photometry with tyramine as the substrate, based on the photometric micromethod (*12*). Tyrosine hydroxylase activity was determined by measuring the formation of [^{14}C]dopa from L-[^{14}C]tyrosine (*13*).

Table 1 shows DBH activity in serum, mesenteric vessels, vas deferens, and adrenals of SH rats and the control Wistar-Kyoto rats. The DBH activity in serum, mesenteric vessels, and adrenals of SH rats was significantly higher than those of the controls, but that in vas deferens was not statistically different in these two strains of rats. Serum DBH is known to arise from sympathetic nerve terminals and not from adrenals (*14*), and a significant fraction of circulating norepinephrine is considered to be derived from blood vessels (*15*). The results, therefore, suggest that the elevated DBH activity in serum of young SH rats is derived mainly from the blood vessels, reflecting the activated peripheral sympathetic nerves. This also agrees with our previous results that serum DBH activity and DBH and TH activities in mesenteric vessels in 10-week-old SH rats increase significantly when blood pressure is increased rapidly by administration of NaCl for 4 weeks (*16*).

The DBH and TH activities in the brain regions of catecholaminergic neurons are given in Table 2; DBH activity was found only in the regions of noradrenergic neurons, such as the locus coeruleus and hypothalamus. Very low DBH activity in the dopaminergic regions, such as caudate nucleus and substantia nigra, may be due to accurate dissection of the anatomical region. Low DBH activity in the caudate nucleus has been reported (*17*). In contrast, TH activity was found in both dopaminergic and noradrenergic brain regions, although it was much higher in the former.

In the locus coeruleus DBH activity of SH rats was significantly lower than that of Wistar-Kyoto rats, but in the hypothalamus there was no statistical difference in DBH activity between the SH and the control rats.

The TH activity in the substantia nigra, caudate nucleus, and locus coeruleus was similar in SH and Wistar-Kyoto rats; the activity in the hypothalamus of SH rats was higher than that of Wistar-Kyoto rats but does not differ significantly ($P < .1$). Since Lamprecht *et al.* (*18*) also reported that after 4 weeks of immobilization stress to rats there was a significant increase in the activity of hypothalamic TH and in the activity of serum DBH, the significance of a slightly higher TH value in the hypothalamus remains to be investigated further.

The present findings on the abnormality of central noradrenergic neurons of SH rats may be significant, since such central changes were detected in young SH rats whose peripheral sympathetic nerve activity, as judged by DBH activity in blood vessels and serum, appears to be increased. After the development of hypertension, the peripheral sympathetic tone of SH rats appears to be decreased, probably by a compensatory mechanism (*8, 19*). The noradrenergic neuron in the brainstem is believed to play a role in depression of peripheral sympathetic nerves through an alpha receptor to decrease the blood pressure (*9*), whereas that in the hypothalamus may play a role in activation of peripheral sympathetic nerves through a beta receptor to increase the blood pressure (*10*). Probable implication of the central noradrenergic inhibitory center in hypertension of SH rats had first been suggested by Yamori *et al.* (*20*). However, we need evidence to show whether there is a relation between changes in catecholamine synthesizing enzymes in the brain and their increase in peripheral tissues.

Although care is necessary in relating the present results to hypertension, they do suggest that central and peripheral sympathetic nerve function may be changed before the onset of essential hypertension, and, therefore, should be examined at this early period, and they support the concept that the nervous system is an important regulator of blood pressure.

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Attachment and Penetration of Miracidia Observed by Scanning Electron Microscopy

Abstract. *Scanning electron microscopy can be utilized to understand more clearly many aspects of the parasite-host relationships of schistosome miracidia and their molluscan intermediate hosts. Specialized structures on the apical papilla of the miracidium, used for attachment and penetration, become visible in greater detail.*

Few biologists, including those working with parasites, have observed actual penetration of a snail by the motile and nonfeeding larval stage of a digenetic trematode called the miracidium. The small size of the parasite, the relatively short penetration time, and the continual movement of the snail intermediate host make this parasite-host contact difficult to witness.

Recent developments with scanning and transmission electron microscopes have made possible more extensive research on the mechanisms of miracidial attachment and penetration. Penetration and devel-

opment of the miracidia of a liver fluke, *Fasciola hepatica* Linnaeus, 1758, and the human blood fluke, *Schistosoma mansoni* Sambon, 1907, were observed with light microscopy in the 1960's by Dawes (*1*) and Wajdi (*2*), respectively. In more recent studies with various species of trematodes the transmission electron microscope has been used to describe morphological features of the miracidial plates and anterior apical papilla (*3–5*). Recently, LoVerde (*6*) demonstrated topographical features of the *Schistosoma mansoni* and *S. haematobium* (Bilharz, 1852) miracidia with the scanning electron microscope (SEM).

Miracidia of two nonhuman schistosomes, *Gigantobilharzia huronensis* Najim, 1950, and *Schistosomatium douthitti* Price, 1931, were selected for this study for the following reasons: (i) the mechanisms of attachment and penetration are probably similar to those of related human schistosomes (*Schistosoma*), the causative agents of a most important subtropical and tropical disease called schistosomiasis; (ii) a condition known as swimmer's itch or cercarial dermatitis, found throughout the world, is produced by these two, or related, species; (iii) attachment and penetration is very dramatic with these species; and (iv) small host snails (*Physa* sp.) are able to survive numerous penetrating miracidia.

In this report we demonstrate various degrees of penetration into a snail's tegument by a miracidium of *G. huronensis* with the SEM. The attachment and penetration apparatus of a rodent schistosome, *Schistosomatium douthitti*, is also shown.

Schistosome miracidia gain entrance into the proper molluscan intermediate host by direct penetration through the tegument. Within the mollusk, the miracidium elongates to form a germinal sac called a sporocyst. A second generation of sporocysts, called the daughter sporocysts, gives rise to cercariae, the infective stage to man. After penetration, the cercariae develop to adults in the venous system of man. The life cycle is completed when eggs from adult worms reach suitable aquatic habitats.

The apical papilla, located at the anterior end of the miracidium, contains the greatest concentration of sensory structures. Brooker (*5*) described three sets of ciliated nerve endings distributed around the apical papilla of a *Schistosoma mansoni* miracidium. Four to six receptors, each bearing a single terminal cilium, are located on each of the two anterior sets. The third set is located just posteriad and is composed of six ciliated-pit nerve endings. It appears that miracidia of *Schistosomatium douthitti* have a similar arrangement. Figure 1 shows the two anterior sets, each with at least four ciliated nerve endings. We were unable to discern the third set (ciliated-pit nerve endings) of sensory structures on the micrographs because of the unsatisfactory orientation of the miracidium. The function of these receptors is still tenuous; however, it is thought that some may function as chemoreceptors and others as tangoreceptors (tactile).

In addition to the sensory structures, the apical papilla contains branching and anastomosing structures at the distal end called microvilli or apical filaments (*3*) that have been described by various investigators (*6, 7*). It has been suggested that these structures can form suckerlike cups (*6*) or a minisucker pad (*3*) that the miracidium uses to attach to the tegument of the snail. At the base of the apical papilla, the microvilli end and the cilia of numerous epidermal plates begin (Fig. 1).

For success in attachment and subsequent penetration, several criteria must be met. First, the miracidium must come in contact with the proper molluscan host. When experimentally placed in a vial of pond water containing a young *Physa gyrina* (Say) snail, the miracidium of *G. huronensis* swims at random until it comes in close contact with the soft parts of the snail. Its pattern of swimming then becomes swifter and more erratic. The "excited" behavior probably enhances its chance for contact with the mollusk. If this snail or clam is a suitable intermediate host, the miracidium will attach, penetrate, and then develop into sporocysts within the mollusk. In some instances, actual attachment and subsequent penetration by the miracidium may occur without any further development in the parasite life cycle.

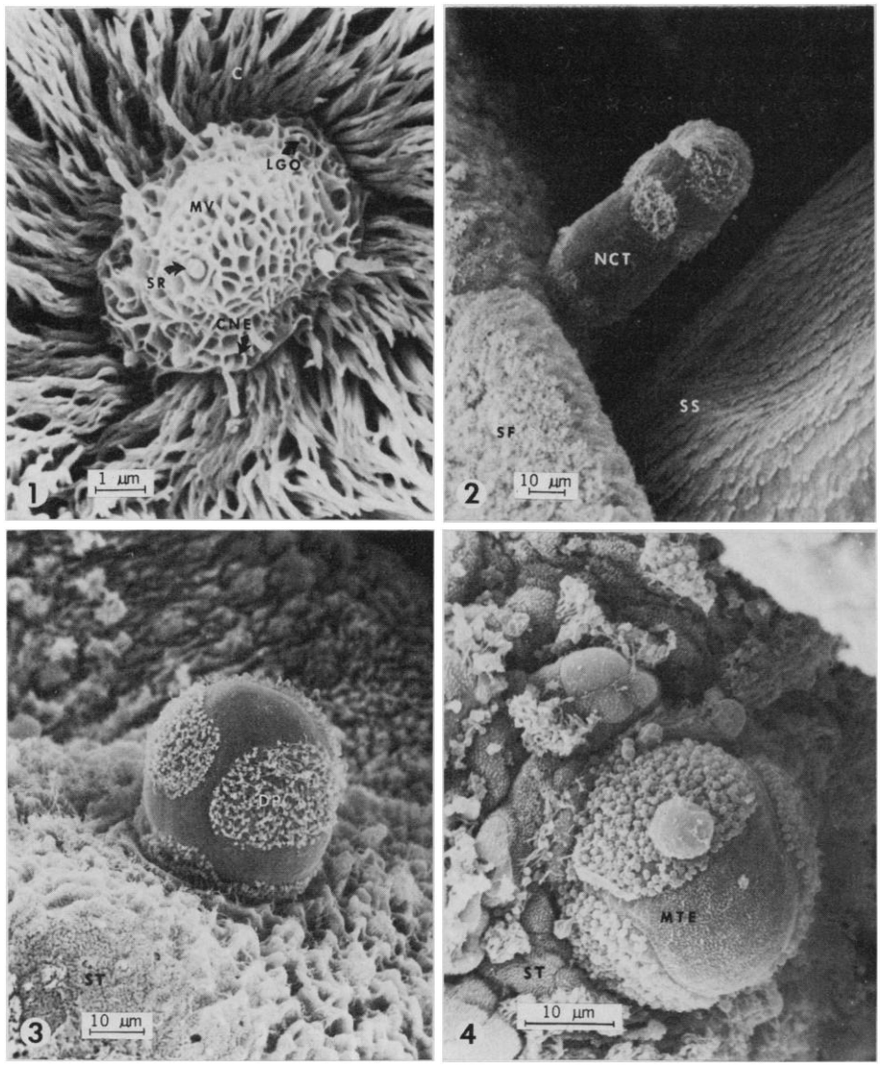

Fig. 1. Anterior view of the apical papilla of the miracidium of *Schistosomatium douthitti* (*8*); *C*, cilia; *CNE*, ciliated nerve ending; *LGO*, lateral gland opening; *MV*, microvilli; *SR*, sensory receptor. Fig. 2. Miracidium of *G. huronensis* on the foot of a young snail, *P. gyrina*, 1 minute after attachment; *NCT*, nonciliated tegument; *SF*, snail foot; *SS*, snail shell. Fig. 3. Miracidium of *G. huronensis* penetrating a young snail, *P. gyrina*, 2.5 minutes after attachment; *DP*, dermal plate of miracidium; *ST*, snail tegument. Fig. 4. Miracidium of *G. huronensis* in a young snail, *P. gyrina*, 3 minutes after attachment; *ST*, snail tegument; *MTE*, tegument evaginations of miracidium.

When the miracidium approaches an unsuitable candidate snail host, or an unsuitable portion such as the shell of a natural intermediate host, it appears to probe the surface with its apical papilla before backing off and swimming away. In laboratory exposures, the snail is sometimes able to dislodge attached miracidia by contraction of its head or foot against the shell. When this happens, the detached miracidia will swim away.

Figure 2 illustrates a *G. huronensis* miracidium on the foot of a young *P. gyrina* snail 1 minute after attachment. While attached, it continues to be very active, extending and contracting in an effort to gain entrance into the snail. Once penetration begins, it can be completely enclosed in the host's tegument within a few minutes. The tegument of the miracidium has many dermal plates covered by cilia (Figs. 2–4). The cilia curl during fixation, appearing as white spheres on the dermal plates. The areas lying between the dermal plates lack cilia but contain small tissue evaginations.

Advanced stages of penetration by the miracidia are shown in Figs. 3 and 4. In Fig. 3 the miracidium appears to be halfway into the host tissue, with only the posterior portion of the middle row of dermal plates and the posterior row still visible. In Fig. 4 penetration is nearly complete, and in less than a minute the miracidium will have disappeared. The tegument of the snail surrounding the miracidium then appears to reseal, with the tissue completely filling the opening left by the penetrating miracidium. Although we examined nearly a hundred snails after penetration, the absence of such openings strongly suggests that penetration leaves no scars.

Scanning electron microscopy not only allows investigators to examine the snail's tegument and the attachment apparatus in topographical detail not seen with light or transmission electron microscopes, but it also may provide information essential to biochemists, malacologists, physiologists, and behaviorists. These specialists need to combine their efforts to assist in finding methods of controlling schistosomiasis, a disease that now is of worldwide significance.

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8. Material was fixed in 3 to 5 percent buffered formalin, gradually dehydrated in ethanol, transferred into increasing concentrations of amyl acetate, and then dried at the critical point with carbon dioxide. Specimens were mounted on metal stubs and then coated with approximately 200 to 300 Å of gold. Micrographs were taken with a Jeol-JSM-U3 scanning electron microscope.
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What Retains Water in Living Cells?

Abstract. *Three types of evidence are presented showing that the retention of cell water does not necessarily depend on the possession of an intact cell membrane. The data agree with the concept that water retention in cells is due to multilayer adsorption on proteins and that the maintenance of the normal state of water relies on the presence of adenosine triphosphate as a cardinal adsorbent, controlling the protein conformations.*

There are two opposing views on the mechanism controlling the retention of water in the cell. In one view the major instruments for cell water retention are the cell membrane and a battery of postulated pumps in the membrane (*1*), which regulate the total amount of free solutes and hence osmotic activity in the cell; osmotic activity in turn determines intracellular water content. With the exception of a small percentage in the form of water of hydration on proteins and other macromolecules, cell water itself is considered to be in the free state. Metabolism provides adenosine triphosphate (ATP) as a fuel to operate the pumps. In this view, the integrity of the cell membrane is essential for the retention of cell water.

According to an alternative view, the association-induction hypothesis, the bulk of cell water is retained in the cell in a physical state different from that in a dilute aqueous solution; the major instrument for the regulation of the cell water is the cell proteins (*2*, *3*). The maintenance of cell proteins in a certain "extended" conformation, where the backbone peptide groups polarize, orient, and retain deep layers of water, depends on the adsorption of ATP on certain controlling "cardinal sites." According to this hypothesis, the function of metabolism is to provide ATP,

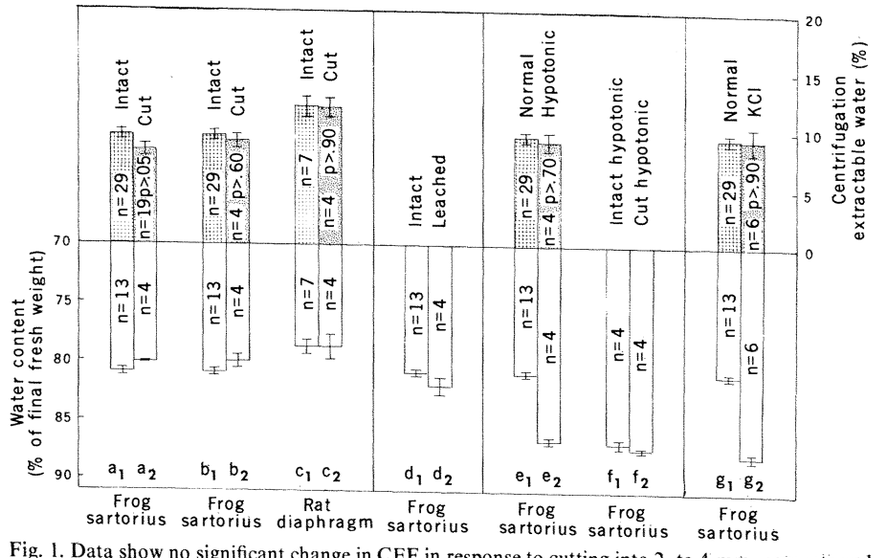

Fig. 1. Data show no significant change in CEF in response to cutting into 2- to 4-mm segments and to swelling brought about by exposure to (e) hypotonic solution (osmolarity 20 percent that of normal Ringer solution, 4 hours at 0°C) and (g) a high KCl concentration (93 m*M*, 72 hours at 4°C). Muscles of b_2 were incubated in a Ca-free Ringer solution containing 1 m*M* ethylenediaminetetraacetic acid (EDTA) for 30 minutes at 4°C before cutting. (d) Removal of intracellular solutes produced no significant change in the amount of water retained in muscles after centrifugation at 1000*g* for 4 minutes. (f) Similar water contents of intact and cut muscles after exposure to hypotonic solution (osmolarity 40 percent that of normal Ringer solution, 5 hours at 0°C) but in this case the muscles were blotted but not centrifuged. The letters a_1, a_2, b_1, b_2, and so on are experiment designations for easier reference.