

SCHISTOSOMA MANSONI ADULTS: UPTAKE AND INCORPORATION OF EPIMERIC MONOSACCHARIDES BY THE TEGUMENT

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The uptake, incorporation, and utilization of labeled glucose, galactose, and mannose by adult *Schistosoma mansoni* was investigated at the light and electron microscope levels. The results confirm that glucose is absorbed by the tegument; however, galactose and mannose appear to be absorbed by the gut. Glucose is incorporated mainly into glycogen with a small percentage being utilized in the tegument. Galactose and mannose are found in high concentrations in the subtegument and tegument. In each instance where a monosaccharide is a structural molecule of the tegument, incorporation occurred first in the Golgi apparatus, then in tegumental inclusions. The rate of uptake into the Golgi apparatus and then into the tegument mimics the data previously reported for membrane turnover.

INTRODUCTION

Numerous recent investigations have demonstrated intimate relationships between carbohydrate-containing molecules in and on the tegument and the structure and functions of the tegument of *Schistosoma mansoni*. Investigations into the absorptive activities of the tegument have shown that carbohydrates are actively, and in some instances exclusively, transported into the organism by that structure (1,2). Also, a role of immunological protection has been assigned to the glycocalyx which coats the tegument of *S. mansoni* (3,4,5,6,7, 8). It is possible that molecules with carbohydrate moieties may act as receptor sites for host antigens which then mask the parasite from any host effector mechanisms (6,7,8). Murrell et al. (9) and Kusel et al. (10,11) have proposed that membrane fragments of the tegument and glycocalyx are being sloughed into the circulatory system of the definitive host at all times. These fragments may then impart selective host immunity to superinfection by invading immature forms (12). Further, it has been demonstrated that glycolipids, glycoproteins, and acid mucopolysaccharides produced and packaged in the Golgi apparatus of the tegument cell bodies (subtegument) contribute to the structure of both the tegumental ground substances and the glycocalyx (6,7,13,14,15).

This investigation was an attempt to correlate the absorption, incorporation, and utilization of three epimeric monosaccharides (glucose, galactose, and mannose) which have been demonstrated to be important to the various functions of the tegument of *S. mansoni*. Glucose was chosen because of its role in schistosome metabolism (16), because it is taken up exclusively via the tegument (2), and because Stein and Lumsden (13) and Wilson and Barnes (6) have reported that mucopolysaccharides are important components of the schistosome tegument. Hughes and Sharon (17), in turn, state that glucose is often a component of membrane mucopolysaccharides. Also, Bennett and Seed (8) reported the presence of α -D-glucosyl carbohydrates in the tegument of *S. mansoni*. Galactose was chosen because of its role in membrane mucopolysaccharides (17,18) and, as with glucose, because of Bennett and Seed's (8) report on the presence of α -D-galactosyl and other related carbohydrates localized in the *S. mansoni* tegument. Mannose was investigated because Bennett and Seed (8) demonstrated that some glycoprotein receptor sites in the *S. mansoni* tegument have mannose as the terminal unit in the polysaccharide moiety. Finally Simpson and Smithers (19) have reported the presence of all three monomers on the surface of the adult schistosome.

MATERIALS AND METHODS

Schistosoma mansoni was maintained in the laboratory. Mice infected with *S. mansoni* were lightly anesthetized with chloro-

form and then sacrificed. The mice were killed 30-60 days post-infection and the abdominal cavity opened by a mid-ventral incision. The intestines were dissected free at the posterior end, carefully stripped upward from the abdominal cavity, cut free at the anterior end, and placed in 0.85% saline. Worms *in copula* were carefully extricated with fine-tipped forceps from the mesenteric and portal veins to glycylglycine-buffered physiological saline (pH 7.8) (2) at 22 C. Forceps were used to strip the vein without damaging the worms. The sexes separated *au naturel* and were subsequently rinsed twice in the glycylglycine-buffered saline. Areas of the worm to be used as experimental slices were not touched; whole worms were handled with extreme care to avoid damage to the tegument.

Whole worms were incubated at 37 C in glycylglycine-buffered physiological saline (2) at pH 7.8, containing 30 mCi/ml concentrations of either D-galactose- $1\text{-}^3\text{H}$ (S.A. 2400 mCi/ml), D-glucose- $1\text{-}^3\text{H}$ (S.A.4900 mCi/ml), D-mannose- $1\text{-}^3\text{H}$ (S.A.562.5 mCi/ml, or D-mannose- $1\text{-}^3\text{H}$ plus 10 mM sodium pyruvate). Sodium pyruvate was added to reduce the conversion of mannose to amino acids (20). Adults were also cut into 0.5-mm slices and incubated for up to 5 hr at 22 C in glycylglycine-buffered physiological saline medium containing the respective radioactive sugar. These samples were then spray-rinsed four times with glycylglycine-buffered saline (pH 7.8).

Additional samples were treated exactly as the above tissues; however, they were then subjected to enzymatic digestion. The following enzymes were used, each at a concentration of 1% in 0.1 M phosphate buffer (pH 7.0): (a) α -amylase (Bacterial crude Type III, Sigma Chemical Co.) (b) β -amylase (Barley, Mann Research Laboratories, Inc.). Tissue sections from both whole worms and 0.5-mm slices were incubated for four hr at 37 C with continuous agitation. After incubation, tissue sections were rinsed twice for 30 min each in the buffer at room temperature. The rationale for using the two enzymes was that α -amylase would completely digest and β -amylase would partially digest α -[1 \rightarrow 4]-linked carbohydrates only to the 1 \rightarrow 6 branches. Comparative data helped to assess the selectivity of the digestions.

Adult worms were fixed whole or as slices in either 5% glutaraldehyde-0.05 M phosphate (pH 7.4) for 30 min or in 1% osmium tetroxide-0.05 M phosphate (pH 7.4) for one hr. The glutaraldehyde-fixed specimens were subsequently rinsed overnight in 0.05 M phosphate buffer (pH 7.0) and post-fixed in 1% osmium tetroxide-0.05 M phosphate (pH 7.4) for one hr. After either osmium tetroxide fixation or postfixation, the tissues were rinsed in two changes of phosphate buffer (0.01 M phosphate, pH 7.0) for 30 min each at 4 C.

All tissues were dehydrated at 4 C from ethanol through to propylene oxide and embedded in Araldite. Sections were cut at 2 μm for light microscopy and 50-60 nm for electron microscopy. Tissues were stained with uranyl acetate and lead citrate (29).

TABLE 1. *Distribution of label from galactose- ^3H incorporation in S. mansoni adults*

Experimental material	Time (min)	Subteg- ument	Cytoplasmic connections	Muscle	Tegument	Tegument surface
Whole worms	5	+ ^a	0	0	0	0
	30	+ ^b	+ ^a	0	0	0
	60	++	+	0	+	0
	300	+++ ^c	+++ ^c	+	++	+
Worm slices	1	0	0	0	0	0
	5	0	0	0	0	0
	15	0	0	0	0	0
	30	+	0	0	0	0
	60	+	+	0	0	0
	120	++	++	+	+	0
	240	++	++	+	++	0
300	++	++	++	++	+	

0 = Absent; + to +++ = Frequency of occurrence as determined by observation.

^a Female worms = No label.

^b Female worms = Slight label.

^c Female worms = Considerably less label.

For light microscope radioautography, slides with sections of plastic-embedded tissue were dipped in Kodak nuclear track emulsion NTB-2 at 60 C, drained, dried, exposed in a light-tight vacuum desiccator for 7 - 12 days at 22 C, and developed (21, 22, 23). Developed slides were then dried, dehydrated, and mounted.

For electron microscope radioautography, pale gold sections (60-80 nm) of the Araldite-embedded tissues were sectioned and placed on uncoated 400-mesh nickel grids. Sections were stained with uranyl acetate-saturated 70% ethanol for 10 min, spray-rinsed, and blotted. Ilford L-4 nuclear emulsion was utilized, prepared according

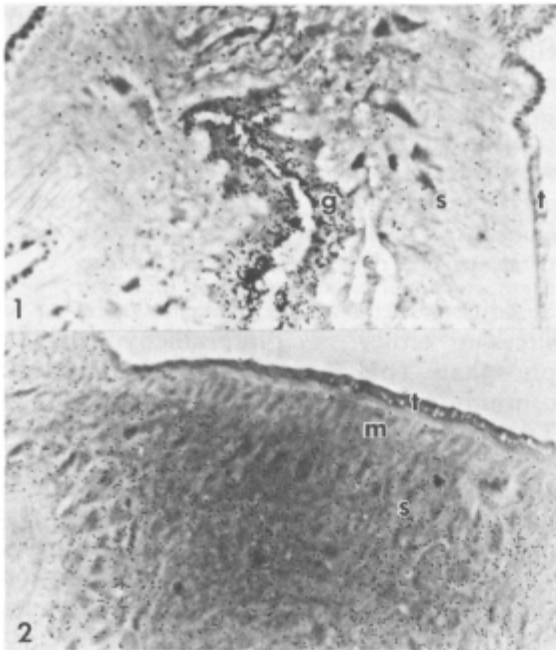


FIGURE 1. Light microscope radioautograph of a section of adult worm incubated whole for 30 min in galactose-³H. Note intense labeling in the area of the gastrodermis. (g) gastrodermis, (s) subtegument, (t) tegument. × 1,400.

FIGURE 2. Light microscope radioautograph of a section of adult worm incubated whole for 60 min. in galactose-³H. (s) subtegument, (m) subtegumental muscles, (t) tegument. × 960.

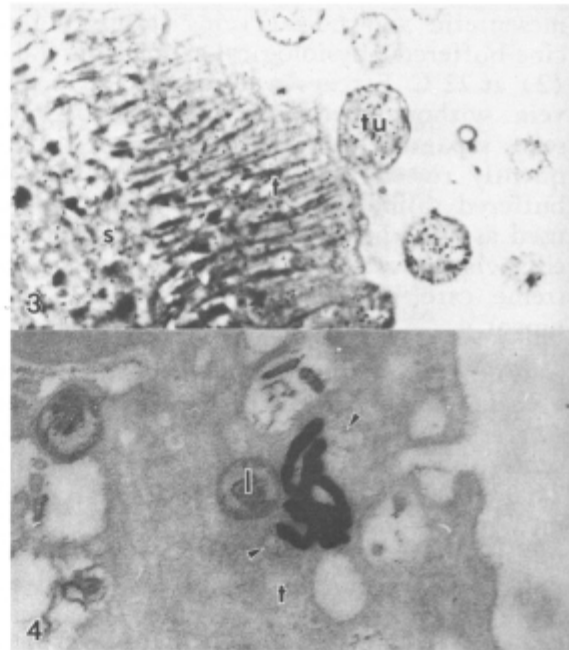


FIGURE 3. Light microscope radioautograph of a section of adult worm incubated whole for 5 hr in galactose-³H. (s) subtegument, (t) tegument, (tu) tubercles. × 1,400.

FIGURE 4. Electron microscope radioautograph of a section of adult worm incubated whole for 5 hr in galactose-³H. (t) tegument, (l) large inclusions, (arrows) small inclusions. Note silver grains associated with small inclusions. × 81,700.

TABLE 2. Distribution of label from glucose-³H incorporation in *S. mansoni* adults

Experimental material	Time (min)	Subtegument	Cytoplasmic connections	Muscle	Tegument	Tegument surface
Whole worms	5	++ ^a	++	++	+ ^b	0
	30	++	++	+++	++	+
	60	++	++	+++	++	+
	300	+++	+++	+++	+++	++
Worm slices	1	0	0	0	0	0
	5	+	0	0	0	0
	15	+	+	0	+	0
	30	+	+	0	+	0
	60	+	+	0	+	0
	120	++	++	+	+	0
	240	++	++	++	++	0
300	+++	+++	+++	+++	0	

0 = Absent; + to +++ = Frequency of occurrence as determined by observation.

^a Female worms = Slight label.

^b Label in tubercles of males.

to the methods of Caro and von Turbergen (24) and Caro (25). Gelled emulsion, showing pale gold interference color, was applied with a platinum loop to single grids (26). Grids were exposed for 50 to 70 days at 22 C in a vacuum desiccator (24). The grids were developed for 3 min in Microdol-X at 24 C followed by a stop in 1% acetic acid (10 sec), a fix in Kodak rapid fixer, and three rinses in distilled water (26,27). The grids were then stained in lead citrate and spray-rinsed with 0.02 N sodium hydroxide (28). Observations were made with Hitachi HS-8 and electron micrographs were made on Kodak plates or Estar base film.

RESULTS

Galactose-³H utilization

The distribution of labeling resulting from galactose-³H uptake is summarized in Table 1. Light microscope radioautographs

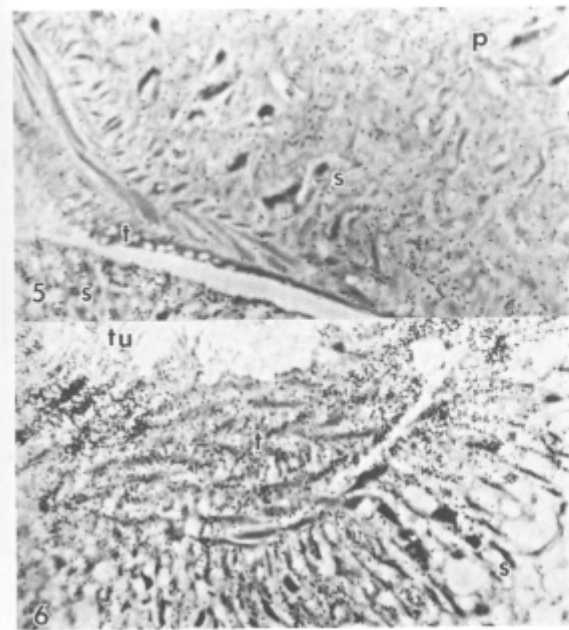


FIGURE 5. Light microscope radioautograph of a tissue slice incubated for 15 min. in glucose-³H. (t) tegument, (s) subtegument, (p) parenchyma. $\times 1,400$.

FIGURE 6. Light microscope radioautograph of a tissue slice incubated for 4 hr in glucose-³H. (tu) tubercle, (t) tegument, (s) subtegument. $\times 1,400$.

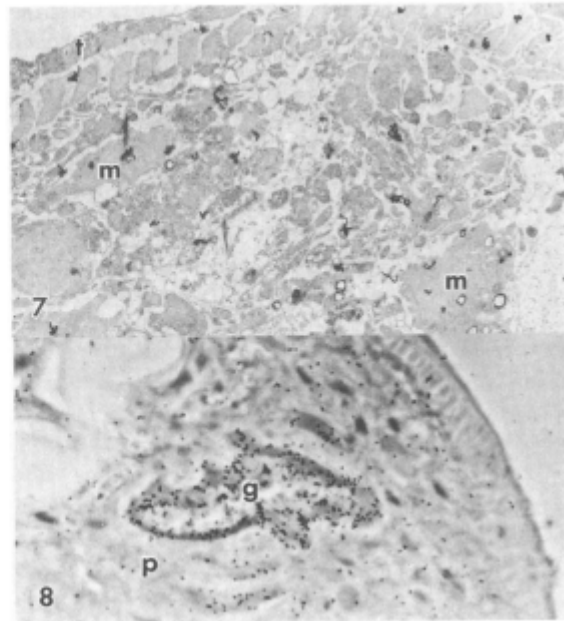


FIGURE 7. Electron microscope radioautograph of a tissue slice incubated for 5 hr in glucose-³H. Note activity throughout the (t) tegument and (m) subtegumental muscles. $\times 7,200$.

FIGURE 8. Light microscope radioautograph of a section of adult worm incubated whole for 60 min. in mannose-³H. Note heavy label over (g) gastrodermis. (p) parenchyma. $\times 1,350$.

TABLE 3. Distribution of label from mannose-³H incorporation in *S. mansoni* adults

Experimental material	Time (min)	Subtegument	Cytoplasmic connections	Muscle	Tegument	Tegument surface
Whole worms	5	+	0	0	0	0
	30	+	0	0	+	0
	60	++	0	0	+	0
	300	+++	+	+	+	+
Worm slices	1	0	0	0	0	0
	5	0	0	0	0	0
	15	0	0	0	0	0
	30	+	0	0	0	0
	60	+	0	0	0	0
	120	+	0	0	0	0
	240	+	+	0	+	0
300	+	+	0	+	0	

0 = Absent; + to +++ = Frequency of occurrence as determined by observation.

of tissue from incubated whole worms showed initial light labeling in the tegument of the male worms after five-min incubation. The females showed some label after 30-min incubation. Labeling was observed over the gastrodermis of the male worm after 30-min incubation (Fig. 1). After one-hr incubation, the label was associated with the subtegumental cells and, to a much lesser extent, the basal area of the tegument (Fig. 2). The tegument, in addition to the subtegument, was heavily labeled after five-hr incubation (Fig. 3). The amount of label over the subtegument of the female worm was considerably less than that observed in the male. Negligible label occurred over the subtegumental musculature of the male and female worms. Ultrastructural radioautographs of tissue from whole worms incubated for five hr revealed some labeling associated with inclusions in the tegument (Fig. 4). Labeling also occurred over the gut lumen, the gastrodermis, and vitelline globules.

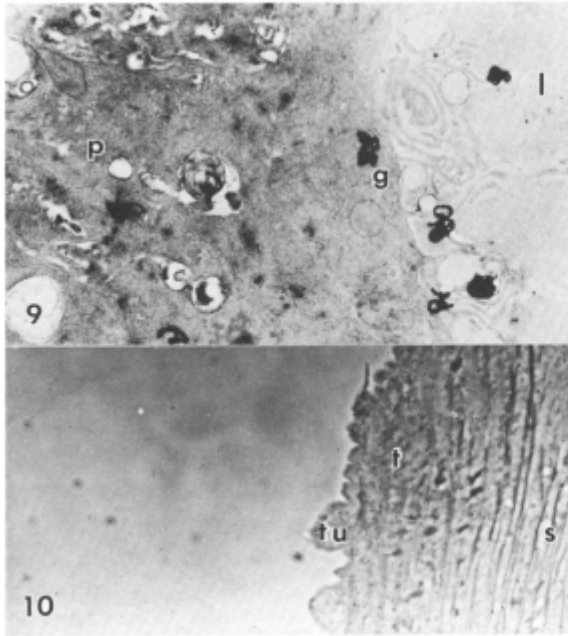


FIGURE 9. Electron microscope radioautograph of a section of adult worm incubated whole for 5 hr in mannose- ^3H . (g) gut, (l) gut lumen, (p) parenchyma. $\times 1,350$.

FIGURE 10. Light microscope radioautograph of a tissue slice incubated for 5 hr in mannose- ^3H . (tu) tubercle, (t) tegument, (s) subtegument. $\times 786$.

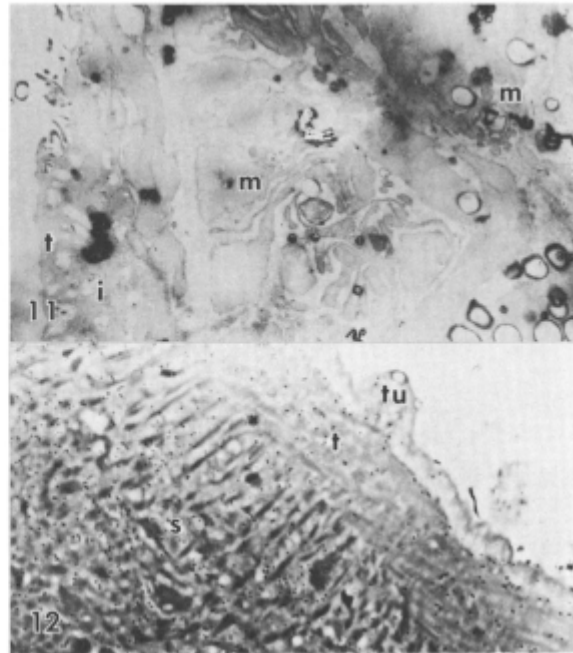


FIGURE 11. Electron microscope radioautograph of a tissue slice incubated for 5 hr in mannose- ^3H . Note labeling over (m) subtegumental muscles, (i) cellular inclusions, and (t) tegument. $\times 7,800$.

FIGURE 12. Light microscope radioautograph of a section of adult worm incubated whole for 5 hr in mannose- ^3H containing sodium pyruvate. (t) tegument, (tu) tubercle, (s) subtegument. $\times 960$.

TABLE 4. Distribution of label from mannose- ^3H incorporation in *S. mansoni* adults with sodium pyruvate added

Experimental material	Time (min)	Subtegument	Cytoplasmic connections	Muscle	Tegument	Tegument surface
Worm slices	1	0	0	0	0	0
	5	+	0	0	0	0
	15	+	0	0	0	0
	30	+	0	0	0	0
	60	+	0	0	0	0
	120	++	0	0	0	0
	240	+++	+	0	+	+
	300	+++	++	0	+	+

0 = Absent; + to +++ = Frequency of occurrence as determined by observation.

Light microscope radioautographs of tissue from worm slices incubated in galactose-³H showed initial labeling over the subtegument after 30-min incubation. After one-hr incubation, silver grains were observed over the subtegument and basal area of the tegument. Between two and three hr of incubation, the tegument began to exhibit some label. After the five-hr incubation, the tegument was heavily labeled. Ultrastructural radioautography of tissue incubated five hr showed labeling in the tegument. Some of the silver grains also appeared to be associated with the basal lamina, while others appeared to be associated with glycogen in the muscle layers or the subtegument.

Light microscope radioautography of tissue slices incubated in galactose-³H for five hr and digested in α -amylase showed a reduction in the amount of label when compared to the above control slices, although some degree of labeling over the subtegument and tegument was still evident. Radioautography at the ultrastructural level showed silver grains over the subtegument and tegument.

Tissues that were incubated in galactose-³H for five hr and then exposed to β -amylase digestion still exhibited heavy labeling. However, tissue between the muscle bundles appeared to have lost considerable label. Examination of electron microscope radioautographs revealed some labeling remaining in the subtegumental muscle and tegument.

Glucose-³H utilization

The distribution of labeling resulting from the uptake of glucose-³H is summarized in Table 2. Light microscope radioautography of whole worms incubated in the isotope-labeled sugar showed label in the tegument and subtegument after five min of incubation. The concentration of silver grains over the tissues increased as the time of incubation increased. After five-hr incubation, all the tissues of the worms appeared labeled. Radioautography at the ultrastructural level revealed label over glycogen deposits in the subtegumental muscles and subtegument after five-hr incubation.

Light microscope radioautography of tissue slices incubated in glucose ³H showed an initial uptake in the tegumental and subtegumental regions of the males after five-min incubation (Fig. 5). Both sexes exhibited labeling over the subtegument and tegument after 30-min incubation. After two-hr incubation, the subtegumental musculature was also labeled. Labeling over the musculature and tegument was considerably increased after four-hr incubation (Fig. 6) and was heavily concentrated in these regions after five hr. The tubercles in the dorsal tegument of the male showed heavy concentrations of label (Fig. 6). Electron microscope radioautographs of tissue slices incubated five hr showed silver grains over glycogen deposits in the subtegument and subtegumental muscles.

Light microscope radioautographs of adult tissue slices incubated five hr in glucose-³H and digested in α -amylase showed an almost complete eradication of labeling as compared to undigested tissue. Only scattered silver grains were seen over the subtegumental and tegumental areas. Ultrastructural radioautography of the tissue showed a few silver grains associated primarily with the subtegumental muscles.

Radioautography at the light microscope level showed β -amylase digestion to have negligible effect on the incorporated glucose-³H. Electron microscopic radioautography of the tissue showed label associated with glycogen deposits in the subtegumental muscles and the tegument (Fig. 7).

Mannose-³H utilization

The distribution of labeling resulting from the uptake of mannose-³H is summarized in Table 3. Radioautography at the light microscope level of tissue from incubated whole worms revealed label in the gut and tegument after five-min incubation. These same areas exhibited heavy labeling after 30 to 60 min of incubation (Fig. 8). After five-hr incubation, labeling was observed over all the areas under investigation including the tegument, with the heaviest concentration appearing over the subtegument. Ultrastructural radioautographs of the tissue demonstrated labeling associated with the tegument, the subtegumental musculature, and the vitelline globules in the underlying tissues. A high concentration of label was also observed in the gastrodermis and over material in the lumen of the gut (Fig. 9).

Worm slices incubated in mannose-³H exhibited label over the subtegument in

light microscope radioautographs after 30-min incubation. The distribution of label remained the same up to four hr of incubation, after which time the label appeared also over the tegument. After five-hr incubation, the label appeared over the same areas and at about the same concentration as seen after four hr of incubation (Fig. 10). Radioautographs at the electron microscope level showed silver grains associated with the subtegumental musculature and tegument inclusions after five-hr incubation (Fig. 11).

Light radioautographs of tissues digested in α -amylase after having been incubated in tritiated mannose for five hr showed negligible labeling when compared to undigested controls. At the ultrastructural level these same tissues exhibited only scattered silver grains associated with the subtegumental musculature.

Radioautographs of mannose- 3H -incubated tissues which were digested in β -amylase exhibited negligible labeling over the tegument and subtegument. Electron microscope radioautographs of these tissues revealed scattered silver grains over the subtegumental musculature.

The distribution of labeling resulting from the uptake of mannose- 3H in the presence of sodium pyruvate by slices of adults is summarized in Table 4. Light microscope radioautographs showed an initial uptake in the subtegument after a five-min incubation period. Label over the subtegument appeared heavier after two-hr incubation. After a four- to five-hr incubation period, tissues showed additional uptake in the cytoplasmic connections, the tegument, and the tegumental surface (Fig. 12). At the ultrastructural level, the labeling was distributed over the subtegument and the tegument.

Light microscope radioautographs of adult tissue slices incubated for five hr, as in the preceding section, and digested in α -amylase showed heavy labeling over the vitellaria, moderate labeling over the subtegument, and some labeling over the tegument. Radioautographs at the ultrastructural level showed silver grains associated with the subtegumental musculature, the tegumental inclusions, and the tegument.

Light microscope radioautographs of tissue slices from the tritiated mannose-sodium pyruvate incubation followed by β -amylase digestion exhibited label over the subtegument and underlying tissues and scattered label over the tegument. Ultrastructural radioautographs exhibited silver grains associated with glycogen deposits in the subtegument and subtegument musculature.

DISCUSSION

The results achieved with galactose utilization strongly suggest that this monosaccharide is absorbed primarily via the gut, although whole adults did show minimal labeling in the tegument after five minutes. The combined data from the whole worm and the tissue slice experiments along with the α -amylase and β -amylase digestions demonstrated that galactose is utilized in at least two ways by *S. mansoni*. First, the sugar is apparently absorbed by several organs including the parenchymal tissues, epimerized into glucose, and then incorporated into glycogen. This conclusion is supported by the fact that label can be removed from the worm tissue in large amounts via α -amylase digestion but not by β -amylase digestion. Such a pattern would be expected since Ressig and Bueding (30) reported that the glycogen in *S. mansoni* is much more highly branched than that found in other helminths and mammals. The digestion experiments also revealed that some label remained after exposure to either of the amylases. Coimbra and Leblond (21) utilizing rat liver exposed to either labeled glucose or galactose reported that label remaining after amylase digestion could be considered to be in glycoprotein, glycolipid, or complex polysaccharides. Since several previous investigations have reported the presence of acid mucopolysaccharides, glycolipids, glycoproteins, α -D-glucosyl carbohydrates, and other related compounds in the tegument and glycocalyx (6,7,8,13), it follows that *S. mansoni* may be utilizing galactose as a component of these tegumental macromolecules. This conclusion is further supported by the time-sequence studies on uptake and incorporation. In whole worms, the labeled molecules appeared first in the gut (30 min), then in the subtegument (60 min), and finally, in the tegument proper (five hr), where they often associated with teg-

umental inclusions. In tissue slices, the incorporation followed a similar pattern with the exception of the gut, thus suggesting absorption directly into the exposed tissue. Such data fit well with previous reports which demonstrated that tegumental components are manufactured in the subtegumental Golgi apparatus and transported to the tegument via inclusions or vesicles (6,7,13). Wilson and Barnes (14) reported that in *S. mansoni* the half-life for such vesicles is two to three hr. The time-sequence movement of label from the gut to the tegument reported here closely parallels Wilson and Barnes' (14) timetable.

The uptake and turnover of labeled galactose in female *S. mansoni* tegument does not follow the above patterns. Such results might be expected since it is known that the female is tightly sealed from the surrounding milieu when *in copula* (31). It is possible that the tegument of the female does not slough as does that of the male since there is much less need for defense against immunological attack. Such a hypothesis is borne out by the data presented, as most of the uptake in the female can be accounted for as glycogen or vitelline substances.

The results of the glucose experiments with whole worms confirm previously published results (1,2). The glucose was taken up by the tegument and accumulated with time. Also, as reported previously by Rogers and Bueding (2), the females absorbed the label at a lower rate than did the males. The tissue slices incubated in glucose absorbed this monosaccharide as expected. The pattern of absorption versus time differed, however, from that in whole worms in that the label accumulated first in the subtegument, then moved to the cytoplasmic connections, and finally to the muscle and tegument. Most of the label could be accounted for as glycogen via examination of the amylase digestion results and electron micrographs of the glycogen deposits. However, as with galactose, the time-interval samples and the lack of total digestion by α -amylase suggest that some of the glucose may be incorporated into tegumental components (21). It is likely though, that most of the glucose is incorporated into glycogen and is subsequently oxidized via glycolysis.

The results achieved utilizing mannose resembled those obtained with galactose but were even more dramatic. In the whole worms, mannose was absorbed by the gut, transported to the subtegument, and finally to the tegument. In tissue slices, as with galactose, uptake occurred first in the subtegument, bypassing the gut, and within four hr the tegument was labeled. Much of this label was associated with tegumental inclusions presumably generated by the subtegumental Golgi apparatus. Much, but not all, of this label can be removed by either α - or β -amylase digestion. These data fits well with the results of Bennett and Seed (8), who demonstrated that the concanavalin A sites on the surface of *S. mansoni* are α -D-glucosyl carbohydrates and that some of these sites have mannose as the terminal sugar; therefore, any digestion would remove the terminal monosaccharide. The gut-to-tegument timetable of four hr also fits well with Wilson and Barnes' (15) data that the half-life of the tegument proper is two to three hr.

The addition of sodium pyruvate to the mannose incubation produced two results. First, the rate of uptake was increased, and second, the tegumental surface was labeled. Such was not the case where pyruvate was lacking. The reasons for these differing results are not clear at this time. It is possible that mannose may be utilized in the formation of amino acids (20). The addition of sodium pyruvate may have flooded this system and thus allowed more mannose to be utilized in its original configuration. The digestion experiments demonstrated that in the presence of pyruvate more label remained in the subtegument, tegument, and tegumental inclusions than was the case in the absence of pyruvate. These experiments did confirm the presence of what is assumed to be labeled mannose in macromolecules on the tegumental surface.

Several conclusions are to be drawn from these experiments. In *S. mansoni* adults, monosaccharides are absorbed by both the tegument and the gut. Absorbed monosaccharides can be incorporated into macromolecules throughout the body of both the male and female worms, often in the form of glycogen. However, some of these labeled macromolecules are incorporated into vesicles by the subtegumental Golgi appar-

atus transported via these vesicles to the tegument, and incorporated into the tegumental matrix and surface. These macromolecules appear to play a role in tegumental structure.

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