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ANATOMY AND DEVELOPMENT OF THE SOMATIC MUSCULATURE OF THE NEMATODE *ASCARIS*

By A. O. W. STRETTON

* *Department of Zoology, University of Wisconsin, Madison, Wisconsin* 53706, *and M.R.C. Laboratory of Molecular Biology Cambridge, England*

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SUMMARY

The musculature of the nematode *Ascaris* has been studied by the examination of serial sections by light and electron microscopy. The muscle cells of nematodes are unusual in that they send branches to the neurones in contrast to the more usual situation in other animals where neurones send processes to the muscles. The neuromuscular synapses are made at the ends of the arms. Muscle cells receive multiple innervations and perform integration of the combined inputs. The action potentials are initiated near the ends of the arms so each arm acts as an integrative centre. It is shown that it is common for a muscle cell to have several arms, raising the possibility that each arm may integrate different combinations of neuronal inputs.

In the second larval stage the total number of muscle cells is 83. The adult has approximately 5×10^4 muscle cells. The very striking increase in cell numbers of the musculature is not matched by a corresponding increase in the number of cells in the nervous system. A model for the way in which a small number of neurones can co-ordinate the activity of an increasing population of muscle cells is presented.

INTRODUCTION

Nematodes, and in particular the human and pig parasite *Ascaris lumbricoides*, were recognized many years ago as animals of great potential interest for the study of nervous systems. Goldschmidt (1908) established that the number of neurones in the central nervous system was very small (162 in the head ganglion), and reproducible from animal to animal. It was his intention to try to understand how the system worked, but after he had completed his very detailed and careful anatomical work on the head and tail ganglia he realized that without physiological techniques for studying the function of recognizable elements, he could gain no further insight and abandoned the project. The necessary electrophysiological techniques are now available and it is now feasible to attempt to complete the original task of understanding the neuronal control of behaviour in terms of the functional anatomy of the nervous system.

The work reported in this paper is part of an attempt to understand how the nervous system of *Ascaris* controls the way the animal moves. Besides studying the details

* Present address.

of the inter-neuronal network and its electrical activity (which will be reported elsewhere) it is important to know the arrangement of the muscle cells that carry out the motor commands generated by the nervous system; in this paper the anatomy of the muscle cells and their neuromuscular contacts is described.

Anatomical background

The somatic musculature consists entirely of longitudinal muscles (Fig. 1); there are no circular muscles. The two lateral lines divide the body musculature into a ventral and a dorsal half (Fig. 2*a*). Each half is innervated exclusively by one of the major nerve cords (the dorsal or ventral cord) which divides each of the halves into two quadrants. In the anterior one third of the body, each half is also innervated by two minor nerve cords, the sub-dorsal or sub-ventral cords, known collectively as the sublateral cords (Fig. 2*c*). The most anterior muscle cells are innervated by the nerve ring, either exclusively or, as will be shown below, in conjunction with the major and minor nerve cords, The most common movements of the animal are the generation of waveforms caused by the concerted contraction of regions of the dorsal or ventral musculature. The waveforms, therefore, are in a dorso-ventral plane. When the waveforms are propagated either anteriorly or posteriorly, and the animal is in an appropriate physical environment (e.g. inside a tube of dimensions resembling those of a pig small intestine) locomotion occurs.

The role of muscle arms

The first part of this paper is concerned with the anatomical description of muscle cell lengths in different regions of adult *Ascaris*, and of the different ways the muscle cells may be contacted by the neurones that control them.

It has been known for many years that the innervation of nematode muscle is unusual. Schneider (1866) concluded that 'the nerves do not branch out to the muscles, but instead branches of the muscle cells reach the nerves'. The muscle arms often originate from the cell body which is generally expanded into a balloon-like structure, the belly (Fig. 2*b*). The arms may also extend from the contractile portion of the cell, the muscle spindle; the use of this term is somewhat unfortunate since there is no analogy with the sensory receptor organs of vertebrate muscles.

As each of the muscle arms approaches the nerve cord it divides into several small fingers which may then subdivide into finer processes which finally make contact with the neurones. Rosenbluth (1965) and Hinz (1963) have shown that the neuromuscular synapses are made at these contact points.

The physiological work of del Castillo and his colleagues (1967) has shown that the muscle cells of *Ascaris* resemble those of other invertebrates in that they receive multiple innervation, both excitatory and inhibitory. Some integration of the neuronal inputs is therefore performed by the muscle cells, unlike the typical situation in vertebrate skeletal muscle. The fingers at the end of the arms diverge fairly widely and then send smaller branches towards different parts of the nerve cord. Therefore, it is likely that these fine processes at the end of each muscle arm make contact with several different neurones, so that the electrical signals carried by the arms to the contractile portion of the muscle cell are related to the integrated activity of the inputs at the end of the arm. Since Looss (1905) had reported that in the nematode *Ancylostoma* each

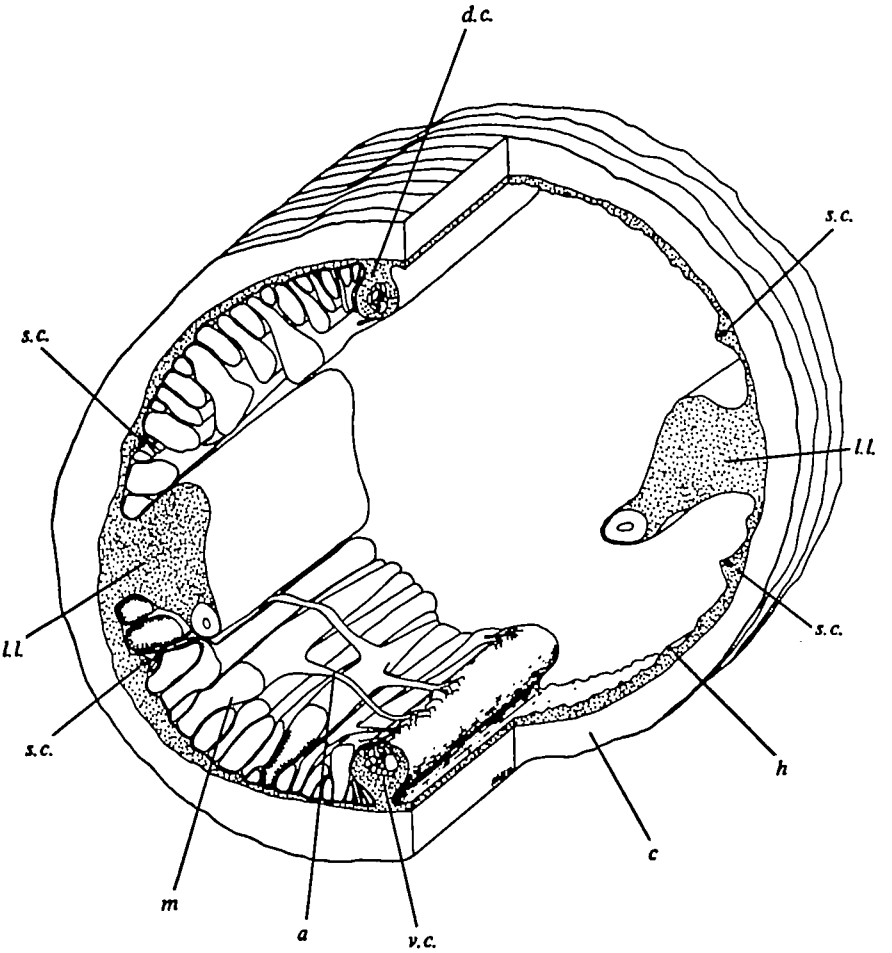

Fig. 1. Diagram of a short-length of the body of *Ascaris* in which the gut and the musculature on the right of the animal has been omitted. The diagram shows the relationship of the muscle cells to the nerve cords. *a*, muscle arm; *c*, cuticle; *d.c.*, dorsal cord; *h*, hypodermis; *l.l.*, lateral line; *m*, muscle cell; *s.c.*, sublateral cord; *v.c.* ventral cord.

muscle cell may send several arms to the nerve cords, it was interesting to see whether there were also multiple arms in *Ascaris*; each arm could represent a separate site where different combinations of inputs are integrated.

Development of the musculature

The second part of the paper is concerned with the development of the musculature from the larval to the adult forms. A study of the length and number of muscle cells in the adult and larva shows that the muscle cells increase dramatically in number as well as in size. The situation in *Ascaris* is contrasted with that in the horse pinworm, *Oxyuris equi*, where the increase in muscle cell number apparently does not occur.

In *Ascaris* the nervous system does not show an increase in cell numbers which parallels the change in the musculature. The nervous system, where cell number remains essentially constant, is required to control a population of muscle cells which

increases nearly a thousand-fold in number. The problem seems to have been solved by linking neighbouring muscle cells with electrical synapses to ensure that their activity is co-ordinated (DeBell, del Castillo & Sanchez, 1963).

MATERIALS AND METHODS

Adult Ascaris

Collection and maintenance of animals

Ascaris were collected from local slaughterhouses and transported to the laboratory in Kronecker's solution (9·0 g/l NaCl; 1·5 mM NaOH) at 37 °C, usually within ½ to 1 h of the death of the host pig. The nematodes were then rinsed with warm Kronecker's solution, and kept at 37 °C in either Kronecker's solution or *Ascaris* Ringer solution [24·5 mM KCl, 11·8 mM $CaCl_2$, 9·8 mM $MgCl_2$, 3·9 mM NaCl and 125 mM sodium acetate (after del Castillo & Morales, 1967)]. The worms were usually used for histology on the same day that they were collected.

Histological preparation for light microscopy

Whole worms were cut transversely into pieces 0·8–1·0 cm long. In order to recognize the polarity (anterior–posterior) of each piece, the posterior end was marked by dipping into a 4% solution of Procion Red M2BS, Procion Blue M3GS Procion Green M2BS or Procion Black. These Procion dyes bind covalently to macromolecules and so survive histological procedures; the polarity can be checked immediately before embedding so that subsequent sectioning can proceed in the same direction through each fragment of the worm.

All histological procedures were carried out at room temperature, except where noted. The fixative used routinely was a freshly prepared mixture of 5 ml double strength *Ascaris* Ringer, 2·5 ml water, 2·5 ml 25% glutaraldehyde and 0·2 ml acrolein. The tissue was fixed overnight, rinsed several times with *Ascaris* Ringer solution, and dehydrated in a methanol series of seven steps to absolute methanol, each step lasting 30 min. After two 1 h soaks in 100% methanol, the material was transferred to 1:1 methanol:propylene oxide for 30 min, then to pure propylene oxide for 1 h. Infiltration of the plastic used for embedding was a prolonged process in which the proportion of plastic to propylene oxide was increased from 30 to 100% in three steps over a period of two days. Freshly mixed plastic was then used for two more soaks in pure plastic at room temperature, and then for two further treatments each for about 1½ h at 60 °C in a vacuum dessicator to eliminate air bubbles. Finally, the tissue was transferred to fresh plastic in aluminium boats and polymerized at 60 °C for 12 h. The formula used for the plastic was Epon 812, 23 ml; dodecynl succinic anhydride, 18 ml; nadic methylanhydride 9, ml; tris (dimethylaminoethyl) phenol, 0·75 ml (Ladd Research Industries).

In cases where the tissue was post-fixed in osmium tetroxide, the second treatment with Ringer following the aldehyde fixation was replaced by a wash in 0·1 M phosphate buffer pH 7·4 for 1 h. After 5 h in 1% osmium tetroxide in 0·1 M phosphate buffer, the tissue was washed with 0·1 M phosphate buffer and then dehydrated and embedded as described above.

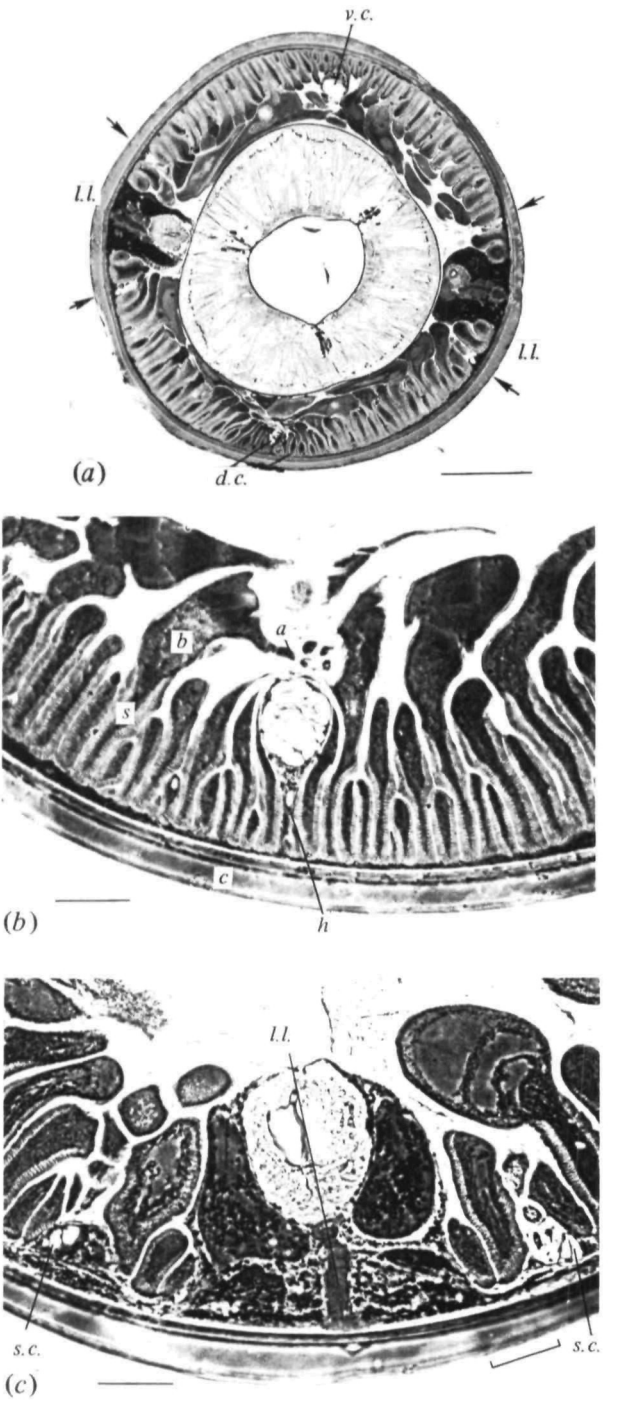

Fig. 2. Light micrographs of transverse sections of *Ascaris*. (*a*) Entire section. *l.l.*, lateral line; *v.c.*, ventral cord; *d.c.*, dorsal cord. The position of the sublateral cords is indicated by the arrows. Bar = 300 μm. (*b*) Detail in the region of the ventral cord. *s*, muscle spindle; *b*, muscle belly; *a*, muscle arm; *h*, hypodermis of the ventral cord; *c*, cuticle. Bar = 50 μm. (*c*) Detail in the region of the lateral line. *l.l.*, lateral line; *s.c.*, sub-lateral cords. The brackets indicate the muscles of the outer octant. Bar = 50 μm.

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Serial 10 μm sections were cut with a steel knife, transferred onto albumenized or gelatinized slides, and sometimes stained with Toluidine Blue before mounting in Permount (Fisher Chemical Company). The sections were examined with a Zeiss microscope at magnifications of 250 ×–400 × using phase optics.

Oxyuris equi

Specimens were obtained through the kindness of Dr Morris Round, Equine Research Laboratory, Newmarket, England. After transporting to the laboratory in 0·9 % saline at 37 °C, the worms were each cut transversely into five pieces which were put into 1 % OsO_4 in sodium phosphate buffer, pH 7·4. Subsequent dehydration and embedding was carried out as described in *Ascaris*.

Larval Ascaris

Ascaris larvae were obtained by the method of Fairbairn (1961). To release the eggs and to remove non-gamete material, the vaginal third of the uterus of adult females was disrupted in 0·1 N NaOH in a homogenizer with a loosely fitting plunger. The eggs were washed with several changes of 0·1 N NaOH, collected by centrifugation, and transferred to 0·1 N H_2SO_4. After being shaken gently for 20 days at 30 °C, the larvae which had developed were released from their shells by squeezing the eggs between a microscope slide and cover glass, The animals were fixed in 1 % osmium tetroxide in 0·1 M phosphate buffer, pH 7·4, washed in 0·1 M phosphate buffer, and then embedded in agar; each animal was cut out in a small cube of agar to facilitate handling, and then dehydrated and embedded in Epon by the usual procedure. Serial 50 nm sections were taken with an LKB Ultratome III, mounted in ribbons on formvar-coated copper grids and stained with uranyl acetate and lead citrate, and examined in an AEI EM6 electron microscope. The block face was trimmed so that one edge had a notch. This enabled the polarity of each ribbon of sections to be determined. Usually three ribbons of 10–15 sections were mounted on each grid. To determine their relative order, low power electron micrographs were taken of the sections at each end of the ribbon (or as close to the end as possible if the last sections lay on a grid bar), and the continuity established by image matching. In most cases there was no ambiguity in the order of the ribbons on a grid, nor in establishing the continuity between grids.

RESULTS

Adult Ascaris

Fig. 3 shows the reconstruction from light micrographs of serial 10 μm sections of the musculature of the anterior 4 mm of a ventral quadrant taken from an adult female *Ascaris*. Every muscle cell which originated within the first 2·5 mm was traced starting at its anterior end. All muscle cells are mononucleate, confirming earlier reports. Posterior to the first 2·5 mm other cells not shown in the diagram were present: in this region of the worm the number of muscle cells in any transverse section increases markedly as one proceeds in a posterior direction.

Within each quadrant, the musculature of *Ascaris* is arranged in two unequal portions: a smaller, outer (i.e. more lateral) octant, bounded by the lateral line and the sub-lateral nerve cord; and a larger inner octant, bounded by the sub-lateral cord and

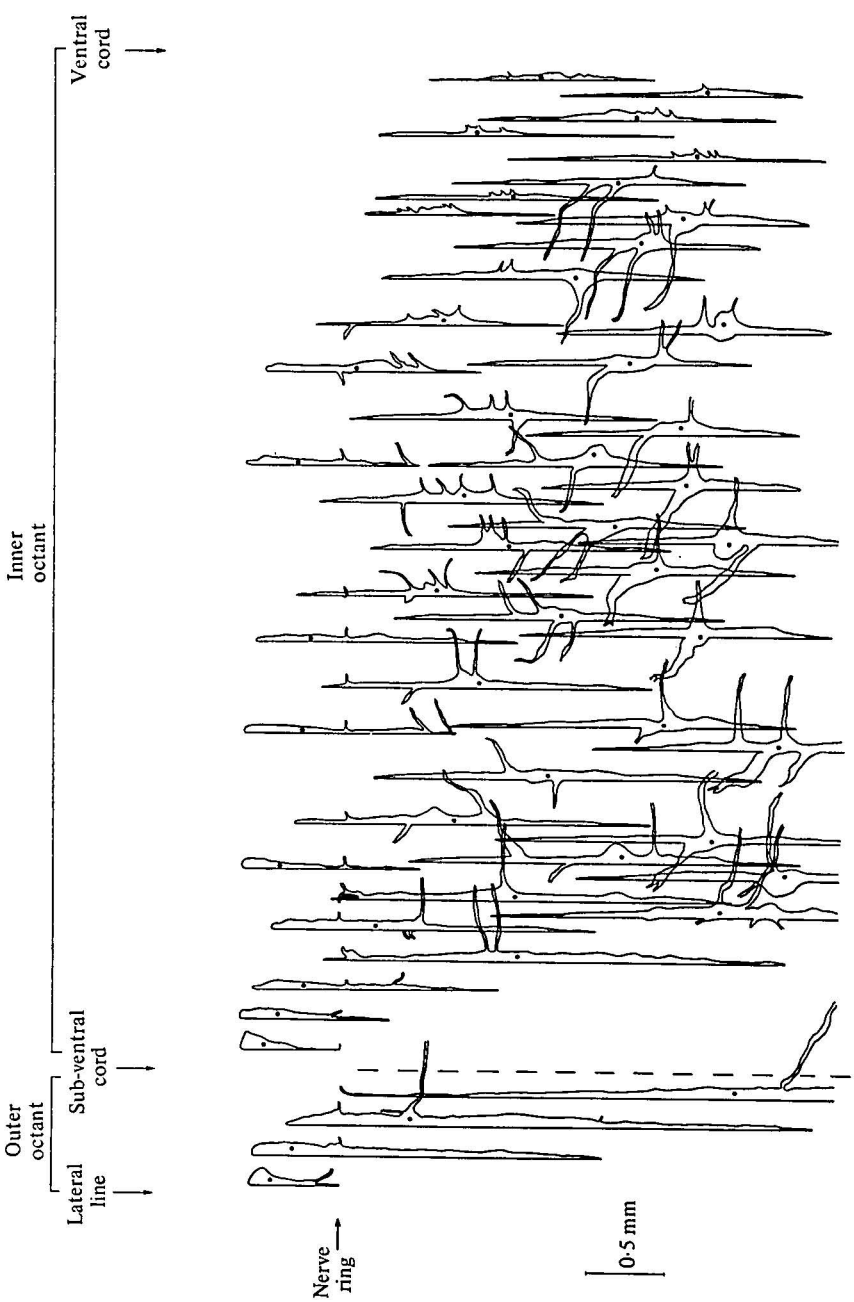

Fig. 3. Reconstruction from serial transverse sections of the 50 most anterior muscle cells in a ventral quadrant of *Ascaris*. Each cell outline is recorded as a projection onto a plane tangential to the cuticle at the base of the cell. The relative longitudinal and lateral positions are shown correctly, but cells have been separated laterally for the sake of clarity. For cells between the ventral and sub-ventral cords, all muscle arms shown to the left of the cell have been traced to the sub-ventral cord, and arms to the right to the ventral cord. For cells between the lateral line and the sub-ventral cord, arms which are shown to the right reach the sub-ventral cord except those shown crossing the cord, which reach the ventral cord. All cells shown giving rise to arms at the level of the nerve ring send these arms into the ring. The anterior part of the animal is at the top of the diagram. The filled circles show the position of the nuclei.

the major (dorsal or ventral) nerve cord. The sub-lateral cord does not extend anteriorly beyond the nerve ring, so the head musculature of the outer octant is defined by reference to the lateral line (which does extend anterior to the ring) and to the other muscle cells. Only the first cell of the outer octant fails to extend beyond the origin of the sub-lateral cord, and its position relative to the other muscles of the outer octant naturally leads to its being included with them, rather than with the cells of the inner octant (Fig. 3).

Muscle arms

The head musculature is defined as those muscle cells which send processes into the nerve ring. In the outer octant two muscle cells are innervated only by the nerve ring, and two by the nerve ring, the sub-lateral nerve cord and the major nerve cord. In the inner octant, there are four cells innervated only by the ring, four triply innervated by the ring, sub-lateral cord and the major cord, and seven doubly innervated by the ring and the major nerve cord.

Nearly all cells have multiple arms, the exceptions being those cells which send processes only into the ring (Fig. 3). Most cells receive innervation from both the major and the minor nerve cords, but cells near the major nerve cord send branches only to that cord. In the region of the worm analysed here, cells may have up to five major arms. The mean number was 2·7. Often the major arms branched near the nerve cord, and it is known from the work of Rosenbluth (1965) that each arm subdivides into many small branches as it reaches the nerve cord. We are currently determining the number of neural inputs that each muscle cell receives to see whether the individual major arms receive different combinations of neural input that could allow different motor commands to be integrated.

Muscle length

Cells tended to become longer the more posterior and lateral they were (Fig. 3). This trend was examined more closely in a serial set of 15,500 sections of a whole adult female *Ascaris*. Fig. 4 shows the lengths (reconstructed from serial sections) of two sets of cells in a ventral quadrant: those in which the muscle spindle is in contact, at any point along its length, with the hypodermis of the ventral cord (Fig. 2*b*), and those which are similarly immediately adjacent to the lateral line. For the 171 cells next to the ventral cord, the cell length increases from just over 1 mm near the head to just over 2 mm in the tail region (Fig. 4*a*). The 66 cells next to the lateral line increased in length from 0·6 mm in the head (this cell, though not strictly next to the lateral line since it was a head muscle was included since it is in the outer octant) to as much as 13 mm in the tail (Fig. 4*b*). The length increases much more markedly in this set of muscles than in those next to the major nerve cord.

It appeared possible from the data of Figs. 3 and 4 that the inner and outer octant musculature were composed of different populations of cells, all the inner octant cells being short, and the outer octant containing the only elongated cells. To test this, the length of muscle cells within a quadrant was determined at six stations, roughly equally spaced along the length of the worm (Fig. 5). Most of the muscle cells in *Ascaris* are of the same length as those next to the major nerve cord, and it is only those cells nearer the lateral line that are elongated. However, elongated cells are by no means strictly

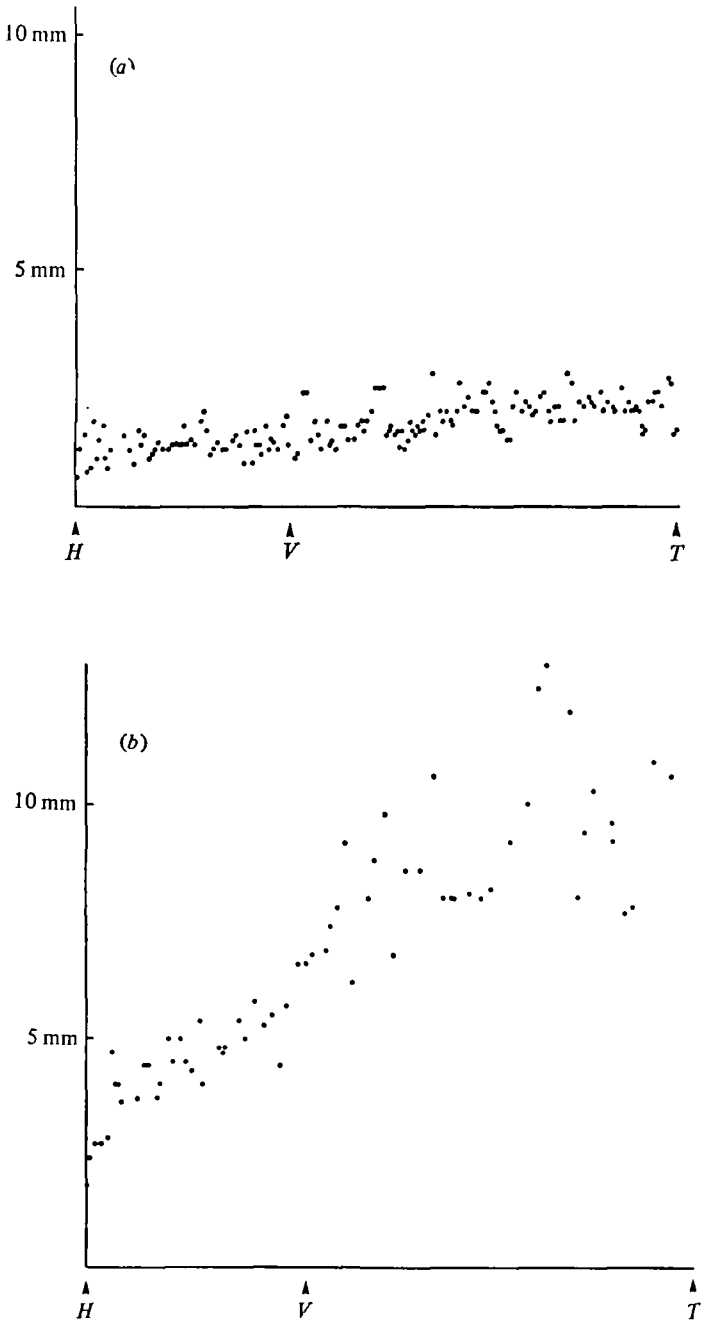

Fig. 4. Length of muscle cells in a ventral quadrant. *H*, head; *V*, vulva; *T*, tail. The position of the cell is recorded as the location of its anterior end. (*a*) Muscle cells which at any point are in contact with the hypodermis of the ventral cord. (*b*) Muscle cells which at any point in their length are in contact with the lateral line.

confined to the outer octant: there is a zone of some 20–30 cells across which the length-increase gradient is found.

The length distribution profile in Fig. 5 enables an estimate of the number of muscle cells in adult *Ascaris* to be made. The average length of the muscle cells is 2·6 mm, the average number at each station is 180, and the reconstructed length of the worm is 15·5 cm, so the total number of muscle cells in the 4 quadrants is 4×10^4. An alternative method for estimating the number of muscle cells, in which the quadrants were divided into zones (see Fig. 6) with muscles of similar length, and the numbers calculated within each zone, gave a total of about 5×10^4 cells.

Although the length distribution profiles were examined in the degree of detail shown in Figs. 4 and 5 in only one worm, other results support the generality of the findings. (*a*) The outer octant musculature has been traced in a total of four worms, two male and two female, and the same trends are seen in each: of the 21 cells shown for each worm (Fig. 7*a* and *b*) the muscle cells are longer the further they are from the head. (*b*) Sometimes in worms that have been treated with collagenase (Stretton, 1976*a*) the muscle cells became detached from the hypodermis and remain together in a clump representing all the musculature of a particular region of the worm. When these muscle cells were gently teased apart, it could be seen that the cells were small and constant in size over most of the region, but towards the lateral edges they became larger, the largest cells being at the periphery.

Musculature of the second stage larva of Ascaris

The reconstructions from serial electron micrographs of the larval muscles (Fig. 8) show a total of 83 muscle cells arranged in four quadrants. The two dorsal quadrants and one ventral quadrant each contain 21 cells and the remaining ventral quadrant contains 20 cells. Dorsal and ventral halves can easily be distinguished by the location of the excretory pore, ventral ganglion, and neuronal cell bodies in the ventral nerve cord. We did not distinguish left from right in the manipulations of the sections, the microscopy, or the photography so the handedness of the exceptional ventral quadrant is unknown.

The length profile of the muscles is shown in Fig. 9, and no position-related variation in length is noticeable.

The musculature of Oxyuris equi

Fig. 10 shows the reconstruction of the muscles of the four quadrants of an entire adult *Oxyuris*. The total number of muscle cells is 65, in agreement with the findings of Martini (1916). There are 17 muscle cells in each of the two dorsal quadrants, 16 in the right ventral and 15 in the left ventral quadrant. The length distribution of these muscle cells, plotted in Fig. 11, shows a large increase in the length of the cells of the tail compared with those of the head, resembling the outer octant muscles, rather than the cells near the major nerve cords, of adult *Ascaris*.

DISCUSSION

Development of the musculature

During the growth of *Ascaris* from the second stage larva to the adult there is an enormous change in the musculature. All the cells in the adult are much larger than

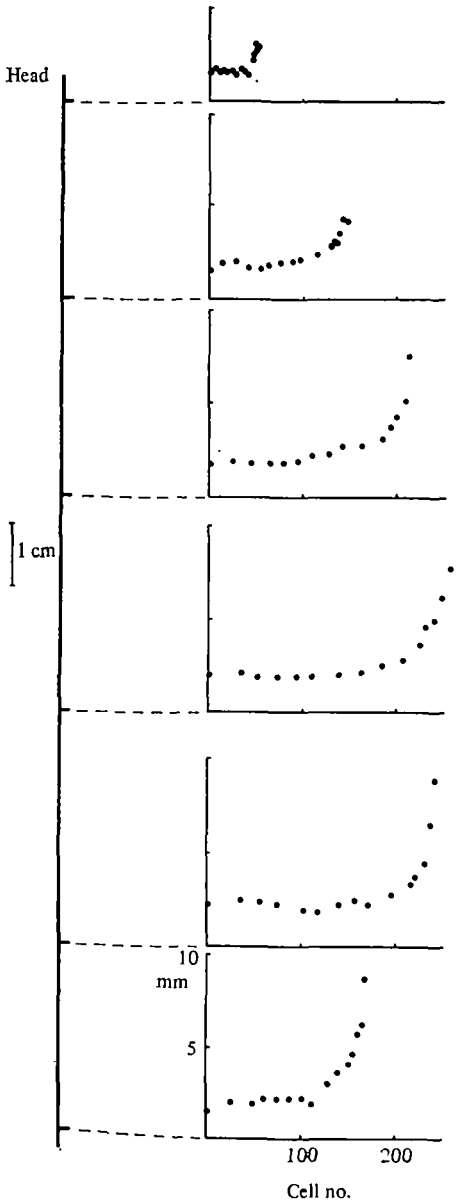

Fig. 5

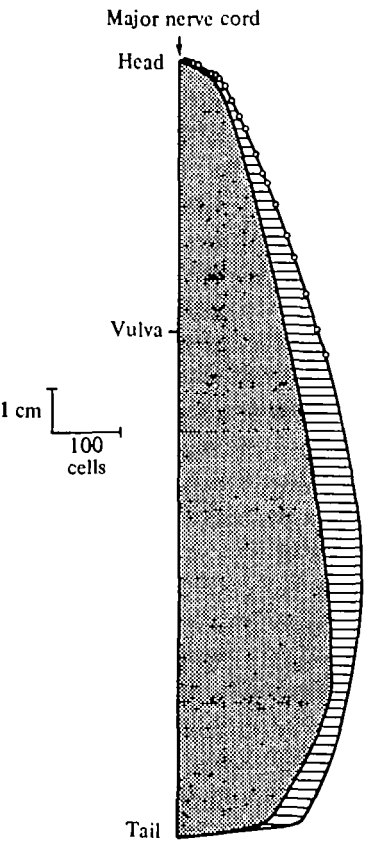

Fig. 6

Fig. 5. Length distribution profiles of muscle cells of one ventral quadrant at six different stations along the worm. The location of these stations is given on the line to the left of the profiles which represents the whole worm. The scales for all the profiles are given on the most posterior one. At each station the cells are numbered serially from the most medial, next to the major nerve cord, to the most lateral, next to the lateral line.

Fig. 6. Zones of muscle cells in one quadrant of adult *Ascaris*. The stippled area includes cells which are up to 2.5 mm long. The hatched zone represents longer cells. The muscle cells of the outer octant (anterior ends marked with a circle) lie in the most lateral positions. The abscissa represents lateral displacement from the major nerve cord, expressed in terms of the number of muscle cells from the nerve cord.

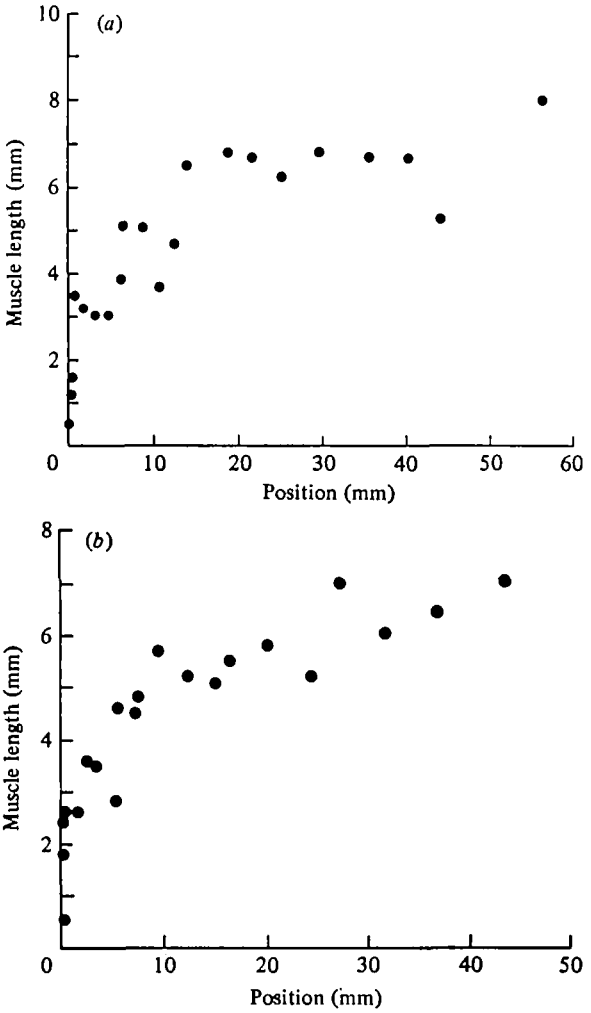

Fig. 7. Length distribution of the outer octant muscle cells of adult *Ascaris* (*a*) female, (*b*) male. The position of each cell is recorded as the location of its anterior end.

those in the larva, so cell growth must be an important factor. Cell multiplication is also very striking: there are nearly a thousand times as many muscle cells in the adult as in the larva.

It seems that nematodes adopt two alternative strategies for dealing with the musculature to attain large size. One is to allow muscle cells to grow in size, keeping the number constant. This is the solution adopted by *Oxyuris*, which is a typical meromyarian nematode, having a small number of muscle cells in the adult. Alternatively, the restriction on cell division may be lifted from the muscle cells or their precursors so that their numbers increase, as in polymyarian nematodes such as *Ascaris* in which the adult has a large number of muscle cells. In nematodes, it seems that the strategy employed by many animals, namely growth by fusion of cells, is not adopted since all the somatic muscle cells are mononucleate.

The fact that the muscle cells are not uniform in length and that the larger muscle

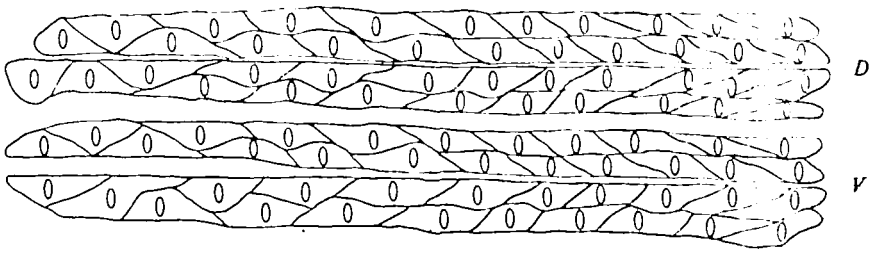

Fig. 8. Reconstruction from 4000 serial sections of the musculature of *Ascaris* larva drawn as a cylindrical projection cut along a lateral line and opened out. The total reconstructed length of the animal was 192 μm. The longitudinal scale is twice the lateral scale. The nuclei are shown as ellipses.

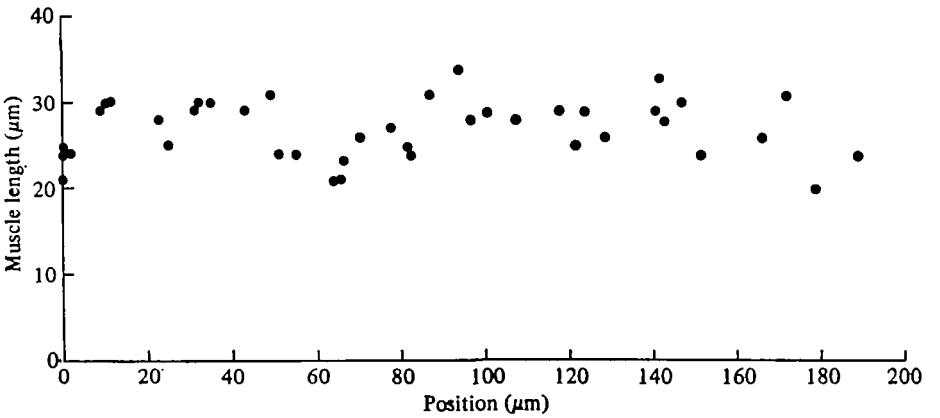

Fig. 9. Length distribution of the ventral muscle cells of larval *Ascaris* as a function of their position along the length of the worm. The position of each cell is recorded as the location of its anterior end.

cells are consistently found in one location, near the lateral lines, is very striking. A convenient explanation for the greater length of the muscle cells in the outer octant is that they are actually the same cells as the original larval set. This would imply that the larval cells which are initially all the same length would start to grow, and at some stage be subject to a differential growth so that the posterior cells become the longest. It also implies that the new cells appearing during the later stages of development are added medially to the original muscle cells. Several observations are consistent with this model.

(*a*) The length profile of the outer octant of adult *Ascaris* is very similar to that of the quadrant musculature of *Oxyuris*.

(*b*) The counts of the muscle cells in the outer octant of *Ascaris* shown in Fig. 7(*a*) and (*b*) was 21 in each outer octant, which is identical with the number in the larval quadrant. This result should be viewed cautiously, however, since one of the boundaries of the outer octant, the sublateral cord, is hard to localize in the posterior part of the body. The 3 or 4 nerve fibres of which it is composed gradually diminish in size until near the vulva where they become difficult and finally impossible to see in the light microscope. In two other adult animals, the counts were 24 and 21, indicating some variability.

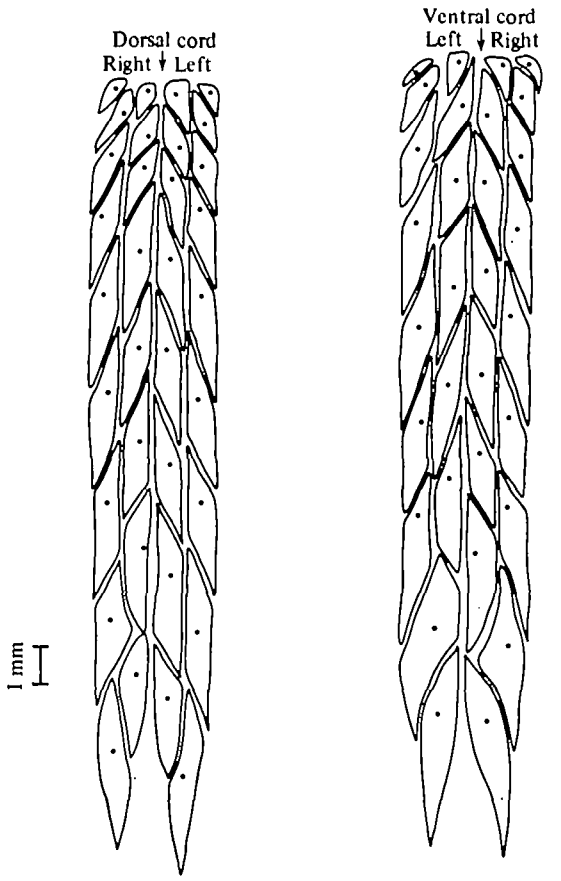

Fig. 10

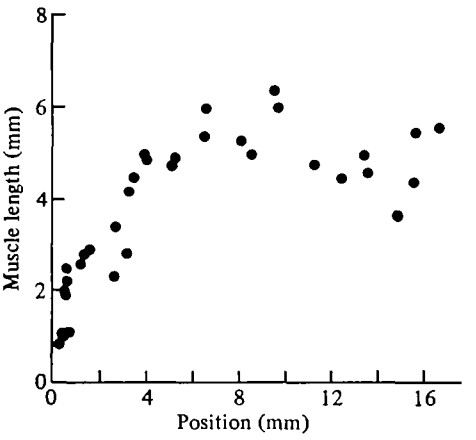

Fig. 11

Fig. 10. Reconstruction from serial sections of the musculature of adult *Oxyuris equi* drawn as if the animal had been slit open along the right lateral line and opened out. The scale refers to the longitudinal axis. The lateral dimensions have been magnified × 2·4. The total reconstructed length of the animal, including the tail which contains no muscle cells, was 25 mm. The dorsal and ventral blocks of muscle cells are widely separated in this animal – the separation is shown to scale.

Fig. 11. Length distribution of the 34 muscle cells of *Oxyuris equi*. The position of each cell is recorded as the location of its anterior end

(*c*) The arrangement of the muscle cells in zones based on their length is also consistent with the hypothesis that the most medial cells are the most recent. In *Ascaris* the cells in a large zone around the major nerve cord are very similar in length and constitute the majority of the muscle cells. Within this zone, the muscle cells immediately adjacent to the major nerve cord have the smallest volume (their cross-sectional area is small, even though they are the same length as the other cells). It is reasonable to assume that these smallest cells nearest the major cord are the most recent and, conversely, that those furthest from the cord are the oldest. We are currently examining the musculature of the other larval stages and of young adults to verify this scheme.

Neuromuscular relationships

In contrast to the large change in cell numbers that occurs in the musculature, the nervous system remains very simple during the course of development. The nervous system of the adult has only some 250 neurones (Stretton, 1976*a*), and although a complete count of the larval neurones has not yet been completed, portions of the system have been examined and found to be very similar – thus there are about 50 sensory neurones in the head in both larva (Brenner, personal communication) and adult (Goldschmidt, 1908), and the ventral cord of the larva has roughly 60–70 neurones which compares with 74 neurones found in the adult. Structures similar to the adult ventral ganglion and lateral ganglia described by Goldschmidt (1908) are also present in the larva, but the cells have not been counted. So at the very least the larval nervous system has half the number of cells found in the adult, and it is not unlikely that the neurones of larva and adult are very close, if not identical, in number. Certainly the nervous system does not undergo the massive cell proliferation shown by the musculature.

There is a problem, then, of how the innervation of an increasing population of muscle cells may be controlled by a small population of neurones, and it is interesting to consider this problem by relating the anatomical results presented in this paper to the known electrical properties of muscle cells.

At the moment we do not know the rules of connectivity between nerve and muscle. It may be that each muscle cell contacts every neurone that is present in the region of the nerve cord where the muscle arm, or arms, of that cell sends its projections. This seems unlikely since some neurones have very few and widely separated points where they make contact with muscle arms (Stretton *et al.* 1976*b*). If all muscle cells were receiving input from these neurones, the arms of many muscle cells would have to send processes along the nerve cord to reach the rare contact areas. We have shown, by injecting Procion Yellow into muscle cells, that there are no such muscle processes travelling along the cord for large distances (Walrond & Stretton, unpublished). Alternatively, the nerve-muscle connexions may be made in a probabilistic way, so that at a given point in the nerve cord each neurone has a certain probablity of making contact with the muscle cells that send arms to that point, some of the probabilities being high and others low. The existence of electrical synapses between the muscle cells (DeBell *et al.* 1963; Rosenbluth, 1965) could be a means of transmitting the electrical signals to neighbouring cells so that their activity tends to be synchronized. The presence of an action potential mechanism which seems to be localized near the ends of the muscle arms near the nerve cords (del Castillo, de Mello & Morales, 1967) may be a means of recruiting muscle cells which do not have direct input from a particular neurone, but may still be activated as long as a critical number of immediate neighbours are stimulated by that neurone. On the other hand, the existence of electrical synapses would tend to decrease the influence of neurones which contact only a few muscle cells since there would be a number of comparatively low-resistance pathways to neighbouring muscle cells which would shunt the charge deposited on the membrane by synaptic input (cf. Getting, 1974). Gap junctions between muscle cells are found within a few microns of the chemical synapses (Rosenbluth, 1965; Richardson & Stretton, unpublished) so they are ideally located to serve this function. The

electrical connectivity between muscle cells may therefore be acting as a buffer system that enables the consequences of sloppy connectivity (cf. Macagno, Lopresti & Levinthal, 1973) between nerve and muscle to be taken care of so that the system still functions correctly even though some muscle cells which ought to be in synaptic contact with a neurone are not, and muscle cells are directly innervated by a neurone when they should not be.

The existence of widespread electrical synapses between the muscle cells could provide an electrical conduction pathway along the nerve cord separate from the neurones themselves. At first sight it seems unlikely that an action potential initiated in the muscle syncytium is propagated along the entire animal by sequential triggering of neighbouring cells: upon stimulation of the ventral nerve cord, only a local region of the dorsal musculature, rather than the whole of the dorsal muscles, are seen to contract. Jarman (1959) reported finding blocks of muscle cells with co-ordinated electrical activities, neighbouring blocks having different patterns of activity. Both of these results argue against the muscle cells being tightly enough coupled electrically to allow continuous propagation. However, in neither case was there any evidence to rule out (*a*) the prevention of propagation by the activation of inhibitory neurones innervating muscles adjacent to active regions so as to block the propagation, or (*b*) the necessity of neuronal excitatory input to the muscle in order to allow the electrical synaptic input from neighbouring muscle cells to bring it above threshold. In either of these views the neuronal input would be acting as a gating mechanism either enabling or disabling the electrical activity of blocks of muscle cells.

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REFERENCES

DeBell, J. T., Castillo, J. del & Sanchez, V. L. (1963). Electrophysiology of the somatic muscle cells of *Ascaris lumbricoides*. *J. cell. comp. Physiol.* **62**, 159.

del Castillo, J. & Morales, T. (1967). The electrical and mechanical activity of the esophageal cell of *Ascaris lumbricoides*. *J. gen. Physiol.* **50**, 603.

del Castillo, J., de Mello, W. C. & Morales, T. (1967). The initiation of action potentials in the somatic musculature of *Ascaris lumbricoides*. *J. exp. Biol.* **46**, 263.

Fairbairn, D. (1961). The *in vitro* hatching of *Ascaris lumbricoide* eggs. *Can. J. Zool.* **39**, 153.

Getting, P. A. (1974). Modification of neuron properties by electrotonic synapses. I. Input resistance, time constant, and integration. *J. Neurophysiol.* **37**, 846.

Goldschmidt, R. (1908). Das Nervensystem von *Ascaris lumbricoides* und *megalocephala*. I. *Z. Wiss. Zool.* **90**, 73.

Hinz, E. (1963). Electronenmikroscopische Untersuchungen an *Parascaris equorum* (Integument, Isolationsgewebe, Muskulatur und Nerven). *Protoplasma* **56**, 202.

Jarman, M. (1959). Electrical activity in the muscle cells of *Ascaris lumbricoides*. *Nature, Lond.* **184**, 1244.

Looss, A. (1905). The anatomy and life-history of *Ancylostoma duodenale*. *Rec. Egypt. Gov. School Med.* **3**, 1.

Macagno, E. R., Lopresti, V. & Levinthal, C. (1973). Structure and development of neuronal connections in isogenic organisms: variations and similarities in optic system of *Daphnia magna*. *PNAS* **70**, 57.

MARTINI, E. (1916). Die Anatomie von *Oxyuris curvula*. *Z. Wiss. Zool.* **116**, 137.
ROSENBLUTH, J. (1965). Ultrastructure of somatic muscle cells in *Ascaris lumbricoides*. *J. Cell Biol.* **26**, 579.
SCHNEIDER, A. (1866). Monographie der Nematoden. Berlin.
STRETTON, A. O. W. (1976*a*). Constancy of the structure of the nervous system of *Ascaris lumbricoides* (in preparation).
STRETTON, A. O. W., FISHPOOL, R. M., DONMOYER, J. E., SOUTHGATE, E., & FLEMANS, M. (1976*b*). Patterns of neuromuscular projections in *Ascaris lumbricoides* (in preparation).