2 h later, the animals received i.p. either 50 µg of dexamethasone/100 g (Oradexon, Organon) (Groups 3 and 4), or 0.2 ml of physiological saline/100 g (Groups 1 and 2). 2 h after the i.p. injection, 1 ml of blood was withdrawn from the saphenous vein, under light ether anaesthesia, within 2 min of removing the animal from its cage. Histamine (1 mg/100 g) was injected i.p., followed by blood withdrawal from the abdominal aorta 30 min later. Pre- and after-stress plasma corticosterone was determined by the competitive protein-binding method[9]. Cortexolone did not interfere with corticosterone determination, since 3 h after administering s.c. the above dose of cortexolone to rats adrenalectomized 24 h earlier plasma corticosterone equalled that in the adrenalectomized controls (0.57 ± 0.08 µg/100 ml v.s. 0.54 ± 0.05 µg/100 ml; number of determinations is 9 in both groups).

Results and discussion. Dexamethasone administration lowered the resting plasma corticosterone level (Table). After histamine, corticosterone level in the dexamethasone-treated rats rose, the increment being significantly smaller than in the controls (Table). Cortexolone exerted no effect on the stress reaction of rats (Group 2 in the Table). This finding seems to be at variance with that of JONES et al.[10], a difference probably due to the fact that we administered cortexolone in 10 times lower doses than they did.

Cortexolone administration prior to dexamethasone did not prevent the decrease in the resting corticosterone level (Group 4 in the Table), but in these animals histamine stress induced an increment in plasma corticosterone similar to that in the vehicle-treated controls (v.s. Group 4 and Group 1 in the Table).

Our interpretation of these data is that cortexolone probably displaces dexamethasone from the hypothalamic and/or extrahypothalamic corticosteroid receptor sites, and thus prevents dexamethasone from inhibiting stress-induced ACTH release. The data favor the assumption that the corticosteroid feedback action depends on the binding of the hormone to specific receptors in the target cells, the mechanism of feedback action being in this respect similar to that of other well-known corticosteroid effects. It was suggested[11,12] that the mechanisms controlling ACTH-release under non-stress and stress conditions are functionally dissociable. Our present data seem to support this assumption by indicating that the site and/or the mechanism of dexamethasone suppression of resting ACTH secretion are distinct from those depressing stress induced ACTH release.

Summary. Cortexolone in a dose of 1 mg/100 g body wt., administered to rats prior to dexamethasone, prevented dexamethasone from suppressing stress-induced ACTH-release without interfering with the effect of dexamethasone on the resting plasma corticosterone level.

ZSUZSANNA ÁCS and E. STARK[13]

Institute of Experimental Medicine, Hungarian Academy of Sciences, P.O. Box 67, H-1450 Budapest (Hungary), 23 June 1975.

[9] B. E. P. MURPHY, J. clin. Endocr. Metab. *27*, 973 (1967).
[10] M. T. JONES, E. M. TIPTAFT, F. R. BRUSH, D. A. N. FERGUSON and R. L. B. NEAME, J. Endocr. *60*, 223 (1974).
[11] E. ZIMMERMAN and V. CRITCHLOW, Am. J. Physiol. *216*, 148 (1969).
[12] J. R. HODGES, Progr. Brain Res. *32*, 12 (1970).
[13] Acknowledgements. Authors wish to thank Mr. G. FOLLY for the mathematical analyses and Mrs. M. PONGRÁCZ for her valuable technical assistance.

Effect of Progesterone on Human Corticosteroid 21-Hydroxylation

The biochemical basis of the genetically inherited metabolic disorders known as the adrenogenital syndrome[1,2] is as yet not fully understood. While two forms, the 'salt-losing' and the 'non-salt-losing' varieties, are known, both of which appear to result from defective adrenal 21-hydroxylation capability, it is not yet known whether these represent a single or two genetic defects[3,4]. Researches on these possibilities have recently been reviewed elsewhere[5]. The present account reports the results of experiments to investigate whether independent active sites for the 21-hydroxylation[6] of progesterone to 11-deoxycorticosterone (DOC) and of 17α-hydroxyprogesterone to 11-deoxycortisol (Reichstein's compound S) are present in human adrenocortical microsomal protein. This investigation formed part of a communication[7] presented at the meeting of the Southern Society for Pediatric Research in 1974.

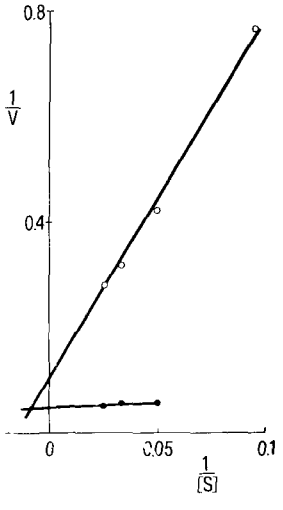
Two normal human adrenals were obtained fresh postmortem and the external fat and connective tissue were trimmed off. The weight of the 2 glands was 7.07 g. They were cut into small pieces in about 100 ml cold 0.25 *M* sucrose. The suspension was homogenized using a motor-driven glass-teflon homogenizer and centrifuged at 600 *g* for 10 min to remove nuclear components, unbroken cells and unwanted debris[8]. The supernatant was centrifuged at 8,720 *g* for 10 min to separate the heavy mitochondria and twice at 26,700 *g* for 30 min to remove light mitochondria. The microsomes were isolated from the supernatant by centrifugation at 78,500 *g* for 90 min. They were gently hand-homogenized in 1–2 ml *Tris*-HCl, 50 m*M*, pH 7.45 containing 3 m*M* $MgCl_2$ and the protein concentration determined by the biuret method. Determination of the cytochrome P450 content of the microsomal protein was performed from the optical extinction at 450 nm after treatment with dithionite and carbon monoxide[9].

Incubations were performed for 5 min with agitation in a Dubnoff incubator maintained at 37 °C. The reaction mixtures contained microsomal enzyme (1.7 mg protein); trisodium isocitrate, 11 µmoles (Sigma); pigheart iso-

[1] K. S. HOLT and D. N. RAINE, *Basic Concepts of Inborn Errors and Defects of Steroid Biosynthesis* (E. and S. Livingstone, Edinburgh 1966).
[2] D. B. VILLEE, *Human Endocrinology, a Developmental Approach* (Saunders, Philadelphia 1975).
[3] F. C. BARTTER, Arch. Dis. Childhood *44*, 138 (1969).
[4] G. T. BRYAN, B. KLIMAN and F. C. BARTTER, J. clin. Invest. *44*, 957 (1965).
[5] R. H. WICKRAMASINGHE, Enzyme *19*, 348 (1975).
[6] K. J. RYAN and L. L. ENGEL, J. Am. chem. Soc. *78*, 2654 (1956).
[7] E. B. NELSON, R. H. WICKRAMASINGHE and G. T. BRYAN, Clin. Res. *22*, 79A (1974).
[8] R. H. WICKRAMASINGHE, Int. J. Peptide Protein Res. *6*, 187 (1974).
[9] T. OMURA and R. SATO, J. biol. Chem. *239*, 2379 (1964).

citrate dehydrogenase (E.C.1.1.1.42), 0.72 units (Sigma); NADP, 0.55 μmoles (Sigma); steroid substrate and *Tris*-HCl, 50 m*M*, pH 7.45 (containing 3 m*M* $MgCl_2$) to a final volume of 1.5 ml. Tritiated tracers of steroid substrates were used as required to quantitate the hydroxylation of the steroid being studied. Other details of the assay procedure were as previously described[10]. Controls showed that, in the absence of NADPH generator, conversion of 17α-hydroxyprogesterone(17α-OHP, 40 μ*M*) to 11-deoxycortisol was about 0.8% under the conditions of the assay procedure. Controls using tritiated progesterone (40 μ*M*) as the sole steroid inclusion showed production of 17α-OHP (2.46%) and 11-deoxycortisol (0.95%) under similar reaction conditions. These control data were taken into account in the calculation of relevant substrate and product values. The cytochrome P450 content was 0.17 nmol/mg protein.

The Figure shows the 21-hydroxylation of 17α-OHP to 11-deoxycortisol in the presence of increasing quantities of substrate. Under the conditions studied the rate of the reaction was limited by the concentration of the substrate. The *Km* for 17α-OHP was 4.3 μ*M* and the V_{max} for the reaction, 2.6 nmol/min/mg protein. It was observed that the addition of 40 μ*M* progesterone (unlabelled) causes a marked decrease in the rate of 21-hydroxylation of 17α-OHP. The double-reciprocal plot demonstrates that the inhibition by progesterone is of the 'mixed type'[11]. Further data on the possibility of 17α-OHP influencing the 21-hydroxylation of progesterone could not be undertaken at the present time due to the presence of the very active 17-hydroxylation of progesterone by the human adrenal microsomal preparations.



21-Hydroxylation of 17α-hydroxyprogesterone by human adrenocortical microsomes in the presence and absence of progesterone. V, nmoles 11-deoxycortisol produced in 5 min incubation; [S], concentration of 17α-OHP, μ*M*; •, only 17α-OHP present; ⊙, 17α-OHP plus progesterone (40 μ*M*) present. Incubation products separated by thin-layer chromatography on silica-gel IB-F (Baker) in benzene:acetone 3:1.

It is concluded from these studies that progesterone does have an inhibitory action, with possible regulatory implications, on the 21-hydroxylation of 17α-OHP by human adrenocortical microsomes. It is not possible to determine unequivocally at the present time as to whether the 21-hydroxylation of these two steroid substrates are effected at the same or at separate active sites. It may well be that the inhibitory effect of progesterone observed results from multiple actions on the multiprotein[5] enzyme system and complex hydroxylation mechanism concerned[12].

Summary. Progesterone inhibits the 21-hydroxylation of 17α-hydroxyprogesterone by human adrenal cortex microsomes. The possible light this finding may shed on the genetic condition, the 'adrenogenital syndrome' is discussed. *Km* and V_{max} data for the above hydroxylation reaction are given.

R. H. Wickramasinghe[13,14] and G. T. Bryan[13]

Pediatric Endocrine Laboratory, Department of Pediatrics and the Clinical Study Center, The University of Texas Medical Branch, Galvestone (Texas, USA), 6 June 1975.

[10] R. H. Wickramasinghe and G. T. Bryan, to be published (1975).
[11] M. Dixon and E. C. Webb, *Enzymes*, 2nd edn. (Academic Press, New York 1964), p.326.
[12] S. Narasimhulu, Arch. Biochem. Biophys. *147*, 391 (1971).
[13] Acknowledgements. Supported by DHEW Grants No. AM-07616 from the National Institute of Arthritis and Metabolic Diseases and No. RR-73 from the General Clinical Research Centers Branch of the Division of Research Resources, National Institutes of Health, USPHS.
[14] Present address: Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, 45 Shattuck Street, Boston, Massachusetts 02115 (USA).

Effect of Cyproheptadine Hydrochloride on Spermatogenesis[1]

While investigating some endocrine effects of cyproheptadine hydrochloride in rats, we observed that chronic administration of the drug to young male rats caused a slight but consistent stimulation of spermatogenesis. But this was not statistically significant as revealed by spermatodynamic study of testicular sections (unpublished data). Since the spermatogenesis in young adult rats is optimal and a further stimulation is difficult to obtain, the effects of a potential stimulant of spermatogenesis may not be very obvious. Hence, we investigated the potentiality of cyproheptadine hydrochloride as a stimulant of spermatogenesis in old rats where the process is normally retarded. The results of these experiments, which prompted us to investigate the effect of this drug on artificial cryptorchid testis, along with some interesting observations in the latter experiment, are presented here.

Materials and methods. A group of 6 male rats aged $2^1/_2$ to 3 years were treated orally with cyproheptadine hydrochloride (5 mg/kg/day) for 48 days. A similar untreated group served as control. In the second experiment, 2 groups of 6- to 8-month-old young male rats, were made unilaterally cryptorchid. One group was treated with cyproheptadine hydrochloride (5 mg/kg/day) for 15 days from the day of operation. At the end of the treatment, all the rats were killed and weights of testis of old rats,

[1] This gives a part of the work done by K. V. Jogi for Ph. D. thesis.