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THE INITIATION OF ACTION POTENTIALS IN THE SOMATIC MUSCULATURE OF *ASCARIS LUMBRICOIDES*

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The results of previous work (DeBell, del Castillo & Sanchez, 1963; del Castillo, de Mello & Morales, 1963) led to the conclusion that the rhythmic action potentials controlling the somatic muscle layer of *Ascaris* are of myogenic origin, being generated at the muscle syncytium. As shown in Fig. 1, this is a strip of tissue formed by the interlacement of the terminal arborizations of the arms (or innervation processes) that each individual muscle cell sends to either the dorsal or the ventral main nerve cords.

It must be admitted, however, that such a conclusion was based largely on indirect evidence, since it had not been possible to obtain direct evidence on the excitability properties of the syncytium. Whereas the depolarization of the nuclear bag, or belly, of a muscle cell (see Fig. 1) gives rise to a single action potential, one should anticipate that the syncytial membrane would show little or no accommodation. Nevertheless, attempts to demonstrate this by injecting electric current into bellies close to the nerve cord had been inconclusive.

As described in this paper, we have recently been able to elicit repetitive discharge of action potentials by direct injection of polarizing current into the syncytial region. Moreover, muscle cells generating rhythmic impulses have been found close to the lateral lines. The influence of the membrane potential on the rate of firing of action potentials can easily be shown in these cells.

The present paper also describes observations on the manner in which the rhythmic depolarizations that occur at the syncytium give origin to action potentials in the terminal portion of the muscle cell arms. Furthermore, the influence of the nerve-cord fibres on the membrane potential of the syncytium and the frequency of those depolarizations has been investigated.

METHODS

Preparations and techniques. All the experiments have been performed on preparations of the body wall of *Ascaris lumbricoides* var. *suum.* Previous papers, in particular that of DeBell *et al.* (1963), should be consulted for details.

Solutions. 30% (v/v) sea water was used as the bath solution throughout this work; see table 1 of del Castillo & Morales (1967) for its ionic composition.

Histology. The photomicrographs shown in Pl. 1 were obtained from tissue fixed in 4% formaldehyde in phosphate buffer (pH 7·0), post-fixed in OsO_4 in 30% (v/v) sea water and embedded in Araldite within acrylic beads, following the technique

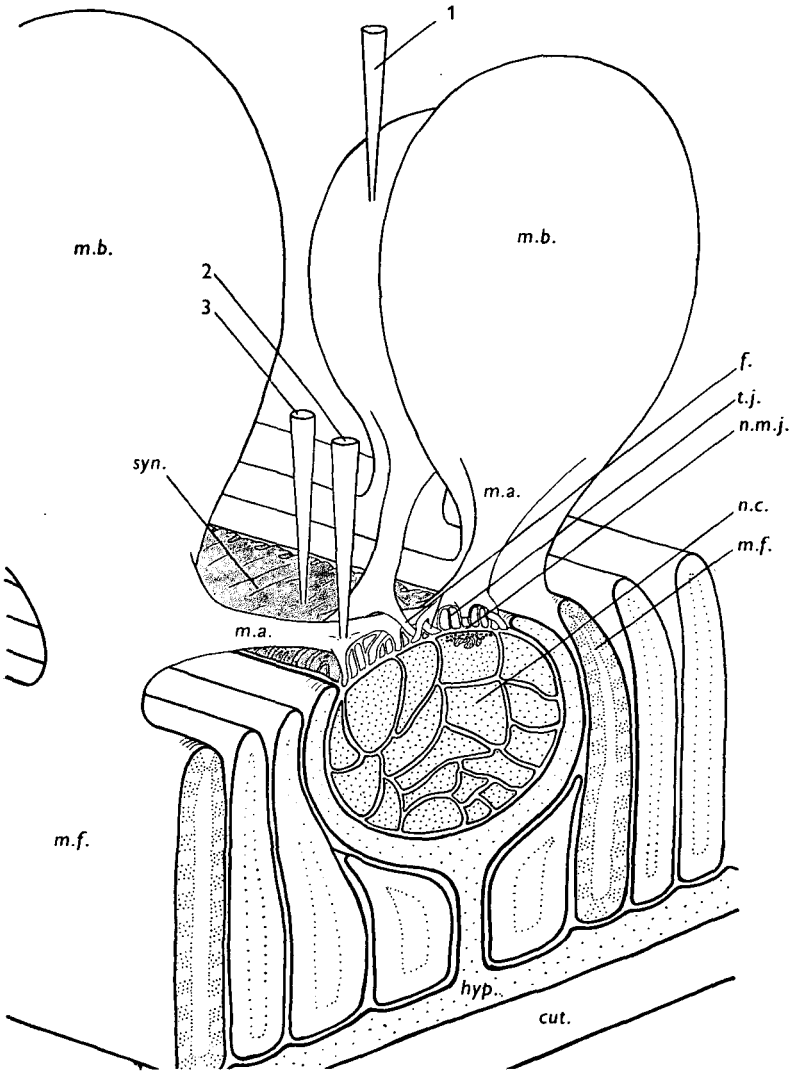


Fig. 1. Drawing based on fig. 1 of Rosenbluth (1965) and fig. 3 of DeBell (1965), illustrating neuromuscular relationships in *Ascaris lumbricoides*. A cross-section and a short segment of one of the main nerve cords (*n.c.*) is seen together with the associated muscle syncytium (*syn.*) drawn as a dark band parallel to the nerve fibres. As shown in the foreground, the syncytium originates from the entanglement of the terminal arborizations or fingers (*f.*) of the muscle arms, or innervation processes (*m.a.*) which each muscle cell sends towards one of the nerve cords. Adjacent muscle cells are electrically interconnected at this level into a functional syncytium across tight junctions (*t.j.*) between the fingers. Conventional neuromuscular junctions are established between the nerve fibres and the syncytium; one of them is represented in the drawing (*n.m.j.*). Large mitochondria and synaptic vesicles exist in the presynaptic cytoplasm. Four muscle fibres (*m.f.*) or muscle *spindles* are shown at each side of the nerve cord, but only three muscle *bellies* (*m.b.*) have been drawn. The nerve-cord fibres are embedded in a trough formed by hypodermal tissue (*hyp*). The lowest layer is the cuticle (*cut.*). 1 is a recording microelectrode inserted in a muscle belly and 2 and 3 indicate the probable position of the polarizing and recording microelectrodes in the experiments described in §§ I and V of the text. In our preparations the muscle syncytium is buried under several layers of muscle bellies. (The terms written in italics are those introduced by Cappe de Baillon, 1911.)

described by del Castillo, Chapeau & Lee (1966). Sections (1 μ) were cut with a Huxley-type ultramicrotome (Cambridge Instrument Company) and stained with methylene blue.

RESULTS

1. *Effects of direct current injection into the muscle syncytium*

Fig. 2. illustrates the effect of a pulse of outward membrane current injected, with help of a micropipette, into the belly of a quiescent muscle cell and recorded with a microelectrode inserted nearby in the same cell. In spite of the prolonged depolarization only a single spike is seen at the beginning of the pulse. The same is observed if both electrodes are inserted in the spindles and arms of the muscle cells.

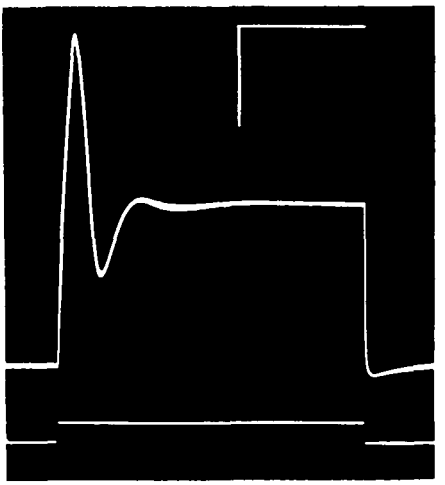


Fig. 2. Effect of a pulse of outward membrane current (4×10^{-7} A) injected into the belly of a somatic muscle cell of *Ascaris*. Upper trace is the transmembrane potential recorded with an intracellular microelectrode. The lower trace monitors the current injected with another micro-pipette inserted nearby. Calibrations: vertical, 20 mV.; horizontal, 10 msec.

In experiments performed on spontaneously active preparations the amplitude of the recorded spikes decreased when outward membrane current was injected into the muscle belly, but only small and inconsistent frequency changes could be produced.

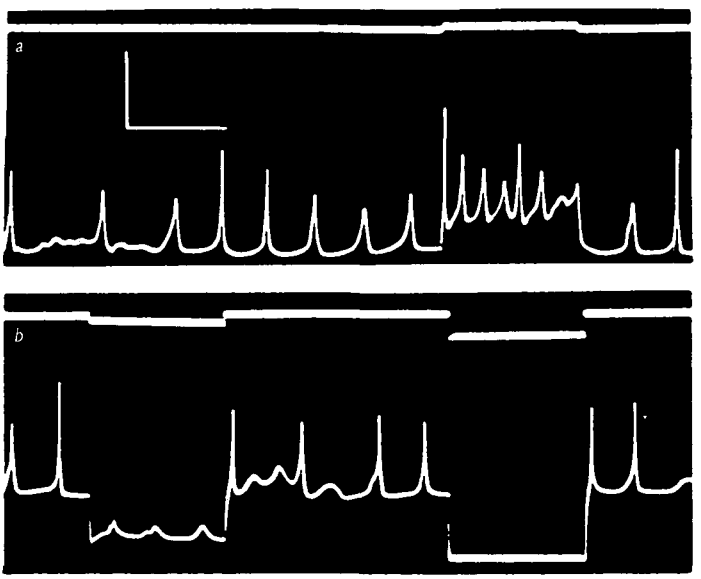
Inward membrane current applied in the same manner also decreased the amplitude of the recorded spikes, and with sufficiently high currents each spike was replaced by a small, slower depolarization on which a number of potential transients were superimposed (see fig. 14 of DeBell *et al.* 1963). These depolarizations and associated bursts of small transients occurred at the same frequency as the spikes.

Although there were reasons to believe that currents injected in this manner do reach the syncytium (see DeBell *et al.* 1963) our failure to influence the frequency of the spikes suggested that the ensuing potential changes across the syncytial membrane were not large enough, or did not extend over a sufficiently large area, to set up new foci of rhythmic spike activity. For this reason we attempted to inject polarizing current directly into the syncytium.

This proved to be difficult, not only because of the very small thickness of the

syncytium, but also because its deep position prevented visual control of the microelectrode tip. The procedure was to insert first the recording microelectrode in a muscle belly (see Fig. 1) and then to drive the polarizing micropipette towards the nerve cord, faintly visible as a dark line. This pipette was moved by very small steps synchronized with the sweeps of the cathode ray oscilloscope and a pulse of depolarizing current was applied with each sweep.

When the micropipette tip reached some critical sites (see Fig. 1 for probable position) electrotonic potentials accompanied by marked changes in the frequency of the action potentials were observed. This is illustrated in Fig. 3. The upper record



Fig, 3. Electrical activity recorded from a muscle belly close to the nerve cord, and changes induced by the injection of current into the syncytial region with help of a micropipette (probably located in positions 1 or 2 of Fig. 1). The upper trace in each record shows the direction and intensity of the polarizing current. Calibrations: vertical, 20 mV. and 2×10^{-6} A. (outward membrane current upwards); horizontal, 0·5 sec.

shows the spontaneous electrical activity, as well as the depolarization caused by an outward membrane current of about 0·1 μA. The spike frequency increased from 5 to about 10/sec. during the catelectrotonus. Two pulses of inward current were applied during the lower record; the first one (0·2 μA) produced a marked decrease in the amplitude of the spikes and the appearance of the small potential transients mentioned above. The second pulse (0·5 μA) caused a hyperpolarization of about 15 mV. and abolished the action potentials.

Similar effects could be observed in many insertions. However, the tip of the polarizing microelectrode could only rarely be kept in such positions for a sufficiently long time to explore the effects of a wide range of current intensities. Some of the records obtained in one of the most successful experiments are illustrated in Fig. 4 which shows that, in contrast to the very rapid accommodation exhibited by most of the muscle cell membrane, depolarization of the syncytial region was accompanied by prolonged changes in the frequency of the action potentials. Records *a–f* inclusive

in this figure show the effects of pulses of outward membrane current of increasing strength.

From *a* to *d* the frequency of the spikes increased with the intensity of the current, but remained almost constant with currents higher than 0·15 μA, as seen in records

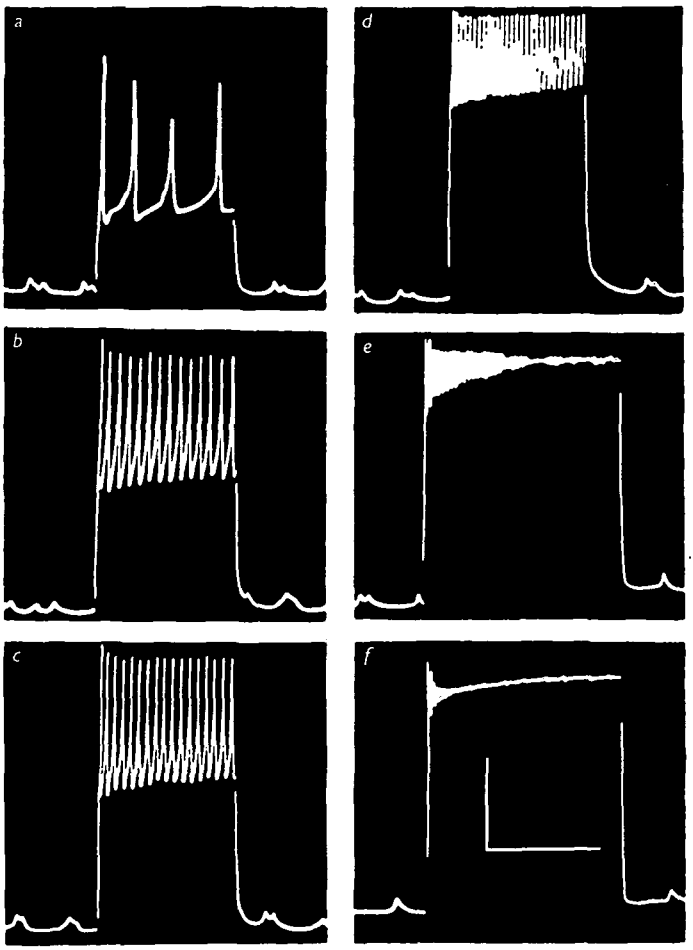


Fig. 4. Changes in the electrical activity recorded from a muscle belly close to the nerve cord caused by injection of current into the syncytial region (see Fig. 1 for probable location of the polarizing micropipette). To expand the range of depolarizing pulses a steady inward membrane current of about $0{\cdot}8 \times 10^{-7}$ A., which increased the resting potential to about 40 mV., was applied throughout the experiment. At this potential level each action potential was replaced by a burst of abortive spikes. Pulses of outward membrane current were superimposed on the d.c. current. As seen in the records, the resulting catelectrotonic potentials were accompanied by the firing of spikes. The intensity of the pulses in each record ($\times 10^{-7}$ A.) and the frequency of the spikes (per sec) are as follows: (*a*) 0·4 and 6·9; (*b*) 0·8 and 25; (*c*) 0·9 and 30; (*d*) 1·5 and 50; (*e*) 2·1 and 50; (*f*) 2·9, frequency of the oscillations was not measurable. Calibrations: vertical, 20 mV.; horizontal, 0·5 sec.

d and *e*. With the lowest current strengths there was some frequency damping, but as the current increased the intervals between the spikes varied little throughout the pulses. With the highest currents, a rapid inactivation, i.e. a damping in the amplitude of the oscillations, was observed.

No evidence was obtained in these experiments, indicating that the rhythmic generation of action potentials might be restricted to specific sites or areas of the syncytium. However, some correlation might exist between pacemaker properties and the presence of synaptic contacts.

II. *Impulse-generating properties of muscle cells adjacent to the lateral lines*

Working with preparations of the anterior end of the worm we observed that the frequency of the action potentials recorded from the muscle cells immediately adjacent to the lateral lines is very sensitive to changes in membrane potential. In quiescent preparations the injection of depolarizing current into these cells has effects similar to those seen in the syncytium, as shown in Fig. 5.

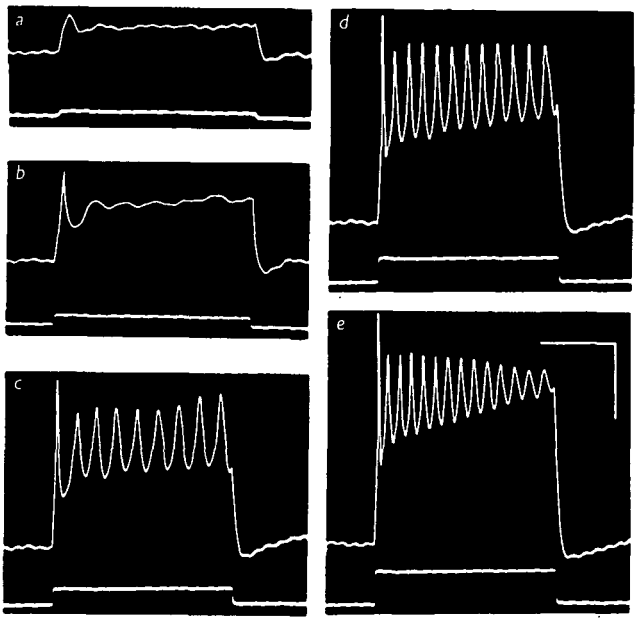


Fig. 5. Effect of depolarizing current pulses of increasing intensity on the electrical activity recorded from one of the muscle cells adjacent to the lateral line. Calibrations: vertical, 10 mV., and 4×10^{-7} A.; horizontal, 0·1 sec.

These observations are in apparent disagreement with previous results, suggesting that the action potentials were initiated at the muscle syncytium, where their frequency is under the nervous control. It was surprising, therefore, to see that spikes could also be produced at the lateral edges of the muscle field, far from the modulatory influence of the nerve cord.

A histological examination of that region revealed the presence of nerve fibres at both sides of the lateral line. Between the muscle fibres immediately adjacent to the lateral line (2 or 3 depending on the sections) and the rest of the muscle layer there exists a distinct gap (see Pl. 1 A, B). The hypodermal tissue protrudes slightly at this level, forming a shallow groove in which 2–4 nerve fibres are seen. These thin nerve strands appear to be those described by Goldschmidt (1909) with the name of sub-dorsal and subventral nerve cords.

We have not been able to discover, in our preparations, synaptic contacts between the muscle cells adjacent to the lateral lines (which belong to the type termed platymyarian; see Hyman, 1951) and the subdorsal and subventral nerve cords. Nevertheless it seems reasonable to assume that such connexions exist and control the activity of these cells.

Furthermore, the lateral nerve cords (i.e. the subdorsal and subventral) seem to be connected to a number of muscle cells situated between them and the medial nerve cords (the dorsal and ventral ones). In fact, the gap in the muscle layer above the lateral cords is occupied by arms of muscle cells converging towards these nerves. In some sections, as shown in Pl. 1 B, it can be seen how one of the cells internal to the gap sends an arm towards the lateral cord.

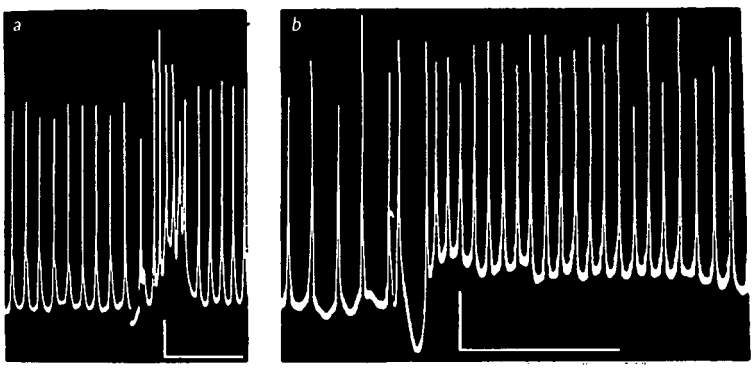


Fig. 6. Depolarization and increased frequency of the action potentials caused by the stimulation of excitatory nerve-cord fibres. Each of the two records shows the activity recorded from a muscle belly close to the nerve-cord region which was stimulated with a pair of wire electrodes. Record *a* shows a purely excitatory effect; a depolarization lasting for about 0·5 sec, accompanied by an increase in the frequency of the spikes. Record *b* shows an effect more commonly encountered; a hyperpolarization, due presumably to the stimulation of inhibitory nerve fibres, precedes the depolarization and increased frequency of firing. Calibrations: vertical, 5 mV. in *a* and *b*; horizontal, 1 sec. in *a* and 0·5 sec. in *b*.

III. *Modulation of myogenic spike activity by nerve action*

The results of pharmacological experiments (del Castillo *et al.* 1963, 1964*a*, *b*) led to the notion that the frequency of the action potentials generated at the syncytium is modulated by chemical transmitters released by the nerve-cord fibres. This view received support from the work of Hinz (1963) and Auber-Thomay (1964) in *Parascaris equorum* (former *A. megalocephala*) and Rosenbluth (1965) in *Ascaris lumbricoides*, demonstrating that synaptic junctions are established between the nerve-cord fibres and the terminal arborizations of the muscle arms.

The small size and tight packing of the nerve fibres makes their dissection exceedingly difficult. Inhibitory and excitatory effects can be produced separately by stimulation of the nerve cord region with a pair of wire electrodes. The effects of the applied pulses depend very critically upon the position of the electrodes and inter-electrode distance, as well as on the strength and direction of the applied current.

Facilitatory effects (see Fig. 6) are seldom observed in isolation; more commonly, they occur after a brief hyperpolarization due, presumably, to the simultaneous stimulation of inhibitory fibres. Record *a*, Fig. 6, shows one of the rare purely ex-

citatory effects, i.e. a depolarization accompanied by an increase in the frequency of the action potentials. In record *b*, the depolarization and enhanced spike frequency are preceded by a transient hyperpolarization with inhibition of the spike potentials. In both records the stimulus artifact is immediately followed by an action potential caused by the direct stimulation of the muscle syncytium (see DeBell *et al.* 1963).

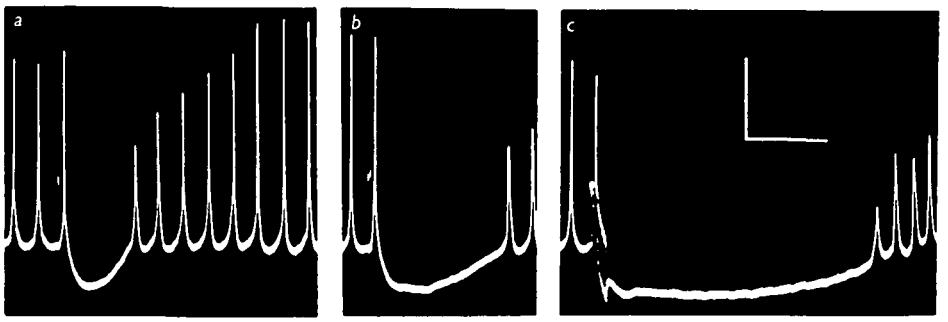


Fig. 7. Inhibitory effects elicited by electrical stimulation of the nerve cord. The records *a*, *b* and *c*, obtained with an intracellular microelectrode inserted into a muscle belly show spontaneous rhythmic action potentials and their suppression following the applied stimuli. Only a single pulse was delivered in record *a*, two in *b* and about eleven in *c*. Notice how the level attained by the membrane potential is the same in all the records, though the duration of the inhibitory effects increases with the number of applied pulses. Calibrations for all records: vertical, 10 mV.; horizontal, 1 sec. Resting potential about 30 mV.

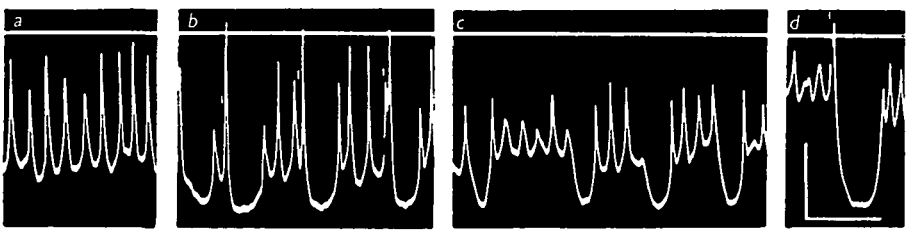


Fig. 8. Spontaneous inhibitory potentials following prolonged electrical stimulation of the nerve cord. Record *a* shows the spontaneous electrical activity recorded from a muscle belly before stimulation. Record *b* shows the effects of electric pulses applied to the nerve-cord region; each stimulus is followed by an action potential (due to direct electrical stimulation of the syncytium) and an inhibitory post-synaptic potential. Stimulation was maintained at a frequency of 1 sec. for about 4 min. (during record *b*, however, the stimuli were controlled manually). When the stimulation was stopped, spontaneous hyperpolarizations were observed (see record *c*); they can be explained as due to spontaneous firing of action potentials in the inhibitory nerve fibres. Soon after record *c* was taken the resting potential decreased spontaneously to about 17 mV. Stimuli applied to the nerve cord elicited inhibitory potentials which reached the same level as in record *b* (about 45 mV.). Calibrations in *d* for all records: Vertical, 20 mV.; horizontal, 1 sec.

Inhibitory effects, which are more frequently obtained, are illustrated in Fig. 7. Records *a*, *b* and *c* show the effects of applying 1, 2 and 11 stimuli, respectively, to the nerve-cord region. The inhibitory potential attained maximal amplitude following a single pulse (see record *a*). As the number of applied pulses was increased, the period during which the membrane potential was held at that level was prolonged from about 100 msec. to nearly 2 sec.

The amplitude of the inhibitory potentials recorded from muscle bellies varied

considerably, depending not only on the applied stimulus and resting potential, but also upon the length of the muscle arm and consequent electrotonic attenuation. Another important factor may be the distance between the syncytial end of the arm and the stimulated inhibitory synapses. For this reason the average value of the potentials is not very significant, the maximal levels of membrane potential elicited by inhibitory stimulation being more informative.

In a number of cells (see Fig. 8*d*) the peak of the inhibitory potentials reached 46 mV., a value in agreement with the average membrane potentials measured in *Ascaris* muscle under the influence of piperazine and γ-aminobutyric acid (GABA), compounds believed to activate inhibitory synaptic receptors. The membrane potential in presence of a 10^{-5} M concentration of GABA was of 46·3 mV., whereas in a concentration of piperazine of just over 10^{-3} M was of 45·2 mV. (see del Castillo *et al.*, 1964*b*).

There are reasons to believe that this potential level is determined by the equilibrium potential for Cl^- ions, an assumption further supported by the fact that in a Na-free solution of choline chloride the membrane potential, now under the exclusive control of Cl^- ions, increases to 47·9 mV. (del Castillo *et al.* 1964*c*).

In some preparations repeated stimulation of inhibitory nerve fibres was followed by spontaneous inhibitory potentials, as shown in Fig. 8. Record *a* shows the spontaneous action potentials recorded from the belly of a muscle cell. Record *b* shows the inhibitory potentials produced by stimulation of the nerve-cord region, and record *c* illustrates spontaneous hyperpolarizations after the stimulation was stopped. These negative potentials, which reach the same level as the inhibitory potentials which follow applied stimuli, are probably due to the firing of inhibitory nerve fibres as a result of damage or depolarization produced by the repeated stimulation. It should be noticed that such spontaneous polarizing potentials begin at different moments of the rhythmic action potentials, suggesting a complete independence from them. After record *e* was taken, the resting potential decreased spontaneously to about 17 mV. Stimuli applied to the nerve-cord region caused inhibitory potentials (see record *d*) which reached the same level as before, a membrane potential of about 45 mV.

IV. *Resumption of spike activity after cooling*

DeBell and Sanchez (unpublished observations) have shown that when the temperature is lowered from 40° to 20° C. the average resting potential of *Ascaris* muscle increases with a slope of 0·53 mV./°C. At the same time the frequency of the action potentials decreases, most preparations becoming silent when the temperature drops below 30° C.

To obtain more information on the manner in which the action potentials are initiated we have followed the resumption of electrical activity of cooled, quiescent preparations as they warmed up. Fig. 9 illustrates this process as recorded with a microelectrode inserted into a muscle belly close to the nerve cord.

When the temperature is lower than 30° C. the increased membrane potential, which in some cells may reach up to 46 mV., i.e. the inhibitory potential level, is extremely steady. The first sign of electrical activity upon warming up the preparation is the appearance of small, slow depolarizations occurring at long and variable

intervals. These depolarizations usually appear when the membrane potential decreases below 38 mV. As illustrated in Fig. 9*a*, a number of small potential transients are superimposed on each of such depolarizations.

As the temperature gradually increases and the resting potential decreases further, the slow depolarizations become larger and more frequent. At the same time the base-line becomes noisier and the size of the superimposed potential transient increases, as seen in records 9*b*, *c* and *d*, until they resemble small abortive spikes,

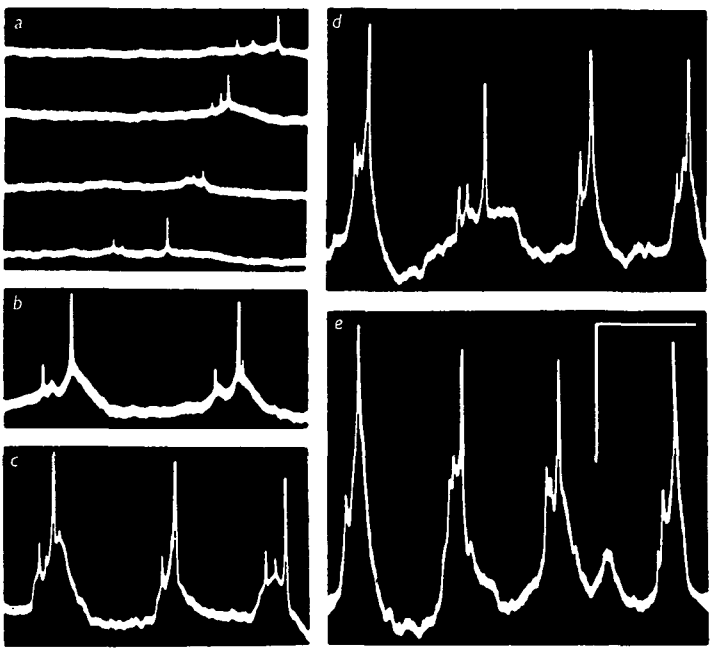


Fig. 9. Intracellular potentials continuously recorded from a muscle belly showing the resumption of spontaneous electrical activity when the preparation, which had been cooled to below 30° C., was allowed to warm up gradually. Records were obtained at the following bath temperatures: *a*, below 35° C.; *b*, 37 C.; *c*, 38° C.; *d*, 39° C.; *e*, 40° C. See text for explanation, and see also Table 1, which includes also data taken at 35·5° C, not illustrated here. Calibrations: vertical, 2 mV.; horizontal, 0·5 sec.

as in 10*e*. Soon after record 10*e* was taken, and without any further increase in the bath temperature, the small, separate abortive spikes merged into single, large action potentials, each of them superimposed upon one of the slow depolarizations.

The fact that the large action potentials originate from the union of a number of small, discrete transients is in agreement with the above-mentioned observations of DeBell *et al.* (1963) on the effects of membrane hyperpolarization on spike activity. Indeed, by raising and lowering the membrane potential with the injected current it is possible to transform each single action potential into a burst of abortive spikes and vice versa (see Figs. 14, 16 and 17 of DeBell *et al.* 1963).

The small transients recorded from any given cell as a result of increasing the membrane potential are characterized by a remarkable constancy. Indeed, if we examine a number of such bursts, as illustrated in Fig. 10, we see that the patterns formed by them are, in general, very similar. Those in Fig. 10*a*, for instance, begin

with the largest transient, whereas in those shown in 10*b*, recorded from other cell, the largest transients occur at the end of each burst.

Furthermore, a closer look at these and similar records reveals not only that the number of transients in each burst is relatively constant, but also that individual transients can be identified in successive bursts on the basis of their relative amplitude and time-course.

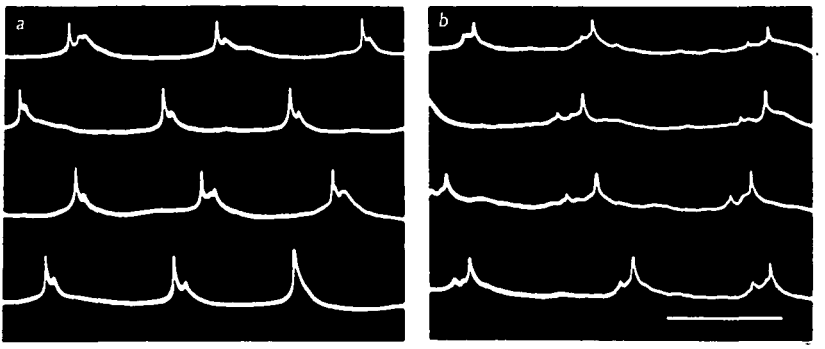


Fig. 10. Records showing a number of rhythmic depolarizations and superimposed bursts of transients taken from two different cells (*a* and *b*). Calibrations: traces are separated by 10 mV. steps. Horizontal bar in *b*, 1 sec. for both records.

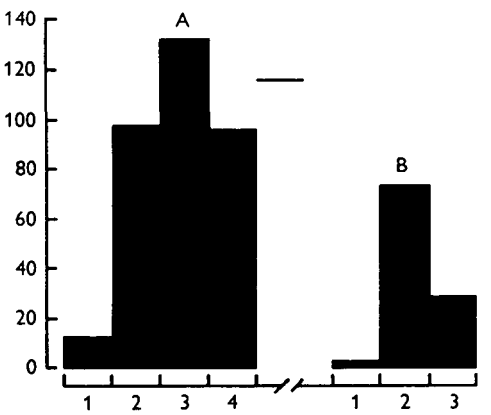


Fig. 11. Histograms showing the statistical distribution of the number of transients superimposed upon each depolarization in two different cells (see text). Abscissae: number of transients per depolarization. Ordinates: number of observations. Thickness of horizontal bar is equivalent to two observations.

The histograms of Fig. 11 show the statistical distribution of the number of transients per depolarization in two different cells. The highest number of transients per burst was 4 in cell A and 3 in cell B. However, the most frequent numbers were 3 and 2 respectively, and in a number of depolarizations only one transient was observed (see, for instance, the lowest trace in Fig. 10*a*). Such variations in number seem to be due to the random summation of single transients.

Although the absolute size of these potential transients depends on the size of the underlying depolarizations, their relative amplitude appears to remain constant, as shown by the measurements summarized in Table 1.

This table is based on records obtained in the experiment illustrated in Fig. 9. Seventy depolarizations were taken, ten for each different temperature, and the following parameters were measured: (*a*) amplitude, (*b*) frequency, (*c*) number of transients superimposed upon each depolarization, and (*d*) ratio of the amplitudes of two transients, one large and one small, which could easily be identified in each of the bursts. The average values, and the S.D., are given in the columns of Table 1.

Table 1. (See text for explanation.)

	Depolarization		Abortive spikes	
Temperature (°C.)	Frequency (c/sec)	Amplitude (mV.)	Number per depolarizations	Ratio of amplitudes (see text)
Initial temperature before cooling (38–39° C.)	1·06	0·76 (±0·16)	3·3 (±0·5)	2·55 (±0·26)
35°	—*	0·12 (±0·09)	3·0 (±0·5)	2·42 (±0·21)
35·5°	0·45	0·23 (±0·08)	3·0 (±0·5)	2·53 (±0·26)
37·0°	0·58	0·36 (±0·07)	3·0 (±0·7)	2·42 (±0·29)
38·0°	0·80	0·72 (±0·12)	3·1 (±0·6)	2·80 (±0·29)
39·0°	1·01	1·11 (±0·23)	2·9 (±0·6)	2·31 (±0·24)
40·0°	1·59	1·49 (±0·12)	3·0 (±0·7)	2·49 (±0·35)

* Not measurable in our records.

Figures in parenthesis are the S.D. of the mean.

It can be seen that the amplitude of the rhythmic depolarizations is a function of the temperature, increasing by a factor of over 12 when the temperature was raised from below 35° to 40° C. By contrast, the number of transients superimposed upon each depolarization remained constant, as does the relative amplitude of the two selected transients.

It should be emphasized, furthermore, that the order or sequence with which the different individual transients appear at each burst is also relatively constant as it can be seen in Fig. 10.

The various observations described in this section suggest that the bursts of potential transients superimposed upon each rhythmic depolarization are not the result of functional factors. On the contrary, the constancy in number, in individual time-course, in relative amplitudes and even in their sequence indicates that structural conditions are more likely to be responsible for their occurrence.

V. *Synaptic noise*

During the experiments described in the previous section, the membrane potentials of a large number of muscle bellies were recorded with high amplification. The baselines obtained were often extremely noisy, even if the microelectrode resistances did not change during the impalement. The noise was particularly large in preparations with a low average resting potential showing widespread spike activity (see Fig. 12).

In electrically quiescent preparations with higher resting potentials the amplitude of the noise was usually much lower. Occasionally, a small residual noise was observed which could be resolved into small discrete depolarizations barely higher than the electronic noise.

The largest of these small transients showed a fast rise and a slow decay. This time-course and the fact that no obvious rhythmicity could be detected in them were reminiscent of the most typical features of the miniature end-plate potentials (M.E.P.P.'s) recorded from vertebrate muscle. Therefore, the possibility was considered that such noise might be of synaptic origin, arising from the spontaneous liberation of depolarizing transmitter substance from the nerve-cord fibres.

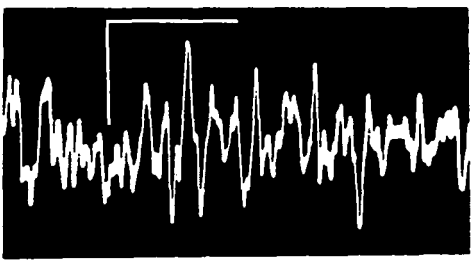


Fig. 12. 'Noise', recorded from a muscle belly situated over the nerve cord (see text). Resting potential was 20 mV. Calibrations: vertical, 2 mV.; horizontal, 0·5 sec.

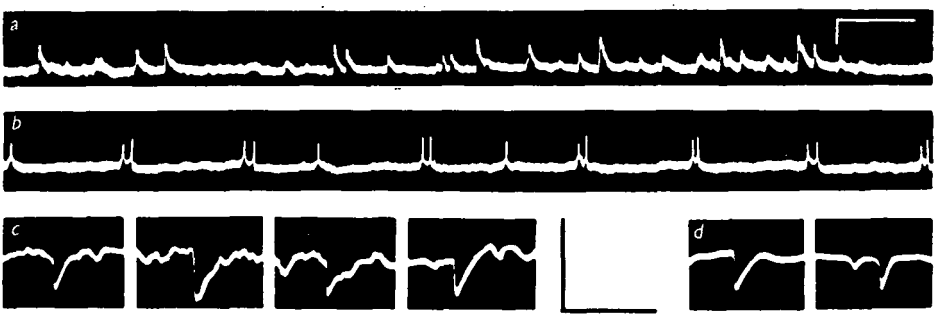


Fig. 13. Record *a* shows spontaneous depolarizing transients recorded with a microelectrode inserted into the syncytial region (see Fig. 1 for location). The possible nature of these transients is discussed in the text. Record *b*, taken at the same amplification and film speed shows, for comparison, small transients such as those illustrated in record *a* of Fig. 9. In this instance, however, the slow depolarizing component was very small. Records *c* and *d* illustrate hyperpolarizing transients with a time-course similar to those shown in record *a*. Because of their low frequency only a few selected potentials are shown, recorded from two different preparations (*c* and *d*). Calibration between *c* and *d*, which applies to all these records, is 1 mV. and 0·5 sec.

If the recording microelectrodes were driven directly towards the muscle syncytium, sites could be discovered where the amplitude of the discrete noise components was much larger than when recorded from the muscle bellies (see Fig. 13*a*). However, the recording tips could seldom be kept in those positions for more than a few seconds, suggesting that they were lodged either in the syncytial end of the muscle arms or, perhaps, within the syncytium itself (see Fig. 1).

Although the transients recorded from these sites (Fig. 13*a*) do resemble M.E.P.P.'s, it could not be ascertained on the basis of their appearance alone whether or not they are generated by spontaneous liberation of transmitter. Moreover, because of their fleeting character, we have not been able to apply the tests which helped to determine the random character and physical nature of the M.E.P.P.'s (see Fatt & Katz, 1952).

Indeed, the longest series of transients so far recorded, up to 125, is too short for a statistical analysis of the intervals. Furthermore, since depolarizations of this type can be recorded briefly in only a few sites in each preparation, it has been impossible to study systematically the influence of temperature, osmotic pressure and cholinergic drugs, factors known to influence either the frequency or the amplitude of M.E.P.P.'s. However, the results of experiments described in § IV are in general agreement with those obtained in vertebrate muscle; the noise does decrease when the temperature is lowered (see Fig. 9).

In the absence of quantitative information on the behaviour of the depolarizing transients under various experimental situations it will be useful to compare them with other potential changes of similar amplitude observed in *Ascaris* muscle. For instance, action potentials are recorded often with considerable attenuation, as a result of decremental conduction or local block in the arms of the muscle cell. Although the size of these potentials may be as low as 2–3 mV. they show a different time-course and a clear rhythmicity.

At other times small abortive spikes, such as those decribed in § IV, also with an amplitude of about 1 mV. are recorded on a rather flat base-line. However, they tend to occur in bursts, at regular intervals, and are superimposed upon slower depolarizations. A record illustrating such transients occurring singly and in pairs on particularly small depolarizations has been included for comparison purposes in Fig. 13*b*.

Another important difference between the depolarizing noise components and the small abortive spikes is the fact that the absolute size of the latter changes with the amplitude of the underlying depolarization (see Fig. 9). Contrariwise, an enlarged version of the potentials illustrated in Fig. 13*a* has never been observed.

All these observations lend support to the assumption that the noise recorded from the somatic muscle cells, arising at the syncytial region, may be of synaptic origin. However, further work is needed to determine its exact nature.

Discrete negative components with a time-course similar to the depolarizing components described above have also been observed in a few instances. A few o such negative transients are illustrated in records *c* and *d* of Fig. 13. The nature of these negative components is not known. It is tempting to suppose that they are due to the release of inhibitory neuromuscular transmitter, but this cannot be established on the basis of our very limited observations.

DISCUSSION

The results described above confirm our previous assumptions on the manner in which the rhythmic action potentials recorded from the somatic muscle cells of *Ascaris* are initiated.

The experiments in which the membrane potential at the syncytial region of the somatic musculature was decreased by direct current injection may be regarded as direct evidence on the myogenic origin of the muscle spikes. Indeed, this area of the muscle membrane shows little or no accommodation and generates continuously action potentials at a rate which is a function of the prevailing membrane potential.

In spontaneously active preparations the syncytial membrane appears to be maintained in a depolarized condition by the continuous release of small amounts of

excitatory transmitter (del Castillo *et al.* 1963). This process may be responsible also for the synaptic noise described in this paper.

Rhythmic oscillations of the membrane potential at the syncytial region, sustained by depolarization of synaptic origin and, presumably reinforced by electrical excitation, set up the action potentials in the arm of each individual muscle cell. Whenever this process fails to reach an all-or-none character, each of the large action potentials is replaced by a number of small, abortive spikes which are seen superimposed upon the crest of the depolarizations.

The individuality, constancy in number and relative amplitude of these potentials and the frequent repetition of the sequence in which they appear within each burst, suggests that structural factors, rather than purely functional ones, are responsible for their occurrence. Indeed, they can be explained in terms of the morphology of the muscle syncytium.

Rosenbluth (1965) has shown that each muscle arm divides, upon reaching the neighbourhood of the nerve cord, into a number of 'fingers' which in their turn branch into the thin terminal arborizations which form the muscle syncytium. As a consequence of this arrangement (see Fig. 1) the electrical excitation of each arm by the syncytial depolarization should take place at a number of different sites, i.e. at each separate 'finger'. It is likely, therefore, that when this process fails to elicit a full-size action potential the separate subthreshold excitation of each finger will be recorded as a small, independent, abortive spike.

Whereas the number of small transients recorded from any given cell should depend upon the number of 'fingers' of its arm, the relative amplitude and time-course of these transients is probably related to the length and relative diameter of each finger. Although the number of fingers at the end of each muscle arm has not been studied in detail, the illustrations published by Rosenbluth (1965) suggest that 3 is a common number. This is in agreement with our observations.

The mechanism proposed above is analogous to that envisaged by Katz (1950) to account for the step-like prepotentials and abortive spikes recorded from sensory axons close to muscle spindles in frog muscle. Katz suggested that the propagation of the 'newborn' impulses from the fine axon terminals into the main axon trunk meets, due to geometrical conditions, a lower safety margin than in the reverse direction. Delays or partial blocks preventing summation may therefore occur at the points of bifurcation. Similar circumstances would be responsible for the abortive spikes recorded from the muscle bellies in *Ascaris* whenever the safety margin for electrical excitation is reduced.

The fact that the intervals between the transients are not constant could be explained by variations in the rate of spread of the waves of depolarization conducted in the syncytium from the pacemaker sites, and also, perhaps, by differences in the pathways followed by each wave.

The observation that action potentials originate also at the muscle cells adjacent to the lateral lines suggests, by analogy with our observations in the main syncytium, that neuromuscular synapses are established between the subdorsal and subventral nerve cords and a number of muscle cells at the edges of both the dorsal and ventral somatic muscle fields. The simultaneous activation of such pacemakers at the same side of the worm would be responsible for movements of the body in a horizontal plane.

SUMMARY

1. The site and mechanism of initiation of the rhythmic action potentials controlling the somatic musculature of *Ascaris* have been reinvestigated.

2. Polarization of the muscle syncytium by direct current injection revealed little accommodation. Action potentials are generated continuously at this region at a frequency which depends on the membrane potential.

3. Excitatory and inhibitory nerve fibres control the membrane potential of the syncytial membrane and, therefore, the frequency of spike firing. The effects of stimulation of these fibres are described.

4. The resumption of electrical activity when cooled, quiescent preparations were warmed up was studied. The first signs of activity are slow rhythmic depolarizations on which bursts of abortive spikes are superimposed. When the amplitude of the transients in each burst increases sufficiently they unite into a large, single action potential.

5. Evidence is presented suggesting that each of the abortive spikes represents the separate, subthreshold excitation of one of the terminal branches of the muscle arm, due to a low safety margin for the conduction of impulses towards the muscle belly.

6. Small (1–2 mV.) spontaneous, apparently random, depolarizations and hyperpolarizations have been recorded with microelectrodes inserted into the syncytial region. Their possible synaptic origin is discussed.

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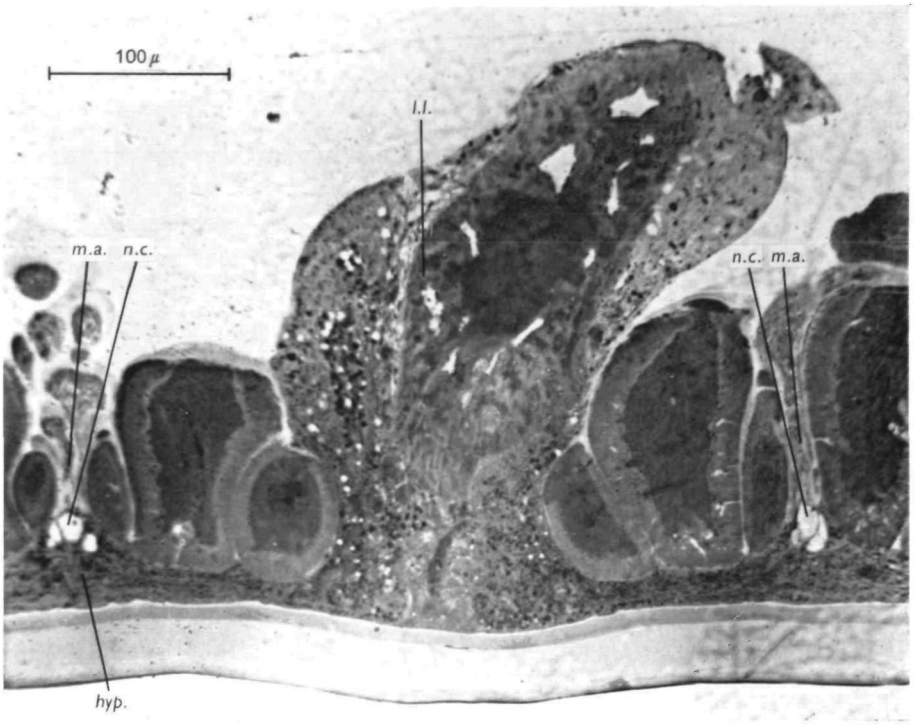
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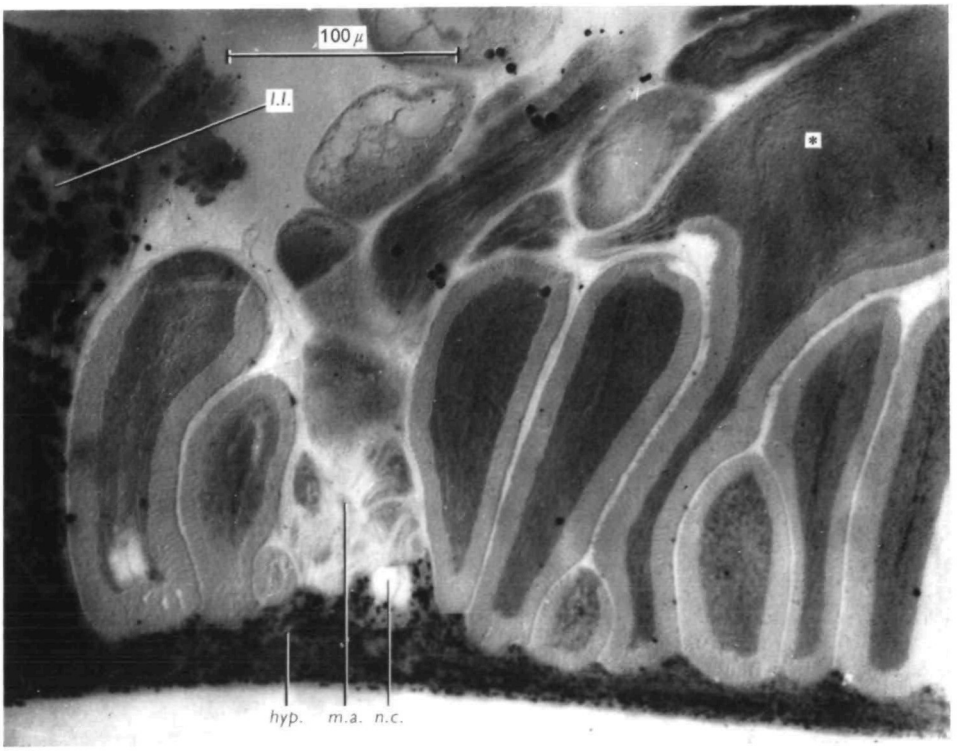
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EXPLANATION OF PLATE

A, Cross-section of the body wall of *Ascaris* showing one of the lateral lines (*l.l.*) as well as the lateral (subventral and subdorsal) nerve cords (*n.c.*) and several muscle cells. The nerve fibres lie in a shallow trough formed by two ridges of hypodermal tissue (*hyp.*). A gap can be seen above each nerve cord which is occupied by muscle cell arms (*ma*). B, Cross-section similar to A, showing only a part of the lateral line and one of the lateral nerve cords formed by two nerve fibres (*n.f.*). One of the muscle cells at the right of the microphotograph (marked by the asterisk) is seen to project its arm towards the gap above the nerve cord (the rest of the abbreviations are the same as for A).