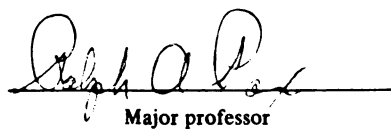


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COMPARATIVE STUDIES OF THE
PHYSIOLOGY OF MALE AND FEMALE
SCHISTOSOMA MANSONI

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COMPARATIVE STUDIES OF THE PHYSIOLOGY OF MALE AND FEMALE

SCHISTOSOMA MANSONI

By

Carla Cress Siefker

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

COMPARATIVE STUDIES OF THE PHYSIOLOGY OF MALE AND FEMALE SCHISTOSOMA MANSONI

By

Carla Cress Siefker

Measurements of surface electrical activity, motor activity and membrane potentials indicate that the physiology of male and female Schistosoma mansoni is, for the most part, similar. The surface electrical activity of the two sexes is similar in form but is of higher frequency in the female parasite. Electrical stimulation, resulting in muscle contraction, is more effective in the female than in the male. These differences, as well as the greater responses of the males to the tension inducing agents, praziquantel, 60 mM K^+ , 2,4-dinitrophenol, ouabain and 5C, may be a result of the recording method or they may reflect the anatomical differences in the sexes. Three membrane potentials, recordable upon penetration of the female with a microelectrode, are essentially identical in order of appearance and in magnitude to those reported from males. Both sexes respond similarly to the putative neurotransmitters 5-HT, dopamine and carbachol. Females are affected to a greater degree by the removal of Ca^{++} from the bathing medium and by increases in external Mg^{++} . There is no significant loss in calcium content, as measured by elemental analysis, in the female after a one hour incubation in a 0 Ca^{++} medium. A greater permeability

Carla Cress Siefker

to Mg^{++} and Ca^{++} in the female and smaller stores of available calcium in the female may account for the differential effects of ionic alterations in the two sexes.

To Jim

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INTRODUCTION

Schistosomiasis, the second ranked parasitic disease in man today, has a long history. Although evidence of the disease has been found in Egyptian mummies (1250 to 1000 BC) the actual discovery of the parasite did not occur until 1815 when Theodor Bilharz found the adult trematode in human mesenteric veins. He later showed a relationship between the parasite, it's ova and the dysentery present in the Egyptian people. Sambon (1907) established that two species of schistosomes are responsible for the disease in Egypt, Schistosoma mansoni and Schistosoma haematobium. Katsurada (1904) described Schistosoma japonicum and more recently Schistosoma mekongi has been described (Voge et al., 1978). Leiper (1916), for S. haematobium and S. mansoni, and Miyairi (1914) for S. japonicum showed that snails were required to complete the life cycles of the schistosomes.

The schistosomes are digenetic trematodes with a complex life cycle. Schistosoma mansoni, responsible for intestinal schistosomiasis, is found as an adult in human mesenteric veins. Eggs are passed from man into water where they hatch and release free swimming miracidia. Miracidia have only several hours to find and penetrate the appropriate snail where they develop into primary sporocysts. The primary sporocyst gives rise to the secondary sporocyst which asexually produces cercariae. Free swimming cercariae emerge from the snail and penetrate

the skin of man where, as schistosomules, they invade blood vessels and migrate through the heart to the lungs and into the liver and mesenteric veins where they reside as mature trematodes.

An estimated 500 million people in 73 countries suffer from schistosomiasis today (Iarotski and Davis, 1981). The primary pathology consists of granulomatous pseudotubercles formed in host tissues in response to the parasite's eggs. Symptoms of the disease vary with the species of parasite. Dysentery, nausea, vomiting, flatulence, fatigue, weight loss, urticaria, chronic cough, eosinophilia and enlarged liver and spleen result from severe infections of S. mansoni (Belding, 1965).

General Anatomy

Schistosoma mansoni is a sexually dimorphic platyhelminth. The larger male has a ventral gynecophoral canal running the length of his body in which the more slender female lies during copulation (Figure 1). Male S. mansoni average 1.0 x 0.11 cm in size while females average 1.4 x 0.016 cm. The female appears darker than the male due to ingested red blood cells visible in her intestine. Both sexes have an oral sucker at their anterior ends. The tegument of the schistosome has an outer anuclear layer connected to a deeper layer of cell bodies. The tegumental surface of the female is smoother than that of the male. Layers of circular and longitudinal muscles lie beneath the tegument. Subjacent to these muscle layers lie the digestive, reproductive and excretory systems of the parasite.

Figure 1. Drawing illustrating the external characteristics of male and female S. mansoni. OS, oral sucker; VS, ventral sucker; GC, gynecophoral canal.

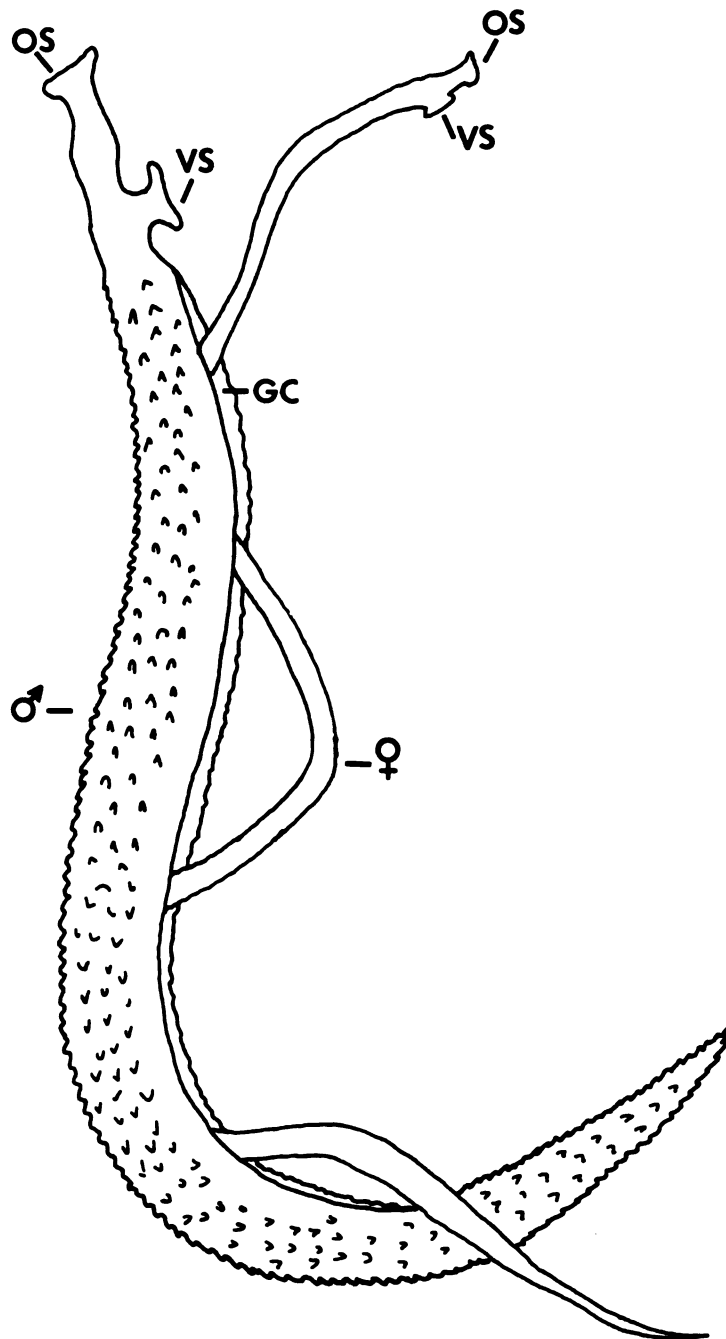


Figure 1

Both male and female digestive systems open at the oral sucker. The mouth, pharynx and esophagus lead into an intestine which bifurcates in the area of the ventral sucker. The cecum reunites and ends blindly at the posterior of the schistosomes.

The male reproductive organs consist of 4-8 testes situated dorsal to the ventral sucker. These lead, via the efferent duct, vas deferens and seminal vesicle, to the genital pore just posterior to the ventral sucker. The female reproductive system includes an elongate ovary situated in the anterior half of her body. The oviduct runs from the ovary and joins with the vitelline duct at the ootype, where fertilization occurs. Mehlis' gland surrounds the ootype. The uterus runs anteriorly from the ootype and opens to the genital pore posterior to the ventral sucker (Figure 2).

The excretory system of the schistosome is protonephridial in nature. Diffusely distributed flame cells empty via two longitudinal collecting tubules into a posteriorly located bladder and excretory pore.

The Tegument

The teguments of male and female Schistosoma mansoni differ in surface characteristics (Morris and Threadgold, 1968; Silk and Spence, 1969c; Silk et al., 1970; Senft and Gibler, 1977). The male tegument is easily differentiated into dorsal and ventral surfaces, the dorsal surface being covered with bosses and spine studded tubercles while the ventral surface is highly spined but lacking bosses and tubercles (Silk et al., 1970; Senft and Gibler, 1977). Many clefts are evident

Figure 2. Drawing comparing the digestive and reproductive systems of male and female *S. mansoni*. OS, oral sucker; VS, ventral sucker; GP, genital pore; U, uterus; OT, ootype; MG, Mehlis' gland; O, ovary; C, cecum; V, vitelline gland; T, testes; GC, gynecophoral canal.

on the surface of the gynecophoral canal. Pores, thought to be sites for transfer of material, and setae, thought to be tactile receptors, are present in some of the clefts (Senft and Gibler, 1977). The tegumental surface of the female is smoother than that of the male and dorsal-ventral differentiation is not easily made. Small spicules, diffusely covering the female tegument are somewhat more dense on the ventral surface (Senft and Gibler, 1977). No bosses or tubercles are present and spines are seen primarily at her posterior (Silk et al., 1969b; Silk et al., 1970; Senft and Gibler, 1977).

Despite the differences in surface characteristics, male and female S. mansoni are very similar in tegumental ultrastructure. The tegument is a cytoplasmic syncytium connected by processes to nucleated cell bodies, the cytons, in and below the muscle layers of the parasite (Morris and Threadgold, 1968; Silk and Spence, 1969a; Smith et al., 1969) (Figure 3).

The outer tegumental membrane measures $110\text{\AA}$ and has deep invaginations into the cytoplasm. Its heptalaminate appearance is caused by the close apposition of two trilaminate membranes (Hockley and McLaren, 1973; Wilson and Barnes, 1974). The inner tegumental membrane has numerous infoldings upward into the cytoplasm and is trilaminate. This inner membrane is separated from the deeper muscle layers by a basal lamina (450-850A). The tegument can vary in thickness from $5.0\text{ }\mu\text{m}$ during a contraction to $.25\text{--}.5\text{ }\mu\text{m}$ when inner and outer membrane invaginations approach each other (Wilson and Barnes, 1974). Males and females show similar variations in tegumental thickness.

Figure 3. Schematic representation of a longitudinal section through male and female *S. mansoni* showing the tegument and subtegumental layers. OM, outer membrane; T, tegument; S, spine; IM, inner membrane; BL, basal lamina; CM, circular muscle; LM, longitudinal muscle; RM, radial muscle; TC, tegumental cyton; N, nucleus; G, glycogen; L, lipid.

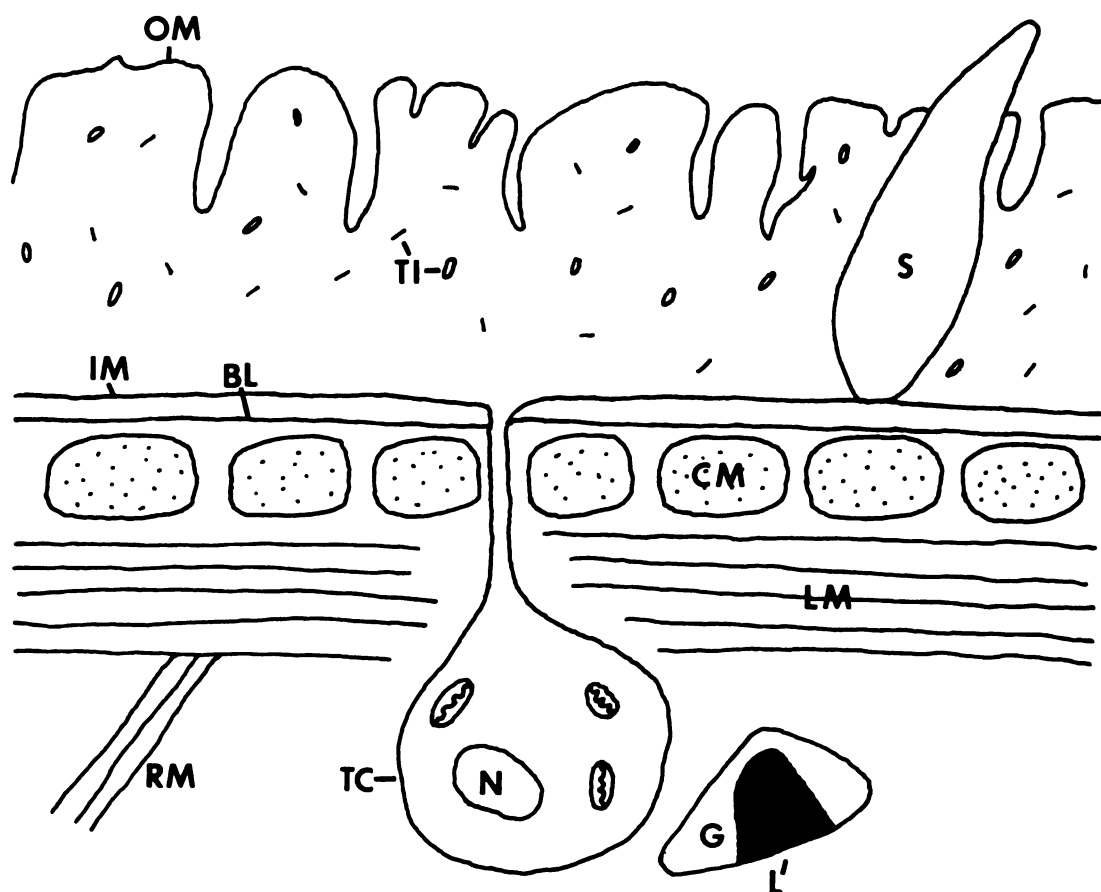


Figure 3

With the exception of a few poorly developed mitochondria the cytoplasmic syncytium is devoid of cellular organelles. Other tegumental inclusions include spines, discoid granules which disperse and become cytoplasmic ground substance and multilaminate vesicles which form part of the outer tegumental membrane (Smith et al., 1969; Hockley and McLaren, 1973; Wilson and Barnes, 1974). The sometimes multinucleate cytons are rich in organelles necessary for the synthesis and packaging of proteins, other macromolecules, discoid granules and multilaminate vesicles. These are transported to the syncytium via inter-nuncial processes (Silk et al., 1969b; Wilson and Barnes, 1974). Junctional complexes occur between cytons and muscle cells but not between adjacent cytons (Silk et al., 1969b).

Studies on the effects of lipophilicity on transintegumental uptake in S. mansoni suggest that the unusual multilaminate outer membrane of the schistosome tegument functions similarly to the more common lipid-bilayer unit membrane (Bocash et al., 1981). As the host parasite interface, the tegument has several functions. It is the site of nutrient uptake; purine and pyrimidine transport (Levy and Read, 1975a,b), amino acid absorption (Asch and Read, 1975; Isseroff et al., 1976; Chappell, 1974) and sugar uptake (Uglen and Read, 1975; Bueding, 1962; Cornford and Hout, 1981) have been demonstrated at the tegumental surface. The tegument also serves to protect the parasite from a host mounted immune attack. It appears that certain host erythrocyte components form antigenic determinants on the tegumental surface thereby hiding the parasite from the host's immune system (Clegg, 1972;

Smithers and Terry, 1969). Regulation of ions may also occur at the tegument. Fetterer et al. (1981) suggest the presence of an active $\text{Na}^+ - \text{K}^+$ pump on the tegumental surface.

The Musculature

The schistosome musculature lies subjacent to the tegumental syncytium and basal lamina. Circular muscle is found directly below the tegument, longitudinal muscle is beneath the circular muscle and radial muscle runs dorsal-ventrally through the circular and longitudinal musculature. The muscle masses are more extensive in the male parasite (Table 1). Often only one layer of longitudinal muscle is present in females while males have several layers (Silk and Spence, 1969a). Fibrous interstitial connective tissue surrounds the muscle layers. All muscle is smooth muscle, typical of that found in invertebrates (Lowy and Hansen, 1962).

The musculature of male and female S. mansoni is structurally similar. Muscle bundles consist of thick and thin filaments. The thick filaments (180-400A) are surrounded by as many as 14 thin filaments (50A). Thin filaments branch and cross linking occurs between thin filaments as well as between thick and thin filaments (Silk and Spence, 1969a).

The muscle cell nuclei are found below the layers of myofibrils. The nuclei are ovoid with clumped chromatin and prominent nucleoli. The sarcoplasmic reticulum is poorly developed though rough elements are present. Free ribosomes and ribosomes bound to the outer nuclear membrane are evident, as are Golgi apparatus and mitochondria.

Table 1
 Depths of Circular and Longitudinal Muscle Masses in Male
 and Female S. manosni

Sex	Circular (μm)	Longitudinal (μm)	Source
Female	1.03 $\pm$.13	.94 $\pm$.30	Silk, Spence and Gear, 1969 Smith, Reynolds and von Lichtenberg, 1969
Male	1.55 $\pm$.40	9.48 $\pm$ 1.91	Bricker, Pax and Bennett, 1981

Values are means $\pm$ one S.E.M. for a minimum of five animals.

Microtubules are not seen. Lipid globules and glycogen particles are present in the muscle cells (Silk and Spence, 1969a).

Junctional complexes exist between adjacent muscle cells as well as between muscle cells and tegumental cytons (Silk and Spence, 1969a).

The Nervous System

The nervous system of S. mansoni is typical of that found in other trematodes. Paired esophageal ganglia are connected by dorsal commissures. Two pairs of dorsal and ventral nerve trunks extend from these ganglia to the posterior of the parasite. Numerous commissures connect these nerve trunks. Fibers originating in the esophageal ganglia supply the oral sucker while fibers from the ventral nerve trunk innervate the ventral sucker (Fripp, 1967).

Neuromuscular junctions and axo-axonic synapses occur within the nervous system of the schistosome. Although nerve processes are closely associated with tegumental cytons and tegumental syncytium in the area of the esophagus, no direct innervation of the tegument occurs (Silk and Spence, 1969b). Sensory receptors formed by nerve fibers extending through the tegument occur over the surface of the schistosome (Silk and Spence, 1969b; Morris and Threadgold, 1967).

A number of compounds have been implicated as neurotransmitters in schistosomes. These compounds, known to be neurotransmitters in other systems, include: norepinephrine and dopamine (Bennett and Bueding, 1971; Gianutsos and Bennett, 1977; Tomosky et al., 1974), acetylcholine (Bueding, 1952; Fripp, 1967; Barker et al., 1966; Machado et al., 1972) and 5-hydroxytryptamine (Bennett et al., 1969).

Male-Female Pharmacological Differences

It is well documented that male and female schistosomes often respond differently to the same drugs (Table 2). Studies indicate that differential effects of drugs may be due to biochemical differences in the sexes rather than differences in membrane permeability (Bocash et al., 1981). Male and female S. mansoni function similarly with respect to the effect of lipophilicity on membrane permeability thus any differential effects of lipophilic drugs are not likely to be due to differences in permeability.

Antimony, thought to reduce glycolysis in schistosomes by inhibiting phosphofructokinase, has long been a treatment for schistosomiasis. Females take up 3 to 5 times as much antimony as males (Browne and Schulert, 1964; Molokhia and Smith, 1969). Tubercidin (7-deazaadenosine), effective against S. mansoni and S. japonicum in primates, kills the sexually mature female parasite but spares most of the males (Jaffe et al., 1973, 1975). More of the anthelmintic niridazole is taken up by the female schistosome (Hess et al., 1966) and females are somewhat more susceptible to the drug than are males (Foster et al., 1970).

Lucanthone and hycanthone, used to treat S. mansoni and S. haematobium, are more active against male schistosomes than against females (Foster et al., 1970; Lee, 1972) though initially the in vivo uptake of hycanthone is greater in the female parasite (Yarinsky et al., 1970). Hycanthone interferes with the normal serotonin metabolism of the schistosome (Chou et al., 1973; Bueding et al., 1974) and drug treated males take up 3 to 4 times more serotonin than drug treated females (Senft et al., 1976). UK-3883, a 2-aminomethyltetra-hydroquinoline

Table 2
A Comparison of Effects of Drugs on Male and Female
S. mansoni

Property	Observation	Source
<u>Drug Uptake</u>		
Amoscanate	f > m	Liu <u>et al.</u> , 1980
Antimony	f > m	Browne and Schulert, 1964
Hycanthone	f > m (initially)	Yarinsky <u>et al.</u> , 1970
Niridazole	f > m	Foster <u>et al.</u> , 1970
Oxamniquine	m > f (<u>in vivo</u>) m = f (<u>in vitro</u>)	Foster <u>et al.</u> , 1973b
<u>Drug Sensitivity</u>		
Emetine	m > f	Walker and Chappell, 1980
Iso-OMPA	f > m	Gear, 1976
Hycanthone	m > f	Foster <u>et al.</u> , 1970 Lee, 1972
Lucanthone	m > f	Foster <u>et al.</u> , 1970 Lee, 1972
Mirasan	m > f (single dose) f > m (mult. dose)	Foster <u>et al.</u> , 1970 Foster <u>et al.</u> , 1970
Niridazole	f > m	Hess <u>et al.</u> , 1966
Oxamniquine	m > f	Foster <u>et al.</u> , 1973b Woolhouse and Kaye, 1977
Praziquantel	f = m	Gonnert and Andrews, 1977
Tubercidin	f > m	Jaffe <u>et al.</u> , 1973, 1975
UK-3883	m > f	Foster <u>et al.</u> , 1970

derivative active against S. mansoni in monkeys and mice, is more effective against male schistosomes (Foster et al., 1970). Males are more susceptible to mirasan, an antischistosomal effective in mice, when given in single doses while females are more or equally susceptible to multiple doses of mirasan (Foster et al., 1970). Oxamniquine, a potent schistosomicide, is more effective against males than against females (Foster and Cheetham, 1973a; Foster et al., 1973b; Woolhouse and Kaye, 1977). Greater amounts of oxamniquine are taken up by male worms in vivo but in vitro there is no difference in the amount of drug taken up by each sex (Woolhouse and Kaye, 1977). Male S. mansoni incubated in vitro for short periods in the antischistosomal praziquantel show greater vacuolization, vesicle formation and ballooning of the uppermost tegumental layers than females (Becker et al., 1980). Though praziquantel in vivo is equally effective against both sexes (Gönnert and Andrews, 1977), more lesions are seen on the males (Melhorn et al., 1981).

Treatment with subcurative doses of the antischistosomal, amoscinate, induces different tegumental surface alterations in male and female schistosomes. Females, who take up more of the drug (Liu et al., 1980), show large tegumental pores, constrictions in the tegumental surface and disruption of sensory structures as well as the swelling of the tegumental surface, collapse of sensory bulbs and loss of surface layers seen in male worms (Voge, 1980).

Drugs other than anthelmintics also show differential effects on the sexes of schistosomes. Females are less sensitive to emetine, an inhibitor of eukaryote protein synthesis, than are males. This is true

in both paired and unpaired worms (Walker and Chappell, 1980). Females are more sensitive to ios-OMPA (iso-octa methyl pyrophosphoramidate), an irreversible inhibitor of cholinesterase (Gear, 1976).

The cells of the female reproductive system are particularly sensitive to the action of drugs. Use of x-ray analysis in the transmission electron microscope has shown antimony to locate in the cells of the ovary and vitelline gland (Erasmus, 1974). Developmental stages of the vitelline cells are affected to different degrees. Treatment with the antimonial astiban results in a large accumulation of the drug in S4 (mature) cells while S2 and S3 cells contain varying smaller amounts of drug. S1 cells remain drug free but are unable to develop further (Erasmus, 1975). Vitelline gland changes in response to hycanthone and lucanthone are somewhat different. Abnormal S4 cells resulting from hycanthone and lucanthone are retained in the vitelline lobule while S4 cells altered by astiban degenerate. S1 cells continue to develop but lose their ability to divide after hycanthone and lucanthone treatment (Erasmus and Popiel, 1980). Vitelline cell damage due to niridazole begins posteriorly and progresses forward. This is in contrast to astiban, hycanthone and lucanthone where the entire gland is damaged simultaneously (Popiel and Erasmus, 1981b). Niridazole accumulates in the vitelline gland, depletes the gland of egg shell-substance and shell formation in the ootype ceases. Though less sensitive than the female gonads, the male gonads are also affected by niridazole; spermatogenesis stops and the testes degenerate (Rollo, 1980).

Male-Female Metabolic Differences

Glycolysis, representing a major source of energy for Schistosoma mansoni, proceeds at a high rate and results in the excretion of lactic acid. Though oxygen is consumed, the oxidative pathway for energy production is not essential for survival (Bueding, 1950). About one-quarter of the parasite's energy comes from oxidative phosphorylation (Coles, 1972). Enzymatic activities suggest that glycolysis is more important to the male schistosome while oxidative metabolism is more important to the female (Table 3). The male produces more lactic acid than the female (Bueding, 1950) and glycolytic enzymes (pyruvate kinase, lactate dehydrogenase, phosphoenol pyruvate carboxykinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) have higher specific activities in the male parasites (Coles, 1973a; Bueding and Saz, 1968). Lactate, malate and glucose-6-phosphate dehydrogenases show different electrophoretic patterns in male and female schistosomes (Coles, 1970). In contrast, females use more oxygen than males (Bueding, 1950). Female cytochrome oxidase activity is higher (Coles, 1972) as is the isocitrate dehydrogenase activity (Coles, 1973a,b). Malate dehydrogenase does not fit this pattern, as its activity is greater in males than in females but it must be remembered that enzyme activity is not always correlated with the importance of the pathway involved (Coles, 1973a; Bueding and Saz, 1968). The suggestion that oxidative metabolism is more important to the female parasite is supported by the observation that schistosomes cultured in nitrogen fail to produce eggs. Oxidative metabolism may be necessary for egg production (Coles, 1973b) or it could be a substrate for phenol oxidase which is involved in egg production.

Table 3
Metabolic Comparisons of Male and Female S. mansoni

Property	Observation	Source
<u>Carbohydrate Metabolism</u>		
Glucose uptake	m > f	Bueding, 1950; Cornford and Hout, 1981
Glycogen content	m : f = 3-5:1 per mg dry wt	Bueding, 1950
Lactate production	m : f = 5.3:1 per mg dry wt	Bueding, 1950
Oxygen uptake	m : f = 0.57:1 per mg dry wt	Bueding, 1950
Pyruvate kinase	m > f	Coles, 1973; Bueding and Saz, 1968
Lactate dehydrogenase	m > f	Coles, 1973; Bueding and Saz, 1968
Phosphoenol pyruvate kinase	m > f	Coles, 1973; Bueding and Saz, 1968
Glucose-6-phosphate dehydrogenase	m > f	Coles, 1973; Bueding and Saz, 1968
6-Phosphogluconate dehydrogenase	m > f	Coles, 1973; Bueding and Saz, 1968
Cytochrome oxidase	f > m	Coles, 1972
Isocitrate dehydrogenase	f > m	Coles, 1973
Malate dehydrogenase	m > f	Coles, 1973; Bueding and Saz, 1968
<u>Other Enzyme Activities</u>		
Acetylcholinesterase	f > m	Gear and Fripp, 1974
Monoamine oxidase	m > f	Nimmo-Smith and Raison, 1967
Proteolytic enzyme	m > f	Timms and Bueding, 1959
Alanine aminotransferase	m > f	Coles, 1973
Aspartate aminotransferase	f > m	Coles, 1973

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Table 3 (continued)

Property	Observation	Source
<u>Amine Acid Uptake</u>		
Ornithine	f > m	Cornford and Oldendorf, 1979
Lysine	f > m	Cornford and Oldendorf, 1979
Arginine	f > m	Cornford and Oldendorf, 1979
Aspartate	m > f	Cornford and Oldendorf, 1979
Proline	m > f	Senft, 1968
	m = f	Cornford and Oldendorf, 1979
Leucine	m = f	Cornford and Oldendorf, 1979
Tyrosine	m = f	Cornford and Oldendorf, 1979
Isoleucine	m = f	Cornford and Oldendorf, 1979
Glutamate	m = f	Cornford and Oldendorf, 1979

Both male and female S. mansoni transport glucose across the tegument (Rogers and Bueding, 1975). Mediated transport, as well as diffusion, is involved in glucose uptake (Uglen and Read, 1975). Copulating pairs take up more labeled glucose than unpaired males or females (Uglen and Read, 1975). Cornford and Hout (1981) confirmed this observation using several labeled hexoses (D-glucose, 3-O-methyl-glucose and 2-deoxy-D-glucose). Uptake of hexose in unpaired males is greater than in females. In copula, uptake of glucose and methyl glucose is greater in the female fluke than in the male (Cornford and Hout, 1981). The rate of incorporation of labeled glucose into glycogen is greater in unpaired males than in females. This is consistent with the female utilizing less glucose, producing less lactic acid and storing less glycogen than the male (Bruce et al., 1974). Glycogen stores are greatest in the unpaired male schistosome. Male glycogen stores decrease when in copula. Direct transtegumental transfer of glucose during copulation has been demonstrated (Cornford and Hout, 1981).

Amino acids are transported across the tegument actively and via diffusion. All of the amino acids tested by Isseroff et al. (1976) (alanine, arginine, aspartate, cycloleucine, glutamate, leucine, methionine, phenylalanine, proline and valine) are transported by mediated systems except proline. In vitro C^{14} labeling studies (Senft, 1968) found proline uptake to be greater in the male schistosome than in the female. Proline accumulated in the tegument, subtegument, paraesophageal area, gut and testes of the male. More proline was found in the ventral tegument than in the dorsal tegument of the male, with autoradiographic studies showing greatest accumulation in the

ventral arms of the gynecophoral canal, the area of the female contact. The female fluke concentrated proline in the posterior ovary and Mehlis' gland.

This differential uptake of proline was not seen in studies by Cornford and Oldendorf (1979). Using a water reference method, Cornford and Oldendorf found similar amounts of proline, leucine, tyrosine, isoleucine and glutamate absorbed by both sexes. Female flukes transported more ornithine, lysine and arginine than males. Aspartate was the only amino acid in which male uptake was greater than that of the female. Uptake of ornithine, lysine and methionine equalled the amount of glucose absorbed by each sex (greater glucose absorption being seen in the male). Arginine was taken up in greater quantities than glucose. The higher metabolic rate of the female schistosome may account for the generally higher amino acid uptake.

Various enzymatic differences are present in male and female schistosomes (Table 3). ATP phosphohydrolase is located in the plasma membrane and nuclear envelope of parenchymal cells in both sexes, but it is also active in the basal plasma membrane of the female tegument (Bogitsh, 1980).

Distribution of alkaline and acid phosphatases, thought to be involved in metabolic exchange with the environment, also vary in the two sexes (Cesari, 1974). Most of the alkaline phosphatase activity is associated with membranous structures and is found in the tegument of both sexes. With the teguments removed by saponin- CaCl_2 treatment, alkaline phosphatase activity is significantly higher in the female

fluke. This difference may be due to internal membrane bound phosphatases or to incomplete removal of the tegument. Alkaline phosphatase activity is also found in the internal tissues of the uterus and vitellaria of the female schistosome and is more active in the female than in the male (Coles, 1973a). Acid phosphatase activity in the male parasite is concentrated in the tegument while females show equal activity in the tegumental fraction and the denuded fraction. Electrophoretic patterns for acid and alkaline phosphatases are identical in male and female parasites (Cesari, 1974).

Marked differences exist in male and female acetylcholinesterase, the enzyme responsible for the hydrolysis of acetylcholine (Gear and Fripp, 1974). Female S. mansoni have a higher concentration of this enzyme than males. K_m , the Michaelis Menten constant, for the female schistosome is twice that of the male. This may indicate different enzymatic arrangements on the cell membrane and an increase in the number of active sites.

The male parasite has a higher monoamine oxidase activity than does the female. This enzyme is responsible for inactivating the putative neurotransmitter 5-hydroxytryptamine. This difference may reflect the greater size of the male and the greater content of nervous tissue (Nimmo-Smith and Raison, 1968).

Phenol oxidase activity appears to be unique to the female parasite. This copper containing enzyme is located in the vitelline gland and catalyzes the polymerization of proteins in the production of egg shells (Clegg and Smyth, 1968; Seed et al., 1978; Bennett et al., 1978).

The presence of this enzyme in females accounts for the greater amount of Cu detectable in females in elemental analyses of male and female S. mansoni (Wolde Mussie and Bennett, 1982). Of the other elements examined (K, Na, Ca, Mg, Fe) only iron was found in unequal amounts in the two sexes. The greater quantity of iron present in females is due to her greater ingestion of red blood cells.

Enzymatic degradation of ingested globin may result in products important to the nutrition of the schistosome (Cheever and Weller, 1958; Timms and Bueding, 1959; Zussman et al., 1970; Lawrence, 1973; Grant and Senft, 1971). These products may be most important in the nutrition of the female parasite. The female contains thirteen times as many red blood cells and ingests these cells nine times faster than males. Females are able to turn over their cecal contents in less time than are males (Lawrence, 1973) and they incorporate greater amounts of L-leucine labeled hemoglobin than do males (Zussman et al., 1970). A proteolytic enzyme that attacks hemoglobin, but not serum proteins, is 4 to 5 times more active in the female parasite (Timms and Bueding, 1959). Studies suggest that the breakdown products of globin, small peptides, might be absorbed and used for yolk formation (Sauer and Senft, 1972; Senft, 1968). The apparent lack of globinase activity in the immature female, evidenced by the undigested globin present in her cecum, and the appearance of globinase activity in the mature female suggests that the breakdown products of globin are necessary for egg production (Grant and Senft, 1971).

Male-Female Interdependence

Female S. mansoni from unisexual infections do not reach sexual maturity nor do they grow to full body size (Vogel, 1941; Moore et al., 1949; Maldonado and Herrera, 1949) while males, in the absence of females, mature normally (Vogel, 1941). The presence of the male not only affects the development of the immature female but also affects the fecundity of the mature female. Females separated from males lay fewer eggs than paired females (Michaels, 1969). Originally, it was thought that a stimulus from the male initiated sexual and somatic development in the female. It appears now that stimulation from the male does not necessarily initiate development but it increases the rate of development of the vitelline gland and coordinates the development of the entire female reproductive system (Shaw and Erasmus, 1981).

Ultrastructurally, females from unisexual infections are different from paired females. The ovary is smaller and produces ova lacking the characteristic cortical granules. The golgi bodies synthesize material but are unable to organize their products (Erasmus, 1973). Mehlis' gland is incompletely developed and the vitelline gland is in varying stages of development (Shaw and Erasmus, 1981). The observation that older unisexual infections showed a higher proportion of mature vitelline cells than did younger infections led to the belief that the male stimulus was not necessarily responsible for the initiation of sexual development in the female (Shaw and Erasmus, 1981). Degeneration of the vitelline cell occurs when previously paired, mature females are transferred to hosts without male parasites (Clough and Schiller, 1979).

Previous sexual experience in the male does not seem to affect the development of the female. Pairing with sexually inexperienced males or with previously paired males results in equivalent development. Development consists primarily of vitelline cell maturation and egg shell substance formation while the ovary remains in its undeveloped state (Shaw, 1977). Unisexual males are seen to pair more readily with unisexual females than are unpaired males from a bisexual infection (Michaels, 1969; Shaw, 1977). This could be the result of a build-up of a stimulatory pairing substance in the unisexual males or a deleterious effect of unpairing on the previously paired males.

The nature of the stimulus given by the male has not been determined. Possible stimuli include: tactile stimulation (Armstrong, 1965), nutritional influences (Senft, 1968; Vogel, 1941), hormonal influences (Moore et al., 1954), insemination and chemicals transferred with sperm (Moore et al., 1954; Vogel, 1941). The ability of anorchid males to pair and stimulate egg laying in females implies that insemination is not necessary for development in the female (Michaels, 1969). Further evidence of this is the absence of sperm in the oviduct of nearly mature paired females (Erasmus, 1973; Shaw, 1977).

In vitro experiments underline the importance of male female apposition in the gynecophoral canal. Close apposition may result in tactile stimulation and may allow transtegumental transport to occur. Development occurs only in females positioned in the gynecophoral canal of a mature male. Unisexual females in the proximity of a male-female pair do not develop. If the stimulus from the male is chemical in nature, the microenvironment in the canal space may be necessary for

maintaining a proper concentration of that substance. The in vitro development of the vitelline gland of unisexual females in response to male extract is evidence for chemical stimulation (Shaw, 1977; Popiel and Erasmus, 1981a).

Electrophoretic studies have shown direct transtegumental transport of protein between male and female S. mansoni. The most abundant protein found in the female is not synthesized by the female. Synthesis of this 66000 MW protein occurs in the male. Although the male makes more of this protein than any other, he retains little. When labeled males are mated with females, a significant amount of label is present in the female in 20 hours. Females also acquire some minor polypeptides common to both male and female parasites (Atkinson and Atkinson, 1980). Studies by Ruppel and Cioli (1977) did not identify this 66000 MW protein though they found a 29000 MW protein that was unique to female schistosomes. More recently, Atkinson and Atkinson (1982), using flouorographic analysis in conjunction with electrophoresis, have been able to detect five polypeptides produced by male S. mansoni and four polypeptides specific to the female. Despite some quantitative differences, they found male and female S. mansoni to be qualitatively similar in polypeptide synthesis; the two sexes having at least 65 polypeptides in common.

Direct tegumental transport of glucose also occurs between the sexes. Labeled hexoses from the copulating male accumulate in the female within minutes. Measurements of glycogen in paired and unpaired males show male glycogen stores to be depleted when in copula. As

glycolysis is the major energy pathway, this exchange of glucose may be highly significant. The female may be dependent on the male for a large portion of her required glucose intake (Cornford and Hout, 1981).

OBJECTIVE

Schistosomiasis is a devastating disease in many parts of the world. When not life threatening, it is debilitating; removing men from the work force and weakening the economy of the region. Schistosoma mansoni, the causative agent of one form of this disease, has been the subject of study for many years. Only recently has the physiology of this trematode been investigated and research on the female of the species has been virtually neglected. This neglect does not reflect the importance of the female, rather it reflects the difficulty inherent in working with a small organism. The female is a necessary part of the schistosome life cycle and as the layer of eggs, the true pathogen, is of considerable interest. This investigation will employ several techniques in exploring the physiology of the female schistosome and comparing it to that of the male.

Specifically, I will examine 1) the role of Ca^{++} in muscle contraction, 2) the effects of electrical stimulation on the musculature, 3) the regional variation in surface electrical activity, and 4) the membrane potentials of the tegument and subtegumental compartments.

MATERIALS AND METHODS

Source and Maintenance of Animals

Female laboratory mice were injected intraperitoneally with 200 cercariae of Schistosoma mansoni (Puerto Rican strain). Fifty to sixty days post-infection, mature schistosome pairs were dissected from the portal and mesenteric veins of the mice. Paired worms were maintained at 37°C in Hank's balanced salt solution (HBS) consisting of 138 mM NaCl, 5.4 mM KCl, 1.4 mM CaCl₂, 0.5 mM MgCl₂, 0.5 mM MgSO₄, 0.5 mM KH₂PO₄, 0.25 mM Na₂HPO₄, 1.0 gm/l glucose and 20 mM Hepes. Only unpaired males and females were used for experimentation. Some females became unpaired prior to use, those that did not were gently teased from the gynecophoral canal of the male schistosome. Parasites were used within 8 hours of dissection.

Experimental Media

The standard medium used in all experiments was HBS. The 60 mM K⁺ HBS consisted of HBS made with 60 mM KCl and 95 mM NaCl rather than the usual 5.4 mM KCl and 138 mM NaCl. The Ca⁺⁺ free medium consisted of HBS with the 1.4 mM CaCl₂ omitted and with 5x10⁻⁴M ethyleneglycol-bis(-amino-ethyl ether)N,N'-tetraacetic acid (EGTA) added. The high Mg⁺⁺ HBS contained 20 mM MgSO₄ rather than the normal 0.5 mM MgSO₄.

All solutions were adjusted to pH 7.4. Experiments were run at 37°C unless otherwise noted.

Pharmacological Agents

Praziquantel (PZ) (supplied by Drs. P. Andrews and H. Stohler of the Bayer Institute for Chemotherapy, Wuppertal, Germany) was dissolved in dimethyl sulfoxide (DMSO) at 10^{-2} M. Further dilutions were made with distilled water. Twenty-five μ l aliquots were added to the 2.5 ml bath to achieve the final concentration desired. 2,4-Dinitrophenol (DNP) was dissolved in HBS to give a 1 mM solution. Ouabain (Sigma Chemical Co., St. Louis, MO) was dissolved in a 50% DMSO-distilled water mixture to give a 10^{-2} M solution then diluted to 10^{-3} M with distilled water. Twenty-five μ l of the 10^{-3} M solution were added to the bath to give a final concentration of 10^{-5} M ouabain in the recording chamber.

Mechanical Recording Apparatus

A suction pipette-balance arm system similar to that developed by Fetterer et al. (1977, 1978) was used to measure muscle tension and tension changes in male and female schistosomes (Figure 4). Parasites were held in a recording chamber by two suction pipettes. Pipettes for securing the parasites were made of polyethylene tubing (Intramedic PE-20 or PE-50, Clay Adams, Parsippany, NJ) drawn out to a tip of either 100 μ m for females or 200 μ m for males. The first pipette, measuring 2 cm, was inflexible. The second pipette was made as flexible as possible by using a longer piece of tubing (10 cm) with two constrictions. One constriction was 2 cm from the tip and the second was 4.5 cm

Figure 4. Schematic representation of the suction pipette-balance arm system used to measure motor activity in S. mansoni.

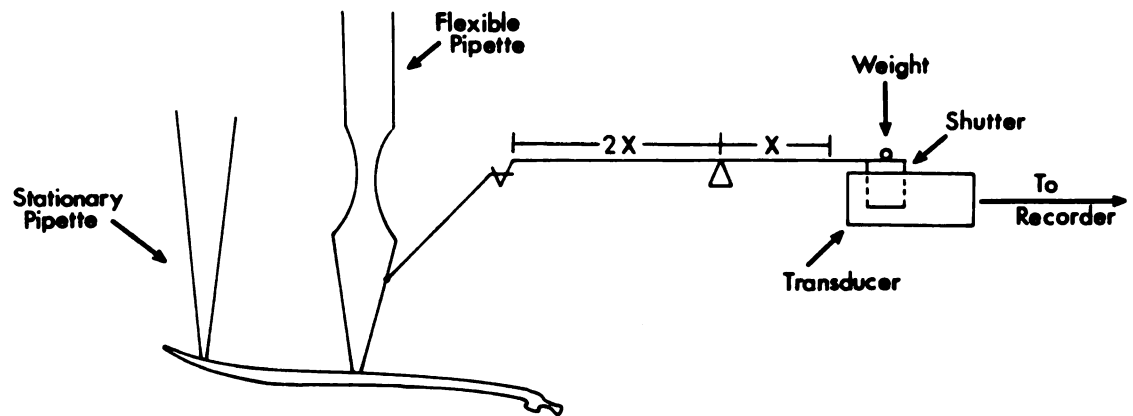


Figure 4

beyond that. For males the inflexible pipette measured 2 cm while the flexible pipette was 3 cm long with a single constriction 1.5 cm from the tip.

Flexible electrodes were attached by means of a 0.25 mm diameter 5 cm long stainless steel wire to a 12 cm balance arm. The balance arm, also of 0.25 mm wire, had a fulcrum set 7.5 cm from the suction pipette. When recording from males a 4 mg weight was added to the black acetate. This weight was not added when recording from females as it appeared to apply excessive load on the female musculature. Any shortening of the parasite caused movement of the balance arm and acetate strip. The acetate strip acted as a shutter for the photocell in a modified Narco Bio-systems (Houston, TX) A-3527 myograph. The output of the transducer was connected to a transducer coupler type 7173 then to a channel amplifier type 7000 (both Narco Bio-systems) and was recorded on a physiograph (Narco Bio-systems).

For electrical stimulation a silver wire inserted into the HBS filled stationary electrode was connected to a Grass S48 Stimulator via a stimulus isolation unit (Grass Instrument Co.). A silver wire in the recording chamber served as ground. The stimulus was monitored by recording the signal from the stimulus isolation unit. This signal was sent to a hi-gain coupler type 7171 and a channel amplifier type 7070 (both Narco Bio-systems) before being displayed on the physiograph. Stimuli consisted of 0.5 msec positive pulses given in 5 sec trains and repeated every 2 minutes.

Recording Procedures

2.5 ml of media were added to the recording chamber. The flexible electrode was filled with HBS and a 4 mg weight was placed on the balance arm. The consequent deflection of the physiography pen made it possible to express tension exerted on the pipette in terms of force needed to displace the acetate shutter. After calibrating, a single unpaired schistosome was added to the chamber. The stationary electrode was placed as close as possible to the tail without allowing the parasite to be drawn up the pipette. The flexible electrode was placed anterior to this so that when stretch was applied to the parasite the distance between the electrode tips ranged from 1.25 mm-2.00 mm. The initial attachment often stimulated the animal so a ten minute equilibration period was allowed before any experimental treatment was applied.

Since force of tension change is relative to the length of parasite over which the change occurs, data are given in mg force per mm of parasite. Tension changes are expressed relative to a control value. This value is the average level of tension recorded for four minutes before application of experimental treatment.

Surface Electrical Recordings

A suction electrode system, previously described by Semeyn (1981) and Semeyn et al. (1982) was used to record surface electrical activity from the anterior, midbody and caudal regions of male and female S. mansoni. The electrode consisted of a 1.8 cm piece of Intramedic PE-50 polyethylene tubing drawn out to a tip of 80 μ m (inner diameter)

and mounted on a 1 cc tuberculin syringe with a 23 gauge needle. During recordings, bathing media, drawn into the syringe was in contact with a silver wire in the syringe. A silver wire in the recording chamber acted as a ground. The signal was sent to a preamplifier with filters set at 0.3 Hz and 0.3 kHz (Model P-15, Grass Instrument Co., Quincy, MA) and displayed on a physiograph (Narco Biosystems, Inc., Houston, TX) and an oscilloscope (Model 5113, Tektronix, Inc., Beaverton, OR). The output was recorded on magnetic tape (Model B, Vetter Instrument Co., Rebersburg, PA) for later analysis.

Male and female parasites were secured to electrodes using a minimal amount of suction. The electrode was placed in the anterior sixth of the animal (posterior to the ventral sucker) for recording from the anterior region. It was placed midway along the length of the animal for midbody recordings and in the posterior sixth of the animal for caudal recordings. Attachment of the electrode stimulated the schistosome so 10 minutes of equilibration time was allowed. Activity was then recorded on the physiograph for 20 minutes. Electrical activity during the eleventh and twentieth minutes were recorded for computer analysis.

These data were analyzed on a Hewlett Packard computer. Tapes were replayed and amplitude histograms of negative potentials over ten second intervals were developed. Potentials less than 10 μ V were not counted since they were indistinguishable from the background noise of the system.

Microelectrode Recordings

Paired schistosomes were immobilized by 10^{-4} M carbachol in HBS. Females were lifted from the gynecophoral canal of the male and the males were discarded. Stainless steel wire (.005 in. diameter) wickets were used to secure the female to the Sylgard (Dow-Corning) lined recording chamber. The chamber was then rinsed and recordings were made every minute for one half hour in HBS.

Microelectrodes were pulled from 1.5 mm capillary tubing (Omega Dot, Frederick Haer) with a horizontal electrode puller (Narishige Instruments). These electrodes were filled with 3 M KCl and had a resistance of 15-20 megohms. A Leitz micromanipulator was used to move the electrodes. A silver wire in the electrode was connected to a preamplifier (M-4A, W.P. Instruments) with a lead wire. Signals from the preamplifier were displayed first on an oscilloscope (Tektronix 5118) and then on a chart recorder (Gilson Medical Electronics). A silver wire was placed in the recording chamber as a ground.

Elemental Analysis

S. mansoni pairs were incubated at room temperature in RPMI/1640 (Grand Island Biological) with 10% horse serum and 0.5% pentobarbital and separated. Females were incubated at 37C in 0 Ca^{++} 5×10^{-4} M EGTA HBS for 1 hour then collected over a glass fiber filter (Whatman GF/B) and washed four times with ice-cold 150 mM choline chloride. Three samples of 160 females were weighed and each was homogenized in 2% HCl. Homogenates were incubated in boiling water for 10 minutes then centrifuged at 12,000 x g for 20 minutes using a Beckman model J-21

centrifuge. The supernatant was decanted and used for determination of Ca levels. A multichannel, direct current plasma spectrometer (Spectrospan III A, Spectrometric Inc., Andover, MA). Values were standardized using the 2% HCl as blank and a multi-element standard solution containing reagent grade KCl, NaCl, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and CuSO_4 (Mallinckrodt, Inc.) and CaCO_3 (Aldrich Chemical Co., Inc.) prepared using 2% HCl as a solvent. To minimize contamination, all glassware was rinsed with double (glass) distilled water and then with 2% HCl.

Statistical Procedures

Unless otherwise noted data are expressed as means plus or minus one standard error of the mean. Statistical significance of difference was determined using Student's t-test.

RESULTS

Normal Activity

Mechanical Activity. Muscle tension changes in male and female S. mansoni were recorded using a suction pipette-balance arm system as described by Fetterer et al. (1977, 1978). Originally female movement was monitored on a system identical to that used on males except that smaller electrode tips were used. When recording from males an external weight of 4 mg is normally added but when this external weight was used when recording from females, very little spontaneous activity was recorded. When the load of the system was lessened by eliminating the 4 mg weight and making the flexible electrode more flexible, larger active contractions were recorded from the female schistosome. This lightened system was used for all studies on female mechanical activity while the system with the 4 mg weight added was used to monitor male activity.

Recordings showed both male and female able to actively contract and to maintain muscle tension under control conditions. Although magnitude and frequency of active contractions varied, those recorded from males were generally greater in frequency and in magnitude than those recorded from females (Figure 5). Because of the size difference between males and females and the impossibility of applying equivalent loads to each, one cannot say with certainty that this difference in

Figure 5. Chart recording of motor activity in S. mansoni. Upper trace, male. Lower trace, female.

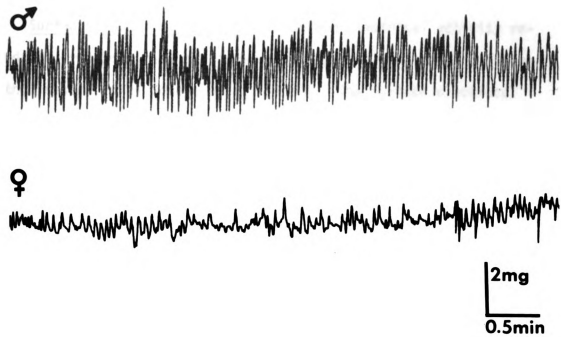


Figure 5

spontaneous activity is due to differences in the sexes alone. Though no external weight was added to the female system and the flexible electrode was made as responsive as possible, the elasticity of the plastic electrodes may still have been enough to dampen, to some extent, the contractions of the female.

Surface Electrical Activity. Surface electrical activity recordable from both male and female schistosomes was analyzed by the method described by Semeyn et al. (1982). The surface electrical activity of female S. mansoni was similar to that of the male in that potentials were bi- and triphasic, occurred in no particular pattern and those less than 40 μV in amplitude were most frequent. For the anterior, midbody and posterior regions, the frequency of potentials between 10 μV and 40 μV was significantly greater in the female than in the male while frequencies of potentials over 40 μV were relatively infrequent in both sexes (Table 4, Figure 6). The maximum potential size, in both the anterior and midbody regions, were greater in males than in females. Potentials in the posterior regions of both sexes occurred within the same range of sizes. The activity I recorded from males was consistently smaller than that collected by Semeyn et al. (1982). Semeyn used an electrode with a tip diameter of 100 μm while in my experiments a smaller tip diameter of 80 μm was used to hold the more slender female securely. This difference in size of suction electrode tips may account for the differences between my data and those reported by Semeyn et al.

Table 4
Regional Variation in Surface Electrical Activity of Adult Male
and Female S. mansoni

	Anterior			Midbody			Posterior		
	10 $\mu V < \text{Amp} \leq 40$ μV	Amp > 40 μV		10 $\mu V < \text{Amp} \leq 40$ μV	Amp > 40 μV		10 $\mu V < \text{Amp} \leq 40$ μV	Amp > 40 μV	
female	329 $\pm$ 38 ^a	1 $\pm$ 1		338 $\pm$ 66 ^a	7 $\pm$ 3		510 $\pm$ 39 ^b	22 $\pm$ 11	
male	177 $\pm$ 50	3 $\pm$ 2		198 $\pm$ 37	4 $\pm$ 3		207 $\pm$ 60	22 $\pm$ 13	44
male (Semeyn, 1982)	424 $\pm$ 23	4 $\pm$ 2		648 $\pm$ 23	98 $\pm$ 15		408 $\pm$ 51	274 $\pm$ 51	

^a .10 $<p\leq$.20

^b $p\leq$.01

Values represent the frequency of potentials in counts/10 sec and are expressed as the mean $\pm$ S.E.M.
for a minimum of six animals.

Figure 6. Amplitude histograms of surface electrical activity recorded from male and female *S. manosni*. Abscissa represents amplitude of potentials in μV . Ordinate represents frequency of potentials in counts/sec. Upper histogram: male. Lower histogram: female.

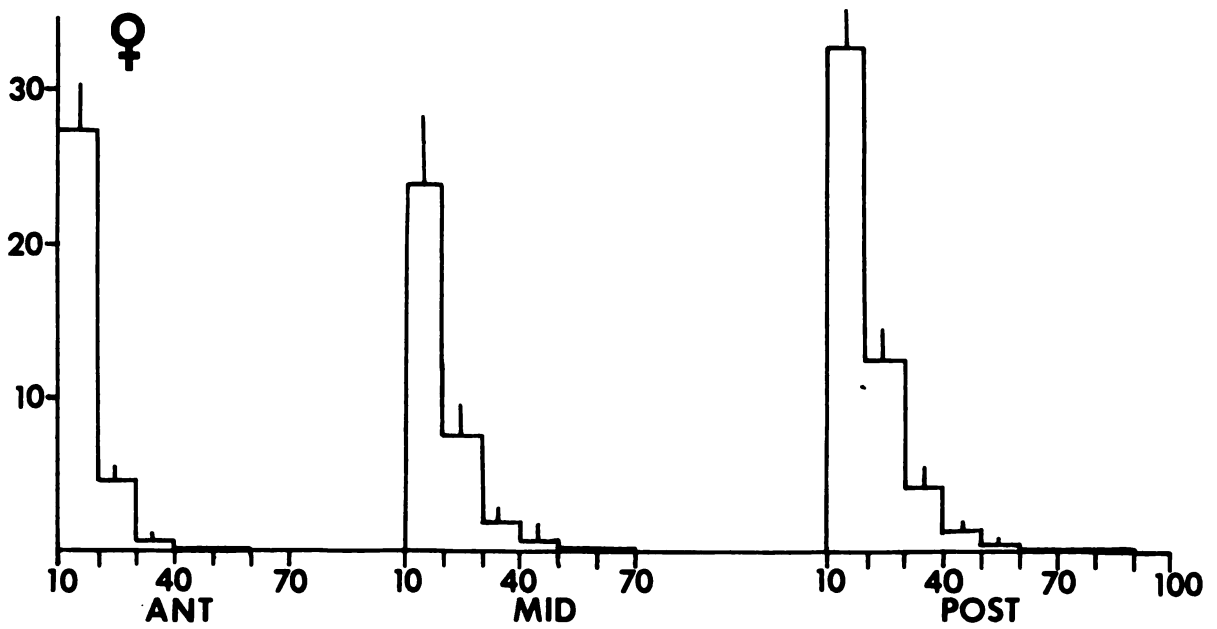
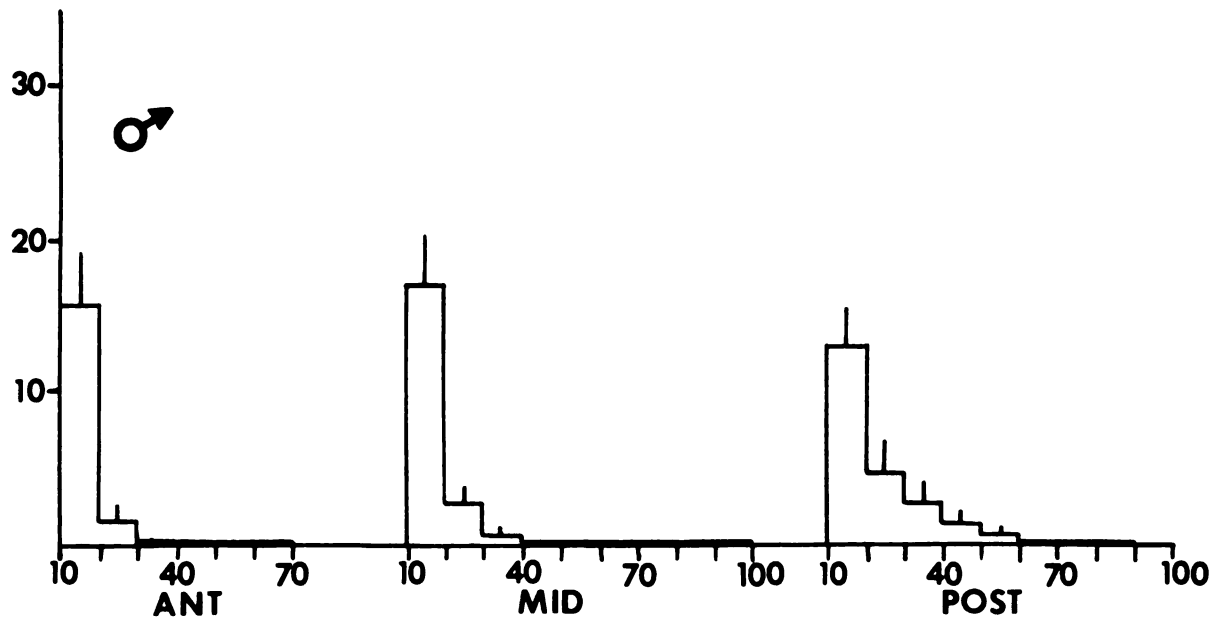


Figure 6

Microelectrode Recordings. Three potentials, all negative to the bath and stable over time, were recorded upon penetration of the female parasite with a microelectrode (Figure 7). Initial penetration of the female surface resulted in a potential ranging from -40 mV to -65 mV with a mean of -50 ± 3 mV. Upon further penetration a second potential averaging -28 ± 2 mV and ranging from -12 to -40 mV was recorded. Advancement of the microelectrode even further resulted in a third potential, ranging from 0 to -15 mV with a mean value of 7 ± 1 mV.

These three potentials, in order of appearance as well as their relative magnitudes, are nearly identical to those reported by Fetterer et al. (1980a) and Bricker et al. (1982) for male S. mansoni arising from tegument, muscle and extracellular spaces (Table 5). Since the structure of the two sexes, as described by Silk and Spence (1969a,b) and Silk et al. (1969c), is essentially the same, it seems likely that these three potentials have the same origin as the three potentials recorded from male S. mansoni by Fetterer et al. (1980a) and Bricker et al. (1982).

Responses to Neurotransmitters

Carbachol eliminates active contractions and reduces muscle tension in male S. mansoni (Fetterer et al., 1977; Barker et al., 1966). A lengthening response and reduction in contractile activity occurs after exposure to high concentrations of dopamine (Tomosky et al., 1974; Pax et al., 1978) while 5-HT increases contractile activity (Barker et al., 1966; Tomosky et al., 1974; Fetterer et al., 1977).

Figure 7. Potential profile obtained as a microelectrode is advanced into an adult female *S. mansoni*. If these potentials are equivalent to those recorded from males, the initial vertical drop indicates penetration of the tegument. The first upward deflection represents E_{musc} . The second upward deflection represents E_3 . Calibration: Vertical, 10 mV; Horizontal, 2 sec.

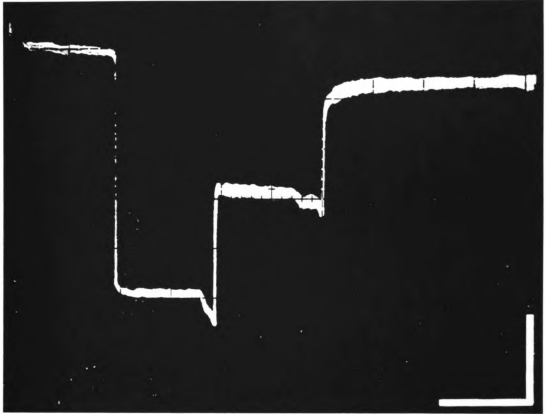


Figure 7

Table 5
 Membrane Potentials from Tegument and
 Subtegumental Compartments in Male
 and Female S. mansoni in mV

	Female	Male
E_{teg}	-50 ± 3	-51 ± 1 (dorsal) -35 ± 7 (ventral)
E_{musc}	-28 ± 2	-28 ± 1
E_3	-7 ± 1	-10 ± 1

Values are means $\pm$ one S.E.M. Male data as reported by Fetterer et al. (1980a) and Bricker et al. (1982).

In a series of experiments the effects of these compounds on motor activity in female S. mansoni was compared to their effects on males. In all cases, the responses of female S. mansoni were essentially identical to those in the male parasite. Application of either 10^{-4} M dopamine or 10^{-4} M carbachol resulted in marked decrease in tension and contractile activity while 10^{-5} M 5-HT caused an increased rate of contraction (Table 6).

Responses to Electrical Stimulation

Electrical stimulation of female schistosomes resulted in contraction of the parasites' musculature (Figure 8). Stimuli were applied to the posterior of the worm and tension changes were measured just anterior to this. Five second trains of different current strengths were given at a frequency of 100 pulses/sec and a duration of 0.5 msec to determine the relationship between stimulus strength and tension change. The maximal tension change was elicited with a 200 μ A stimulation. The effect of stimulus frequency on tension change was tested using 5 sec trains of 30 μ A, 0.5 msec pulses. Tension increased with increasing frequency of stimulation and at 200 pulses/sec (the highest frequency tested) the response was still increasing. A similar relationship between stimulus frequency and strength and muscle tension is found in male S. mansoni (Pax et al., 1981).

Table 7 compares various aspects of the responses of male and female S. mansoni to 30 μ A, 0.5 msec, 100 pulses/sec stimulus trains. Latency and time to one-half maximum response are not significantly

Table 6
Effects of Neurotransmitters on Adult Male and
Female S. mansoni

	Maximum Responses (mg)		Contractions/min	
	10^{-4} M dopamine	10^{-4} M Carbachol	Control	10^{-5} M 5-HT
Male	-1.76 .44	-2.60 .71	9.67 2.00	25.20 3.61
Female	-1.84 .58	-2.42 .74	3.17 1.28	10.17 4.28

All values are means one S.E.M. (N=6). Male data previously collected in the laboratory of R.A. Pax.

Figure 8. Examples of the responses of male and female S. mansoni longitudinal muscle to electrical stimulation. Stimuli consisted of 30 μ A, 100/sec, 0.5 msec pulse trains delivered to the posterior region of the parasite. Upper trace, male. Lower trace, female.

Table 7
Comparison of Male and Female *S. mansoni* Responses
to 30 μ A, 100/sec, 0.5 msec Pulse Trains

	Female	Male
Latency (msec)	86 $\pm$ 12	102 $\pm$ 8
$t_{1/2}$ (msec)	559 $\pm$ 88	670 $\pm$ 9
Maximum response (mg)	3.67 $\pm$.51	2.13 $\pm$.17
Prolonged stimulation	transient	maintained

All values are means $\pm$ one S.E.M. (N=6). Male data from Pax et al., 1981.

different for male and female but total tension induced is significantly greater ($.01 < p < .02$) in the female. With prolonged stimulation of males the tension increase is maintained (Pax et al., 1981) but in females the tension begins to decrease after a few seconds and is at one half its maximum value after $6.43 \pm .84$ sec.

Responses to Tension Inducing Agents

Male and female parasites were exposed to a variety of agents (PZ, 60 mM K^+ , DNP, ouabain and 5C) which cause sustained contractures in male S. mansoni (Pax et al., 1978; Fetterer et al., 1980b, 1981). Tables 8 and 9 summarize the results. All agents tested had similar contracture inducing abilities in both sexes, but the maximum tension increases were generally smaller in the female parasite. Other significant differences between the sexes with respect to responses to these contracture inducing agents are noted in Tables 8 and 9.

Praziquantel. Though the maximum tension induced in males by PZ was larger than that in females, the dose response curves of male and female S. mansoni to PZ indicate sensitivity to PZ is nearly the same in the two sexes. In both, higher concentrations of PZ gave lesser responses (Figure 9). Treatment of the male parasite with $10^{-6}M$ PZ resulted in an immediate large contracture while in females there was often a transient relaxation before the contracture developed (Figure 10). This relaxation occurred only with the application of $10^{-6}M$ PZ. The time to one-half peak tension was significantly greater in the female than in the male.

Table 8
Responses of Male and Female S. mansoni to Tension Inducing Agents and to the Loss
and Reintroduction of Calcium to the Bathing Medium

Agent	Sex (m = male f = female)	Peak Tension (mg)	t _{1/2} Peak (sec)	Tension, Decrease in 0 Ca ⁺⁺ Medium (mg)	Tension Change With Ca ⁺⁺ Add'n (mg)	t _{1/2} Peak (sec)
PZ (10 ⁻⁶ M)	m	6.70± .36	12± 2	6.04±1.34	3.02±1.05 ^e	40±10
	f	3.42± .24 ^a	22± 4 ^a	2.90± .73	2.89± .47	10± 2 ^a
60 mM K ⁺	m	5.71± .69 ^b	6± 1 ^d	7.90± .80	6.21± .59 ^g	46± 6
	f	3.80± .24 ^b	69±46 ^d	2.70± .55	2.75± .53 ^f	8± 1 ^a
DNP (1 mM)	m	6.46±1.34 ^c	9± 1 ^a	7.73±1.11	4.84± .97	59±12 ^a
	f	3.68± .64 ^c	2± 1 ^a	3.33±1.03	3.49± .91	14± 4 ^a

All values are means ± one S.E.M. for a minimum of five animals.

a-d compares males and females.

e-g compares tension changes after treatment to tension
changes after the addition of Ca⁺⁺.

^ap<.001

^ep<.001

^b.01<p<.02

^f.02<p<.05

^c.02<p<.05

^g.10<p<.20

^d.10<p<.20

Table 9
Responses of Male and Female *S. mansoni* to Ouabain and Low
Temperature and to the Removal of Calcium from the Bathing Medium

Treatment	Sex (m = male f = female)	Tension at 15 min (mg)	Tension at 20 min in 0 Ca ⁺⁺ Medium (mg)	Tension Decrease in 0 Ca ⁺⁺ Medium (mg)
Ouabain (10 ⁻⁵ M)	m	1.66±1.04	1.21±.91	.45±1.54
	f	1.72±.57	.47±.70 ^a	1.66±.68
5C	m	5.96±1.03	5.05±1.33	.98±.62
	f	3.11±.71	1.19±.79 ^a	1.92±.54

All values are means ± one S.E.M. for a minimum of five animals.

^a Compares female tension at 15 min with tension at 20 minutes in 0 Ca⁺⁺ = .05<p<.10.

Figure 9. The effects of various concentrations of praziquantel on the tension produced by the musculature of male and female S. mansoni. Open circles, males; closed circles, females.

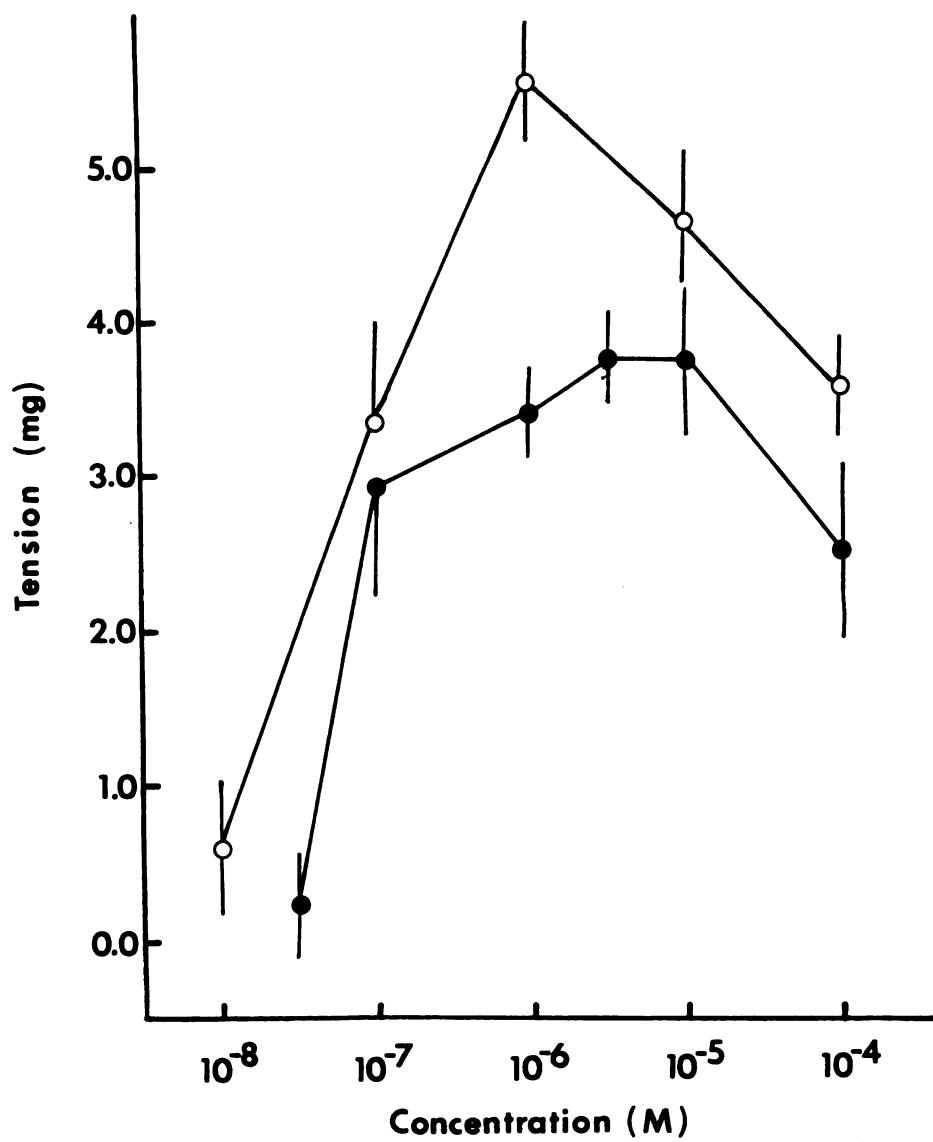


Figure 9

Figure 10. Chart recording of male and female *S. mansoni* muscle tension responses to 10^{-6} M PZ. At second arrow parasites were exposed to a 0 Ca^{++} medium. At third arrow 1.4 mM Ca^{++} was reintroduced to the medium. Upper trace, male; Lower trace, female.

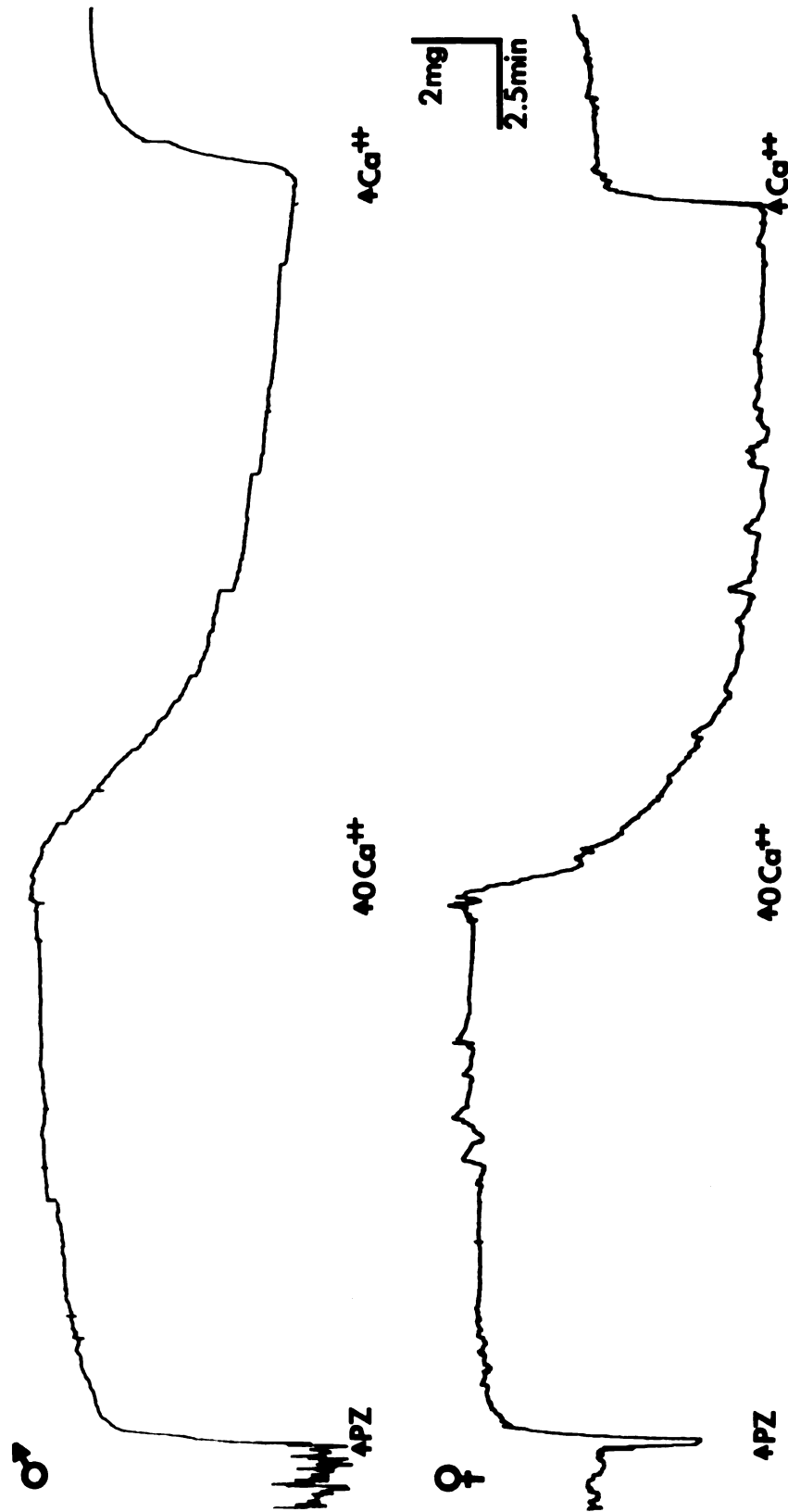


Figure 10

Figure 11. The effects of 10^{-6} M PZ on muscle tension of male and female S. mansoni. At second arrow parasites were exposed to a 0 Ca^{++} medium. Values are means with one standard error for a minimum of six animals. Open circles, males; closed circles, females.

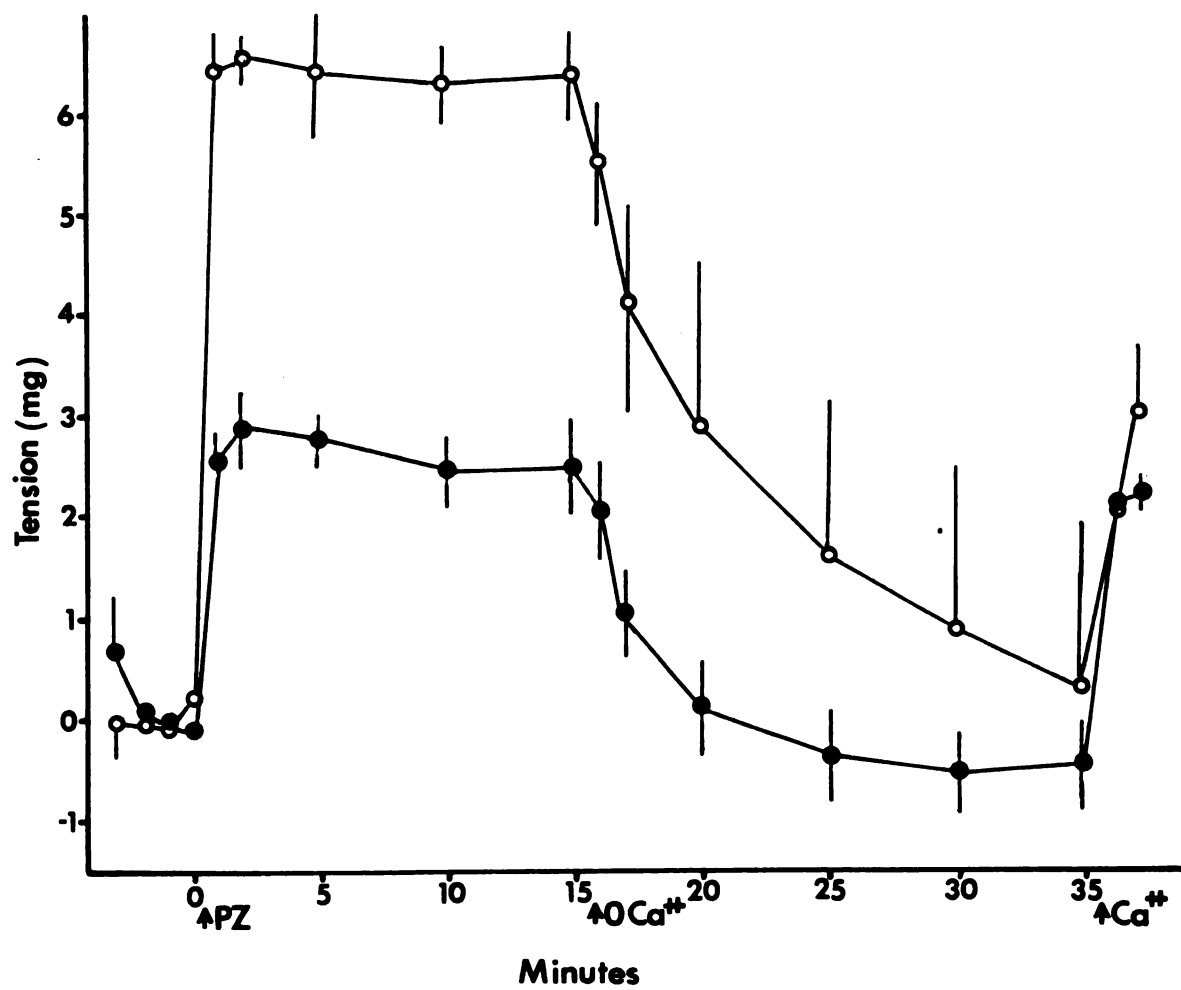


Figure 11

Elevated Potassium. Exposure of S. mansoni males and females to 60 mM K^+ HBS resulted in large biphasic contractures in both (Figure 12). The male responded with an initial phasic contraction, reaching a maximum tension change of $5.65 \pm .68$ mg in 13 ± 2 sec followed by a tonic contraction whose peak of $5.86 \pm .73$ mg was reached in 71 ± 27 sec. The female also contracted in response to the high K^+ but differed from the male in that the initial phasic contraction, reaching peak of $2.37 \pm .70$ mg in 10 ± 1 sec, was more pronounced and was followed by a slower tonic contraction which did not reach a maximum even after 15 minutes. The average maximum tension increase due to elevated potassium was only about 60% that of the males, but the time to one-half maximum was more than ten times greater.

2,4-Dinitrophenol. Large contractures were experienced by both male and female schistosomes exposed to 1 mM DNP (Figure 13). The maximum tension increase was significantly greater in males than in females, as was the time needed to achieve a maximum response. In both sexes there was a gradual relaxation so that after 15 min the tension had decreased by 53% for the female parasite and 58% for the male.

Ouabain. The responses of male and female S. mansoni to $10^{-5}M$ ouabain were virtually identical in magnitude and average time course (Figure 14). In both sexes, the contractures were gradual and continued to increase throughout the 15 minute monitoring period.

5C. Lowering the bath temperature to 5C resulted in large tension increases of both the male and female musculature (Figure 15).

Figure 12. The effect of 60 mM K^+ on the muscle tension of male and female *S. mansoni*. At the second arrow parasites were exposed to a 0 Ca^{++} medium. At the third arrow 1.4 mM Ca^{++} was reintroduced to the medium. Values are means with one standard error for a minimum of 6 animals. Open circles, males; closed circles, females.

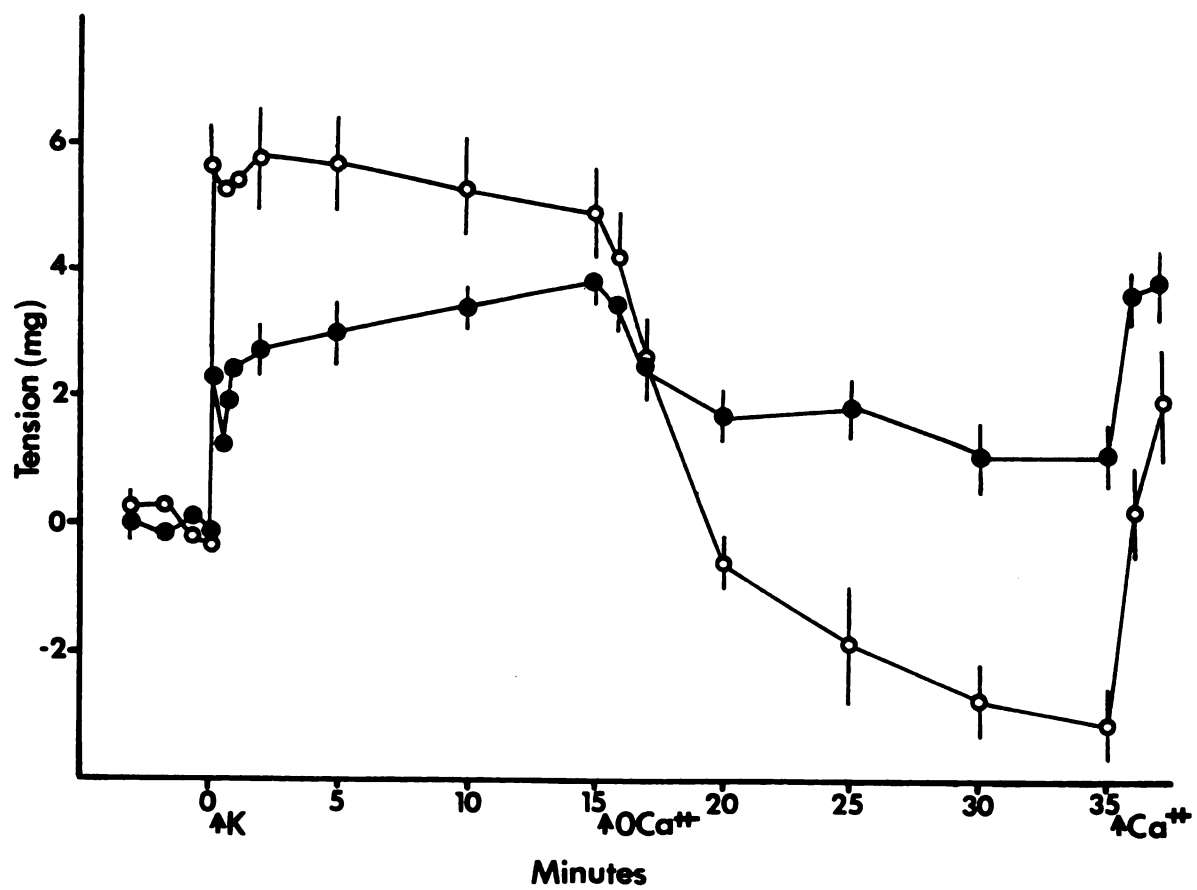


Figure 12

Figure 13. The effects of 1 mM DNP on the muscle tension of male and female *S. mansoni*. At second arrow parasites were exposed to a 0 Ca^{++} medium. At third arrow 1.4 mM Ca^{++} was reintroduced to the medium. Values are means with one standard error for a minimum of six animals. Open circle, males; closed circles, females.

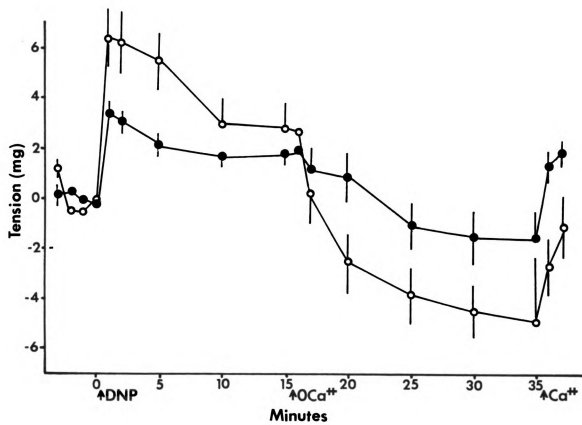


Figure 13

Figure 14. The effect of 10^{-5} M ouabain on the muscle tension of male and female *S. mansoni*. At second arrow parasites were exposed to a 0 Ca^{++} medium. At third arrow 1.4 mM Ca^{++} was added back to the medium. Values are means with one standard error for a minimum of six animals. Open circles, males; closed circles, females.

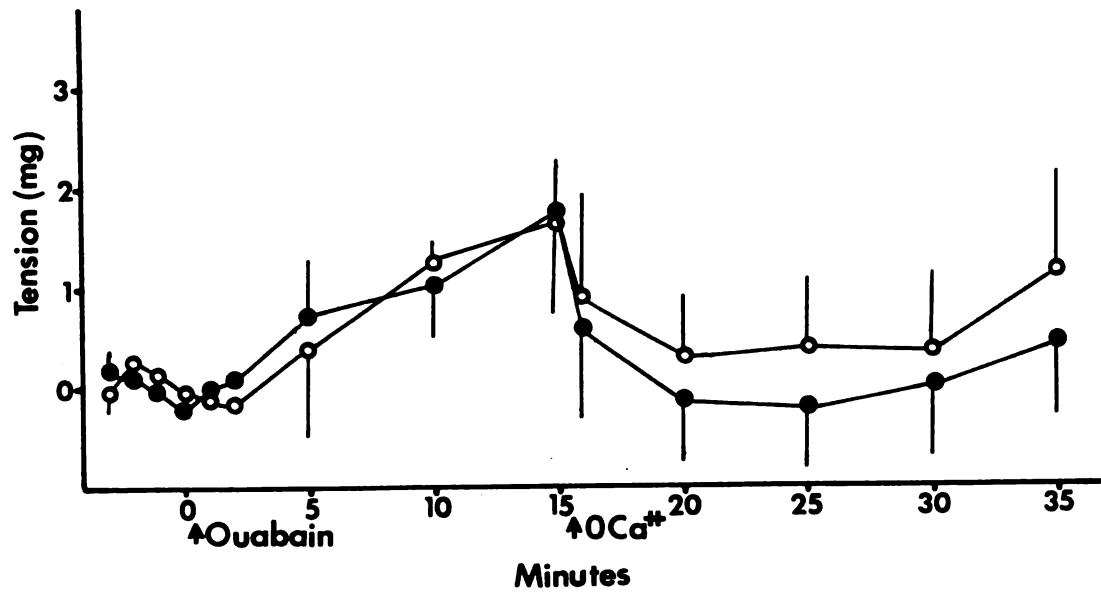


Figure 14

Figure 15. The effect of lowering the bath temperature to 5C on the muscle tension of male and female *S. mansoni*. At second arrow parasites were exposed to a 0 Ca⁺⁺ medium. Values are means with one standard error for a minimum of five animals. Open circles, males; closed circles, females.

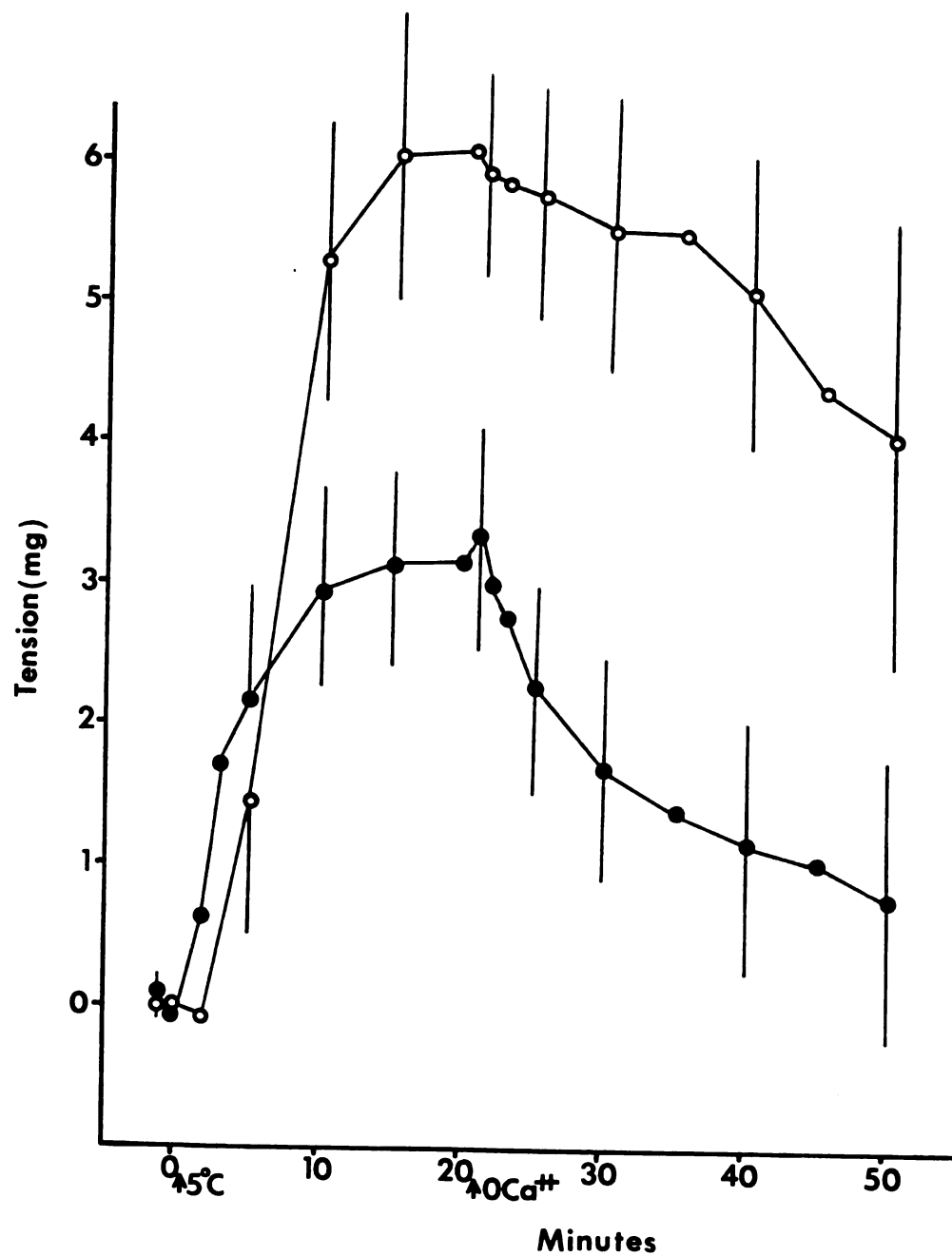


Figure 15

The cold induced contracture was significantly greater in the male parasite than in the female but the timing of the contracture was somewhat quicker in the female.

Responses to Ionic Alterations

Calcium. To assess the role of calcium in schistosome muscle contraction, male and female parasites were exposed to 0 Ca^{++} $5 \times 10^{-4}\text{M}$ EGTA HBS during treatment with contracture inducing agents. Tables 8 and 9 detail the responses of both sexes to this manipulation. Though still in the presence of tension inducing agents, the parasites, with the exception of males exposed to ouabain or low temperature, experienced significant relaxations within 20 minutes. Females exposed to these same two treatments did relax but this relaxation of the female musculature was considerably less than that occurring after the removal of calcium from PZ, 60 mM K^+ or DNP treated females.

In these same experiments, calcium was reintroduced into the recording medium 20 minutes after the removal of Ca^{++} from PZ, 60 mM K^+ and DNP treated parasites. Reintroduction of calcium in all cases resulted in relatively rapid contractions of the musculature in both males and females but in every instance, the female schistosome responded more quickly, the $t_{1/2}$ being four to six times shorter than for the males (Table 8). The responses of both male and female high K^+ and DNP treated parasites to the reintroduction of Ca^{++} were similar in magnitude to the contractures induced by these treatments under normal calcium conditions. The magnitude of the response to Ca^{++} reintroduction in PZ treated females was similar to that induced by PZ

in normal calcium, while the response of the males to the reintroduction of Ca^{++} was significantly less than in normal calcium.

Effects of calcium removal were also tested by exposing male and female *S. mansoni* to a Ca^{++} free medium for various lengths of time prior to applying tension inducing agents (Table 10). Parasites were preincubated for 1-2 hours in 0 Ca^{++} 5×10^{-4} M EGTA HBS before treatment with PZ, 60 mM K^+ and DNP while those exposed to ouabain and low temperature were preincubated for 20 and 30 minutes, respectively.

The responses of both sexes to the calcium free medium were highly variable. Some parasites experienced a decrease in muscle tension while others experienced an increase. Active contractions increased in size and frequency in some animals while in others they were attenuated or eliminated. Despite this variability, the responses of the two sexes were not significantly different.

Treatment of female schistosomes with 10^{-6} M PZ after preincubation in 0 Ca^{++} HBS gave a peak tension increase not significantly different from that in normal Ca^{++} but the decline in tension following the peak was far greater than that seen in the control animals (Figure 16, Compare with Figure 11). At 10 minutes the tension had decreased by 98% in the 0 Ca^{++} treated females but only by 21% in the controls. In contrast, preincubation of the males resulted in a PZ response significantly lower than that seen in normal calcium medium but the response was maintained (Figure 16, Compare to Figure 11).

Table 10

Maximum Responses of Male and Female S. mansoni to
Tension Inducing Agents Under Various Conditions

Agent	Treatment	Male	Female
PZ (10^{-6} M)	Control	6.70± .36	3.44±.42
	1-2 hr in 0 Ca ⁺⁺ HBS	2.00± .94 ^a	2.98±.63
60 mM K ⁺	Control	5.71± .69	3.80±.24
	1-2 hr in 0 Ca ⁺⁺ HBS	.16± .17 ^a	.79±.56 ^a
DNP (1 mM)	Control	6.46±1.34	3.68±.64
	1-2 hr in 0 Ca ⁺⁺ HBS	5.60±1.11	2.06±.69 ^b
Ouabain (10^{-5} M)	Control	2.58± .93	2.10±.54
	20 min in 0 Ca ⁺⁺ HBS	1.94± .26	1.13±.31 ^b
5C	Control	6.44± .96	3.52±.48
	30 min in 0 Ca ⁺⁺ HBS	4.86±1.35	.28±.22 ^a

All values are means ± one S.E.M. for a minimum of six animals.

^ap<.001

^b.02<p<.05

Statistics compare control peaks to peaks in 0 Ca⁺⁺.

Figure 16. The effect of 1-2 hours preincubation in a 0 Ca^{++} medium on the responses of male and female S. mansoni to 10^{-6}M PZ. At the second arrow 1.4 mM Ca^{++} was added back to the medium. Compare to Figure 11. Values are means with one standard error for a minimum of six animals. Open circles, males; closed circles, females.

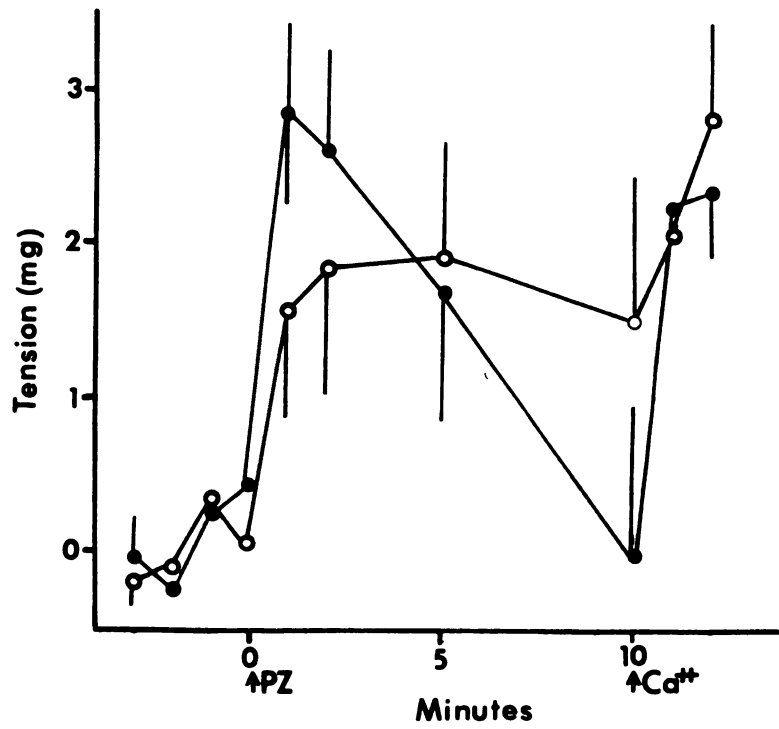


Figure 16

Both male and female schistosomes showed attenuated responses to high potassium after preincubation in 0 Ca^{++} . The response to elevated potassium was virtually blocked in both sexes (Figure 17, Compare with Figure 12).

Male and female parasites responded differentially to DNP after incubating in 0 Ca^{++} medium (Figure 18, Compare with Figure 13). Males reached a peak tension not significantly different from that achieved in normal Ca^{++} while females contracted significantly less than controls.

Preincubation in 0 Ca^{++} prior to treatment with ouabain resulted in similar increases in muscle tension for both sexes (Figure 19, Compare with Figure 14) but the maximum tension increase achieved by females in 0 Ca^{++} was significantly less than that in the presence of Ca^{++} . The maximum response in the males was similar with and without calcium in the medium.

Male and female S. mansoni were preincubated in 0 Ca^{++} medium 30 minutes prior to lowering the bath temperature to 5°C . Though the male response to cold temperature in the absence of Ca^{++} was not significantly different in magnitude from that in the presence of Ca^{++} , it was significantly slower. The time to one-half the peak response was 396 ± 80 sec under control conditions but it was 996 ± 55 sec after incubation in 0 Ca^{++} medium ($p < .001$). In contrast, the female response was virtually blocked by the preincubation in 0 Ca^{++} (Figure 20, Compare with Figure 15).

Figure 17. The effect of 1-2 hours preincubation in a 0 Ca^{++} medium on the responses of male and female S. mansoni to 60 mM K^+ . At the second arrow 1.4 mM Ca^{++} is added back to the medium. Compare to Figure 12. Values are means with one standard error for a minimum of six animals. Open circles, males; closed circles, females.

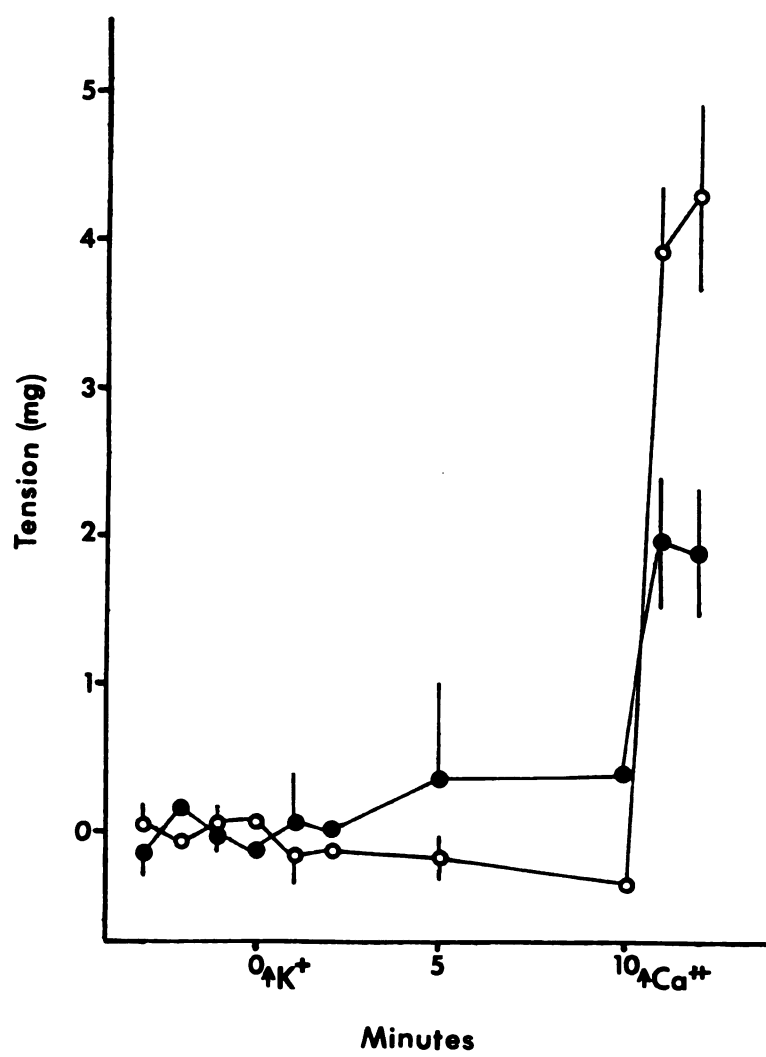


Figure 17

Figure 18. The effect of 1-2 hours preincubation in a 0 Ca^{++} medium on the responses of male and female *S. mansoni* to 1 mM DNP. At the second arrow 1.4 mM Ca^{++} is added back to the medium. Compare to Figure 13. Values are means with one standard error for a minimum of six animals. Open circles, males; closed circles, females.

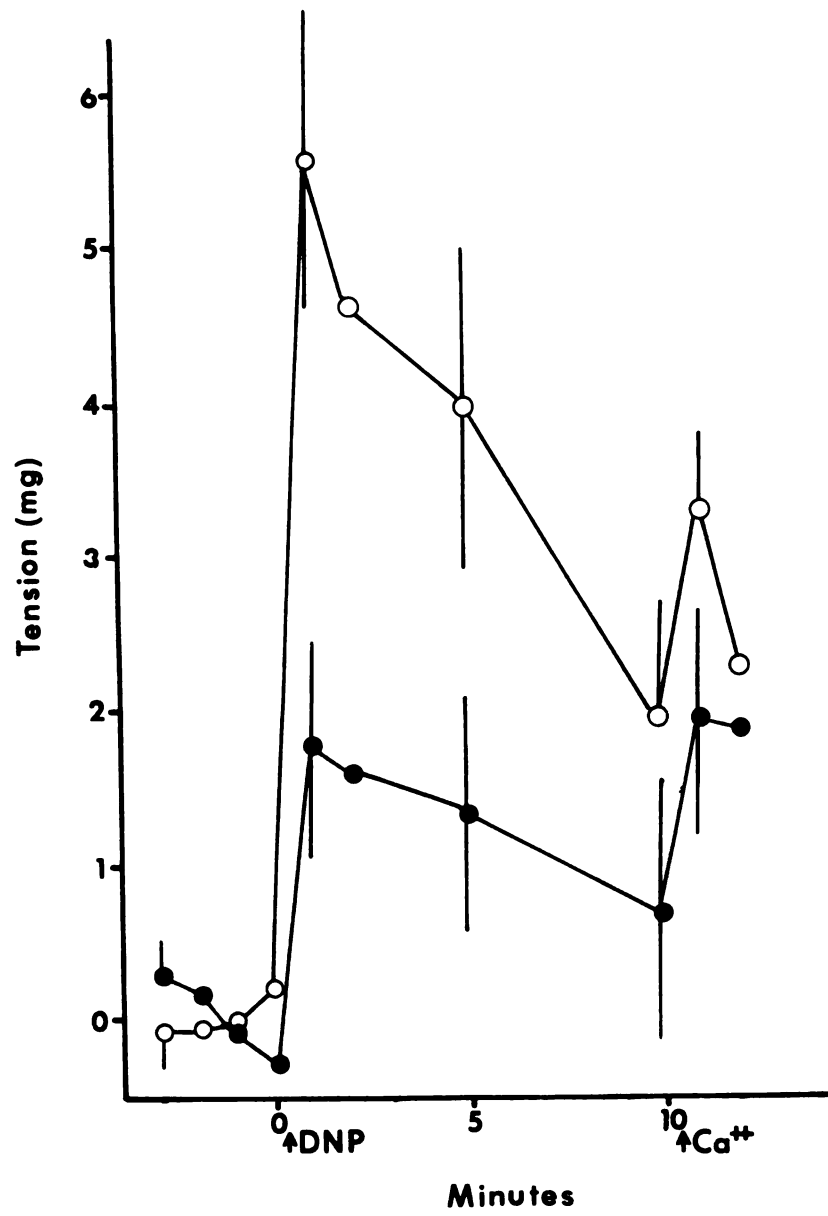


Figure 18

Figure 19. The effect of 20 minutes preincubation in a 0 Ca^{++} medium on the responses of male and female S. mansoni to 10^{-5}M ouabain. At the second arrow 1.4 mM Ca^{++} was added back to the medium. Compare with Figure 14. Values are means with one standard error for a minimum of six animals. Open circles, males; closed circles, females.

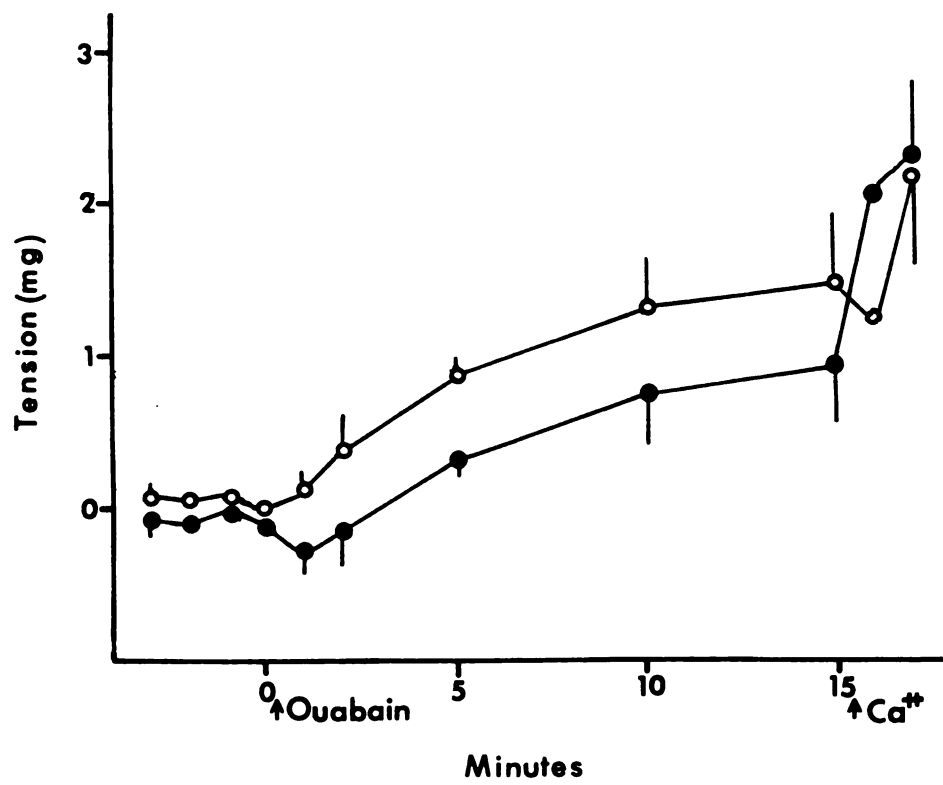


Figure 19

Figure 20. The effect of 30 minutes preincubation in a 0 Ca^{++} medium on the responses of male and female *S. mansoni* to a bath temperature of 5C. At the second arrow 1.4 mM Ca^{++} was added back to the medium. Compare to Figure 15. Values are means with one standard error for a minimum of five animals. Open circles, males; closed circles, females.

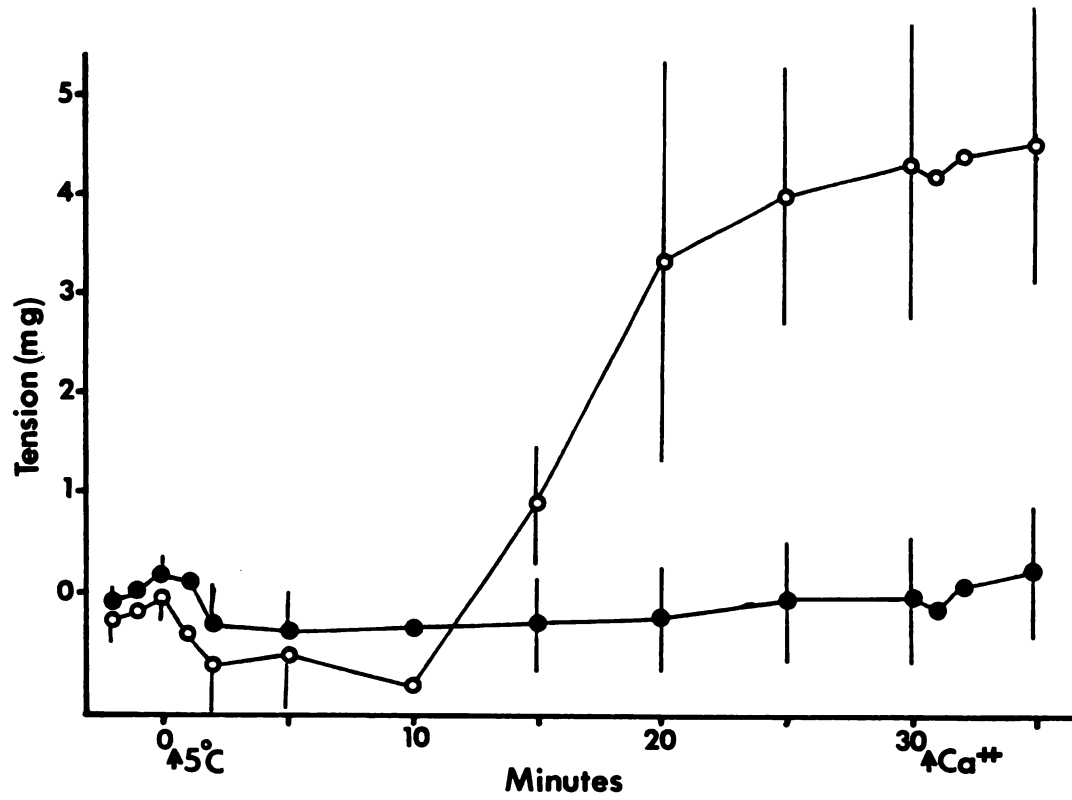


Figure 20

Elemental Analysis. Elemental analysis of the female schistosome after a one hour incubation in a 0 Ca^{++} medium showed no significant loss of calcium. Calcium content in control females was $5.35 \pm .81 \text{ mmol/kg}$ ($n=3$) while the calcium content of 0 Ca^{++} treated females was $5.16 \pm .12 \text{ mmol/kg}$ ($n=3$). In contrast, the level of calcium in males after the same incubation had decreased significantly (Wolde Mussie et al., 1982).

Magnesium. Since Mg^{++} is known to inhibit Ca^{++} dependent contractile mechanisms, the effects of contraction inducing agents were examined in the presence of Mg^{++} . Data from male S. mansoni suggest that PZ allows both Ca^{++} and Mg^{++} to enter the muscle of the parasite, while 60 mM K^+ allows only the entry of Ca^{++} (Fetterer et al., 1980b; Wolde Mussie et al., 1982). In these experiments, male and female S. mansoni were incubated in a high Mg^{++} solution for 15 minutes prior to treatment with 60 mM K^+ or 10^{-6} M PZ .

Application of high potassium to parasites in an elevated Mg^{++} solution resulted in a marked contraction in males not unlike that elicited in normal HBS (Table 11, Figures 21 and 22). In contrast, the female response to 60 mM K^+ was smaller than that seen under control conditions. Both male and female schistosomes experienced a decrease in tension with the addition of 10^{-6} M PZ to the bath.

Male and female S. mansoni experienced transient contractions when exposed to PZ in the presence of elevated Mg^{++} (Table 11, Figure 23). Though the peak tensions of these contractions were not significantly different from those in normal HBS, both contractures were more transient than under normal conditions. The one-half time of the relaxation

Table 11

The Effects of a High Magnesium Solution on the Responses
of Male and Female *S. mansoni* to Praziquantel and
Elevated Potassium

	PZ		60 mM K ⁺	
	Male	Female	Male	Female
Peak Tension in Normal HBS (mg)	6.70±.36	3.44±.42	5.71± .69	3.80±.24
Peak Tension in 20 mM Mg ⁺⁺ (mg)	6.06±.80	2.64±.49	6.43±1.24	1.60±.20 ^b
t _{1/2} Decline in 20 mM Mg ⁺⁺ (sec)	341±34	90±9 ^a	---	---

Data are expressed as means ± one S.E.M. for a minimum of six animals.

^aCompares male and female: p<.001.

^bCompares female in normal HBS with female in 20 mM Mg⁺⁺ HBS: p<.001.

Figure 21. Chart recordings of tension responses of male and female *S. mansoni* to 60 mM K^+ . (A) Response of male to 60 mM K^+ in normal HBS. At the second arrow $10^{-6}M$ PZ was added to the bath. (B) Response of male to 60 mM K^+ in 20 mM Mg^{++} HBS. At the second arrow $10^{-6}M$ PZ was added to the bath. (C) Response of female to 60 mM K^+ in normal HBS. At the second arrow $10^{-6}M$ PZ was added to the bath. (D) Response of female to 60 mM K^+ in 20 mM Mg^{++} HBS. At the second arrow $10^{-6}M$ PZ was added to the bath.

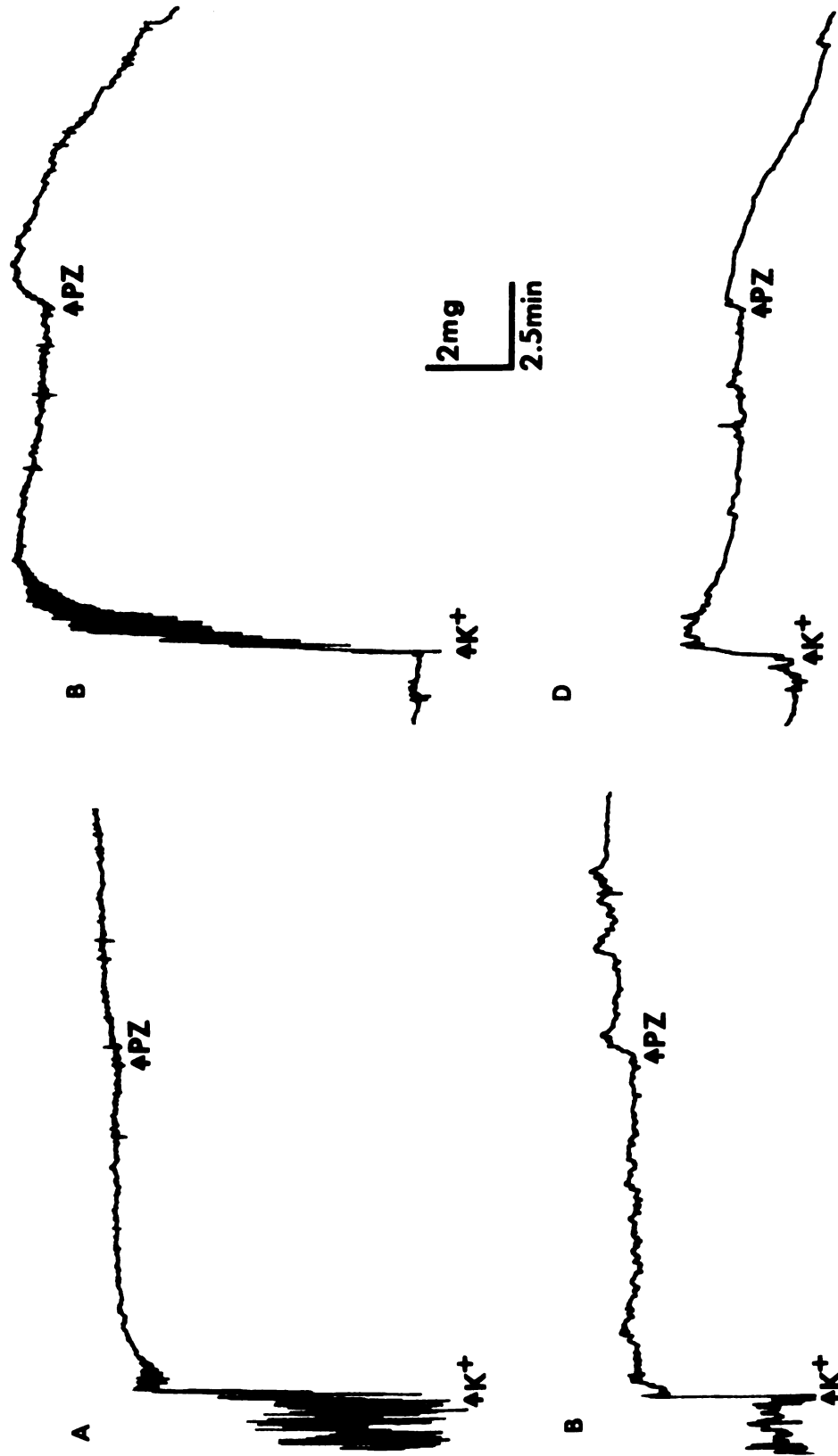


Figure 21

Figure 22. The responses of male and female *S. mansoni* to 60 mM K^+ in 20 mM Mg^{++} HBS. At the second arrow $10^{-6}M$ $\overline{PZ}$ was added to the bath. Compare to Figure 12. Values are means with one standard error for a minimum of six animals. Open circles, males; closed circles, females.

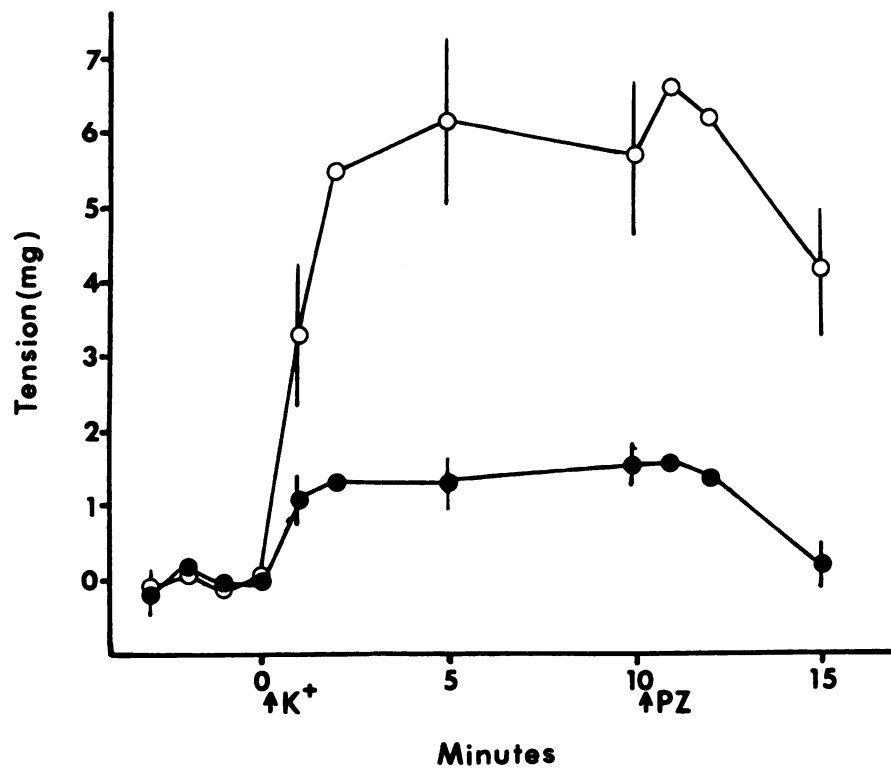


Figure 22

Figure 23. The responses of male and female *S. mansoni* to 10^{-6} M PZ in 20 mM Mg^{++} HBS. At the second arrow the parasites were exposed to 60 mM K^{+} . Compare to Figure 11. Values are means with one standard error for a minimum of six animals. Open circles, males; closed circles, females.

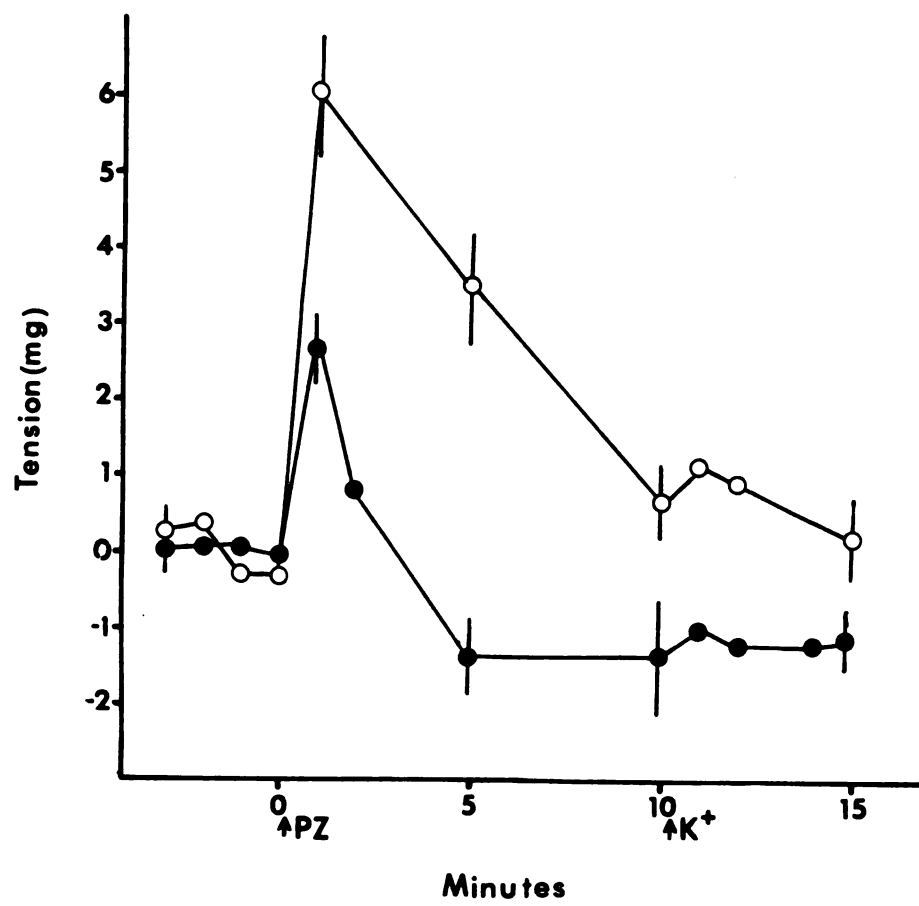


Figure 23

of the PZ response in high Mg^{++} was significantly greater in the male than in the female. There was no increase in tension with the addition of 60 mM K^+ to the bathing medium. The prior treatment with PZ and 20 mM Mg^{++} apparently blocked the expected increase.

DISCUSSION

Despite the differences in the roles and structures of male and female Schistosoma mansoni, the results of this study indicate that their physiology is, for the most part, similar. Recordings of their surface electrical activity show both male and female parasites emitting bi- and triphasic potentials with the lower amplitude potentials being more frequent. Males and females both experience spontaneous active contractions of varying frequency and amplitude. Similar latencies and halftimes are experienced by males and females in response to electrical stimulation. Both sexes are contracted by the same tension inducing agents and respond similarly to the putative neurotransmitters, 5-HT, dopamine and carbachol. The potential profile and the relative magnitudes of these potentials, as recorded from tegumental and subtegumental compartments of both sexes are essentially identical.

The differences which were seen can probably be accounted for on the basis of anatomy and my methods of recording. Surface electrical activity recorded from females has a higher frequency than that recorded from males. There may, in fact, be electrical activity occurring within the male that is not being recorded because of his large size. Potentials may have more distant origins than any originating within

the female and, in the process of being conducted to the recording site, may decrement to such an extent that they cannot be recorded. The smaller size of the female results in shorter pathways for the conductance of potentials from within the parasite than those present in the male. This allows a greater proportion of existing electrical activity to be recorded. Since the female schistosome is much smaller than the male and the same suction pipette tip was used to record surface electrical activity, the higher frequency seen in the females may also be due to the relatively larger area over which the activity was recorded.

The active contractions of the female were generally smaller and less frequent than those of the male. This may be a direct result of the lesser developed musculature of the female or it may reflect a load problem in the monitoring system. Though the suction pipette-balance arm system used to record female activity was lightened, it was impossible to insure that the loads of the systems were equal on both sexes. The load may still have been large enough to dampen the contractions of the female.

Though both male and female S. mansoni contract in response to electrical stimulation the degree of contracture differs in the two sexes. The largest contracture elicited by electrical stimulation of females is a maximal response, that is, the response is similar in magnitude to a PZ induced contracture and the female probably can contract no further. In contrast, the largest contracture experienced by males in response to electrical stimulation is significantly less than a PZ induced contracture. The less extensive musculature of the

female may allow for more efficient stimulation in that all fibers would be stimulated. The differential responses of the sexes to electrical stimulation may also be due, in part, to the size of pipette tip through which the stimulus was delivered. Though smaller pipette tips were used to secure the female during electrical stimulation, the stimulus may have been delivered over a proportionately larger area of the female than in the male. It is difficult, if not impossible to say whether anatomical differences or technical problems play a greater part in causing the differential responses seen in the two sexes.

The results of this study suggest that the female is more sensitive to ionic alterations than the male parasite. The female is seen to lose muscle tension more quickly than males when in a zero calcium medium and she is affected to a greater degree by increases in external magnesium. If these differences were due solely to anatomical differences in the sexes, that is, if they were due to the larger surface to volume ratio in the male, one would expect passive calcium efflux and passive magnesium influx under these experimental conditions to be greater in the male. Since the data reported here do not support this view, it is possible that permeability differences do exist in the two sexes. If the females were more permeable to magnesium and calcium, the decrease of the high K^+ response in 20 mM Mg^{++} HBS in the female and the more rapid loss of muscle tension in 0 Ca^{++} HBS in the female would be expected.

An alternate explanation would be that smaller stores of calcium are available to the female musculature than to the male. Elemental

analysis of female schistosomes showed no significant loss in the total calcium content after one hour in a zero calcium-EGTA solution. This suggests that only a small fraction of the calcium in the female is free. In contrast, Wolde Mussie et al. (1982) show a significant loss of calcium under the same conditions in male S. mansoni, indicating that a greater proportion of the total calcium in males is free or at least readily available. If the bulk of calcium in females is sequestered and released only under highly specified conditions, the musculature may be very dependent on the calcium external to the parasite as a means of replenishing internal stores. Calcareous corpuscles in the vitelline lobules of female S. mansoni may represent a strongly sequestered store of calcium (Erasmus and Davies, 1979). Though the presence of these corpuscles in the vitelline gland is unique to the genus Schistosoma, they have been found in the excretory organs of the trematode Cyathocotyle bushiensis (Erasmus, 1967) and the cestode Taenia taeniaeformis (Nieland and von Brand, 1969) as well as in other phyla (Dunkelberger and Watabe, 1974; Richardot and Wautier, 1972). Many functions have been ascribed to these corpuscles including the storage of calcium for use in metabolic activities and the regulation of intracellular calcium concentration (Simkiss, 1976). Perhaps, in female schistosomes, calcareous corpuscles do not contribute greatly to the regulation of intracellular calcium concentration, but do contribute significantly to calcium levels as measured by elemental analysis.

Though this study has shown male and female Schistosoma mansoni to be very similar physiologically, previously documented differences

in enzyme activities and drug sensitivities suggest that significant physiological differences do exist between the two sexes. The greater abundance of acetylcholinesterase in the female parasite, despite her less extensive musculature is indicative of this. The acetylcholinesterase activity associated with the female reproductive system (Fripp, 1967) is unlikely to account for the large differences in the two sexes. Differential effects of lipophilic drugs such as oxamni-quine (Foster and Cheetham, 1973a; Foster et al., 1973b) and amoscanate (Liu, 1980) may have their basis in biochemical or physiological differences. It is hoped that further study will define the male-female differences in greater detail. Since single sex infections have been shown to be less pathogenic (Warren, 1961) and to confer some immunity against further infections in rhesus monkeys (Smithers, 1962; Hsü, 1969), the interdependence of the two sexes may be an important factor in controlling these parasites.

SUMMARY

1. Measurements of surface electrical activity, motor activity and membrane potentials indicate that the physiology of male and female Schistosoma mansoni is, for the most part, similar.
2. Many of the differences which are seen may be a result of anatomical differences in the sexes or they may be due to the methods of recording.
 - a) The surface electrical activity of the two sexes is similar in form but is of higher frequency in the female parasite.
 - b) Electrical stimulation, resulting in muscle contraction, is more effective in the female than in the male.
 - c) The males experience a greater contracture when exposed to 10^{-6} M PZ, 60 mM K^{+} , 1 mM DNP, 10^{-5} M ouabain or 5C.
3. Three membrane potentials, recordable upon penetration of the female with a microelectrode, are essentially identical in order of appearance and in magnitude to those reported from males.
4. Both sexes respond similarly to the putative neurotransmitters 5-HT, dopamine and carbachol. Lengthening responses are experienced with the application of 10^{-4} M carbachol or 10^{-4} M dopamine while 10^{-5} M 5-HT causes an increase in contractile activity.

5. Females are more sensitive to ionic alterations in the medium than males. A greater permeability to Mg^{++} and Ca^{++} in the female and smaller stores of available calcium in the female may account for the differential effects of ionic alterations in the two sexes.
- a) Removal of calcium from the medium and increases in external Mg^{++} affect the female more than the male.
 - b) There is no significant loss in Ca^{++} content, as measured by elemental analysis, in the female after a one hour incubation in a 0 Ca^{++} medium.

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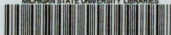
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