This is to certify that the

thesis entitled

L-glutamine : An amino acid required for maintenance of the tegumental membrane potential of Schistosoma mansoni.

presented by

Carolyn A. Lane

has been accepted towards fulfillment of the requirements for

Masters degree in Zoology

Ralph A Pax
Major professor

Date May 13, 1987

L-GLUTAMINE: AN AMINO ACID REQUIRED FOR MAINTENANCE OF THE TEGUMENTAL MEMBRANE POTENTIAL OF SCHISTOSOMA MANSONI

By

Carolyn Arlene Lane

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Zoology

1987

ABSTRACT

L-GLUTAMINE: AN AMINO ACID REQUIRED FOR MAINTENANCE OF THE TEGUMENTAL MEMBRANE POTENTIAL OF SCHISTOSOMA MANSONI

By

Carolyn Arlene Lane

The tegumental membrane potential (-63±2.9 mV) of adult male Schistosoma mansoni is significantly depolarized (-25±7.5 mV) when the parasite is incubated in inorganic media (Hank's Balance Saline or RPMI-1640 without organic constituents). Of nine amino acids (L-glutamine, D-glutamine, L-arginine, L-proline, L-glutamate, L-asparagine, L-aspartate, L-isoleucine, and L-methionine), L-glutamine alone is sufficient to repolarize the membrane potential to the value (-56±4.5 mV) not significantly different from that found in RPMI-1640. Repolarization by glutamine is dose-dependent with significant effects obtained as low as 0.10 mM L-glutamine. The concentration of phosphate in the medium significantly alters the membrane potential. Physiological levels of phosphate (5.6 mM) are necessary in conjunction with L-glutamine, to obtain the full polarization of the membrane potential. In the absence of organic constituents, the membrane potential is strongly dependent on the external medium pH. When L-glutamine is present in the medium, the membrane potential becomes virtually independent of the external pH.

ACKNOWLEDGEMENTS

I would like to thank Dr. Ralph A. Pax, for his guidance and assistance throughout the course of this study.

I would also like to thank Dr. James L. Bennett, and Dr. Neal Band for their encouragement, and for serving on my committee.

A special thanks to C.C. Siefker for her expert assistance in the laboratory.

I would also like to thank all my colleagues in both Dr. Bennett's and Dr. Pax's laboratory. They made this an enjoyable experience.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

There are over 200 million people in the world infected with the digenean parasites of the genus Schistosoma, making schistosomiasis second only to malaria in occurrence among infectious diseases. Schistosoma mansoni is a classic schistosome with mature parasites living in the human mesenteric and portal veins. Eggs are passed from humans via the feces into water, where they hatch and release the free swimming form, miracidia. The miracidia penetrate specific snail hosts and develop into primary sporocysts, which in turn give rise to the cercariae-producing secondary sporocysts. Free swimming cercariae emerge from the snail and penetrate the skin of humans. Transformed into schistosomula, they invade blood vessels and migrate through the heart to the lungs and into the liver and mesenteric vein network, where they mature. Initially distributed in Africa, S. mansoni is now found in much of the Middle East, Egypt, and Libya, and has spread to Brazil, Surinam, Venezuela, and some Caribbean islands.

General Anatomy

Adult male Schistosoma mansoni average 1.0 cm long, 0.2 cm wide, while females are slightly longer (1.5 cm) and much thinner (0.02 cm). Both sexes have an oral sucker located near the anterior end. The body of the male has a ventral, longitudinal gynecophoral canal in which the mature female normally resides. Paired intestinal ceca converge and fuse at the midpoint of the adult animal, and then continue as a single gut posteriorly (Schmidt and Roberts, 1977).

The Tegument

The external covering of Schistosoma mansoni forms an interface with the host environment and is a site where considerable biochemical, physiological, and immunological interplay takes place. Knowledge of the nature of this covering layer is therefore fundamental to an understanding of the host-parasite relationship.

The body of the adult S. mansoni is covered by a syncytial epithelium, generally referred to as the tegument (Figure 1). The surface membrane of the tegument measures approximately 11 nm and has deep invaginations into the cytoplasm. It is composed of a multilayer structure which is essentially heptalaminate, consisting of two closely opposed trilaminate membranes (McLaren and Hockley, 1977). 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. The thickness of the tegumental epithelium can vary from 5.0 μm to 1.0 μm, depending on the contractile state of the parasite and the region in which it is measured (Wilson and Barnes, 1977).

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, Reynolds and von Lichtenburg, 1969). 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 internuncial processes. Junctional complexes occur between cytons and muscle cells but not between adjacent cytons (Silk and Spence, 1969).

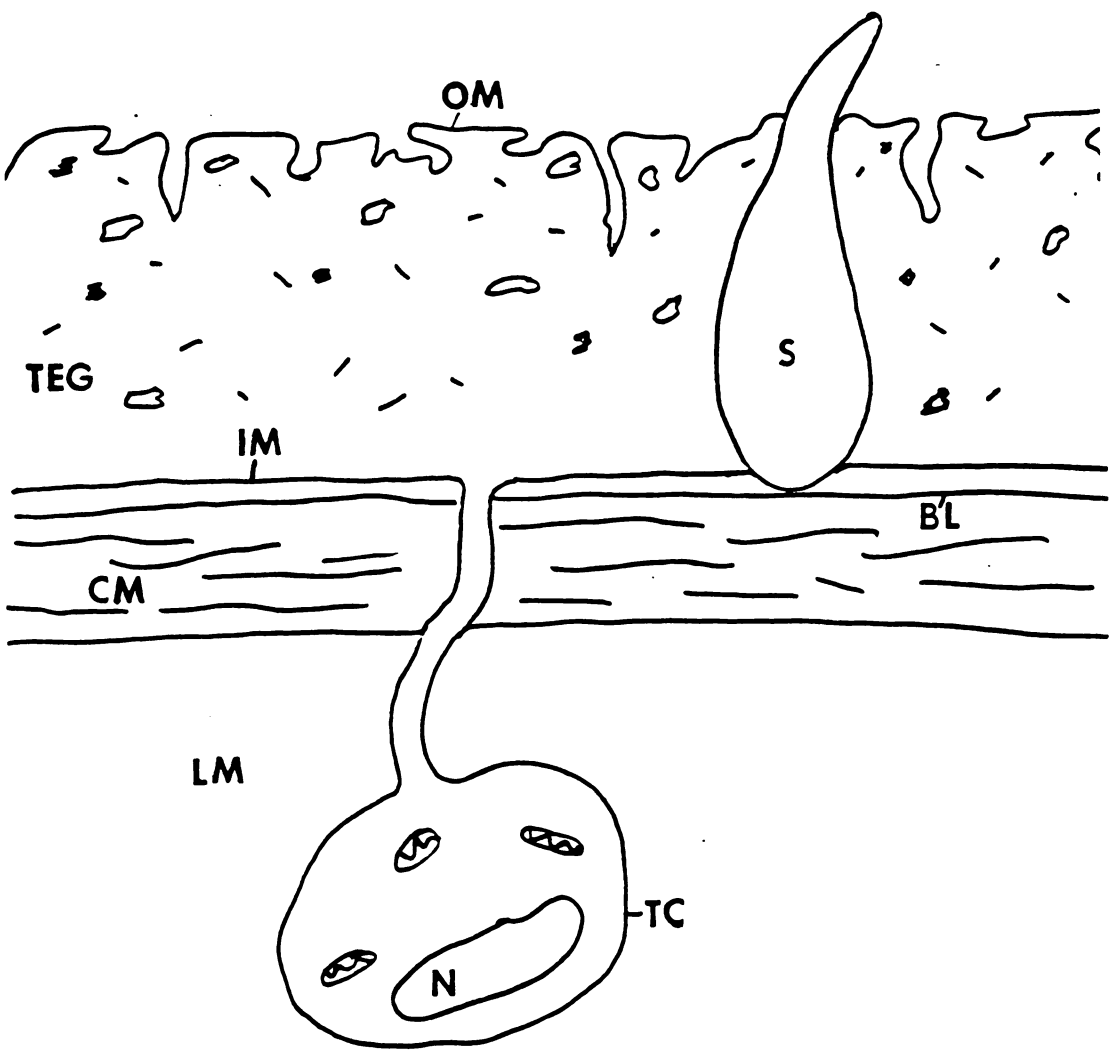

Figure 1. Schematic of tegument and muscle layers in Schistosoma mansoni. TEG, tegument; S, tegumental spine; OM, outer membrane; IM, inner membrane; BL, basil lamina; TC, tegumental cyton; N, nucleus; CM, circular muscle.

Tegumental Transport

Although the tegument can absorb substances of low molecular weight, its importance, relative to the gut, is unclear. There is some information to indicate that the tegument of the schistosome may be the major route for the absorption of host-blood glucose and amino acids, while the gut in this parasite may function primarily in macromolecular digestion and the subsequent absorption of soluble digestive products. The transport of low molecular weight compounds across the tegument of the adult male Schistosoma mansoni has been studied extensively. Both diffusion and mediated processes have been shown to contribute to the absorption of various substrates. In schistosomes, glucose, 2-deoxyglucose, galactose, glucosamine, and mannose are absorbed by a combination of mediated transport and diffusion; fructose and 3-O-methylglucose apparently enter by diffusion alone (Uglem and Read, 1975). The influx of glucose is Na^+-dependent and inhibited by ouabain and phlorizin, suggesting the presence of an energy-dependent, active transport mechanism similar to that found in tapeworms and vertebrates (Cornford and Oldendorf, 1979; Podesta and Dean, 1982).

Altough the schistosome appears to rely heavily on carbohydrate metabolism for energy production, these helminths do not prosper, in vitro, on an exclusively carbohydrate diet. Additional substrates are required for growth and reproduction. Adult schistosomes appear to be highly dependent on an external source of preformed bases for nucleotide biosynthesis. The tegumental uptake of several purines and pyrimidines has been demonstrated to follow saturation kinetics, and five distinct transport sites for these compounds have been postulated (Senft, 1972; Levy and Read, 1975).

Amino acid requirements of S. mansoni have been under investigation for many years. Amino acid uptake in schistosomes occurs by both diffusion and mediated transport, and evidence that many amino acids are actively transported

has been reported (Isseroff, Ertel and Levy, 1976). The incorporation and utilization of amino acids by schistosomes has been described biochemically (Bruce, Ruff, Belusko and Werner, 1972; Chappel and Walker, 1982) and transport systems for acidic, neutral, or basic amino acids have been postulated and characterized (Asch and Read, 1975; Cornford, 1985).

Tegumental Membrane Potential

Despite the unique structure of the schistosome surface membrane, its bioelectrical characteristics do not appear to be dissimilar from conventional cell membranes. Fetterer, Pax and Bennett (1980, 1981), using microelectrode recording techniques, established the presence of a membrane potential in S. mansoni originating from the electrochemical gradient across the parasite's outer multilaminate membrane. Membrane potential, resistance, and capacitance all appear to be well within the range of values recorded from a variety of cells. The tegumental membrane appears to be electrically inexcitable. The tegumental potential is similar to that found in transport epithelia, such as the lining of the small intestine or the bladder in vertebrates, in that it appears to be sensitive to the external K^+ concentration. In its response to external Na^+ concentrations, it differs from vertebrate epithelial cells, becoming depolarized upon lowering the external Na^+ (Fetterer and Pax, 1980). Inhibition of K^+ uptake and Na^+ efflux by ouabain or low temperature, and the effects of these treatments on the tegumental membrane potential indicate the importance of an active Na^+/K^+ transport system in the tegument of the adult male schistosome (Fetterer and Pax, 1981).

Objective

This study was begun as an effort to extend the work of Fetterer and Pax, and characterize more fully the effect ionic alterations have on the tegumental membrane potential. To do this, a medium composed of the inorganic salts of a commonly used cell culture medium (RPMI-1640) was utilized for incubation. However, initial data indicated that schistosomes incubated in this inorganic medium did not maintain a membrane potential equivalent to that recorded in the full complement medium, even when glucose was added as an energy source. It has been well documented that in vitro culturing of schistosomes requires a complex organic medium for lengthy survival and egg production (Mercer and Chappell, 1985), but this was the first suggestion that the absence of organic compounds also perturbed the tegumental membrane potential.

In addition to the inorganic salts, RPMI-1640 contains sixteen vitamins, twenty amino acids, glucose, and glutathione. The most significant proportion of the organic constituents are the amino acids (Table 1), and as such are the logical topic of investigation. The importance of amino acids to the schistosome is not known, although the previously mentioned uptake across the tegument implies a possibly crucial role in the normal functioning of the parasite. The focus of this work will be to consider the possibility of a relationship between the tegumental membrane potential and the presence of amino acids in vitro. Such a relationship could become a potential target for chemotherapeutic intervention in human schistosomiasis.

TABLE 1

The Amino Acid Components of RPMI-1640

Amino Acid	mM	Amino Acid	mM
L-arginine	1.15	L-leucine	0.38
L-asparagine	0.38	L-lysine	0.22
L-aspartic acid	0.15	L-methionine	0.10
L-cystine	0.21	L-phenylalanine	0.09
L-glutamic acid	0.13	L-proline	0.17
L-glutamine	2.05	L-serine	0.19
glycine	0.13	L-threonine	0.25
L-histidine	0.09	L-tryptophan	0.02
L-hydroxyprolone	0.15	L-tyrosine	0.01
L-isoleucine	0.38	L-valine	0.17

MATERIALS AND METHODS

Source and Maintenance of Parasites

Mature adult pairs of Schistosoma mansoni (St. Lucian strain) were isolated 45-60 days post-infection from the mesenteric and portal veins of infected female white mice obtained from the laboratory of Dr. J. L. Bennett, Department of Pharmacology and Toxicology, Michigan State University. Paired worms were maintained in RPMI-1640 (Grand Island Biological, New York, NY) with 20 mM Hepes (N-2-hydroxyethylpiperazine-N-2-ethene sulphonic acid), pH 7.4, with 100 units/ml penicillin-streptomycin. The worms were kept at 37°C and used for experiments within 8 hours of removal from mice.

Experimental Media

The standard medium used in all experiments consisted of 102.74 mM NaCl, 0.44 mM $Ca(NO_3)_2 \cdot 4H_2O$, 5.36 mM KCl, 0.41 mM $MgSO_4$, 5.64 mM Na_2HPO_4, 11.1 mM glucose, and 20 mM Hepes (N-2-hydroxyethylpiperazine-N-2-ethene suplphonic acid) added as buffer at pH 7.4. This medium is identical to RPMI-1640 except that it does not contain any of the various amino acids and other organic constituents normally present in that medium. This medium will be referred to as inorganic RPMI. For those experiments using amino acids, one of the following amino acids was added to the medium before incubation of the parasites: L-glutamine, D-glutamine, L-arginine, L-glutamate, L-asparagine, L-proline, L-isoleucine, L-methionine, or L-aspartic acid (Sigma Chemical Co.).

Some experiments were performed on parasites incubated in Hank's Balance Saline (HBS), a commonly used incubation medium. HBS contains 13.8 mM NaCl, 5.4 mM KCl, 1.4 mM $CaCl_2 \cdot 2H_2O$, 0.5 mM $MgCl_2 \cdot 6H_2O$, 0.5 mM KH_2PO_4, 0.25 mM Na_2HPO_4, 11.1 mM glucose and 20 mM Hepes added as buffer at pH 7.4. In those experiments in which the phosphate concentration was altered, the Na_2HPO_4 concentration was adjusted to 5.64 mM, the same concentration as in inorganic RPMI, and the KH_2PO_4 was eliminated. For all experiments, parasites were preincubated in appropriate medium for 1 hour at 37°C unless otherwise stated.

Microelectrode Recordings

For microelectrode recordings of the membrane potential, 15-40 megaohm micropipettes pulled from 1.5 mm "Kwik-fill" capillary tubing (WPI, New Haven, CT) and filled with 3 M potassium chloride were used. The electrodes were connected to a microelectrode preamplifier and the results were displayed on an oscilloscope (Tektronix 5113) and a chart recorder (Gould model 220). A silver-silver chloride wire or a KCl agar bridge placed in the preparation bath served as a ground.

The recording chamber consisted of a 5 ml disposable microbeaker with a screen platform, placed in a water bath maintained at 37°C. Schistosomes were placed on the screen platform within the recording chamber, which contained 2.5 ml of incubation medium and 1×10^{-4}M carbachol (carbamyl choline chloride) to immobilize the worm. Six microelectrode recordings were taken from each animal in different areas of the worm, and averaged. Where indicated, n refers to the number of animals used in each experiment.

L-(U-^{14}C)-glutamine Uptake

Studies on L-(U-^{14}C)-glutamine uptake were performed by placing parasites and heat-killed controls (5°C for 10 minutes) in vials (15/vial) containing 5 ml of inorganic RPMI with 0.5 mM L-glutamine, and preincubating them for 15 minutes at 37°C. After the preincubation period, 0.50 µCi of L-(U-^{14}C)-glutamine (Amersham Radiochemicals, Arlington Heights, IL) was added to each vial (288 mCi/mmol). The parasites were then incubated for an additional 5, 20, and 60 minutes. Samples (100 µl) of the medium were taken, followed by separation of the parasites from the incubation medium by filtration over a 25 mm glass-fiber filter placed under vacuum on a Millipore filtration apparatus. The trapped parasites were then washed 3 times in 5 ml of inorganic RPMI, weighed, placed in a vial containing 1 ml of 4% TCA and homogenized. Samples (100 µl) of the homogenate, and the medium samples, were then placed in scintillation vials containing 7 ml of aqueous counting scintillant and counted with a scintillation spectrometer. Quench correction was used by the H method (Horrocks, 1974) and all samples were corrected against heat-killed controls.

Statistical Analysis

One-way analysis of variance and Dunnett's procedure were used to determine statistical differences between control mean potentials and mean potentials measured under various experiments conditions.

RESULTS

Effect of Amino Acids on Membrane Potential

When parasites were preincubated in normal RPMI-1640 for a minimum of one hour at 37°C, the membrane potential recorded in RPMI-1640 averaged -63±2.9 mV (n=27). For animals incubated under identical conditions in our inorganic RPMI, the membrane potential averaged only -25±7.5 mV (n=47). From this it appears that one or more of the constituents normally present in RPMI-1640 but lacking in our inorganic medium is necessary for the maintenance of normal membrane polarization. In other experiments the time course of the depolarization of the tegument that occurs when animals are transferred from RPMI-1640 to inorganic RPMI was examined. In these studies, recordings of membrane potentials were taken from 3 to 92 minutes after transfer of animals from RPMI-1640. For the parasites tested, the membrane potential at 3 minutes was not significantly different from that recorded in RPMI-1640 before the transfer. The potential slowly decreased to -48±2 mV at 35 minutes, and by 1 hour fell to -29±4 mV. Since it took a full hour for the depolarization to become complete, in all subsequent experiments, incubations were a minimum of 1 hour.

In an effort to determine if the missing component in inorganic RPMI was an amino acid, the membrane potential was recorded from parasites incubated in inorganic RPMI's to which various of the amino acids normally present in RPMI-1640 were added. The most concentrated amino acid in human or mouse serum is glutamine being present at 0.50 mM in human serum (Senft, 1966). Amino acids were added to the media at this concentration.

Of the nine amino acids tested, only four (L-glutamine, L-glutamate, L-asparagine, and D-glutamine) were able to sustain a membrane potential significantly greater ($p < 0.01$) than that in inorganic RPMI alone (Table 1). Of these, only L-glutamine maintained the membrane potential at a level not significantly different from that of RPMI-1640, while the potential in the D-glutamine, L-asparagine, or the L-glutamate media was significantly less than that in RPMI-1640 ($p < 0.01$).

With the exception of L-arginine (1.15 mM) and L-glutamine (2.05 mM) all of the amino acid concentrations in RPMI-1640 are below 0.5 mM. The membrane potentials of parasites incubated in inorganic RPMI with concentrations of individual amino acids equal to those in RPMI-1640 was recorded. The membrane potentials were significantly less ($p < 0.01$) than that measured in parasites incubated in RPMI-1640 in every case except for those parasites incubated with L-glutamine.

Since L-glutamine was the most effective in maintaining the membrane potential at a level similar to that in RPMI-1640, its effects on the potential were examined more closely. In RPMI-1640 the concentration of L-glutamine is 2.05 mM. Its addition to the inorganic medium at this concentration was sufficient to restore the membrane potential to its normal value (Figure 2). When the glutamine concentration was decreased to 0.05 mM, the potential was no longer significantly different from the potential in the inorganic RPMI incubations, but at a concentration as low as 0.10 mM the potential was significantly greater than that in inorganic RPMI alone. Between 0.05 mM and 0.10 mM, the slope of the line was very steep, with a 220 mV change per 10-fold increase in L-glutamine concentration. Raising the concentration above 2.05 mM did not alter the membrane potential significantly from the value found in RPMI-1640.

TABLE 2

The Mean Membrane Potential of Schistosoma mansoni when Incubated in Medium with Amino Acids

Media	Amino Acid Concentration (mM)	n	Membrane Potential (mV) (mean ± S.D.)
RPMI-1640	----	27	-63±2.9**
Inorganic RPMI	----	47	-25±7.5
+L-glutamine	0.50	11	-56±4.5**
	2.05	24	-60±3.6**
+D-glutamine	0.50	6	-43±7.9*
	2.05	17	-37±7.6*
+L-arginine	0.50	6	-21±4.6
	1.15	11	-40±7.5*
+L-proline	0.50	7	-26±4.5
	0.17	7	-29±5.6
+L-glutamate	0.50	9	-44±4.0*
	0.14	8	-36±3.0*
+L-asparagine	0.50	7	-48±5.4*
+L-aspartate	0.50	6	-26±4.4
+L-methionine	0.50	8	-29±2.2

*Significantly different ($p < 0.01$) from RPMI-1640 and inorganic RPMI.

**Significantly different ($p < 0.01$) from inorganic RPMI.

The remaining values are significantly different ($p < 0.01$) from RPMI-1640, but not from inorganic RPMI.

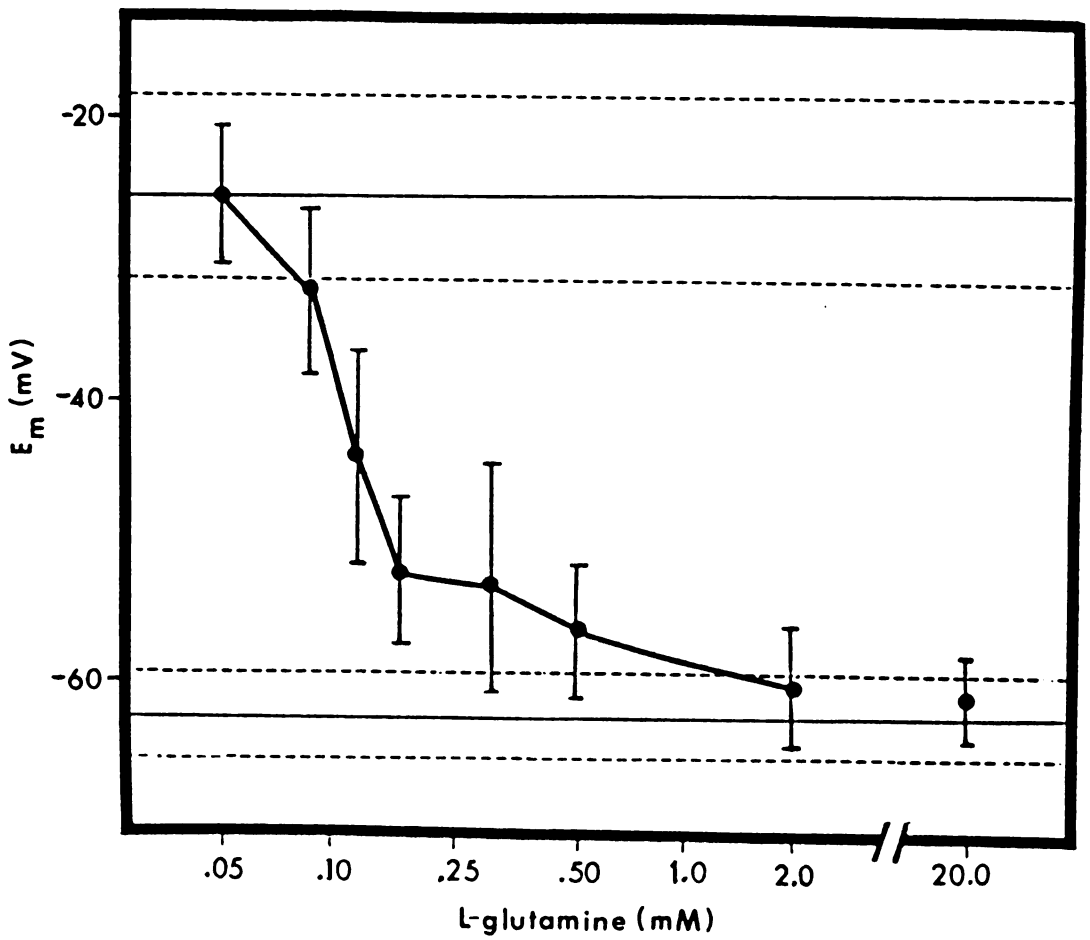

Figure 2. The effect of varying the L-glutamine concentration in inorganic RPMI on the tegumental membrane potential of Schistosoma mansoni. All points represent the mean and standard deviation of the membrane potential for a minimum of 10 animals. The lower set of horizontal lines represents the mean ± S.D. for control animals in full compliment RPMI-1640 (n=27). The upper set of horizontal lines represents the mean ± S.D. for animals in inorganic RPMI.

Effect of Phosphates on Membrane Potential

Hank's Balanced Saline (HBS) is a commonly used incubation medium for S. mansoni. The membrane potential of parasites incubated in HBS for 1 hour at 37°C was depolarized to -33±8.9 mV (n=11), significantly different from that in RPMI ($p < 0.01$) (Table 3). L-glutamine was added to HBS at the same concentration as in RPMI (2.05 mM) to determine if glutamine would render the same effect in HBS as in inorganic RPMI. The membrane potential was increased to -48±4.8 mV, but was still significantly less than the RPMI value. Since one of the differences between HBS and inorganic RPMI is the amount of phosphate in the medium, the phosphate concentration in HBS (0.25 mM Na_2HPO_4 and 0.50 mM KH_2PO_4) was changed to that present in inorganic RPMI (5.64 mM Na_2HPO_4), and the membrane potentials were recorded. In HBS with the higher sodium phosphate levels, there was no significant change from the membrane potentials of parasites incubated in normal HBS. However, when L-glutamine was added (2.05 mM) to the medium with the higher phosphate, the membrane potential dropped to -56±4.2 mV (n=13). This is significantly different ($p < 0.01$) from all of the potentials obtained in the HBS variations detailed above, but not from the membrane potentials obtained in RPMI-1640 or in inorganic RPMI with 2.05 mM glutamine.

When the phosphate concentration of inorganic RPMI was lowered to the same concentration as is present in HBS (0.25 mM Na_2HPO_4) and 0.5 mM KH_2PO_4), the membrane potential of schistosomes incubated for one hour in the medium was -44±11.3 mV (n=6). Upon the addition of 2.05 mM L-glutamine to the medium, the membrane potential increased slightly (-48±5.0; n=5), but was not sigificantly greater than in the medium without glutamine. When both media had the same phosphate and L-glutamine concentrations, the membrane potentials

TABLE 3

The Mean Membrane Potential of Schistosoma mansoni when Incubated in Different Phosphate and L-Glutamine Concentration

Medium	Na_2HPO_4:KH_2PO_4 (mM)	L-glutamine (mM)	n	Membrane Potential (mV) (mean ± S.D.)
RPMI-1640	(5.6:0)	(2.05)	27	-63± 2.9
HBS	(5.6:0)	----	11	-30±11.7
	(0.25:0.5)	----	8	-33± 8.9
	(0.25:0.5)	(2.05)	17	-48± 4.8
	(5.6:0)	(2.05)	13	-56± 4.2*
Inorganic RPMI	(5.6:0)	----	47	-25± 7.5
	(0.25:0.5)	----	6	-44±11.3
	(0.25:0.5)	(2.05)	5	-48± 5.0
	(5.6:0)	(2.05)	24	-60± 3.6*

*Not significantly different from RPMI-1640.

obtained by incubating parasites in inorganic RPMI showed no significant difference from those of parasites incubated in HBS medium. On the basis of these data it appears that the crucial difference between inorganic RPMI plus glutamine and HBS plus glutamine is the higher phosphate content of the inorganic RPMI and that the differences between the two media with respect to the other inorganic salt concentrations is of no consequence with respect to maintaining the tegumental membrane potential.

Effect of pH on Membrane Potential

In a variety of tissues, glutamine has been postulated as part of an intracellular cycle involved in pH homeostasis (Sies, 1984). In view of the findings described above, the relationship between membrane potential and extracellular pH was also examined. To do this, parasites were incubated in media at extracellular pH levels between 7.0 and 8.0 while the membrane potential was monitored.

In the absence of amino acids, the membrane potential was strongly dependent on the external medium pH, showing a 30 mV increase in potential when pH was increased by one unit (Figure 3A). Changes in the membrane potential were complete within 3 minutes of the change in external pH, the shortest time after the change that reliable measurements were obtainable. The membrane potential of the parasites incubated in normal RPMI-1640 with its full complement of amino acids, by contrast, showed little pH dependence, increasing by only 8 mV with a 10-fold decrease in proton concentration (Figure 3B). When L-glutamine (0.50 mM) was present in the incubation medium, the membrane potential was virtually independent of the external pH over the range tested, becoming only 2 mV more negative with a one unit increase in pH (Figure 3C). This ability of glutamine to render membrane potential insensitive to external pH

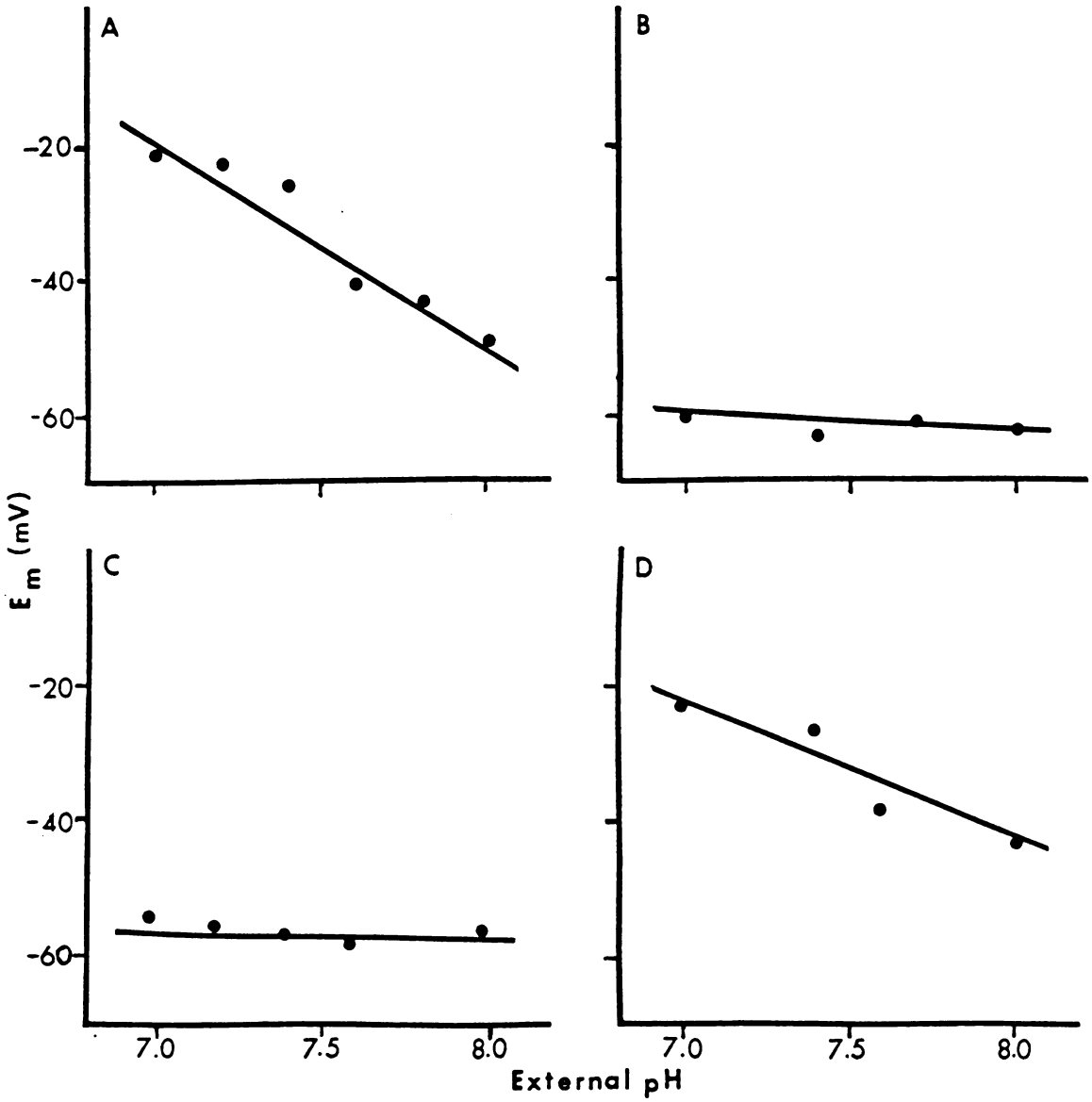

Figure 3. The effect of changes in external pH on the tegumental membrane potential of Schistosoma mansoni. All points represent the mean for a minimum of 10 animals. (A) Inorganic RPMI. (B) RPMI-1640. (C) Inorganic RPMI + 0.50 mM L-glutamine. (D) Inorganic RPMI + 0.50 mM L-proline.

changes appears to be at least somewhat specific since with the addition of proline to the inorganic RPMI, the membrane potential changed by -21 mV as the pH increased by one unit (Figure 3D).

Uptake of L-(U-^{14}C)-Glutamine

Glutamine uptake was determined by incubating worms for various times in inorganic RPMI with 0.50 mM L-glutamine containing 0.50 μCi L-(U-^{14}C)-glutamine. Figures 4A and 4B show the results of these studies. At 5 minutes, the DPM/gram wet weight of ^{14}C was 216,600±60,000. By 20 minutes the DPM/gram wet weight had increased to 342,400±32,000. The uptake appears to equilibrate after 20 minutes, increasing by only 46,000 DPM between 20 and 60 minutes, to 388,400±52,000 DPM/gram wet weight (Figure 4A). The decrease in ^{14}C from the medium follows a similar pattern, with the DMP/ml decreasing from 672,600±2,000 at time 0 to 537,400±9,600 DPM/ml at 20 minutes, a decrease of 135,200 DPM. Between 20 and 60 minutes, the DPM/ml drops by only 18,800 DPM to 518,600±17,600 DPM/ml (Figure 4B). From this study it appears that glutamine can be taken up by the parasite.

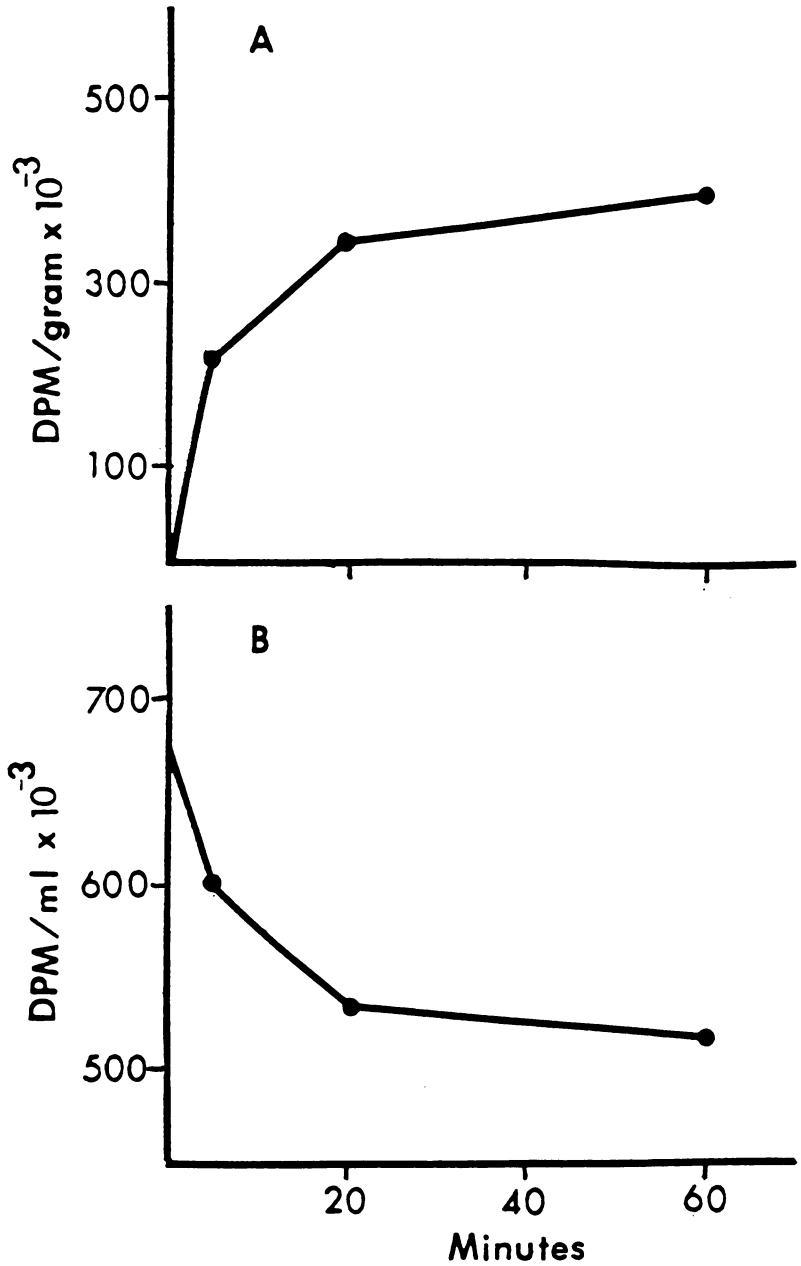

Figure 4. The uptake (A) of L-(U-^{14}C)-glutamine by Schistosoma mansoni incubated in inorganic RPMI + 0.50 mM L-glutamine. The simultaneous decrease (B) of L-(U-^{14}C)-glutamine from the incubation medium.

DISCUSSION

From the data presented here, it is clear that incubation media devoid of organic components are wholly inadequate, even on a short-term basis (less than one hour), for maintenance of the normal potential gradient across the tegument of adult Schistosoma mansoni. This inability to maintain a normal potential also implies the disruption of a variety of other crucial functions of the tegumental membrane, such as transport of Na^+ and amino acids (Heinz and Geek, 1978), sugars (Kimmich, Randles, Restrepo and Montrose, 1985), H^+ and catecholamines (Johnson, Carty and Scarpa, 1985). With this consideration in mind, it appears any studies on tegumental transport utilizing totally inorganic incubation media must be interpreted with considerable caution.

With the normal complement of inorganic salt present in RPMI-1640, the deficit in tegumental potential can be remedied by the addition of a single amino acid, L-glutamine. This ability of L-glutamine to maintain normal polarization of the tegument is highly effective (a concentration as low as 0.10 mM producing significant beneficial effects), and it appears to be specific. No other amino acids tested, aside from L-asparagine and D-glutamine, are able to produce this effect.

Glutamine is a neutral amino acid under physiological conditions. The membrane potential recorded from the tegument is produced by a charge gradient. The transport of glutamine across the tegument therefore should not affect the membrane potential unless it is linked to a change in the internal charge concentration. From the results presented (Figure 3), it appears that the

presence of glutamine is a crucial factor in the ability of the parasite to cope with external pH changes. In the absence of L-glutamine, the H^+ gradient is an important determiner of the tegumental potential but when L-glutamine is present, this gradient is no longer significant. This independence of tegumental potential from the proton gradient in the presence of L-glutamine would be consistent with the presence of an intracellular L-glutamine cycle for pH control in S. mansoni.

An intracelluar L-glutamine cycle has been implicated in the maintenance of pH homeostasis in a variety of animal tissues (Sies, 1984). In this cycle, L-glutamine within the cytoplasm is deaminated by the enzyme glutaminase (E.C. 3.5.1.2.) to form L-glutamate and, with the addition of a proton, ammonium. In this way the cycle could serve as a mechanism for buffering of free protons in the cytoplasm. Most of the glucose metabolized by Schistosoma mansoni is converted to lactate (Smyth and Halton, 1983). For every mole of lactate produced by the anaerobic metabolism of glucose, two moles of protons are generated (Hochachka, 1983). The glutamine cycle would buffer the excess protons, enabling the parasite to regulate the potential decrease in intracellular pH caused by the products of anaerobic glycolysis. This could be especially important in S. mansoni, which has a high rate of glucose metabolism (Bruce, Ruff, Davidson and Crum, 1974).

Glutaminase is a widely distributed enzyme (Kvamme, Torgner, and Svenneby, 1985). It occurs in a number of mammalian tissues and has also been found in bacteria (Holcenberg, 1985). The glutaminase reaction in mammals is fast, energy-independent, and independent of any known cofactor. However, it does require a polyanionic activator, and phosphate is one of the major physiological activators (Kovacevic and McGivan, 1983). The results obtained in the use of HBS point to the need for substantial levels of phosphate (5.6 mM) in the incubation

media for maintenance of the membrane potential in the inorganic RPMI media with glutamine (Table 2).

While the mammalian enzyme utilizes L-glutamine alone as substrate, the bacterial glutaminases catalyze the hydrolysis of both the L- and D-isomers of glutamine and asparagine. Holcenberg (1985) states that at 20 mM, the relative activities are L-glutamine, 1:00; L-asparagine, 0.77; D-glutamine, 0.35; and D-asparagine, 0.26. The membrane potential of S. mansoni appears to show a similar graded response to the presence of D-glutamine and L-asparagine (D-asparagine was not tested) suggesting the ability of the parasite to utilize these amino acids in the absence of L-glutamine (Table 1). Arginine and glutamic acid, while appearing to affect the membrane potential to some extent, did so only at a concentration 10 times higher than normally found in human serum.

The results of the glutamine uptake studies suggest that, in the absence of other amino acids, a substantial amount of L-glutamine can enter the adult S. mansoni. The fate of the labeled L-glutamine was not determined in these studies. The purpose was to simply ascertain whether the parasite was able to remove this radioactive amino acid from the medium and incorporate the label into tissue. The high levels of glutamate found by Chappel and Walker (1982) in the free amino acid pool of the adult parasite and incorporated into the proteins of all the stages of S. mansoni, may reflect an enzymatic metabolism of the absorbed glutamine to glutamate by the enzyme glutaminase. This could also account for the inhibition of glutamate uptake by glutamine reported by Asch and Read (1975), and Isserhoff et al. (1976). In closing, it is interesting to note that Schnell (1985) reported that glutamine is one of the few amino acids in Biomphalaria glabrata to undergo a significant reduction in concentration when the snail is infected by the sporocysts of S. mansoni. This could indicate there may be a need for the amino acid in other stages of the parasite.

SUMMARY

1. The tegumental membrane potential of adult male Schistosoma mansoni is significantly depolarized when the parasite is incubated in inorganic media (Hank's Balance Saline or RPMI-1640 without organic constituents).
2. Of nine amino acids (L-glutamine, D-glutamine, L-arginine, L-proline, L-glutamate, L-asparagine, L-aspartate, L-isoleucine, and L-methionine), L-glutamine alone is sufficient to polarize the membrane potential to the value found in a full complement medium. The polarization is dose-dependent with significant effects obtained as low as 0.10 mM L-glutamine.
3. The concentration of phosphate in the medium significantly alters the membrane potential. Physiological levels of phosphate (5.6 mM) are necessary in conjunction with L-glutamine, to obtain the full polarization of the membrane potential.
4. In the absence of organic constituents, the membrane potential is strongly dependent on the external medium pH. When L-glutamine is present in the medium, the membrane potential becomes virtually independent of the external pH. Only L-asparagine produces a similar (although reduced) response.

BIBLIOGRAPHY

BIBLIOGRAPHY

Altman, P.L. and Dittmer, D.S. (1971) Blood and Other Body Fluids. Bethesda: Federation of American Societies for Experimental Biology.

Asch, H. and Read, C. (1975) Membrane transprot in Schistosoma mansoni: Transport of amino acids by adult males. Experimental Parasitology 38: 123-135.

Bricker, C.S., Pax, R.A., and Bennett, J.L. (1982) Microelectrode studies of the tegument and sub-tegument compartments of male Schistosoma mansoni: Anatomical locations of sources of elecrical potentials. Parasitology 85: 149-161.

Bruce, J.I., Ruff, M.D., Belusko, R.J., and Werner, J.K. (1972) Schistosoma mansoni and Schistosoma japonicum: Utilization of amino acids. International Journal for Parasitology 2: 425-430.

Bruce, J.I., Ruff, M.D., Davidson, D.E. and Crum, J.W. (1974) Schistosoma mansoni and Schistosoma japonicum: Comparison of selected aspects of carbohydrate metabolism. Comparative Biochemistry and Physiology 49B: 157-164.

Chappell, L.H. and Walker, E. (1982) Schistosoma mansoni: Incorporation and metabolism of protein amino acids in vitro. Comparative Biochemistry and Physiology 73B: 701-708.

Cornford, E.M. (1985) Schistosoma mansoni, S. japonicum, and S. haematobium: Permeability to acidic amino acids and effect of separated and unseparated adults. Experimental Parasitology 59: 355-363.

Cornford, E.M. and Oldendorf, W. (1979) Transintegumental uptake of metabolic substrates in male and female Schistosoma mansoni. Journal of Parasitology 65: 357-363.

Fetterer, R.H., Pax, R.A. and Bennett, J.L. (1980) Schistosoma mansoni: Characterization of the electrical potential from the tegument of adult males. Experimental Parasitology 49: 353-365.

Fetterer, R.H. and Pax, R.A. (1981) Na-K transport, motility, and tegumental membrane potential in adult male Schistosoma mansoni. Parasitology 82: 97-109.

Heinz, E. and Geek, P. (1978) The electrical potential difference as a driving force in Na^+ linked cotransport of organic solutes. In Membrane Transport Processes, Vol. 1 (Ed. J.F. Hoffman), pp. 13-18. New York: Raven Press.

Hochachka, P.W. and Mommsen, T.P. (1983). Protons and anaerobiosis. Science 219: 1391-1397.

Holcenberg, J.S. (1985) Glutaminase from Acinetobacter glutaminificans. In Glutamate, Glutamine, Glutathione, and Related Compounds (Ed. A. Meister). Methods in Enzymology 113: 257-263.

Horrocks, D.L. (1974) Applications of Liquid Scintillation Counting. London, Academic Press.

Isseroff, H., Ertel, J.C., and Levy, M.G. (1976) Absorption of amino acids by Schistosoma mansoni. Comparative Biochemistry and Physiology 54B: 125-135.

Johnson, R.G., Carty, S.E., and Scarpa, A. (1985) Coupling of H^+ gradients to catecholamine transport in chromaffin granules. In Membrane Transport Driven by Ion Gradients (ed. G. Seminza and R. Kinne), pp. 254-267. New York: New York Academy of Sciences.

Kimmich, G.A., Randles, J., Restrepo, D., and Montrose, M. (1985) The potential dependence of the intestinal Na^+ dependent sugar transport. In Membrane Transport Driven by Ion Gradients (ed. G. Seminza and R. Kinne), pp. 63-76. New York: New York Academy of Sciences.

Kovacevic, Z. and McGivan, J.D. (1983) Mitochondrial metabolism of glutamine and glutamate and its physiological significance. Physiological Reviews 63: 547-605.

Kvamme, E., Torgner, I., and Svenneby, G. (1985) Glutaminase from mammalian tissues. In Glutamate, Glutamine, Glutathione, and Related Compounds (ed. A. Meister). Methods in Enzymology 113: 241-257.

Levy, M.G. and Read, C. (1975) Purine and pyrimidine transport in Schistosoma mansoni. Journal of Parasitology 61: 627-632.

McLaren, D.J. and Hockley, D.J. (1977) Blood flukes have a double outer membrane. Nature 269: 147-149.

Mercer, J.G. and Chappell, L.H. (1985) Schistosoma mansoni: Effect of maintenance in vitro on the physiology and biochemistry of adult worms. Parasitology 90: 339-349.

Pappas, P. and Read, C. (1975) Parasitological review: Membrane transport in helminth paasites: A review. Experimental Parasitology 37: 469-530.

Pax, R.A., Bricker, C.S., Thompson, D.P., Semeyn, D.R., and Bennett, J.L. (1983) Neurophysiology of adult male Schistosoma mansoni. Pharmacological Therapeutics 22: 117-125.

Podesta, R. and Dean, L. (1982) Uptake of galactose by the epithelial syncytium of Schistosoma mansoni. Molecular Biochemistry and Parasitology 5: 353-360.

Schnell, S. (1985) Amino acid metabolism in the freshwater pulmonate Biomphalaria glabrata infected with the trematode Schistosoma mansoni. Comparative Biochemistry and Physiology 81B: 1001-1008.

Senft, A.W. (1966) Studies in arginine metabolism by schistosomes. I. Arginine uptake and lysis by S. mansoni. Comparative Biochemistry and Physiology 18: 209-216.

Senft, A.W. (1972) Purine metabolism in Schistosoma mansoni. International Journal for Parasitology 2: 249-260.

Sies, H. (1984) Hepatic glutamine and ammonia metabolism nitrogen redox balance and the intracellular glutamine cycle. In Glutamine Metabolism in Mammalian Tissues (ed. D. Haussinger and H. Sies), pp. 78-98. Berlin: Springer-Verlag.

Silk, M.H. and Spence, I.M. (1969) Ultrastructural studies of the blood fluke Schistosoma mansoni. III. The nerve tissue and sensory structures. South African Journal of Medical Science 34: 93-104.

Smith, J.H., Reynolds, E.S., and von Lichtenburg, F. (1969) The integument of Schistosoma mansoni. American Journal of Tropical Medicine and Hygiene 18: 28-42.

Smythe, J.D. and Halton, D.W. (1983) The adult - general metabolism. In The Physiology of the Trematodes, 2nd ed. Cambridge: Cambridge University Press.

Thompson, D.P., Pax, R.A., and Bennett, J.L. (1982) Microelectrode studies of the tegument and sub-tegument compartments of male Schistosoma mansoni. Parasitology 85: 163-178.

Uglem, G. and Read, C.P. (1975) Sugar transport and metabolism in Schistosoma mansoni. Journal of Parasitology 61: 390-397.

Wilson, R.A. and Barnes, P.E. (1977) The formation and turnover of the membranocalx of the tegument of S. mansoni. Parasitology 74: 11-72.