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ELECTROPHYSIOLOGICAL STUDIES ON THE TEGUMENTAL AND SUBTEGUMENTAL POTENTIALS OF ADULT MALE SCHISTOSOMA MANSONI

presented by

Connie Sue Bricker

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Major professor

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ELECTROPHYSIOLOGICAL STUDIES ON THE TEGUMENTAL AND SUBTEGUMENTAL POTENTIALS OF ADULT MALE *SCHISTOSOMA MANSONI*

By

Connie Sue Bricker

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ABSTRACT

ELECTROPHYSIOLOGICAL STUDIES ON THE
TEGUMENTAL AND SUBTEGUMENTAL POTENTIALS
OF ADULT MALE SCHISTOSOMA MANSONI

By

Connie Sue Bricker

Histological studies using HRP as a marker injected iontophoretically through a recording electrode have indicated the origins of potentials encountered upon advancement of the electrode into and beneath the dorsal surface of adult male Schistosoma mansoni. The first potential encountered, having a value of -51 ± 0.6 mV, originates across the outer tegumental membrane. The next potential usually recorded has a value of -28 ± 0.6 mV and originates in the muscle masses underlying the tegument. A potential having the value -10 ± 0.5 mV originates within the basal lamina, and the interstitial fibers and extracellular spaces surrounding the muscle.

Altering ion concentrations in the bathing medium (i.e., high K^+, low Na^+, 0 Ca^{++}, low Cl^-, high Li^+) depolarizes all three potentials. External applications of ouabain, 2,4-dinitrophenol, and the antischistosomal praziquantel also cause depolarization of the potentials. There appears to be a close correlation between changes in E_{teg}, E_2, and E_3.

Active transport appears to be important in maintenance of both tegumental and muscle potentials.

To Mom

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Schistosomiasis is one of the most important and widespread of human parasitic diseases, infecting millions of people in many parts of Africa and tropical America. One of the worms causing this disease is Schistosoma mansoni, a digenetic trematode living in the mesenteric veins of its human host. The pathology of this schistosome's infection includes diarrhea or dysentery, enlargement of the spleen, cirrhosis of the liver, abdominal pain, anemia and lesions around the eggs (Chandler and Read, 1961; Brown, 1975).

Control of the muscles of the suckers and body of this endoparasite are necessary for its movement within the host. A peristaltic or wave-like movement that occurs on the surface of the worm combined with a coordinated effort between the oral and ventral suckers propels the worms through the veins (Fetterer, Pax and Bennett, 1977). When the worm has reached a suitable feeding location it must maintain this position by attaching itself to the host with the ventral sucker (Rew, 1978). Proper muscular coordination is also essential to enable the male to encircle the female, holding her in copula within the gynecophoric canal. For these reasons interfering with the muscular coordination of the schistosome may prove to be an effective means of action against this parasite.

Previous studies involving the musculature have included visual

observations comparing the motor activity between control and experimental animals (Tomosky, Bennett and Bueding, 1974). Photoelectric and ultrasonic methods have also been used to measure overall worm movements (Brown, Koura, Bell and Gilles, 1973; Hillman and Senft, 1973). More recently a method developed by Fetterer et al. (1977) allows direct quantitative recordings of muscle tension and contractile activity.

To the best of my knowledge no attempts have been made previously to make intracellular electrode recordings of the schistosome musculature, though this technique has proved valuable in the study of many vertebrate and a few invertebrate smooth muscles. This technique has proved feasible in the study of the tegument of the schistosome (Fetterer, Pax and Bennett, 1980a). My research was undertaken to determine if microelectrode recording techniques could also be utilized to characterize the musculature of Schistosoma mansoni.

The electrophysiological studies were done on the dorsal surface of the adult male rather than the ventral surface as in the studies of Fetterer et al. (1980a), since the dorsal tegument and muscle layers are thicker, thus increasing the likelihood that successful recordings could be made.

General Anatomy

The adult male Schistosoma mansoni has a maximum length of about one centimeter, a width of 0.2 centimeter and a thickness of about 300 microns. A gynecophoric canal is formed by the ventrally inrolled sides of the male's body. The adult female lies in the gynecophoric canal.

Oral and ventral suckers are present anteriorly. The genital pore lies posterior to the ventral sucker. A short duct leads from the genital pore to the seminal vesicle, which has ducts (vasa efferentia) leading to the seven testes.

The digestive system originates within the oral sucker and continues posteriorly as the prepharynx, pharynx, and esophagus which divides near the ventral sucker to form two ceca. The ceca extend posteriorly for a considerable distance and then unite to form a single intestine. The digestive system often appears brown from ingested hemaglobin (Erasmus, 1972).

The excretory system is protonephridial. Flame cells are connected to paired protonephridial tubules which open to the exterior through a posterior excretory pore (Senft, Philpott and Pelofsky, 1961; Meglitsch, 1972).

The reproductive, digestive, and excretory systems are located in the parenchyma. The muscle layers lie above the parenchyma and beneath the tegumental epithelium which covers the entire body of the worm.

The Nervous System

The nervous system of Schistosoma mansoni follows the basic trematode pattern with a circumesophageal commissure joining two pairs of ganglia. Fibers extend from these ganglia anteriorly into the oral sucker, and posteriorly into the ventral sucker and into four lateral nerve trunks (Fripp, 1967). The trunks connect at the posterior end of the worm and have transverse connections along their length.

Neurons of the central ganglion give rise to non-myelinated

axons. Synapses between axons occur within the circumesophageal commissure and in other regions. Neuromuscular synapses similar to the axo-axonal synapses are found. Nerve trunks are seen in close proximity to the tegumental cell bodies but no nerve processes are directly associated with the tegument except in the esophageal region (Silk et al., 1969b) or where they penetrate the tegument to form a bulblike structure which Morris and Threadgold (1967) assume detects the flow of the surrounding medium.

Various neurotransmitters have been shown to be present in Schistosoma mansoni. These include 5-HT (Bennett, Bueding, Timms and Engstrom, 1969; Chou, Bennett and Bueding, 1972), dopamine and norepinephrine (Gianutos and Bennett, 1977), and catecholamines (Bennett and Bueding, 1971; Machado, Machado and Pellegrino, 1972).

Tegument

The tegument of adult Schistosoma mansoni is a syncytium consisting of the superficial anuclear layer connected by cytoplasmic channels to the nucleated subtegumental cell bodies located beneath the muscle layers (Morris and Threadgold, 1968; Smith, Reynolds and von Lichtenberg, 1969; Hockley, McLaren, Ward and Nermut, 1975) (Figures 1-4). The tegument, being the interface between the schistosome and its host, is important in its functions of nutrient absorption (Lumsden, 1975; Pappas and Read, 1975), and as the site of immunological attack by the host (Cox, 1979; Lee, Elhelu and Aboko-Cole, 1979).

The outer membrane of the tegument, which has a heptalaminate appearance (Hockley and McLaren, 1973), is composed of two trilaminate

Figure 1. Diagrammatic representation of a cross section of dorsal tegument and musculature of adult male Schistosoma mansoni. CM, circular muscle; LM, longitudinal muscle; DVM, dorso-ventral muscle; MC, muscle cyton; DT, dorsal tegumental epithelium; S, spine; TI, tegumental inclusions; TC, tegumental cyton, CC, cytoplasmic channels of tegument; BI, basal invaginations of tegument; BL, basal lamina; L, lipids; G, glycogen.

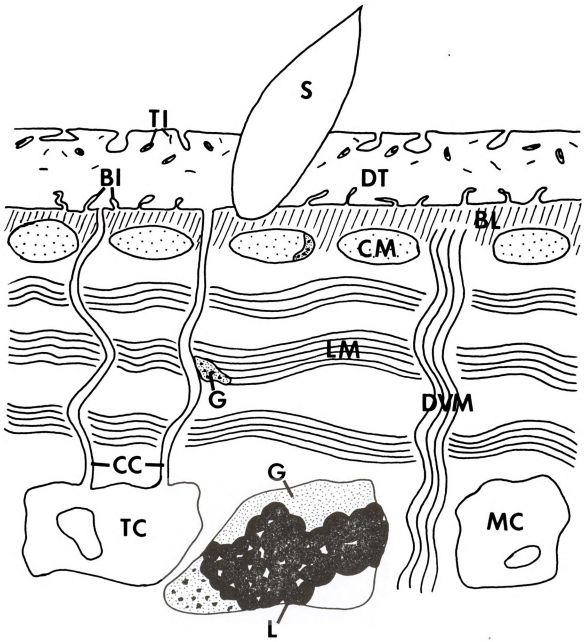

Figure 1

Figure 2. Electron micrographs of cross sections of dorsal tegument and musculature of adult male Schistosoma mansoni. CM, circular muscle; LM, longitudinal muscle; DVM, dorso-ventral muscle; MC, muscle cyton; DT, dorsal tegumental epithelium; S, spine; TC, tegumental cyton; CC, cytoplasmic channels of tegument; BL, basal lamina, L, lipids; G, glycogen.

a. calibration: 2 microns

b. calibration: 3 microns

c. calibration: 4 microns

d. calibration: 2 microns

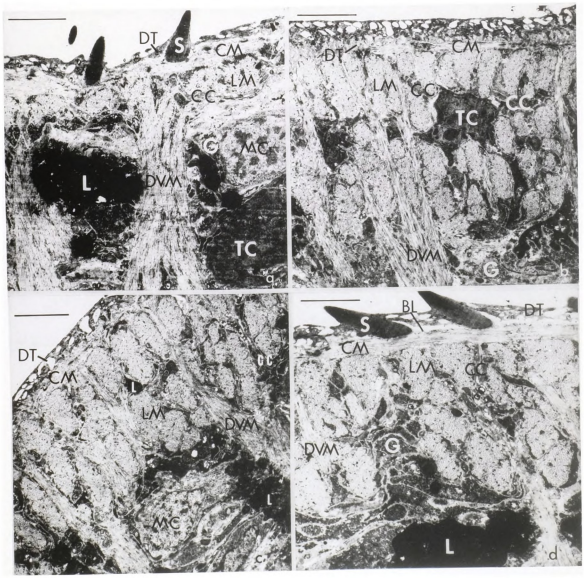
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Figure 2

Figure 3. Diagrammatic representation of a longitudinal section of dorsal tegument and musculature of adult male Schistosoma mansoni. CM, circular muscle; LM, longitudinal muscle; DVM, dorso-ventral muscle; MC, muscle cyton; DT, dorsal tegumental epithelium, S, spine; TI, tegumental inclusions; TC, tegumental cyton; CC, cytoplasmic channels of tegument; BI, basal invaginations of tegument; BL, basal lamina; L, lipids; G, glycogen.

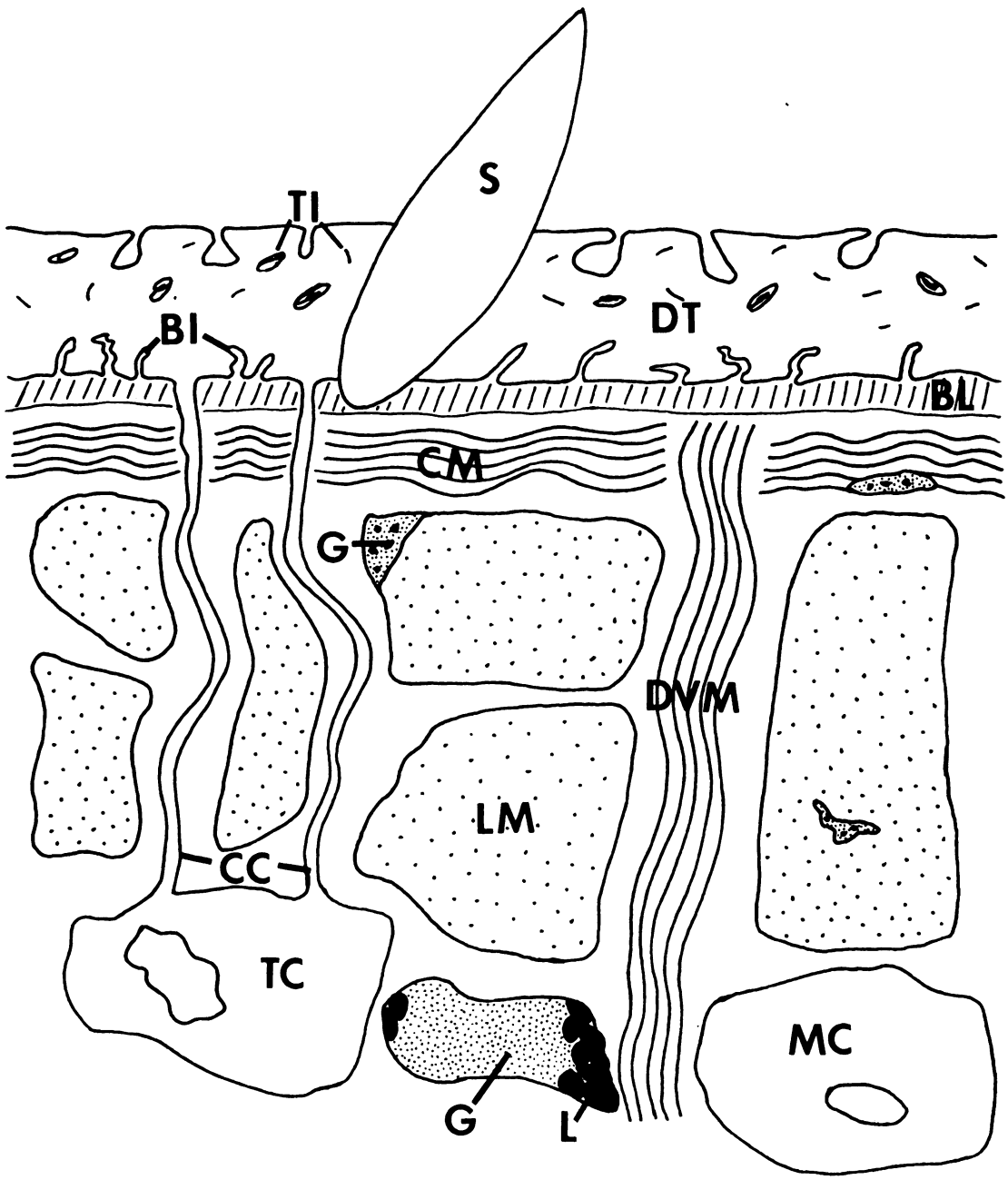

Figure 3

Figure 4. Electron micrographs of longitudinal sections of dorsal tegument and musculature of adult male Schistosoma mansoni. CM, circular muscle; LM, longitudinal muscle; DVM, dorso-ventral muscle; MC, muscle cyton; DT, dorsal tegumental epithelium; S, spine; TC, tegumental cyton; CC, cytoplasmic channels of tegument; BL, basal lamina; L, lipids; G, glycogen.

a. calibration: 4 microns

b. calibration: 4 microns

c. calibration: 5 microns

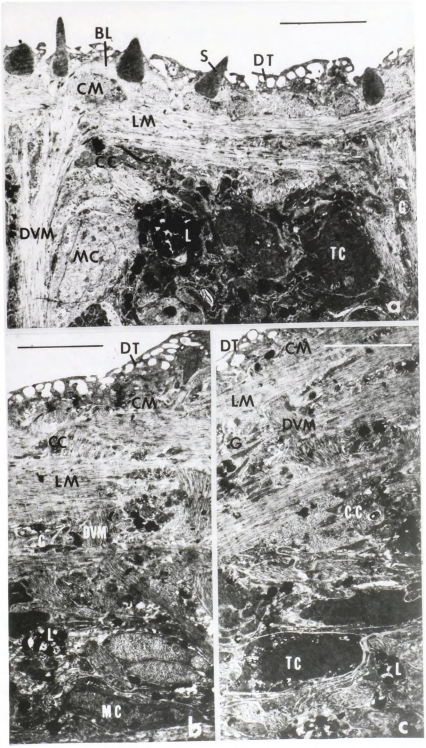
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Figure 4

membranes in close contact (Hockley et al., 1975; McLaren, Hockley, Goldring and Hammond, 1978). This outer membrane has many invaginations forming channels into the cytoplasm of the tegumental epithelium. The outer tegumental membrane is covered with a surface coat, the glycocalyx. The glycocalyx contains acid mucopolysaccharides (Stein and Lumsden, 1973) and may serve a protective function by the adsorption of cellular elements from the host, disguising the worm from immunological recognition (Clegg, 1972). The inner plasma membrane also has many invaginations which project upward into the tegument (Smith et al., 1969; Silk, Spence and Gear, 1969; Wilson and Barnes, 1974b). The thickness of the tegumental epithelium varies from one to five microns depending on the contractile state of the worm (Wilson and Barnes, 1974a, 1977).

The tegumental epithelium contains several different types of inclusions: mitochondria, spines, the discoid granules which disperse to form the ground substance of the tegument, and the multilaminate vesicles which contribute to the multilaminate plasmalemma of the surface. The discoid granules and multilaminate vesicles are formed in the Golgi of the tegumental cell body and then are transported upward through the cytoplasmic channels to the tegumental epithelium (Wilson et al., 1974a, 1974b). The crystalline spines, which are more numerous on the dorsal surface, project through the tegumental epithelium (Senft et al., 1961; Morris et al., 1968; Silk et al., 1969; Hockley et al., 1973).

Lying beneath the inner plasma membrane is the basal lamina which extends upward into the basal invaginations of the tegument. Interstitial fibers lie under the basal lamina and separate the

circular and longitudinal muscle bundles (Morris et al., 1968; Silk et al., 1969; Hockley et al., 1975).

The cell bodies of the tegument lie within the lower muscle bundles or beneath the muscle layers in the parenchyma and are connected to the tegumental epithelium by cytoplasmic channels (Silk et al., 1969; Smith et al., 1969; Wilson et al., 1974b; Hockley et al., 1975). These channels vary in diameter from 1 to 1.5 microns at their junction with the cell bodies, narrowing down to a diameter of 0.3 microns where they pass between fibers of the muscles. Since the muscle layer on the dorsal surface is thicker than on the ventral surface because of a larger number of longitudinal muscles, the channels vary in length from 8 to 30 microns on the dorsal surface while those on the ventral surface are only about 3 microns long. The channels on the dorsal surface are also more convoluted. The channels are 0.2 to 4 microns apart, lying in the gap between the dorso-ventral muscles. The gaps are 0.1 to 0.5 microns wide and the muscles are 1.0 micron wide. There may be as many as 50 channels per tegumental cell body (Wilson et al., 1974b). These channels contain several of the same inclusions, the discoid granules and the multilaminate vesicles, as the tegumental epithelium (Morris et al., 1968).

The tegumental cell bodies are roughly ovoid in shape and vary in size from 3 to 6 by 6 to 16 microns. Their spacing, which is dependent on the state of contraction, varies from 5 to 20 microns (Wilson et al., 1974b). The cell bodies may be uninucleate or multinucleate. The nucleus has a scalloped, irregular shape. The nucleoplasm has one to three nucleoli. The tegumental cell body contains discoid granules and multilaminate vesicles (Morris et al.,

1968; Silk et al., 1969; Smith et al., 1969).

Junctional complexes are found between tegumental cell bodies and the adjacent muscle cell bodies, but no junctions occur between adjacent tegumental cell bodies (Silk et al., 1969).

Muscles

Smooth muscle is the only kind of muscle in the schistosome. The musculature is arranged in circular, longitudinal, and dorso-ventral fashion. The circular muscle lies below the tegument and is arranged perpendicularly to the long axis of the body. The longitudinal layer is located beneath the circular muscle and extends in an anterior-posterior direction. The dorso-ventral muscle (also referred to as radial by Silk and Spence (1969a), and transverse by Wilson et al. (1974a)) extends through the worm in a dorsal-ventral direction (Figures 1-4).

The musculature is surrounded by a fibrillar interstitial connective tissue. Branching of fiber bundles occurs and these fibers are surrounded by the same connective tissue (Silk et al., 1969a).

In transverse section each muscle bundle is seen to consist of an array of thick and thin filaments. The arrangement of the myofilaments in each fibril bundle is typical of invertebrate smooth muscle (Lowy and Hamon, 1962; Lumsden and Byram, 1967). An array of discontinuous 18-40 nanometers thick tapered filaments is surrounded by 8-14 thinner 5 nanometer filaments. Cross linkages between thin filaments and between thick and thin filaments occur. Amorphous osmiophilic material continuous with the thin filaments is seen associated with the sarcolemma. No transverse invaginations of the

sarcolemma are observed. Cross banded tubular elements of agranular reticulum are located at the fiber periphery (Silk et al., 1969a).

The muscle cell bodies are located beneath the muscle layers in the parenchyma and are connected to the muscle bundles by cytoplasmic processes (Silk et al., 1969a; Smith et al., 1969). The muscle cell body contains the nucleus with prominent nucleoli. The nuclei are generally ovoid but sometimes are irregular with a clumped distribution of chromatin. The sarcoplasmic reticulum is rough and poorly defined, and no microtubules are found. Golgi complexes and numerous mitochondria are distributed peripherally in the perikaryon and in sac-like distentions of the sarcolemma along each myofibril bundle. The sarcoplasm of each cell contains alpha-glycogen and numerous beta-glycogen particles. Lipid globules are common, often surrounded by beta-glycogen particles (Silk et al., 1969a).

Junctional complexes (gap junctions) occur between adjacent muscle cells. The membranes are opposed at a distance of 7-9 nanometers. Similar junctions are formed between muscle and tegumental cell bodies (Silk et al., 1969a).

METHODS

Source and Maintenance of Animals

Adult Schistosoma mansoni (45-55 days post infection) were dissected from the hepatic and mesenteric veins of female laboratory mice (Mus musculus) provided by the laboratory of Dr. James Bennett, Department of Pharmacology, Michigan State University. The mice were killed by cervical fracture.

The schistosomes were placed either in Fetal Calf Serum/ Earle's medium (FCS/Earle's) or Horse Serum/Earle's (HS/Earle's) medium, consisting of a 1:1 mixture of Earle's balanced salt solution (Grand Island Biological) and heat inactivated fetal calf serum or horse serum (Grand Island Biological) buffered at pH 7.4 with 20 mM Hepes (N-2-hydroxymethyl piperazine, Sigma), 100 units/ml penicillin-streptomycin (Grand Island Biological) and a final glucose concentration of 0.2%. The schistosomes were maintained at 37°C and used for experiments within 12 hours after removal from the mice.

Recording Media

For microelectrode recordings and marker injection experiments the worms were placed in a recording chamber containing Hank's Balanced Salt Solution (HBS) and 50 mg% (weight/volume) pentabarbitol-Na (PB, Sigma).

The recording chamber consisted of a 10 ml glass petri dish with Sylgard resin (Dow-Corning) covering the bottom of the dish.

The HBS contains 138 mM Na^+, 5.9 mM K^+, 1.4 mM Ca^{++}, 1.0 mM Mg^{++}, 0.5 mM PO_4^-, 147 mM Cl^-, 0.5 mM SO_4^-, 5 mM glucose, and 20 mM Hepes. The osmolality was 300 mOsm. The pH was adjusted to 7.4 using 6 N NaOH. The temperature of the solutions in the recording chamber was maintained at 37°C by a thermoelectric heater (Cambion Electrics) and the temperature of the solutions monitored by a thermistor.

Microelectrode Recordings

After the worms were immobilized by the PB/HBS, the females were removed from the gynecophoral canal and discarded. Minuten insect pins were used to pin the males flat against the sylgard, so that the ventral surface of the worm was against the bottom of the dish and the dorsal surface was up. After the worm was pinned flat, microelectrode penetrations were made in a mid-dorsal region, lateral to the gut and medial to the edge of the worm. Movements of the microelectrodes were controlled by a Leitz micromanipulator.

The microelectrodes were pulled from 1.5 mm capillary tubing (Omega Dot, Fredrick Haer) with a horizontal electrode puller (Narashige Instruments). These microelectrodes were filled with 3 M KCl and had resistances between 20 and 40 megaohms. A Ag-AgCl wire in the microelectrode was connected to a lead wire leading to a microelectrode preamplifier (M-4A, WP Instruments). The signal from the preamplifier was displayed on an oscilloscope (Tektronix 5118) and recorded on a chart recorder (Gould Model 220). A KCl-agar bridge was placed in the chamber as a ground.

Histological Studies

Horseradish Peroxidase Studies. In order to determine the location of the microelectrode tip, horseradish peroxidase (HRP) was injected through the recording electrode.

The microelectrode was filled with a 4% (weight/volume) HRP solution (Sigma, Type VI). The HRP solution was made by dissolving HRP in an injection buffer consisting of 0.05 M Tris (tris-hydroxy-methyl-aminomethane hydrochloride, Sigma) and 0.3 M KCl (Snow, Rose and Brown, 1976; Graybiel and Devor, 1974), and adjusted to pH 8.6 using 6 N NaOH. HRP filled electrodes had resistances between 60 and 100 megaohms.

The HRP was ejected from the microelectrode by 10 nA positive current pulses supplied by a microiontophoresis programmer (WP Instruments, Model 160). Ten to fifteen current pulses with durations of one second were used. The equipment available did not permit the potential to be monitored during the injection period, but the potential was recorded immediately before and immediately after injection.

After injection the worms were fixed immediately in the recording chamber by replacing the PB/HBS with cold 4% glutaraldehyde (in 0.1 M phosphate buffer pH 7.4). After five minutes, each worm was transferred to a small vial containing glutaraldehyde to continue fixation at 4°C for a minimum of three hours. No post fixation in osmium tetroxide was used since this interfered with the visualization of the HRP reaction product.

After fixation and a phosphate buffer rinse, the worms were placed in a solution of 30 mg% 3,3',diaminobenzidine (DAB, Sigma) in

0.1 M phosphate buffer pH 7.4 for 20 minutes. The reaction was continued for 15 minutes after the addition of 0.06% H_2O_2 (Graham and Karnovsky, 1966). The reaction of the DAB and H_2O_2 with the HRP produced an electron dense reaction product. After another phosphate buffer wash the worms were dehydrated in a graded series of ethanols (25-100%). Before infiltration the worms were taken through a series of acetones as an intermediate solvent. The worms were then embedded in Epon-Araldite/Spurrs resin. Sections were cut using a glass knife on a Sorvall Porter-Blum ultra-microtome. Thin sections (100 nm) were collected on 200-400 mesh copper grids and some were stained with uranyl acetate and lead citrate. Most sections, however, were left unstained since the stain made it more difficult to locate the HRP. Thin sections were examined in a Philips 201 or 300 transmission electron microscope at 45 kV or 60 kV.

Triton X-100. Triton X-100 (Research Products International) was employed to remove the tegument. Worms were incubated in a test tube of a solution of 0.2% Triton X-100 in HBS at 4°C for ten minutes (Oaks, Knowels and Cain, 1978). The worms were agitated gently with a Vortex mixer (Vortex Genie, Scientific Products, Inc.), then allowed to settle and the supernatant containing the teguments was removed and discarded. The worms were then incubated in FCS/Earle's at 37°C for at least one hour. Control worms were treated in the same manner except that the 0.2% Triton X-100 was omitted from the HBS. Microelectrode recordings were made of the treated and control worms. The worms were also prepared as described above for examination in the transmission electron microscope.

Ion Substitution Experiments

The time course of the effect of altered ion concentrations on membrane potentials was determined by first measuring the potentials in worms in PB/HBS at one minute intervals for five minutes, then exchanging a modified saline with the altered ion concentrations for the PB/HBS. Potentials were then measured in the worms in the altered saline solution at one minute intervals for 15 minutes. Recovery was determined by exchanging the altered saline solution for HBS and recording potentials at one minute intervals for ten minutes. The methods of Fetterer, Pax, Strand and Bennett (1978) were used to change the ion concentrations of HBS.

Potassium: A concentration of 60 mM K^+ was obtained by adding KCl. The chloride concentration was maintained by decreasing NaCl to 85 mM.

Sodium: A modified HBS solution with a sodium concentration of 37 mM was obtained by lowering the NaCl concentration and adding Tris-HCl. In this modified HBS, Hepes was omitted and Tris was used as a buffer (pH 7.4).

Calcium: Calcium chloride was eliminated from the HBS to give a calcium concentration of 0 mM.

Chloride: HBS with an altered chloride concentration of 100 mM was obtained by decreasing NaCl and replacing the Na^+ with Na_2SO_4.

Pharmacological Agents

Ouabain: Ouabain (Sigma), a cardiac glycoside, was dissolved at a concentration of 10^{-2} M in dimethylsulfoxide (DMSO) and then diluted to a final concentration of 10^{-5} M in HBS. Potentials

were measured in PB/HBS at one minute intervals for five minutes and then again in ouabain at one minute intervals for 15 minutes after an exchange of solutions was made.

Praziquantel: The antischistosomal compound praziquantel (PZ, Bayer Corp.) was dissolved at a concentration of 10^{-2} M in DMSO and then diluted to a final concentration of 10^{-6} M in HBS. The time course of the effect of PZ was determined by measuring the potentials in PB/HBS at one minute intervals for five minutes and then exchanging this saline for HBS containing PZ and measuring the potentials at one minute intervals for 15 minutes.

Lithium: The concentration of Li^+ was adjusted to 138 mM by a complete substitution of LiCl for NaCl. The time course of the effect of 138 mM Li^+ on the potentials was determined by first measuring the potentials in worms in PB/HBS at one minute intervals for five minutes, then exchanging the modified saline for the PB/HBS. Potentials were then measured in the worms in the 138 mM Li^+ saline solution at one minute intervals for 15 minutes. Recovery was determined by exchanging the altered Li^+ saline solution for HBS and recording potentials at one minute intervals for ten minutes.

Carbachol: The cholinergic agonist carbachol (carbamylcholine chloride, Sigma) was dissolved at a concentration of 10^{-2} M in double distilled water and diluted to a final concentration of 10^{-4} M in HBS. The time course of the effect of carbachol on the potentials was determined by measuring the potentials first in PB/HBS at one minute intervals for five minutes and then exchanging this saline for the saline containing 10^{-4} M carbachol and again measuring the potentials at one minute intervals for 15 minutes.

The effect of carbachol on a complete substitution of Li^+ for Na^+ was determined by adding carbachol (final concentration 10^{-4} M) to the bathing medium after the worm had been exposed to the Li^+ altered HBS for 15 minutes.

Dinitrophenol: The metabolic inhibitor 2,4-dinitrophenol (DNP) was dissolved in HBS at a concentration of 10^{-4} M. Potentials were measured in HBS at one minute intervals for five minutes and then again at one minute intervals for 15 minutes in 10^{-4} M DNP after an exchange of solutions was made. Recovery was determined by exchanging the DNP saline solution for HBS and recording potentials at one minute intervals for ten minutes.

Statistical Procedures

Results from experiments are expressed as means with one standard error. Statistical differences between means were determined by the Student t-test.

Tests for significance were performed by comparing the mean potential of each animal during the five minute control period with its means for the consecutive five minute periods after an exchange of solutions was made.

Preparation of Micrographs for Introduction

Micrographs for the Introduction were prepared using standard electron microscopy procedures. Parasites were fixed in cold 4% glutaraldehyde a minimum of 3 hours, post-fixed in 1% osmium tetroxide, and dehydrated using a graded series (25% increments) of ethanols. The specimens were embedded in Epon-Araldite/Spurrs resin,

using acetone as an intermediate solvent. Sections were cut using a glass knife on a Sorvall Porter-Blum ultra-microtome. Thin sections were collected on 300 mesh copper grids and stained with uranyl acetate and lead citrate. Thin sections were examined in a Philips 201 transmission electron microscope at 60 kV.

RESULTS

Microelectrode Recordings

Upon penetration of the dorsal surface of the adult male Schistosoma mansoni with a microelectrode, a rapid negative potential change was recorded (Figure 5). This potential appears to correspond to the tegumental potential as described by Fetterer et al. (1980) for the ventral surface. The value of this potential relative to the bath was -51.4 $\pm$ 0.6 mV (N = 130) with a range of -32.2 mV to -66.0 mV, compared to -35 mV reported by Fetterer et al. (1980) for the ventral tegument.

When the microelectrode was advanced several microns deeper into the animal, another potential change was recorded (Figure 5). This potential will be referred to as E_2. In my studies this potential had a value with respect to the outside bathing medium of -27.6 $\pm$ 0.6 mV (N = 130) with a range of -13.0 mV to -44.0 mV.

Upon further advancement of the microelectrode into the worm, a third potential change was observed. This potential will be referred to as E_3. It had a value as referenced to the outside bathing medium of -9.9 $\pm$ 0.5 mV (N = 130) with a range of -1.3 mV to -21.8 mV. A sharper change in potential often occurred with the recording of E_3 than with the recording of E_2; this potential would then drift gradually upward until a steady value was reached and maintained.

Figure 5. Potential profile obtained while penetrating into and beneath the dorsal tegument of adult male Schistosoma mansoni with a microelectrode. The sharp vertical drop indicates penetration of the dorsal tegument. The first upward potential change represents E_2. The second upward potential change represents E_3.
Calibration: Vertical, 10 mV; Horizontal, 2 sec.

In order to facilitate the recording of potentials, the values described above were obtained from worms bathed in HBS to which 50 mg% pentabarbitol (PB/HBS) had been added. In order to determine if the pentabarbitol had deleterious effects on the potentials, potentials obtained when animals were incubated in PB/HBS were compared to those obtained when HBS was used. Worms were first bathed in PB/HBS and ten microelectrode penetrations of each worm were made to obtain values for each of the three potentials. The PB/HBS was then exchanged for HBS and ten more microelectrode penetrations per animal were made. Mean values for each potential for ten measurements per animal were then determined for each bathing medium. PB/HBS has no significant effect on any of the three potentials (Table 1).

To determine how stable the three potentials are over time, recordings of the potentials were made at one minute intervals from worms in PB/HBS for a period of five minutes. The PB/HBS was then replaced with HBS and the potentials were recorded at one minute intervals for 25 minutes. Results are shown in Figure 6. These data show that the potentials are stable with time, and that return of worms to drug free HBS after a time in PB/HBS has no measureable effects on the potentials recorded.

Histological Studies

Tegument. To determine if the first potential recorded from the dorsal surface was in fact originating in the dorsal tegument, HRP was injected into ten worms when the recording electrode was placed in what was thought to be the dorsal tegument. Treatment of the worms to make the HRP electron dense revealed that HRP had actually been

Table 1. A comparison of potentials recorded in PB/HBS and HBS. Values are means ± one S.E.M. for 10 measurements per animal. Diff. is the difference between potentials in PB/HBS and HBS.

	E_{teg}			E_2			E_3		
Animal #	PB/HBS	HBS	Diff.	PB/HBS	HBS	Diff.	PB/HBS	HBS	Diff.
1	-54.9±0.4	-54.8±0.5	0.1	-35.2±0.4	-35.1±0.5	0.1	-3.1±0.5	- 2.9±0.6	0.2
2	-47.8±1.2	-46.8±0.9	1.0	-22.6±0.8	-22.7±0.7	-0.1	-6.4±0.6	- 6.1±0.5	0.3
3	-48.3±2.9	-50.5±2.3	-2.2	-26.9±0.7	-27.3±1.1	-0.4	-9.5±0.6	-10.5±1.0	-1.0
4	-45.1±0.9	-45.5±0.8	-0.4	-23.7±1.3	-22.0±1.0	1.7	-5.7±1.1	- 7.0±0.9	-1.3
5	-47.9±1.0	-46.1±1.0	1.8	-19.0±1.8	-21.9±1.2	1.2	-1.3±1.3	- 1.7±1.1	-0.4
6	-58.3±0.4	-58.0±0.3	0.3	-27.4±0.4	-27.9±0.5	-0.5	-7.5±0.4	- 7.9±0.5	-0.4
$\bar{X}$	-50.4	-50.3	0.1	-25.8	-26.2	-0.4	-5.6	-6.0	-0.4
SE	2.1	2.1		2.3	2.1		1.2	1.3	

Figure 6. Stability of potentials over time. Values are means $\pm$ one S.E.M. (N = 6). Worms were preincubated in PB/HBS with 50 mg% PB. At the arrow the medium was replaced with HBS without PB. Closed circles, E_{teg}; Open circles, E_2; Triangles, E_3.

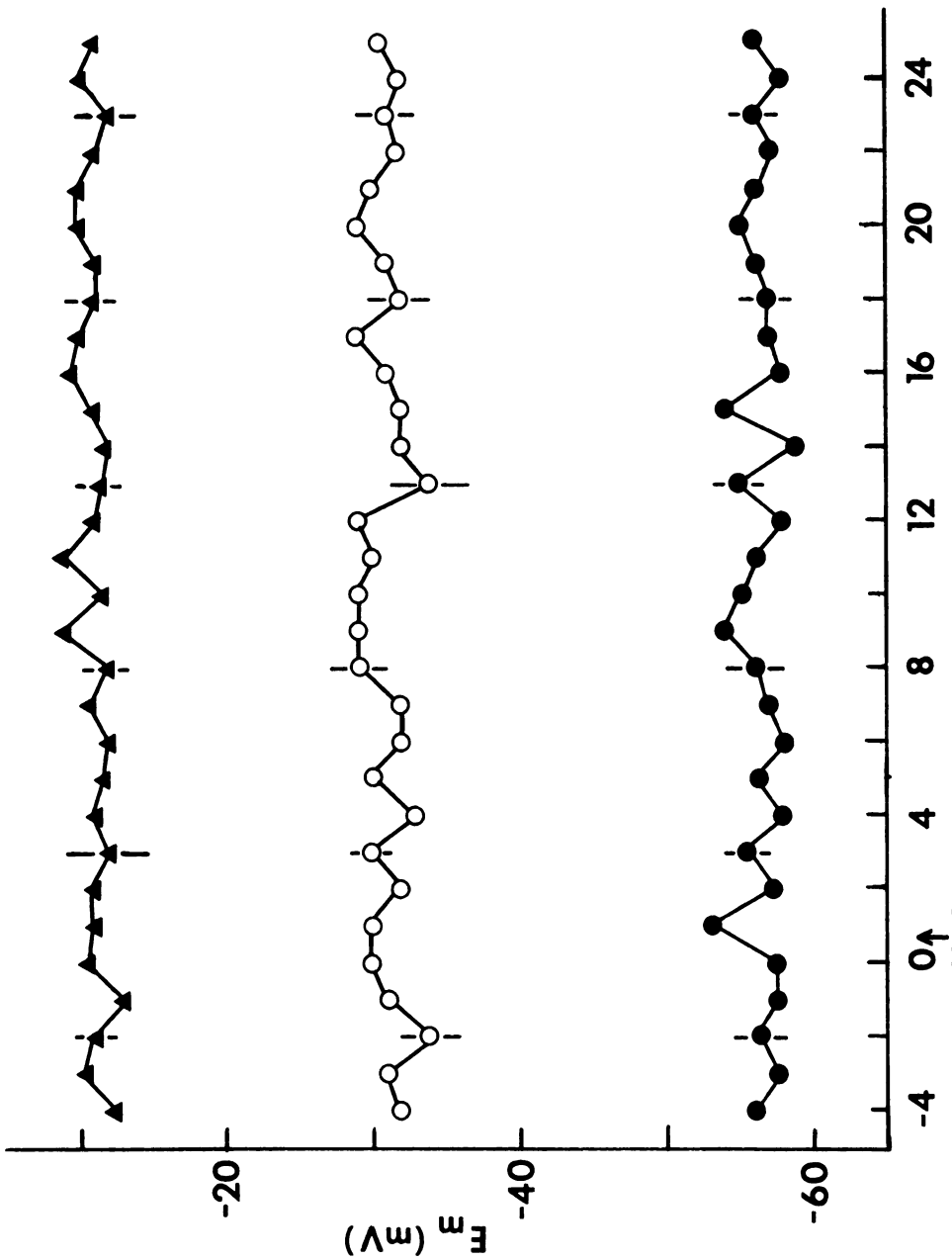

Figure 6

injected in seven of the ten worms. In all seven of these worms, electron micrographs of sections cut through the area in which HRP had been deposited showed the HRP to be confined to the dorsal tegument, its processes and cytons (Figure 7). On the basis of these results I will refer to this first potential as the tegumental potential (E_{teg}).

$\underline{E_2}$. To determine the anatomical correlate of E_2, HRP injections were attempted in 25 worms through a microelectrode which was used to record E_2. This was done by first penetrating the dorsal surface with the HRP injecting electrode, recording E_{teg} and then advancing the electrode until E_2 was recorded. Since the equipment used did not permit the potential to be monitored during the injection period, the potential was monitored immediately before and after the injection of HRP. Subsequent reaction of the HRP with DAB showed that 15 of the 25 attempted injections had resulted in HRP being deposited within the body of the worm.

In eight of the 15 worms with HRP deposits, sections cut through the area of injection showed that the HRP was confined to the longitudinal muscle (five worms, Figure 8a) or to the circular muscle (three worms, Figure 8b). In the other seven worms the HRP was localized in the tegument, its processes and cytons (Figure 8c).

In the above experiments, penetration into the dorsal tegument and dorsal E_2 area was made from the dorsal side of the animal. HRP injections were also done by penetrating the worm from the ventral side. As an electrode is advanced into a worm through the ventral surface, the potentials observed are the same as those described above for the dorsal surface, i.e. E_{teg}, E_2, and then E_3. If the

Figure 7. Localization of HRP injected while recording E_{teg}. DT, dorsal tegument; CM, circular muscle; LM, longitudinal muscle; DVM, dorso-ventral muscle; TC, tegumental cell body; CC, cytoplasmic channels of tegument. Calibration: 4 microns.

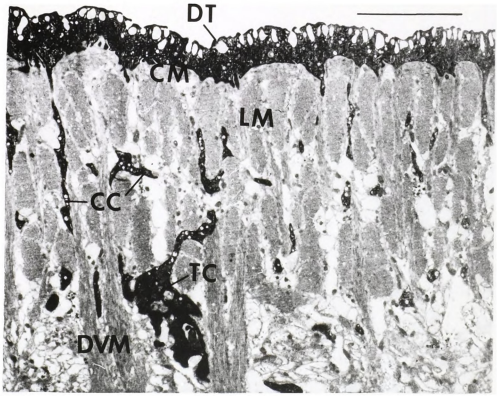

Figure 7

Figure 8. Localization of HRP injected while recording E_2.

a. HRP in longitudinal muscle. DT, dorsal tegument; CM, circular muscle; LM, longitudinal muscle. Uranyl acetate and lead citrate stained.
Calibration: 3 microns.

b. HRP in circular muscle. DT, dorsal tegument; CM, circular muscle; LM, longitudinal muscle; DVM, dorso-ventral muscle. Calibration: 3 microns.

c. HRP in tegument. DT, dorsal tegument, CM, circular muscle; LM, longitudinal muscle; CC, cytoplasmic channels of tegument. Calibration: 5 microns.

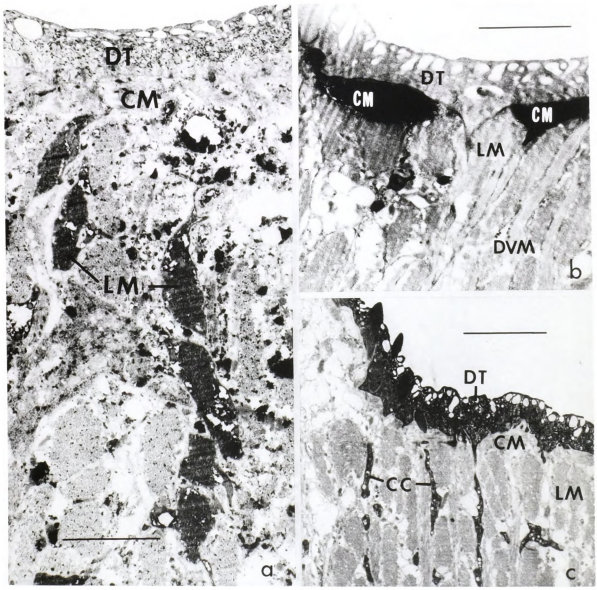

Figure 8

electrode is advanced further, two more potentials, each more negative than the potentials before it, are recorded before the electrode passes completely through the worm. I interpret these two potentials to be the dorsal E_2 and the dorsal E_{teg}.

Using the ventral approach, HRP was injected into two worms when the dorsal E_{teg} was recorded. The following potentials were recorded as the electrode advanced through the worm: -42 mV, -26mV, -12 mV, -25 mV, -45 mV; and in the other worm -52 mV, -30 mV, -25 mV, -8 mV, -23 mV, -56 mV. Sections cut through the area of injection showed HRP specifically in the dorsal tegument, its processes and cytons (Figure 9).

In four worms, HRP injections were made while the electrode was recording the dorsal E_2. In two of these worms sections cut through the site of injection showed the HRP in dorsal muscle (Figure 10a). The following potentials for these two worms were recorded as the electrode was advanced through the animals: in one worm -50 mV, -24 mV, -8 mV, -10 mV, -26 mV; and in the other worm -48 mV, -30 mV, -12 mV, -30 mV. In one worm the HRP was localized in the dorsal tegument and its processes (potentials were -45 mV, -25 mV, -10 mV, -10 mV, -25 mV) (Figure 10b) and in the other it was scattered throughout the worm from dorsal to ventral in the extracellular spaces, and in the interstitial fibers and basal lamina (potentials were -46 mV, -32 mV, -22 mV, -6 mV, -20 mV) (Figure 10c).

$\underline{E_3}$. To determine the origin of E_3, HRP was injected into four worms when the injecting electrode was in a position to record E_3. This was done by first penetrating the dorsal surface and recording E_{teg}, advancing the electrode until E_2 was recorded,

Figure 9. Localization of HRP injected while recording dorsal E_{teg} after electrode penetration through the ventral surface. DT, dorsal tegument; CM, circular muscle; LM, longitudinal muscle; CC, cytoplasmic channels of tegument; TC, tegumental cell body. Calibration: 4 microns.

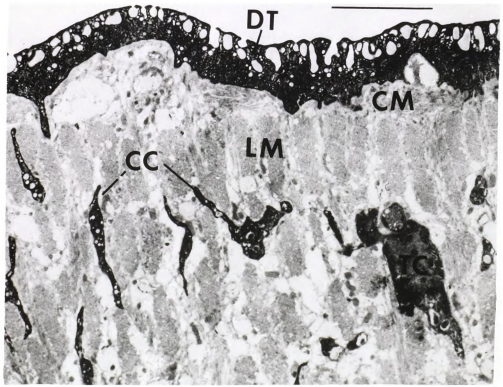

Figure 9

Figure 10. Localization of HRP injected while recording dorsal E_2 after electrode penetration through the ventral surface.

a. HRP in dorsal muscle. DT, dorsal tegument; LM, longitudinal muscle. Calibration: 4 microns.

b. HRP in dorsal tegument. DT, dorsal tegument; CM, circular muscle; LM, longitudinal muscle; CC, cytoplasmic channels of tegument; TC, tegumental cell body. Calibration: 5 microns.

c. HRP in basal lamina. DT, dorsal tegument; BL, basal lamina; CM, circular muscle; LM, longitudinal muscle. Calibration: 2 microns.

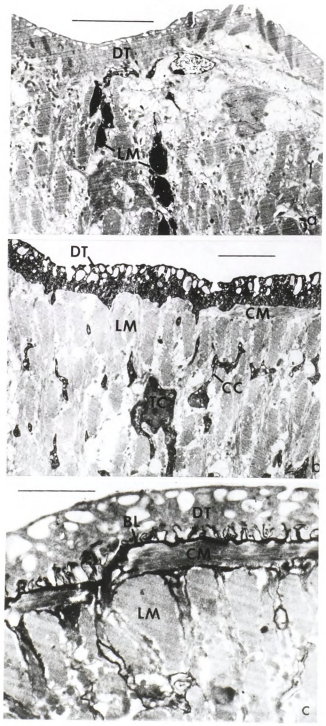

Figure 10

and then advancing the electrode until E_3 was recorded. In all cases sections cut through the HRP injection site showed the HRP to be localized in basal lamina, interstitial fibers and extracellular spaces (Figure 11). Some sections showed HRP in the dorsal basal lamina and also in the ventral basal lamina directly opposite, suggesting that the HRP had diffused from one side of the worm to the other.

Triton X-100. After Schistosoma mansoni were treated with Triton X-100 following the procedures described in the Methods section, only two discrete potentials could be recorded. The first potential change observed when the microelectrode penetrated the dorsal surface was a potential of -25.6 ± 2.1 mV ($N = 4$). If the electrode was advanced further a potential of -7.4 ± 1.2 mV ($N = 4$) was recorded.

Electron microscopic examination of the worms treated with Triton X-100 showed that the tegument in these animals had been removed, but that the underlying muscles were intact (Figure 12a). As controls for these studies a group of four worms were treated in the same manner as the experimental animals except that the Triton X-100 was omitted. These worms had three distinct potentials: E_{teg} was -46 ± 2.3 mV ($N = 4$); E_2 was -27 ± 1.9 mV ($N = 4$); E_3 was -8.8 ± 1.3 mV ($N = 4$). Electron microscopic examination of these worms showed that the worms were intact (Figure 12b).

The first potential recorded in the Triton treated worms was not significantly different from E_2 as recorded in the control worms ($0.3 < p \leq 0.4$) and the second potential recorded in the treated worms was not significantly different from E_3 recorded in the control worms ($0.3 < p \leq 0.4$). These results confirm that E_{teg} originates in the tegument, and suggest that E_2 is a muscle potential.

Figure 11. Localization of HRP injected while recording E_3. DT, dorsal tegument, BL, basal lamina; CM, circular muscle; LM, longitudinal muscle. Calibration: 2 microns.

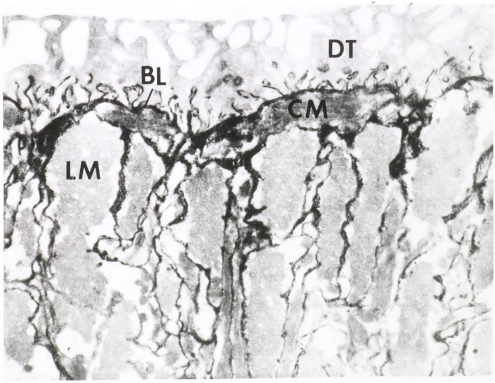

Figure 11

Figure 12. Electron micrographs of Triton X-100 experiment.

a. Triton X-100 treated worm. TR, tegumental remains; BL, basal lamina; CM, circular muscle; LM, longitudinal muscle; DVM, dorso-ventral muscle. Calibration: 2 microns.

b. Triton X-100 experiment control worm. DT, dorsal tegument; CM, circular muscle; LM, longitudinal muscle. Calibration: 2 microns.

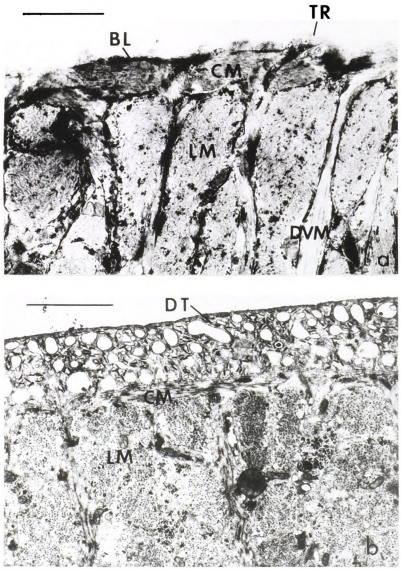

Figure 12

External Ion Substitutions

In an effort to determine some of the characteristics of the potentials, various external ion substitutions were made. First, repeated recordings of the three potentials were made at one minute intervals from worms in PB/HBS for a period of five minutes. The PB/HBS was then replaced with HBS containing varying ion concentrations and the potentials were again recorded at one minute intervals for 15 minutes. The altered HBS was then replaced with HbS and the potentials were again recorded at one minute intervals for ten minutes.

Potassium: When external K^+ concentration was raised to 60 mM, all potentials (E_{teg}, E_2, and E_3) were decreased. Within one minute, the shortest time possible to record, E_{teg} was reduced from -54 ± 1.8 mV to -24 ± 1.6 mV; E_2 was reduced from -30 ± 1.0 mV to -13 ± 1.0 mV; E_3 was reduced from -14 ± 1.2 mV to -4.0 ± 1.0 mV. Little change in any of these potentials was seen after these first minute changes. When the high K^+ medium was replaced with HBS, all potentials fell to near pre-treatment levels within one minute (Figure 13 and Table 2).

Sodium: Lowering the sodium concentration of the bathing medium to 37 mM caused the potentials to decrease, but only gradually (Figure 14). After 15 minutes the tegument depolarized from a control value of -60 ± 3.1 mV to -38 ± 2.6 mV; E_2 depolarized from -38 ± 2.1 mV to -27 ± 1.4 mV; and E_3 depolarized from -18 ± 2.0 mV to -8 ± 1.0 mV (Table 2). The effects of low sodium were not readily reversed when the worms were returned to HBS.

Calcium: When worms were exposed to HBS without Ca^{++} there

Figure 13. Time course of effect of 60 mM K^+. Values are means $\pm$ one S.E.M. (N = 6). Animals were preincubated in PB/HBS. At the first arrow the medium was exchanged with HBS containing 60 mM K^+. At the second arrow the high K^+ medium was replaced with HBS. Closed circles, E_{teg}; Open circles, E_2; Triangles, E_3.

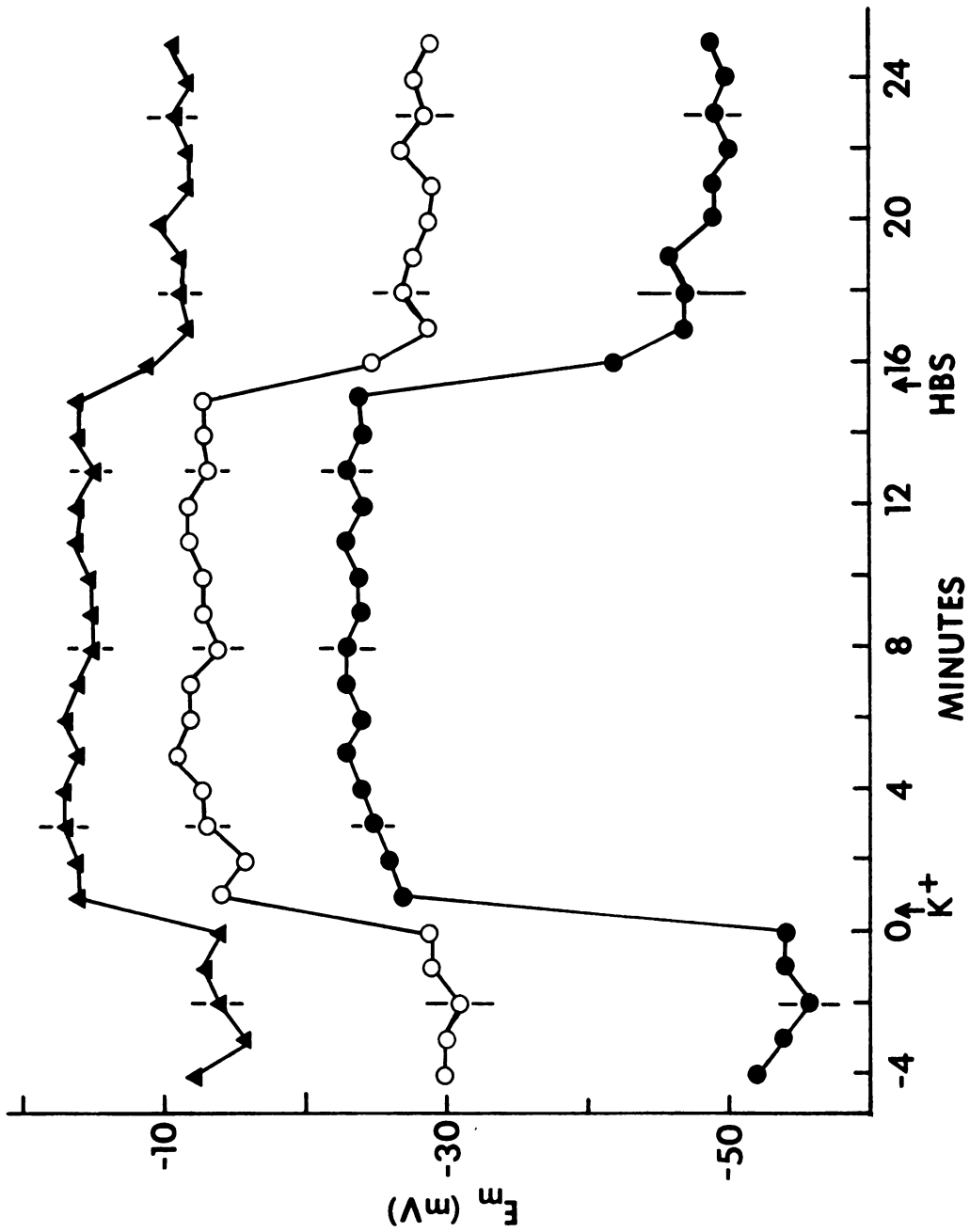

Figure 13

Figure 14. Time course of effect of 37 mM Na^+. Values are means $\pm$ one S.E.M. (N = 6). Animals were preincubated in PB/HBS. At the first arrow the medium was exchanged with the HBS containing 37 mM Na^+. At the second arrow the low Na^+ medium was replaced with the HBS.

Closed circles, E_{teg}; Open circles, E_2; Triangles, E_3.

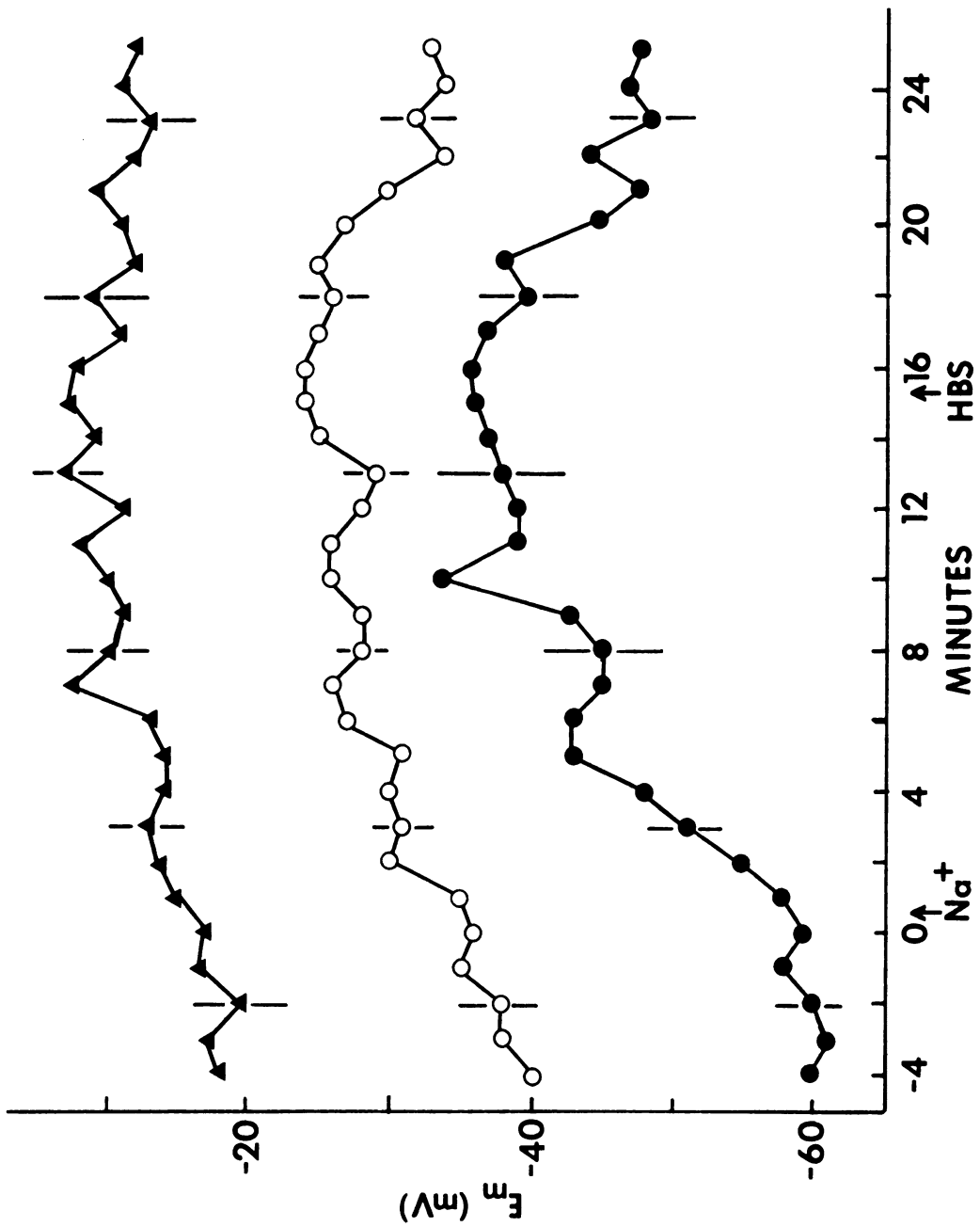

Figure 14

was a large depolarization in all the potentials. In contrast to the high K^+ HBS response, the depolarizations began only after several minutes and reached a peak after about ten minutes. At that time E_{teg} had been reduced by 37 mV; E_2 by 20 mV; and E_3 by 11 mV. No recovery occurred after the addition of Ca^{++} to the bathing medium (Figure 15 and Table 2).

Chloride: Lowering the chloride concentration to 100 mM reduced E_{teg} from -55 $\pm$ 1.4 mV to -48 $\pm$ 1.4 mV; E_2 from -34 $\pm$ 1.8 mV to -26 $\pm$ 1.0 mV; and E_3 from -14 $\pm$ 1.4 mV to -9 $\pm$ 1.0 mV. These values were reached within the first five minutes (Figure 16 and Table 2). When the low Cl^- medium was replaced with HBS, the potentials increased to near pretreatment values within five minutes.

Pharmacological Agents

Ouabain: The addition of 10^{-5} M ouabain to the bathing medium caused a gradual decrease of all the potentials, so that after 15 minutes E_{teg} was depolarized from a control value of -48 $\pm$ 1.3 mV to -23 $\pm$ 1.7 mV; E_2 was depolarized from -21 $\pm$ 1.0 mV to -9 $\pm$ 1.0 mV; and E_3 was depolarized from -7 $\pm$ 1.0 mV to -1 $\pm$ 0.4 mV (Figure 17 and Table 3).

Praziquantel: When worms were exposed to 10^{-6} M PZ HBS a large depolarization occurred in E_{teg} and E_2 but, as with ouabain, this effect was gradual, so that after 15 minutes E_{teg} had been reduced from -49 $\pm$ 1.9 mV to -24 $\pm$ 4.2 mV, and E_2 from -24 $\pm$ 1.9 mV to -11 $\pm$ 2.7 mV. E_3 did not begin to depolarize until after ten minutes. After 15 minutes E_3 had been reduced from

Figure 15. Time course of effect of 0 mM Ca^{++}. Values are means $\pm$ one S.E.M. (N = 6). Animals were preincubated in PB/HBS. At the first arrow the medium was exchanged with HBS with Ca^{++} eliminated. At the second arrow the 0 Ca^{++} medium was replaced with HBS.

Closed circles, E_{teg}; Open circles, E_2; Triangles, E_3.

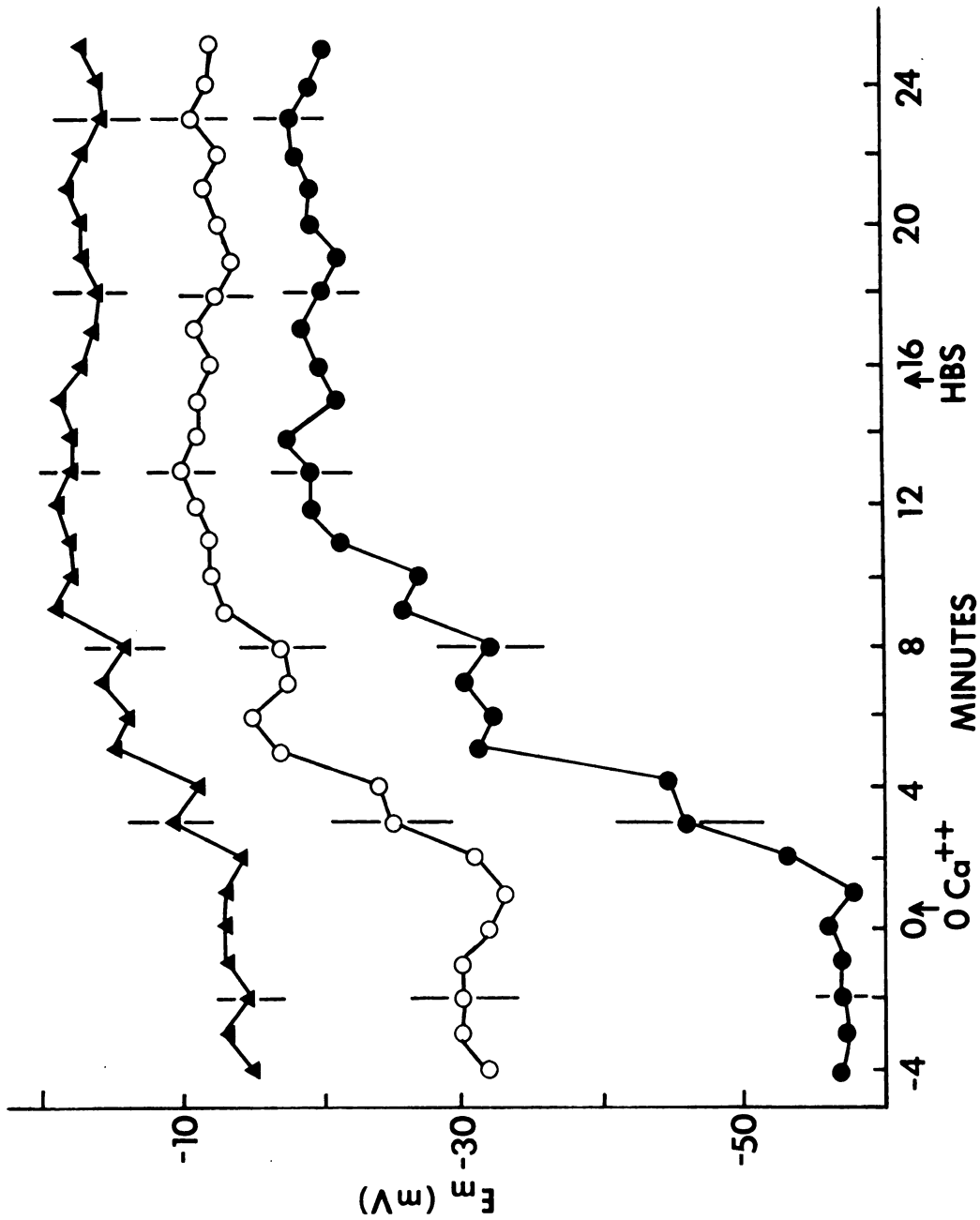

Figure 15

Figure 16. Time course of effect of 100 mM Cl^-. Values are means $\pm$ one S.E.M. (N = 6). Animals were preincubated in PB/HBS. At the first arrow the medium was exchanged with HBS containing 100 mM Cl^-. At the second arrow the low Cl^- medium was replaced with HBS. Closed circles, E_{teg}; Open circles, E_2; Triangles, E_3.

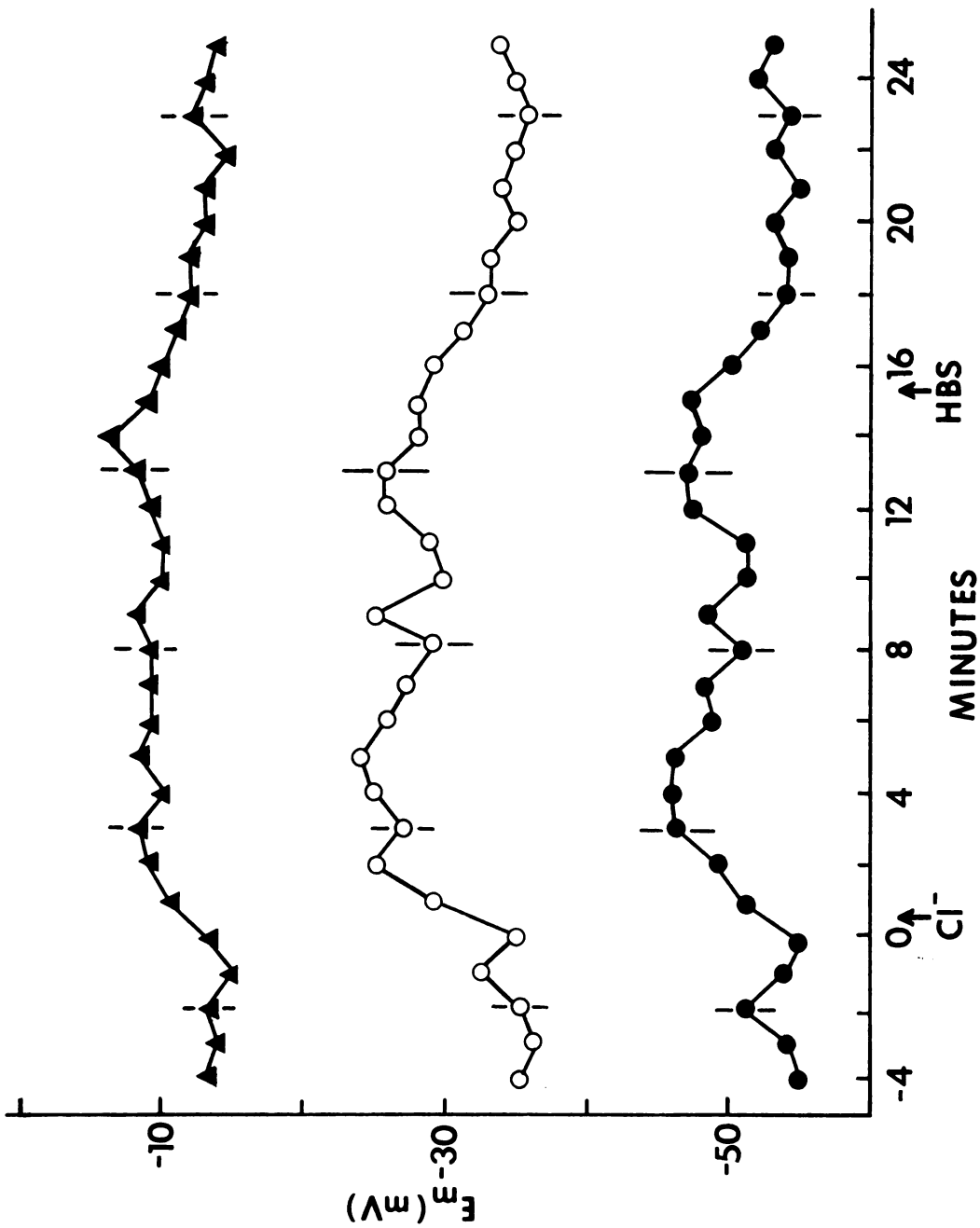

Figure 16

Table 2. Effects of ion substitutions on potentials in adult male Schistosoma mansoni. All values are means ± one S.E.M. (N = 6). E_R is the potential during the five minute control period in PB/HBS. E_{Exp} is the potential during the period 11-15 minutes after exchange to the altered HBS medium. E_R-E_{Exp} is the difference between control and experimental potentials.

ION	E_{teg}			E_2			E_3		
	E_R(mV)	E_{Exp}(mV)	E_R-E_{Exp}	E_R(mV)	E_{Exp}(mV)	E_R-E_{Exp}	E_R(mV)	E_{Exp}(mV)	E_R-E_{Exp}
60 mM K^+	-54±1.8	-24±1.6[a]	30	-30±2.0	-13±1.0[a]	17	-14±1.2	-4±1.0[a]	10
37 mM Na^+	-60±3.1	-38±2.6[a]	22	-38±2.1	-27±1.4[b]	11	-18±2.0	-8±1.0[a]	10
0 mM Ca^{++}	-57±1.7	-19±1.3[a]	38	-31±1.7	-10±1.0[a]	21	-14±1.8	-3±0.7[a]	11
100 mM Cl^-	-55±1.4	-48±1.4[a]	7	-34±1.8	-26±1.0[a]	8	-14±1.4	-9±1.0[b]	5

a $p \leq 0.01$
b $0.01 < p \leq 0.05$

Figure 17. Time course of effect of ouabain. Values are means $\pm$ one S.E.M. (N = 6). Animals were preincubated in PB/HBS. At the first arrow the medium was replaced with HBS containing 10^{-5} M ouabain. Closed circles, E_{teg}; Open circles, E_2; Triangles, E_3.

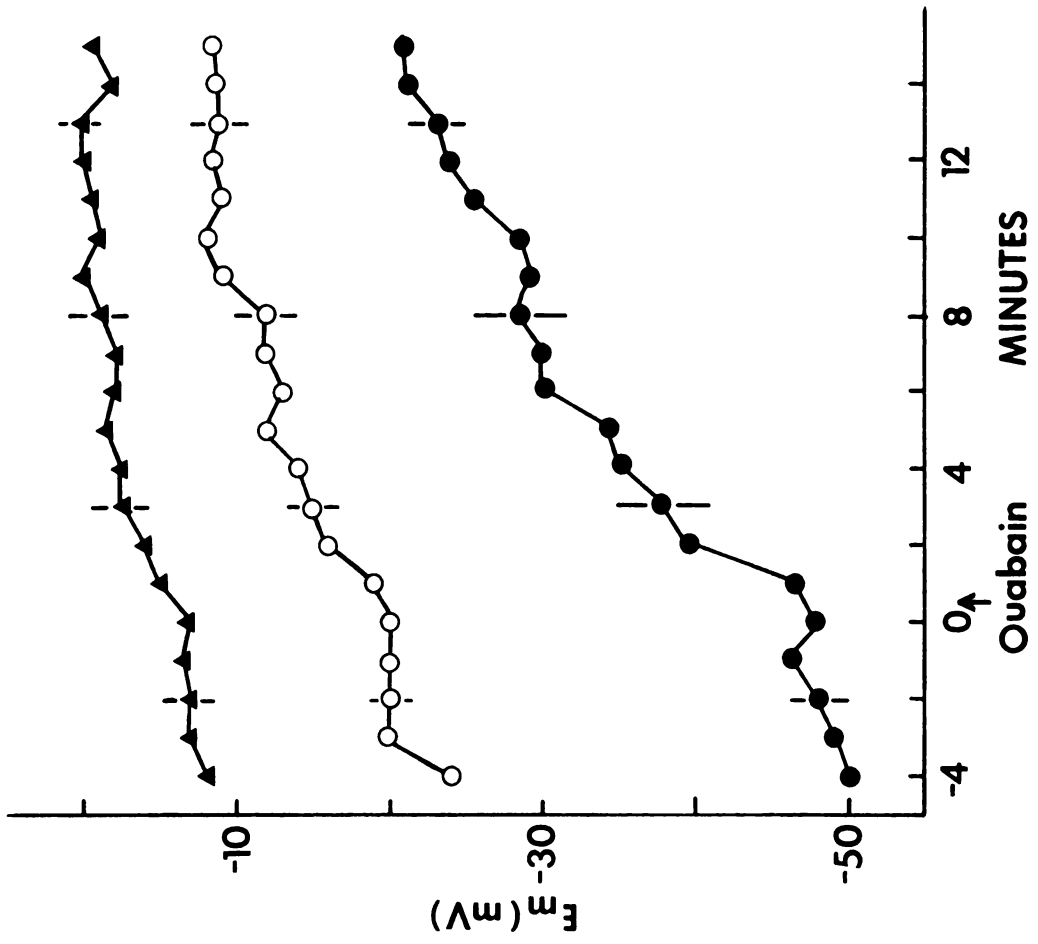

Figure 17

-7 ± 1.3 mV to -2 ± 1.6 mV (Figure 18 and Table 3).

Lithium: A complete substitution of Li^+ for Na^+ caused a large depolarization of all potentials, so that after 15 minutes E_{teg} had been reduced from -52 ± 1.5 mV to -33 ± 1.4 mV; E_2 from -30 ± 1.4 mV to -18 ± 1.4 mV; E_3 from -13 ± 1.5 mV to -6 ± 1.4 mV. As with low Na^+ and ouabain these depolarizations were gradual. The effects of 138 mM Li^+ were not readily reversed when the worms were returned to HBS (Figure 19 and Table 3).

Carbachol: The addition of 10^{-4} M carbachol to HBS caused a slight hyperpolarization of E_{teg} which reached a peak after ten minutes. At this time E_{teg} had been increased from -52 ± 0.5 mV to -55 ± 1.2 mV. Carbachol had similar but lesser effects on E_2 and E_3 (Figure 20 and Table 3).

Carbachol and Lithium: When carbachol was added to the bathing medium after the worm had been exposed to 138 mM Li^+ HBS for 15 minutes, the effect of the high Li^+ medium was partially reversed so that E_{teg} increased from -30 ± 2.6 mV to -41 ± 1.7 mV; E_2 increased from -16 ± 1.2 mV to -26 ± 1.4 mV; E_3 increased from -7 ± 1.0 mV to -10 ± 1.1 mV (Figure 21 and Table 3).

Dinitrophenol: The addition of 10^{-4} M dinitrophenol to the bathing medium caused a rapid depolarization of all three potentials, which was not reversed when the DNP medium was exchanged for normal HBS. E_{teg} depolarized from -47 ± 2.8 mV to -28 ± 2.7 mV; E_2 depolarized from -26 ± 2.0 mV to -16 ± 1.7 mV; E_3 depolarized from -9 ± 0.8 mV to -6 ± 1.1 mV (Figure 22 and Table 3).

Figure 18. Time course of effect of praziquantel. Values are means $\pm$ one S.E.M. (N = 6). Animals were preincubated in PB/HBS. At the arrow the medium was replaced with HBS containing 10^{-6} M praziquantel. Closed circles, E_{teg}; Open circles, E_2; Triangles, E_3.

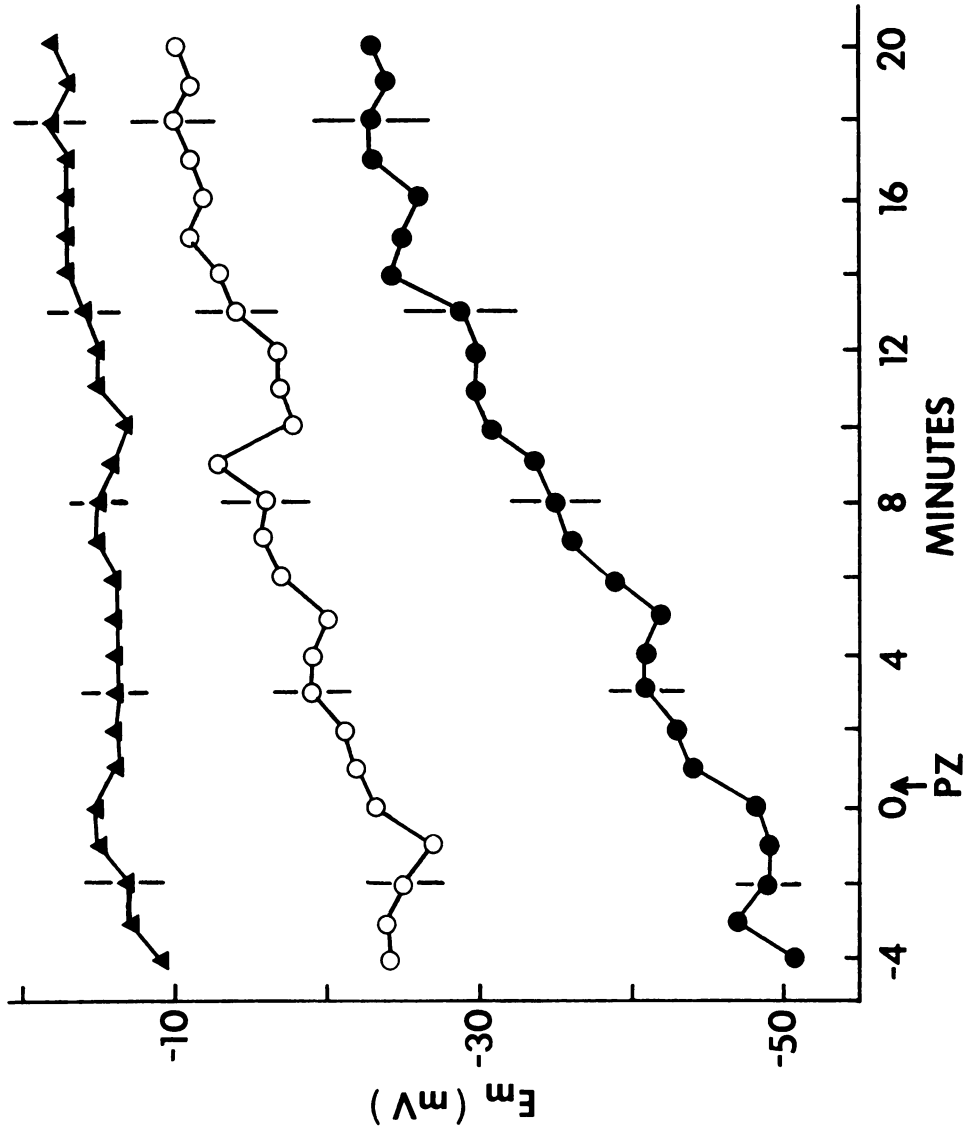

Figure 18

Figure 19. Time course of effect of 138 mM Li^+. Values are means $\pm$ one S.E.M. (N = 6). Animals were preincubated in PB/HBS. At the first arrow the medium was exchanged with HBS containing 138 mM Li^+ (a complete substitution of Li^+ for Na^+). At the second arrow the high Li^+ medium was replaced with HBS.
Closed circles, E_{teg}; Open circles, E_2; Triangles, E_3.

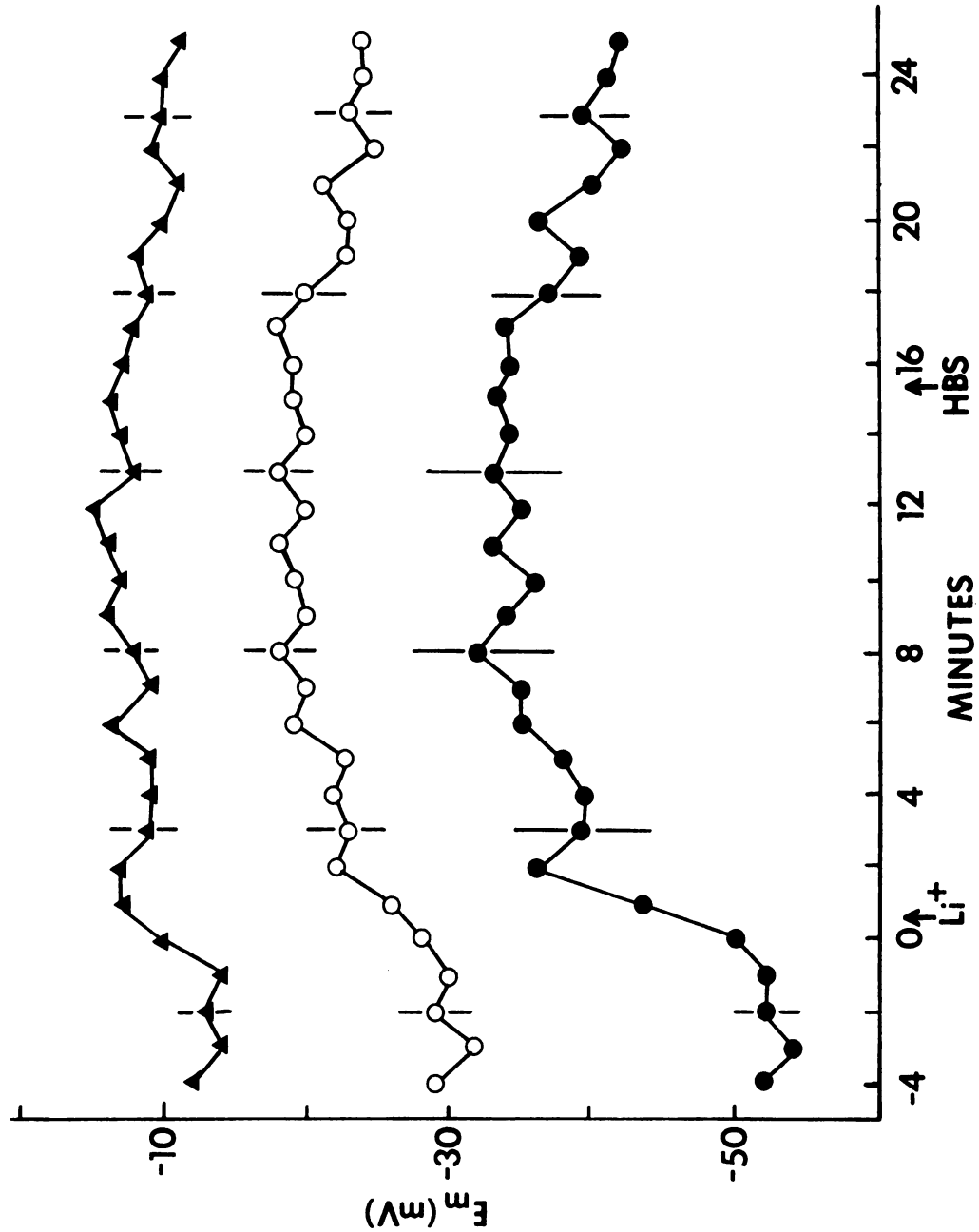

Figure 19

Figure 20. Time course of effect of carbachol. Values are means ± one S.E.M. (N = 6). Animals were preincubated in PB/HBS. At the arrow the medium was exchanged with HBS containing 10^{-4} M carbachol.

Closed circles, E_{teg}; Open circles, E_2; Triangles, E_3.

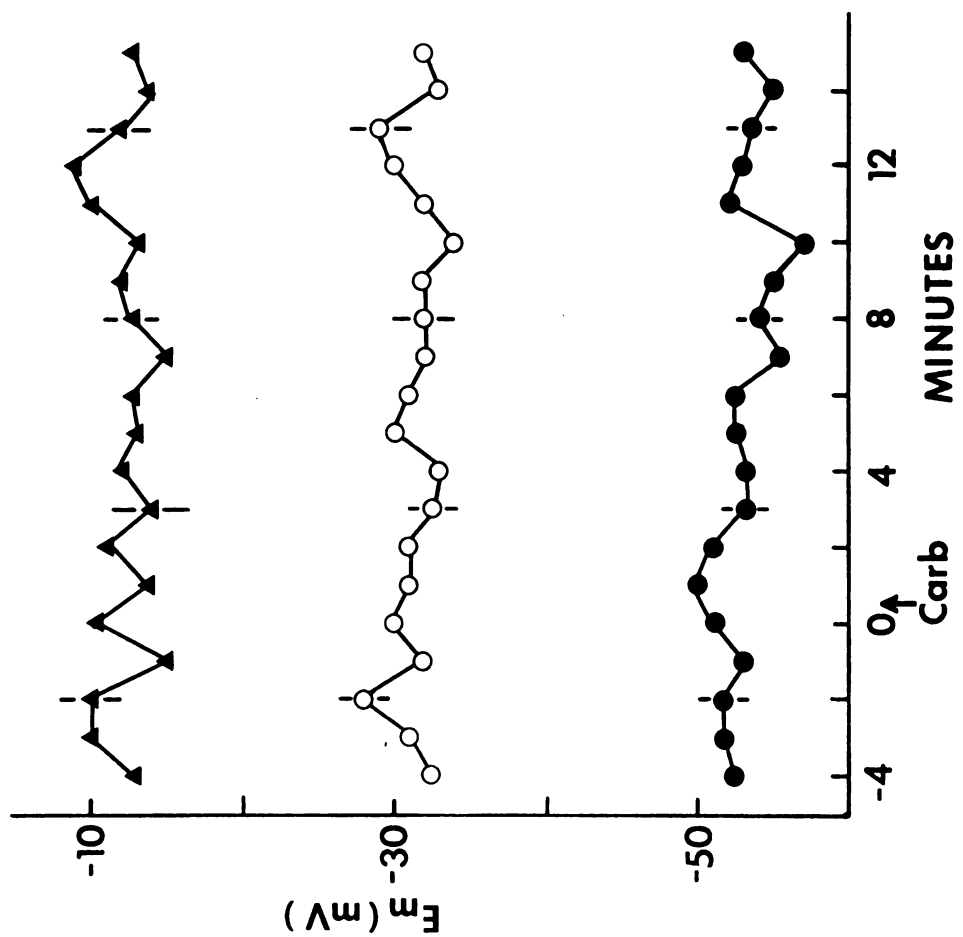

Figure 20

Figure 21. Time course of effect of carbachol on effect of 138 mM Li^+. Values are means $\pm$ one S.E.M. (N = 6). Animals were preincubated in PB/HBS. At the first arrow the medium was exchanged with HBS containing 138 mM Li^+. At the second arrow the high Li^+ medium was replaced with medium containing 138 mM Li^+ and 10^{-4} M carbachol.

Closed circles, E_{teg}; Open circles, E_2; Triangles, E_3.

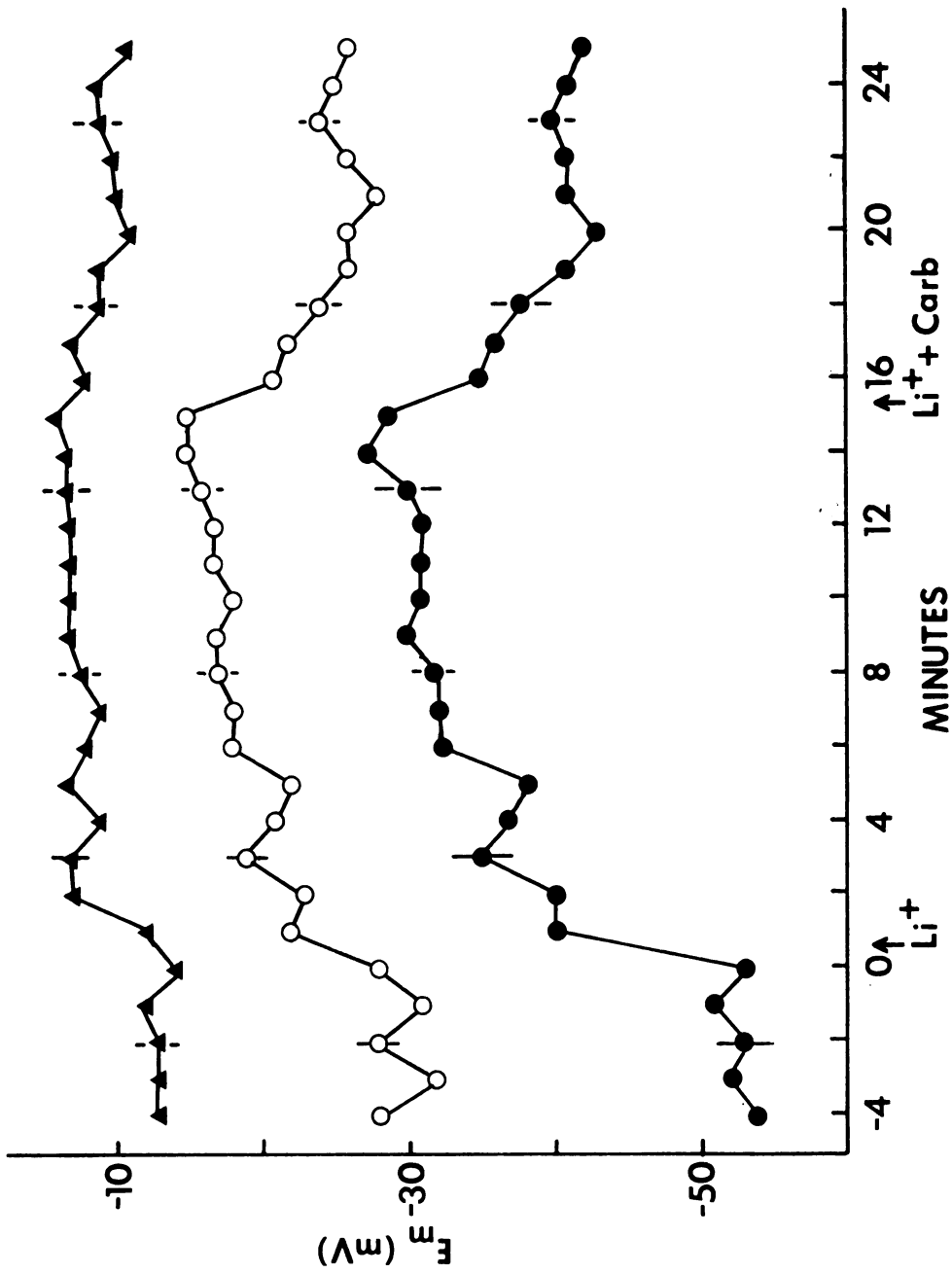

Figure 21

Figure 22. Time course of effect of DNP. Values are means $\pm$ one S.E.M. (N = 6). Animals were preincubated in PB/HBS. At the first arrow the medium was exchanged with HBS containing 10^{-4} M DNP. At the second arrow the DNP medium was replaced with HBS.
Closed circles, E_{teg}; Open circles, E_2; Triangles, E_3.

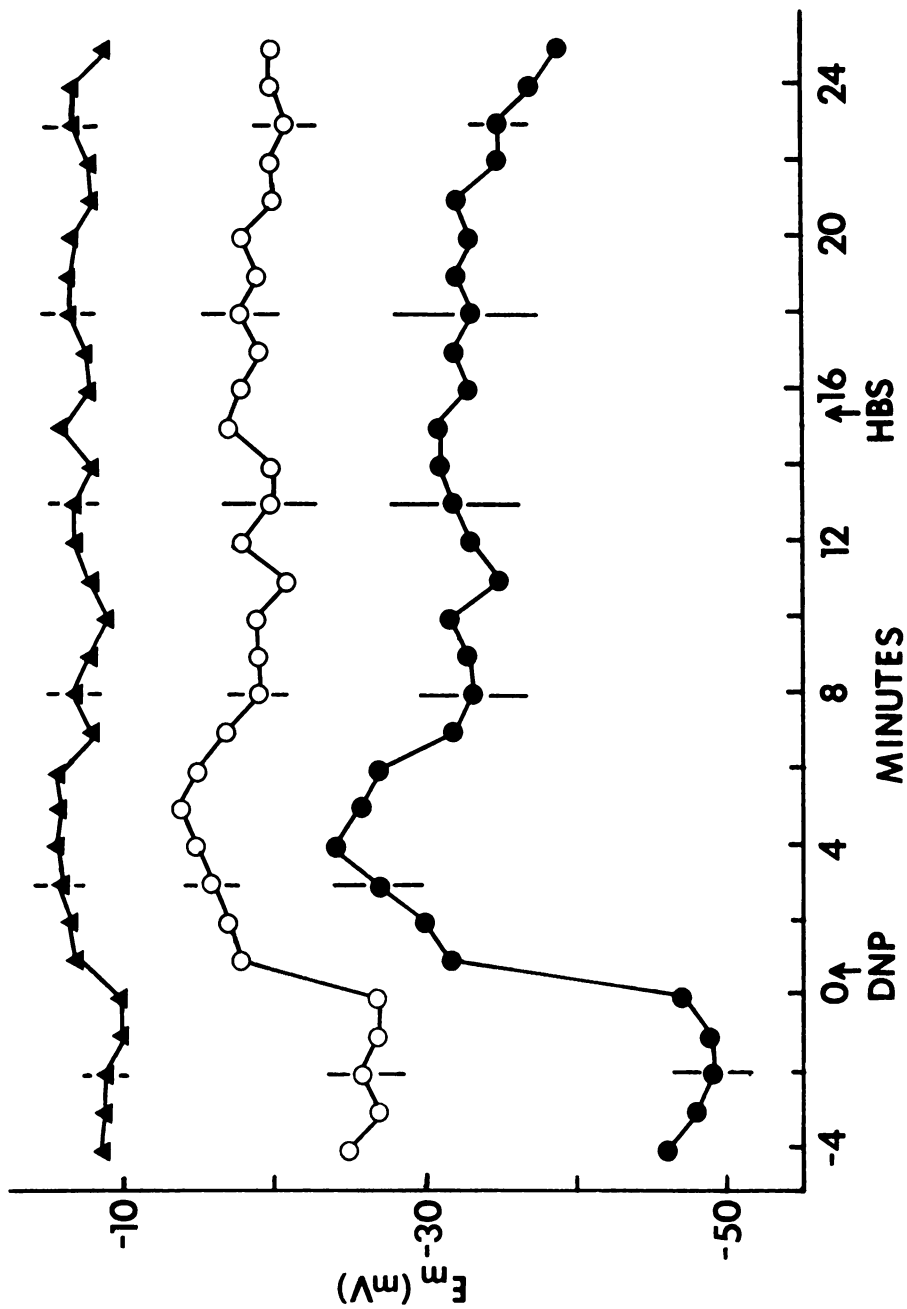

Figure 22

Table 3. Effects of pharmacological agents on potentials in adult male Schistosoma mansoni. All values are means ± one S.E.M. (N = 6). E_R is the potential during the control period in PB/HBS (E_R for Li^+ + Carb is for the period 11-15 minutes after exchange to 138 mM Li^+). E_{Exp} is the potential during the period 11-15 minutes after exchange to HBS with a pharmacological agent (E_{Exp} for PZ is for the 16-20 minute period after exchange; for Li^++ Carb the 11-15 minute period after addition of carbachol to the high Li^+ medium). E_R-E_{Exp} is the difference between control and experimental potentials.

Pharm. Agents	E_{teg}			E_2			E_3		
	E_R(mV)	E_{Exp}(mV)	E_R-E_{Exp}	E_R(mV)	E_{Exp}(mV)	E_R-E_{Exp}	E_R(mV)	E_{Exp}(mV)	E_R-E_{Exp}
Ouabain	-48±1.3	-23±1.7[a]	25	-21±1.0	- 9±1.0[a]	12	- 7±1.0	-1±0.4[a]	6
PZ	-49±1.9	-24±4.2[a]	25	-24±1.9	-11±2.7[b]	13	- 7±1.3	-2±1.6[b]	5
138 mM Li^+	-52±1.5	-33±1.4[a]	19	-30±1.4	-18±1.4[a]	12	-13±1.5	-6±1.4[a]	7
Carb	-52±0.5	-55±1.2[b]	- 3	-31±1.0	-32±1.0[c]	- 1	-11±1.3	-13±1.9[c]	-2
Li^+ + Carb	-30±2.6	-41±1.7[a]	-11	-16±1.2	-26±1.4[a]	-10	- 7±1.0	-10±1.1[b]	-3
DNP	-47±2.8	-28±2.7[a]	19	-26±2.0	-16±1.7[a]	10	- 9±0.8	- 6±1.1[b]	3

a $p \leq 0.01$
b $0.01 < p \leq 0.05$
c $0.10 < p$

DISCUSSION

Source of Potentials

Fetterer et al. (1980a) have shown that the potential change observed upon penetration of the ventral surface of the adult male schistosome originates across the outer tegumental membrane. My research with HRP and Triton X-100 confirms this observation for the dorsal surface of the adult male schistosome.

The musculature of the adult male schistosome forms an almost continuous mass directly beneath the basal lamina. The compact muscle bundles and the limited amount of interstitial space between them are interrupted only by the cytoplasmic channels connecting the tegumental cytons with the tegumental epithelium. On inspection such channels and the interstitial spaces occupy less than 5% of the total area, the rest being devoted exclusively to the muscle itself. On the basis of this anatomy one would expect that a microelectrode driven into the region immediately below the tegument would penetrate muscle cells and that potentials recorded there (E_2) would originate in the muscle. This appears to be borne out since HRP injection while recording E_2 shows the HRP to be localized in the musculature in the majority of worms tested.

Electrode displacement during the injection may account for the failure to localize the HRP in the muscle in some experiments.

Injection of HRP involves the application of relatively large amounts of positive currents. One would expect this to depolarize the cell and cause its contraction. A contraction during injection could cause the position of the electrode tip to change so that it is no longer in the cell. Upon termination of the current, the contraction would cease and the electrode tip would once again be in muscle. Thus when the potential is monitored immediately before and after injection, E_2 is recorded, even though the electrode tip was not in this compartment during the injection itself (a period when the potential could not be recorded). Thompson (1980, personal communication) has been able to observe through a dissecting microscope contractions caused by the injection of positive current while E_2 is being recorded.

After removal of the tegument by Triton X-100, E_{teg} no longer appears to be present. Instead the first potential recorded has a value of -25.6 mV, a value indistinguishable from E_2 recorded in control animals. The continued presence of this potential after the tegument has been removed further supports the conclusion that E_2 has its origin in the muscle. Subsequently E_2 will be referred to as E_{musc}.

HRP injections made while recording from the E_3 compartment result in a localization of the HRP in basal lamina, interstitial fibers and in extracellular spaces. From this I conclude that E_3 probably originates in the extracellular spaces around and beneath the muscles.

The ultrastructure shows that the tegument and underlying muscle are not in direct apposition, but rather are separated by basal lamina

and interstitial fibers. It might be expected then that as an electrode is driven into the worm, a potential, E_3, would be recorded after recording E_{teg} and before E_{musc}. This does occur but extremely infrequently. This is probably due to the small size of the areas involved, the tegument and muscle being separated by a space less than 0.5 micron in thickness, an area too small in which to locate the tip of the electrode. On those rare occasions when the E_3 potential was recorded immediately after recording E_{teg} it seems reasonable to assume that the electrode had penetrated the region between the circular muscle bundles where the interstitial fibers separate the muscles from one another and larger spaces are involved.

An electrode in the tegument records a potential of -51 mV; when in the muscle masses, a potential of -28 mV; and when in extracellular spaces a potential of -10 mV. With the potential in the extracellular spaces being apparently -10 mV with respect to the bathing medium, the potential gradient across the muscle membranes themselves must be -18 mV. The negative potential in the extracellular space also implies that the gradient at the basal tegumental membrane is -41 mV and not the -51 mV seen at the surface of the tegument.

Ion Effects

My studies show external ionic alterations have effects on the dorsal tegument similar to those reported for the ventral tegument (Fetterer *et al.*, 1980a; 1981). These alterations also have effects on the muscle membrane potential similar to the effect they have on

the tegumental membrane potential. High K^+ reduces both tegument and muscle potentials by about 60%; lowered Na^+ reduces both by about 30%; 0 Ca^{++} reduces both by 68%; low Cl^- reduces the tegumental potential by 13% and the muscle potential by 24%.

It appears that E_{musc}, as is the case for E_{teg}, is primarily a K^+ potential since altering the K^+ concentration produces such large changes in the potential.

Drug Effects

My studies show that external applications of ouabain, high Li^+ and PZ have effects on the dorsal tegument similar to those reported for the ventral tegument (Fetterer et al., 1980a, 1981).

As with external ionic alterations, external drug applications produce similar effects on both the tegumental membrane potential and the muscle membrane potential.

LiCl and DNP each reduce both potentials by about 40%; ouabain reduces both by about 55%. The depolarization seen with DNP reaches a peak in about 5 minutes and then shows a partial recovery. This parallels the effects seen on muscle tension (Fetterer et al., 1980b).

Praziquantel causes a large rapid increase in muscle tension (Pax, Bennett and Fetterer, 1978; Fetterer et al., 1980b). Though the contraction induced by PZ is complete within 20 seconds, depolarization of the muscle occurs only gradually over a period of minutes. From this it is clear that the contracture inducing ability of PZ is not a membrane depolarization dependent phenomenon. The muscle membrane potential parallels the tegumental potential in its depolarization. The mechanism of this depolarization appears to be a change in Na^+

and K^+ permeabilities or an increase in Ca^{++} permeabilities. Pax et al. (1978) have shown that PZ stimulates the uptake of Na^+ and Ca^{++} but inhibits the uptake of K^+.

Carbachol, which inhibits active contractions and produces a general relaxation of the schistosome muscle (Fetterer et al., 1977), causes only a slight hyperpolarization of the muscle and tegumental membrane potentials. The ability of carbachol to hyperpolarize the tegument and muscle and to partially reverse the effects of high Li^+ HBS may imply that cholinergic receptor sites are present on tegument or muscle membrane. But, since the concentration of carbachol used (10^{-4} M) was quite high, the effect may be non-specific. Further studies are needed to answer this question.

Active Transport

If a Na^+-K^+ pump is present, decreasing the Na^+ concentration would be expected to inactivate this pump and thus cause membrane depolarization (Thomas, 1972; Casteels, Droogmans and Hendrickx, 1973) Ouabain is a specific inhibitor of active Na^+-K^+ transport (Glynn, 1964) and also causes depolarization. Lithium inhibits the Na^+-K^+ pump by uncoupling Na^+-K^+ ATPase from the pump activity (Willis and Fang, 1970).

The depolarizing effects of ouabain, LiCl, and low Na^+ observed in Schistosoma mansoni suggest active transport is important in maintenance of the muscle potential just as is the case for the tegumental potential (Fetterer et al., 1981). Fetterer, Van deWaa and Bennett (1980) have found Na^+-K^+ pump sites in both tegument and muscle.

Characteristics of Other Smooth Muscles

The E_{musc} I have recorded, though somewhat low, appears comparable to that recorded for a variey of smooth muscles, both vertebrate and invertebrate (Table 4).

In smooth muscle none of the major ions are at equilibrium with the resting potential, so that changes in any of these ion permeabilities will cause a net ion movement which leads to a change in membrane potential (Brading, 1979). Changes in membrane potential in Schistosoma mansoni were observed in my research with changes in ion permeabilities caused by changes in ion concentration.

The control systems of smooth muscles bear a resemblance to those of striated muscle such that the cells have a resting potential which is determined largely by the potassium ratio (Carlson and Wilkie, 1974). In the schistosome the muscle potential is largely K^+ dependent. An increase in external K^+ concentration causes a depolarization of the muscle, which is accompanied by a maintained contraction (Fetterer et al., 1978). A depolarization caused by an increase in external K^+ concentration is also seen in Mytilus edulus (Twarog, 1967; Hidaka, Yamagachi, Twarog and Muneoka, 1977), in Poneroplax albida (Burnstock et al., 1967), the guinea pig taenia coli (Holman, 1958; Shimo and Holland, 1966) and the guinea pig stomach (Kuriyama, Osa, Ito, Suzuki and Mishima, 1974). This depolarization has been shown to increase the influx of Ca^{++} and the release of sequestered Ca^{++}, thus causing the contracture (Briggs, 1962; Edwards and Lorkovic, 1967; Kuriyama et al., 1974; Suano, 1976). Fetterer et al. (1980b) have shown that high K^+ also produces an influx of Ca^{++} into the schistosome.

Table 4. Comparison of Smooth Muscle Membrane Potentials

Animal	Muscle	Potential (mV)	Reference
Schistosoma mansoni	dorsal muscle	-18	this study
Poneroplax albida (mollusc)	posterior intestine	-38	Burnstock, Greenberg, Kirby and Willis, 1967
rat, mouse, cat, guinea pig	non-pregnant myometrium	-35	Marshall, 1959 Kuriyama, 1961 Bulbring, Casteels and Kuriyama, 1968
guinea pig	urinary bladder	-37	Creed, 1971
	gall bladder	-37	Creed and Kuriyama, 1971
Mustelus canis (dogfish)	mesenteric muscle	-20 to -40	Parnas, Prosser and Rice, 1974

Pax et al. (1978) have shown that normal spontaneous contractile activity may be dependent on extracellular Ca^{++}. An absence of extracellular Ca^{++} causes a depolarization in the smooth muscle of Schistosoma mansoni. Reduction of the external Ca^{++} concentration also depolarizes the smooth muscle of Mytilus edulus (Mizonishi, 1976) and the membrane of various verebrate smooth muscles (Liu, Prosser and Job, 1969; Kuriyama, 1971; Prosser, 1974; Tomita, 1975). This effect could be due to a destabilization of the membrane (Bulbring and Kuriyama, 1963) or to an increase in Na^{+} permeability indicated by the dependence of the depolarization on the presence of external Na^{+} (Tomita, 1975). Further research is necessary to determine the basis of the depolarization in the schistosome musculature.

Lowering the Cl^{-} concentration causes a depolarization of the muscle in the schistosome. A slight depolarization is also observed in Mytilus edulus (Hidaka and Twarog, 1977) and a depolarization occurs in some vertebrate smooth muscles (Holman, 1958; Kuriyama, 1963; Casteels and Kuriyama, 1966).

As in Schistosoma mansoni a decrease in external Na^{+} concentration causes a depolarization in mouse uterine smooth muscle (Osa, 1971, 1973; Tomita, 1975). The decrease in external sodium leads to a reduction of intracellular Na^{+} which may depress a Na^{+}-K^{+} pump (Osa, 1971). Just as an active Na^{+}-K^{+} pump may be important in maintaining the muscle potential in the schistosome, Na^{+}-K^{+} pumps are also important in many smooth muscles (Thomas, 1972; Prosser, 1974).

DNP inhibits the Na^{+}-K^{+} pump (Prosser, 1974; Brading, 1979), causes depolarization (Marshall and Miller, 1964), and increases the

internal Ca^{++} concentration in some smooth muscles (Prosser, 1974). In Schistosoma mansoni DNP causes depolarization and contraction of the muscle, but no significant accumulation of Ca^{++} from the medium (Fetterer et al., 1980b).

Coupling and Genesis of Potentials

In all of my studies there appears to be a close correlation between changes in E_{teg} and E_{musc}. This may indicate that the effects of external medium alterations act directly on both muscle and tegument and that the membrane characteristics of the tegument and muscle are similar. A direct effect on muscle is possible since it has been shown that many solutes and nutrients cross the tegument into the worm (Lumsden, 1975; Pappas et al., 1975; Uglem and Read, 1975), ouabain has been shown to penetrate the tegument (Fetterer et al., 1980), and the schistosome has been shown to be permeable to Na^{+}, K^{+} and Ca^{++} (Pax et al., 1978; Fetterer et al., 1980b). Since the tegument is permeable to these ions and ouabain binding sites are present (Fetterer et al., 1980) it also appears that a direct effect on E_{teg} occurs.

Ultrastructural studies have shown the presence of junctional complexes between tegumental cell bodies and muscle cell bodies (Silk et al., 1969). If these junctions represent low resistance pathways between tegument and muscle, then one might expect alteration of either tegumental or muscle potentials to be reflected by the other potential. Such coupling might explain why changes in E_{musc} do not lag changes in E_{teg} to any appreciable extent when the external medium is altered.

It is of interest to note that when the tegument is removed by Triton X-100, E_{musc} does not appear to be altered. Destruction of the tegument in this way might be expected to depolarize the muscle if tegument and muscle are electrically coupled. It is possible that the muscle potential is depolarized initially, but has time to recover during the one hour incubation between tegument removal and electrical recordings.

Just as there appears to be a close correlation between E_{teg} and E_{musc}, there appears to be a close correlation between E_3 and these two potentials. E_3 appears to represent the electrical potential in the extracellular spaces within the animal. Extracellular space within adult male schistosomes appears to be limited. In such a case significant changes in the ionic concentrations in this space may be brought about by activity in tegument or muscle and it is this change in ionic concentrations that might be responsible for the concomitant changes in E_{musc}, E_{teg}, and E_3 that I observe. Changes of this type have been shown to be of importance in the leech and Necturus (Kuffler and Potter, 1964; Cohen, 1970; Somjen, 1975) where ion fluxes across the membranes of glial cells and neurons give rise to potential differences in the extracellular spaces.

E_3 might be analogous to the transepithelial potential measured in frog skin (Koefoed-Johnson and Ussing, 1958), toad bladder (Frazier, 1962; Kimura, Urakabe, Yauasa, Miki, Takamitsu, Orita and Abe, 1977) or gills (Mantel, 1967; Kerstetter and Kirschner, 1972). However, after Triton X-100 treatment, which removes both inner and outer tegumental membranes, E_3 remains.

Further studies of this potential under various experimental conditions will be necessary to determine the details of its genesis and its significance.

SUMMARY

Three potentials can be observed upon advancement of an electrode into and beneath the dorsal surface of an adult male Schistosoma mansoni. Histological studies using horseradish peroxidase and Triton X-100 indicate the origins of these potentials. The first potential, having a value of -51 $\pm$ 0.6 mV, originates across the outer tegumental membrane. The next potential encountered has a value of -28 $\pm$ 0.6 mV and originates in the dorsal musculature. A potential having a value of -10 $\pm$ 0.5 mV originates in extracellular spaces.

Altering external ion concentrations (i.e., high K^+, low Na^+, 0 Ca^{++}, low Cl^-, high Li^+) depolarizes all three potentials. The tegument and muscle potentials are primarily K^+ potentials.

External applications of ouabain, 2,4-dinitrophenol and praziquantel depolarize all three potentials.

Carbachol causes a slight hyperpolarization of all the potentials and partially reverses the effects of a complete substitution of Li^+ for Na^+.

The depolarizing effects of ouabain, LiCl and low Na^+ suggest that an active Na^+-K^+ pump is important in maintenance of both tegumental and muscle potentials.

There appears to be a close correlation between changes in E_{teg}, E_{musc} and E_3.

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