used. The total incorporation of $^{14}C$ was similar in samples labeled early during inhibition of protocollagen hydroxylase and in samples labeled after reversal of the inhibition, an indication that the rate of polypeptide synthesis was essentially the same during the two labeling periods. After reversal of the inhibition, the synthesis of hydroxyproline-$^{14}C$ proceeded at about the same rate in both sets of samples, and there was no significant difference in the rate at which the ratios of hydroxyproline-$^{14}C$ to total $^{14}C$ increased during the reversal period (Fig. 2).

In tibiae that were pulse-labeled after reversal of the inhibition (Fig. 2), essentially all the proline-$^{14}C$ was in polypeptides synthesized in the presence of active protocollagen hydroxylase. In tibiae that were pulse-labeled early in the inhibition period, all the proline-$^{14}C$ should have been in complete polypeptides released from ribosomal complexes. Under the conditions of the experiment, it is unlikely that the separate pools of growing polypeptides on ribosomes and the complete polypeptides released from ribosomes would be hydroxylated at the same rate. If the hydroxylation of proline occurred preferentially in nascent polypeptides attached to ribosomal complexes, the proline-$^{14}C$ in tibiae pulse-labeled after reversal of the inhibition of protocollagen hydroxylase should have been hydroxylated more rapidly than the proline-$^{14}C$ in tibiae pulse-labeled early in the inhibition period. In that both sets of pulse-labeled polypeptides were hydroxylated at essentially the same rate, the results suggest that the hydroxylation of both the newly synthesized and the accumulated polypeptides occurred in the same intracellular pool. From these observations as well as from our previous studies on the effects of puromycin (*8*) and with pulse-labeling techniques in cartilage (*9*), it appears that, even when no measures are taken to inhibit protocollagen hydroxylase, most of the hydroxyproline in collagen is synthesized after complete protocollagen polypeptides are released from ribosomes.

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## Polycystic Renal Disease: A New Experimental Model

Abstract. *A single injection of any one of several long-acting adrenal corticosteroids at birth induces progressive cystic changes in nephrons which develop in the subcapsular zone of the rabbit kidney until 10 days after birth. These cystic lesions enlarge progressively and become visible within a few days after birth. When the animal is 2 weeks old, renal size has become three times as large as that of uninjected littermates. Adrenal corticosteroids prolong the duration of nephrogenic activity in the renal cortex. The cysts are blind and represent dilatation of the developing end of the collecting ducts. When the steroid-induced hypokalemia is prevented with repeated potassium chloride injections, renal cystic disease is almost completely prevented. Certain long-acting steroids induce cystic renal changes without systemic signs of toxicity.*

In the search for an experimental model of polycystic kidney disease (*1*), several investigators have produced cystic lesions in kidneys of experimental animals (*2*) and induced tubular dilatation in rats and rabbits through repeated injections of large doses of steroids in young adult animals (*3*). We studied the effect of a single injection of long-acting adrenal corticosteroids in newborn rabbits and observed persistence and progression of the cysts in the renal cortex and prolongation of the period during which new nephrons developed. Furthermore, we found that 9-fluoroprednisolone acetate (*4*) induces cystic renal changes without signs of systemic toxicity when injected in the proper dosage during the neonatal period; this permits study of the progression of the cystic disease in otherwise intact animals. We obtained evidence which strongly suggests potassium deficiency as the cause for the cystic lesions.

Litters of New Zealand white rabbits (40 to 50 g) were obtained on the day of birth. Some of each litter were given an intramuscular injection of one of nine long-acting steroids (Table 1), while others were not injected and served as controls. After injection, the animals were returned to the care of their mother. One group, injected with 0.5 mg of 9-fluoroprednisolone acetate at birth, was given an intraperitoneal injection of 0.25 meq of potassium chloride (KCl) daily for 14 days. They were provided with as much Purina rabbit chow and water as they wanted. Qualitative urinalyses were done on fresh urine obtained by manually compressing the lower abdomen of the young rabbits. Concentrations of sodium, potassium, creatinine, and urea nitrogen in the serum were determined by routine methods. Urinary osmolalities were measured (on a Fiske Osmometer) in 6-week-old rabbits after they had been deprived of water and food for 36 hours. Microdissection was performed with the method of Oliver *et al.* (*5*). Cystic changes observed on kidney sections stained with hematoxylin and eosin were subjectively graded as zero to four pluses. The majority of long-acting steroids induced cystic changes in renal cortical tubules (Table

1); this effect was clearly related to dosage. With a single exception, all the drugs with this activity led to marked runting characterized by muscular atrophy, dry skin, hair loss, and marked failure in general development. In the case of the highest doses, the animals died within 2 to 3 weeks after injection. When cortisone acetate was used, marked runting was observed, but no cystic lesions were induced. On the other hand, 9-fluoroprednisolone acetate clearly induced cystic lesions in the renal cortex; yet this was not accompanied by runting, and the animals receiving this drug developed as well as uninjected littermates. Methylprednisolone acetate (*6*) induced minimum runting and some cystic disease. None of the short-acting steroids induced either runting or renal lesions at the doses studied. The most severe disease was observed in rabbits given 2.5 mg of prednisolone *tert*-butyl acetate at birth; kidneys were greatly enlarged and weighed three times as much as those of uninjected littermates when compared at 2 weeks of age. This dose produced severe systemic toxicity, however, and the animals failed to survive beyond that age. Cystic lesions were restricted to the kidneys; other effects consisted of variable degrees of atrophy of lymphoid tissues and adrenal cortex. The kidneys were enlarged, with small cysts visible on the surface beneath the capsule and in the cortex in cut sections. Microdissection and serial sections of the cystic kidneys indicated that the cysts represent dilatations of collecting ducts (resembling cul-de-sacs) which apparently failed to establish continuity with developing nephrons. Histological sections revealed cysts arising in the nephrogenic zone of the outer renal cortex as early as 3 days after drug injection; the cysts were lined by cuboidal epithelium early in their development, but, as they enlarged, the epithelial lining became flattened (Fig. 1). They were not observed in the renal medulla. Normally, a nephrogenic zone can be easily identified up to 10 days after birth; in animals with steroid injections, this zone is still present at 15 days of age. Cysts are filled with fluid material which exhibits slight eosinophilia after staining with hematoxylin and eosin; but as cysts enlarge, this fluid no longer stains.

Table 1. Effects of synthetic adrenal corticosteroids in newborn rabbits. Each drug was injected intramuscularly into rabbits within 24 hours after birth. The amount of renal cystic disease and runting were observed during the first 3 weeks after birth. Degrees of renal cystic changes were graded on a subjective basis. PTBA, prednisolone *tert*-butyl acetate; TD, triamcinolone diacetate; 9-FPA, 9-fluoroprednisolone acetate.

| Dosage (mg/kg of body weight) | Runting | Renal cystic disease |
|---|---|---|
| *PTBA (Hydeltra TBA*)* | | |
| 50 | ++++ | ++++ |
| 20 | ++++ | +++ |
| 10 | ++ | + |
| 5 | + | 0 to + |
| 2.5 | 0 to + | 0 |
| *TD (Aristocort*)* | | |
| 20 | +++ | + to ++ |
| 10 | + | 0 to + |
| *Triamcinolone acetonide (Vetalog*)* | | |
| 12 | ++ | 0 to + |
| *Hydrocortisone acetate* | | |
| 250 | ++++ | +++ |
| 500 | ++++ | +++ |
| *Cortisone acetate* | | |
| 200 | ++ | 0 |
| *Dexamethasone (Azium*)* | | |
| 4 | 0 to + | 0 to + |
| *Prednisolone (crystalline)* | | |
| 50 | 0 | 0 |
| *Methylprednisolone acetate (Depomedrol*)* | | |
| 20 | 0 to + | + to ++ |
| *9-FPA (Predef 2x*)* | | |
| 10 | 0 | +++ |
| 20 | 0 to + | ++++ |

*Trade names.

In animals given KCl as described above, cyst formation was almost completely prevented. The few cysts which developed were near the corticomedullary junction and probably arose during the period when the steroid was most active. Cystic disease was not produced with repeated injections of 1.25 mg of deoxycorticosterone acetate 2, 3, and 5 days after birth, but because the degree of hypokalemia induced was not measured, we do not know if this dose was adequate.

Although it has been known for many years that adrenal cortical steroids given in high doses for prolonged periods of time will produce dilatation of tubules in fully differentiated nephrons, we believe that effects of these agents on developing nephrons have not been investigated. The effect of steroids is apparently related to their mineralocorticoid activity; it is postulated that they prevent nephrons from establishing their usual connection with the ureteral bud and that the latter undergoes cystic dilatation. Although the lesions are not as widespread as those described by Osathanondh and Potter (*7*) in type II renal cystic disease, the number of developing nephrons in this experimental model form only a small percentage of total nephrons. Perhaps in human disease, damage to developing nephrons occurs before any nephrons have differentiated completely. The significance of the association between the retardation of development of nephrons (as indicated by the persistence of the nephrogenic zone beyond 10 days) and the induction of cystic changes is not known. By some means, the action of steroids might disrupt the timetable of events and thereby prevent junction of the two major nephric elements. A decrease in serum or tissue potassium, or both, seems to be of importance in the pathogenesis of cyst formation, since prevention of hypokalemia significantly retards this formation. The role of steroids in inducing these cystic lesions may be related to a direct pharmacological action of these drugs as well as

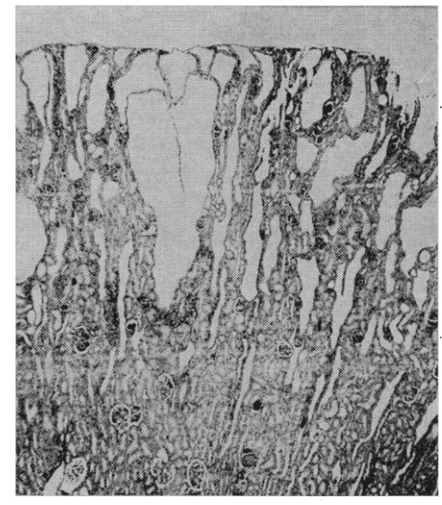

Fig. 1. Renal cortex from a 2-week-old rabbit which had been injected with 2.5 mg of prednisolone *tert*-butyl acetate intramuscularly at birth. Cysts predominate in outer cortex, and some appear to communicate with renal troubles. (Stained with hematoxylin and eosin; magnification × 23.)

to an effect secondary to electrolyte disturbances. Whatever the mechanism involved, our evidence strongly suggests that potassium deficiency is quite important in inducing the formation of cysts. Indeed hypokalemia induces diffuse tubular dilatation in older animals (*8*).

This experimental model brings new perspectives to the study of cystic disease of the kidney not only by producing the disease rapidly and easily but also by initiating it during the early stages of renal development so that it parallels at least certain forms of human cystic renal disease. In addition, with this model, therapeutic approaches are possible. Our studies failed to reveal any abnormality of renal function early in the disease; this abnormality may arise later with the progression of cystic changes, but animals have not yet been studied at advanced stages. Potassium deficiency in the pathogenesis of this model has obvious clinical implications in that this electrolyte disturbance might be encountered or produced iatrogenically during pregnancy.

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## Enzyme Induction by Corticosteroids in Embryonic Cells: Steroid Structure and Inductive Effect

Abstract. *Glutamine synthetase in the developing retina of the chick embryo can be induced to increase by certain corticosteroids. The inductive effectiveness of various natural corticosteroids has been examined in organ cultures of embryonic retina and correlated with specific groupings on the steroid molecules.*

Glutamine synthetase in the neural retina of the chick has a characteristic and unique developmental pattern that coincides with other aspects of retinal development and provides a quantitative marker of differentiation in this tissue (*1*). This pattern and other aspects of retinal development can be modified by corticosteroids (*2*). In normal embryonic development, glutamine synthetase in the retina begins to increase at a very sharp rate after the 16th day of incubation, during the period of final differentiation and maturation of this tissue; however, glutamine synthetase can be induced to rise at a sharp rate several days ahead of time, in cultures of retina tissue and in the retina in the embryo by treatment with hydrocortisone (*2, 3*). This precocious induction of glutamine synthetase is accompanied by acceleration of other developmental features in the retina; it requires RNA and protein synthesis (*4*) and appears to be specific for this embryonic tissue. Exploratory tests indicated that, in addition to hydrocortisone, other corticosteroids had an inductive effect in this system. We have, therefore, examined the correlations between the molecular structure of various natural adrenal steroids and their effectiveness as inducers of glutamine synthetase in organ cultures of embryonic retina in vitro.

Single, whole retinas from 12-day chick embryos were explanted and cultured in 3 ml of medium in 25-ml erlenmeyer flasks. The culture medium consisted of 20 percent fetal bovine serum in Tyrode's solution, 1 percent penicillin-streptomycin mixture (Microbiological Associates), and $10^{-8}$ g of steroid per milliliter. Stock suspensions of the steroids were prepared in 1 ml of Tyrode's solution with 0.04 ml of Tween 80; controls did not contain the steroid. A mixture of 5 percent $CO_2$ in air was passed through the cultures which were then incubated at 38°C on a rotary shaker (70 rev/min). The retinas were harvested after 24 hours in culture and assayed for glutamine synthetase activity (*4*).

The results (Table 1) show that, at the concentration tested, hydrocortisone, corticosterone, and aldosterone are the strongest inducers of glutamine synthetase in the embryonic retina. These three steroids have in common the 11$\beta$-hydroxyl group and the 17$\beta$-side chain with the 20-ketone and the 21-hydroxyl groups. The activity of the strongest inducers was not appreciably lowered by esterification of the 21-hydroxyl group with phosphoric acid. Molecules with a methyl group in the 21-position (11$\beta$-hydroxyprogesterone and 11$\beta$,17$\alpha$-dihydroxyprogesterone) had intermediate activities. On the other hand, molecules without hydroxyl on carbon 11 or with the 11-hydroxyl in the $\alpha$-configuration had only a very slight or no effect. In addition to the steroids listed in Table 1, 11$\alpha$-hydrocortisone was also tested ($10^{-8}$ g per ml) and was essentially ineffective (5 percent increase in glutamine synthetase activity); this is in marked contrast to the high effectiveness of the natural, 11$\beta$-hydrocortisone. All of this suggests that the 11$\beta$-position is of primary significance in the activity of these molecules in inducing retinal glutamine synthetase in this system. This conclusion is further supported by the fact that cortisone, which has a ketone group in the 11-position, had no effect under these conditions.

While the detailed mechanisms whereby these corticosteroids exert an inductive effect on glutamine synthetase in the embryonic retina and enhance the developmental program of this tissue remain to be determined, it is of interest that the steroid molecules which are strong inducers in this system also have a high glucocorticoid activity (*5*). The significance of this remarkably close correlation to the differential effects of these steroids on protein synthesis in various embryonic and adult tissues (*6*) is unknown. Evidence from other systems (*7*) suggests that the function of these molecules involves their association with specific receptors on or in the target cells, and subsequent effects on translative and transcriptive processes (*6*). If the specific inductive effect of the corticosteroids on the glu-