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^{3}HOH-Osmotic Water Fluxes and Ultrastructure of an Epithelial Syncytium

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Summary. Ultrastructural changes associated with osmotically-induced water transport and water permeability were examined in two flatworm species, *Schistosoma mansoni* and *Hymenolepis diminuta*. The structure of the surface layer of these parasites is unusual in that it is a syncytial epithelial layer that lacks tight junctions and lateral extracellular spaces. The permeability coefficients observed in this study are therefore necessarily associated only with the transcellular route of transepithelial transport. The ultrastructural changes associated with volume transport across the epithelial syncytium were also unusual in that the basally located channels extending distally from the inward-facing membrane into the syncytial layer remained open regardless of the direction of water flow.

Despite the structural differences, most of the features of diffusive (P_d) and osmotic (P_{osm}) water fluxes across the syncytium resembled those observed in other epithelia: (i) Low water permeability with maximum values of 4.1×10^{-5} for P_d and 9.6×10^{-5} for P_{osm}. (ii) $P_{osm} > P_d$ by 2.0- to 3.2-fold. (iii) Outward water permeability less than inward water permeability. This asymmetry could not be attributed to collapsing channels when net volume transport was directed outward since channels in the syncytium remained open regardless of the direction of water flow. The asymmetry could be explained by tissue contraction or swelling when bathed in anisotonic fluids. (iv) P_{osm} values were not significantly altered by tissue unstirred layers but both P_{osm} and P_d values were underestimated when the bulk fluid was not vigorously stirred.

The lower permeability in *S. mansoni* relative to *H. diminuta* may be attributed to the membranous surface coat of the former species.

Key words. *Schistosoma mansoni, Hymenolepsis diminuta,* epithelial transport, water transport.

The flow of water across epithelial tissues has been the subject of intensive research over the past two decades, resulting in the discovery of a remarkable correlation between water transport and the geometry of fluid compartments within the epithelial layer. One of the unsolved problems discussed recently by Diamond (1979) is the inability of present techniques to distinguish between water flowing across the epithelial layer either by the paracellular route (across the tight junctions) or by the transcellular route (across the outward- and inward-facing membranes of the epithelial cells). One approach to this problem is to compare fluid movement across cellular epithelia with an epithelial layer that does not have the morphological prerequisites of a paracellular pathway. The present investigation uses an epithelial membrane system with the latter qualities. Although this approach will not aid in distinguishing the relative roles of paracellular and transcellular routes of water transport across epithelial with "tight" or "leaky" cell junctions, it does serve to characterize water transport across epithelia in the absence of tight junctions.

The epithelial layer covering the external surface of parasitic flatworms is a continuous layer of cytoplasm, bounded by outward- and inward-facing unit membranes that are uninterrupted by tight junctions and associated lateral extracellular spaces. This epithelial syncytium appears to be an adaptation to parasitism in this group of acoelous organisms in that, while the surface of parasitic forms is syncytial, the surface of free-living flatworms is "typically" cellular (Podesta, 1980*a, b*). However, the epithelial syncytium behaves similar to other solute transporting epithelia in having outward- and inward-facing membranes with asymmetrical chemical and functional properties capable of achieving vectorial transport of solutes across the epithelial layer. The epithelial syncytium therefore behaves in a manner consistent with the two membranes in series model of transporting

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epithelia proposed by Koefoed-Johnsen and Ussing (Podesta, 1980*a, b*).

Water transport across the epithelial syncytium has not been examined in detail. We previously examined the relationship between solute and water transport in the rat tapeworm, *Hymenolepis diminuta, in vivo* and found that the results could be interpreted in terms of the standing gradient, osmotic-flow hypothesis (*see* Diamond, 1979) (Podesta & Mettrick, 1975). However, the relationship between solute and water transport was unusual in that as both flows increased there occurred a disproportionate increase of solute inflow relative to the net inward flow of water, such that the absorbate became increasingly hypertonic. In addition, there appeared to occur a substantial inward movement of solute in the absence of net inward movement of water. This led us to interpret our results in terms of a fluid circuit through two sets of blind-ending channels, one set being "backward" or secretory, the other being "forward" or absorptive. Since this interpretation was not substantiated by morphological studies or in vitro determinations of water transport, the present study was undertaken.

Materials and Methods

Hymenolepis diminuta was obtained from the small intestine of male Wistar rats that had been infected with 30 cysticercoids from flour beetles (*Tribolium confusum*) 11 days prior to removal, as described previously (Podesta et al., 1977). At this age these parasites are each 30–70 mg wet wt and are approximately 20 cm in length.

Schistosoma mansoni (Puerto Rican strain) was obtained from the perfused hepatic-portal veins of hamsters that were infected 40 days previously by intraperitoneal injection of cercariae shed by infected snails (*Biomphalaria glabrata*), using conventional techniques. At this age these blood flukes are less than a cm in length, the females weighing 83 ± 1.0 µg, the males 251 ± 2.7 µg wet wt.

The diffusion of water across the epithelial syncytium of both parasite species was determined by monitoring the changes in the levels of ^{3}HOH in the parasite tissues during a second 30-min interval where the ^{3}HOH was present in the incubation fluid (10 µCi in 2 ml incubation fluid) during the second incubation (inward rate of exchange) or the tissues were loaded with ^{3}HOH during the first 30 min incubation (outward rate of exchange). Parasites were weighed at the beginning of the second incubation and six independent groups of parasites (one *H. diminuta,* five pairs of *S. mansoni*), were removed at 1-min intervals up to 10 min and at 5-min intervals thereafter. A balanced electrolyte solution (Podesta et al., 1977) was used throughout except in the second incubation in some experiments where the solution was diluted by 50 mOsm/liter or made hypertonic by the addition of 50 mM sucrose. The osmolality of the fluids was monitored with an osmometer (Instrumentation Laboratories). A nonabsorbable marker, ^{14}C-polyethylene glycol 4,000 (0.5 µCi in 2 ml) was used to correct the ^{3}HOH fluxes for label in the adherent surface volume (Podesta, 1977*a*; Podesta et al., 1977). After weighing and solubilizing (NCS, New England Nuclear) the parasites, ^{14}C and ^{3}H were determined by liquid scintillation using an equation derived previously to convert cpm determinations to units of volume flux, J_v (ml cm^{-2}) (Podesta et al., 1977). A weight to surface area relation determined in another study for *H. diminuta* was used in these calculations (Podesta, 1977*b*). Estimates of the outward diffusive permeability (P_d^o) and inward diffusive permeability (P_d^i) were determined as the rate coefficients ± SE of the slope from linear regressions of ln J_v against time, giving P_d in units of ml cm^{-2} sec^{-1}. The osmotic permeability of the epithelial layers (P_{osm}) were estimated by the steady-state volume fluxes determined from weight changes, giving P_{osm} in units of ml cm^{-2} sec^{-1} osmol^{-1} (cm sec^{-1} osmol^{-1}, *see* below). In some experiments using *H. diminuta,* the incubation fluid was vigorously stirred (710 rpm) using a magnetic stirring bar (Podesta, 1977*b*), but in all other experiments the parasites were incubated in a shaking water bath maintained at 37 or 21°.

For the morphological studies, parasites were incubated for 30 min in a shaking water bath at 37° in isotonic or anisotonic fluids as described in Table 1. Specimens were then removed and immersed in 2.5% glutaraldehyde with 2% sucrose in 0.05 M Na cacodylate at pH 7.4, and diced into 2–3 mm lengths. After 5–20 hr fixation, the tissues were washed in 0.05 M Na cacodylate with 4% sucrose and then post-fixed in Na cacodylate-buffered 1% OsO_4. This same procedure was followed for isotonic fixation and the osmolality of all fixatives were monitored with the aid of an osmometer. After post-fixation, the tissues were washed with distilled water and stained "en bloc" for 4–17 hr in aqueous 3.5% uranyl acetate, followed by dehydration in ethanol and propylene oxide and embedment in Araldite. Silver sections stained with uranyl acetate and lead citrate were viewed at 80 kV on a Philips 300 electron microscope.

Results

The ultrastructure of the epithelial syncytium of flatworms has been extensively reviewed (Hockley, 1973). The present ultrastructural studies confirmed our previous suggestion (Podesta & Mettrick, 1975) that the most obvious change in the structure of the epithelial syncytium in response to water transport would involve the space occupied by the infoldings of the inward-facing membrane (IFM). Figure 1 represents a section of the body surface of *S. mansoni* after removal from the portal system without subsequent incubation. The anucleate syncytial layer is 2–5 µm deep, bounded on the outward-facing aspect by a plasma membrane (OFM outward-facing membrane of epithelial syncytium) with numerous invaginations and covered by membranous layers. The IFM (inward-facing membrane of epithelial syncytium) consists of numerous finger-like projections (channels) extending into the syncytial layer of cytoplasm. The epithelial syncytium lies over a thin basal lamina and is connected by narrow internuncial processes to the nucleated cells which lie beneath and among the underlying muscle fibers. Although few mitochondria are located in the epithelial syncytium, numerous multilammelar bodies and elongate bodies are present. Spines are also present in the syncytium connecting at their base with the IFM and covered on the apical surface by the OFM.

Following 30-min incubations in isotonic saline,

the parasites were fixed for morphological examination in fixatives that were hypertonic (Fig. 2) or isotonic with respect to the incubation fluids (Fig. 3). As indicated in these figures and Table 1, incubation of *S. mansoni in vitro* resulted in an increase in the space occupied by the channels, and channel diameters were not altered by the tonicity of the fixative when compared to the changes observed in response to anisotonic incubations. Isosmotic replacement of saline with 150 mM sucrose (Fig. 4) caused a significant increase in channel diameters over control values (Fig. 2, hypertonic fixation after isotonic incubation), while the largest changes occurred in response to hypotonic (Fig. 5) and hypertonic (Fig. 6) incubations. Isosmotic replacement of saline with 20 mM glucose increased channel diameters to the same extent as that observed with 150 mM sucrose (Fig. 7).

The epithelial syncytium on the body surface of *H. diminuta* is similar to that of *S. mansoni* except that it is thicker (10–20 μm), spineless, and the OFM is a brush border rather than having apical invaginations as seen with *S. mansoni* (Lumsden, 1975). The basal region of the syncytium of *H. diminuta* is shown in Fig. 8 to lie over a basal lamina that is thicker (0.5–1.0 μm) than observed with *S. mansoni.* Swelling of the channels was observed to occur when the parasites were bathed in either hypotonic (now shown) or hypertonic fluids, although the channels do not extend as far into the much thicker syncytial layer of *H. diminuta.*

Since the channels swelled regardless of the direction of water movement across the epithelial syncytium, we looked without success at other tissue compartments. There were no consistent changes observed in the depth of the syncytial layer or in the underlying tissues. With *S. mansoni* the size of the spaces made by the invaginations of the OFM varied considerably but not with any consistent pattern. As described in the experiments to follow, the tissues as a whole undoubtedly swelled or contracted depending on the tonicity of the bathing fluids, but these changes could not be associated with any specific tissue compartment.

The ^{3}HOH and osmotic transport studies indicated that the epithelial syncytium of *H. diminuta* and, especially, *S. mansoni* may be characterized as having a low permeability to water (Table 2) – consistent with the hypothesis that the syncytium is an extreme example of a "tight" epithelium (Podesta, 1980*a*). For example, P_{osm} for "leaky" epithelia are in the 10^{-2}–10^{-3} range while tight epithelia have P_{osm} values in the range of 10^{-5}–10^{-6} (Phillips, 1977; Diamond, 1979).

Comparisons between the species indicated that the P_d^o of *H. diminuta* was three- to fourfold (unstirred) greater than that observed with *S. mansoni*, while the P_d^i values were two- to threefold greater and P_{osm} threefold greater in the former species (Table 2). Due to the small size of *S. mansoni* it was not possible to hold this species stationary in a well-stirred incubation fluid.

In both species the P_d^i was slightly higher than the P_d^o when the parasites were bathed in isotonic fluids at 37 °C. To test whether the P_d values were altered by osmotic flow of water, glucose was added to the incubation fluid. Glucose increased the inward and outward rate of exchange of ^{3}HOH ($P<0.05$), which is consistent with the well known observation of glucose-stimulated ion transport in both these species (Podesta, 1980*a, b*). However, when the temperature of the incubation fluid was lowered to 21°, the effect of glucose (hence, osmotic water flow) was minimized since both the P_d^i and P_d^o were reduced to levels equivalent to or below those observed without glucose at 37°. All further experimentation was carried out at 21° to minimize the effect of solute-coupled water fluxes. In this respect, the P_d^i values were generally greater than P_d^o values, except when the direction of osmotically induced water movement favored the outward flow of ^{3}HOH.

When the incubation fluid was unstirred the P_{osm}/P_d ratios (where P_d is in the same direction as volume flow) were approximately 2.1 in *S. mansoni* and 2.9 in *H. diminuta* (Table 2). In both species the osmotic permeabilities were asymmetrical, the inward P_{osm} exceeding the outward P_{osm} by 2.7-fold in *S. mansoni*

Table 1. Diameters of channels in the epithelial syncytium of *Schistosoma mansoni* in response to anisotonic incubations

Incubation fluid	Osmolality of fixative	Diameter of channels	
		X ± SE (nm)	(*n*)
Unincubated	Hypertonic	13 ± 0.64[a]	(22)
Balanced Electrolyte Solution	Hypertonic	187 ± 26.8	(25)
	Isotonic	137 ± 24.4	(25)
1/2 Balanced Electrolyte Solution plus 150 mM Sucrose	Hypertonic	306 ± 38.6[a]	(25)
1/2 Balanced Electrolyte Solution (150 mOsm liter^{-1})	Hypertonic	647 ± 77.2[a]	(25)
Balanced Electrolyte Sucrose	Hypertonic	414 ± 41.2[a]	(25)
Balanced Electrolyte Solution plus 20 mM Glucose	Hypertonic	311 ± 78.6[a]	(25)

[a] Significantly different (at least a 5% level of significance using Student *t* test) from results using balanced electrolyte solution and hypertonic fixative.

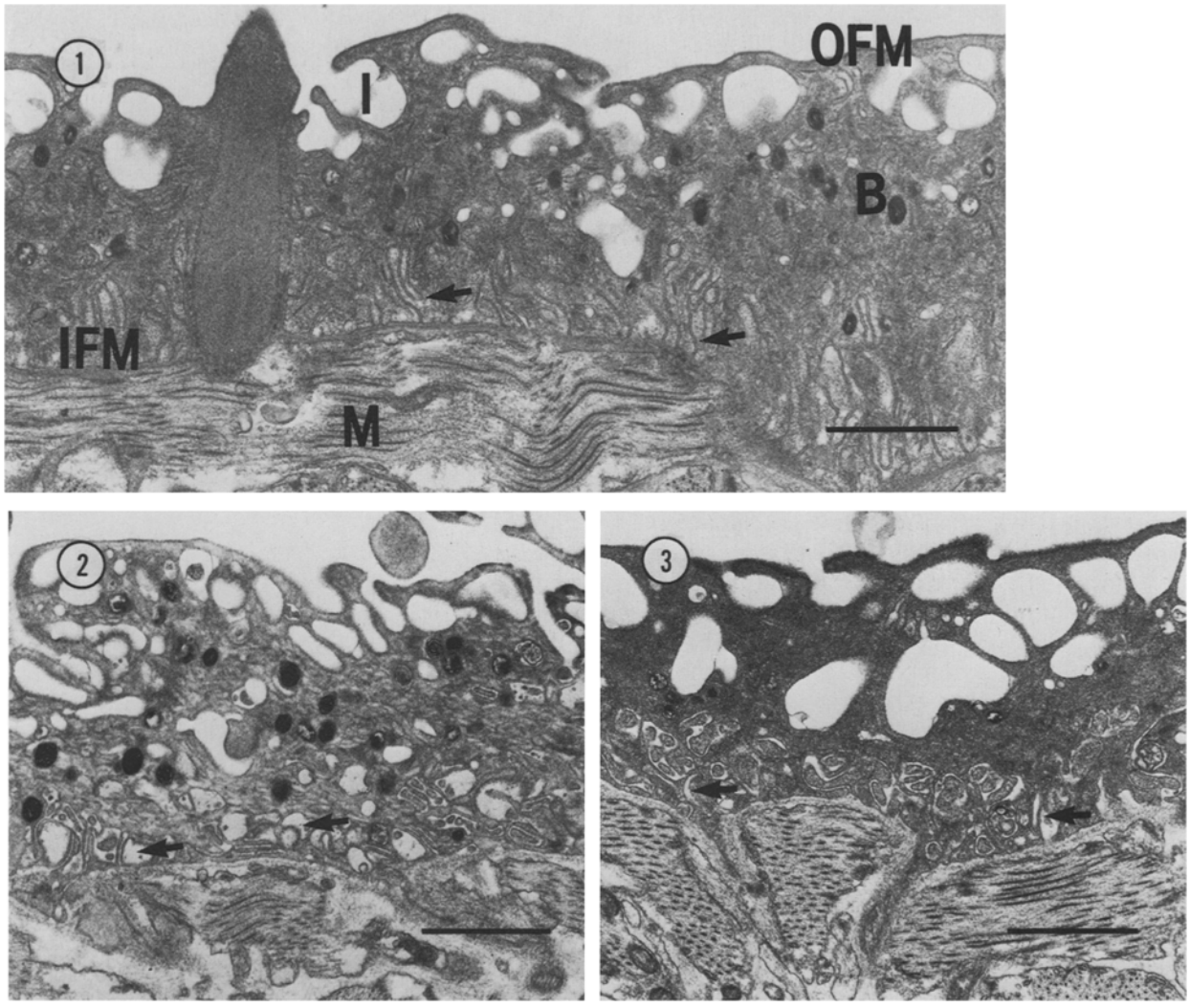

Fig. 1. General ultrastructure of syncytium of *S. mansoni* fixed without prior *in vitro* incubation. Note numerous channels (arrows) of *IFM*. *OFM* invaginations, *I;* multilamellar bodies, *B*; underlying musculature, *M*. ×18,000. Bar = 1 μm

Fig. 2. Syncytium of specimens fixed in hypertonic fixative after incubation in balanced electrolyte solution. The channels (arrows) are swollen slightly when compared to unincubated control tissue. ×18,000. Bar = 1 μm

Fig. 3. Syncytium of specimens fixed in isotonic fixative after incubation in balanced electrolyte solution. The extent of channel swelling (arrows) is not significantly different from hypertonic fixation. ×18,000. Bar = 1 μm

and by 1.7- and 2.3-fold in *H. diminuta* in unstirred and stirred experiments, respectively.

Stirring the fluid bathing *H. diminuta* increased P_d^o by only 5% but the P_d^i was increased by 74% when there was no accompanying volume flow. When ^{3}HOH exchange was examined in the presence of net outward volume flow stirring increased P_d^o by 78% and P_d^i by 2.2-fold when accompanied by net inward volume flow. Stirring the incubation fluid also increased the outward P_{osm} by 26% and the inward P_{osm} by 71%.

The transient changes in osmotic water flow observed in other epithelia, where the initial rates (measured over the first 5 min of incubation) of volume transport in response to osmotic gradients were several times greater than the steady-state values (*see* Diamond, 1979), were also observed in the present study. However, the initial rates of water flow during the first few minutes of incubation never exceeded the steady-state values reported in Table 2 by more than a factor of 1.8. To determine whether our values for P_{osm} were underestimated by unstirred layers in the tissues underlying the epithelial syncytium, we used the analysis published recently by Pedley and Fisch-

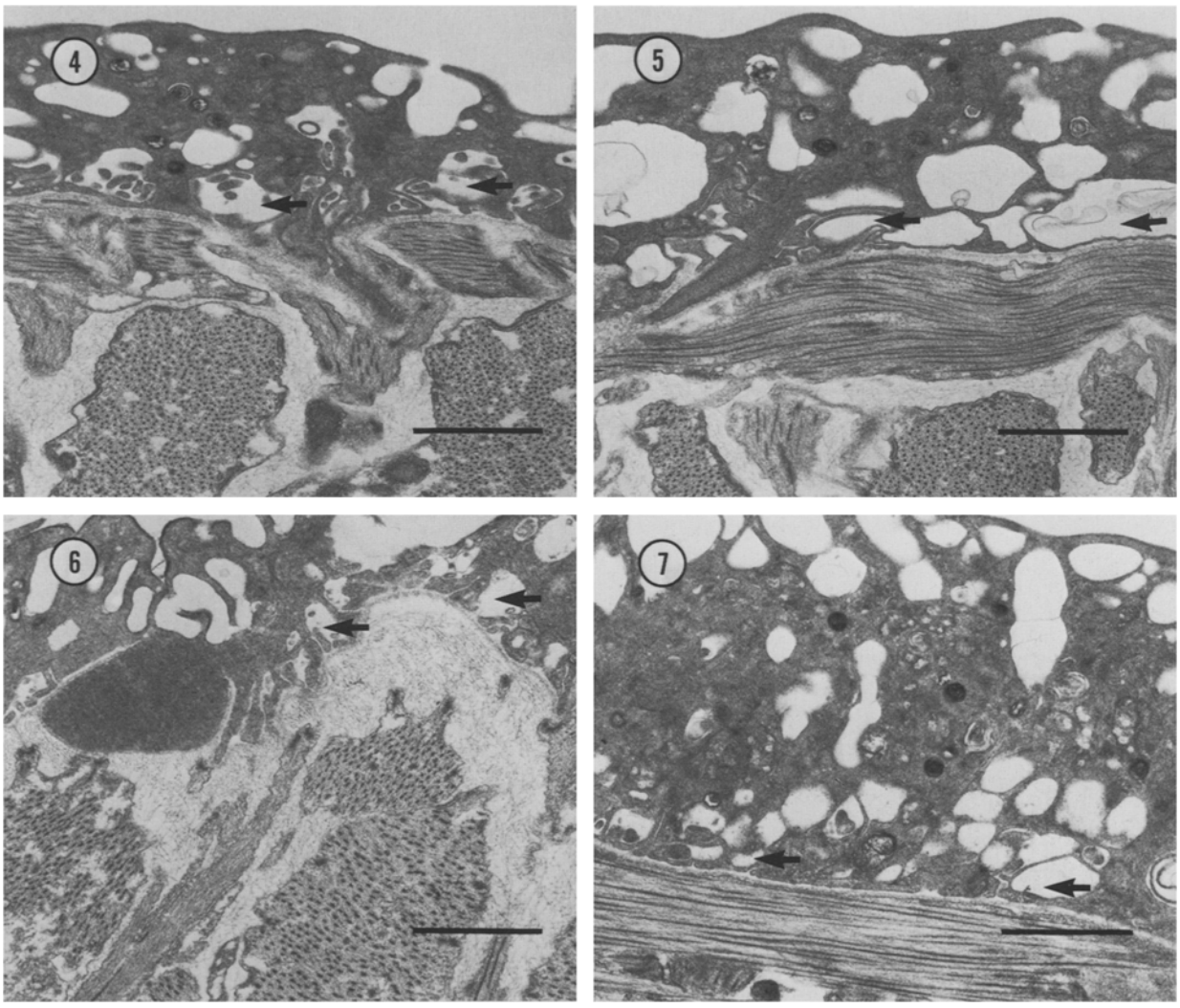

Fig. 4. Isosmotic replacement of saline with 150 mM sucrose in the incubation medium caused a significant increase in channel diameter (arrows) when compared to control images (Fig. 2). ×18,000. Bar=1 μm

Fig. 5. Syncytium exposed to a hypotonic incubation medium. Note the very large channel diameters (arrows). ×18,000. Bar=1 μm

Fig. 6. Syncytium exposed to a hypertonic incubation medium. Large channel diameters (arrows) are characteristic. ×18,000. Bar=1 μm

Fig. 7. Isosmotic replacement of saline with 20 mM glucose in the incubation medium caused a significant increase in channel diameter (arrows) when compared to control images (Fig. 2). ×18,000. Bar=1 μm

barg (1980) to calculate γ – the ratio of the measured water flux to the water flux which would be expected to occur in the absence of tissue unstirred layers. Using this analysis and assuming unstirred layers in the range of 50–500 μm (well within the range expected for the two parasite species), the steady-state osmotic water fluxes observed in the present study were all within 5% of those predicted on the basis of no tissue unstirred layers. This indicated that tissue unstirred layers may not affect the determination of water permeability in the flatworms examined in the present study.

Discussion

Despite the unusual structure of the surface epithelial syncytium of *S. mansoni* and *H. diminuta*, several features of fluid transport across this barrier are similar to those which characterize fluid transport across more intensively studied vertebrate epithelia.

As in other "tight" epithelia (Frömter & Diamond, 1972; Phillips, 1977), the P_d and P_{osm} values obtained in the present study for the epithelial syncytium are lower than those obtained from epithelia whose junctions are leaky. Compared to *Dugesia dor-*

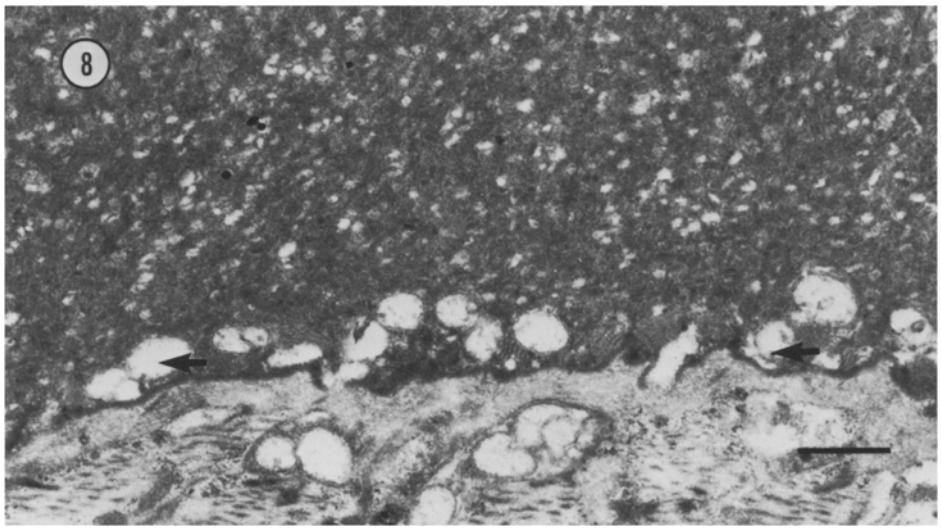

Fig. 8. Syncytium of *H. diminuta* exposed to a hypertonic incubation medium. Swollen channels (arrows) are evident. ×18,000. Bar = 1 μm

Table 2. Outward (P_d^o) and inward (P_d^i) diffusive and osmotic (P_{osm}) water permeability of the surface epithelial syncytium of *Hymenolepsis diminuta* and *Schistosoma mansoni*

Incubation fluid	P_d^o	P_d^i	P_{osm}	Ratio[a]
	($\times 10^{-6}$ cm sec^{-1})		($\times 10^{-6}$ cm sec^{-1} osmol^{-1})	
S. mansoni				
300 mOsm liter^{-1}, 37°	2.4±0.16	4.9±0.17		
300 mOsm liter^{-1}, 37° (5 mM glucose)	3.6±0.13	5.5±0.12		
300 mOsm liter^{-1}, 21° (±5 mM glucose)	1.7±0.10	5.1±0.11		
350 mOsm liter^{-1}, 21°	4.1±0.18	3.8±0.11	9.9±1.22	2.4
250 mOsm liter^{-1}, 21°	2.9±0.14	13.8±0.18	27.1±3.36	2.0
H. diminuta				
300 mOsm liter^{-1}, 37°	8.5±0.19	10.9±0.16		
300 mOsm liter^{-1}, 37° (5 mM glucose)	9.8±0.15	11.6±0.12		
300 mOsm liter^{-1}, 21° (*US*)[b] (± 5 mM glucose)	7.8±0.09	10.1±0.17		
300 mOsm liter^{-1}, 21° (*S*)[b]	8.2±0.15	17.6±0.13		
350 mOsm liter^{-1}, 21° (*US*)	10.3±0.10	7.9±0.15	32.7±3.32	3.2
350 mOsm liter^{-1}, 21° (*S*)	18.3±0.13	6.0±0.15	41.1±3.28	2.3
250 mOsm liter^{-1}, 21° (*US*)	8.8±0.11	19.2±0.18	56.4±4.34	2.9
250 mOsm liter^{-1}, 21° (*S*)	9.6±0.12	41.3±0.22	96.2±6.41	2.3

[a] Ratio of P_{osm} to P_d when the latter is in same direction as P_{osm}.
[b] *US*, unstirred; *S*, stirred bulk fluid.

otocephala, a free-living flatworm with a cellular surface epithelial layer, the outward diffusive permeability of the syncytial layers of *S. mansoni* and *H. diminuta* are also low with a value of 2.38×10^{-5} cm sec^{-1} for the former species (Prusch, 1976) and $1.7–2.4 \times 10^{-6}$ cm sec^{-1} and $7.8–8.5 \times 10^{-6}$ cm sec^{-1} for the latter two species, respectively. However, due to the absence of a paracellular pathway in the epithelial syncytium, we are assured in the present study that the values obtained represent the combined permeabilities of the OFM and IFM. Pedley and Fischbarg (1980) have recently discussed the possibility that the steady-state measures of P_{osm} in rabbit gallbladder may represent the osmotic permeabilities of the cell membranes. If this is correct, then the cell membranes of the leaky gallbladder epithelium will be much more permeable ($1.4–4.7 \times 10^{-3}$ cm sec^{-1}) than the membranes examined in the present study ($9.9–27.1 \times 10^{-6}$ cm sec^{-1}, *S. mansoni;* $3.27–9.62 \times 10^{-5}$ cm sec^{-1}, *H. diminuta*).

Another characteristic of fluid transporting epithelia with which the data of the present study are

consistent, is the observation that osmotic permeabilities are greater than the diffusive permeabilities (Frömter & Diamond, 1972; Schafer & Andreoli, 1972). The ratios of P_d/P_{osm} obtained for *S. mansoni* and *H. diminuta* were 2.0–3.2 compared to 4.8 in toad bladder and 5.3 in toad skin (Schafer & Andreoli, 1972).

The asymmetric osmotic and diffusive permeability properties of the epithelial syncytia is another feature of water permeability in *S. mansoni* and *H. diminuta* that has been observed in other fluid transporting epithelia. In the rabbit gallbladder, the asymmetry has been attributed to the collapsing of lateral intercellular spaces when fluid traverses the epithelial layer in a serosal to mucosal direction. This results in the osmotic permeability of the epithelial layer being less in the secretory direction than in the absorptive direction (van Os, Wiedner & Wright, 1979; Diamond, 1979; Pedley & Fischbarg, 1980). However, this cannot be the explanation for the similar asymmetry observed in the present study since the epithelial syncytium lacks a paracellular pathway and the channels stemming from the IFM remained open regardless of the direction of the net fluid movement. As suggested recently by van Os et al. (1979) and Pedley and Fischbarg (1980) with reference to rabbit gallbladder, the asymmetric osmotic permeabilities in the present study are probably a reflection of tissue swelling and contraction when the parasites were incubated in anisotonic fluids (*see* below).

The lower water permeability obtained with *S. mansoni* relative to *H. diminuta,* is probably a reflection of the unusual "surface coat" covering the surface of the OFM. Although the composition of the surface coat is not known, it appears ultrastructurally as stacks of trilaminate membranes up to 15 layers but is usually pentalaminate or heptalaminate (Hockley, 1973). Recent studies have indicated that the surface coat of *S. mansoni* is predominantly phospholipid (S.S. McDiarmid & R.B. Podesta, *unpublished*) and may therefore be expected to lower the water permeability of the epithelial syncytium in this parasite species. Indeed, the P_{osm} and P_d values obtained for *S. mansoni* are lower than those reported from phospholipid membranes (Schafer & Andreoli, 1972).

The most notable exceptions to current models of fluid transport across epithelia (*see* Diamond, 1979) were the lack of junctions in the epithelial syncytium and the observation that basal channels remained open regardless of the direction of fluid movement across the epithelial syncytium. In a previous study using *H. diminuta*, the latter observation was predicted as the only explanation for the increasing solute to volume flux ratios obtained as net inward ion transport was accelerated in response to CO_2 and glucose in the bathing fluid (Podesta & Mettrick, 1975). However, since the channels formed by the IFM were assumed to be secretory (Diamond, 1971), it was also necessary for the channels to work in conjunction with another tissue compartment to achieve a hypertonic absorbate. In the present study we were not able to attribute a fluid transport role to any single compartment.

On the basis of weight determinations, there appeared to be a general contraction or swelling of the tissues in response to osmotically induced water movement across the epithelial syncytium. This would be expected if, as suggested recently (Podesta, 1980*a, b*), the tissues underlying the epithelial syncytium were connected to it and to each other by electrically coupled gap junctions. This appears to occur in both *H. diminuta* and *S. mansoni* (Podesta, 1980*a*; Thompson, Bricker & Pax, 1980) and would tend to evenly distribute volume changes throughout the cells of the parasite tissue.

Summarizing, the major contribution of this study appears to involve the observation that blind-ending channels in an epithelial syncytium remain open regardless of the direction of osmotic water flow across the epithelial layer. The asymmetric P_{osm} valves obtained in this membrane system cannot therefore be due to collapsing channels as suggested for other epithelia (*see* Diamond, 1979). The explanation for this effect is incumbent upon a detailed analysis of the whole tissue swelling or contraction when the parasites are bathed in anisotonic fluids.

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