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COMPARATIVE PHYSIOLOGICAL STUDIES OF SCHISTOSOMA JAPONICUM AND SCHISTOSOMA MANSONI

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Timothy C. Martin

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COMPARATIVE PHYSIOLOGICAL STUDIES OF SCHISTOSOMA JAPONICUM AND SCHISTOSOMA MANSONI

By

Timothy C. Martin

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ABSTRACT

COMPARATIVE PHYSIOLOGICAL STUDIES OF SCHISTOSOMA JAPONICUM AND SCHISTOSOMA MANSONI

By

Timothy C. Martin

Physiological comparisons of Schistosoma japonicum and Schistosoma mansoni reveal several important differences. Ouabain (10^{-5}M), lithium substitution and elevated K^+ all increase the muscle tension of S. mansoni, but have little or no effect on the muscle tension of S. japonicum. In contrast, praziquantel (10^{-6}M) and low temperature (5°C) produce an equal increase in muscle tension in both species.

S. japonicum and S. mansoni differ in their dependence of external Ca^{++} for the mediation of longitudinal muscle contraction. Muscle contraction induced in S. mansoni by elevated K^+, praziquantel, ouabain and low temperature are all attenuated by a 5 minute preincubation in media containing zero Ca^{++} (plus 10^{-4}M EGTA). Similar pretreatment of S. japonicum has no effect on praziquantel or low temperature induced contractions.

The mechanical threshold for longitudinal muscle contraction is higher in S. japonicum than in S. mansoni. Microelectrode recordings showed that elevated K^+ (60 mM) caused depolarization of the muscle in both parasites, but produced an increase in muscle tension only in S. mansoni. This difference in mechanical threshold appears to be due to a

specific difference in calcium permeability, as $^{45}Ca^{++}$ accumulation in S. mansoni is nearly twice that in S. japonicum while uptake of $^{42}K^{+}$ is the same in both species. Microelectrode recordings show that elevated K^{+} and Li^{+} substitution both caused muscle depolarization in S. japonicum and S. mansoni, indicating that lithium and potassium ions are able to penetrate through the tegument to the level of the muscle in both parasites.

The tegument appears to be a greater permeability barrier to calcium ions in S. japonicum than in S. mansoni. Upon removal of the tegument with triton X-100, exposure of S. japonicum to elevated K^{+} produces a large muscle contraction that is similar to that observed in control S. mansoni. This contraction is dependent on the presence of Ca^{++} in the bathing medium. The reduced movement of Ca^{++} through the tegument of S. japonicum may be caused by fewer voltage sensitive calcium channels or more active extrusion of Ca^{++} by a Ca^{++}-Mg^{++} ATPase. S. japonicum were less responsive to the muscle relaxing effects induced by the voltage-sensitive Ca^{++} channel blocker, D-600. Enzyme analysis reveals a higher level of Ca^{++}-Mg^{++} ATPase in the tegument of S. japonicum.

While both parasites have an active Na^{+}-K^{+} transport system, ouabain or lithium induced inhibition produced a greater degree of muscle depolarization and a larger increase in muscle tension in S. mansoni.

To My Mother and Father

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INTRODUCTION

Schistosoma japonicum and S. mansoni are two medially important trematode parasites of man. It is estimated that these parasites infect well over 500 million people. S. japonicum is endemic to Southeast Asia, China, Japan and the Phillipines, while S. mansoni is distributed throughout most of Africa and parts of South and Central America.

S. japonicum and S. mansoni belong to the same genus and are similar in several respects. Superficially adults appear to be similar though S. japonicum is somewhat larger and is devoid of the tegumental spines characteristic of S. mansoni. The complicated life cycle of the two parasites is almost identical. Free swimming cercariae penetrate the skin of the human host and migrate through the body. Both parasites finally develop to maturity in the mesenteric veins and portal system. Females then lay large numbers of eggs, some of which penetrate the intestinal wall to be expelled in the feces (Noble and Noble, 1976). In general, S. japonicum causes more pathology than S. mansoni.

While the parasites appear to be quite similar in some respects, there are several important differences between them. The host-parasite interface, i.e. the tegumental surface, is quite different in the two worms. The tegument of S. mansoni has spines and bosses while

the tegument of S. japonicum lacks both of these structures and instead is composed of a multifolded membrane which gives its surface a sponge-like appearance (Sakamoto and Ishii, 1977). Both parasites depend primarily on anaerobic respiration and both utilize many of the same sugars and amino acids for energy, but recent evidence indicates that the TCA cycle and electron transport may be more important in the metabolism of S. japonicum than in S. mansoni (Huang, 1980).

S. japonicum is resistant to several antischistosomal drugs which are quite effective against S. mansoni. Three of these drugs, hycanthone, metrifonate and Roll-3128 are thought to act by interfering with normal nerve or muscle function. While this would suggest that the two parasites may differ with respect to nerve or muscle function, virtually no work has been undertaken to determine the underlying physiological cause or causes for these differences in drug sensitivity.

There have been no previous attempts to compare the basic physiology of these two schistosomes. This is due in part to the prevalent belief that an understanding of the physiology of S. japonicum can be inferred from studies of other species of human schistosomes. It was also only recently that techniques were developed which could quantitatively measure mechanical and electrical activity in schistosomes. Using these techniques, I have undertaken studies comparing S. japonicum and S. mansoni in the hope that these studies might help to clarify the underlying causes for the differential sensitivity of these two parasites to various antischistosomal drugs.

Pathology

Most of the pathology associated with schistosomiasis is caused by the deposition of parasite eggs in the host's tissue and the subsequent immune response. Female worms release eggs into the fine mesenteric vessels of the small and large intestine. Approximately half of the eggs penetrate the intestinal wall to be passed in the feces (Kang and Fan, 1973). Under proper conditions these eggs hatch into miracidia and penetrate the skin of an aquatic snail, thus maintaining the parasite's life cycle. Eggs which are not expelled in the feces are washed into the liver, or become lodged in the intestinal wall, forming granulomas. It is this pathology which is responsible for the symptoms of schistosomiasis; i.e., diarrhea, abdominal pain, anemia and spleen and liver enlargement.

While the etiology and symptomology of S. mansoni and S. japonicum infections are the same, there is a difference in the severity of disease caused by the two parasites. Infections caused by S. japonicum are more destructive than those caused by S. mansoni. There are two reasons for this. First, each female S. japonicum can lay ten times the number of eggs laid by a female S. mansoni (Kang and Fan, 1973). Second, the tissue reaction to the ova of S. japonicum is more acute than the reaction to S. mansoni eggs. The immune response to S. japonicum eggs occurs more rapidly and appears to be a generalized reaction. S. mansoni eggs cause a delayed but more specific immune response (Smither, 1972).

Morphology

General Anatomy. Adult male S. japonicum average 15 mm in length, while the adult male S. mansoni average 10 mm in length. Both

parasites are about 1 mm wide. Female S. japonicum and S. mansoni are longer, 20 mm and 16 mm respectively, and thinner, approximately 0.2 mm wide. The males of both species have a groove, the gynecophoral canal, along most of their body length. The female usually lies within this groove (Figure 1).

Much of the general anatomy is the same for the two male schistosomes. Both species are covered with a tegumental epithelium. The tegument covers the parasite's outer circular and inner longitudinal muscle layer. Beneath the muscle layers, the nephridia, testes and intestinal cecae are suspended in a parenchyma matrix. The nervous system of both species consists mainly of two paired circumesophageal ganglia and four lateral nerve trunks. Small fibers from the lateral nerve trunks branch to innervate muscle bundles and tegumental sensory receptors (Silk and Spence, 1969).

Tegument. The body surfaces of both S. japonicum and S. mansoni are covered by an anuclear tegument. The dorsal tegument of male worms is usually from 3 to 5 microns in thickness, while the ventral tegument is relatively thin, measuring only 1 to 3 microns. The tegument is composed of many dense secretion granules, vacuoles of varying size and a few mitochondria, which originate in underlying nucleated epithelial cells. Protoplasmic channels connect the tegument with the nucleated epithelial cells which are located beneath the muscle layers. In contrast to the tegument, the epithelial cells of both schistosomes, contain many mitochondria (Morris and Threadgold, 1968; Inatomi et al., 1970).

S. japonicum and S. mansoni have small (1x4 μm) crystalloid spines embedded in their tegument. These spines are distributed over the

Figure 1. Scanning electron micrograph of paired S. japonicum and S. mansoni. Female is lying within the gynecophoric canal of the male. OS, oral sucker; VS, ventral sucker; GC, gynecophoric canal; F, female; M, Male. Calibration, 0.6 mm. Kindly supplied by D. P. Thompson, Dept. of Zoology, Michigan State University.

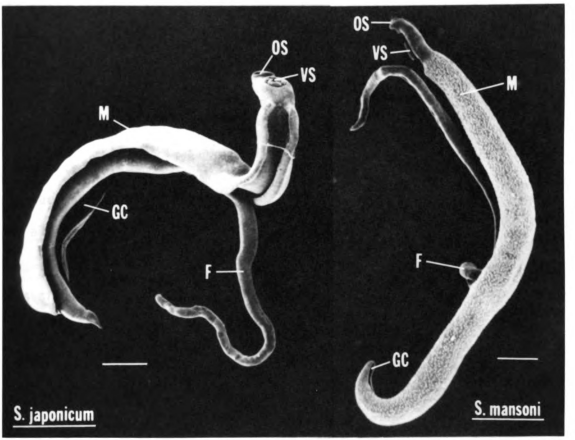

Figure 1

entire tegumental surface of S. mansoni, while the spines are located only near the ventral sucker and in the gynecophoral canal of S. japonicum. The dorsal tegument of S. mansoni is also invested with numerous tegumentary bosses, which are not found on S. japonicum. Scanning electron micrographs (4000X, kindly supplied by C. S. Bricker) show that except for the spines, bosses and what appear to be small sensory bulbs, the tegument of S. mansoni is smooth (Figure 2). S. japonicum also has the small sensory bulbs, but the actual tegumental surface is quite different from that of S. mansoni. S. japonicum's tegumental surface is composed of many complicated ridges and depressions, giving it a sponge-like appearance. The complexity of the tegumental surface of S. japonicum actually increases its surface area, relative to S. mansoni (Sakamoto and Ishii, 1977; Voge et al., 1978; Ma and He, 1981).

Recent work by Fetterer et al. (1981a) and Bricker et al. (1982) has shown that a distinct electrical potential can be recorded across the outer tegumental membrane of S. mansoni. This potential has a value of -35±7.4 mv across the ventral tegument (Fetterer et al, 1981) and -51±0.6 mv across the dorsal tegument (Bricker et al., 1982). Iontophoretic injection of horseradish peroxidase (HRP) when these large potentials are recorded, show that the electrical potential originates from the tegument and associated structures; i.e., tegumental cytons and cytoplasmic channels (Panel A, Figure 3).

Muscle. The muscle of S. japonicum and S. mansoni appears to be smooth muscle, having no striations. The arrangement of this muscle, i.e. circular, longitudinal and dorsal-ventral, is the same in the two parasites. Circular muscle is located beneath the tegument with the

Figure 2. Scanning electron microscopy comparing the tegumental surface of S. japonicum and S. mansoni. A and B show low magnification (600X) of the tegument of S. japonicum and S. mansoni, respectively. C and D show high magnification (4000X) of the tegument of S. japonicum and S. mansoni, respectively. SB, sensory bulbs; S, spine; B, bosses. Calibration bar for A and B, 13 μM; calibration bar for C and D, 2.5 μM. Kindly supplied by C. S. Bricker, Dept. of Zoology, University of Vermont.

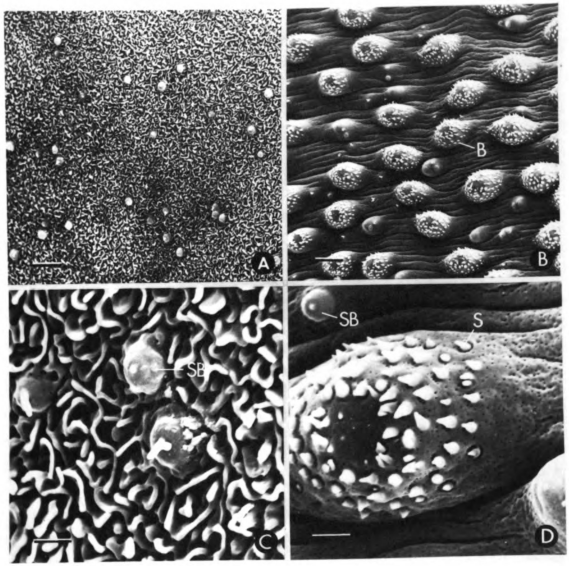

Figure 2

Figure 3. Transmission electron microscopy of tegument, muscle and extracellular space of male S. mansoni injected with HRP. A, injection of HRP into dorsal tegument and associated structure (E_1 = -51+0.6 mv). B, longitudinal muscle and C, circular muscle injected with HRP (E_2 = -10+0.5 mv). T, tegument; C, tegumental cyton; LM, longitudinal muscle; CM, circular muscle, ES, extracellular space. Calibration, 8 microns. Kindly supplied by C. S. Bricker.

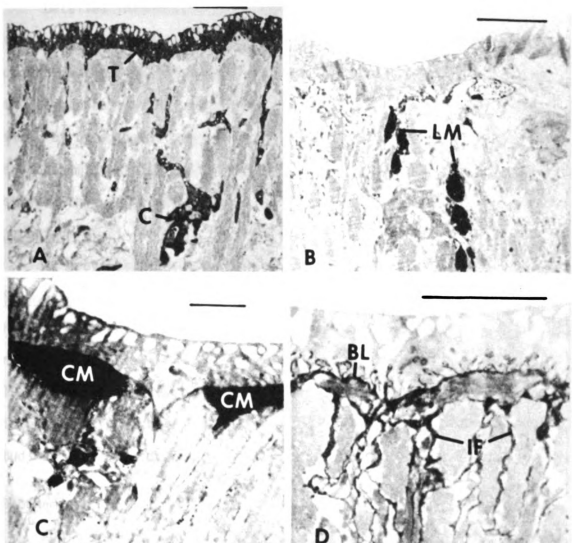

Figure 3

fibers arranged perpendicular to the long axis of the body. Longitudinal muscle lies below the circular muscle and is oriented in an anterior-posterior direction. The dorsal-ventral muscle extends through the body of the worm in a dorsal-ventral fashion. The muscles are surrounded by fine collagen-like fibers of connective tissue.

Detailed information about muscle structure is available only for S. mansoni (Silk et al., 1969). In this parasite, the cell body of the muscle fiber is located below the fibers at the same level as the tegumental cytons. The muscle cell body connects to the muscle fiber by cytoplasmic processes. While there is a poorly defined sarcoplasmic reticulum in the muscle of S. mansoni, no one has demonstrated that a sarcoplasmic reticulum is present in the muscle of S. japonicum.

As previously described, when a microelectrode is advanced into S. mansoni, the large tegumental (E_1) potential is encountered. Further advancement of the microelectrode shows that another, less negative potential (E_2) lies below the tegument (Bricker et al., 1982). This potential has a value of -28±0.6 mv. Iontophoretic injection of HRP shows that this potential originates from longitudinal and circular muscle (Panel B and C, Figure 3).

Biochemistry

Adult schistosomes have a functional digestive tract, the surface of which is modified for absorption. It is generally believed that the adult parasite ingests erythrocytes along with small organic compounds circulating through the host's portal system (Read, 1972). Uptake of these smaller nutrient molecules can also take place through

the tegument. In S. mansoni, trans-tegumental uptake of a variety of sugars and amino acids had been demonstrated (Cornford and Oldendorf, 1979). Indirect evidence for the tegumental uptake of sugars and amino acids in S. japonicum comes from experiments which show that these compounds are metabolized to CO_2 at rates that approximately equal to that in S. mansoni (Bruce et al., 1972, 1974).

Metabolism of carbohydrates is the major source of energy for adult S. mansoni and S. japonicum (Read, 1972; Bruce et al., 1974). While both parasites metabolize glucose, fructose, mannose and glucosamine, there are species differences in the amount of catabolism of each of these sugars. For example, male S. japonicum will produce approximately equal amounts of CO_2 from glucose, fructose and mannose, while male S. mansoni produces most CO_2 from glucose, and lesser amounts from mannose and fructose. Less CO_2 is produced from glucosamine than any of the other sugars, and the amount of glucosamine utilized by S. mansoni and S. japonicum is approximately the same.

Amino acids are used by both adult S. mansoni and S. japonicum for energy production, but this energy source appears to be secondary to carbohydrate metabolism. Bruce et al. (1972) found that the amino acids alanine, arginine, histidine, glutamine and aspartic acid are all metabolized to CO_2 by both schistosome species. The amino acid proline, however, was metabolized to CO_2 only by S. mansoni (Bruce et al., 1972).

Energy metabolism in adult schistosomes appears to be primarily anaerobic. Both parasites have active pyruvate kinase and lactic dehydrogenase (Bueding and Saz, 1968). In the case of S. mansoni, carbohydrate is converted to lactic acid which is then excreted (Read, 1972).

Additional evidence for anaerobic metabolism comes from studies which demonstrate that the activity of TCA cycle enzymes is very low in both species (Smith and Brown, 1977a). More recent evidence by Huang (1980) shows that S. japonicum has an active TCA cycle and several electron transport proteins. Huang provides two explanations for this discrepancy. First, he suggests that electron transport and oxidative phosphorylation may be more important for energy production in adult S. japonicum than in adult S. mansoni. A more likely explanation, according to Huang, is that both species have an active electron transport and oxidative phosphorylation system, in vivo. During worm collection and subsequent enzyme determination, other investigators failed to maintain appropriate oxygen tension in the incubation media, and thus caused the parasites to shift from the normal aerobic to anaerobic metabolism.

Pharmacology

Many of the comparative studies of S. japonicum and S. mansoni have involved the parasite's sensitivity or resistance to anthelmintic drugs. Both parasites reside in the mesenteric veins, and much of their general biology is the same. It is therefore not surprising that both parasites are equally sensitive to several antischistosomal drugs; e.g., tubercidin, praziquantel and several cyanide compounds. What is surprising, is the fact that S. japonicum is resistant to several drugs which are quite effective against S. mansoni. These compounds include hycanthone, metrifonate, oxamniquine, the complex antimonials and the benzodiazepine, Roll-3128.

Hycanthone. The mechanism of hycanthone's action has not been clearly shown, but it may interfere with either the serotonin (5-HT) system (Chou et al., 1973) or the cholinergic system (Hillman et al., 1977). The drug has also been shown to irreversibly damage the tegument of S. mansoni. The tegument of S. japonicum is not affected (Hillman et al., 1977). Clinical trials have shown hycanthone to be effective in the treatment of S. mansoni infections (Goodman and Gilman, 1976). In contrast, the drug is ineffective in treatment of S. japonicum infestations (Yarinsky, 1972). Recent evidence that the drug may be mutagenic (Meadow et al., 1973) has caused a decline in its widespread use.

Metrifonate. This drug is an organophosphate and as such is thought to work by inactivating the acetylcholinesterases. It is the drug of choice for treatment of infections caused by S. haematobium (Blair, 1977), another human schistosome which lives in the blood vessels of the urinary bladder. Metrifonate is also effective, although to a lesser degree, against S. mansoni (James and Webber, 1972). It does not, however, affect S. japonicum (James and Webbe, 1974).

Oxamniquine. The mechanism of action for oxamniquine is unknown. In general, S. mansoni are susceptible to oxamniquine, although different strains of this species do exhibit varying degrees of drug resistance. S. japonicum, however, seems to be totally resistant to oxamniquine.

Antimony. The antimony compounds are thought to inhibit phosphofructokinase, the enzyme that catalyzes the rate-limiting step in the glycolytic pathway (Goodman and Gilman, 1976). Antimonial compounds were the first effective antischistosomal drugs used for the treatment

of schistosomiasis. Antimony potassium tartrate and antimony sodium tartrate are effective in treatment of both S. japonicum and S. mansoni infections. Both drugs are also toxic and are responsible for a variety of side effects. More recently, a complex antimony compound has been developed which is less toxic, antimony sodium dimercaptosuccinate. This new drug will cure diseases caused by both species, but is less effective in treatment of S. japonicum infections.

Roll-3128. The mechanism of action for Roll-3128 is unknown. Clinical studies have shown that it is effective against S. mansoni but of little use against S. japonicum (Stohler, 1978). The in vitro response of the two schistosomes is also different. S. mansoni typically reacts to the drug with a large muscle contracture, while S. japonicum do not contract (Pax et al., 1978). The use of this drug therapeutically is questionable as like some other benzodiazepines, it causes sedation at effective antischistosomal doses.

Objectives

Schistosoma japonicum and S. mansoni are two medically important trematode parasites of man. While comparative studies of the two animals have shown that they differ with respect to drug sensitivity, i.e. S. japonicum is resistant, while S. mansoni is sensitive, to certain antischistosomal drugs, virtually no work has been undertaken to determine the underlying physiological cause or causes for these differences. A knowledge of the basic physiology of S. japonicum and S. mansoni, which may be responsible for the variability in drug response, is essential, not only for describing the mechanism of action

of known antischistosomals, but also for the rational development of new effective drugs.

I have undertaken this study to determine if S. japonicum and S. mansoni differ in some basic physiological way, and if these differences may be related to variability in drug response. The objectives of this study are:

(1) To determine what physiological differences exist between S. japonicum and S. mansoni, with respecto to motility.

(2) To determine the effect of environmental conditions and drugs, which alter motility, on the electrophysiology of the parasite's muscle.

MATERIALS AND METHODS

Source and Maintenance of Animals

Female white mice (Mus musculus) infected with Schistosoma japonicum (Formosa, Chinese and Philippine strain) were obtained from the laboratory of Dr. Y. S. Liang, University of Lowell, Lowell, MA. Female white mice infected with Schistosoma mansoni (Puerto Rican strain) were obtained from the laboratory of Dr. J. L. Bennett, Michigan State University, East Lansing, MI. All parasites were collected from the mesenteric and portal veins, 45 to 60 days post-infection and were maintained at 37°C in RPMI/1640 (Grand Island Biological Company) for mechanical and electrical studies. For biochemical or uptake studies, parasites were collected in Eagle's medium (Grand Island Biological Company) containing 0.5% pentobarbital to permit separation of male and female worms. All experiments were carried out on adult male schistosomes within 12 hours of their removal from the mice.

Recording Media

Hank's Balanced Salt Solution. All experiments were carried out in Hank's Balanced Salt Solution (HBS) or a modified HBS. The concentration of constituents of HBS are: 138 mM NaCl, 5.4 mM KCl, 0.5 mM $MgSO_4$, 1.4 mM $CaCl_2$, 0.5 mM KH_2PO_4, 0.25 mM Na_2HPO_4, 20 mM Hepes (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid) and 0.1% glucose. The pH was adjusted to 7.4 using 6 N NaOH and osmolality was 310 mOSM.

Potassium. The effect of elevated K^+ HBS on mechanical and electrical activity of the schistosomes was tested. HBS with 60 mM or 177 mM K^+ was made by increasing KCl concentration while decreasing NaCl concentration. All other constituents in the medium were unchanged. The modified KCl and NaCl concentrations were as follows: 60 mM K^+ HBS, 60 mM KCl and 87 mM NaCl; 177 m M K^+ HBS, 177 mM KCl and zero NaCl.

Lithium. In order to determine the effect of Li^+ on mechanical and electrical activity of the parasites, a modified HBS, in which LiCl was completely substituted for NaCl, was used. Final concentration of LiCl was 138 mM.

Calcium. The effects of elevated Ca^{++} and zero Ca^{++} on the muscle tension of the two parasites were tested. Zero Ca^{++} HBS was prepared by omitting $CaCl_2$ from the HBS and adding 10^{-4}M ethylene glycol bis(β-aminoethyl ether) N,N'-tetracetic acid (EGTA). Elevated Ca^{++} HBS was made by raising $CaCl_2$ to 14 mM and omitting phosphate and sulfate ($MgSO_4$, KH_2PO_4 and Na_2HPO_4) to prevent precipitation of Ca^{++}. Zero Ca^{++}-60 mM K^+ HBS was prepared as zero Ca^{++} HBS with 60 mM KCl and 87 mM NaCl.

Mechanical Recordings

Changes in the muscle tension of S. japonicum and S. mansoni were measured using a suction pipette-balance arm system as described by Fetterer et al. (1977, 1978). Figure 4 shows a schematic diagram of this system. The two suction pipettes were made from polyethylene tubing (i.d. 0.3 mm, o.d. 1.0 mm) which was drawn out to give an inside

Figure 4. Schematic diagram of apparatus used to make mechanical recordings from male Schistosoma japonicum and S. mansoni.

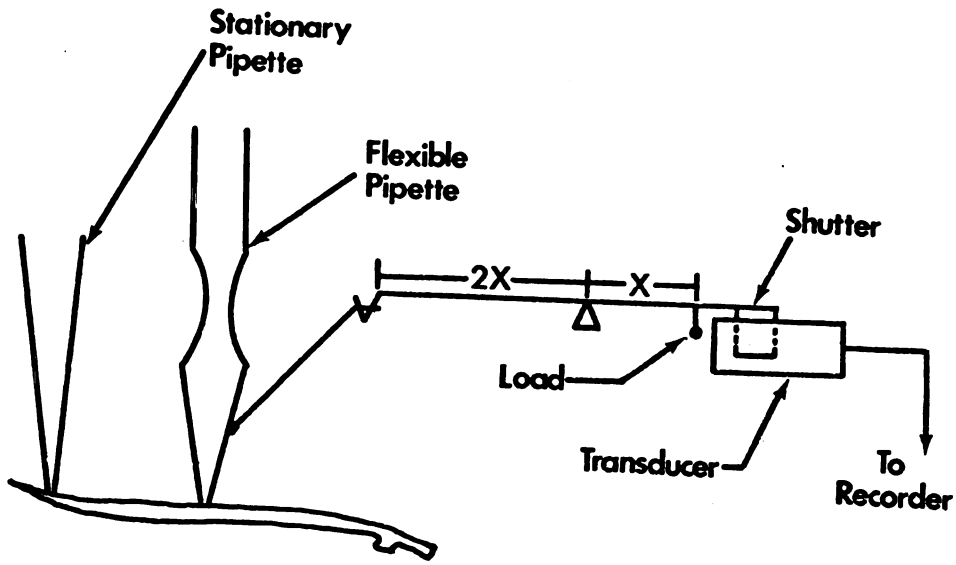
Stationary
Pipette
Flexible
Pipette
Shutter
2X
X
Load
Transducer
To
Recorder

Figure 4

diameter of 0.1 mm. One pipette was 2.5 mm long and inflexible. The other pipette was constructed to make it as flexible as possible. First, the length of tubing from which it was drawn was longer (5.5 mm) and second, it was drawn out so that a second constriction was placed about 1.0 cm above its tip. A 0.25 mm stainless steel wire, 5 cm long was connected to the end of the flexible pipette. The end of this wire rested on the balance arm, which was also made of 0.25 mm stainless steel wire and was 11 cm long. The fulcrum for the balance arm was placed 6 cm from the contact to the flexible pipette. The other end of the balance arm was attached to a blackened acetate strip which served as a shutter for the phototube in a modified "A" myograph (Narco Biosystems, Inc., Houstin, TX). The output of this transducer was connected to a type 7173 transducer coupler and amplified by a type 7000 amplifier (both by Narco Biosystems, Inc.). Output from the amplifier was displayed on a physiograph (Narco Biosystems, Inc.) as a pen deflection.

At the beginning of each experiment, 2.5 ml of buffered medium was placed in the polyethylene dish, which served as the recording chamber. The temperature of the recording chamber was maintained at 37°C, except in the low temperature experiment. The recording apparatus was calibrated by adding a 4 mg weight to the shutter end of the balance arm and observing the magnitude of the pen deflection on the physiograph. The tension exerted on the flexible pipette by the schistosome could later be expressed in terms of force necessary to cause an equivalent displacement of the shutter.

After the system was calibrated, a worm was placed in the recording chamber. The inflexible pipette was attached to the dorsal surface, about 1 mm from the posterior end of the parasite. The flexible pipette was then placed on the parasite anterior to the inflexible pipette. The worm was then stretched to produce an overall tension of 8 to 16 mg and a distance of 1.25 to 1.75 mm between pipettes. The 4 mg load was then applied to the shutter end of the balance arm. After placement of the suction pipettes, a minimum of 10 minutes of equilibration time was allowed before recordings were begun. The force exerted by the schistosome is dependent upon the length of the worm over which the measurements were taken. In this way, tension changes can be expressed in terms of milligrams per millimeter of worm, as compared to baseline tension; i.e., the tension level just prior to drug or altered ion treatment.

Microelectrode Recordings

The microelectrode recordings were made using the procedure described by Fetterer et al. (1981) and Bricker et al. (1982). Microelectrodes were pulled with a horizontal electrode puller (Narashige Instruments) from 1.5 mm capillary tubing (WPI, New Haven, CT). These microelectrodes had resistances between 20 and 40 MOhms. The microelectrodes were filled with 3 M KCl and connected to a preamplifier (M-4A, WPI) via a Ag-AgCl wire. The output of the preamplifier was displayed on an oscilloscope (Tektronix 5118) and recorded on a chart recorder (Gould Model 220). Fluid in the recording chamber was grounded with a 3 M KCl-Agar bridge with an Ag-AgCl wire.

Parasites were immobilized in 50 mg% pentobarbital-HBS to permit removal of the female from the gynocophoral canal. Males were then pinned to the sylgard with minuten insect pins with their ventral side down. After securing the parasite, the chamber was washed three times with drug-free HBS. Parasites were incubated in fresh HBS at 37°C for an additional 10 minutes before any measurements were taken. Microelectrode penetrations were made on the mid-dorsal surface lateral to the gut and medial to the edge of the worm. Recordings were made from electrical compartments (E_1 and E_2) at approximately one minute intervals. Electrical potentials were monitored for five minutes before addition of drugs or ions, and for another 10 to 20 minutes afterward. Advancement of the microelectrodes were controlled by a Leitz micromanipulator.

Pharmacological Agents

Ouabain. The effect of the cardiac glycoside, ouabain, on the mechanical and electrical activity of the parasites was also tested. Ouabain (Sigma) was dissolved in a 50-50 mixture of dimethylsulfoxide (DMSO) and distilled water (dH_2O). A subsequent dilution (1:10) was made with dH_2O. Twenty-five µl of this solution was then added to 2.5 ml of bathing fluid, bringing the final concentration of ouabain to 10^{-5}M.

Praziquantel. Praziquantel (kindly supplied by Drs. P. Andrews and H. Thomas of the Bayer Institute of Chemotherapy, Wuppertal, West Germany) was initially dissolved in DMSO to a concentration of 10^{-2}M. Two subsequent dilutions (1:10) were made with dH_2O. Twenty-five µl

of this solution (10^{-4}M) was then added to the bathing fluid, bringing the final concentration of praziquantel to 10^{-6}M.

D-600. The D-600 (Knoll A.G., Ludwigshafen am Rhein) was dissolved in DMSO at a concentration of 10^{-2}M. In the experiments measuring the effect of D-600 on muscle tension, 25 µl of this concentration was added to 2.5 ml of bathing fluid, bringing the final concentration of D-600 to 10^{-4}M.

Temperature

The effect of lowered temperature (5°C) on the muscle tension of the two parasites was determined. The temperature of the incubation chamber was lowered by circulating ice-water, rather than warm water (37°C), through the temperature regulating system. After 30 minutes, the incubation temperature was returned to 37°C.

Triton X-100

Triton X-100 (Research Products International) was used to remove the tegument of S. japonicum and S. mansoni according to the technique of Oaks et al. (1981). Parasites were separated and males were incubated in 0.2% triton X-100 at 4°C for 10 minutes. The worms were gently shaken with a vortex mixer (Vortex Genie, Scientific Products, Inc.) for 30 seconds). The worms were then allowed to settle, and the supernatant containing the tegumental fragments was removed and discarded. Parasites were then washed (7X) with cold (4°C) HBS and afterward, incubated for at least one hour in fresh HBS at 37°C, before any experiments were performed.

Ion Flux Studies

Potassium. Studies on $^{42}K^{+}$ uptake by schistosomes were performed by placing male S. mansoni (20 per vial) in each of 8 vials and male S. japonicum (15 per vial) in each of 8 vials containing 2 ml of HBS. Parasites were then pre-incubated at 37°C for at least one hour. Half of the parasites from each group, were then incubated in 10^{-5}M ouabain for 10 minutes. After this incubation period, 2.0 ml of HBS containing 8 μCI of $^{42}K^{+}$ (courtesy of the Nuclear Reactor Lab, Michigan State University) was exchanged for normal HBS in the 4 control vials of each group while the 4 vials of each group containing HBS with ouabain was exchanged for HBS containing both ouabain (10^{-5}M) and $^{42}K^{+}$. The above procedure was repeated for each time point tested (i.e., 2, 5, 10, 30, 60 and 120 minutes). The parasites were then separated from the incubation medium by filtration over a 25 mm glass fiber filter (Whatman GF/B) placed under vacuum on a millipore filtration apparatus (Millipore Co., Model XX10-024-00). The trapped parasites were then washed three times in 5 ml ice-cold HBS, weighed, placed in a vial containing 0.5 ml tissue solubilizer (NCS, Amersham Copr., Arlington Hts., IL) and incubated at 50°C for 60 minutes. Acetic acid (150 μl) was added to all samples. The solubilized parasites were then placed in a scintillation vial containing 5 ml of scintillation fluid (ACS, Amersham Corp.) and counted with a scintillation spectrometer (Beckman, Model 7000). Prior to the filtration process, a 20 μl aliquot was collected from each vial and the activity in it counted. In this way it was possible to express $^{42}K^{+}$ accumulation as mM K^{+} per kilogram wet weight.

Calcium. Parasites were prepared for studies of $^{45}Ca^{++}$ accumulation by first incubating worms (15 per vial) in 2.0 ml HBS for a minimum of one hour (37°C). The HBS was then removed and replaced with 2.0 ml of HBS containing 4 μCi $^{45}Ca^{++}$ (New England Nuclear, 15.1 mCi/mg). The worms were incubated in label containing media (37°C) for 15, 30 or 60 minute intervals. At the end of the incubation period, the parasites were filtered using glass fiber filters (Whatman GB/F) on a Millipore filter apparatus (Millipore Inc.) and rinsed three times with 5 ml of ice-cold HBS. After the parasites were trapped on the GB/F filters, they were treated as described above for the $^{42}K^{+}$ experiments. The activity of $^{45}Ca^{++}$ was expressed as counts per minute per milligram wet weight.

ATPase Assay

Ca^{++}- and Mg^{++}-dependent ATPases (i.e., Ca^{++}-Mg^{++} ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase) were measured according to the procedure described by Sulakhe et al. (1973). Parasites (40 per vial) were rinsed (3X) in appropriate buffer and then homogenized at 4°C. The concentration of the constituents in the buffered mediums were as follows: for Ca^{++}-Mg^{++} ATPase buffer - 50 mM tris-HCl, 5 mM MgCl and 40 μM $CaCl_2$ (pH=7.0); for Ca^{++}-ATPase buffer - 50 mM tris-HCl, 5 mM $CaCl_2$, 0.5 mM EDTA (pH=7.0); and for Mg^{++}-ATPase buffer - 50 mM tris-HCl, 5 mM $MgCl_2$ and 0.5 mM EGTA (pH=7.5).

The homogenized tissue was then centrifuged at 3000 RPM in a Beckman centrifuge (4°C) for 15 minutes. The supernatant was then removed and discarded and the tissue was resuspended in fresh buffer, and again

centrifuged. The tissue was washed using this procedure two additional times. After the washing, the vials containing the suspended tissue were incubated at 37°C. To start the reaction, ATP was added to each sample (final concentration, 5 mM). After a 10 minutes incubation period, 250 µl of trichloroacetic acid was added to each sample and the vial immediately removed from the warm water bath and placed in an ice bath. The vials were centrifuged at 3000 RPM for 15 minutes and an aliquot of the supernant removed. Enzymatic activity was determined by measuring PO_4 released into the supernatant using a spectrophotometer (Spectronic 20, Bausch and Lomb). Tissue samples were taken before addition of ATP and protein content was determined by the method of Lowry et al. (1951) with bovine serum albumin (Sigma) as the standard. All enzymatic activity was expressed as PO_4 released per hour per milligram of protein.

Statistical Procedure

Unless otherwise noted, results are expressed as the mean of a minimum of 6 $\pm$ one standard error of the mean. Standard two-tailed t-tests were used to determine statistical differences between means.

RESULTS

Normal Activity

Mechanical Activity. The mechanical activity recorded from adult male Schistosoma japonicum normally consisted of spontaneous contractions of variable frequency (5-50 per minute) and amplitude (Figure 5). This normal motility was indistinguishable from that observed in S. mansoni (Fetterer et al., 1977). Both parasites showed, over time, a gradual relaxation in baseline tension. During the first ten minutes, after parasites were hooked up, the muscle tension of S. japonicum decreased by -0.73±0.26 mg (N=12) while that of S. mansoni decreased by -1.05±0.25 mg (N=12). This muscle relaxation of the two parasites was not significantly different ($P \geq 0.2$).

Electrical Activity. The two species also possess similar electrical characteristics. As demonstrated by Fetterer et al. (1981a) and Bricker et al. (1982), S. mansoni contains three discrete electrical compartments. These electrical compartments have been anatomically identified as originating from (1) tegument (E_1), (2) muscle (E_2) and (3) extracellular space (E_3).

Figure 6 shows the potential changes observed as a microelectrode is advanced into S. japonicum. The most negative potential, E_1, was the first potential encountered. It had a value of -60.2±2.1 mv. This compares to the values I recorded for S. mansoni of -53.3±1.4 mv.

Figure 5. Sample chart recordings of spontaneous contractile activity from schistosomes. The top trace represents activity recorded from S. japonicum. The bottom trace represents activity recorded from S. mansoni.

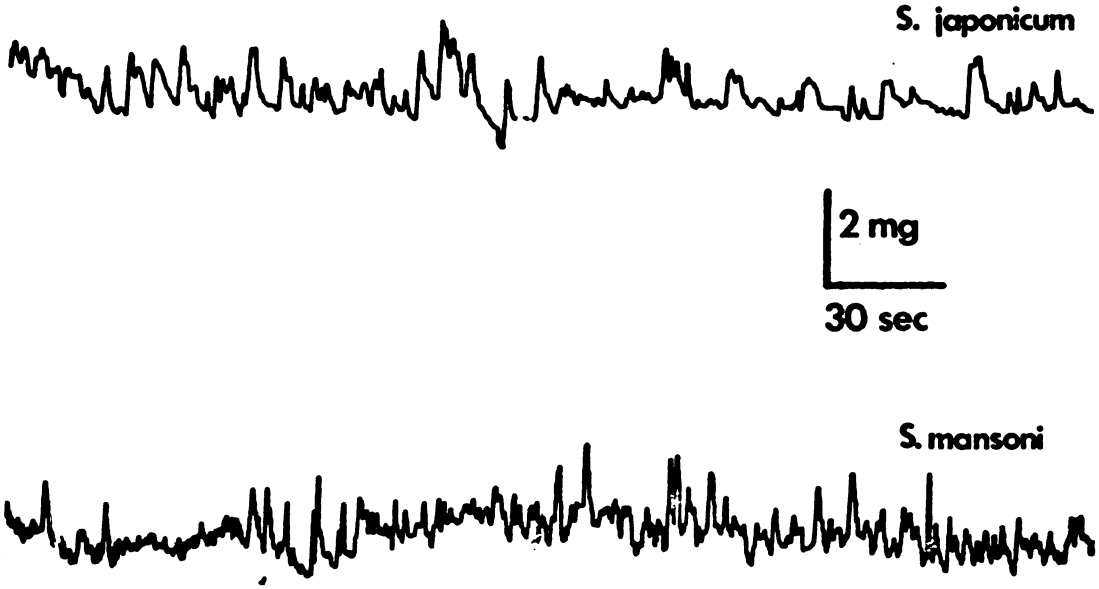

Figure 5

Figure 6. Potential profile obtained while penetrating into the dorsal surface of an adult male S. japonicum with a microelectrode. The sharp vertical drop represents penetration of E_1. The first upward potential change represents E_2. The second upward potential change represents E_3.

The next potential recorded was E_2. It had a value of -27.8±1.4 mv in S. japonicum and -23.5±1.2 mv in S. mansoni. E_3 was also recorded from S. japonicum but no quantifiable data concerning this potential was collected.

Table 1 compares average values recorded for E_1 and E_2 in S. japonicum and S. mansoni. Though the values recorded for E_1 and E_2 were significantly more negative in S. japonicum than in S. mansoni ($P \leq .01$) it seems clear that these potentials in S. japonicum represent the same potentials as identified in S. mansoni as tegument and muscle.

Effect of Altered Ion Concentration

Potassium. Fetterer et al. (1978) demonstrated that exposure of S. mansoni to an elevated K^+ HBS (60 mM) incubation medium caused a rapid, sustained increase in the muscle tension. I have measured a tension increase of 2.9±0.6 mg at two minutes in this parasite. The muscle tension continued to increase and by the end of the 20 minute test period reached a value of 3.9±0.6 mg. In contrast, when 60 mM K^+ was applied to S. japonicum, only a small increase in tension was observed (Figures 7 and 8). Even after 20 minutes, the tension was increased by only 1.5±1.1 mg; after 60 minutes by only 2.1±1.3 mg. The tension at 60 minutes was still significantly less than that achieved by S. mansoni at 20 minutes ($P \leq .01$).

Tension changes in response to 177 mM K^+ was also examined (Figure 9). HBS with 177 mM K^+ caused a large, sustained muscle contraction in both parasites. The large tension increase induced by 177 mM K^+ was not significantly different in the two schistosomes

TABLE 1

The Effects of Elevated K^+ Concentrations on the Mechanical and Electrical Activity of S. japonicum and S. mansoni

	S. japonicum	S. mansoni
Mechanical	**Tension Change**	
60 mM K^+ at 20 min	0.92 ± 1.10 mg	3.80 ± 0.50 mg[a]
177 mM K^+ at 20 min	2.84 ± 0.83 mg	3.24 ± 0.85 mg[b]
60 mM K^+ at 60 min	2.08 ± 1.30 mg	Not Performed
Electrical	**Electrical Potential**	
E_1 (tegument) (N=24)	-60.2 ± 2.1 mv	-53.3 ± 1.4 mv[a]
E_2 (tegument) (N=24)	-27.8 ± 1.4 mv	-23.5 ± 1.2 mv[a]
	Depolarization of Electrical Potential	
E_{musc} in 60 mM K^+ at 5 min	28.2 ± 7.0 mv	13.3 ± 1.7 mv[a]
E_{musc} in 177 mM K^+ at 5 min	31.7 ± 2.9 mv	21.7 ± 3.0 mv[a]

N=6 unless otherwise noted. Statistical analysis compares S. japonicum and S. mansoni.

[a] ($P \leq .01$)

[b] ($P \geq .20$)

Figure 7. Chart recordings comparing the effects of elevated K^+ on the muscle tension of S. japonicum and S. mansoni. At the arrow the medium (HBS) was replaced by 60 mM K^+ HBS. Top trace, S. japonicum; bottom trace, S. mansoni.

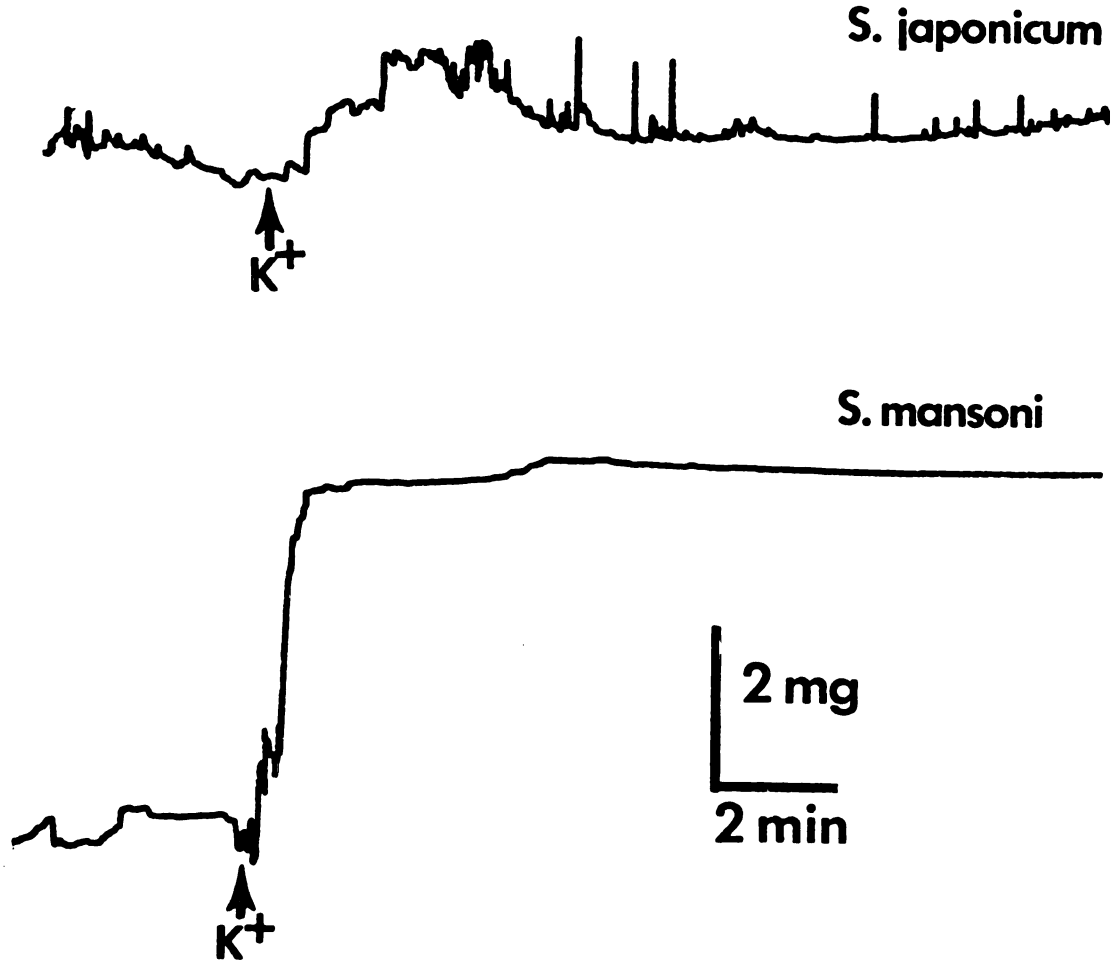

Figure 7

Figure 8. The effects of 60 mM K^+ on the muscle tension of schistosomes. Values are means ± one S.E.M. (N=6). At the arrow the medium was replaced with HBS containing 60 mM K^+. Open circles, S. japonicum; closed circles, S. mansoni.

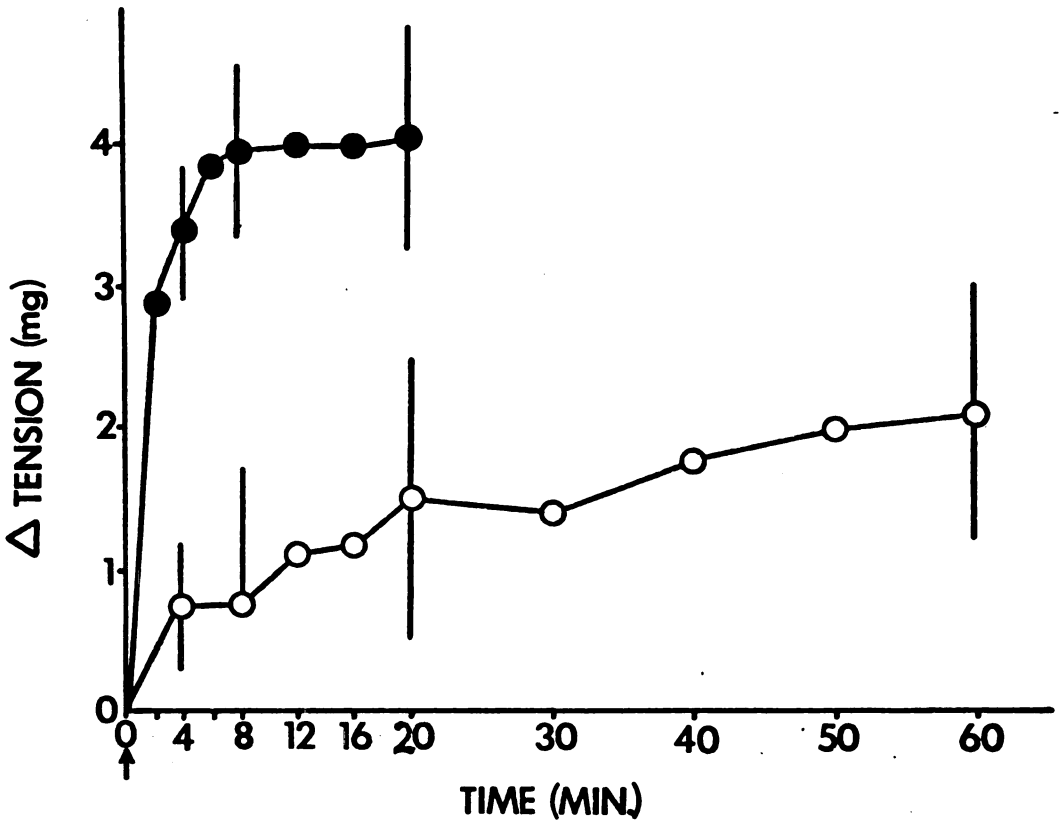

Figure 8

Figure 9. The effects of various concentration of K^+ on muscle tension of schistosomes. Values are means ± one S.E.M. (N=6). All measurements were taken five minutes after medium was replaced with HBS containing elevated K^+. Open circles: S. japonicum; closed circles: S. mansoni.

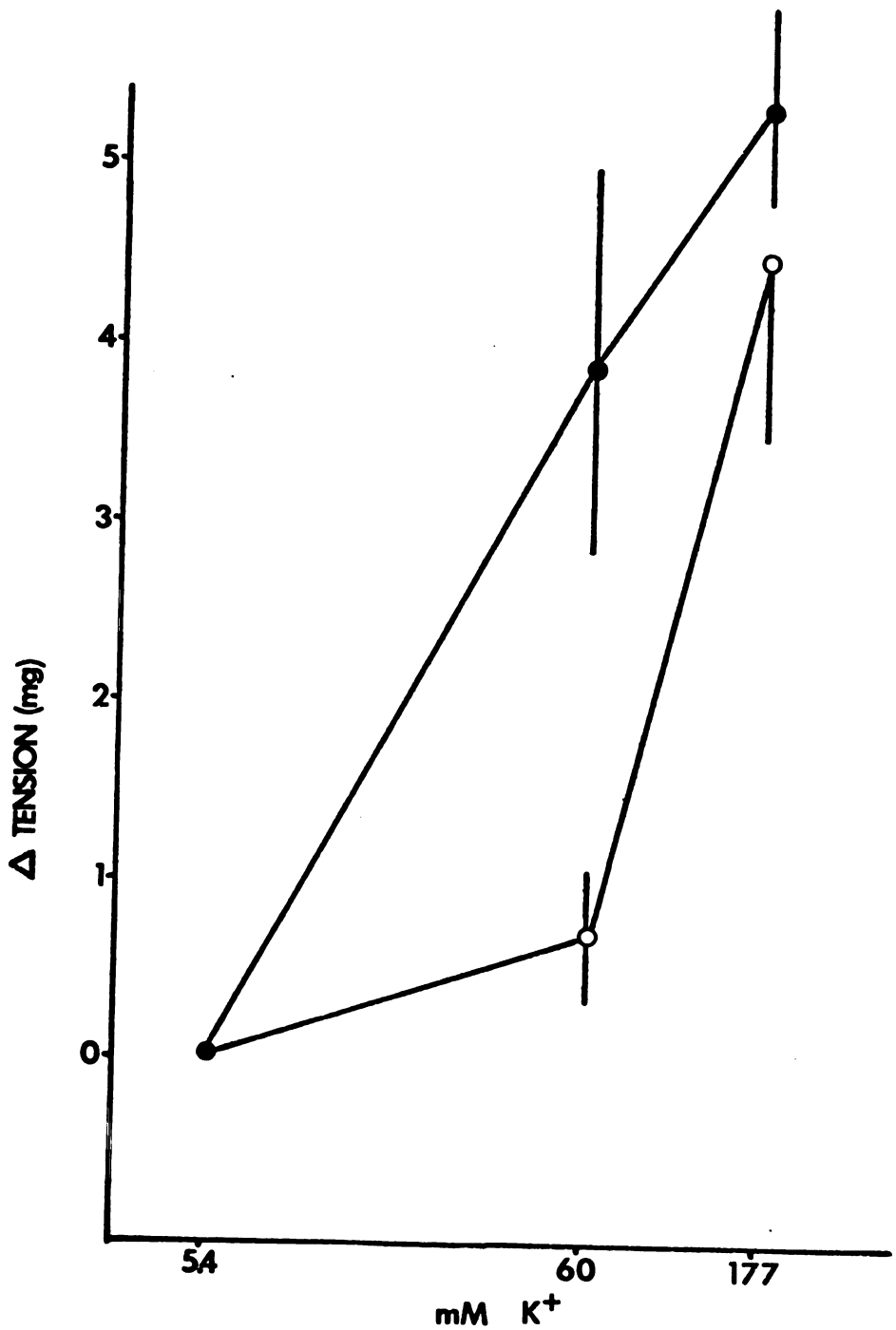

Figure 9

($P \geq .20$). Only at the concentration of 60 mM K^+ was the tension increase significantly greater in S. mansoni than in S. japonicum ($P \leq .01$).

The effect of elevated K^+ concentrations on tegumental and muscle potentials in the two schistosomes is shown in Figure 10. It should be noted that 60 mM K^+ HBS, while it had little effect on the muscle tension of S. japonicum, caused a large depolarization of both tegument and muscle in this parasite. Even within the first minute after exposure to 60 mM K^+, tegument of S. japonicum was depolarized from -56.0±3.5 mv to -26.0±3.0 mv, while muscle was depolarized from -13.0±2.0 mv to +1.0±7.0 mv (Figure 11). The effect of elevated K^+ medium on the mechanical and electrical activity of the two parasites is summarized in Table 1.

Lithium. When S. mansoni was exposed to an HBS in which LiCl had been substituted for NaCl, there is a gradual increase in tension (Fetterer et al., 1981a). Maximum tension reached in my experiments averaged 4.2±0.8 mg, and the half-time for response was six minutes. By contrast, when S. japonicum was exposed to the LiCl substituted HBS, there was a drop in maintained tension, so that after five minutes it reached a minimum of -1.34±0.30 mg below the control level. The half-time for this tension drop was three minutes. After this transient relaxation, the tension of S. japonicum gradually increased (Figures 12 and 13). If permitted to incubate in LiCl HBS for one hours, the tension in the musculature of S. japonicum eventually reached a tension level of 2.5±0.5 mg (Figure 13). This maximum increase in tension of S. japonicum which occurred between 50 and 60

Figure 10. The effects of various concentrations of K^+ on E_1 and E_2 of schistosomes. Values are means $\pm$ one S.E.M. (N=6). All measurements were taken five minutes after medium was replaced with HBS containing elevated K^+. E_1 is shown by broken lines. E_2 is shown by solid lines. Open circles: S. japonicum; closed circles: S. mansoni.

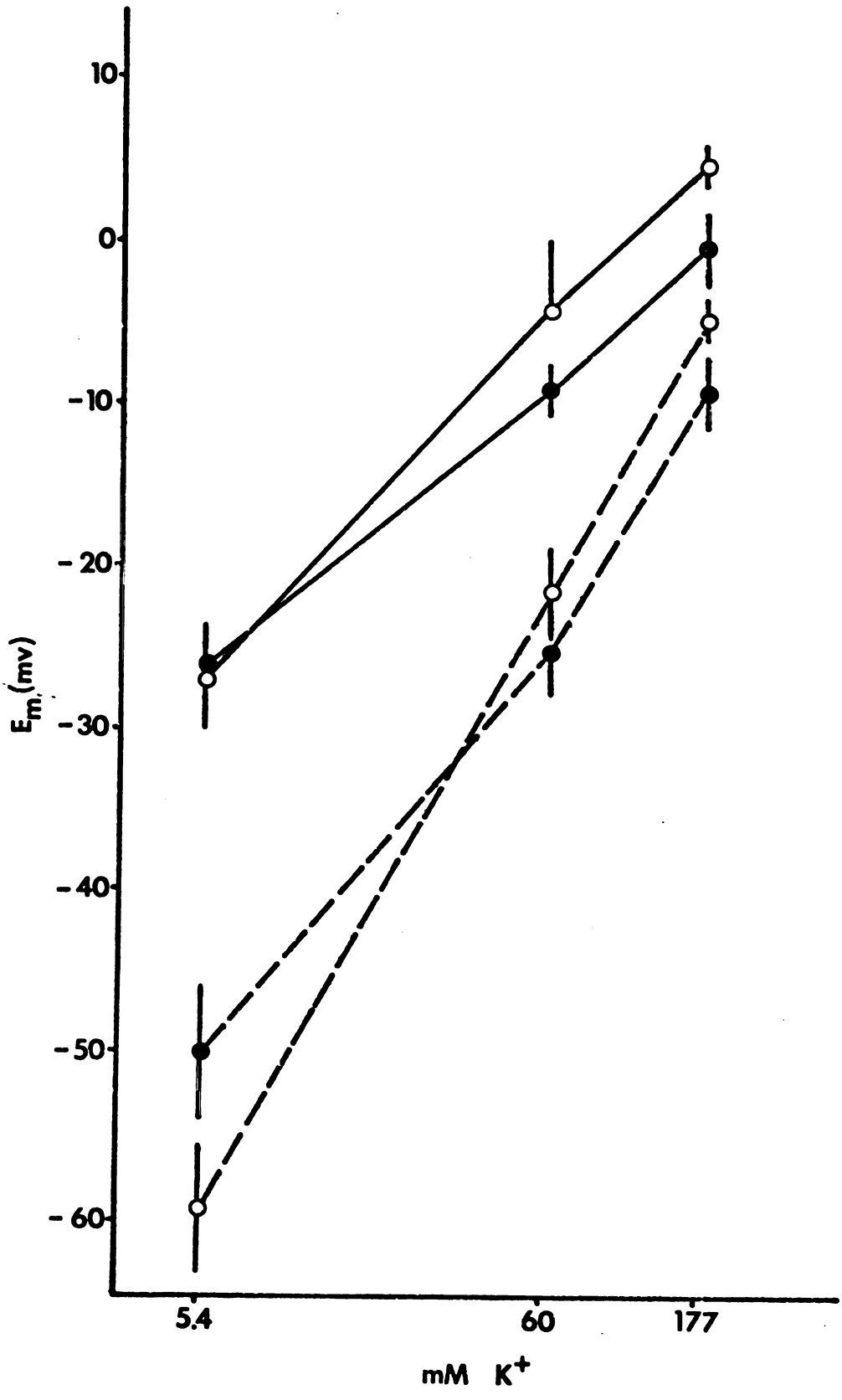

Figure 10

Figure 11. Time course of effect of 60 mM K^+ on E_1 and E_2 in S. japonicum. Values are means ± one S.E.M. (N=6). Animals were pre-incubated in PB/HBS. At the arrow the medium was exchanged with HBS containing 60 mM K^+. Open circles, E_1; closed circles, E_2.

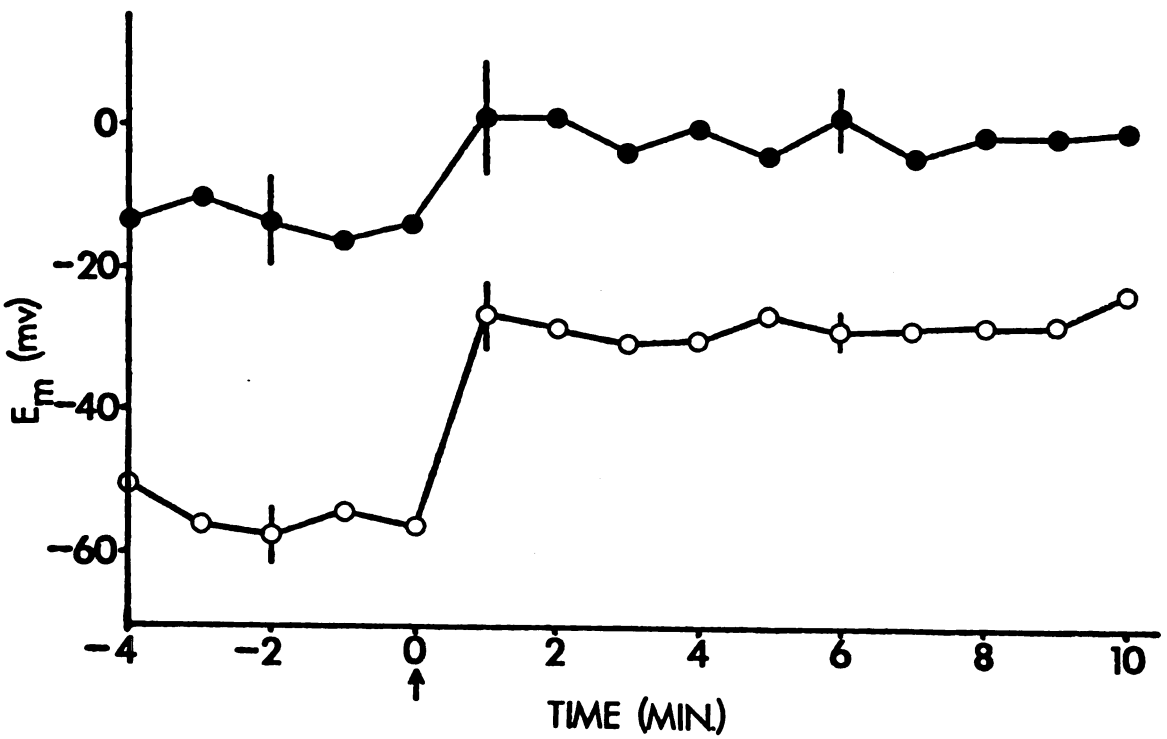

Figure 11

Figure 12. Chart recordings comparing the effects of LiCl HBS on muscle tension of S. japonicum and S. mansoni. At the arrow the medium was replaced by HBS containing 138 mM Li^+ (a complete substitution of Li^+ for Na^+). Top trace, S. japonicum; bottom trace, S. mansoni.

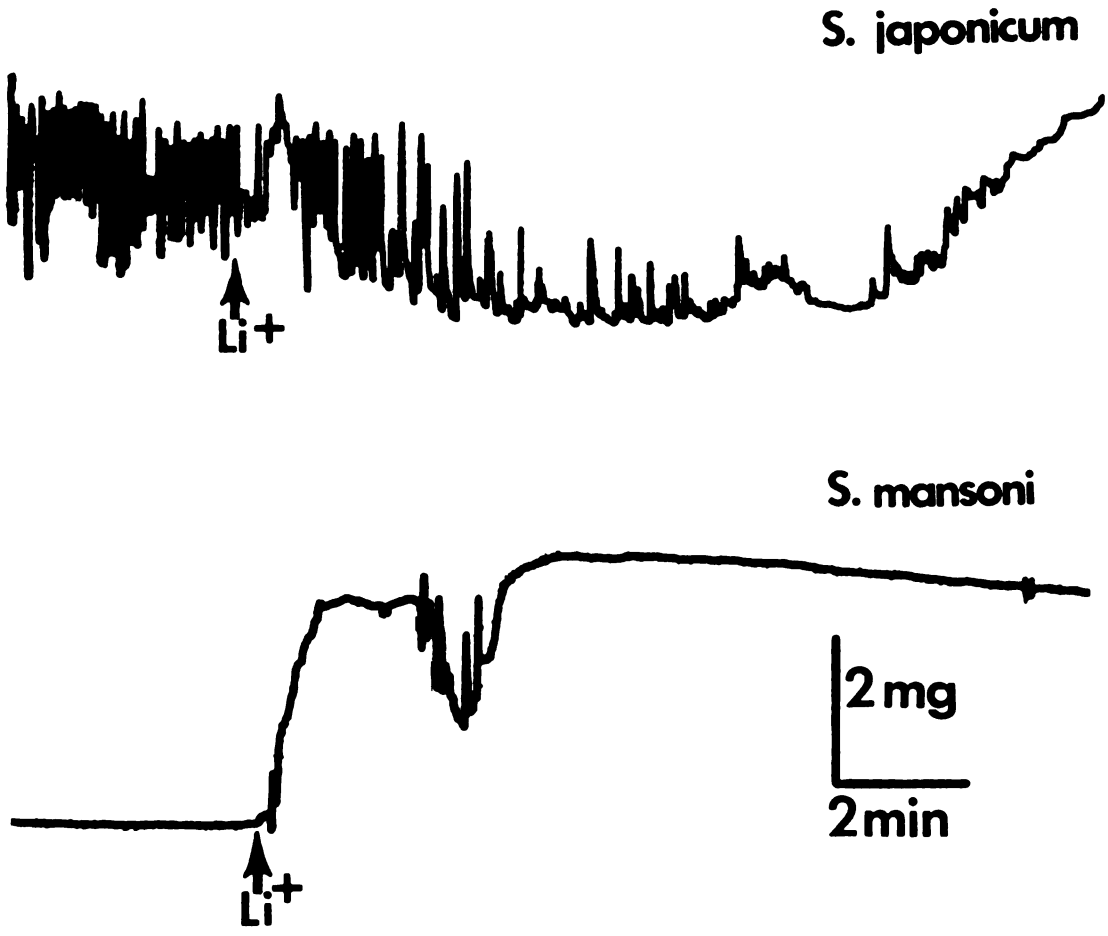

Figure 12

Figure 13. The effects of 138 mM Li^+ on the muscle tension of schistosomes. Values are means ± one S.E.M. (N=6). At the arrow the medium was replaced with HBS containing 138 mM Li^+. Open circles, S. japonicum; closed circles, S. mansoni.

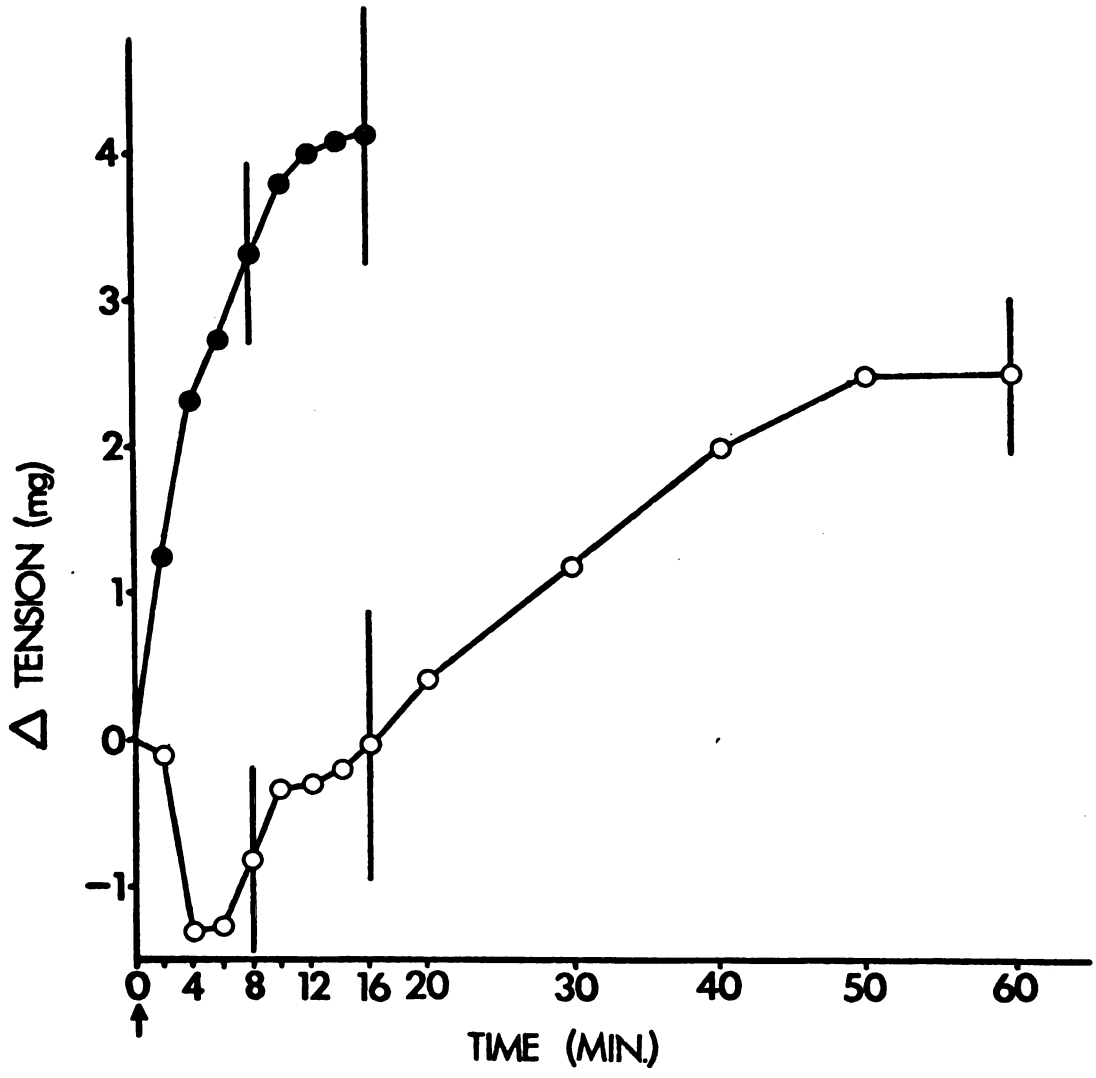

Figure 13

minutes, was still significantly less than the tension increase of S. mansoni at 16 minutes ($P \leq .01$).

LiCl HBS, though it induced no tension increase in S. japonicum until after 15 minutes, did cause depolarization of both the tegument and muscle at times considerably shorter than this. Even after only ten minutes, the tegument was depolarized from -60.0±3.0 mv to -28.0± 5.0 mv, while the muscle was depolarized from -20.0±3.0 mv to -12.0± 3.0 mv (Figure 14). This is similar to the Li^+ effect on S. mansoni in which tegument and muscle are both depolarized (Bricker et al., 1982). The effects of LiCl substitution on mechanical and electrical activity of the two parasites is summarized in Table 2.

Low Temperature

To test the effects of lowered temperature on the muscle tension of the two parasites, the temperature of the circulating fluid in the bathing chamber was lowered from 37°C to 5°C. Both S. japonicum and S. mansoni responded to the low temperature with a maintained increase in muscle tension (Figure 15). In both, the increase in tension was slow in onset, reaching a value of only 0.4±0.2 mg for S. japonicum and 0.1±0.1 mg for S. mansoni at two minutes. Between two and ten minutes the tension increased considerably for both S. japonicum (2.9±0.3 mg) and S. mansoni (2.7±0.3 mg). After ten minutes the muscle tension continued to increase, but as a much slower rate. By 30 minutes, muscle tension for the two parasites was approximately 3.5 mg. When bathing temperature was returned to 37°C, both parasites relaxed at the same rate. Most of this relaxation occurred during the first five minutes; i.e., the muscle tension of S. japonicum dropped

Figure 14. Time course of effect of 138 mM Li^+ on E_1 and E_2 in S. japonicum. Values are means ± one S.E.M. (N=6). Animals were pre-incubated in PB/HBS.$_+$ At the arrow the medium was replaced with HBS containing 138 mM Li^+. Open circles, E_1; closed circles, E_2.

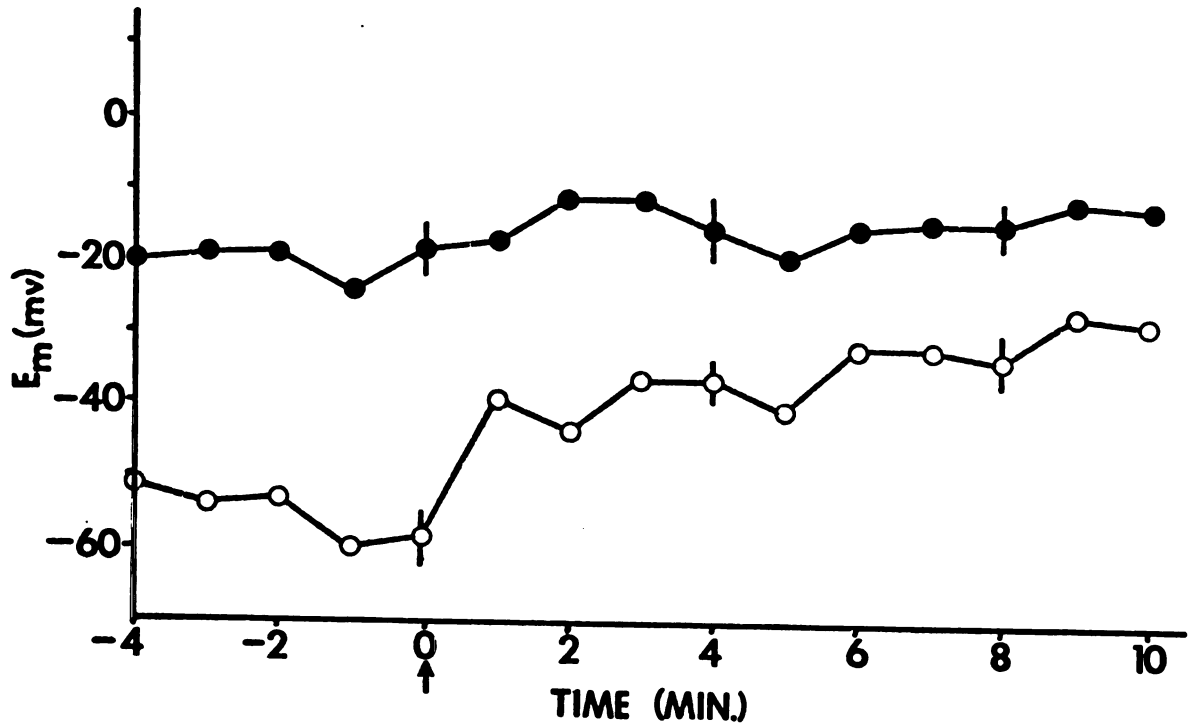

Figure 14

TABLE 2

A Comparison of the Effects of LiCl HBS on the Mechanical and Electrical Activity of S. japonicum and S. mansoni

	S. japonicum	S. mansoni
Mechanical	Tension Change	
LiCl HBS for 20 min	0.40 ± 0.73 mg	3.19 ± 0.82 mg[a]
LiCl HBS for 60 min	2.47 ± 0.51 mg	Not Performed
Electrical	Depolarization of Electrical Potential	
E_{teg} -LiCl at 10 min	23.7 ± 1.5 mv	19[b]
E_{musc} -LiCl at 10 min	8.1 ± 1.0 mv	12[b]

N=6 for all experiments.

[a] ($P \leq .01$)

[b] Values determined by C. S. Bricker et al. (1982) 11 to 15 minutes after Li^+ substitution.

Figure 15. The effect of lowered temperature on the muscle tension of schistosomes. Values are means ± one S.E.M. (N=6). At the first arrow, the temperature of the bathing medium was lowered to 5°C. At the second arrow the temperature of the bathing medium was returned to 37°C. Open circles, S. japonicum; closed circles, S. mansoni.

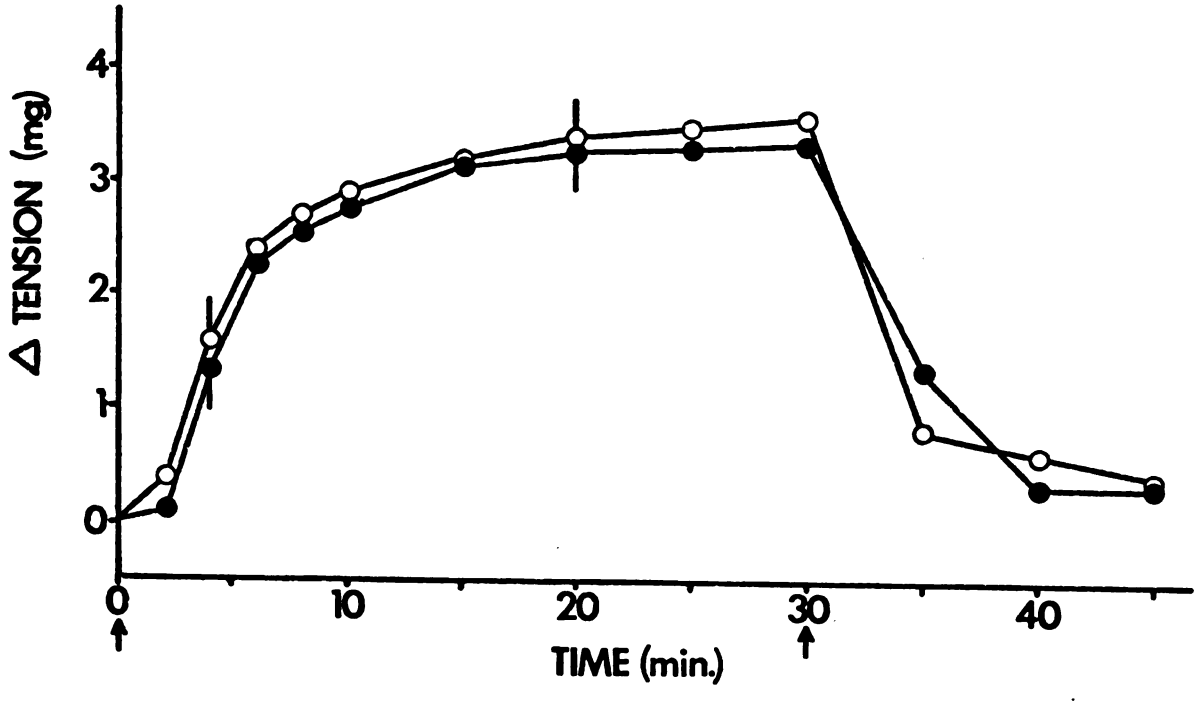

Figure 15

to 0.8±0.5 mg while that of S. mansoni dropped to 1.4±0.5 mg. Fifteen minutes after returning the temperature to 37°C, the muscle tension of S. japonicum (0.4±0.3 mg) and S. mansoni (0.4±0.2 mg) was only slightly elevated above control levels.

When the two parasites were preincubated in zero Ca^{++} plus EGTA, they responded differently to low temperature (Figure 16, Table 3). S. japonicum responded with a gradual increase in muscle tension, reaching 3.49±0.86 mg by 20 minutes. In contrast, incubation of S. mansoni in zero Ca^{++} rendered this parasite unresponsive to the tension inducing effects of low temperature. Addition of Ca^{++} back into the bath, (final concentration of Ca^{++} being 1.4 mM), produced no further increase in S. japonicum's muscle tension, but did cause an immediate increase in the muscle tension of S. mansoni (4.60±0.87 mg two minutes after addition of Ca^{++}).

Pharmacological Agents

Ouabain. A sample chart recording comparing the response of S. japonicum and S. mansoni to ouabain (10^{-5}M) is shown in Figure 17. In my experiments for S. mansoni, the maximum tension increase observed was 4.5±0.7 mg and the half-time for maximum contraction was six minutes. The responses of S. japonicum to ouabain was much different than that of S. mansoni. Tension did not begin to increase until after 15 minutes and even after 60 minutes the maximum tension increase was only 1.5±0.4 mg (Figure 18).

Neither the tegumental or muscle potential of S. japonicum was affected by ouabain (Figure 19). This is in contrast to ouabain's effect on electrical potentials in S. mansoni (Bricker et al., 1982)

Figure 16. The effect of zero Ca^{++} on the low temperature induced muscle contracture in schistosomes. Values are means + one S.E.M. (N=6). Bathing medium (HBS) was exchanged for zero Ca^{++} HBS plus 10^{-4}M EGTA, five minutes prior to lowering the temperature. At the first arrow, the temperature of the bathing medium was lowered to 5°C. At the second arrow $CaCl_2$ was added to the bath, bringing the concentration of Ca^{++} to 1.4 mM. Open circles, S. japonicum; closed circles, S. mansoni.

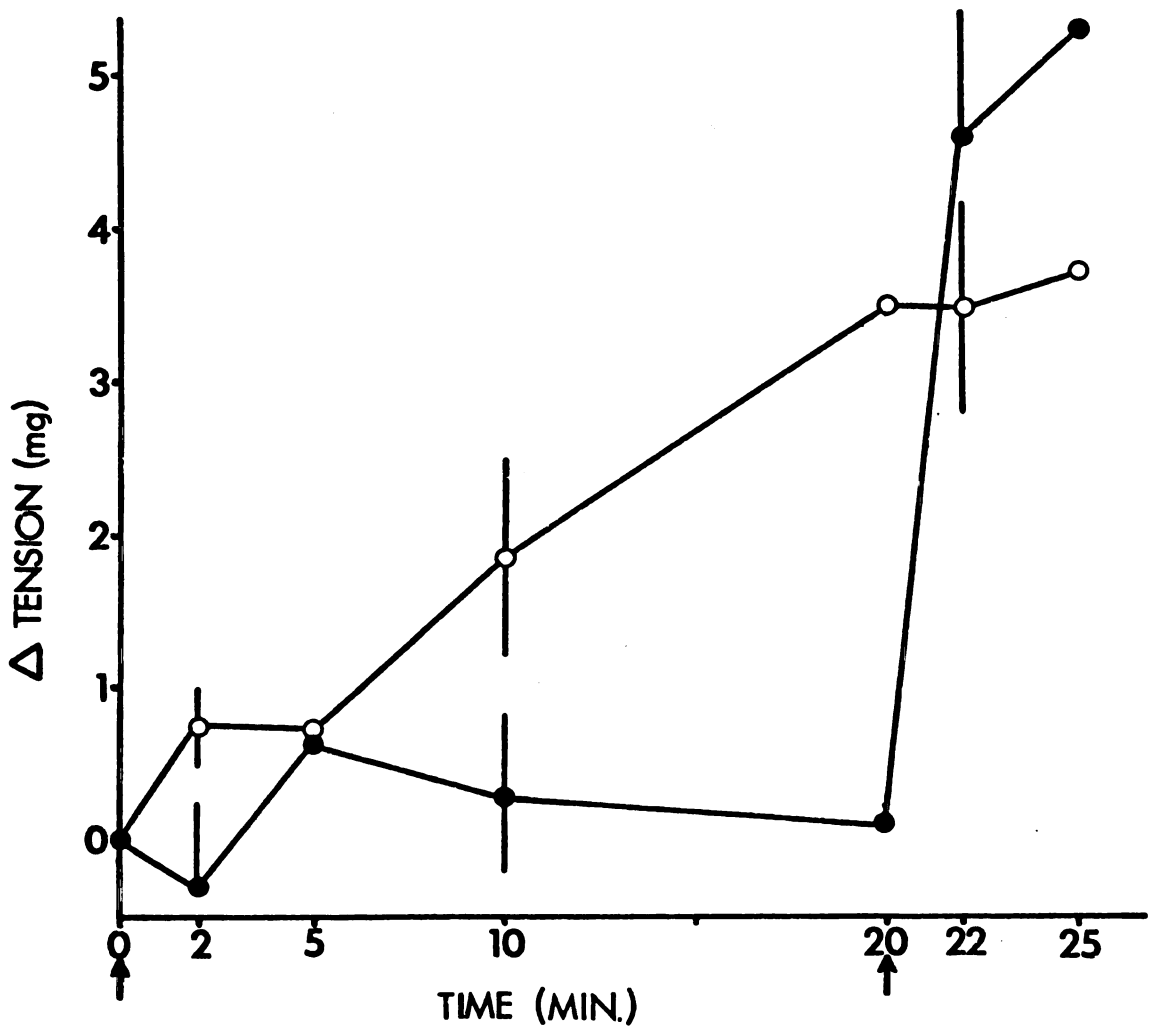

Figure 16

TABLE 3

A Comparison of the Effects of Ouabain, Low Temperature, D-600 Zero Ca^{++} and Praziquantel on the Mechanical Activity of S. japonicum and S. mansoni

	S. japonicum	S. mansoni
Mechanical	Tension Change	
Ouabain (10^{-5}M) at 20 min	0.31 ± 0.82 mg	3.64 ± 0.98 mg[a]
Ouabain (10^{-5}M) at 60 min	1.46 ± 0.44 mg	Not Performed
Low Temperature at 30 min	3.57 ± 0.38 mg	3.35 ± 0.31 mg[c]
Zero Ca^{++} and Low Temperature at 20 min	3.49 ± 0.86 mg	0.13 ± 0.69 mg[a]
D-600 (10^{-4}M) at 20 min	0.10 ± 0.60 mg	-1.00 ± 0.50 mg[b]
Zero Ca^{++} and Praziquantel (10^{-6}M) at 5 min	3.60 ± 0.84 mg	0.73 ± 1.35 mg[a]
Electrical	Depolarization of Electrical Potential	
E_{teg} in Ouabain at 10 min	-1.8 ± 1.8 mv	25 mv[d]
E_{musc} in Ouabain at 10 min	2.6 ± 5.3 mv	12 mv[d]

N=6 for all experiments.

[a] ($P \leq .01$)

[b] ($.01 < P < .05$)

[c] ($P \geq .20$)

[d] Values determined by C. S. Bricker et al. (1982) 11 to 15 minutes after Li^{+} substitution.

Figure 17. Chart recordings comparing the effects of ouabain on muscle tension of S. japonicum and S. mansoni. At the arrow the media was replaced by HBS containing 10^{-5}M ouabain. Top trace, S. japonicum; bottom trace, S. mansoni.

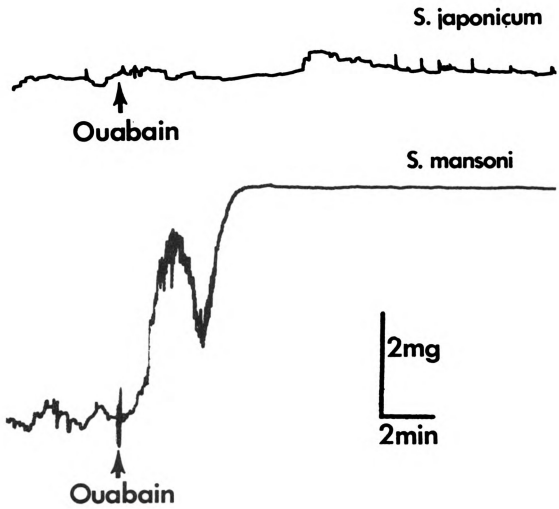

Figure 17

Figure 18. The effects of ouabain on the muscle tension of schistosomes. Values are means + one S.E.M. (N=6). At the arrow the medium was replaced by HBS containing 10^{-5}M ouabain. Open circles, S. japonicum; closed circles, S. mansoni.

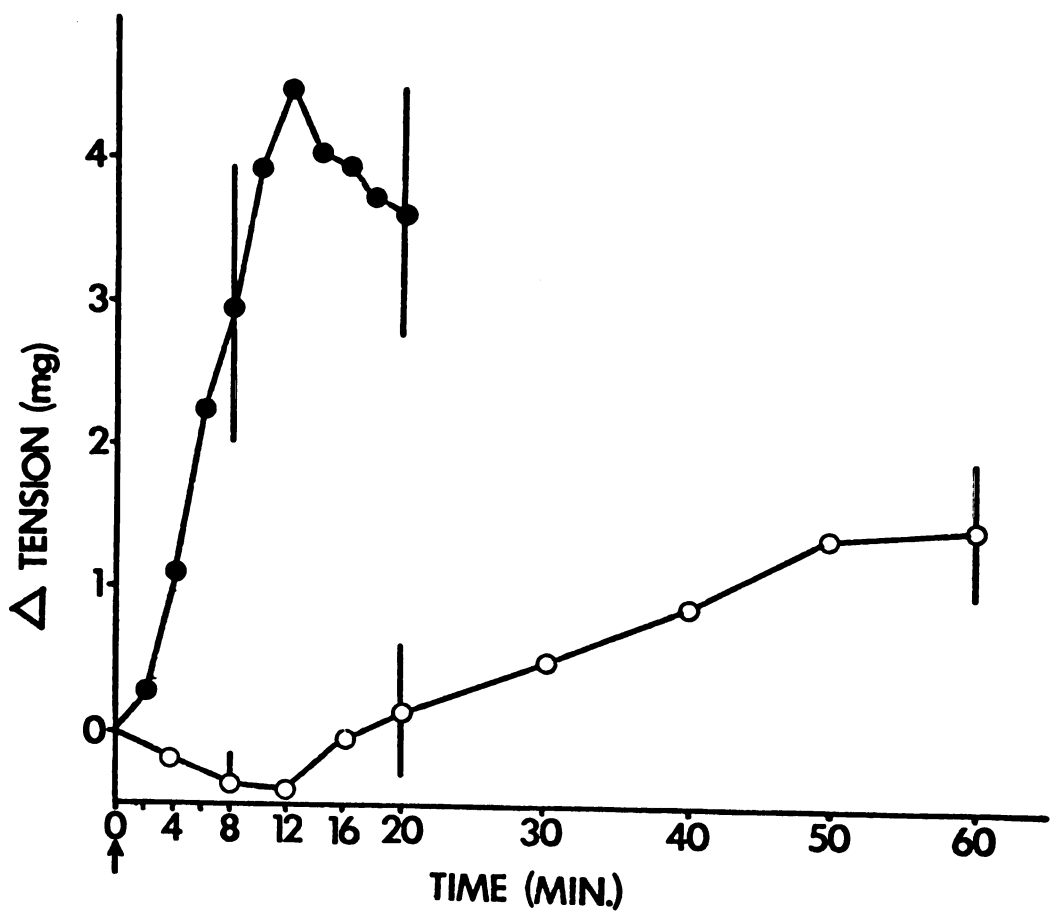

Figure 18

Figure 19. Time course of the effect of ouabain on E_1 and E_2 in S. japonicum. 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^{-5}M ouabain. Open circles, E_1; closed circles, E_2.

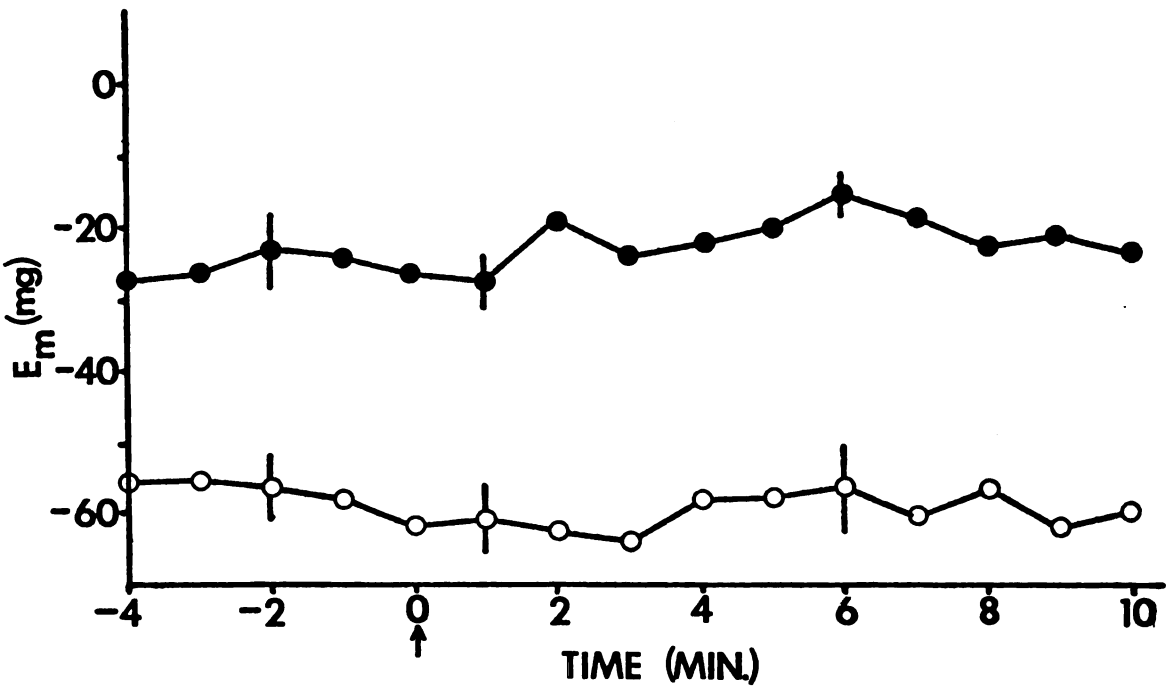

Figure 19

in which the tegument and muscle both show a large but gradual depolarization. The effects of ouabain on the mechanical and electrical activity of the two parasites is summarized in Table 3.

D-600. The effect of the voltage dependent Ca^{++} channel blocker, D-600 (10^{-4}M), on the muscle tension of the two parasites is presented in Figure 20. In both parasites, D-600 elicited a transient increase in muscle tension followed by a relaxation. The maximum increase in tension was similar in magnitude in S. japonicum (1.0±0.8 mg) and S. mansoni (1.2±0.8 mg), but this maximum occurred at two minutes in S. mansoni and at five minutes in S. japonicum. By 20 minutes, the muscle tension of S. mansoni dropped by -1.0±0.5 mg while the tension of S. japonicum decreased only 0.1±0.6 mg (Table 3).

Praziquantel. The potent antischistosomal, praziquantel, has been shown to produce a large, rapid muscle contraction in both S. japonicum and S. mansoni, in vitro (Pax et al., 1978). After a 10 minute incubation period in zero Ca^{++} HBS plus 10^{-4}M EGTA, the praziquantel induced contracture in S. mansoni was greatly reduced (0.73± 1.35 mg after 5 min), while the drug induced contracture in S. japonicum was unaffected by the absence of Ca^{++} (3.6±0.84 mg after 5 min) (Figure 21, Table 3).

Ion Accumulation

Potassium. Potassium uptake was determined by incubating parasites for various periods of time in HBS containing $^{42}K^{+}$. After the incubation period the worms were filtered, rinsed, weighed and solubilized. The $^{42}K^{+}$ activity was then measured in a scintillation

Figure 20. The effect of D-600 on the muscle tension of schistosomes. Values are means ± one S.E.M. (N=6). At the arrow the medium was replaced with HBS containing 10^{-4}M D-600. Open circles, S. japonicum; closed circles, S. mansoni.

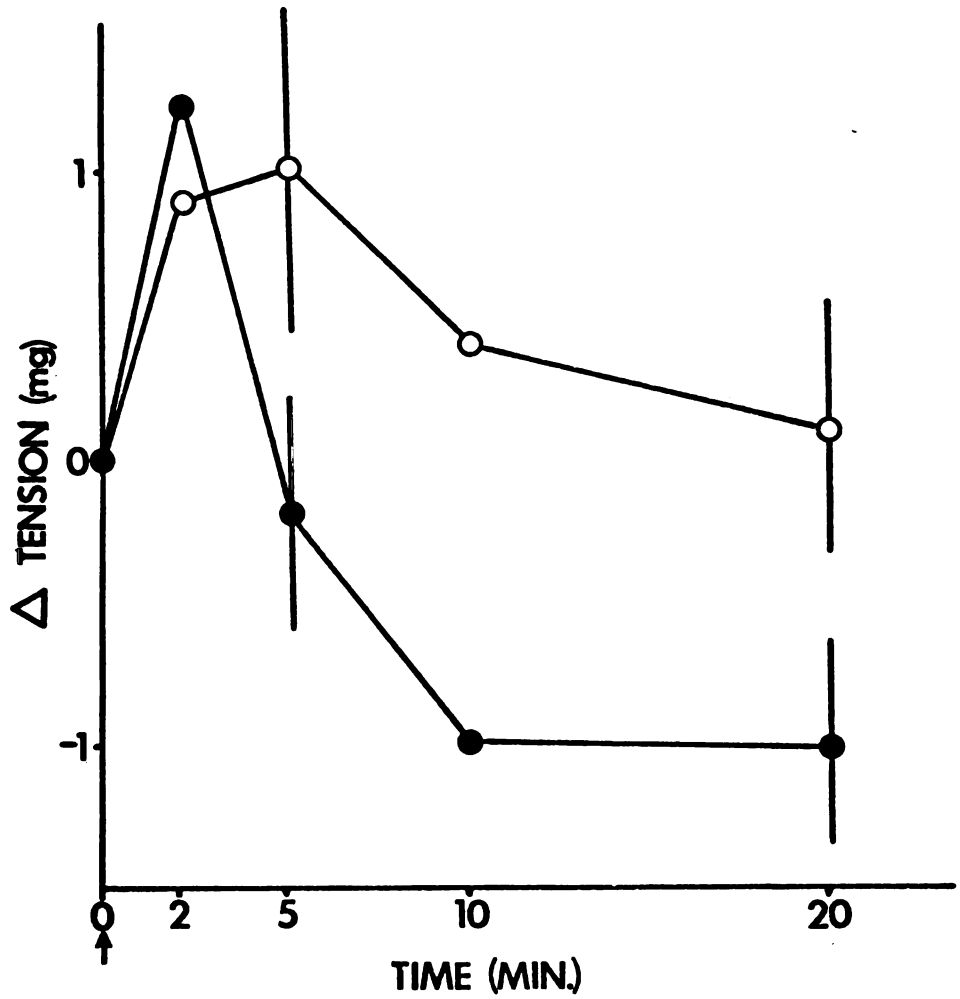

Figure 20

Figure 21. The effect of zero Ca^{++} on the praziquantel-induced muscle contraction in schistosomes. Values are means ± one S.E.M. (N=6). At the first arrow, HBS was exchanged for zero Ca^{++} HBS plus 10^{-4}M. EGTA. At the second arrow praziquantel was added to the bathing medium, bringing the concentration of this drug to 10^{-6}M. Open circles, S. japonicum; closed circles, S. mansoni.

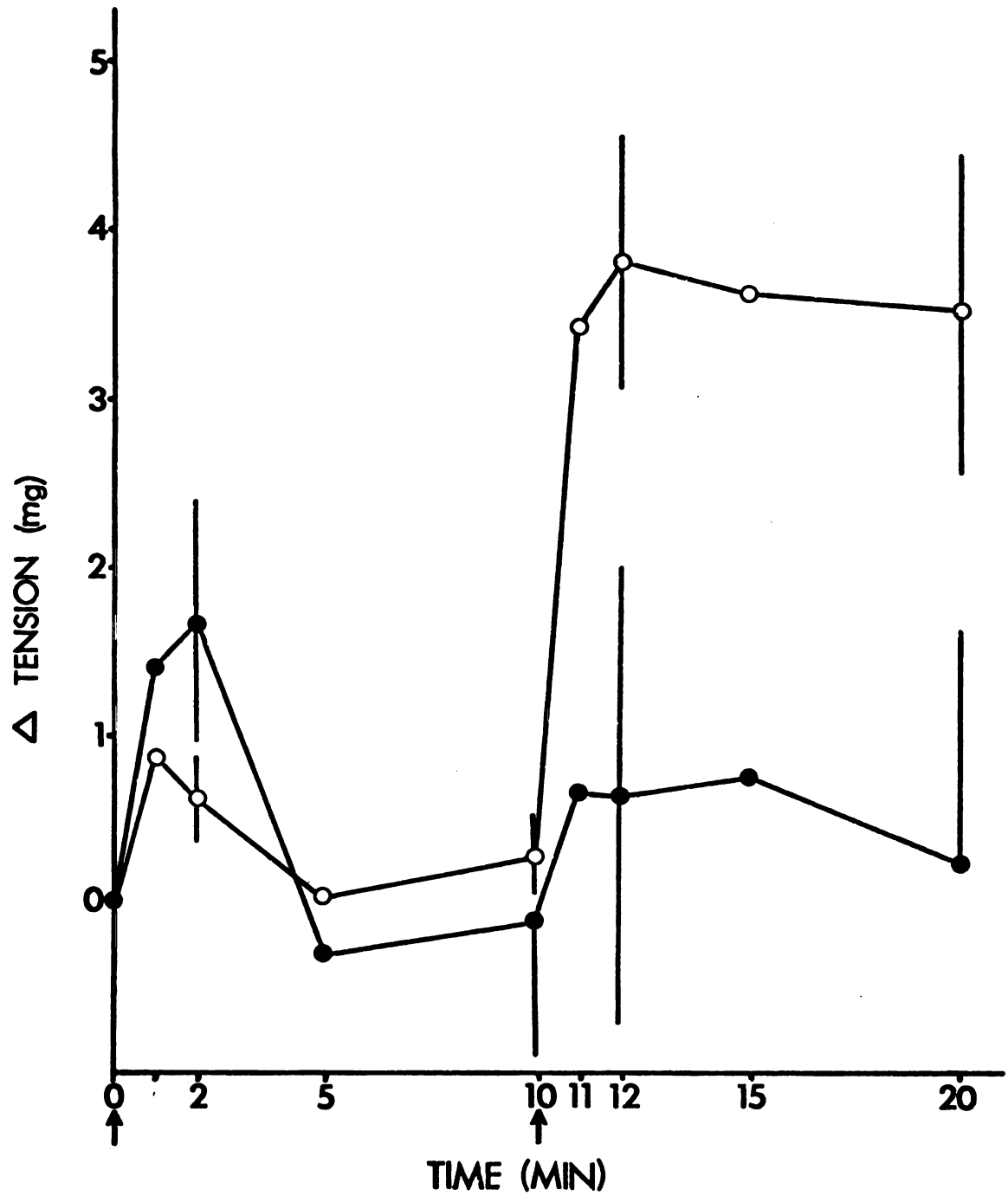

Figure 21

counter. The net accumulation of radioactive K^+ from the bathing medium was not significantly different for S. mansoni and S. japonicum at 2, 10, 30 and 60 minutes ($P>0.2$ (Figure 22). Only at the five minute time point did S. mansoni accumulate significantly more $^{42}K^+$ than did S. japonicum ($.05\leq P\leq .10$). In another experiment, $^{42}K^+$ was monitored over a two hour period. In this experiment also there was no significant difference between S. japonicum and S. mansoni (Figure 23). $^{42}K^+$ attributed to active transport (via a Na^+,K^+-ATPase) was eliminated by preincubating some parasites in ouabain ($10^{-5}M$) for 10 minutes. This concentration of ouabain was also maintained in the incubation fluid during the $^{42}K^+$ uptake measurement. Ouabain caused a significant reduction in $^{42}K^+$ uptake in both worms ($P\leq .01$) (Figure 22).

Calcium. The data showing the accumulation of $^{45}Ca^{++}$ over a one hour time period are shown in Figure 24. At all three time points measured (15, 30 and 60 minutes), S. mansoni accumulated a significantly greater amount of $^{45}Ca^{++}$ than did S. japonicum ($P<.01$). The rate of $^{45}Ca^{++}$ accumulation was greater for S. mansoni than S. japonicum during the first 30 minutes of incubation but after 30 minutes the rate was the same. Table 4 summarizes the uptake of $^{42}K^+$ and $^{45}Ca^{++}$ by the two worms.

Triton Treatment

The tegument of S. mansoni can be effectively removed by incubation in 0.2% triton X-100 (5°C) for ten minutes followed by 30 seconds of gentle vortexing (Oaks et al., 1978). My results have shown that this technique is equally effective in removing the tegument of

Figure 22. Uptake of $^{42}K^{+}$ by male schistosomes over one hour. Values are means $\pm$ one S.E.M.)N=4). Total $^{42}K^{+}$ accumulation is shown by solid lines. $^{42}K^{+}$ accumulation in the presence of 10^{-5}M ouabain is shown by broken lines. Open circles, S. japonicum; closed circles, S. mansoni.

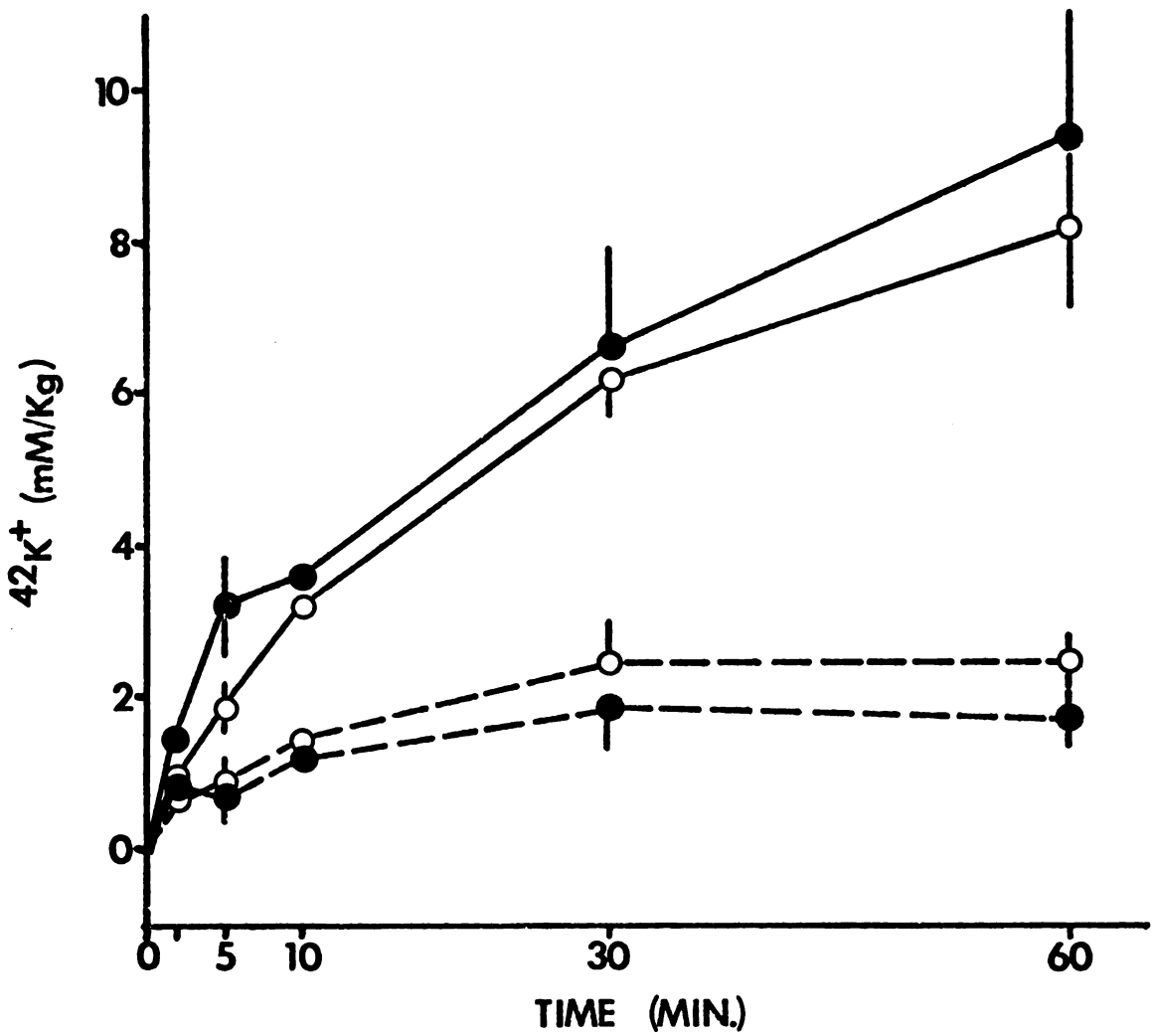

Figure 22

Figure 23. Uptake of $^{42}K^{+}$ by male schistosomes over two hours. Values are means ± one S.E.M. (N=4). Open circles, S. japonicum; closed circles, S. mansoni.

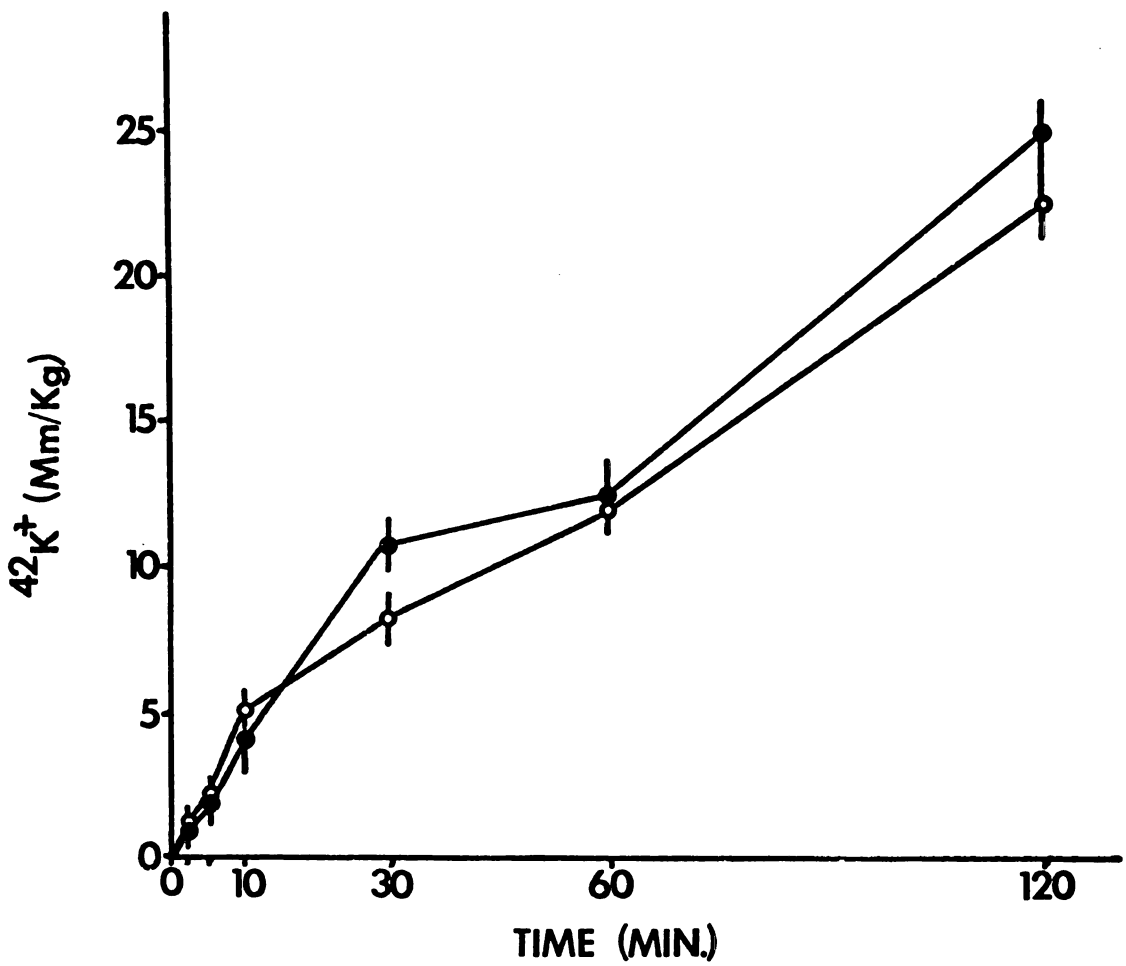

Figure 23

Figure 24. Uptake of $^{45}Ca^{++}$ in male schistosomes. Values are means ± S.E.M. (N=8). Open circles, S. japonicum; closed circles, S. mansoni.

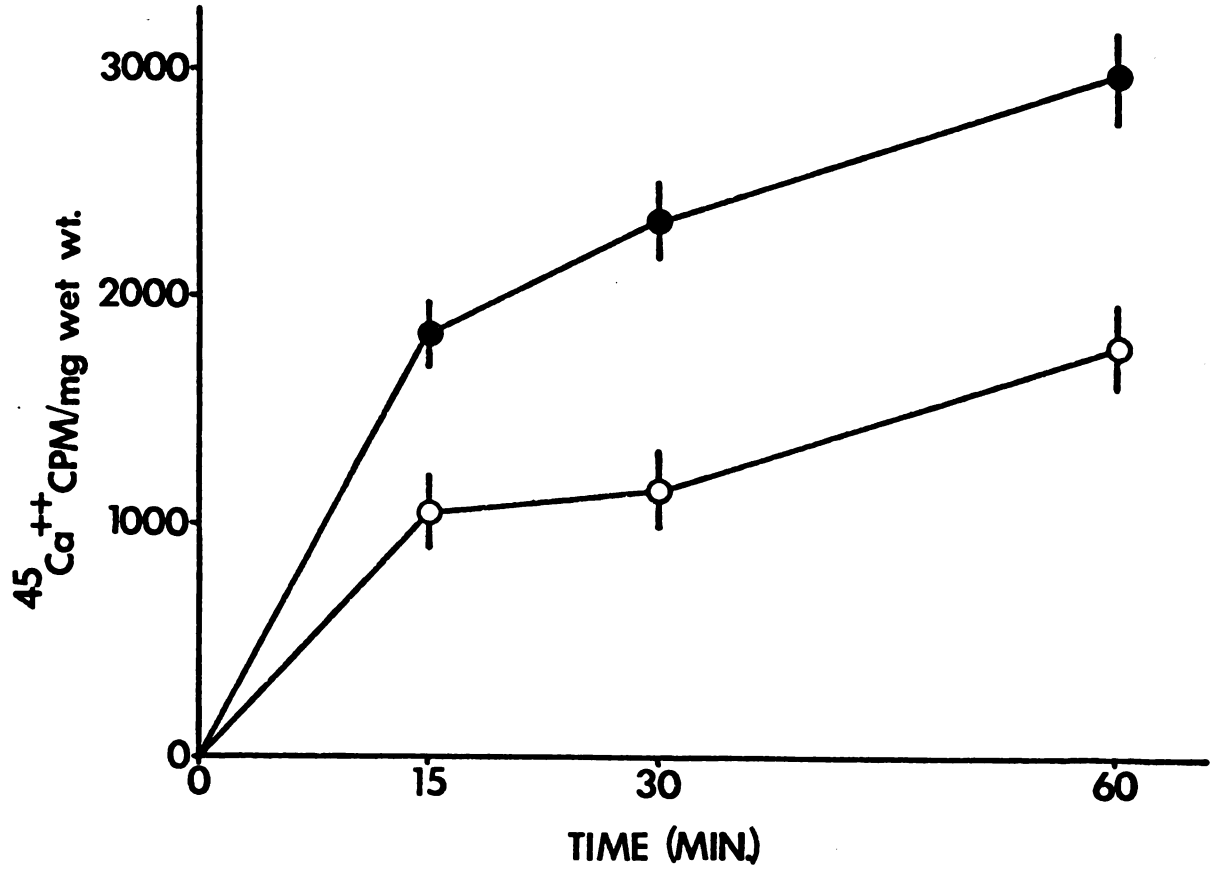

Figure 24

TABLE 4

A Comparison of Ion Uptake in S. japonicum and S. mansoni

Ion	Amount of Isotope Accumulated	
	S. japonicum	S. mansoni
$^{42}K^{+}$-60 min (N=4)	8.23 ± 1.0 mM K^{+}/kg	9.35 ± 1.9 mM K^{+}/kg[b]
$^{42}K^{+}$-120 min (N=4)	25.0 ± 1.1 mM K^{+}/kg	22.0 ± 0.9 mM K^{+}/kg[a]
$^{45}Ca^{++}$-60 min (N=8)	1796 ± 172 CPM/mg wt	3000 ± 193 CPM/mg wt[a]

[a] ($P<.01$). Statistical analysis compares S. japonicum and S. mansoni values.

[b] ($P\geq.02$)

S. japonicum. Scanning electron microscopy (kindly supplied by C. S. Bricker) showed that the convoluted surface of normal S. japonicum was absent in the triton treated worms. These worms, instead exhibited a striated surface resembling muscle fibers (Figure 25).

Mechanical Activity. Triton treated S. japonicum in HBS exhibited less spontaneous activity than did untreated S. japonicum. In over half the parasites tested, there were no spontaneous contractions, whatsoever. Triton treated S. japonicum showed a gradual relaxation in baseline muscle tension, similar to that observed in untreated worms.

Electrical Activity. Microelectrode recordings from S. japonicum after triton treatment, showed that two electrical potentials could usually be measured. The first electrical potential encountered when a microelectrode was advanced into the worm, had a value of -28.3 ± 1.3 mv. This was not significantly different from the muscle values recorded from untreated S. japonicum (-28.9 ± 1.0 mv) ($P \geq .2$) (Table 5). A large negative potential, similar in magnitude to tegument was not present in S. japonicum after tegument removal.

Potassium. A typical chart recording showing the effect of 60 mM K^+ HBS on normal and triton-treated S. japonicum is shown in Figure 26. As already described, normal parasites are affected little by 60 mM K^+. However, after tegument removal, S. japonicum responded with a large rapid increase in muscle tension. Within 15 seconds, for the six animals tested, the tension level was increased by an average of 3.2 ± 0.7 mg and the muscle always remained contracted for the duration of the 10 minutes test period (Figure 27). This tension increase

Figure 25. Scanning electron microscopy showing the effect of triton X-100 treatment on the tegument of S. japonicum. Upper micrograph: low magnification (600X) of the tegument of normal (left) and triton treated (right) S. japonicum. Lower micrograph: high magnification (4000X) of the tegument of normal (left) and triton treated (right) S. japonicum. Calibration bar for A and B, 13 μM; calibration bar for C and D, 2.5 μM. Kindly supplied by C. S. Bricker, Dept. of Zoology, University of Vermont.

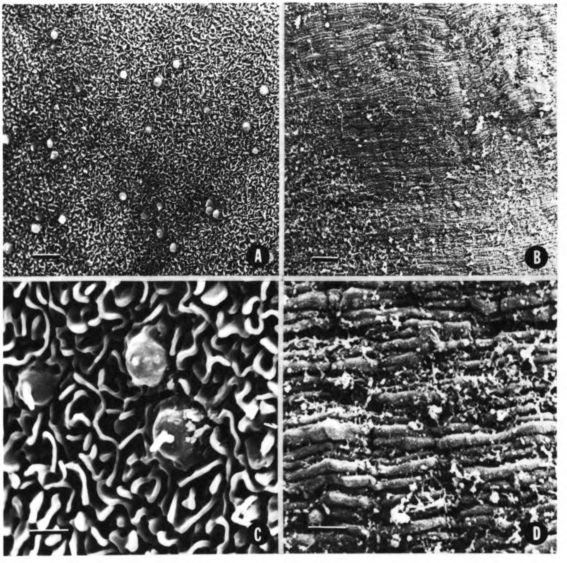

Figure 25

TABLE 5

A Comparison of Normal and Triton Treated S. japonicum; The Effects of Ouabain, LiCl HBS and 60 mM K^+ on Mechanical Activity

	S. japonicum (normal)	S. japonicum (triton)
Mechanical	Tension Change	
60 mM K^+ at 10 min	0.92 ± 1.10 mg	2.77 ± 0.89 mg[a]
Ouabain at 20 min	0.31 ± 0.82 mg	0.39 ± 0.31 mg[c]
LiCl at 20 min	0.40 ± 0.73 mg	2.43 ± 0.34 mg[a]
Electrical	Electrical Potential	
E_{teg}	-60.3 ± 2.5 mv	
E_{musc}	-28.9 ± 1.0 mv	-28.2 ± 0.5 mv[b]

N=6 for all experiments.

[a] ($P \leq .01$)

[b] ($P.10 \geq P > .05$)

[c] ($P \geq .20$)

Figure 26. Chart recordings showing the effect of 60 mM K^+ on the muscle tension of normal and triton-treated S. japonicum. At the arrow the medium was exchanged with HBS containing 60 mM K^+. Top trace, normal S. japonicum; bottom trace, S. japonicum after tegument removal.

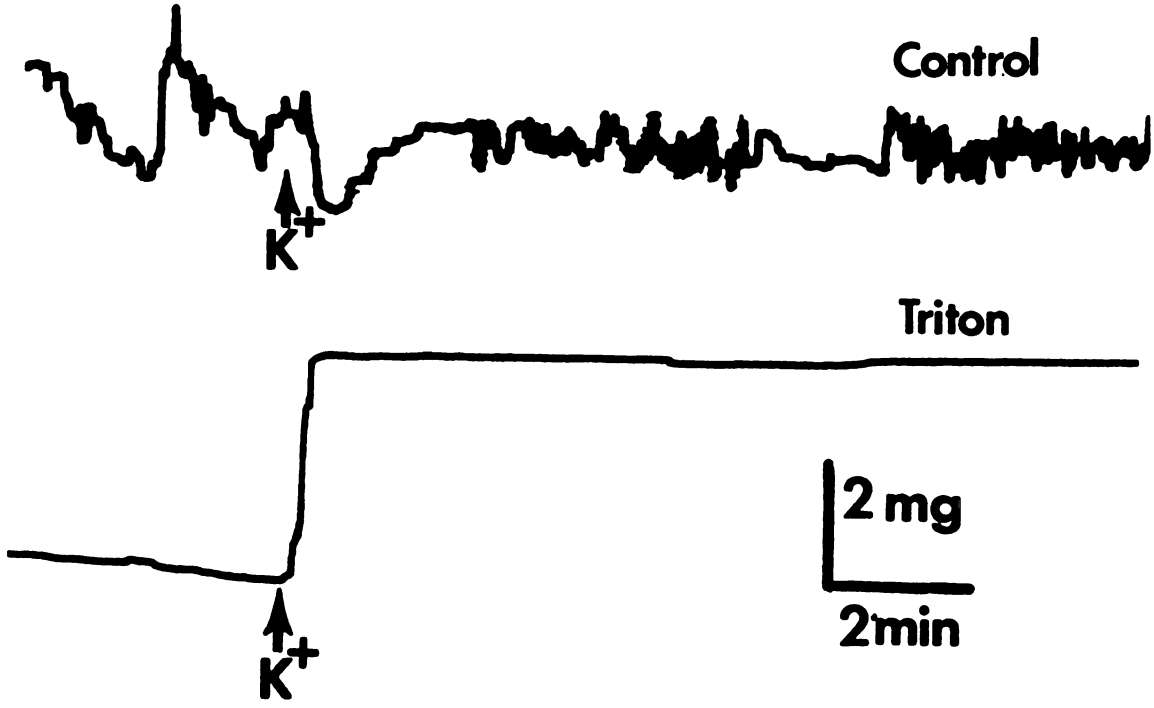

Figure 26

Figure 27. The effect of 60 mM K^+ on the muscle tension of normal and triton-treated S. japonicum. Values are means ± one S.E.M. (N=6). At the arrow the medium was replaced with HBS containing 60 mM K^+. Open circles, normal worms; closed circles, triton-treated worms.

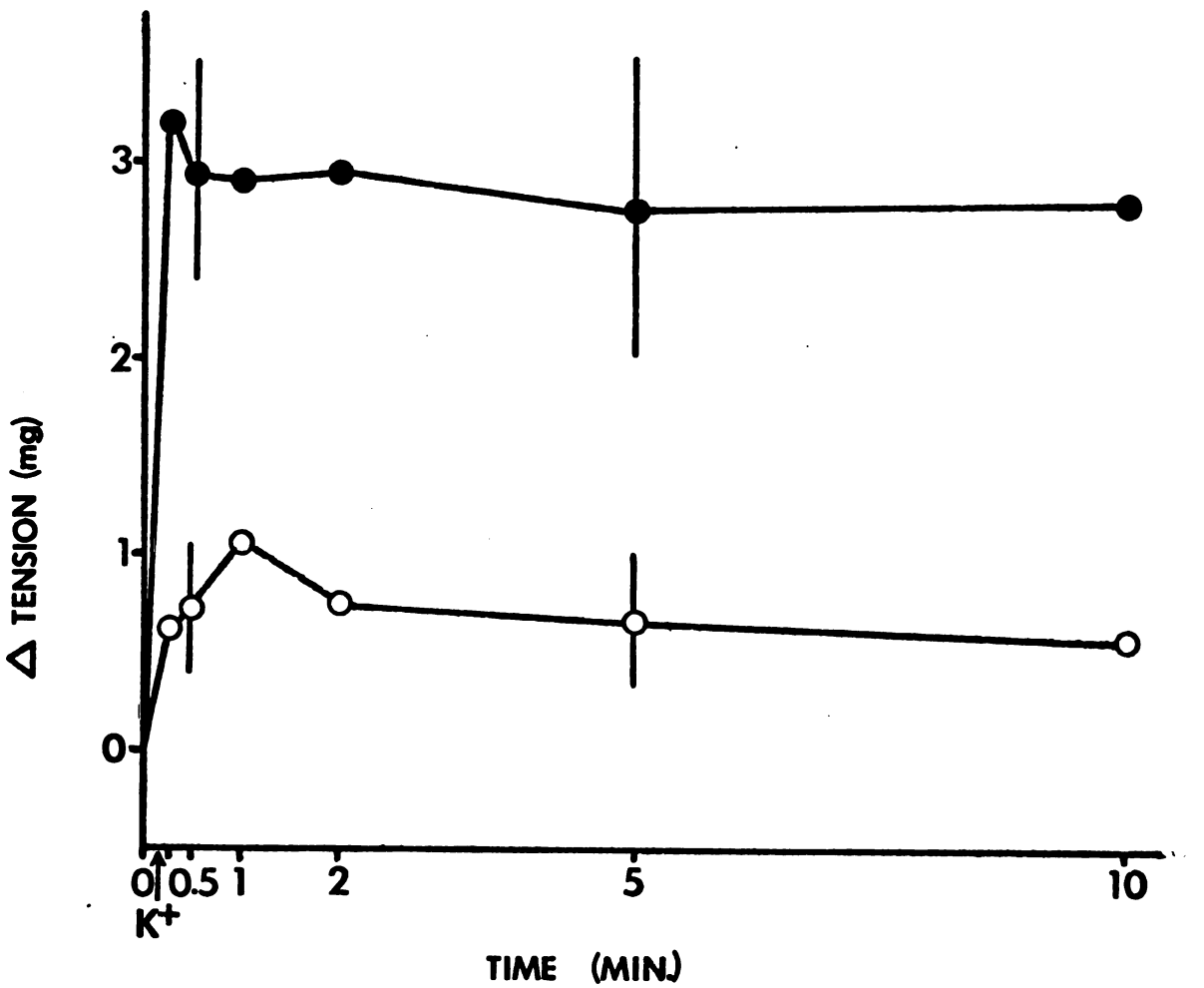

Figure 27

in triton-treated S. japonicum was not significantly different from K^+ induced tension increase in untreated S. mansoni ($P \geq .2$).

Calcium. Incubation of triton-treated S. japonicum in zero Ca^{++} plus 10^{-4}M EGTA HBS caused a decrease in muscle tension, reaching a minimum of -3.74±0.71 mg by 10 minutes (N=6) (Figure 28). Exchange with 60 mM K^+-zero Ca^{++} media caused only a slight transient change in muscle tension during a two minute test period but addition of 1.4 mM Ca^{++}, back into the medium, caused a large, rapid increase in muscle tension (4.72±0.79 mg after one minute).

In several types of muscle, elevated levels of Ca^{++} reduce the size of the K^+ induced contraction, presumably by stabilizing the muscle membrane (Luttgau, 1963; Nasledov et al., 1966). This possibility was tested for S. japonicum by incubating triton-treated parasites in 14 mM Ca^{++} and measuring the effect of 60 mM K^+ on muscle tension. In both 1.4 mM Ca^{++} and 14 mM Ca^{++}, S. japonicum responded to 60 mM K^+ with a large, sustained muscle contraction of comparable magnitude (Figure 29).

Ouabain. Tegumental removal using triton alters S. japonicum's responses to both ouabain and LiCl. As described above, S. japonicum responds to ouabain with only a gradual increase in tension. After removal of the tegument, these parasites were even less responsive to ouabain. After 20 minutes, triton-treated S. japonicum reached a tension level of less than 0.4±0.3 mg, while the tension increase of normal worms was 1.4±0.5 mg (Figure 30).

Lithium. As already described, normal S. japonicum respond to LiCl HBS with a transient decrease in muscle tension followed by an

Figure 28. Chart recording of the effect of zero Ca^{++} HBS, 60 mM K^{+} HBS and 1.4 mM Ca^{++} on the muscle tension of a triton-treated S. japonicum. At the first arrow the medium was exchanged with HBS containing zero Ca^{++} plus 10^{-4}M EGTA. At the second arrow, the zero Ca^{++} medium was replaced with zero Ca^{++}-60 mM K^{+} medium. At the third arrow, Ca^{++} was added back into the bath to bring the final Ca^{++} concentration to 1.4 mM.

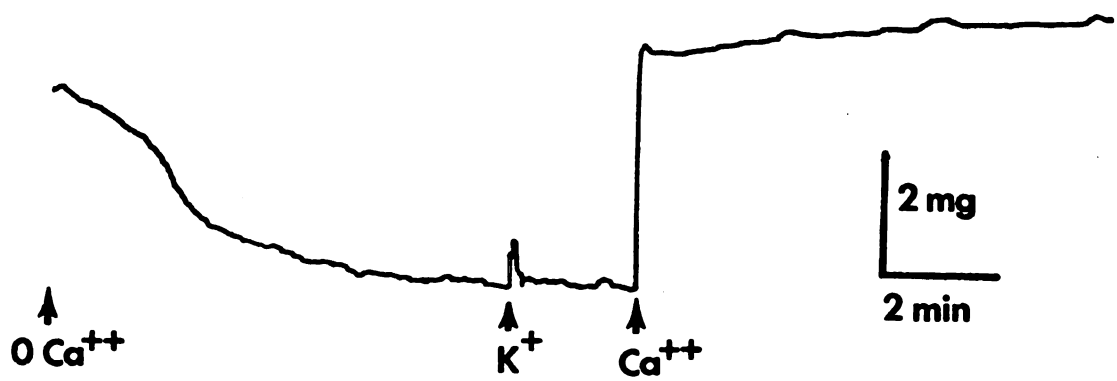

Figure 28

Figure 29. Chart recording showing the effect of 14 mM Ca^{++} on the K^{+} contracture of triton treated S. japonicum. Upper trace: K^{+} induced contracture of parasite bathed in 1.4 mM Ca^{++}. Lower trace: K^{+} induced contracture of parasite bathed in 14 mM Ca^{++}.

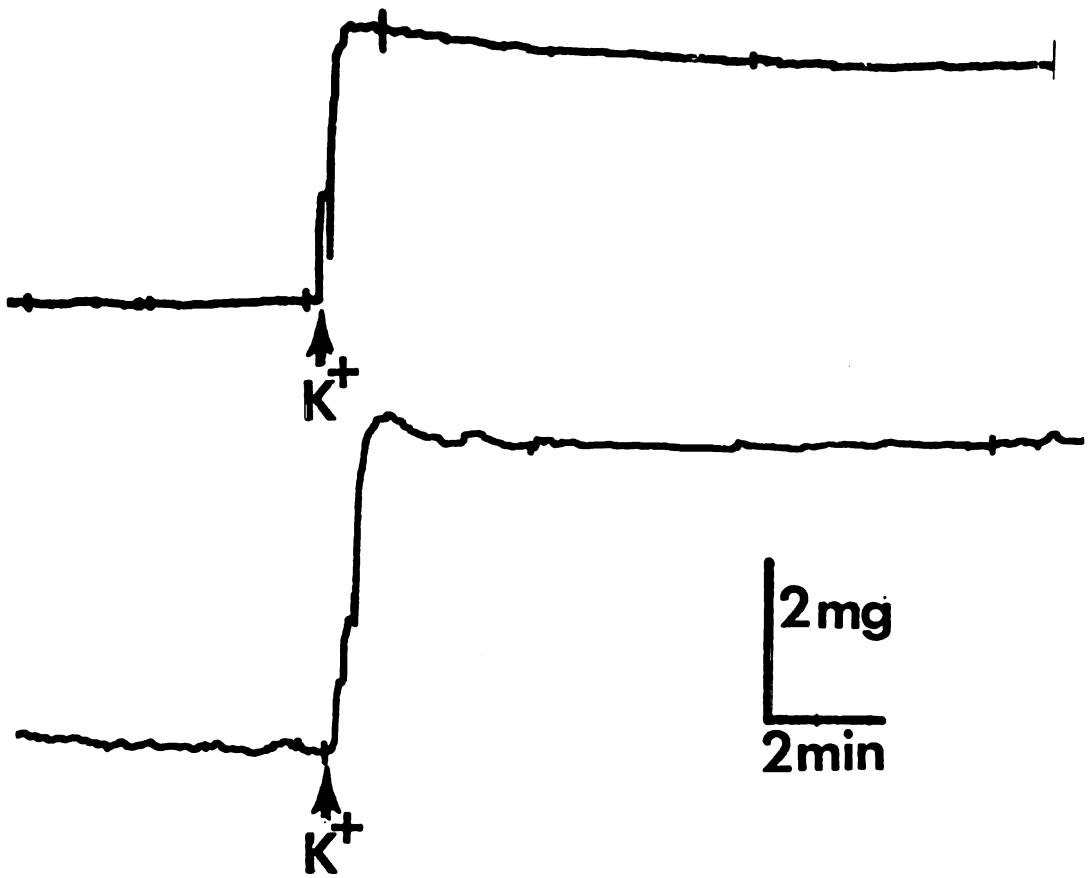

Figure 29

Figure 30. The effect of ouabain on the muscle tension of normal and triton-treated S. japonicum. Values are means ± one S.E.M. (N=6). At the arrow the medium was replaced by HBS containing 10^{-5}M ouabain. Open circles, normal S. japonicum; closed circles, S. japonicum after tegument removal.

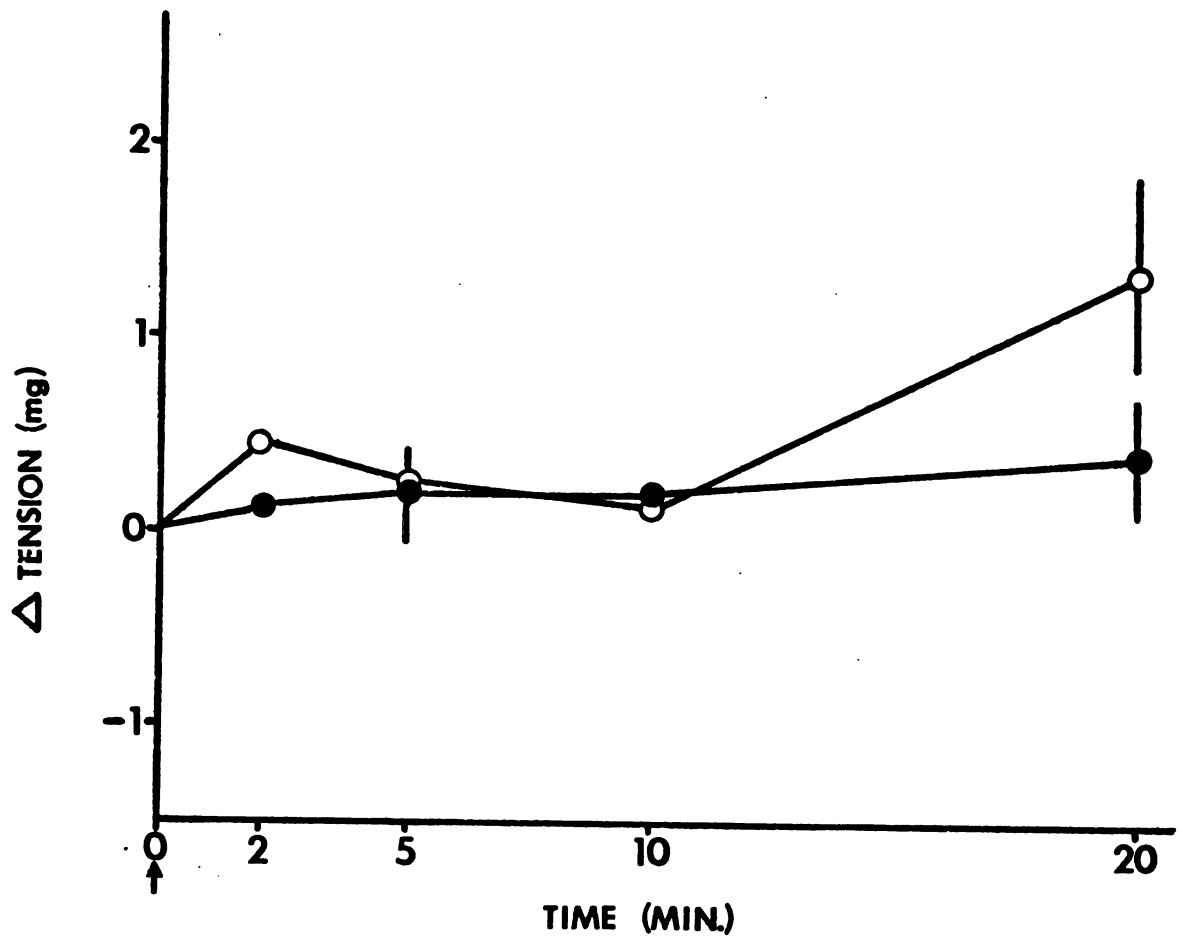

Figure 30

increase in muscle tension. In contrast, triton-treated S. japonicum exhibited an increase in tension throughout the 20 minute test period. At 20 minutes the tension was significantly higher for these parasites (2.4±0.3 mg) than for untreated S. japonicum (1.2±0.3 mg) ($P \leq .01$). The transient relaxation seen in normal worms was not observed in triton-treated ones (Figure 31). A comparison of the effects of high K^+, ouabain and LiCl on the muscle tension of normal and triton-treated S. japonicum is given in Table 5.

Ca^{++}-Mg^{++} ATPase

The amount of Ca^{++} and/or Mg^{++}-activated ATPase activity in crude homogenates of S. japonicum and S. mansoni is given in Table 6. The amount of total Ca^{++}-Mg^{++} ATPase was similar in the two parasites but S. japonicum contained significantly more Ca^{++}-ATPase than did S. mansoni. After tegumental removal, total Ca^{++}-Mg^{++} ATPase activity in S. japonicum stayed the same as control, while the activity in triton-treated S. mansoni increased when compared to its control.

Figure 31. The effect of Li^+ on the muscle tension of normal and triton-treated S. japonicum. Values are means ± one S.E.M. (N=6). At the arrow the medium was replaced by HBS containing 138 mM Li^+. Open circles, normal S. japonicum; closed circles, S. japonicum after tegument removal.

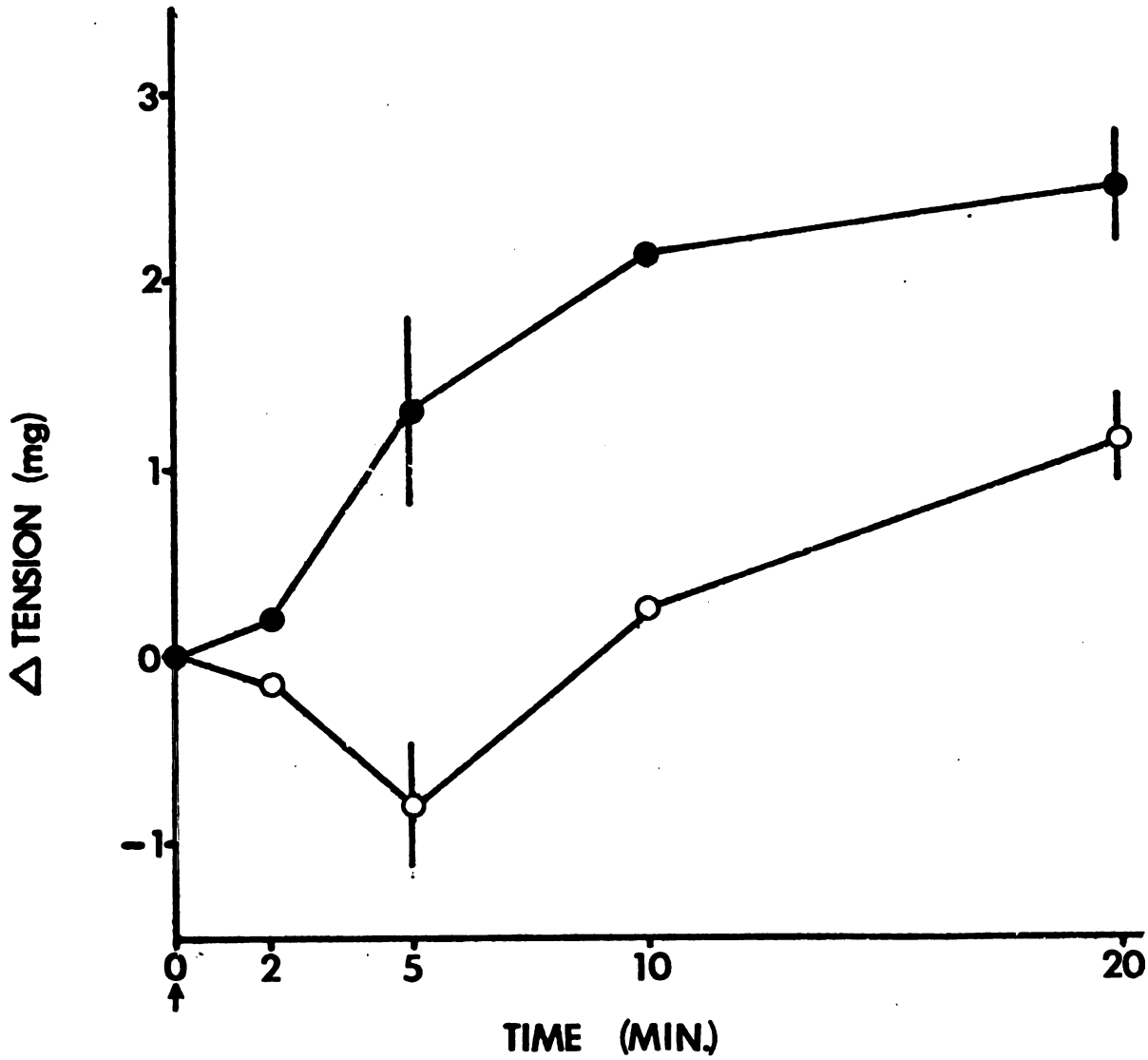

Figure 31

TABLE 6

A Comparison of Ca^{++} and/or Mg^{++} Dependent Enzymes in *S. japonicum* and *S. mansoni*

	S. japonicum	*S. mansoni*
Enzyme	Enzyme Activity Measured as mM PO_4/mg protein/hr	
Total Ca^{++}-Mg^{++} ATPase	696 ± 17	730 ± 10[c]
Ca^{++}-ATPase	414 ± 36	288 ± 29[a]
Mg^{++}-ATPase	560 ± 11	604 ± 15[b]
Total Ca^{++}-Mg^{++} ATPase in triton-treated worms	658 ± 26	992 ± 13[a]

N=4. Statistical analysis compares *S. japonicum* and *S. mansoni* values.

[a] ($P \leq .01$)

[b] ($.10 < P \leq .20$)

[c] ($P \geq .20$)

DISCUSSION

Calcium Permeability

My data clearly show that contraction of the longitudinal muscle of S. japonicum and S. mansoni differs in its dependence on external Ca^{++}. The muscle contractions induced in S. mansoni by elevated K^{+}, praziquantel, ouabain and low temperature are all attenuated by a brief preincubation (2-5 min) in zero Ca^{++} HBS with 10^{-4}M EGTA (WoldeMussie et al., 1982). Addition of Ca^{++} to the bath (final concentration 1.4 mM) elicits an immediate increase in tension comparable to magnitude to the normal response of S. mansoni to these four conditions. In contrast, the same pretreatment (5 min in zero Ca^{++}-10^{-4}M EGTA) of S. japonicum has no effect on the only two conditions which normally induce a muscle contraction in this parasite, e.g., low temperature and praziquantel (Figures 16 and 21). Addition of Ca^{++} back into the bath produces no additional increase in S. japonicum's muscle tension.

The difference in the two parasites' dependence on external Ca^{++} appears to be associated with a specific difference in permeability to Ca^{++}. While accumulation of $^{45}Ca^{++}$ in S. mansoni is nearly twice that in S. japonicum (Figure 24), uptake of $^{42}K^{+}$ is the same in both species (Figures 22 and 23). In addition, the microelectrode recordings show that both elevated K^{+} and Li^{+} substitution cause

depolarization of the muscle of S. japonicum, indicating that lithium and potassium ions are able to penetrate through the tegument to the level of the muscle.

That the muscle of the two parasites may differ in its dependence on external Ca^{++} is also indicated by comparing the mechanical thresholds for muscle contraction of S. japonicum and S. mansoni. The mechanical threshold is determined by plotting the change in muscle tension caused by various concentrations of elevated K^{+} (Figure 9) vs. change in muscle membrane potential under the same conditions (Figure 10). As shown in Figure 32, the mechanical threshold for activation of S. japonicum muscle is shifted to the right of the mechanical threshold for S. mansoni muscle. There may be two explanations for this difference: (1) the Ca^{++} concentration in the extracellular fluid around the muscle fibers of S. japonicum may be higher and this would tend to stabilize the membrane (Luttgau, 1963; Nasledov et al., 1966) or (2) Ca^{++} may be unable to enter the muscle fiber to permit interaction of contractile proteins. A comparison of the elevated K^{+} response to triton-treated S. japonicum bathed in either 1.4 mM Ca^{++} or 14 mM Ca^{++} (Figure 29) shows that incubation in elevated Ca^{++} does not reduce the muscle contraction caused by 60 mM K^{+}. This suggests that the non-responsiveness of normal S. japonicum to 60 mM K^{+} is not caused by increased stabilization of its muscle membrane by higher than normal concentrations of Ca^{++}, but rather by a decrease in Ca^{++} available to the muscle.

The decreased availability of Ca^{++} to the muscle of S. japonicum appears to be caused by the impermeability of this parasite's tegument. Once the tegument is removed by triton, S. japonicum responds

Figure 32. Mechanical threshold for the activation of muscle contraction in S. japonicum and S. mansoni. The plot was made by combining data from the effects of elevated K^+ on muscle tension (Figure 9) vs. the effects of elevated K^+ on the muscle membrane potential (Figure 10). 5.4 mM K^+, left hand points; 60 mM K^+, center points; 177 mM K^+, right hand points. Open circles, S. japonicum; closed circles, S. mansoni. (N=6).

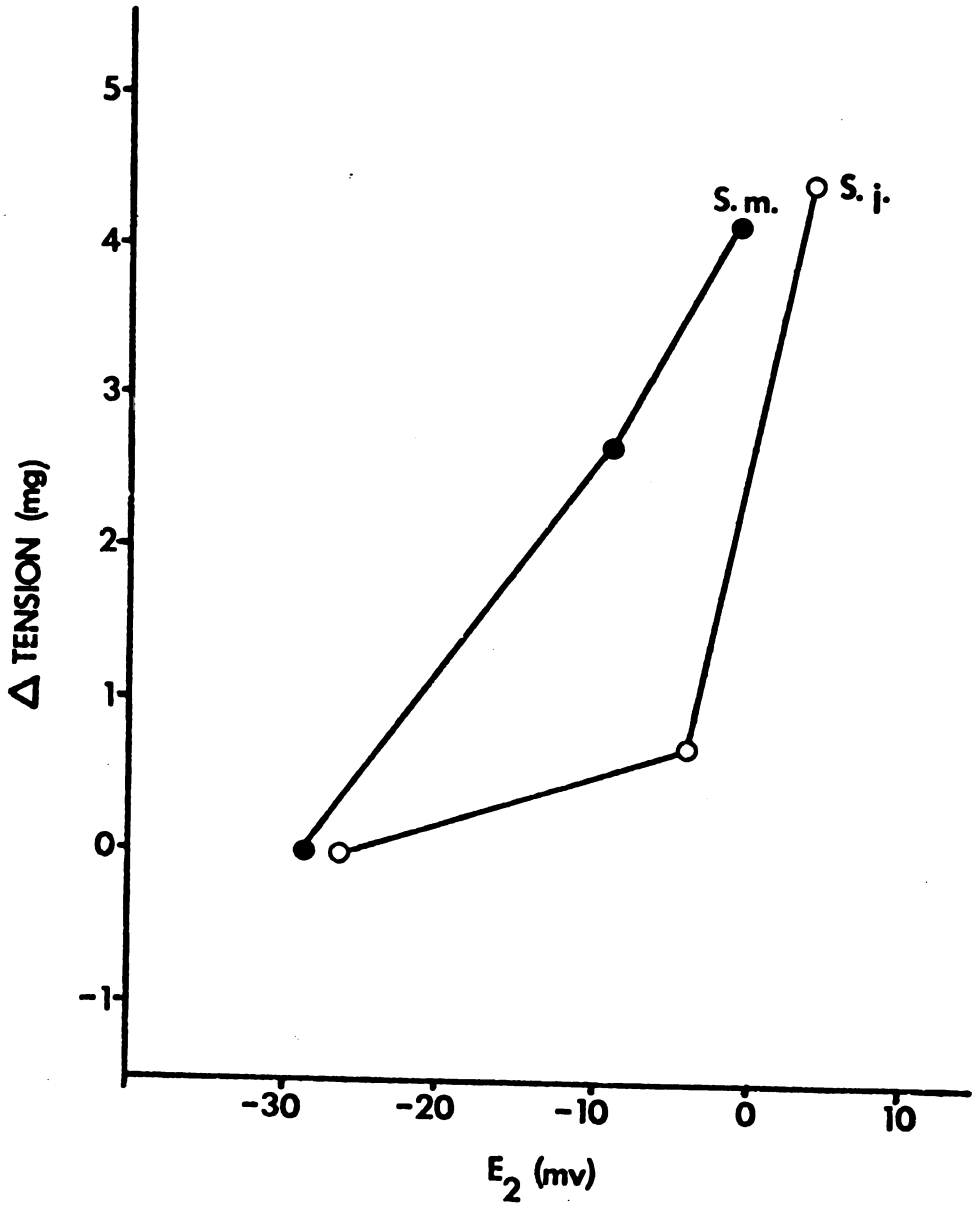

Figure 32

to high K^+ medium with a large, rapid increase in muscle tension (Figures 26 and 27) but only if Ca^{++} is present in the bathing medium. When compared to S. mansoni, normal S. japonicum exhibits only a small, delayed increase in muscle tension to Li^+ substitution. After removal of S. japonicum's tegument, however, this parasite will respond to LiCl with an increase in muscle tension, with no transient relaxation as seen in normal S. japonicum. This difference in Li^+ response of normal and triton-treated S. japonicum would be expected if the tegument of this parasite were prohibiting free external Ca^{++} from penetrating to its muscle.

Regulation of Calcium

From the above discussion it appears that one difference between S. japonicum and S. mansoni is a difference in Ca^{++} fluxes through the tegument. There are several ways in which this difference could be brought about. Ca^{++} movement into the worm either by non-specific movement or by voltage-sensitive Ca^{++} channels, may be less in S. japonicum. In addition, the active extrusion of Ca^{++} by a Ca^{++} pump, located in the tegument, may be more important in S. japonicum.

Calcium channels which open upon membrane depolarization, i.e. voltage-sensitive channels, are reported to be specifically blocked by the drug, D-600 (Flechenstein, 1977). When S. japonicum and S. mansoni are incubated in this drug (10^{-4}M) they respond differently. The transient increase in muscle tension of the two parasites is similar in magnitude, but the maximum increase in muscle tension occurs earlier in S. mansoni (2 min), relative to S. japonicum (5 min). After this transient increase in tension, both parasites relax,

but by 20 minutes, S. mansoni exhibits significantly more relaxation than S. japonicum.

D-600 will block voltage dependent Ca^{++} influx into both cardiac and smooth muscle (Hagiwara and Byerly, 1981). In this way, the drug can block the normal excitation-contraction coupling of muscle (Andersson, 1978). If the muscle and/or tegument of S. mansoni contained a more active voltage-sensitive Ca^{++} channel system, this animal should also be more sensitive to blockage of these channels. After blockage with D-600, less Ca^{++} would pass through the channels, and one would eventually see a greater degree of relaxation. That is, what is observed in S. mansoni, relative to S. japonicum. So it appears one reason for the lesser Ca^{++} flux in S. japonicum is due to a less active voltage-sensitive Ca^{++} channel system in this parasite.

Fewer voltage-sensitive Ca^{++} channels in S. japonicum than in S. mansoni could also explain the difference in elevated K^{+} response. In the face of equal muscle depolarization, less Ca^{++} would pass through the tegument to the extracellular space or directly into the muscle of S. japonicum. The result would be a large increase in muscle tension in S. mansoni, but little or no change in muscle tension in S. japonicum. This is what is observed when these parasites are incubated in 60 mM K^{+}.

My studies comparing Ca^{++}-Mg^{++} ATPase in normal parasites and in parasites after tegumental removal indicate that the tegument of S. japonicum contains more active Ca^{++}-Mg^{++} ATPase than the tegument of S. mansoni. Membrane bound Ca^{++}-Mg^{++} ATPase has been found in a variety of tissues (Devine et al., 1973; Endo, 1977; Fozzard, 1977; Leninger et al., 1978; Matsumura et al., 1980), and in all of these

tissues the enzyme functions in actively sequestering Ca^{++}, which results in a low free cytoplasmic Ca^{++} concentration. The Ca^{++}-Mg^{++} ATPase located in the schistosome's tegument may function in the active removal of Ca^{++} from inside the tegumental compartment and therefore from inside the worm. This could well explain the differences in $^{45}Ca^{++}$ accumulation in the two parasites. In order for $^{45}Ca^{++}$ in the bathing medium to come into equilibrium with Ca^{++} in the parasite there must be $^{45}Ca^{++}$-Ca^{++} exchange across at least three different membranes, the outer tegumental membrane, the inner tegumental membrane and the muscle membrane. An active Ca^{++}-Mg^{++} ATPase in the outer or inner tegumental membrane of S. japonicum could prevent $^{45}Ca^{++}$ exchange across the muscle membrane.

Active Transport

Active Na^{+}-K^{+} transport has been well characterized in S. mansoni (Fetterer et al., 1981a; Bricker et al., 1982). The evidence for the presence of a Na^{+},K^{+}-ATPase is derived from both physiological and biochemical studies. Lowered Na^{+} and K^{+}, Li^{+} substitution, ouabain (10^{-5}M) and low temperature (5°C) all cause depolarization of tegument (Fetterer et al., 1981a) and muscle (Bricker et al., 1982) and all elicit a muscle contracture in S. mansoni (Fetterer et al., 1978, 1981a). Biochemical assays of intact schistosomes and homogenates, indicate that a specific ouabain receptor represents the Na^{+}-K^{+} pump site (Fetterer et al., 1981b).

Na^{+},K^{+}-ATPase is also present in S. japonicum, however, its physiological role in this parasite is less clear. Biochemical assays have shown that S. japonicum have a Na^{+},K^{+}-ATPase with activity

approximately equal to that found in S. mansoni (R.H. Fetterer, personal communication). My data showing that most of the $^{42}K^+$ accumulation in S. japonicum and S. mansoni is inhibited by ouabain (Figure 22) further indicates the importance of Na^+,K^+-ATPase in both of these parasites. Low temperature, which also inhibits Na^+,K^+-ATPase, causes an increase in S. japonicum's muscle tension. This treatment, however, is a non-specific inhibitor of Na^+,K^+-ATPase, and other active processes are likely to be affected (Lippert and Schultz, 1980). In contrast, my experiments measuring the mechanical response of S. japonicum to Li^+ and ouabain indicate that Na^+,K^+-ATPase may have a less important role in control of the physiology of this parasite. Inhibition of the Na^+,K^+-ATPase with either ouabain or Li^+ causes only small changes in muscle tension. After removal of the tegument, S. japonicum show less response to ouabain and no transient relaxation to Li^+ substitution. These results suggest that the difference in S. mansoni's and S. japonicum's response to these drugs is not based on a permeability difference alone, either to Ca^{++}, Li^+ or ouabain.

All of the above differences could be explained if the two parasites differed with respect to an electrogenic Na^+-K^+ pump; i.e., a pump that contributed to the membrane potential (Thomas, 1972). A series of tests were carried out on S. mansoni to determine whether or not the Na^+,K^+-ATPase in its tegument was electrogenic (Fetterer et al., 1981). Fetterer's results, which showed that ouabain, low Na^+ and K^+ and low temperature depolarized tegument, all suggest that the pump is electrogenic. Because of the large surface area and small tegumental volume, however, the observed depolarizations could also

have been caused by inhibition of an electrically neutral pump and a subsequent loss of internal K^+. If the Na^+-K^+ pump in S. mansoni is more electrogenic, relative to the pump in S. japonicum, one would expect to see a more rapid mechanical response in S. mansoni to ouabain and LiCl as the tegument and/or muscle is depolarized. This possible explanation is further supported by the fact that tegument and muscle of S. japonicum show less depolarization to ouabain and Li^+ than does S. mansoni (Bricker et al., 1982). One could still measure a similar amount of Na^+,K^+-ATPase activity and still see a substantial inhibition of $^{42}K^+$ uptake with ouabain, in the two schistosomes.

My results indicate that ouabain and LiCl are having different effects on the mechanical activity of S. japonicum. In normal parasites both ouabain and Li^+ cause a small increase in muscle tension, but Li^+, unlike ouabain, produces a transient relaxation first. After tegumental removal, ouabain produces less tension increase while Li^+ causea a more rapid contraction. This difference may be due simply to the fact that ouabain and LiCl exert their action by two different mechanisms. Ouabain inhibits Na^+,K^+-ATPase by binding to an extracellular site on the pump. When LiCl is substituted for NaCl, Li^+ will move through the membrane, as does Na^+, however, Li^+ is not pumped back out of the cell by the Na^+,K^+-ATPase.

Significance of the Physiological Difference

What becomes clear from my comparative study is that the muscle physiology of S. japonicum and S. mansoni is quite different. This difference is caused by a greater Ca^{++} permeability in S. mansoni relative to S. japonicum. This physiological difference may also be

related to another well documented difference between the two parasites, that of drug sensitivity.

My work has shown that S. japonicum is resistant to the tension increasing effect of high K^+, ouabain and LiCl. S. japonicum is also resistant to a variety of antischistosomal drugs, including some of which are thought to act by interfering with the nervous system of the parasite; e.g., hycanthone, metrifonate and Roll-3128. Of these drugs, two have a measurable effect on the muscle tension of S. mansoni. Metrifonate (10^{-5}M) causes a relaxation of -1.5 mg (Pax et al., 1981) while Roll-3128 causes an increase in tension of 3.0 mg (Fetterer et al., 1978). This is not surprising, as many antiparasitic drugs act by interfering with the normal motility of the parasite (Rew, 1979). What is surprising is that two closely related organisms like S. japonicum and S. mansoni exhibit such marked differences in drug sensitivity. If the motility altering effects of drugs such as metrifonate and Roll-3128 depend on free Ca^{++}, one would expect S. mansoni to be sensitive while S. japonicum would be resistant to them. In vivo (James and Webbe, 1974; Stohler, 1978) and in vitro (Pax et al., 1978, 1981) studies support this. These findings suggest that the difference in the effectiveness of some antischistosomal drugs is caused by a basic physiological difference; i.e., Ca^{++} availability.

SUMMARY

1. Muscle tension of S. mansoni is increased by ouabain (10^{-5}), lithium substitution and elevated K^+, while muscle tension of S. japonicum is unaffected by these treatments. Praziquantel (10^{-6}M) and low temperature (5°C) produce equal increases in muscle tension in both species.
2. All induced muscle contractions in S. mansoni are dependent on free Ca^{++} in the bathing medium, while those induced in S. japonicum by praziquantel and low temperature are not dependent on external Ca^{++}.
3. The mechanical threshold for a muscle contraction is higher in S. japonicum than in S. mansoni. Elevated K^+ (60 mM) caused an equal muscle depolarization in both species, but produced muscle contraction in S. mansoni only.
4. The difference in mechanical threshold in the two schistosomes appears to be due to a specific difference in calcium permeability. The tegument of S. japonicum appears to present a greater barrier to Ca^{++} entry than that of S. mansoni. Tegument removal with triton X-100 eliminates this difference and renders the parasite susceptible to the tension inducing effects of elevated K^+ and Li^+ substitution. These conditions promote muscle tension increase only if Ca^{++} is present in the bathing medium.

5. The specific impermeability to Ca^{++} exhibited by S. japonicum may be attributed to fewer voltage sensitive calcium channels and/or a more active extrusion of Ca^{++} via a Ca^{++}-Mg^{++} ATPase located in the tegumental membrane of S. japonicum. S. japonicum were less responsive to the muscle relaxing effects induced by a voltage-sensitive Ca^{++} channel blocker, D-600, and enzyme analysis revealed a higher level of Ca^{++}-Mg^{++} ATPase in the tegument of S. japonicum.
6. Both parasites have an active Na^{+}-K^{+} transport system. Inhibition of this Na^{+}-K^{+} transport, however, with ouabain or lithium produces a greater degree of muscle depolarization and a larger increase in muscle tension in S. mansoni than in S. japonicum.

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