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.

DANIEL Y. E. PEREY
ROGER C. HERDMAN
ROBERT A. GOOD

Department of Pediatrics, University of Minnesota, Minneapolis

References and Notes

1. G. A. Schrader, C. O. Prickett, W. D. Salmon, *J. Nutr.* **14**, 85 (1937); A. Kornberg and K. M. Endicott, *Amer. J. Physiol.* **145**, 291 (1946); H. W. Deane, J. H. Shaw, R. O. Greep, *Endocrinology* **43**, 133 (1948); J. H. Meyer, R. R. Grunert, M. T. Zepplin, R. H. Grummer, G. Bohstedt, P. H. Phillips, *Amer. J. Physiol.* **162**, 182 (1950); E. M. Darmady and F. Stranack, *Brit. Med. Bull.* **13**, 21 (1957).
2. J. O. Thomas, A. J. Cox, Jr., F. DeEds, *Stanford Med. Bull.* **15**, 90 (1957); S. W. Kime, Jr., J. J. McNamara, S. Luse, S. Farmer, C. Silbert, N. S. Bricker, *J. Lab. Clin. Med.* **60**, 64 (1962).
3. T. J. Baxter, *Brit. J. Exp. Pathol.* **41**, 140 (1960); P. M. Newberne, *Amer. J. Vet. Res.* **25**, 1256 (1964).
4. Predef, the Upjohn Company, Veterinary Division, Kalamazoo, Michigan.
5. J. Oliver, M. MacDowell, L. G. Welt, M. A. Holliday, W. Hollander, Jr., R. W. Winters, T F. Williams, W. E. Segar, *J. Exp. Med.* **106**, 563 (1957).
6. Depomedrol, the Upjohn Company, Kalamazoo, Michigan.
7. V. Osathanondh and E. L. Potter, *Arch. Pathol.* **77**, 474 (1964).
8. R. H. Follis, Jr., E. Orient-Keiles, E. McCollum, *Amer. J. Pathol,* **18**, 29 (1942); B. Spargo, *J. Lab. Clin. Med.* **43**, 802 (1954); *Arch. Pathol.* **70**, 599 (1960).
9. Aided by grants from The National Foundation, American Heart Association, PHS (HE-02085), and Minnesota Chapter of Arthritis and Rheumatism Foundation. D.Y.P. is Advanced Research Fellow of the American Heart Association. We thank J. Rundquist for valuable technical assistance. Microdissection studies were performed by Dr. R. L. Vernier.

14 August 1967

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.*

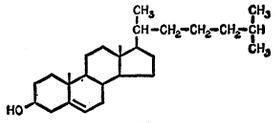
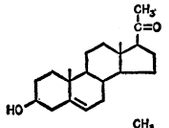
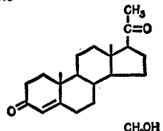
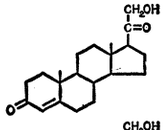
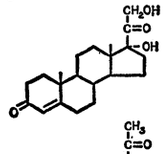
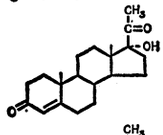
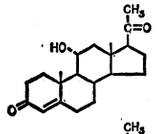
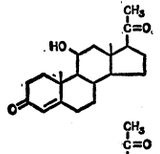
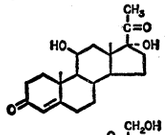
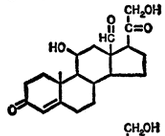
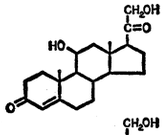
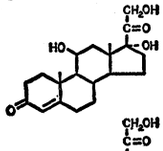
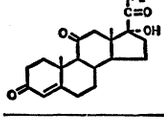
Glutamine synthetase in the neural retina of the chick has a characteristic and unique developmental pattern that coincides with other aspects of retinal development and provides a quantitative marker of differentiation in this tissue (*1*). This pattern and other aspects of retinal development can be 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 11β-hydroxyl group and the 17β-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 (11β-hydroxyprogesterone and 11β,17α-dihydroxyprogesterone) had intermediate activities. On the other hand, molecules without hydroxyl on carbon 11 or with the 11-hydroxyl in the α-configuration had only a very slight or no effect. In addition to the steroids listed in Table 1, 11α-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, 11β-hydrocortisone. All of this suggests that the 11β-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-

Table 1. Effect of different adrenal steriods on glutamine synthetase (GS) activities in cultured embryonic retina. Numbers in parentheses indicate the number of assays.

Structural formula	Steroid (10^{-8} g/ml)	Specific activity (12-day retina cultured for 24 hr)	Increase in GS activity (%)
	Control	0.95 ± .28 (12)	
	Cholesterol	0.84 ± .05 (2)	
	Pregnenolone	0.86 ± .03 (2)	
	Progesterone	0.97 ± .12 (4)	2.0
	Deoxycorticosterone	1.22 ± .28 (4)	28.4
	11-Deoxycortisol	1.16 ± .15 (4)	22.1
	17α-Hydroxyprogesterone	0.84 ± .19 (4)	
	11α-Hydroxyprogesterone	1.39 ± .14 (4)	46.3
	11β-Hydroxyprogesterone	2.62 ± .10 (3)	175.7
	11β,17α-Dihydroxy-progesterone	3.59 ± .16 (3)	277.8
	Aldosterone	4.34 ± .08 (2)	356.8
	Corticosterone	4.36 ± .30 (4)	358.9
	Hydrocortisone	5.06 ± .89 (4)	432.6
	Cortisone	0.94 ± .18 (4)	

tamine synthetase system in the embryonic retina depends initially on specific interactions with retinal receptors, our data suggest that the position and orientation of the 11β-hydroxyl group is critical for the specificity of these interactions and that the 21-position has a secondary role in this sequence. However, the possibility also exists that one of these positions is more important for interactions with receptors, while the other is more closely involved in the regulation of biosynthetic processes relevant to the sequence of induction in these cells (*4*).

The present system lends itself well to detailed studies of these problems with respect to the mechanism of induction in embryonic cells. It provides a rather unique correlation between the structural, chemical, and physiological properties of an inducer molecule and its effect on the development of an enzyme characteristic of differentiation in a well-defined population of embryonic cells. Of added interest is the possibility that induction in vitro of glutamine synthetase in the embryonic retina may offer a sensitive and practical bioassay system for testing glucocorticoid activity of various steroids (*8*).

A. A. MOSCONA
R. PIDDINGTON
Department of Zoology, University of Chicago, Chicago, Illinois

References and Notes

1. A. A. Moscona and J. L. Hubby, *Develop. Biol.* **7**, 192 (1963); R. Piddington and A. A. Moscona, *J. Cell Biol.* **27**, 246 (1965).
2. A. A. Moscona and R. Piddington, *Biochim. Biophys. Acta* **121**, 409 (1966); R. Piddington, *Develop. Biol.*, in press.
3. R. Piddington and A. A. Moscona, *Biochim. Biophys. Acta*, in press.
4. D. L. Kirk and A. A. Moscona, *Develop. Biol.* **8**, 341 (1963); A. A. Moscona and D. L. Kirk, *Science* **148**, 519 (1965); D. L. Kirk, *Proc. Nat. Acad. Sci. U.S.* **54**, 1345 (1965).
5. J. Fried and A. Borman, *Vitamins and Hormones* **16**, 303 (1958).
6. F. Moog, in *The Biochemistry of Animal Development*, R. Weber, Ed. (Academic Press, New York, 1965), vol. 1, p. 307; P. Feigelson, M. Feigelson, O. Greengard, *Recent Prog. Hormone Res.* **18**, 491 (1962); L. D. Garren, R. R. Howell, G. M. Tomkins, R. M. Crocco, *Proc. Nat. Acad. Sci. U.S.* **52**, 1124 (1964); E. H. Davidson, *Sci. Amer.* **212**, 36 (1965); C. Kidson, *Biochem. Biophys. Res. Commun.* **21**, 283 (1965); E. B. Thompson, G. M. Tomkins, J. F. Curren, *Proc. Nat. Acad. Sci. U.S.* **56**, 296 (1966)
7. I. E. Bush, *Pharmacol. Rev.* **14**, 317 (1962).
8. Research supported by grants from USPHS, Institute for Child Health and Human Development (HD-01253), and from NSF (G-23852). We acknowledge the assistance of N. Saenz and M. Gundersen. We thank Dr. W. J. Cole, Abbott Laboratories, and Dr. P. W. O'Connell, Upjohn Company, for gifts of steroids, and Dr J. Fried of the University of Chicago for counsel and discussion.

7 August 1967