

## ELECTROPHYSIOLOGY OF ACANTHOCEPHALAN BODY WALL MUSCLES

By B. S. WONG, DONALD M. MILLER AND T. T. DUNAGAN

*Department of Physiology, Southern Illinois University,  
Carbondale, Illinois 62901*

(Received 29 September 1978)

### SUMMARY

Body wall muscles of an acanthocephalan *Macracanthorhynchus hirudinaceus* were studied by means of scanning and light microscopy and intracellular recording of potentials. Three types of spontaneous potential changes were found: larger (L) potentials which usually exhibited overshoot and were as large as 65 mV; smaller symmetric (A) potentials approximately 15 mV in amplitude; and even smaller asymmetric (S) potentials which sometimes reached 10 mV. The potentials recorded depended upon the position of the electrode in the anterior–posterior, as well as the medial–lateral, axis. Tetrodotoxin eliminated L but not S potentials. Ouabain lengthened the time for depolarization of L potentials and depolarized the membrane potentials. It is suggested that the rete system activates the body wall muscles in Acanthocephala.

### INTRODUCTION

The nervous system of helminths, particularly acanthocephalans, has not been studied to the same extent as the nervous system of molluscs or arthropods. Acanthocephala are obligate endoparasites in all stages of their life-cycle. As a result of this habitat, the nervous system and other organ systems are extensively reduced such that the worms are little more than reproductive ‘sacks’. The body wall, consisting of an outer tegument, a middle circular muscle layer and an inner longitudinal muscle layer, is permeated by an intricate system of canals which has been thought to act as a primitive ‘circulatory’ system (Miller & Dunagan, 1976). In the invertebrate host the worm measures only a few millimetres in length or less. However, upon establishment in the vertebrate host the worm may attain a length of 50 cm or more. Because acanthocephalans are eutelic, i.e. the number of nuclei is determined in the immature stage, this increase in length means that the majority of larval cells undergo extreme hypertrophy to form adult cells. For example, longitudinal muscles of the body wall may reach a length of 50 cm. It is difficult to understand how this organism maintains control of the muscles to effect its behaviour while the cells are expanding. This study is an attempt to understand the nature of muscular control by investigating cellular potentials.

## METHODS AND MATERIALS

For light microscopy we collected specimens of *Oligacanthorhynchus tortuosa* from opossum (*Didelphis virginiana*) in the vicinity of Southern Illinois and *Macracanthorhynchus ingens* from raccoons (*Procyon lotor*) on St Catherine's Island, Georgia. For microscopy and electrophysiological work, specimens of *Macracanthorhynchus hirudinaceus* were obtained from swine (*Sus scrofa*) at either Hunter Packing Company or Swift Fresh Meats Company, East St Louis, Illinois, and placed in Dewar flasks along with minimal amounts of gut contents for transport to the laboratory. Afterwards, worms were transferred at 24 h intervals into a physiological saline solution (PSS) consisting of 30% sea water (artificial), 0.1 M glucose, 0.1 M sucrose, pH 7.0 (Denbo, 1971), and maintained at 30 °C.

Preparations for scanning electron microscopy included several washings in PSS solution to clean the worm and then fixation was in cold 0.3% glutaraldehyde solution for 24 h. Razor-blade sections were made and re-fixed in osmium tetroxide, dehydrated in a Pearse-Edwards vacuum dryer and mounted on aluminium stubs. The stubs were coated in a Denton vacuum evaporator and examined in a Cambridge Stereoscan IIA microscope.

Photomicrographs of unstained body wall muscles were accomplished by first fixing the worms in cold 0.3% glutaraldehyde for 24 h. The tegument was stripped from the body wall. Hand sections of the body wall were mounted on microscope slides, trans-illuminated with light through an opal glass filter, and photographed using phase contrast.

The PSS was used as the external medium for all preparations except the one for which pseudocoelomic fluid was used. To record potentials from muscles without extensive dissection we resorted to a technique of inverting the worm. The most posterior end of the worm was cut, the small end of a tapered plastic strip (15 cm long, 0.05 cm thick, 1.0 cm at the base and 3 mm at the tip) was applied to the apex of the head, and the body rolled over the plastic strip. In this preparation the muscles are all exposed and intact, movements of the worm are minimized, and electrophysiological recording is facilitated. Potentials from inverted worms were recorded by means of glass-capillary micropipettes (filled with 3.0 M-KCl and with resistances measuring 20 M $\Omega$  or greater), amplified by a Mentor intracellular probe system, displayed on an oscilloscope and recorded with an oscilloscope camera. Electrode placement was determined by placing neutral red solution on the organism at the end of the recording to outline the area.

## RESULTS

*Morphological observations*

The rete network in *M. hirudinaceus* was originally described by Miller & Dunagan (1976) and in this species is an anastomosing network of thin-walled muscle superimposed over the medial surface of the longitudinal muscles and may simply be a modification of the longitudinal muscle (Fig. 1). However, it does not have the thick radial muscle fibres observed in longitudinal muscles. In stained sections it appears to extend processes into both longitudinal and circular muscles and in living

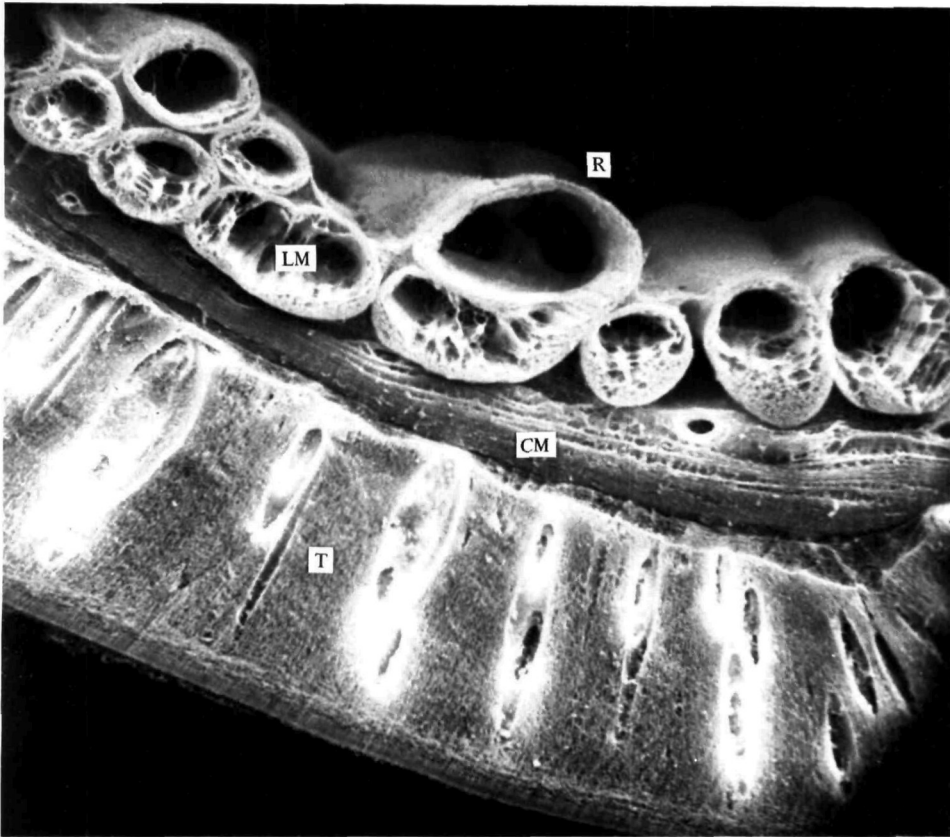


Fig. 1. Scanning electron micrograph of a cross section of the body wall of *Macracanthorhynchus hirudinaceus*. Rete channel (R) is a thinner walled structure which overlies the thicker walled longitudinal muscles (LM). CM is circular muscle and T is the tegument. Magnification 188  $\times$ .

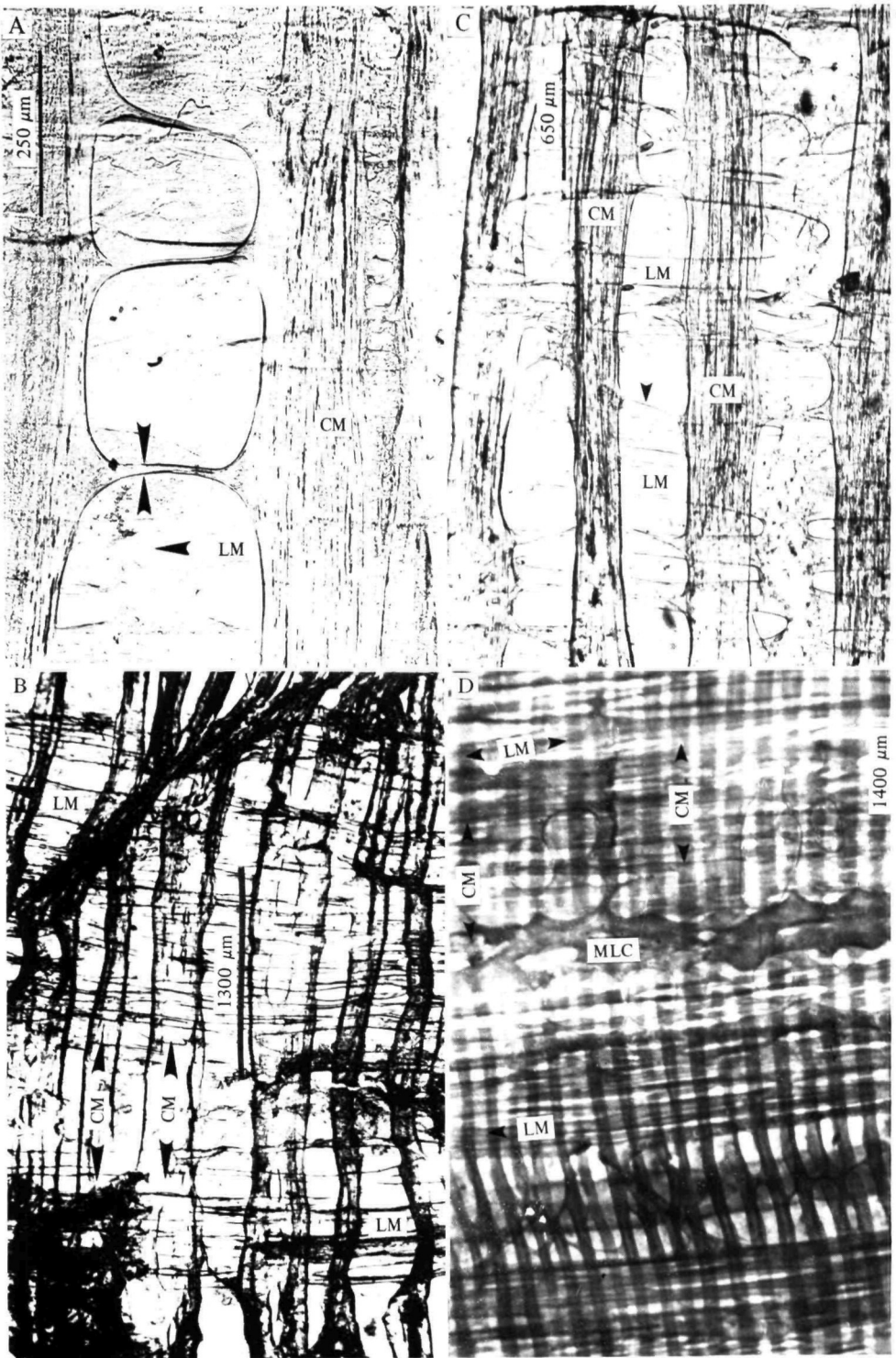


Fig. 2. Photomicrographs using transmitted light of body wall muscles of acanthocephalans. The tegumental layer has been removed and the syncytial nature of circular muscles (CM) and longitudinal muscles (LM) is apparent. (A, C) *Oligacanthorhynchus tortuosa*, (B) *Macracanthorhynchus ingens*, (D) *Macracanthorhynchus hirudinaceus*.

B. S. WONG, D. M. MILLER AND T. T. DUNAGAN

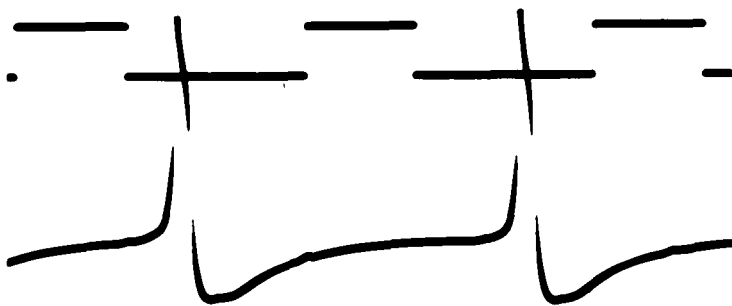


Fig. 3. Spontaneous potentials recorded from the rete system of *Macracanthorhynchus hirudinaceus*. Calibration: base-line = 0 mV; pulse = +10 mV, 2 s.

preparations it appears as a system of transparent gelatinous tubules on the inner surface after being injected (Miller & Dunagan, 1976) with Procion Red.

Transmitted light microscopy of the body wall muscles, looking from outside in and with the muscles stripped free of tegument, indicates extensive interconnexions, not only in *M. hirudinaceus* (Fig. 2D) but also in *O. tortuosa* (Fig. 2A and C) and in *M. ingens* (Fig. 2B). It is not possible in transmitted light microscopy to differentiate between the rete system and the longitudinal muscles. Thus, the muscle cells of this organism have tubes, lacunar channels, coursing through their interior.

#### Electrophysiological observations

Resting membrane potentials recorded from the rete system averaged  $35.1 \pm 1.5$  ( $n = 20$ ) mV, those from the longitudinal muscles averaged  $33.6 \pm 1.2$  ( $n = 10$ ) mV, and those from the circular muscles  $34.2 \pm 1.8$  ( $n = 10$ ) mV. Membrane potentials were measured in either PSS or pseudocoelomic fluid and did not vary by more than 5 mV.

In the region of the medial longitudinal channel (MLC) large numbers of spontaneous depolarizations or L potentials were observed in the rete system. These potentials began with a depolarization of approximately 10 mV in 2.2 s, which was succeeded by a more rapid depolarization (Fig. 3) which lasted 0.4 s, reached zero, then usually overshoot approximately +10 mV. The repolarization process which lasted 0.4 s had two phases: a slower one which re-established the potential to approximately zero and then a faster one which returned the potential to the level approximating the original membrane potential. Following this, there was an undershoot which lasted 0.3 s and polarized the membrane potential by about another -10 mV. These L potentials were always larger than 15 mV.

Depending upon the point of placement of the electrode on the rete system along the anterior-posterior axis of the MLC, small potentials can be detected which may or may not be correlated with L potentials. When they are correlated, they may either precede L potentials (Fig. 4C) or follow them. Moving the electrode slightly along the MLC produced a change in spacing between alternate L potentials (Fig. 4D). Moving the electrode over the surface of the body wall in a direction perpendicular to the MLC resulted in considerably different potential recordings. As the electrode was placed further away from the MLC, alternate L potentials decreased



Fig. 4. Spontaneous potentials recorded from the rete system of *Macracanthorhynchus hirudinaceus*. Calibration: base-line = 0 mV; pulse = +10 mV, 2 s. (A) L potentials recorded in PSS. (B) L potentials recorded in pseudocoelomic fluid. (C) L and S potentials recorded in PSS. (D) Superposition of L and S potentials recorded near medial longitudinal channel in PSS.

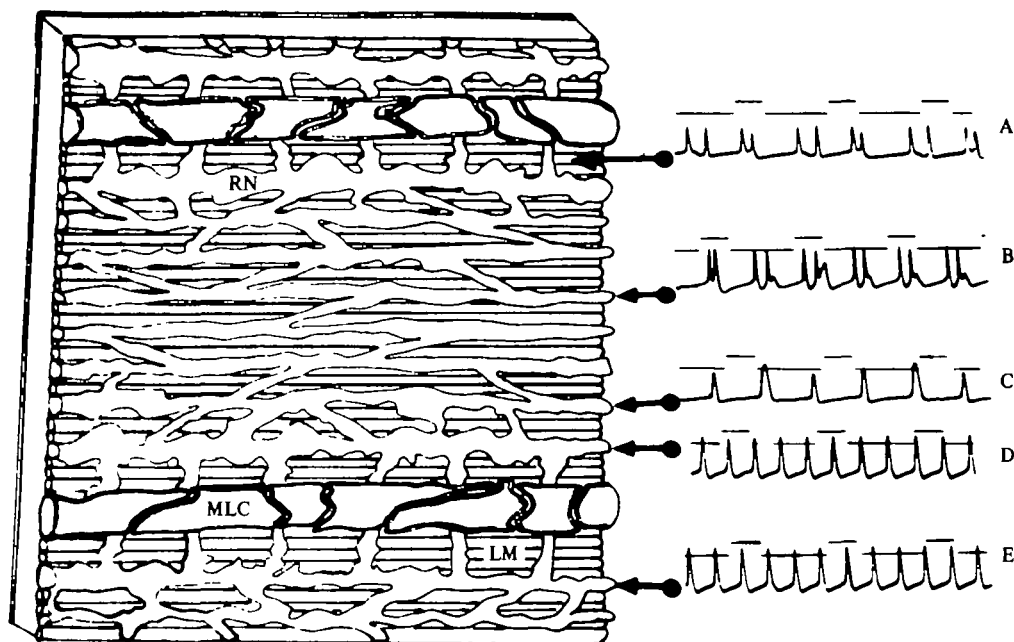


Fig. 5. Spontaneous potentials recorded from the rete system (RN) of *Macracanthorhynchus hirudinaceus*. The rete system overlies the longitudinal muscles (LM). Calibration: base-line = 0 mV; pulse = +10 mV, 2 s. Waveform of potentials varies as a function of distance of the electrode from medial longitudinal channel (MLC) and/or position along a line parallel to the MLC.

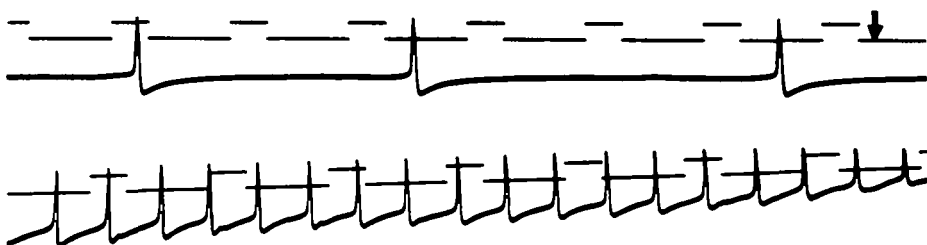


Fig. 6. Effect of acetylcholine ( $10^{-4}$  M) on spontaneous L potentials from the rete system of *Macracanthorhynchus hirudinaceus*. Acetylcholine was added at arrow. Calibration: base-line = 0 mV; pulse = +10 mV, 2 s.

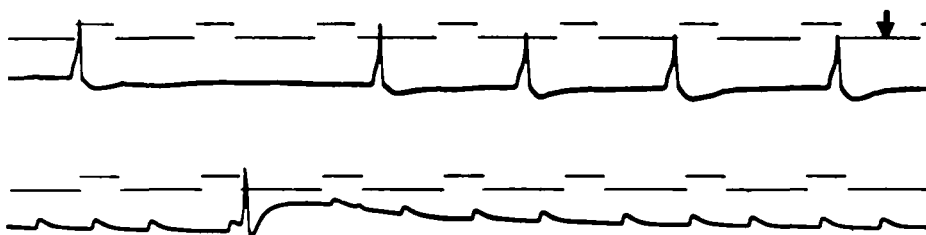


Fig. 7. Effect of tetrodotoxin ( $10^{-4}$  M) on spontaneous L potentials from the rete system of *Macracanthorhynchus hirudinaceus*. Toxin was added at arrow. Calibration: base-line = 0 mV; pulse = +10 mV, 2 s.

in amplitude (Fig. 5). When records were made midway between the MLCs, all spontaneous potentials were greatly decreased in amplitude and the pattern of potentials became less regular (Fig. 5B). In one type of small potential, the S type, (less than 10 mV in amplitude) the rise time is faster than the repolarization, giving the potentials a skewed shape (Fig. 4C). In a second type of small potential, the A type, the rise and fall times are similar (Fig. 5A).

Acetylcholine in concentrations as low as  $10^{-4}$  M will initiate all three types of potentials as well as increase their frequency (Fig. 6). In addition, the application of ACh always results in some degree of depolarization of the membrane potential.

Tetrodotoxin applied to the muscles completely abolished the L potentials, but did not affect the S potentials (Fig. 7). Washing out the tetrodotoxin returned the L potentials but only after a very long wait and several washings. Application of ouabain ( $10^{-5}$  M) to the preparation had the interesting effects of abolishing the overshoot, lengthening the time for depolarization but not significantly affecting the time for repolarization until some 60 s later. At the same time the membrane potential shifted towards zero (Fig. 8). This effect could be reversed by repeated washing.

In the presence of Ca-free PSS, all spontaneous activity was inhibited while the membrane potential stayed essentially unchanged (Fig. 9).

While membrane potentials could be recorded from all muscle cells, only the rete system provided L, S, and A type potentials. Moreover, attempts to electrically stimulate any of the muscle types by means of intracellular current injection was without success.

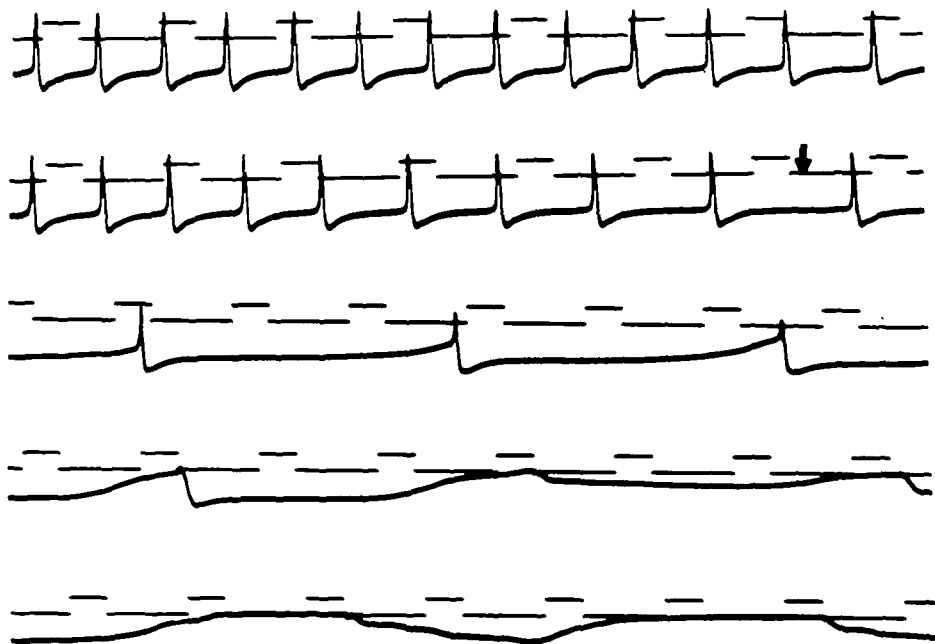


Fig. 8. Effect of ouabain ( $10^{-4}$  M) on spontaneous L potentials from the rete system of *Macracanthorhynchus hirudinaceus*. Ouabain was added at arrow. Calibration: base-line = 0 mV; pulse = +10 mV, 2 s.

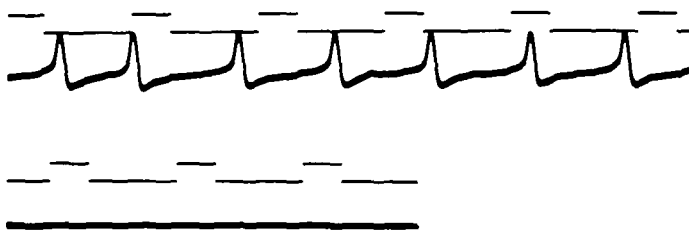


Fig. 9. Inhibition of spontaneous activity in *Macracanthorhynchus hirudinaceus* in the presence of Ca-free PSS. Calibration: base-line = 0 mV; pulse = +10 mV, 2 s.

#### DISCUSSION

Those parameters which characterize the L potentials are the rapid rate of depolarization compared to the other potentials, the usual presence of overshoot and undershoot, the fact that they are usually larger than 15 mV, are inhibited by the presence of tetrodotoxin and are stimulated by acetylcholine. The S potentials are less than 10 mV in amplitude and are usually correlated with the L potentials, have a faster rise time than recovery time, are not inhibited by the presence of tetrodotoxin, but are stimulated by the presence of acetylcholine. The A type potentials differ from the S potentials in that they do not necessarily correlate with L potentials, do not have a faster rise time, and tend to increase in number as the electrode is moved away from the MLC.

The nearest organism in a phylogenetic sense to Acanthocephala whose muscle potentials have been studied is *Ascaris lumbricoides*, and the muscle potentials in this species range, as found in *M. hirudinaceus*, from  $-30$  to  $-40$  mV (DeBell, 1965; Del Castillo, De Mello & Morales, 1967). Nevertheless, ascarids are quite distant from acanthocephalans, so this may be a poor comparison.

We believe the spontaneous L potentials associated with the rete system of Acanthocephala are action potentials, albeit slow ones. The fact that tetrodotoxin inhibits the generation of the L potentials would support this concept. This toxin from the Tetraodontidae is one which reversibly blocks sodium ion channels in muscles and nerves. In excitable tissues which use sodium currents to produce their action potential, tetrodotoxin blocks the electrical conductance needed to produce this potential (Narahashi, 1974). The form and duration (0.1–0.5 s) of the L potentials are comparable to the all-or-none potentials recorded in hydroids (Josephson, 1961).

The observation that L but not S potentials were abolished in the presence of tetrodotoxin suggests that the L potentials are indeed regenerative events, and dependent upon sodium ion influx. Additionally, ouabain may inhibit L potentials by allowing an accumulation of sodium ions inside the cell. The increased sodium concentration would change both the concentration and electrical gradients for sodium so that the peak depolarization reached during periods of increased permeability would diminish, thereby accounting for the decreased amplitude of the events recorded in the presence of ouabain. This would also account for the depolarization of the membrane potential in the presence of ouabain. Spontaneous, rhythmic depolarizations that are either slow (0.2 s) or fast (30–40 ms) have been recorded in the cell body of longitudinal muscles of *Ascaris* (DeBell, Del Castillo & Sanchez, 1963). The slow potentials were shown to originate from a syncytium between the lateral nerves and the muscles in the body wall. The rete system of *M. hirudinaceus* is an analogous structure to this syncytium. The rete system may possibly be composed of a specialized form of muscle cell which is activated by extensions from the body wall nerves and in turn serves to initiate contractions in both longitudinal and circular muscle layers.

S potentials did not display an overshoot, suggesting that they may either be attenuated potentials or synaptic (junction) potentials resulting from permeability changes involving channels that are not regenerative Na channels. Junction potentials which depolarize and activate local areas of muscles, have been widely found in crustaceans (Lockwood, 1967), slow postural frog muscles, many insect and molluscan muscles, and most invertebrate slow muscle (Prosser & Brown, 1961). S potentials may represent synaptic potentials since they are absent in the presence of Ca-free PSS. Since A potentials are not seen in the presence of tetrodotoxin they probably represent attenuated L potentials conducted electrotonically from the rete system; and the multitude of interconnections that we have found in this study point to this as a distinct possibility.

Hightower, Miller & Dunagan (1976) reported that acetylcholine initiated contraction in both intact worms and sections of worms. Similarly, we have found that acetylcholine will initiate potentials in quiescent cells and surmise that the rete network may be activated by acetylcholine mediated synapses. While the concentration of acetylcholine used may be considered high, it is usual for parasites to have much higher concentration requirements (von Brand, 1973).

This work was supported by the National Institutes of Health, Research Grant AI-128833, the Graduate School of Southern Illinois University, and the American Museum of Natural History. The authors thank Dr Yoromi Matsumoto for constructive criticism and advice.

## REFERENCES

- DEBELL, J. T. (1965). A look at neuromuscular junctions in nematodes. *Q. Rev. Biol.* **40**, 233-251.
- DEBELL, J. T., DEL CASTILLO, J. & SANCHEZ, V. (1963). Electrophysiology of the somatic muscle cells of *Ascaris lumbricoides*. *J. cell comp. Physiol.* **62**, 159-178.
- 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**, 263-279.
- DENBO, J. R. (1971). Osmotic and ionic regulation in *Macracanthorhynchus hirudinaceus* (Acanthocephala). Masters Thesis, Southern Illinois University, Carbondale, 111.
- HIGHTOWER, K., MILLER, D. M. & DUNAGAN, T. T. (1975). Physiology of the body wall muscles in an acanthocephalan. *Proc. Helm. Soc. Wash.* **42**, 71-79.
- JOSEPHSON, R. K. (1961). Repetitive potentials following brief electric stimuli in a hydroid. *J. exp. Biol.* **38**, 579-593.
- LOCKWOOD, A. P. M. (1967). *Aspects of the Physiology of Crustacea*. San Francisco: W. H. Freeman.
- MILLER, D. M. & DUNAGAN, T. T. (1976). Body wall organization of the acanthocephalan, *Macracanthorhynchus hirudinaceus*: A reexamination of the lacunar system. *Proc. Helm. Soc. Wash.* **43**, 99-106.
- NARAHASHI, T. (1974). Chemicals as tools in the study of excitable membranes. *Physiol. Rev.* **54**, 813-889.
- PROSSER, C. L. & BROWN, F. A. (1961). *Comparative Animal Physiology*. Philadelphia: Saunders Press.
- VON BRAND, T. (1973). *Biochemistry of Parasites*, 2nd ed. New York: Academic Press.