known about the factors involved in the morphogenesis of the proximal tubule, further studies on the effect of corticoids on the development of the proximal tubule could be very interesting for a better understanding of this problem. Finally, our results emphasize the importance of the combination of microdissection and SEM analysis for a better knowledge of the nephron alterations. The study of the human polycystic kidney could give important information about the possible occurrence of proximal tubule alterations accompanying this congenital malformation.

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Fluorescent antibody study of the post-cysticercoid development of *Moniezia expansa*

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Summary. The strobila of *Moniezia expansa* was separated into developmental areas, and these were compared using immunological techniques. Agar double diffusion plates and immunoelectrophoresis showed differing antigenic composition or concentration between the strobilar regions studied. Conjugation of the antisera with rhodamine lissamine-200 aided in localization of common antigens on tissue sections of the various developmental stages. What appeared to be unique localizations were observed.

The developing cells and tissues of an organism are characterized by selective gene activity and the production of progressively different structural proteins and enzymes. These molecules are often antigenic, and appropriately labelled antibodies may serve as a useful indicator of differentiation.

Accounts of the antigenicity of helminths are common in the literature. Many deal with comparisons of antigenic components of various life cycle stages of parasites. The tapeworm strobila has been likened to a series of genetically identical embryos, each showing increasing degrees of maturity[2]. A progressive acquisition of antigens in *Schistosoma mansoni* from egg to larva to adult has been postulated[3]. In an antigenic analysis of *Toxocara canis,* the author concluded that adjacent developmental stages have more similar antigen composition than stages more distant in development[4].

Upon this foundation, agar double diffusion, immunoelectrophoresis and direct immunofluorescence were collectively used to investigate changes in antigenic protein composition during the post-cysticercoid development of *Moniezia expansa.*

Materials and methods. Specimens of *Moniezia expansa* were collected from the intestines of freshly slaughtered lambs. The tapeworms were washed in barbitone acetate buffer, separated into scolex and neck, immature, mature and gravid regions, and frozen at $-10\,^{\circ}C$ to be used as supernatant material, or fixed in Carnoy's fixative. The proglottids were thawed and homogenized with an equal volume of barbitone acetate buffer (pH 8.8, ionicity 0.05) in all-glass tissue homogenizers. Centrifugation, followed by gravity and Seitz filtration yielded a supernatant, and this was merthiolated at a 1:10,000 dilution, and stored at 4 °C. 4 rabbits, 2 each, were immunized with mature and gravid proglottid tissue supernatants. Reactivity of control sera and antisera was tested by the Crowle[5] modification of the agar double diffusion method of Ouchterlony[6] using 1% purified agar. Immunoelectrophoresis was for 30 min at

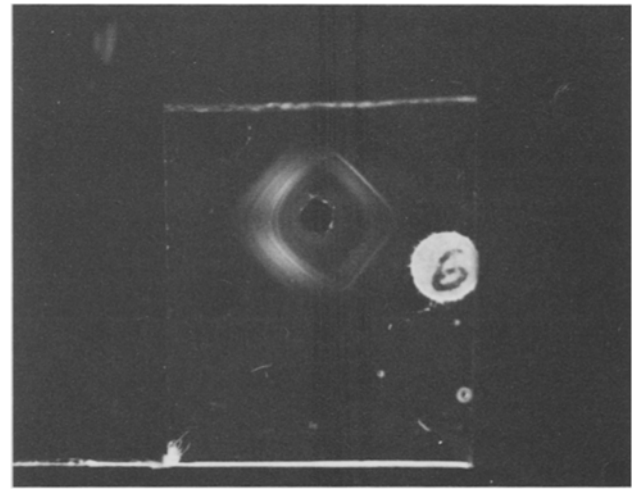

Figure 1. Double immunodiffusion of rabbit antimature proglottid supernatant material (center well) against gravid proglottid supernatant (left 2 wells) and scolex supernatant (right 2 wells). Note reactions of identity.

200 V, with 1-1/2% agar in barbitone acetate buffer. Antiserum was conjugated to rhodamine lissamine-200 using the method of Nairn[7]. Tapeworm proglottids were embedded in paraffin, sectioned at 10 µm, and affixed to slides with glycerin-albumin affixative. Standard histological procedures for paraffin removal and rehydration were followed by fluorescent antiserum conjugate treatment for 1 h at 37 °C. 4 controls were treated simultaneously as follows: a) Rhodamine lissamine-200 buffer solution; b) unconjugated antiserum; c) untreated; d) unconjugated antiserum, followed by conjugated antiserum. Slides treated with rhodamine lissamine-200 were washed 2.5-3 h in 3 changes of barbitone acetate buffer. Glycerine and a coverglass were placed on the sections, and storage was at 4 °C in a moist chamber until viewing. Viewing of slides and photomicrographs were taken using a dark contrast phase condenser on an AO Spencer Microstar. UV-light was provided by an AO Spencer Fluorolume Illuminator with an Osram HBO-200 mercury vapor arc lamp. The exciter filter was a Schott BG-12 and the barrier filter an E.K. No. 15.

Results. Antigenicity of the various developmental stages was established with agar double diffusion and immunoelectrophoresis. Gravid and mature proglottid antisera clearly differentiated scolex and immature, as well as gravid and mature supernatants, manifested by diffusion line quality and number. Figure 1 demonstrates common antigens between scolex and gravid supernatants. Gravid antiserum showed a similar differentiating reaction. In general, agar double diffusion and immunoelectrophoresis demonstrated common and differing antigenic components between developmental stages of the strobila.

Fluorescent localization occurred in the inner and outer muscle layers of the acetabulum, in the excretory ducts, and in what appeared to be glandular tissue of scolex sections treated with mature as well as gravid antiserum conjugates. Vivid antigen localization was observed in the cuticle and developing eggs of mature proglottids with homologous antiserum conjugate. Similar sections treated with gravid antiserum conjugate showed slight cuticular fluorescence, and granular fluorescence on developing eggs. Strong localization occurred on the eggshell, embryophore, and pyriform apparatus of the eggs within the uterine sacs of gravid proglottid sections, with mature conjugate (fig. 2). There was a significant lack of cuticular fluorescence in those sections. A similar control section demonstrates autofluorescence (fig. 3). In contrast, treatment of gravid sections with homologous conjugate resulted in intense localization in the cuticle. The eggshells, embryophore and pyriform apparatus were also observed to be fluorescent.

Discussion. The scolex region appeared to have the least amount of antigens in common with the other developmental regions, manifested by the smallest number of reactions of identity. The scolex is a fully differentiated body area of the tapeworm[8]; detection of common proteins with the more actively developing parts of the strobila would probably be less likely. In a similar vein, the immature region, characterized by 2 sets of male and female genital rudiments in *Moniezia expansa,* would be expected to share some common protein components with mature proglottids, with fully functional reproductive structures. The results obtained support this observation.

A concentration of fluorescent localization occurred in the cuticle of all sections treated, with some exceptions. The tegument of cestodes is an absorptive-digestive surface, involved with high levels of protein synthesis and secretion, and has been found to be quite antigenic[9,10]. The scolex cuticle in this study showed localization with gravid, though not notably with mature conjugate. The cuticle of the scolex region of *Hymenolepis diminuta* has been reported to differ markedly from the cuticle of the remainder of the strobila[11]; present results suggest the cuticle of *Moniezia expansa* may manifest similar heterogeneity. The cuticle of the mature tissue sections gave an intense fluorescent localization reaction with mature conjugate, and less, if at all, with gravid conjugate. Some antigenic difference in the cuticle of mature and gravid proglottids is indicated here. Differential enzyme activity has been found[12] between mature proglottids and other developmental regions of *Moniezia expansa.* The results of the present study suggest there may be some chemical differences in the metabolic progression from sexually active to inactive proglottids.

The eggs of *Moniezia expansa* are very antigenic, and localization on the embryophore and pyriform apparatus of fully developed eggs with whole worm antiserum conjugate has been reported[13]. The results of this study were consistent, and showed an additional localization on the egg capsule, with both mature and gravid conjugates. In mature proglottid sections, the homologous conjugate gave rise to an intense localization on the developing eggs, while identi-

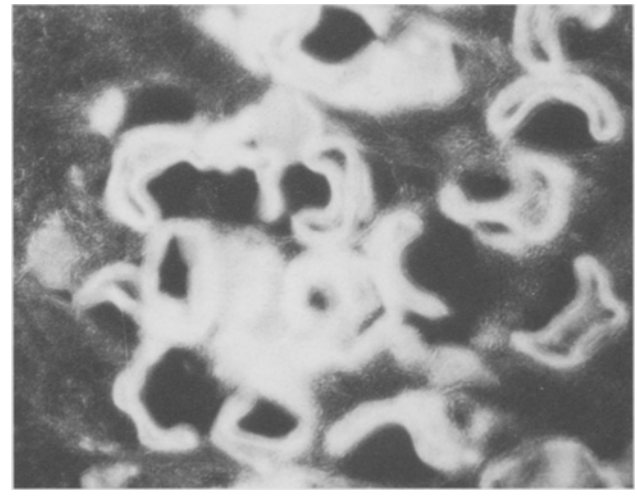

Figure 2. Fluorescent localization of tissue antigens in sections of *Moniezia expansa* proglottids, with rhodamine lissamine-200 labelled antibodies. Gravid proglottid section treated with antimature serum conjugate. Note egg membranes.

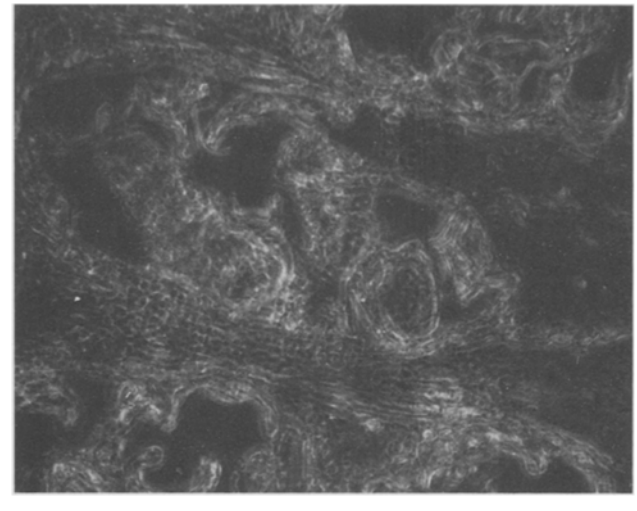

Figure 3. Gravid proglottid control section, demonstrating tissue autofluorescence.

cal treatment with gravid conjugate showed a more granular, diffuse reaction. This suggests a difference in egg antigen composition or concentration, as development proceeds.

Conclusions. In this study, the variety of structures on which localization occurred reveals the extreme complexity of antigen-antibody systems present. Structures particularly manifesting a difference in antigenic protein content are the cuticle and eggs. Exhaustive serum absorption studies in conjunction with antigen separation may elucidate the number and specific location of common and unique antigens involved. To summarize, general antigenic differences in the strobila of *Moniezia expansa* were noted, by an immunologic monitor of development.

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Weight changes in mice after intrauterine treatment with MPG (2-mercaptopropionylglycine) against I^{131} irradiation

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Summary. MPG, administered in utero against I^{131}-irradiation, protected young mice to some extent from loss of body weight at different postnatal intervals. Increase in the tissue weight induced by the internal emitter was enhanced at 6 weeks of age by the drug.

MPG (2-mercaptopropionylglycine) has been shown to be a radioprotector effective at a very low dose (20 mg/kg b.wt) in mice[2]. A number of reports from this laboratory provide further evidence to its protective role[3,4]. Of late, Uma Devi and Jagetia[5] have shown that MPG protects mice by lowering the thyroid metabolic rate. Thyroid function is increased during pregnancy[6]. It was, therefore, thought worthwhile to study the radioprotective effect of MPG on the development of mice where the mother's thyroid had been suppressed by irradiation with internally administered I^{131}.

Material and methods. 10 female mice at day 11.5 of pregnancy (considering that mating took place after midnight and treatment was done on the following day at 14.00–15.00 h) were injected i.p. with a single dose of 150 µCi I^{131} per animal to serve as the control group. Another 10 mice of the same pregnancy received MPG (dissolved in double distilled water, with the pH adjusted to 6.4 with the addition of 0.1 N NaOH solution; each pregnant animal received 0.5 mg MPG in 0.25 ml solution) i.p. at the rate of 20 mg/kg b.wt, 30 min before, in addition to the same dose of I^{131}, to serve as the experimental group. The latter group of animals received the same dose of MPG daily, on all the subsequent gestation days till parturition. The normal group consisted of pregnant mothers similarly treated with double distilled water.

All the 3 groups were allowed to breed. The body weight of at least 2 animals from each sex for each litter was recorded from the day of birth to 6 weeks of age, at weekly intervals. Abnormalities, if any, in litters were also noted. At the age of 6 weeks, at least 1 male and 1 female from each litter were sacrificed and the wet weights of various tissues like liver, spleen, thymus, testes, pituitary, brain and kidney were recorded.

Results. All the female bred normally with the completion of term (19 days under these laboratory conditions). The offspring of the control group were lethargic throughout their postnatal development. The hind limbs of control animals were extremely weak as compared to the experimental ones (fig. 1). No death was recorded up to 6 weeks of age.

The body weights of both males and females were consistently less, except at 3 weeks old, in both control and experimental groups when compared to the normal. But the experimental group showed values which were closer to the normal values (fig. 2).

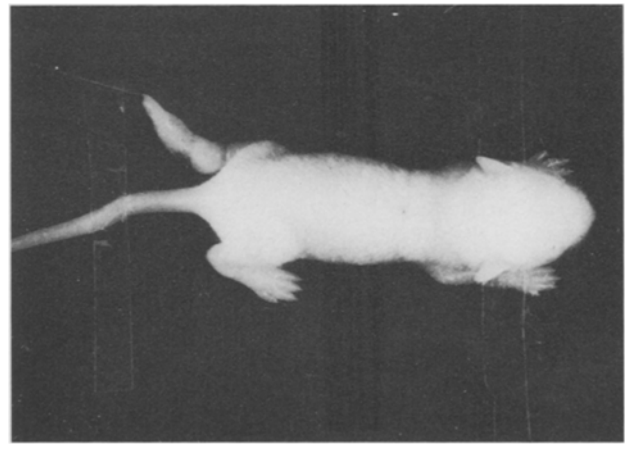

Figure 1. 2-week-old control mice (I^{131}-treated in utero) showing weak hind limbs and inflamed toes.