of five graded doses of either control (0.001–0.1 mg FSH) or assay (0.01–0.9 mg); the mice also received 40 I.U. of chorionic gonadotropin[7]. After appropriate time the ovarian and uterine weights were recorded. Typical dose-response regressions were obtained with 0.03 mg and more FSH, whereas the highest dose (0.9 mg) of the RDE-treated FSH used did not cause any significant increase in ovarian and uterine weights. Similar results were obtained with rats (without simultaneous application of chorionic gonadotropin).

The following conclusions may be drawn from the experimental results. (a) All sialic acid residues in FSH are terminal, linked α-ketosidically to their partner and are accessible to α-neuraminidase. (b) Enzymic release of the sialic acid residues reduces the biological activity of FSH by 97 % or more. A more exact estimate of the residual biological activity, if any, would have required amounts of pure FSH not available at present. The data presented taken together with the well known capacity of certain mucoproteins to inhibit in high dilution haemagglutination by influenza viruses, a capacity lost on treatment with α-neuraminidase, are some indication of the range of biological activities imparted to mucoproteins by the presence in them of terminal sialic acid residues.

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[1] W. K. WHITTEN, *Australian J. Sci. Research Ser. B.*, 1 (1948) 388.
[2] A. GOTTSCHALK, *Biochim. Biophys. Acta*, 23 (1957) 645.
[3] A. GOTTSCHALK, *Nature*, 181 (1958) 377.
[4] R. BROSSMER AND K. WALTER, *Klin. Wochschr.*, 36 (1958) 925.
[5] L. SVENNERHOLM, *Arkiv. Kemi.*, 10 (1957) 577.
[6] A. GOTTSCHALK, *Biochem. J.*, 61 (1955) 298.
[7] S. L. STEELMAN AND F. M. POHLEY, *Endocrinology*, 53 (1953) 604.

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Metabolism of [4-^{14}C]corticosterone by fibroblasts, strain U12-79

The physiological importance of the fibroblast and its ability to resist destruction in the presence of corticosteroids in an inflammatory process has been shown by DOUGHERTY *et al.*[1,2]. It has been demonstrated that the ability of corticosteroids to inhibit inflammation is a function of structure and that compounds which have a 17α-OH group are the most potent anti-inflammatory steroids[3]. The metabolism of various steroids by connective tissue[4] and fibroblasts propagated *in vitro*[5,6] have been studied in these laboratories. The data to be presented in this report indicate that a permanent strain of fibroblasts converts corticosterone to 11β,20β,21-trihydroxypreg-4-en-3one.

Strain U12–79[7,8] was propagated in solution S103[9] fortified with 5 % dialysed

horse serum (medium 79) according to method described previously[7,8]. In expt. 1, 10 ml of medium 79 supplemented with 0.3 μg/ml [4-^{14}C]corticosterone (specific activity, 4.2 μC/mg) was added to each of 5 T60 flasks containing a total of $3.4 \cdot 10^7$ cells. Expt. 2 was identical with 1 except that 0.05 μg/ml unlabeled cortisol was added to the 79 in addition to [4-^{14}C]corticosterone. After 72 h at 37° the cells and medium in each experiment were combined and stored at —20° until analysed. Aliquots of each medium incubated with [4-^{14}C]corticosterone for 72 h at 37° in the absence of cells served as controls. Each reaction mixture was extracted with acetone–chloroform[4]. This fraction was chromatographed according to the methods of ZAFFARONI[10]. Isotope techniques for the identification and quantitation of steroids were used as described previously[11,12].

The isotope data indicated that at least 90 % of the steroid was recovered in the chloroform fraction from each of the experimental and control incubation mixtures. In Expts. 1 and 2 only one radioactive peak other than that of the un-metabolized corticosterone was observed when the extracts were chromatographed in the chloroform–formamide system[10]. In the control incubations, only [4-^{14}C]corticosterone was observed upon chromatography. This compound was checked by chromatography in chloroform–formamide (R_F, 0.7) and in benzene–formamide (R_F, 0.1). The acetate derivative was prepared[11], and it migrated at the same rate as authentic non-radioactive corticosterone acetate in benzene–formamide (R_F, 0.5).

The unknown metabolite migrated at a rate (R_F, 0.29) in chloroform–formamide which is slower than cortisol and faster than substance "E" of REICHSTEIN (11β, 17α,20β,21-tetrahydroxypregn-4-en-3-one). These results suggested that the compound could be either 11β,20β,21-trihydroxypregn-4-en-3-one or 20 epi substance "T" of REICHSTEIN (20α,21-dihydroxypregn-4-ene-3,11-dione.)

Unlabeled 11β,20β,21-trihydroxypregn-4-en-3-one was added to an aliquot of radioactive unknown and the mixture was chromatographed in chloroform–formamide. Both compounds migrated at the same rate. The mixture was then acetylated and chromatographed in benzene–formamide. Again the radioactivity migrated at the same rate, indicating the formation of 11β-hydroxy-20β,21-diacetoxypregn-4-en-3-one. This mixture was further oxidized with CrO_3[11] and chromatographed in the benzene–formamide system. The polarity of both the radioactive unknown and authentic 11β,20β,21-trihydroxypregn-4-en-3-one diminished, without separating and forming 20β,21-diacetoxypregn-4-ene-3,11-dione as final product. The foregoing data indicate that corticosterone is converted to 11β,20β,21-trihydroxypreg-4-en-3-one by the U12-79 strain of fibroblasts. There was a 9.8 % and 8.0 % conversion in Expts. 1 and 2 respectively.

Authentic, unlabeled 11β-hydroxyandrost-4-ene-3,17-dione, 20 epi substance "T" of REICHSTEIN and cortisol were added as carriers to the initial chloroform extract in all cases. Upon chromatography and formation of derivatives, no radioactivity was observed in the same zone of the chromatogram where the carrier was present.

The U12-79 strain of fibroblasts[5], loose connective tissue[4], and osteosarcoma cells from beagle dogs [3] convert cortisol to corticosterone. In earlier studies with U12-79[5] employing cortisol as substrate, 11β,20β,21-trihydroxypregn-4-en-3-one did not accumulate in sufficient quantities to be identified. The data obtained in Expt. 2 indicate that corticosterone is not converted to cortisol by U12-79. It appears that a similar situation may exist *in vivo* since the antiphlogistic activity of corticosterone

is much less than that of cortisol. Similarly, compounds reduced in the 20 position lose their antiphlogistic activity[14]. It is of interest in this connection, that most of the products of cortisol and progesterone metabolism by U12-79 are much less inhibitory to the growth of this strain than are the corresponding hormones[15]. It would therefore appear that both cells *in vivo* and those propagated *in vitro* convert the active hormone to less active products. The relationships between these transformations and the physiological effects of the corticosteroids on fibroblasts[2] remain to be elucidated.

The results obtained with corticosterone are in agreement with earlier observations[5,6,16,17] which indicate that the steroid nucleus is not metabolized. Similarly the data are in agreement with other observations which indicate that neither cells propagated *in vitro* nor extra hepatic tissues can actively reduce the 3 position of the steroid molecule. This reaction and conjugation of steroids appear to be carried out mainly by the liver[18,19].

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1 T. F. DOUGHERTY AND G. L. SCHNEEBELI, *Proc. Soc. Exptl. Biol. Med.*, 75 (1950) 854.
2 T. F. DOUGHERTY, R. BIGLER, G. L. SCHNEEBELI AND H. A. SALHANICK, *Ann. N.Y. Acad. Sci.*, 64 (1956) 466.
3 T. F. DOUGHERTY AND G. L. SCHNEEBELI, *Ann. N.Y. Acad. Sci.*, 61 (1955) 328.
4 D. L. BERLINER AND T. F. DOUGHERTY, *Proc. Soc. Exptl. Biol. Med.*, 98 (1958) 3.
5 M. L. SWEAT, B. I. GROSSER, D. L. BERLINER, H. E. SWIM, C. J. NABORS, JR. AND T. F. DOUGHERTY, *Biochim. Biophys. Acta*, 28 (1958) 591.
6 B. I. GROSSER, H. E. SWIM AND M. L. SWEAT, *Federation Proc.*, 17 (1958) 235.
7 H. E. SWIM AND R. F. PARKER, *Am. J. Hyg.*, 2 (1957) 235.
8 H. E. SWIM AND R. F. PARKER, *Ann. N.Y. Acad. Sci.*, special publication, 5 (1957) 303.
9 H. E. SWIM AND R. F. PARKER, *J. Lab. Clin. Med.*, 52 (1958) 309.
10 A. ZAFFARONI, *Recent Progr. in Hormone Research*, 8 (1953) 51.
11 D. L. BERLINER AND H. A. SALHANICK, *Anal. Chem.*, 28 (1956) 1608.
12 D. L. BERLINER, O. V. DOMINGUEZ AND G. WESTENSKOW, *Anal. Chem.*, 29 (1957) 1797.
13 A. VAN DOOREN AND J. DOUGHERTY, *Proc. Am. Assoc. Cancer Research*, 2 (1957) 257.
14 T. F. DOUGHERTY, unpublished data.
15 B. I. GROSSER AND H. E. SWIM, *Proc. Am. Assoc. Cancer Research*, 2 (1958) 304.
16 T. F. DOUGHERTY AND D. L. BERLINER, in *Connective Tissue, Thrombosis, and Atherosclerosis*, Ed. I. PAGE, Acad. Press Inc., N.Y. 143, 1959.
17 D. L. BERLINER AND T. F. DOUGHERTY, *Proceedings Reticulo Endothelial Soc.*, in the press.
18 D. L. BERLINER AND W. WIEST, *J. Biol. Chem.*, 221 (1956) 449.
19 D. L. BERLINER, B. I. GROSSER AND T. F. DOUGHERTY, *Arch. Biochem. Biophys.*, 77 (1958) 81.

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