PN-ABF-130 936-5053

66160

BIODETERIORATION RESEARCH 2: 661-682
Plenum Press, New York, 1989

SURFACE ULTRASTRUCTURAL CHANGES IN THE TEGUMENT OF *CYSTICERCUS FASCIOLARIS* AND *SCHISTOSOMA MANSONI* FOLLOWING *IN VITRO* LOW DOSES OF THE ANTIHELMINTHIC DRUG, OLTIPRAZ

BETTY R. JONES*, Department of Biology, Parasitology and Electron Microscopy Laboratory and the Atlanta University Center Scanning Electron Microscopy Research Laboratory, Morehouse College, Atlanta, GA 30314, USA
RANDOLPH TAYLOR, Department of Biology, Atlanta University, Atlanta, GA 30314, USA

INTRODUCTION

Cysticercus fasciolaris is the larval form of the adult cat tapeworm, *Hydatigera taeniaeformis* (Taenia taeniaeformis). It is of increasing interest because of the considerable pathological conditions produced in a rat or mouse host in which it is normally found. The larvae encapsulate and develop on the liver of rats and mice and as a result of this infection, a serious cancer-like growth may develop (Noble and Noble, 1974).

The surface tegument of cestodes like that of schistosomes is a highly active layer in which many processes occur (Smyth 1969, 1966). It is a metabolically active syncytial, protoplasmic layer formed by cytoplasmic extensions from tegumental cells lying in the subtegumental zone. The tegument has two major components, namely the distal cytoplasmic zone (surface syncytium) and the perinuclear cytoplasmic zone (cytoplasm in the region of tegumental cells). Under the light microscope the surface of the distal cytoplasmic zone appears as a fringe of fine hair-like processes (Smyth, 1969, Jones et al. 1977a, 1977b, Lumsden and Specian, 1980, Threadgold, 1984). At the electron microscope level, the distal surface is revealed as spine-like processes which morphologically resemble the brush border of many vertebrate and invertebrate cells and have been referred to as microtriches, microvilli, or tegumental projections (Smyth, 1969, Shivers et al., 1986).

The tegument functions to: (1) mediate the absorption of nutrients by the worm from the host environment, (2) protect the integrity of the worm against the alien environment of its host, and (3) bear the

immunological mechanisms whereby the parasite is able to resist inactivation by antibodies produced against the invader by its host (Lumsden and Specian, 1980).

As with most active drugs for parasitic diseases, the active drug form, structure-function relationships, metabolites, mode and site of action on the parasite's surface tegument are poorly understood (Fifth Annual Report on Research and Training in Tropical Diseases, 1981).

In recent years, interest has developed in two new antihelminthic drugs, praziquantel (2-cyclohexylcarbonyl-1, 2, 3, 6, 7 11b-hexahydro-4-H-4-pyrazinol-[2,1a]-isoquinolin-4-one) and oltipraz (4-methyl-5-[2-pyrazinyl]-3H-7,2-dithide-3-thinoel). Praziquantel has been found effective in a large number of cestodes; Hymenolepis nana cysticercoids; C. fasciolaris in mice; C. pisiformis in rabbits; Echinococcus granulosus in dogs; C. tenuicollis in sheep and pigs; C. ovis in sheep; C. bovis in cattle and C. cellulosae in pigs (Thomas et al., 1978; Hochner et al., 1979 and Anderson et al., 1985).

Praziquantel has also been reported to have high activity against schistosomes and other parasites; Becket et al., 1980; Andrews, 1981; Harinasuta et al., 1984; Wegner, 1984; Massoud et al., 1984a, 1984b; Marcos et al., 1985; and Schmahl et al., 1985.

Though Oltipraz has received less attention than praziquantel, recent clinical and biological tolerance studies have shown that patients administered the drug have shown a high proportion of egg reduction. The mechanism of action of oltipraz is unclear but there are indications that it might inhibit the parasite's glutathione levels (Cerf et al., 1982; Bella et al., 1982; Lapierre et al., 1983; Rey et al., 1984; Kardaman et al., 1985; Bieder and Jumeau, 1985).

The effects of radioactively labeled praziquantel has been reported in experimental cysticercosis (Andrews it al., 1980). However, the effects of oltipraz on the tegument of cestodes and schistosomes has received little attention. Thus, the objective of this preliminary study is to show the in vitro effects of oltipraz on the tegument of C. fasciolaris in relation to dose and time using scanning electron microscopy. Though experiments were also designed and carried out to examine the effects of the drug on the tegument of S. mansoni, the data presented in this paper will focus on C. fasciolaris. Both C. fasciolaris and S. mansoni appear to be excellent model systems for studying the effects of oltipraz on the surface tegument. Experiments are underway to determine the effects of the drug on the tegument using ultrathin sectioned material, in addition to the

-7'

incorporation of radioactively labeled oltipraz at the transmission electron microscopy level.

MATERIALS AND METHODS

Maintenance of the Infection

Animals used in this study were housed and maintained at the Center for Laboratory Animal Resources at the Science Research Institute of the Atlanta University Center. A cat was used for maintaining the adult tapeworm, *H. taeniaeformis*. Gravid segments or proglottids were collected weekly from an infected cat and macerated with a mortar in order to liberate the onchospheres. This material was then administered to twenty-day old male albino sprague-dawley and long evan rats. After 10 to 60 weeks, infected rats were sacrificed at intervals as needed for drug experiments. Adult schistosome worms (*S. mansoni*) were obtained from the Helminthic Disease Branch, Parasitology Section, Centers for Disease Control in Atlanta, Georgia.

Treatment of Worms with Oltipraz

The drug, oltipraz, was obtained from Miles Pharmaceutical Company in New Jersey. The cysts of tapeworms were dissected intact from infected livers of rats and washed in cold 0.85% physiological saline. The strobilocerci were liberated by gently rupturing the cyst walls with fine point scissors. Worms were divided into several groups, incubated and gently agitated in a 37°C water bath in solutions of oltipraz at the following concentrations: 25, 50, 100, 250, 500, 1000 and 1500 micrograms. Time intervals ranged from 30-150 minutes. Control worms were incubated parallel to experimental worms in an equivalent amount of physiological saline. Following incubation, worms were prepared for scanning electron microscopy.

Scanning Electron Microscopy

The scolex, strobila and bladder of the strobilocerci were fixed in 2.5 - 4% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.4, rinsed in buffer, postfixed for two hours in buffered 2% osmium tetroxide and dehydrated in a graded series of ethyl alcohol (i.e., 30, 50 70, 80, 90, 95 and 100%). Specimens were then critical point dried with carbon dioxide and coated with gold-palladium. The worms were examined in an Etec-Omniscan Scanning Electron Microscopy and pictures were taken at desirable magnifications using Polaroid 55 P/N film. Schistosome worms

- 3

were processed for scanning electron microscopy according to the same procedure.

RESULTS AND DISCUSSION

Control Worms

Electron scans of control tapeworms revealed certain distinct surface features of the scolex, strobila and bladder of C. fasciolaris. The suckers were comprised of large folds, depressions and ridges. Numerous short-studded and regularly distributed microtriches were apparent over the entire scolex and neck region (Figures 1 and 2). At higher magnifications microtriches could be seen within the cavities and well distributed around the rims of the suckers (Figure 2).

When the scolex of the worm is in a projected state, the double crown of hooks embedded in the rostellar sac of the rostellum can be seen (Figures 1 and 3). Rees (1951) published the first detailed paper on the anatomy of C. Fasciolaris at the light microscopic level and found that there were 22 biconcave hooks present in the rostellar sac. Control worms in this study showed that the surface cushion of the rostellum consisted of pit-like depressions and microtriches. The surface cushion of the rostellum also exhibited a porous-like appearance (Figure 3).

Further studies are needed on the retractability of the scolex and rostellum of C. fasciolaris. Previous studies have revealed that when the rostellum is in a fully retracted state, all four suckers around the scolex can be seen; however, the rostellar hooks are no longer visible and the muscular cushion of the rostellum shows a hexagonal-like shape. The anterior surface of the scolex also shows constriction when the rostellum is in a retracted state (Jones et al., 1977a; 1977b).

In the neck region or zone of proliferation of the worm at low and high magnifications an interesting structure was observed (Figures 1 and 4). This structure appeared as a distinct and well-defined center "opening" surrounded by two lateral lobe-like features. Extraneous material which could possibly be food and other foreign matter was observed at some of the site openings (Figure 4). This is the first time such a surface opening has been reported in the neck region of C. fasciolaris.

The tegument of the strobila was studded with bosses of uniform size which were revealed as microtriches at higher magnifications (Figures 5 and 6). A new structure was also observed on the lateral surface of the proglottids near the bladder. This structure had the appearance of an opening, circular in shape surrounded by a ruffled-like microvillus surface

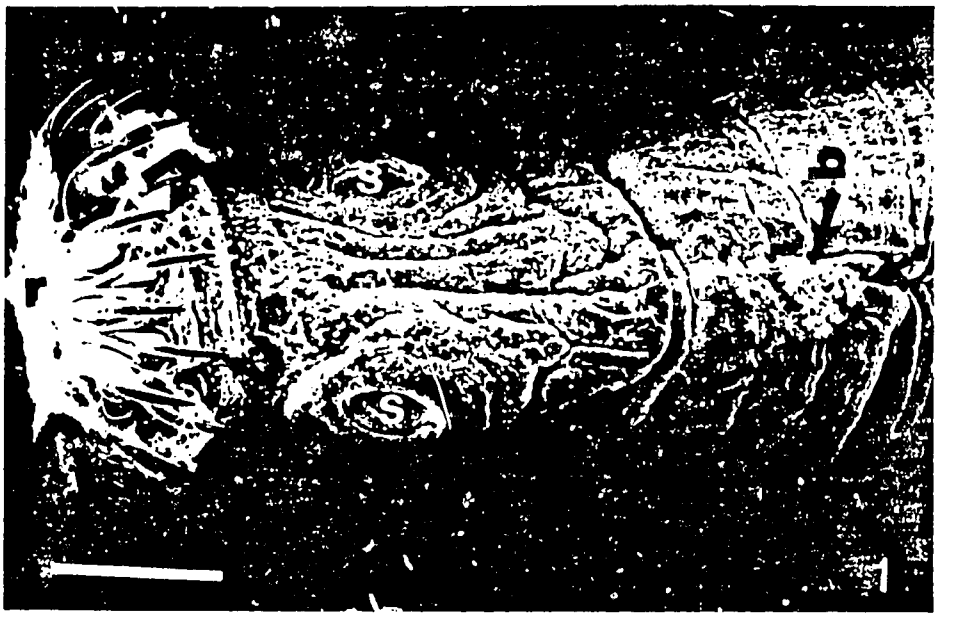


Figure 1. Control Scanning Electron Micrograph (SEM) of Scolex Region of Cysticercus fasciolaris: (r). Rostellum; (arrows-a), Hooks; (s), suckers; (arrows-b), New Unidentified Structure in the Neck Region. Marker, 264 μm.

Figure 2. Higher Magnification of Scolex Showing Micotriches (Microvilli). Marker, 310 μm.

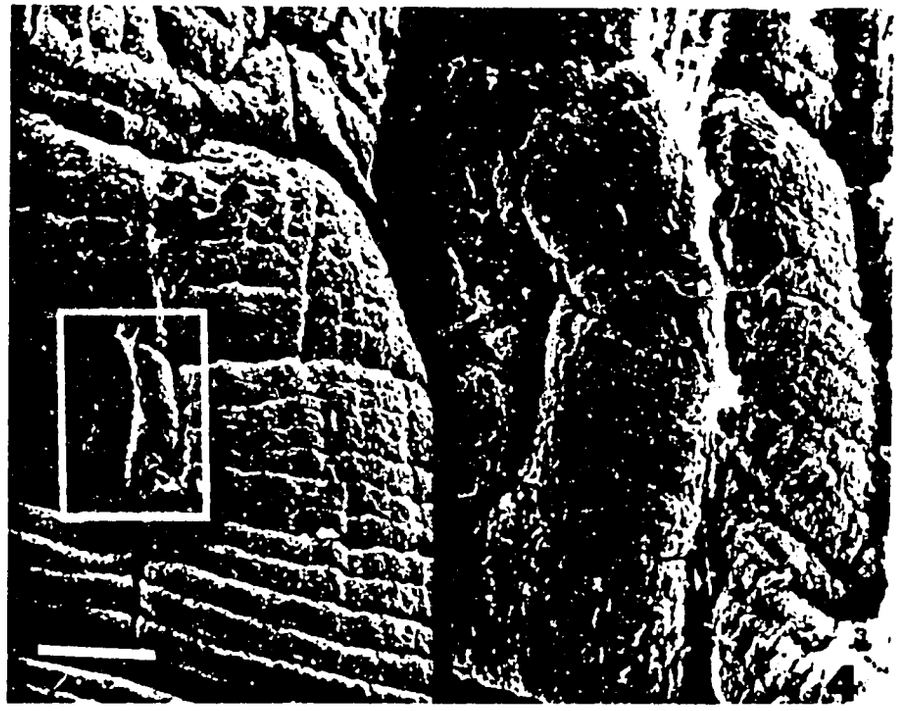
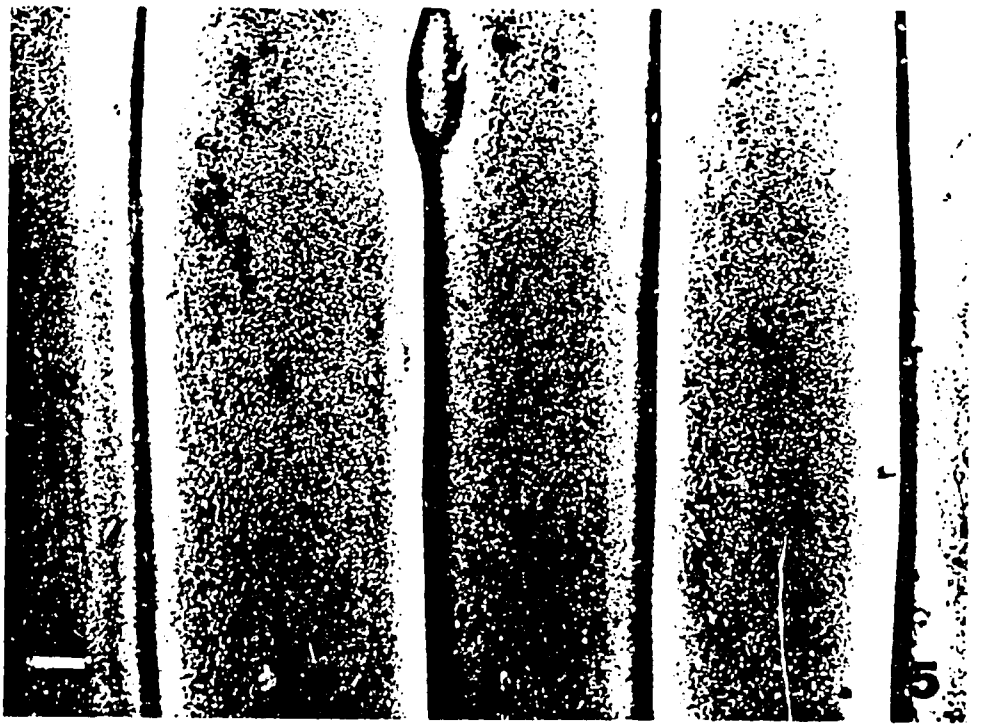


Figure 3. Rostellum (r) showing Double Crown of Hooks. Marker, 169 µm.

Figure 4. Close-up Views of New Unidentified Structure in Neck Region (box). Marker 166 µm.



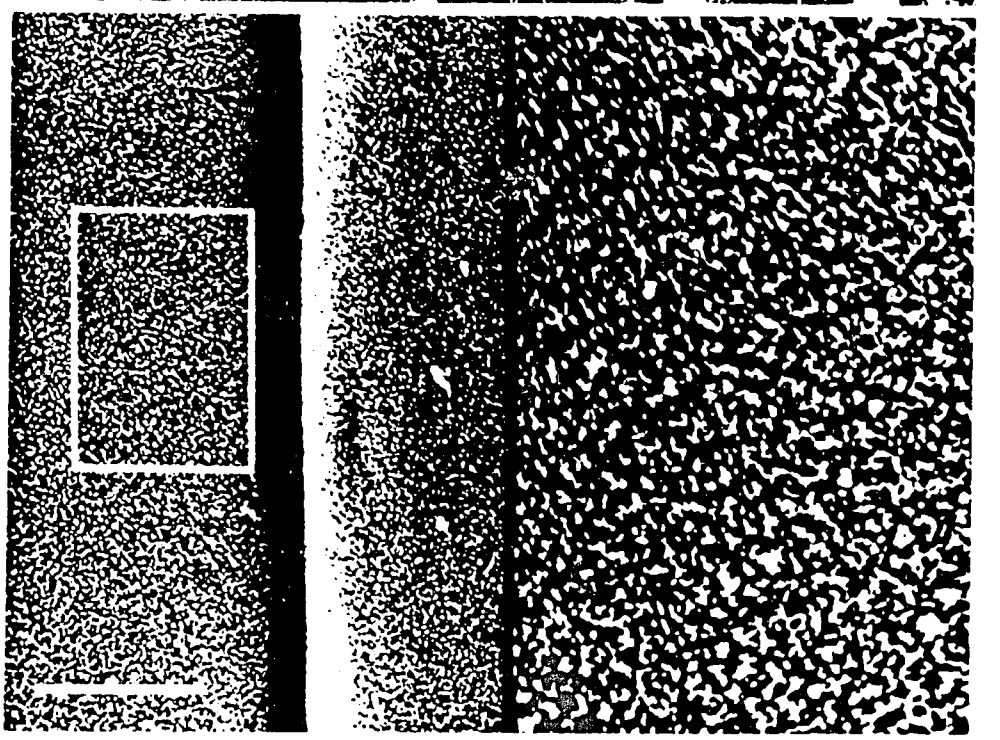


Figure 5. Strobila showing Proglottids of Segments. Marker, 1 μm.

Figure 6. Close-up view of Strobila (Box) and Higher Magnification showing Microtriches. Marker, 4 μm.

(Figures 7 and 8). Extraneous material was observed from the opening (Figures 7 and 8). Such material may be excretions of waste products.

In younger tapeworms, microtriches were less apparent on the strobilar surface (Figure 9). The bladder of C. fasciolaris showed a pear-like shape and was located at the terminal posterior end of the worm. Previous studies by Jones et al. (1977b) have revealed that the bladder of older worms (i.e., 6-12 month infections) consist of lateral and terminal "excretory pores" and densely compacted microtriches. However, such structures were not always seen on the bladder of younger tapeworms (Figure 10). Electron scans of control worms were somewhat in accord to previous studies by Jones et al., 1977a, 1977b, 1979, 1980 and 1982.

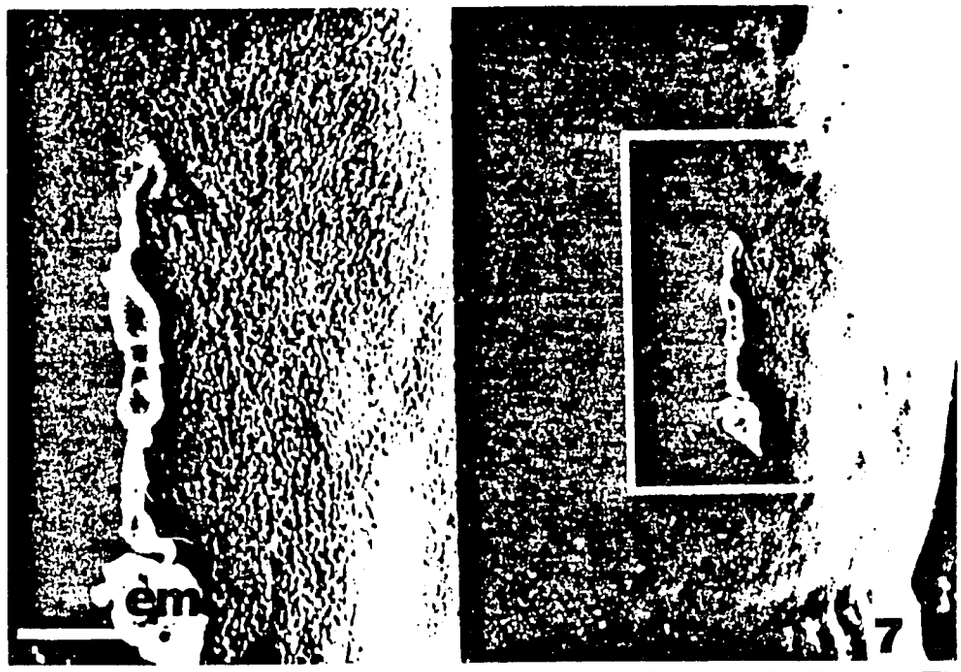
Experimental Worms

Worms incubated in both low and high concentrations of oltipraz at varying time intervals revealed certain distinct surface tegument changes or alterations that were not found in controls. Such morphological changes varied in the intensity of damage in relation to the drug concentration and incubation time. As the concentration of oltipraz was increased, the more surface tegumental damage was observed. Worms treated in concentrations of the drug lower than 25 micrograms at time intervals ranging from 30 to 60 minutes showed little damage. The tegumental damage observed with the use of both low and high concentrations of the drug was more pronounced on the scolex and strobilar regions of the worm (Figures 11-22).

The scolex showed a moderate amount of hypertrophy and the formation of numerous, irregularly distributed spherical knob-like structures heavily concentrated on the surface of the rostellum and sucker areas (Figure 11). We postulate that these structures are microtriches that have become altered due to the drug. The surface of the scolex, neck and strobilar region showed progressive degeneration and necrosis after the incorporation of low concentrations of the drug (Figures 12-14 and Figure 19).

Other pronounced changes on the surface strobilar tegument of the tapeworm consisted of the following: irregularly distributed patch-like structures (Figures 15 and 16), formation of spine-like spicules (Figure 17), large irregular shaped goblet-like cells adhering to the strobila (Figure 18) and pronounced shrinkage, degeneration, presence of crater-like structures and necrosis (Figures 19, 20 and 21). Scans of inner tegument cells of the worms using low concentrations of the drug revealed no apparent damage (Figure 22).

The results reported in this study are somewhat in accord with the effects of praziquantel on the surfaces of C. fasciolaris and S. mansoni. Thomas et al. (1982) observed that radioactively labeled ^{14}C-praziquantel



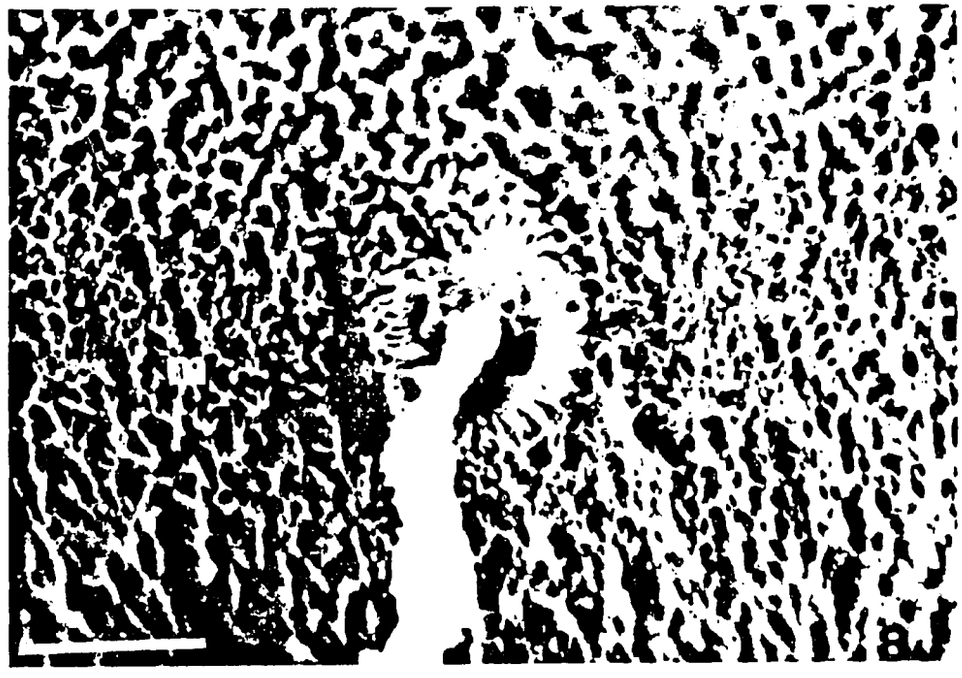


Figure 7. New Unidentified Structure (arrow) on the Lateral Surface of a Proglottid believed to be Excreting Material (em). Marker, 2 µm.

Figure 8. Higher Magnification of New Unidentified Structure showing Microtrichial-like Outer Surface (arrows) and Micotriches (m) on the Proglottid. Marker 4 µm.

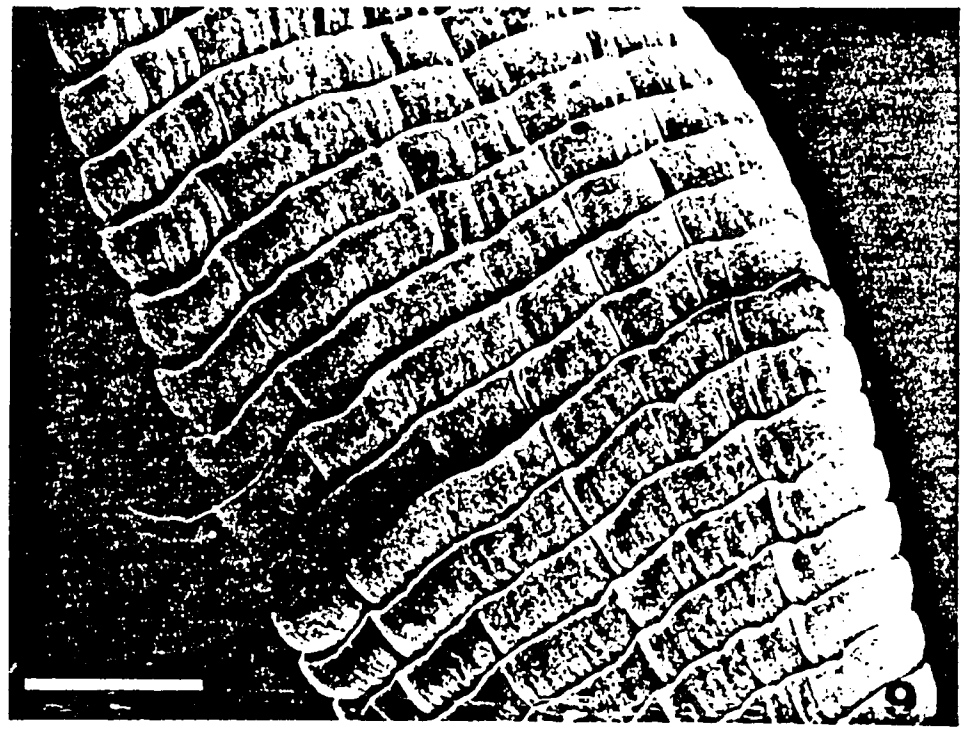


Figure 9. Strobila of a Younger Tapeworm. Marker, 317 μm.

Figure 10. Bladder of C. fasciolaris (arrows). Marker, 428 μm.

Figure 11. Scolex Region of C. Fasciolaris, Oltipraz-treated, 500 Micrograms/60 Minutes. Note the formation of bleb-like structures believed to microtriches (arrows). Marker, 170 µm.

Figure 12. Scolex, Oltipraz-treated, 150 Micrograms/90 Minutes showing Tegumentary Damage to the Suckers. Marker, 183 µm.

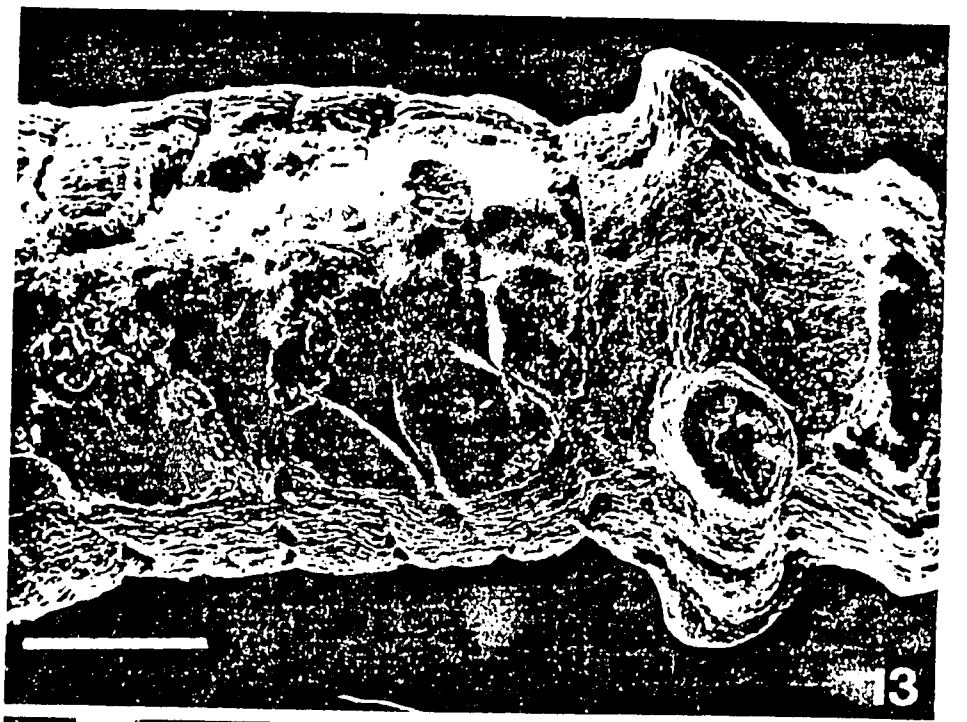


Figure 13. Scolex, Neck and Strobilar Region, Oltipraz-treated, 100 Micrograms/120 Minutes, showing Necrosis. Marker 313 µm.

Figure 14. Scolex, Oltipraz-treated, 25 Microgram/120 Minuets. Marker 175 µm.

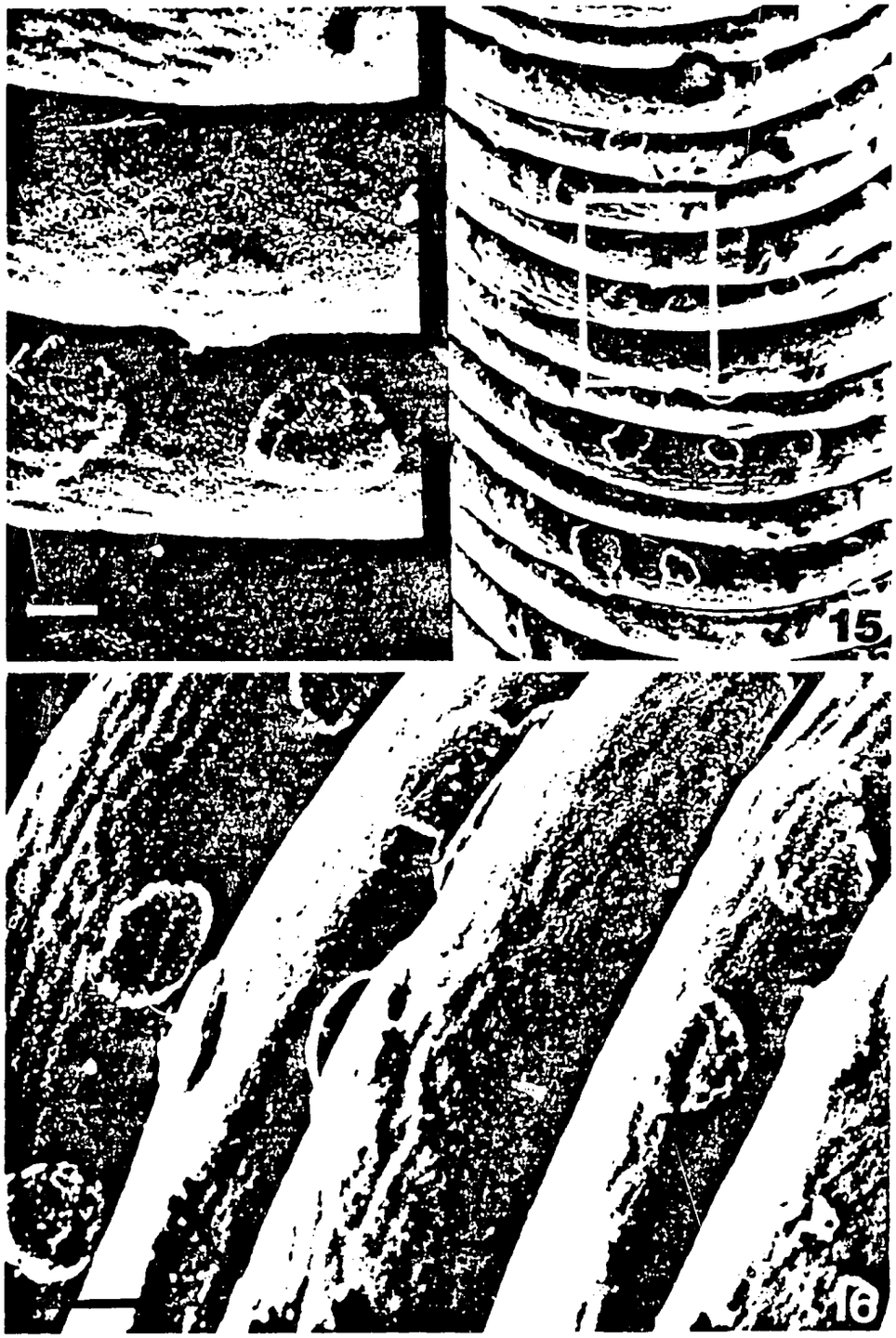


Figure 15 and 16. Strobilar Region Oltipraz-treated, 50 Micrograms/150 Minutes showing Pronounced Patch-like Structures on the Tegumentary Membrane Surface. Marker, 2 µm, 1 µm.

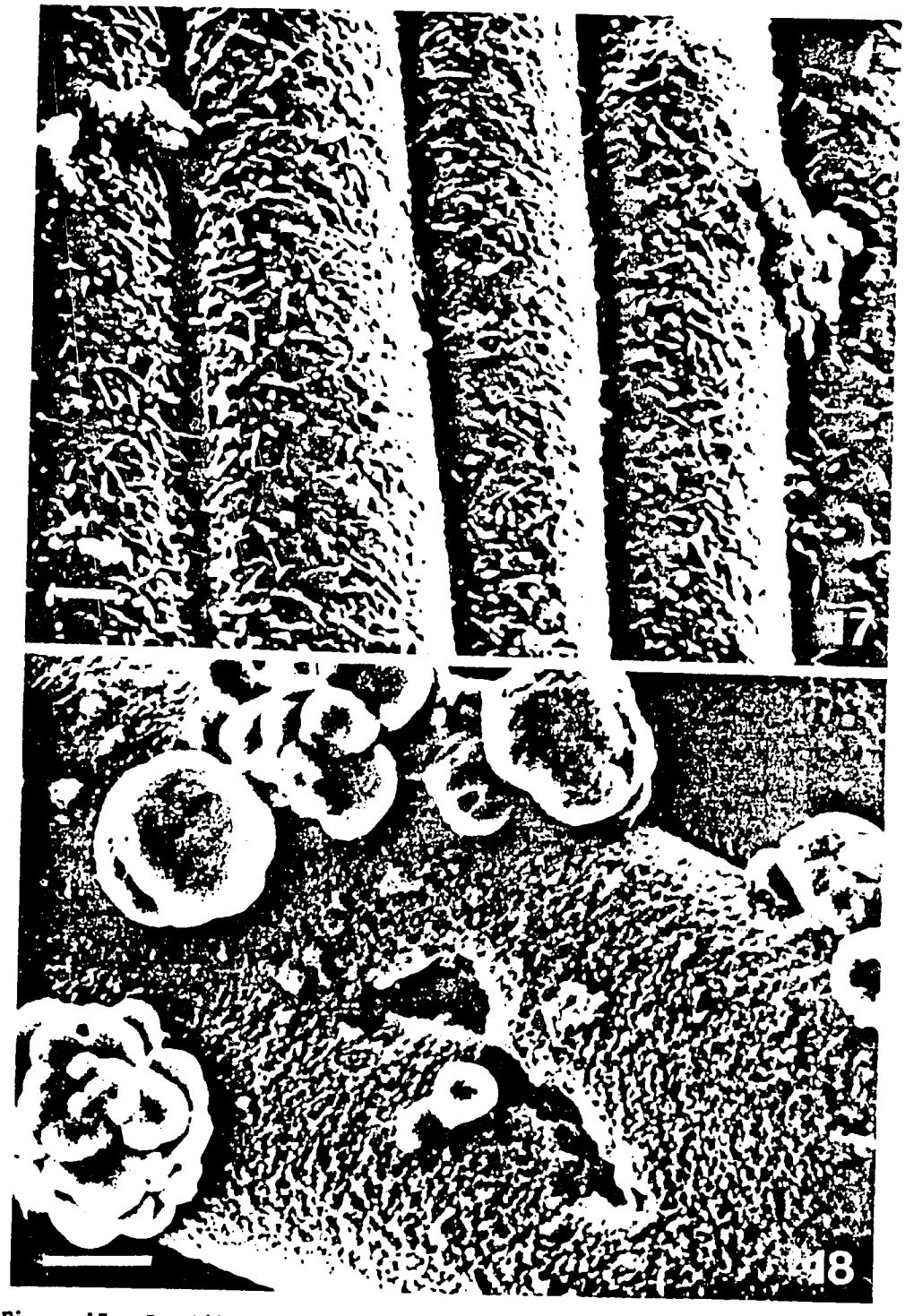


Figure 17. Strobilar Region, Oltipraz-treated 50 Micrograms/150 Minutes showing the Formation of Spicule-like Structures. (Microtriches). Marker, 1 µm.

Figure 18. Strobilar Region, Oltipraz-treated, 1500 Micrograms/60 Minutes showing unusual Goblet-like Structures Adhering to the Surface. Marker, 2 µm.

14

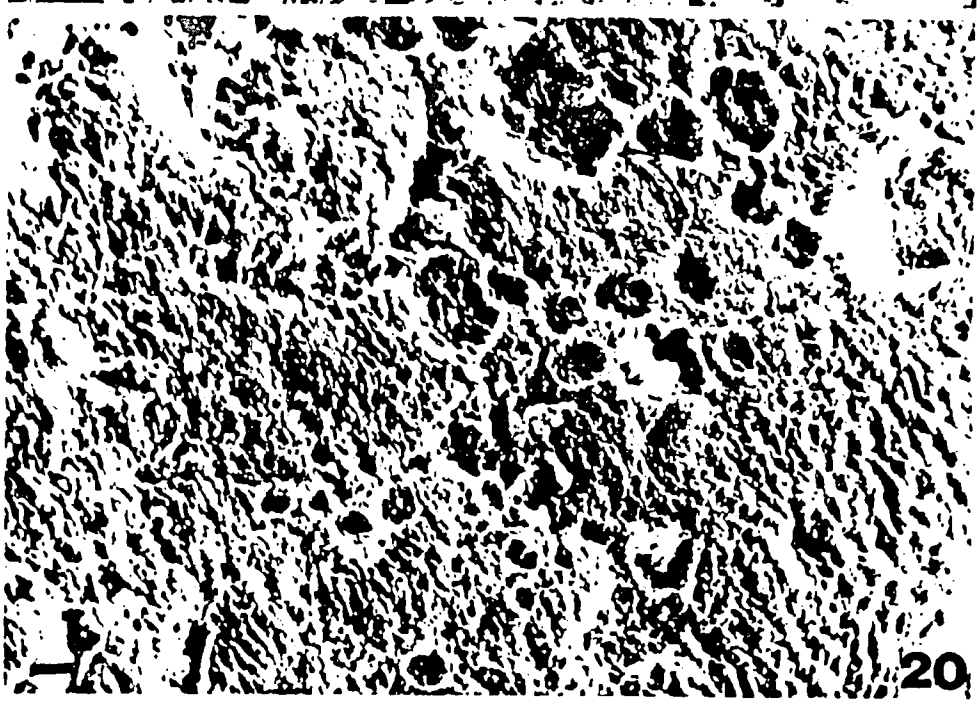


Figure 19. Strobilar Region, Oltipraz-treated, 25 Micrograms/90 Minutes showing Necrosis. Marker, 171 µm.

Figure 20. Strobilar Region, Oltipraz-treated, 100 Micrograms/60 Minutes showing Irregular Distributed Crater-like Structures (arrows). Marker, 82 µm.

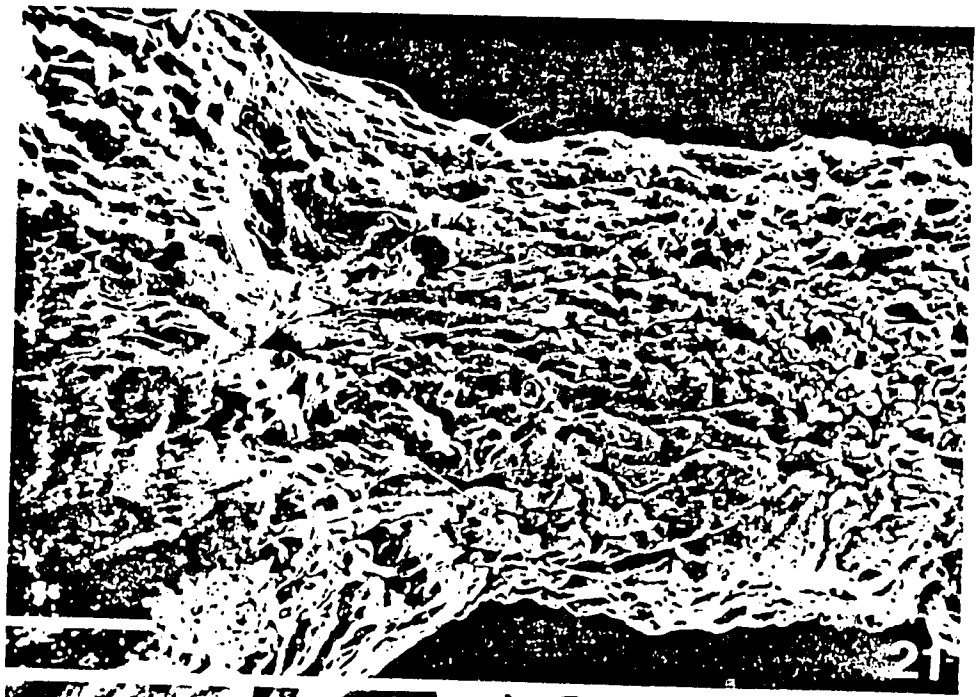


Figure 21. Strobilar Region, Oltipraz-treated, 50 Micrograms/150 Minutes. Marker 223 μm.

Figure 22. Scan of Inner Tegumental Cells, Oltipraz-treated, 25 Micrograms/60 Minutes showing Little Apparent Damage. Marker 91 μm.

- 16

in vitro and in vivo caused marked destruction of the tegument along the whole pseudo-strobila and scolex of C. fasciolaris. These investigators also found that the drug had similar effects on the tegumental surface of schistosomes, liver flukes and adult tapeworms. In addition, their studies showed that when the drug was taken up by the parasites, it was distributed evenly throughout the tegument and no selective accumulation in certain organs was exhibited autoradiographically. As the concentration of the drug was increased, surface tegumental damage was more pronounced. Similar findings were exhibited with the use of oltipraz on the surface tegument of C. fasciolaris. However, low concentrations of the drug revealed marked surface changes on the tegument.

Studies by Thomas et al. (1982) showed that the cyst wall was a barrier to the penetration of ^{14}C-praziquantel. The concentration of the drug was 4 to 10-fold higher in the cyst wall than larval tissue contained in the cyst. Experiments are underway in our laboratory to examine the uptake of radioactively labeled oltipraz (i.e. in vitro and in vivo) on the cyst wall and the larval parasite.

Data in this paper are in agreement with studies by Shaw et al (1983). They reported that praziquantel administered in vivo causes tegumental damage to S. mansoni at concentrations ranging from 10, 25, 50, 100, 200 and 500 mg/kg body weight of mouse. Such changes included surface bleeding, swelling, wrinkling, constriction and surface lesions. It was found that in male worms, in particular, the number of worms exhibiting damage and the amount of tegumental surface damage depended, mainly, on the concentration of praziquantel, although, at any given dose level, the extent of the damage increased with post-treatment. In the current study similar changes were exhibited after in vitro treatment of S. mansoni with oltipraz. Andrews (1981) and Becker et al. (1980) reported that intensive vacuolation occurred in distinct sites of S. mansoni tegument after in vivo and in vitro treatment of praziquantel. Such data was revealed in thin sectioning material.

The findings in our study are similar to the effects of other antihelminthic drugs such as mebendazole on the tegument of C. fasciolaris using scanning electron microscopy. Verheyen et al. (1978) reported time-related topographical changes in mature cysticerci of Taenia taeniaeformis induced after medication of infected mice with 250 ppm of mebendazole. The changes included the gradual disappearance of microtriches and progressive degeneration of the tegument resulting in an irregular surface with grooves, holes, and crater-like structures. Host cells were reported as adhering to the altered areas and the number of these cells increased when more severe changes became apparent. Finally, the necrotized cysticerci, which lost their tegument completely, were almost entirely covered with

host cells. A difference in the time sequence of the reported changes occurred between the scolex, the pseudoproglottids and the bladder.

We found similar changes in oltipraz treated worms. The unusual, large goblet-like structures adhering to the surface of the strobila tegument in figure 18 are practically identical to the host cells reported by Verheyen et al (1978) in C. fasciolaris. However, since experiments in this study were designed to examine the effects of oltipraz in vitro in the worms, it is possible that such structures are accumulations and aggregates of the altered destructed tegument of the parasite.

The mechanisms by which various antihelminthic drugs such as praziquantel, oltipraz and mebendazole cause changes in the tegument of cestodes and schistosomes are not clear. The data in this study suggest that oltipraz in vitro at low and high concentration in relation to time induced surface ultrastructural changes in the tegument of C. fasciolaris. The microtriches on the tegument undergo pronounced and marked changes. These structures increase the absorptive surface area of the worm, thereby aiding in certain metabolic processes that take place across and throughout the tegument. Therefore, any interference or changes in these structures may effect the overall absorption, transport mechanisms and process of the parasite.

SUMMARY

The tapeworm, Cysticercus fasciolaris appears to be an excellent model system for studying the effects of anti-schistosomal drugs and for correlative studies on schistosomes. Preliminary data in this study revealed that the drug, oltipraz at low and high concentrations (i.e. 25, 50, 100, 150, 250, 500, 1000 and 1500 micrograms) in relation to time (i.e. 30, 60, 90, 120 and 150 minutes) induces pronounced changes on the surface tegument of the tapeworm. The most marked changes were observed on the scolex proper and strobilar regions. Such changes included some of the following: hypertrophy of the scolex, numerous spherical knob-like structures heavily concentrated on the rostellum and suckers, formation of blebs, craters and spiny, spicule-like structures on the tegument; and large, aggregated goblet-like cells adhering to damaged tegumental areas. The entire surface tegument of the worm showed progressive degeneration and finally necrosis as the concentration of the drug and time was increased. More detailed data are needed to further examine these changes in vitro and in vivo. Studies incorporating the use of labeled oltipraz and transmission electron microscopic studies are underway.

References

Anderson, F. L., Short, J. A. and McCurdy H. D. (1985). Efficacy of a Combined Paste Formulation of Praziquantel/Febantel against Immature Echinococcus granulosus and Immature Echinococcus multilocularis. Am. J. Vet. Res., 46, 253-255.

Andrews, P. (1981). A Summary of the Efficacy of praziquantel against Schistosomes in Animal Experiments and notes on its Mode of Action. Arzneim-Forsch Drug Res., 34, 538-539.

Becker, B., Merlhorn, H., Andrews, P., Thomas, H. and Eckert, J. (1980). Light and Electron Microscopic Studies on the Effect of Praziquantel on Schistosoma mansoni, Dicrocoelium dendriticum and Fasciola hepatica (Trematoda) in vitro. Z. Parasitenkd., 63, 113-128.

Bella, H., Rahim, A., Mustafa, M., Ahmed, M., Wasfi, S. and Bennett J. (1982). Oltipraz Antischistosomal Efficacy in Sudanese Infected with Schistosoma mansoni. Amer. J. Trop. Med. Hyg., 31, 775-778.

Bieder J. and Jumeau, H. (1985). Disposition of ^{14}C-Oltipraz in Animals. Pharmacokinetics in Mice, Rats and Monkeys; Comparison of the Biotransformation in the Infected Mouse and in Schistosomes. Arzneim-Forsch, 35, 1431-1436.

Cerf P., Moyround, J. and Coulanges, P. (1982). The Treatment of Schistosomiasis mansoni with RP 35972 (Oltipraz). Bull. Soc. Pathol. Exot. Filiates., 75, 174.

Fifth Annual Report of the United Nations Development Program, World Bank and World Health Organizations; Special Programme for Research and Training in Tropical Diseases, 1981, pp. 61-95.

Harinasuta T., Bunnag, D. and Radomyos, P. (1984). Efficacy of Praziquantel on Fasciolopsiasis. Arzneim-Forsch, 34, 1214-1215.

Hayashi, M., Matsuda, H., Tormis, L. C., Nosenas, J. S. and Blas B. L. (1984b). Clincal study on Hepatosplenomegalic Schistosomiasis japonica on Leyte Island: Follow-up study Four Years after Treatment with Praziquantel. S. E. Asian J. Trop. Med. Pub. Hlth.

Hayashi, M., Matsuda, H., Tormis, L. C., Nosenas, J. S. and Blas, B. L. (1984a). Clinical Study on Cerebral Schistosomiasis japonica on Leyte Island, Phillipines. Follow-up Study six years after Treatment with Antischistosomal Drugs. S. E. Asian J. Trop. Med. Pub. Hlth., 15, 502-506.

Horchner, F., Langness, A. and Oguz, T. (1979). Die wirkung von mebendazole and praziquantel auf larvale Taenienstadien bei maus, Kaninchen und Schwein. Tropenmed. Parasit., 28, 44-50.

19

Jones, B. R., Smith, B. F. and Leflore, W. B. (1979). Scanning electron microscopy of the infective egg of Hydatigera taeniaeformis, Microb. Lett., 24, 185-193.

Jones, B. R. (1980). Three dimensional surface topography ultrastructure and x-ray microanalysis of the cyst wall of Cysticercus fasciolaris, Rivista Di Parassitol., 61, 697-411.

Jones, B. R. (1982). Freeze-fracture replication of the tegument of Cysticercus fasciolaris. Rivista Di Parassitol., 63, 281-285.

Jones, B. R. and Taylor, R. (1988). Effects of oltipraz on experimental cysticercosis. Proc. of the SE Electr. Micros. Soc., May (Abstract).

Jones, B. R. and Taylor, R. (1988). Surface tegumentary membrane alterations in Cysticercus fasciolaris following low doses of the antihelminthic drug, oltipraz. First Ann. F. E. Mapp Sympos., Morehouse College, Atlanta, GA, April, p. 25 (Abstract).

Jones, B. R., Smith, B.F. and Leflore, W.B. (1977a). Scanning electron microscopy of the scolex of Cysticercus fasciolaris. Microb. Lett., 4, 71-78.

Jones, B. R., Smith, B.F. and Leflore, W.B. (1977b). Electron scan of the bladder of the cysticercus of Hydatigera taeniaformis, Microb. Lett., 4, 145-150.

Kardaman, M., Fenwick, A., el Igail, A., el Tayeb, M., Bennett, J. and Daffalla, A. (1985). Field trials with oltipraz against Schistosoma mansoni in the Gezira irrigated area, Sudan. Amer. J. Trop. Med. Hyg., 88, 95-100.

Lapierre, J., Keita, A., Faurant, C., Heyer, F., Tourte-Schaefer, C., Ancelle, T. and Dupouy-Camet, J. (1983). Treatment of 700 cases of bilharziasis with the new drugs oxaminiquine, oltipraz, praziquantel. Bull. Soc. Pathol. Exot Filiates, 76, 526-533.

Lumsden, R. and Specian R. (1980). The Morphology, histology and fine structure of the adult stage of the cyclophyllidean tapeworm, Hymenolepis diminuta. In: Biology of the Tapeworm Hymenolepis diminuta (ed. H. Arai), pp. 157-280, Academic Press, NY.

Massoud, A. A., Hafez, A. S., Hussein, M. M., Abdel, K. and Nahaal, A. (1984b). Pharmacokinetic studies on praziquantel and oxaminiquine in intestinal schistosomiasis. J. Egypt. Soc. Parasitol., 14, 599-608.

Massoud, A. A., Kholy, A. M. and Anwar, W. A. (1984a). Assessment of efficacy of praziquantel against Schistosoma mansoni infection. J. Trop. Med. Hyg., 87, 119-121.

Marcos, S. H. Khayyal, M. T., Mansour M. M., Saleh, S., Ishah, E. ., Girgis, N. I. and Dunn, M. A. (1985). Reversal of hepatic fibrosis after praziquantel therapy of murine schistosomiasis. Am. J. Trop. Hyg., 34, 314-321.

Noble, R., Noble, A. (1974). Parasitology: The Biology of Animal Parasites, Third Addition, p. 244, Lea and Fibiger, Philadelphia, PA.

Rees, G. (1951). The Anatomy of Cysticercus fasciolaris taeniaeformis (Batsh 1786, Cysticerus fasiolaris Rud. 1808) from the liver of Rattus norvegicus, including an account of spiral torsion in species and some minor abnormalities in structure. Parasitol., 41:46-59.

Rey, J., Sellin, E., Sellin, B., Simonkovich, E. and Mouchet, F. (1984). Comparative efficacy of oltipraz (1 dose, 30 mg/kg) and the combination of niridazole (25 mg/kg) and metrifonate (10 mg/kg) against Schistosoma haematobium. Med. Trop., 44, 155-158.

Schmahl, G. and Mehlhorn, H. (1985). Treatment of fish parasites. 1. praziquantel effective against monogenea Dactylogyrus vastator, Dactylogyrus extaesus, Diplozoon paradoxum. Z. Parasitenkd., 71, 727-737.

Shaw, M. and Erasmus, D. (1983). Schistosoma mansoni: Dose-related tegumental surface changes after in vivo treatment with praziquantel. Z. Parasitenkd., 69, 643-653.

Shivers, R., Siddiqui, A. and Podesta, R. (1986). Integument of the tapeworm scolex. 1. freeze-fracture of the syncytial Layer microvilli and discoid bodies. Tissue and Cell, 18, 869-885.

Smyth, J. (1966). The Physiology of Trematodes. W. H. Freeman and Company, San Francisco, CA.

Smyth, J. (1969). The Physiology of Cestodes. W. H. Freeman and Company, San Francisco, CA.

Thomas, H., Andrews, P. and Mehlhorn, H. (1982). New results on the effect of praziquantel in experimental cysticercosis, Am. J. Trop. Med. Hyg., 31:803-810.

Thomas H. and Gonnert, R. (1978). The efficacy of praziquantel against cestodes in animals. Z. Parasitenkd., 52, 117-128.

Thomas, H., Gonnert, R., Pohlke, R. and Seubert, J. (1985). Investigations about treatment of larval tissue stages with a new compund. J. Proc. 7th Int. Conf. Wld. Assa. Parasit., p. 49.

Verheyen, A., Vanparijs O., Borgers, M. and Thienpont, D. (1978). Scanning electron microscopic observations of Cysticercus fasciolaris (Taenia taeniaeformis) after treatment of mice with mebendazole. J. Parasit., 64, 411-425.

21

Wegner, D. H. (1984). A profile of the trematodicidal compound praziquantel. Arzneim-Forsch, 34, 1132-1136.

Acknowledgments

We wish to thank Mr. Thomas Pettigrew, President, Mr. Bernard Cameron, Vice-President and Mr. Joseph V. Busby, Word Processor of SA-PACK Enterprises for the word processing work associated with this manuscript. We are also pleased to acknowledge Mr. Dayton Funk and Ms. Karen Gurty of Georgia Institute of Technology for some of the technical work in this manuscript. This research is supported by the following agencies: Agency for International Development, Grant Number DAN-5053-G-SS-7078-00; National Institutes of Health/Minority Biomedical Research Support, Grant Number RR-8006 and the National Science Foundation, Grant Number RII-80-6958.