

of neural cell cluster, and of a notochord which appears in 3 sections only (fig. 5).

Transverse section through the head region of the blastoderm presented in figure 2 shows an open neural plate but no identifiable brain structure (fig. 6a). A more posterior section shows a discontinuous neural plate, a notochord, and also lateral mesoderm with some indication of segmentation (fig. 6b).

Histological examination of the blastoderms pictured in figures 3b and 4 shows normal development.

Discussion. The effect of thymidine on chick blastoderm differentiation depends on the stage of development and on the length of time blastoderms are exposed to thymidine. Our results indicate that it is the process of PS formation which is sensitive to thymidine. We assume that the severely defective neural plate is an expression of the thymidine interference with determination of the axial mesoderm and its inductivity. Blastoderms at stage 4⁵ with a mature PS escape the effect of thymidine and develop normally,

probably because the subsequent differentiation and inductive role of the different PS components are already determined.

It is likely that thymidine exerts its effect on young blastoderms by increasing their thymidine triphosphate pool, thus disturbing the delicate balance that must exist among the nucleotide pools and initiating a chain of events which leads to interference with differentiation.

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Proximal tubule changes in the polycystic kidney induced by methylprednisolone acetate in the newborn rabbit. A microdissection-SEM study¹

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Summary. A single i.m. injection of methylprednisolone acetate, given to rabbits within 24 h after birth, produced dilations and modifications in the proximal tubule convolutions of the nephrons during the elongation stage. These changes were not accompanied by alterations in the surface morphology of the epithelial cells of the proximal tubule.

Dilation of the different nephron segments and also of the collecting tubules induced by repeated injections of cortisol have been reported in the young rabbit³. On the basis of that study Perey et al.⁴ have developed an experimental model of renal polycystosis, which is produced in the newborn rabbit by a single administration of corticoids, and only 2 kinds of cystic alterations have been described to be present⁵⁻⁷: tubular cysts affecting the ampular portion of the collecting tubules and glomerular cysts consisting of dilation of Bowman's capsular space. No alterations have been reported until now in other nephron segments.

In the present work we have undertaken a SEM study of microdissected proximal tubules in corticoid-induced renal polycystosis, to assess possible structural alterations.

Materials and methods. Newborn rabbits were injected i.m. once with methylprednisolone acetate (20 mg/kg), as previously described⁵. Some animals of each litter, injected with an equal volume of saline solution, were employed as a control. Rabbits from 2 to 20 days old, anaesthetized with ether, were fixed by perfusion through the aorta with 3% glutaraldehyde made in 0.1 M cacodylate buffer at pH 7.3. Small kidney fragments were then digested by HCl and collagenase⁸ and then carefully microdissected. The isolated nephrons were attached to a gelatin-coated coverslip, dehydrated in acetone, dried by the critical point method, using liquid CO₂, then ion-sputtering coated with gold and observed with a Philips SEM-501.

Results. Although all the segments of the nephron were microdissected we only report here the alterations observed in the proximal tubule.

In approximately 10% of the nephrons studied the convoluted portion of the proximal tubule, especially its terminal

segment, showed a conspicuous spiral twisting with short convolutions, taking the appearance of a corkscrew (fig. 1a). This morphology contrasted with the normal appearance of this portion (fig. 1b). Alterations were never observed in the straight portion of the proximal tubule. In some instances the terminal segment of the convoluted portion displayed eccentric dilation separated by normal segments or, more rarely, by constrictions (fig. 2a, b). All these alterations were observed as early as 10 days after treatment and affected only the nephrons of the outer cortex. No evolutive changes of the lesion were observed in the older animals.

The observation of fractures of the proximal tubules did not reveal any difference between the cell morphology of the normal animals and that of the corticoid treated animals, even in the segments displaying abnormal dilation. As can be seen in figure 3a, the epithelial cells were pyramidal in shape with a brushed luminal surface and a large basal surface attached to the basal lamina. When digestion of the extracellular matrix was carried out, this basal surface showed its normal rough appearance (figs 2b and 3b). The lateral cell infoldings, described in the normal cells, were also prominent (fig. 3a).

Discussion. Our results show that in addition to the glomerular and tubular cysts described in the corticoid-induced polycystic kidney⁵, alterations of the convoluted portion of the proximal tubule are also present. Similar alterations of the proximal tubule were produced by repeated injections of corticoids in older rabbits³, but they did not develop renal polycystosis.

The location of the affected nephrons in the outer cortex of the kidney, and the fact that in the postnatal period studied an important morphogenetic process takes place, suggest

that these alterations could be due to a disturbance of the normal elongation of the nephrons.

Different factors, not mutually exclusive, can be proposed to explain the proximal tubule modifications. Firstly, the collecting tubule cysts could press down on the proximal tubules, thus preventing their normal growth. Since the elongation of the proximal tubules is dependent on the presence of a critical amount of blastemic cells⁹, the alteration reported here could also be due to a reduction in the mass of blastemic cells caused by the necrotic process present in those cells after the administration of corticoids⁶. On the other hand a direct effect of corticoids on cell division¹⁰ and extracellular material production¹¹⁻¹³, which play an important role in morphogenesis and differentiation¹⁴, could also explain the alteration. Since little is

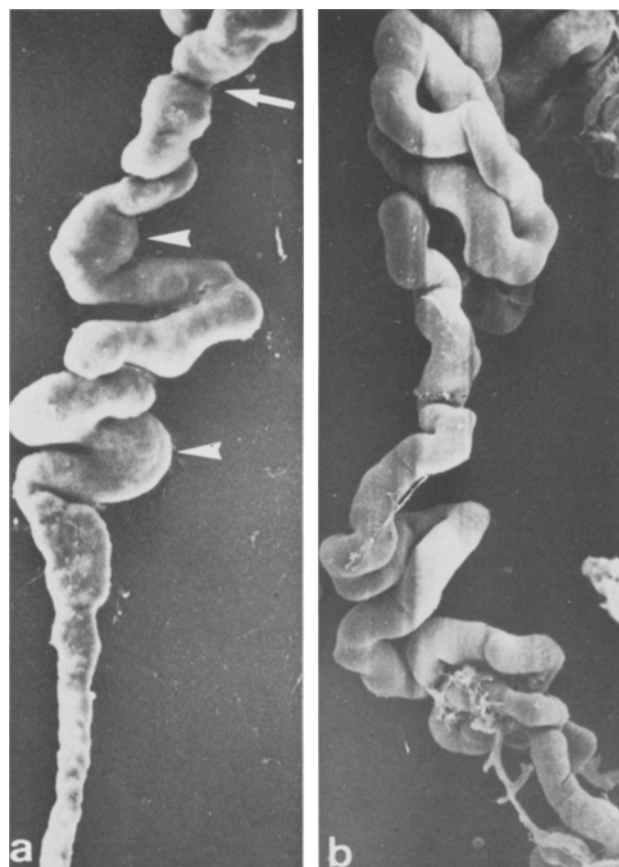
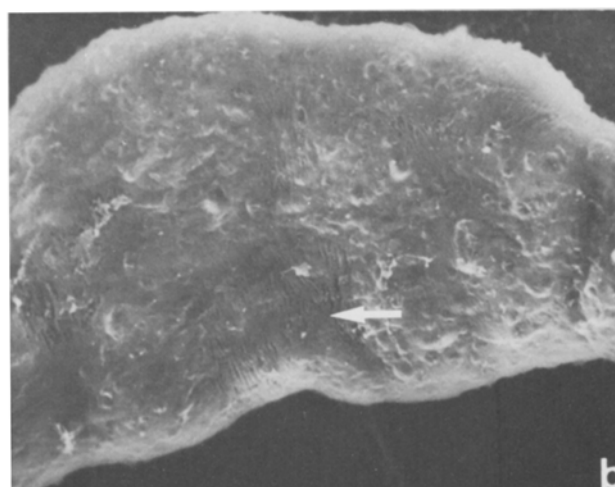


Figure 1. *a* Convoluted and straight portions of a proximal tubule. 10-day-old experimental animal. *b* Control animal. Note the peculiar appearance of the convoluted portion and compare with that of a control animal. Constrictions (arrow) and dilations (arrow head) are clearly recognizable. SEM, $\times 160$.



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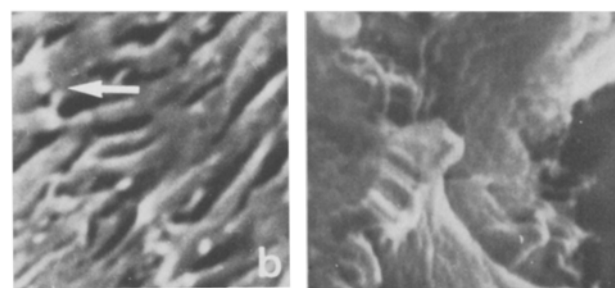
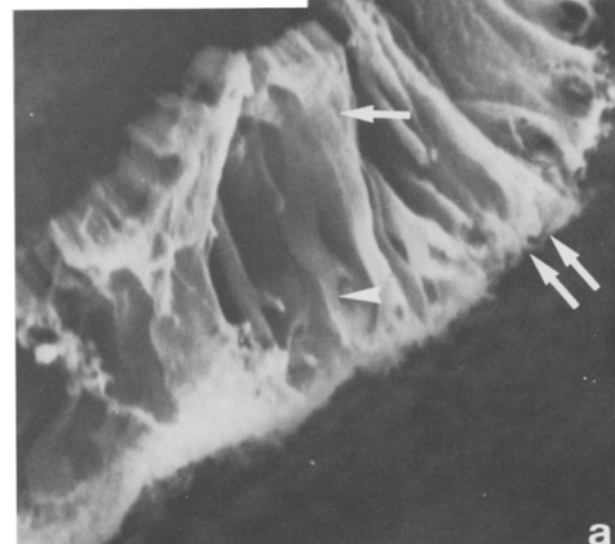


Figure 2. *a* Terminal segment of the convoluted portion of a proximal tubule. 10-day-old experimental animal. Eccentric dilation and one constriction (arrow) can be observed. SEM, $\times 320$. *b* Basal view of an eccentric dilation showing linearly arranged lateral-basal processes (arrow). SEM, $\times 1250$.



3

Figure 3. *a* Fracture of the proximal convoluted cells at the level of a dilation. The cells broaden from apex to base. Numerous lateral ridges (arrow), lateral-basal processes (arrow head) and basal villi (double arrow) can be observed. 15-day-old experimental animal. SEM, $\times 5000$. *b* Basal view of a dilation showing extensive interdigitation of the lateral-basal processes and the presence of basal villi (arrow). SEM, $\times 10,000$.

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 combi-

nation 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 maturity². A progressive acquisition of antigens in *Schistosoma mansoni* from egg to larva to adult has been postulated³. In an antigenic analysis of *Toxocara canis*, the author concluded that adjacent developmental stages have more similar antigen composition than stages more distant in development⁴.

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°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 Crowle⁵ modification of the agar double diffusion method of Ouchterlony⁶ using 1% purified agar. Immunoelectrophoresis was for 30 min at

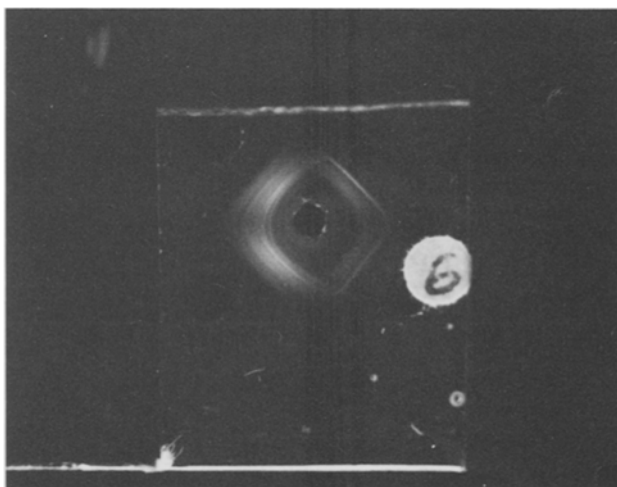


Figure 1. Double immunodiffusion of rabbit antimature proglottid supernatant (center well) against gravid proglottid supernatant (left 2 wells) and scolex supernatant (right 2 wells). Note reactions of identity.