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Ultrastructural Investigations on the Effect of Praziquantel on the Tegument of Five Species of Cestodes

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Abstract. Adult *Hymenolepis diminuta, H. microstoma, H. nana, Echinococcus multilocularis,* and *Taenia* (*Hydatigera*) *taeniaeformis* have been exposed in vitro in media containing 0.1 to 100 µg praziquantel/ml. Already after 5 min characteristic tegumental lesions, that were restricted to the growth zone of the neck region, were recognized using both scanning and transmission electron microscopy. Within the tegument numerous vacuoles were formed that released their contents to the exterior and finally caused destruction of the tegument. Proglottides of the central or posterior strobilar portions were never damaged.

Larvae of *T. taeniaeformis* (cysticerci) and *E. multilocularis* (alveolar cysts) were studied employing the same methods both after in vitro exposure to and after in vivo treatment of their hosts with praziquantel. Strobilocerci of *T. taeniaeformis* developed identical tegumental lesions after contact with praziquantel whether incubated in vitro or treated in vivo. The wall of the bladder containing the larva remained unaffected. Evaginated protoscolices of *E. multilocularis* were damaged by in vitro contact with praziquantel while invaginated protoscolices remained intact. After in vivo exposure there were some fully developed evaginated and damaged protoscolices whereas all invaginated protoscolices and the cyst wall with its germinative layer were unaffected.

Key words: Praziquantel – Cestodes – *Hymenolepis* – *Echinococcus* – *Taenia*

Ultrastrukturelle Untersuchungen zur Wirkung von Praziquantel auf das Tegument von fünf Bandwurmarten

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Zusammenfassung

Adulte *Hymenolepis diminuta, H. microstoma, H. nana, Echinococcus multilocularis* und *Taenia* (Hydatigera) *taeniaeformis* wurden in vitro in Medien inkubiert, die 0,1–100 µg Praziquantel/ml enthielten. Mit Hilfe der Scanning- und der Transmissionselektronenmikroskopie konnten charakteristische Schäden des Teguments ausschließlich im Bereich der Sprossungszone schon nach 5 min nachgewiesen werden. Es entstanden hier zahlreiche Vakuolen, die ihren Inhalt nach außen abgaben und so schließlich das Tegument zerstörten. Proglottiden der mittleren und hinteren Strobilaabschnitte wurden niemals derartig geschädigt.

Larven von *T. taeniaeformis* (Strobilocercen) und *E. multilocularis* (alveoläre Cysten) wurden mit den gleichen Methoden in vitro sowie nach in vivo-Behandlung in ihren Wirten untersucht. Es zeigte sich, daß der Strobilocercus von *T. taeniaeformis* sowohl nach Inkubation in vitro wie nach in vivo-Behandlung mit Praziquantel gleichartige charakteristische Schäden aufwies, die umhüllende Blasenwand allerdings niemals. Bei *E. multilocularis* ergab sich, daß bereits evaginierte Protoscolices in vitro geschädigt wurden, nicht dagegen die invaginierten Protoscolices.

In vivo wurden einige voll entwickelte evaginierte und geschädigte Protoscolices gefunden, während die invaginierten und die Brutkapsel mit dem Keimepithel offenbar intakt blieben.

Abbreviations.

B	Bubbles	S	Sucker
BL	Basement layer	SC	Scolex
CM	Circular musculature	SEM	Scanning electron microscopy
LM	Longitudinal musculature	TD	Tegumentary disks
M	Microtriches	TEM	Transmission electron microscopy
MI	Mitochondria	TG	Tegument
P	Proglottis	TGC	Tegumental cell
R	Rostellum	V	Vacuoles

Introduction

Praziquantel (Droncit, Biltricide, Cesol$^+$) is a new chemotherapeutic agent with high activity against juvenile and adult cestodes and also against a broad spectrum of trematodes (Thomas and Gönnert 1977, 1978a, b; Gönnert and Andrews 1977). First in vitro studies on the morphological changes induced by praziquantel in the dwarf tapeworm, *Hymenolepis nana,* have shown that contact with praziquantel results very rapidly in a vacuolization and ballooning of the tegument which is restricted to the neck region only and subsequent disruption of the apical tegumental layer (Becker et al. 1980a). The present paper summarises the morphological changes of the cestode tegument obtained after in vitro exposure of adult *Hymenolepis diminuta, H. microstoma, H. nana;* adults and cysts of *Echinococcus multilocularis;* and adults and strobilocerci of *Taenia* (*Hydatigera*) *taeniaeformis* to various concentrations of praziquantel. The larval stages of the two latter species were also studied after in vivo treatment of their hosts.

Material and Methods

1. Experimental Animals

Hymenolepis diminuta were isolated from the intestine of female Wistar rats that had been infected three weeks previously with cysticercoids taken from *Tenebrio molitor* beetles. *Hymenolepis microstoma* were obtained from the bile duct and intestine of male CF_1/W74 mice that had been infected 4.5 months previously with 10 cysticercoids taken from *Tribolium castaneum* beetles. Two week old *Hymenolepis nana* were isolated from the intestine of male CF_1/W74 mice that had been infected with 70 eggs of *H. nana*. *Echinococcus multilocularis* worms were isolated from the intestine of a dog 28 days after infection, whilst the alveolar cysts were removed from the peritoneal cavity of cotton rats (*Sigmodon hispidus*) which had been infected 3.5 months before. Adult *Taenia* (*Hydatigera*) *taeniaeformis* were recovered from a cat that had been infected with strobilocerci contained in mouse liver 17 months before. Cysts containing the strobilocerci of *T. taeniaeformis* were isolated from the livers of male CF_1/W74 mice that had been infected 4.5 months before.

2. In Vitro Exposure

After isolation from the host the parasites were rinsed in physiological saline and incubated for 5, 15, 30, or 60 min at 37° C in culture medium containing 0.1, 1, 10, or 100 µg praziquantel/ml. The larvae of *T. taeniaeformis* and protoscolices of *E. multilocularis* were removed from their cysts before incubation. Only fully developed protoscolices were used. Protoscolices that had evaginated spontaneously in the incubation medium and not evaginated ones were incubated separately. *H. diminuta* worms were also incubated for 5 min in medium containing only 0.1, 0.05, 0.01, 0.005, or 0.001 µg praziquantel/ml. The medium used was TC 199 with Earle's balanced salt solution (Serva, Heidelberg, no. 47770 A). One extra gram of glucose and 2.2 g $NaHCO_3$ were added to each litre of medium.

3. In Vivo Treatment

Mice infected with larval *T. taeniaeformis* were treated with 1×500 mg praziquantel/kg on the first and with 1×250 mg praziquantel/kg on the following four days. The bladder was removed at predetermined times and fixed as described below.

Cotton rats containing alveolar cysts of *E. multilocularis* were orally treated with four doses of 250 mg praziquantel/kg each given on consecutive days. They were killed 6 h after the last dose. The cysts were removed and fixed as described below.

4. Light and Electron Microscopy

For light and electron microscopic investigations the incubated worms were fixed for 24 h in 5% glutaraldehyde buffered with 0.1 M sodium cacodylate pH 7.4 at 4° C. Postfixation (2% OsO_4; 2 h) took place in the same buffer. For light and transmission electron microscopy the specimens were then dehydrated in graded ethanol, transferred to propylene oxide, and embedded in Araldite. Sections were stained with methylene blue and Azur A for light microscopy, and with uranyl acetate and lead citrate for electron microscopy. Ultrathin sections were examined in a Zeiss EM 9-S2 transmission electron microscope. For scanning electron microscopic observations the fixed and osmium-treated material was dehydrated in graded acetone, transferred to ethyl ether, and air dried (Boyde and Wood 1969), or transferred to liquid CO_2 and critical-point-dried. The dried specimes were mounted on metal stubs, sputtered with gold, and examined with a Leitz-AMR 1,000 scanning electron microscope.

Results

Scanning Electron Microscopic (SEM) Investigations

Figures 1–11 show SEM micrographs of drug-incubated and control worms of *Hymenolepis diminuta, Taeniae taeniaeformis,* and *Echinococcus multilocularis.* The parasites contracted immediately after being placed in media containing

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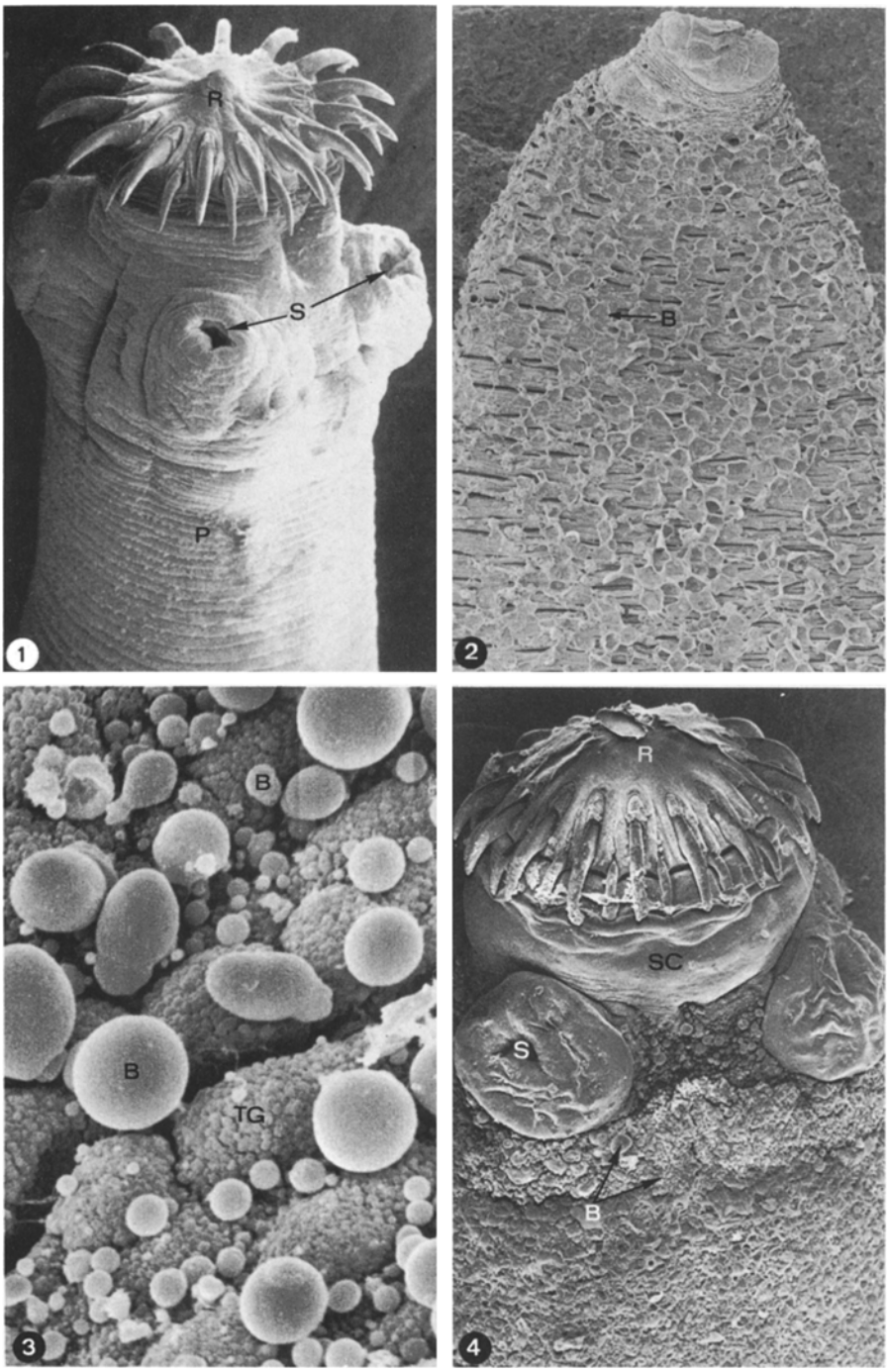
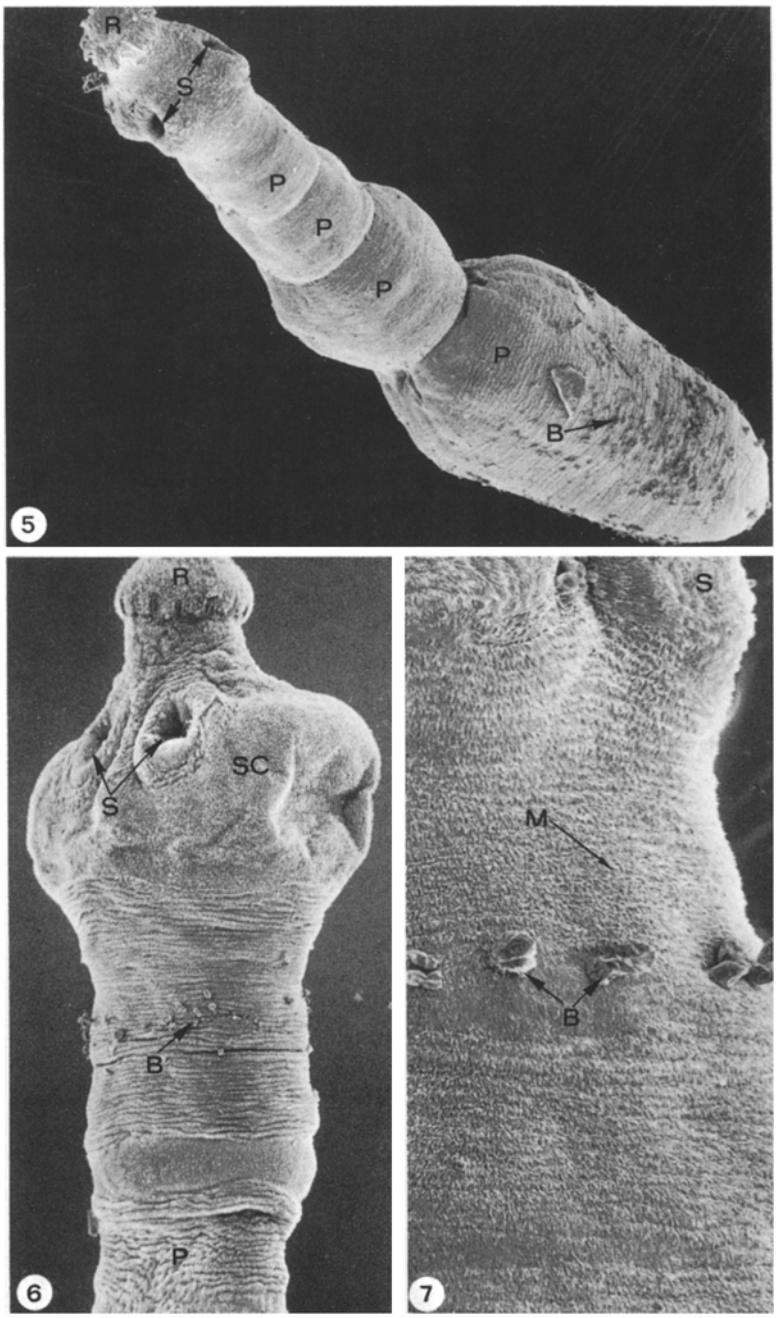


Fig. 1. SEM of untreated adult *Taenia taeniaformis* from cat intestine. × 65

Fig. 2. SEM of adult *Hymenolepis diminuta* incubated for 60 min in 10 µg praziquantel/ml. × 110

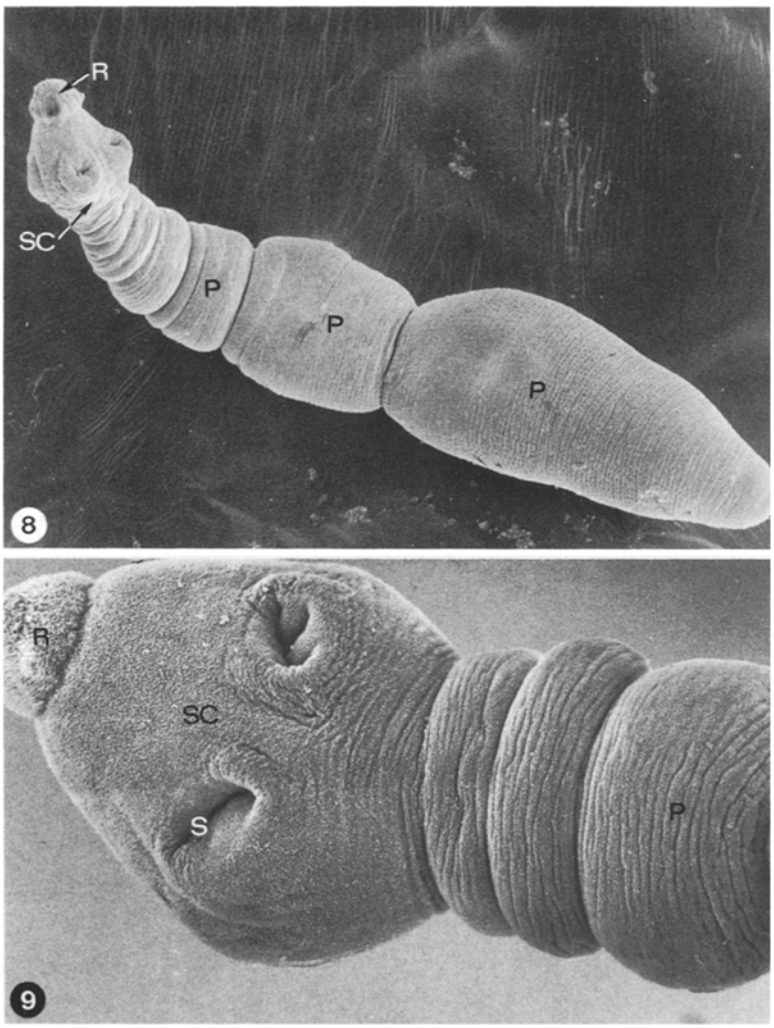
Figs 3 and 4. SEM of adult *T. taeniaeformis* incubated for 60 min in 100 µg praziquantel/ml. **Fig. 3.** Higher magnification of bubbles (*B*) in the neck region. × 2,750. **Fig. 4.** × 65



Figs 5–9. SEM of *Echinococcus multilocularis* from dog intestine.

Fig. 5. Incubated for 5 min in 100 µg praziquantel/ml. Note bubbles (*B*) on the terminal proglottis. ×180

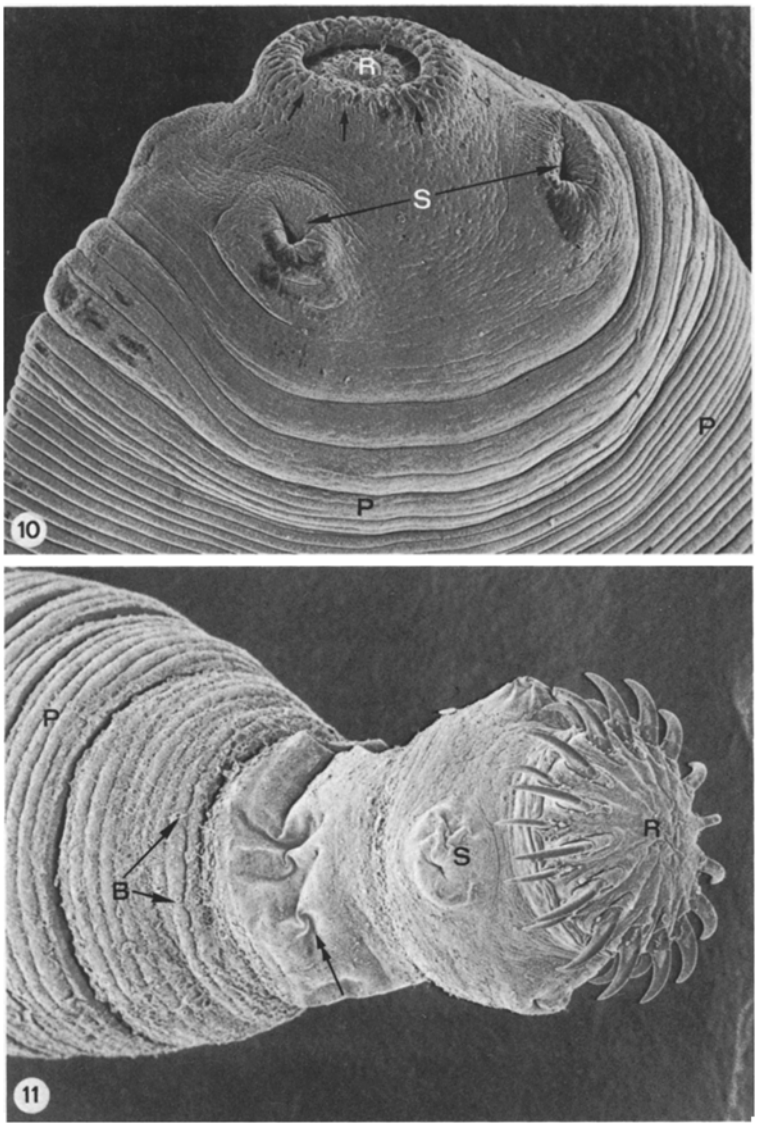
Figs. 6 and 7. Incubated for 30 min in 100 µg praziquantel/ml. **Fig. 6.** ×360. **Fig. 7.** ×1,100



Figs. 8 and 9. Incubated for 5 min (**Fig. 8.** × 190) or 30 min (**Fig. 9.** × 780) in media without praziquantel

praziquantel. The contraction of the scolex, of the neck, and of the immature strobilar region was much stronger than that of the gravid strobila, where only slight contractions were observed. In the controls the hooks of the rostellum were often retracted, whereas they protruded in drug-incubated worms. Marked changes were present after exposure to 0.1–100 μg praziquantel/ml as compared with the controls. Depending on increasing incubation times (5–60 min) areas of the tegumental surface appeared to degenerate progressively and were covered with many balloon-shaped structures of different size indicating bursting of and leakage from the tegument.

In the three *Hymenolepis* species and in adult *T. taeniaeformis* the tegumental surface of the neck region was covered with many blebs and this bubble forma-



Figs. 10 and 11. SEM of strobilocercus of *T. taeniaeformis* from mouse. Incubated in drug-free medium (**Fig. 10.** × 150) or in medium containing 100 µg praziquantel/ml (**Fig. 11.** × 90) for 60 min. Note that the praziquantel-incubated scolex (*S*) shows protruding rostellar (*R*) hooks. Its neck shows depressions and numerous bubbles (*B*)

tion extended to the immature proglottides (Figs. 2–4). Scolex and mature proglottides appeared to be unaffected by the drug. However, in the strobilocercus of *T. taeniaeformis* scolex and all pseudoproglottides showed bubble formation at the tegumental surface (Fig. 11). The bladder, however, was not affected; not even after a 60 min exposure to 100 µg praziquantel/ml. The same changes were also observed in strobilocerci that had been isolated after their mouse host had been orally treated with praziquantel. In adults of *E. multilocularis*

a distinct collar of blebs was observed at the tegumental surface behind the scolex, possibly indicating that the neck region might be very narrow in this parasite (Figs. 6 and 7). The whole surface of the last proglottis was covered with many blebs after exposure to 0.1–100 µg praziquantel for 5 min (Fig. 5). However, in worms which were incubated for 30 or 60 min these lesions of the terminal proglottis had nearly disappeared and only a few blebs were noted in this area. In other parts of the parasite (scolex, second and third proglottides) single blebs were occasionally found.

The outer surface of the brood capsules of *E. multilocularis* did not show significant ballooning during the experiments nor were other changes observed. The microtriches which cover the entire surface of all parasites studied did not seem to be affected by praziquantel, and no differences in morphology or density were found as compared with the controls.

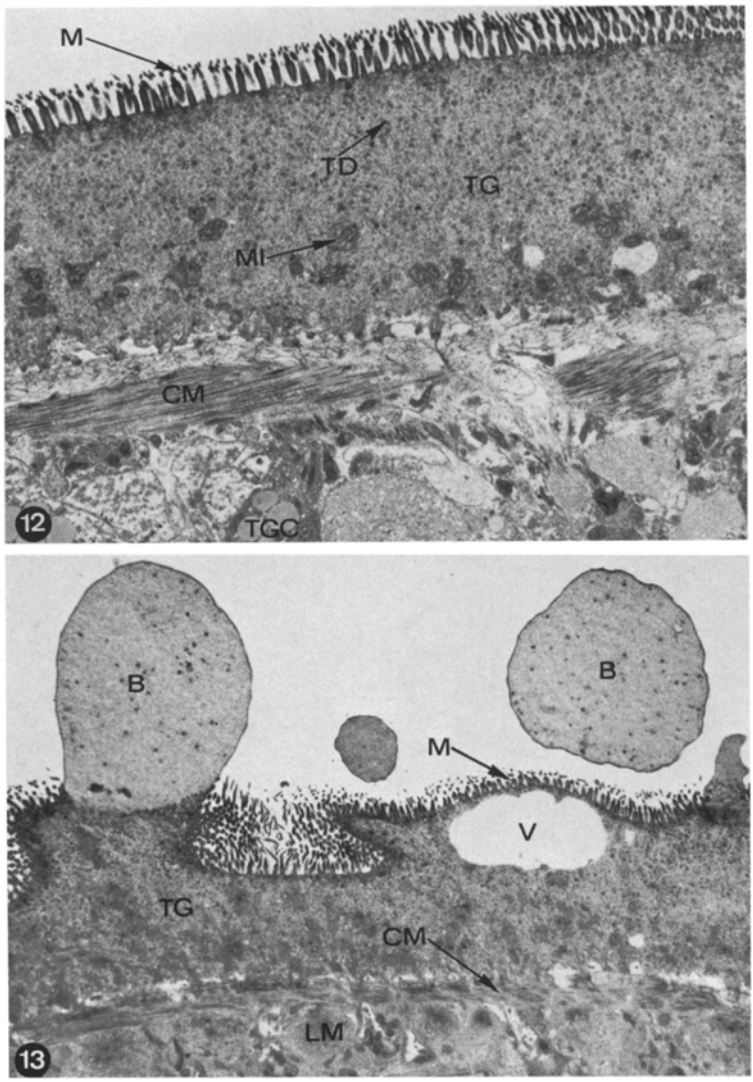
In order to establish the minimal concentration for a drug induced reaction adult *H. diminuta* were incubated in very low concentrations of praziquantel for 5 min. It was found that vacuolization was significant at a concentration of 0.1 µg and infrequent at 0.05 µg/ml. In both instances the worms were contracted, whereas, at lower concentrations (0.01 – 0.001 µg/ml) the worms neither contracted nor were tegumental vacuoles formed.

Light and Transmission Electron Microscopic (TEM) Observations

1. Controls. The cytological ultrastructure of the tegument in the investigated tapeworm-species is very similar and is typical of that described for cestodes (Lumsden 1966; Ubelaker et al. 1973; Verheyen et al. 1978). In principle, the tegument does not differ in structure along the surface of the whole tapeworms, except for thickness and shape of microtriches which may differ along the surface of a single worm.

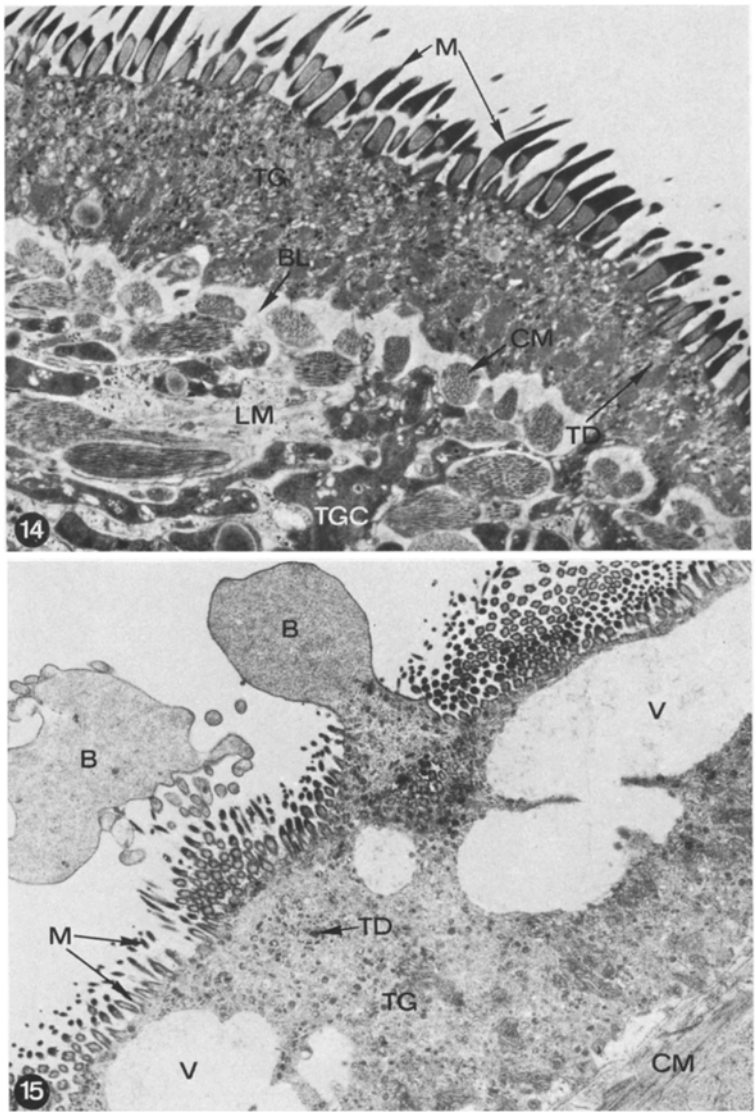
The distal part of the tegument was a continuous cytoplasmic syncytium with microtriches on the free surface (Figs. 12 and 14). The cytoplasm had a compact granular appearance and was filled with numerous vesicles, tegumentary discs, and mitochondria. The nuclei of the syncytium were located in the basal part of the tegument in elongated cells, the tegumental cells, which were situated below the basement layer and the longitudinal and circular musculature in the medullary parenchymal tissue (Figs. 12 and 14). These tegumental cells were connected to the anucleate syncytium by slender cytoplasmic processes; they were rich in endoplasmic reticulum, free ribosomes, and Golgi apparatus. In the controls (0 µg praziquantel/ml, 60 min) blebs or other artificially produced structures were not observed in light or electron microscopy.

2. Treated Tapeworms. The fine structure of incubated controls was first studied in those areas of the tegument showing bubble formation in SEM and then compared to that of more posterior regions of each worm. Incubation in the presence of praziquantel caused a marked change in the cytological structure of the tegument of adult parasites. All tapeworm species studied showed the same reaction to the drug in the neck region. In the range from 0.1–100 µg praziquantel/ml number and size of tegumental vacuoles seemed to depend



Figs. 12 and 13. Thin section of tegument (*TG*) of adult *Hymenolepis diminuta*. **Fig. 12.** Control ×9,300. **Fig. 13.** Worms incubated for 60 min in 100 µg praziquantel/ml ×4,200

mainly on the exposure time and not on the drug concentrations (Fig. 13). A strong initial effect of praziquantel (5 min, 0.1–100 µg/ml) on the tegument of the terminal proglottis of *E. multilocularis* was reduced at longer incubation times. After 60 min only a few vacuoles and bubbles were found along the syncytial tegumental layer (Fig. 15). Muscle and tegumental cells seemed to be unchanged when compared to the controls. The 5 µm thick tegument of the *Hymenolepis* species was more strongly affected by praziquantel using short incubation times (5 min) and low drug concentrations (0.1–10 µg/ml) than the



Figs. 14 and 15. Thin section of tegument of *Echinococcus multilocularis*. **Fig. 14.** Control. × 12,000. **Fig. 15.** Worms incubated for 60 min in 100 µg praziquantel/ml. × 7,200

thicker tegument (7–12 µm) of adult *T. taeniaeformis*. This gradual difference disappeared when higher praziquantel concentrations (100 µg/ml) were used for 30 or 60 min. The strobilocercus of *T. taeniaeformis* reacted in vivo and in vitro in the same way as the adult whereas the tegument of the brood capsules as well as the invaginated protoscolices of *E. multilocularis* showed no significant alterations during the course of the experiment. However, the evaginated protoscolices of *E. multilocularis* had the typical bubbles just posterior to the suckers in all concentrations of the drug used in vitro as well as in vivo.

In all in vitro experiments the development of vacuoles and tegumental lesions was studied during the course of a 60 min exposure period. After a 5 min incubation in media containing 0.1–100 µg praziquantel/ml vesicle formation was only observed in the syncytial zone of the tegument but not in cell bodies. Vesicles with a diameter of up to 15 µm and filled with cytoplasm of various densities and some granules were observed protruding from the outer surface indicating leakage from the syncytium. Often many small vacuoles (0.2–1 µm) were scattered along the basement layer. In *H. diminuta* it was shown that a concentration between 0.1 and 0.05 µg praziquantel/ml is required to induce the typical vacuole formation within 5 min of exposure. After 15 to 30 min of exposure to 0.1–100 µg praziquantel/ml similar but more pronounced changes in the tegument were observed. Vesicle formation and vacuolization had increased. After a 60 min incubation in media containing 0.1–100 µg praziquantel/ml excessive degenerations were noted in the tegument. Vacuolization was so pronounced that nearly the whole syncytium consisted of vacuoles. At the outer surface vesicles filled with cytoplasm or cellular debris were found. The tegumental cell bodies were swollen and muscle cells had burst. Often, the basement layer was disrupted or had disappeared.

Discussion

The results obtained during our experiments are in agreement with previous ultrastructural studies on the effect of praziquantel on cestodes and trematodes in vitro, as well as on schistosomes in vivo (Becker et al. 1980a, b,; Mehlhorn et al. to be published a, b). It can be concluded that the similarity of the reaction to the drug praziquantel indicates that a system common to all these platyhelminths is affected by it. Damage to that system causes destruction of the surface by an enormous vacuolization and thus finally leads to the death of the parasites. Cestodes and trematodes differ with respect to the sites of reaction. In schistosomes and other trematodes the vacuolization is scattered over numerous places all over the surface and differed in intensity, e.g. no reaction in *Fasciola hepatica* and only a very slight one in *Paragonimus westermani.*

In cestodes it is always restricted to the neck of the strobila, tegumental destruction never occurs along mature proglottides. In both groups, however, vacuolization is preceeded by contraction. This is also shown by our experiments with low doses, where only those worms reacted, that were visibly contracted.

In trematodes as well as in the cestodes studied here the extent of tegumental damage appears to depend mainly on the time of exposure and not on the drug concentration in the range from 0.1–100 µg praziquantel/ml. The tegumental alterations that are caused by praziquantel are not artifacts due to isolation of the worms. Proof for this is the normal tegumental structure of the incubated controls. Furthermore, the larva of *T. taeniaeformis* showed the same vacuolization whether incubated in vitro or exposed to the drug in vivo by treating the infected host.

It is not yet understood, why the tegument of the brood capsules and the still invaginated protoscolices of *E. multilocularis* showed no reaction, not even after exposure to 100 µg praziquantel/ml for 60 min, although their tegument

is identical in fully developed and developing protoscolices and in the germinative layer. The observation that the developing protoscolices and the walls of the brood capsules are not significantly damaged may explain the observation of Eckert et al. (1977) that praziquantel lacks a curative effect in *Meriones unguiculatus* intraperitoneally infected with cysts of *E. multilocularis*. Some of the fully developed protoscolices evaginated after treatment of the host with four daily doses of 250 mg praziquantel/kg and then showed vacuolizations identical to those produced by in vitro exposure to the drug of pre-adult worms or spontaneously evaginated protoscolices. A loss of infectivity to dogs of protoscolices recovered from cotton rats that had been treated intraperitoneally with 2× 250 mg praziquantel/kg has been recorded (Thomas and Gönnert 1978a).

Morphological changes to the cestode tegument comparable to the ones caused by praziquantel have been also described for other drugs. Thus Miracil D brought about similar alterations in schistosomes (Gönnert 1955) and mebendazole in several species of cestodes (Borgers et al. 1975; Verheyen et al. 1978; Laclette et al. 1978). However those effects were only obtained after much longer incubation times of at least 8 h as compared to 5 min with praziquantel. In the case of praziquantel the onset of drug action, contraction and tegumental vacuolization, is extremely rapid and directly related to the death of the worm.

One must conclude that praziquantel attacks cestodes at an extremely sensitive region of the tegument, the neck region of the strobila, which also shows high metabolic activity. However, mature proglottides have never been seen to be affected by praziquantel. This is an important observation because it reduces the possibility that parasite ova may be set free within the intestinal tract of the final host. This is even true for *E. multilocularis* where an initial vacuolization of the terminal proglottis was observed. However, this vacuolization was only temporary and had receded within 30 min of incubation in the continued presence of praziquantel. The processes causing this ephemeral event are not yet understood.

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