected. These facts suggest strongly that we are dealing here with nerve fibres of different conduction rates, but the possibility of repetitive discharge cannot definitely be excluded in all cases.

Since there is good reason to believe that we have two types of fibre in the nerve trunk, it is pertinent to enquire if these have different actions upon the muscle as has been shown for Crustacea (Marmont & Wiersma, 1938 and earlier publications therein referred to), for the cockroach (Pringle, 1939) and for the clam Mya (Pumphrey, 1938). It can only be stated, bluntly, that no physiological evidence even remotely suggestive of this conception has been obtained. Throughout most of the physiological work the histological differentiation of the nerve fibres was kept in mind, and any peculiarity of behaviour observed in the muscle was carefully studied from the point of view of double innervation. But in the end there was nothing to suggest that the response of the muscle, electrical or mechanical, was fundamentally different when the nerve carried two impulses or only one.

The following explanation is suggested as being in reasonable accordance with the facts as at present known. It is assumed that the nerve fibres leaving the central nervous system are of the thick type and that on their way to the muscle they divide up into fibres of the thin type: and that the level at which this division occurs varies from one fibre to another. It is not unjustifiable to assume further that the rate of conduction in the thick part of the fibre is more rapid than in the thinner branches. Thus if the nerve is stimulated at the central end and led off near the muscle, the impulse in a neurone which divides up near the muscle will reach the lead off before the impulse in a neurone which divides up early and so on. Further, this arrangement might account for an observation which can be made upon the results in general, namely, that, with the stimulating electrodes at the central end of the nerve and the leading off electrodes near the muscle, the nerve impulse volleys are more ragged than with the opposite arrangement.

If, then, the nerve trunk is composed of fibres differing widely in diameter and in physiological properties correlated with this, attributes of excitable tissues such as chronaxie, refractory period and rate of conduction cannot readily be defined except as maximum and/or minimum values.

**Chronaxie.** Lapicque (1935) has made determinations of chronaxie upon the nerve supply to the columella muscle of the snail. Contraction of the muscle was taken as index of nerve activity and single shocks were used. The value for chronaxie is given as 10 msec. and it is further claimed that the chronaxie of the muscle has normally the same value. When one remembers that in many invertebrate neuromuscular systems a single impulse in the nerve is not sufficient to cause visible contraction in the muscle, it is difficult to accept Lapicque's results without reservation. Attempts were made in the present work to determine the chronaxie of the nerves running to the retractor muscle: contraction of the muscle was taken as an index of nerve activity, but in this case series of shocks at various frequencies were used. It was found difficult to determine the rheobase satisfactorily: as the duration
of the shock was increased, the minimal intensity sank slowly but steadily until the
duration of each shock became of the same order as the interval between successive
shocks. It was decided that the contraction of the muscle was not a suitable index
of nerve activity for this purpose, and in the absence of an adequate moist chamber
technique attempts to use the nerve action current instead were not made.

Refractory period. Five determinations were made of the refractory period of
the nerve using the contraction of the muscle as index of activity. Two induction
shocks, at varying intervals controlled by the multiple rheotome, were given and the
intensity of the second shock could be varied. Strictly speaking, this method
determines the refractory period of the neuro-muscular system as a whole, but the
existence of a relative refractory period and its dependence upon the intensity of the
second shock may be taken as showing that the refractory period of the nerve is the
limiting factor.

<table>
<thead>
<tr>
<th>Absolute refractory period (msec.)</th>
<th>Relative refractory period (msec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>5.5</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Rate of conduction. In view of the short length of nerve available and the
slowness of the mechanical response of the muscle, no attempt was made to deter-
mine the rate of conduction by the classical technique of measuring the latent
period of contraction. From the records of the experiment referred to on pp. 105–6,
in which two pairs of leading off electrodes were laid upon the nerve, it is possible to
estimate the conduction velocities with an accuracy of about ±10%: they are
70 cm./sec. for the slow impulse and 140 cm./sec. for the fast impulse. These values
compare with values obtained by Jenkins & Carlson (1903) for the pedal nerves of
other Gasteropods:

- **Ariolimax columbianus**: 44 cm./sec.
- **Limax maximus**: 124 cm./sec.
- **Pleurobranchaea californica**: 78 cm./sec.

Mention might be made in passing of another finding by the same authors
(Jenkins & Carlson, 1904), that the rate of conduction is hardly at all affected by
stretching the nerve up to double its resting length.

**B. The muscle**

The histology of the muscle fibres of the snail has been described by Plenk
(1924) according to whom the retractor of the buccal mass is composed of typical
smooth muscle fibres, the total length of a stretched fibre being about 1 mm.
Bozler (1930) reports that his attempts at maceration were not attended with great
success, but that he succeeded in isolating fibres which in the stretched condition
were equivalent to half the length of the muscle, thus suggesting that there are two sets of fibres arranged end to end. This suggestion is adumbrated by the general distribution of the nerves, whose bundles tend to break up at the two ends of the muscle, and by the following observation. If the last 0.5 cm. of the columellar end of a stretched muscle is killed by heat, it is noticed that stimulation of the nerve evokes a contraction of the buccal end of the muscle only: this contraction is observed as far as the middle of the muscle, and beyond the middle the columella end remains relaxed.

Notwithstanding the precautions described on p. 103 and that only well-extended, active animals were taken, the muscles were often in obviously different condition. When the dissection was performed some of the animals showed no more than a few transitory contractions of the musculature, and when the muscle was set up with a small weight (2 g.) it extended to its full length in a few seconds. But others would respond to the first cut with a violent and persistent retraction, and when set up the muscle might take 20 min. to relax fully. This may have been due to high viscosity, but with good mechanical magnification it could be seen that there were also very small spontaneous contractions, though it could not be decided with certainty in all cases that these did not arise from the musculature of the buccal mass. Generally, but not always, the muscles of the first type, i.e. those which relaxed readily, responded well to a single shock: in the second type the response to a single shock might be minute, but facilitation was more pronounced than in the first type (see later).

Bozler (1930 a) obtained simultaneous electrical and mechanical records from this muscle. He stimulated it directly and led off the two ends to a string galvanometer. Fig. 8 is taken from his paper and it is clearly the record from a muscle belonging to the first type: some of his records show no sign of facilitation at all. Bozler was mainly concerned with the mechanical properties of the muscle and he showed (Bozler, 1931) (1) that the form of the record of fall in tension after contrac-
tion ("erschläffung") was the same as that for fall in tension after stretching ("relaxation"), and (2) that the rates of both these processes were greatly decreased by CO$_2$. In another communication (Bozler, 1930b) he studied the heat production and found $H/Tl=0.14$ as for frog's skeletal muscle. There is nothing abnormal in the mechanical properties of this muscle, and, in general, stimulation at a frequency of 10 per sec. will cause it to shorten completely against a load of 2 g.

The type of electrical record obtained from this muscle varies, as to some extent it must do, with the position of the electrodes. The most consistent results were

Fig. 9. The upper line shows the degree of shortening of the muscle; the lower line is the electrical record. Associated with each effective stimulus one can distinguish (1) the stimulus artefact, (2) the nerve action current (rapid, diphasic), (3) the muscle action current (slow, monophasic). These records are cuttings from a long series. Throughout a and up to stimulus no. 4 in $b$ the intensity of stimulation was gradually increased up to $2 \times$ threshold. Thereafter it was lowered to threshold—stimulus no. 2 in $c$—and increased again. Direct-coupled amplifier. A 1 Megohm variable resistance was used in series with the leads from the muscle and served to reduce the amplitude of its action current to the same order as that of the nerve. Electrodes $b$ and $d$ (Fig. 3), but columellar end of muscle not killed.

obtained in the following way. The columellar end of the muscle was killed with a jet of steam and the silver pin, $d$ (Fig. 3), was stuck into the muscle rather on the buccal side of the half way line. The other electrode was the silver plate, $b$, which was in contact with the buccal mass. In this way almost completely monophasic records were obtained. If prolonged stimulation is to be applied to this muscle it must be allowed to shorten, otherwise it tears itself away from its attachments. The process of shortening necessarily alters the disposition of the electrodes and may lead to modification of the electrical record: this is not readily to be avoided.

Bozler's figure shows a very distinct spike followed by a slower potential change of lesser amplitude. Records of this type have been obtained in the present work.
also, but not consistently. The spike is always present, and may show indications of a double nature as in the latter part of records \(a\) and \(b\), Fig. 10, or it may be followed by positive or negative after-potentials of varying duration. Even in the same preparation the form of the record is not consistent. Fig. 9 illustrates this very well: most records were very much more regular. Up to the present no correlation seems indicated between the precise form of the action current and the nature of the contraction.

It was mentioned above that neuro-muscular facilitation is evident in this preparation and that, like most of the other physiological properties, it is manifested in varying degree. In general, when two shocks are administered about 50 msec. apart the response to the two shocks is very much more than double the response to either alone. The relation of the interval between the shocks to the magnitude of the response has been studied from smoked drum records, but owing to the extremely small size of the contractions it was found more convenient to adopt the following modification of technique. Apparatus A was used with an "isometric" lever carrying a galvanometer mirror, and a beam of light was reflected from this on to a scale 5 ft. away. The excursions were read off the scale directly. The stimuli were condenser discharges controlled by thyратrons and the multiple rheutome, and the intensities of the two shocks were adjusted to give equal effects. The most consistent results were obtained with the following procedure. As zero, a point was chosen upon the scale which indicated a slight tension in the muscle: as the latter's

\[\text{Fig. 10. Electrical and mechanical records from muscle only. Three different frequencies of stimulation, intensity } 1.5 \times \text{threshold. Direct-coupled amplifier.}\]
gradual relaxation from a previous contraction reached this value the next pair of stimuli was given. The intervals between successive contractions were of the order of 30 sec. A representative graph reproduced in Fig. 11 shows an optimum interval of about 50 msec.; the refractory period is about 10 msec. and the facilitatory effect of the first stimulus is prolonged for well over a second. Other results suggest that where the response to a single shock is good the optimum interval is perhaps a little longer: the prolonged facilitatory effect is a feature of all the results so far obtained.

The electrical responses to two stimuli have also been studied. The muscle was mounted in apparatus B and the stimuli were given by the system illustrated in Fig. 4. Fig. 12 is a record of the effects of two stimuli given at increasing time-intervals apart. An interval of 11 msec. clearly falls inside the refractory period, but at 13 msec. a second spike appears and sums with the first to produce a peak not quite double the height of the single spike. As the interval is increased further and the second spike starts during the descending phase of the first, the total height reached becomes less and less and finally two separate and more or less identical spikes are shown.
This stage—at which the two spikes become identical in height—is reached when the interval between the stimuli approaches 100 msec. At shorter intervals it seems that some kind of summation does occur, but reference to Fig. 11 shows that the facilitatory effect of the first stimulus upon the contractile mechanism is prolonged over a much longer period than in the case of the action current mechanism, suggesting that these two mechanisms are not so closely associated as is often supposed. This suggestion is supported by other evidence: if a series of stimuli is given at high frequency the spikes rapidly dwindle in size, almost to extinction, while the contraction of the muscle is sustained more or less indefinitely (Fig. 10).

In another experiment the muscle was stimulated at a frequency of about 1 per sec. which gave normal action currents but a barely perceptible contraction. The frequency was increased to about 100 per sec. for half a second, and this reduced the action currents almost to nothing. The frequency was returned to 1 per sec. which was just sufficient to maintain the contraction which had developed in response to the higher frequency. Under these conditions the action current showed considerable recovery in about 2 sec.

No method has yet been devised whereby the muscle can be stimulated directly and the possibility of indirect stimulation via intramuscular nerves excluded.

V. DISCUSSION

It must be admitted that the results presented above are lacking in completeness and, in some cases, in precision also. But it is the object of this communication to present a brief survey of the behaviour of this nerve-muscle preparation and it was decided to publish the results in their present form before attempting further investigation of the various problems which have arisen and of which many may prove to be somewhat difficult. More especially this applies to the histological side where the question of double innervation cannot be considered as settled. Attempts to develop silver impregnation methods for studying the nerve fibres and their distribution failed completely and were abandoned. On the physiological side the effects of ions, drugs, etc. have barely been touched upon.

At first one might imagine that, of all the smooth muscles so far examined, the anterior retractor of the byssus of Mytilus (Fletcher, 1937a, b, c) would be the most similar in behaviour. This however, is not the case: there are several distinct differences in behaviour between the muscles of Helix and of Mytilus and not all these differences can be explained as due to differences in technique.

(1) In Mytilus the muscle fibres run the whole length of the muscle: in Helix probably not more than half-way.

(2) In Mytilus the motor nerve supply is as yet unknown and therefore there is no evidence of neuro-muscular facilitation as there is in Helix.

(3) With a series of stimuli at about 10 per sec. in Helix the muscle action currents rapidly decline: in Mytilus they show a staircase effect.

(4) In Mytilus alternating current and direct current elicit different mechanical responses: this does not appear to be the case in Helix.
The *Mytilus* muscle seems more comparable to the abductor of *Pecten* (Bozler, 1930a) and perhaps to the adductor of *Mya* (Pumphrey, 1938) than to the *Helix* muscle.

On the other hand the electrical records of Fig. 10 show an obvious similarity to the records published by Rosenblueth *et al.* (1936) for the nictitating membrane of the cat. Lambert & Rosenblueth (1935) in an earlier study have analysed the rather complex electrogram of this muscle, recognizing three components; I, an obvious spike, II, a slower and smaller potential change of much the same type, closely following upon I, and III a very slow potential change occurring much later. Eccles & Magladery (1937a, b) have also identified two potential changes, A and B, which may be the same as Rosenblueth’s I and II. In the latter part of records a and b, Fig. 10, it is evident that the spike is of a double nature and that as stimulation proceeds the second component appears to increase at the expense of the first. Bozler’s records (Fig. 8) also show two components, but so far no evidence of a third has been found in the snail. The interpretation of the electrogram of the cat’s nictitating membrane is still controversial (see also Bacq & Monnier, 1934), and in view of the relative lack of information about the snail’s muscle it would be unprofitable at this stage to discuss the findings herein reported in relation to the various theories advanced for the nictitating membrane.

Such investigation as has been made of the effects of ions and drugs upon this preparation is not worth reporting in detail. As mentioned in the introduction, this muscle is sensitive to acetylcholine. In the presence of eserine (1/15,000) it will respond to a concentration of 1/1,500,000 acetylcholine with a powerful and prolonged contraction; without eserine it will respond to a concentration of 1/800,000 acetylcholine. Bacq (1937) reports contraction of the foot in *Buccinum* to 1/50,000 acetylcholine. In a later paper by Bacq & Coppée (1937) eserine (1/50,000), atropine (1/1000) and curare (1/1000) were found to be without effect upon the contraction of the foot of *Buccinum* in response to stimulation of the pedal nerves. In *Helix* atropine in concentrations of about 1/1000 does have some effect in depressing the response to the nerve and to acetylcholine, but this is admittedly a high concentration. Crude curare is without demonstrable effect. Eserine in moderate concentrations (1/15,000) weakens the response to the nerve: in high concentrations (1/3000) it evokes spontaneous contractions of the muscle. Veratrine (about 1/300,000) causes repeated action currents and a sustained contraction in response to a single shock applied to the nerve. Precipitation of calcium by sodium oxalate gives rise to irregular spontaneous activity which can be suppressed by the addition of calcium chloride. Potassium in slightly increased concentration potentiates the response to the nerve: as the concentration is further increased the response to the nerve weakens and then the muscle goes into a state of contraction spontaneously. It should be emphasized that the findings recorded in this paragraph are based on a very limited number of experiments and require confirmation.

It is proposed, next, to investigate more fully the role of acetylcholine in neuromuscular transmission and to extend the range of the facilitation experiments to more than two stimuli. One of the main objects in presenting the results in their
A Nerve-muscle Preparation from the Snail

present form is to draw attention to the possibilities of this preparation and it is hoped that some of its problems will be considered worthy of investigation by other workers.

VI. SUMMARY

1. A nerve-muscle preparation from the snail and its method of removal from the animal are described.
2. Further description is given of the apparatus used for obtaining records of its activity.
3. The nerve appears to contain fibres which fall into two groups according to their diameters. It is suggested that the thin fibres are derived from the thick ones.
4. No physiological evidence of double innervation has been found.
5. There is clear evidence of neuro-muscular facilitation.
6. The muscle contracts in response to low concentrations of acetylcholine.

ACKNOWLEDGEMENTS

My thanks are due to Dr C. F. A. Pantin for suggesting the possibility of making the preparation described above, and to him and to Dr W. A. H. Rushton for reading through and criticizing the manuscript of this paper. I am also much indebted to Mr H. P. Whiting for advice about histological methods and for various reagents which he most kindly supplied. My thanks to Dr R. J. Pumphrey I have already recorded, but it is a pleasure to do so again.

REFERENCES

—— (1930b). J. Physiol. 69, 442.
Pumphrey, R. J., Schmidt, O., & Young, J. Z. (1939). In the Press.