STEROID BIOSYNTHESIS BY CULTURES OF NORMAL HUMAN ADRENAL TISSUE

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SUMMARY The metabolism of [7α-^{3}H] 17α-hydroxypregnenolone (3β,17α-dihydroxypregn-5-en-20-one) by fascicular and reticular tissue, separated from a human adrenal gland, and maintained in organ culture, has been studied. The percentage conversions to three metabolites, cortisol, DHA (dehydroepiandrosterone) and DHA sulphate were measured. Cortisol was produced by both zones but an increased production under the influence of ACTH only occurred in the fascicular tissue. DHA and DHA sulphate were produced mainly by the reticular tissue and there was a marked stimulation of their synthesis in this tissue under the influence of ACTH.

Assessment of steroid biosynthesis by adrenal tissue maintained in culture systems has thus far been confined to tumour tissue grown in monolayers (Stollar, Buonassisi & Sato, 1964; Kowal & Fiedler, 1968; Neville, Anderson, McCormick & Webb, 1968). No report has yet been made on the organ culture of normal adult human adrenal tissue although Block, Romney, Klein, Lippiello, Cooper & Goldring (1965) have succeeded in maintaining normal foetal human adrenal tissue in organ culture. Jones & Griffiths (1968) and Cameron, Jones, Jones, Anderson & Griffiths (1969) have shown that there is a qualitative difference in steroid production between the zona fasciculata and the zona reticularis of the adrenal cortex of both the guinea pig and human glands in that the sulphation of DHA takes place only in the compact cell of the zona reticularis. This work was carried out using an ultramicrochemical technique. In an attempt to continue the

study of the biosynthetic activity of the small amounts of normal human adrenal tissue available, it was decided to investigate the metabolism of 17α-hydroxypregnenolone by separated fascicular and reticular cells maintained in long term organ culture under conditions which are sometimes considered to be more physiological than the established conditions employed in the usual short term in vitro incubation techniques.

MATERIALS AND METHODS

Normal adrenal tissue was obtained from a patient undergoing adrenalectomy for mammary carcinoma and was transported to the laboratory in a sterile container surrounded by crushed ice. In the laboratory, all work on the tissue was carried out in a Microflow tissue culture cabinet. The gland was stripped free of fat, and unfolded to reveal the dark brown reticular zone. This was scraped away gently, to give tissue consisting mainly of the compact cells of the zona reticularis. The light yellow coloured fascicular tissue was then removed in a similar manner. Four explant cultures of tissue from each zone were then established separately in organ culture dishes (Falcon Plastics), in Eagle's minimum essential medium fortified with 10% calf serum, 292 μg./ml. glutamine, 200 units/ml. penicillin and 100 μg./ml. streptomycin. Explants were cultured on lens paper rafts supported by stainless steel grids, a modification of the Trowell (1954, 1959) technique.

After two days the medium was changed, the fresh medium introduced containing 1 μC $\left[7\alpha\text{-}^{3}H\right]$ 17α-hydroxypregnenolone (19,900 mC/mM). Long-acting ACTH (Organon, corticotrophin Zn) was added to two fascicular and two reticular cultures (0.1 i.u.). After four more days, the media were collected for analysis

and the tissue allowed to wash in fresh medium for another four day period. The medium for the final four days again contained [7α-^{3}H] 17α-hydroxypregnenolone, and ACTH was added to the same cultures as for days 3-7. Media from identical cultures were then pooled and all were analysed for labelled cortisol, DHA and DHA sulphate after the addition of 500 μg. each of non-labelled carrier steroids. The tissue was dried on filter paper and weighed.

The two neutral steroids were extracted with ether, and the DHA sulphate with ethyl acetate after saturation of the media with ammonium sulphate. Table 1 shows the derivatives formed and the thin layer systems used for purification. Thin layer solvent systems used for these studies were as follows:- I Chloroform: methanol: water (187:12:1); II Cyclohexane; ethyl acetate (7:3); III Hexane: ethyl acetate (1:1); IV Benzene: ethyl acetate (9:1); V Cyclohexane: ethyl acetate (9:11); VI tert-butanol: ethyl acetate: 5 N NH_4OH (41:50:20). Derivatives were prepared as described previously (Griffiths, Grant & Whyte, 1963) and the DHA sulphate solvolysed by the method of Burstein & Lieberman (1958). The specific activities of the steroids and their derivatives were measured after elution from thin layer plates by the procedures of Griffiths, Grant, Browning, Cunningham & Barr (1966), and the mean values used to determine percentage conversions. Radioactivity was determined using a Nuclear Chicago Liquid Scintillation Spectrometer (Model 6860).

RESULTS

Table 1 shows the evidence for the identification of steroids isolated from the incubation of [7α-^{3}H] 17α-hydroxy-

pregnenolone with the cultured tissues. The percentage conversion of [7α-^{3}H] 17α-hydroxypregnenolone to the three hormones investigated are also represented diagrammatically in fig. 1.

Steroid and Derivative	Solvent System used for Purification	Specific Activities (dpm/mμM) 1	2	3	4	5	6	7	8
Cortisol	I	27.0	12.6	58.5	30.7	50.4	19.4	30.0	13.2
Cortisol acetate	I	26.7	12.1	57.6	32.3	49.5	20.1	28.3	12.8
Adrenosterone	II	28.8	11.6	59.6	30.6	50.6	21.0	30.8	12.9
DHA	III	11.4	9.6	12.8	7.3	11.6	8.0	20.9	8.5
DHA acetate	IV	12.4	10.0	12.8	6.8	11.9	8.2	21.2	9.0
Androst-5-ene-3β,17β-diol	V	12.6	10.6	14.1	6.8	12.4	8.4	21.3	9.4
DHA sulphate	VI								
DHA	III	21.5	9.0	25.6	8.1	15.8	8.6	50.0	24.8
DHA acetate	IV	21.4	8.7	24.5	7.5	16.0	7.8	51.1	23.6
Androst-5-ene-3β,17β-diol	V	21.8	8.5	25.5	8.0	16.3	8.1	52.6	22.6

The culture flasks, numbered 1-8 were set up as follows, the type of tissue being given and in parentheses the period of culture in days: 1, fascicular (3-7); 2, fascicular (11-15); 3, fascicular and ACTH (3-7); 4, fascicular and ACTH (11-15); 5, reticular (3-7); 6, reticular (11-15); 7, reticular and ACTH (3-7); 8, reticular and ACTH (11-15). The tissue was found to be less active during days 11-15 than during days 3-7, but the pattern of cortisol,DHA and DHA sulphate formation was maintained. No attempt was made to measure the production of these steroids from endogenous precursors.

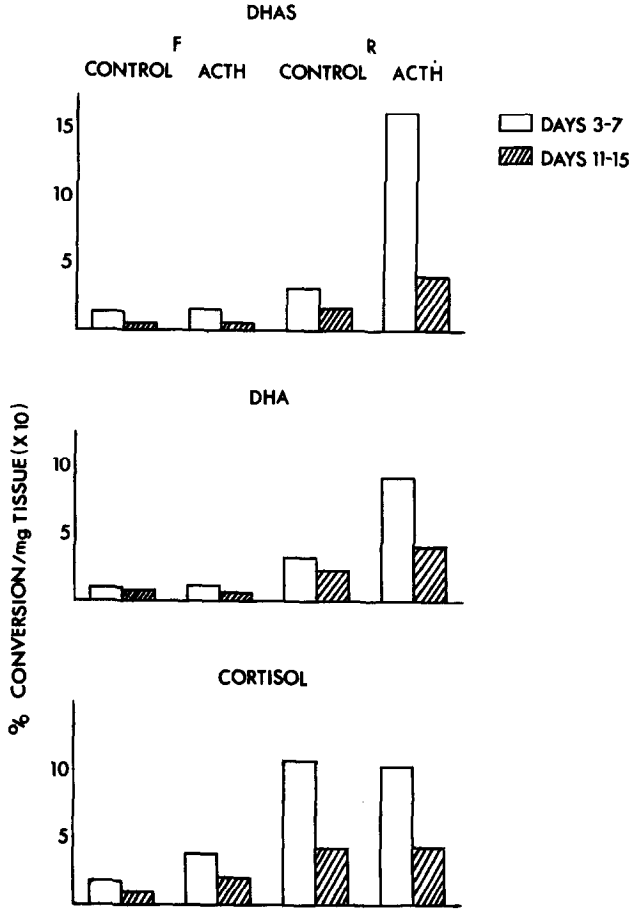

Fig. 1

DISCUSSION

Earlier studies (Griffiths, Grant & Symington, 1963) showed that both the fascicular and reticular tissue of the human adrenal cortex synthesized and secreted cortisol, corticosterone and 11β-hydroxyandrostenedione. However, only the clear cells of the zona fasciculata responded to corticotrophin *in vitro* with an increased synthesis of cortisol. Ultramicrochemical techniques employed by Jones & Griffiths (1968) and Cameron et al (1969) provided a more refined procedure for the separation of fascicular and reticular tissue, and experiments on the sul-

phation of DHA, in the guinea pig and human adrenal tissue, showed a qualitative difference between the zones. The sulphation of DHA was confined to the compact cells of the zona reticularis. A similar pattern has been provided by these organ culture studies, the formation of small amounts of DHA sulphate by the fascicular tissue probably being due to contaminating compact cells. It is of interest to note that in tissue culture, the pattern of DHA synthesis was similar to that of DHA sulphate, suggesting that DHA production may occur predominantly in reticular tissue.

Cortisol was synthesized from $\left[7\alpha\text{-}^{3}H\right]$ 17α-hydroxypregnenolone. by both zones, although no attempt was made to measure the production of cortisol from endogenous precursors. It is probably reasonable to assume however, that the presence of substantial amounts of cholesterol in the fascicular tissue and its metabolism by the tissue may have diminished the uptake of the radioactive precursor from the medium. The total amount of cortisol synthesized by the fascicular tissue may have been greater therefore, than that produced by the reticular tissue, although the incorporation of label into cortisol was greater in the reticular tissue. The results reported here show that the action of corticotrophin is not confined to the one zone. Whereas conversion of $\left[7\alpha\text{-}^{3}H\right]$ 17α-hydroxypregnenolone to cortisol doubled in the fascicular tissue under the influence of corticotrophin, there was no stimulation of the reticular tissue to synthesize more cortisol. This contrasts with the effect on DHA and DHA sulphate production, which was stimulated in the reticular tissue. Unpublished results from this laboratory have shown that the in vivo administration of corticotrophin does not stimulate the in vitro conversion of DHA to DHA sulphate in the guinea pig. The increase in the synthesis

of DHA sulphate observed here after corticotrophin stimulation may have been due to the increased production of its immediate precursor, DHA, from the $[7\alpha\text{-}^{3}H]$ 17α-hydroxypregnenolone.

These results provide further evidence therefore that DHA sulphate synthesis and its control by corticotrophin is confined to the compact cell of the zona reticularis of the human adrenal cortex. Moreover, the study emphasizes the importance of working with well defined groups of cells when investigating biosynthetic pathways and the control mechanisms involved.

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ERRATUM

Volume 37, No. 3 (1969), in the Communication "Fluorescence Study of Interactions Between Valyl-tRNA Synthetase and Valine-Specific tRNAs from E. Coli," by C. Helene, F. Brun, and M. Yaniv, pp. 393-398, the relation, p. 394, should read

$$I_F = I_M \frac{d}{do} \frac{1 - 10^{-do}}{1 - 10^{-d}}$$ instead of $I_F = I_M \frac{do}{d} (1-10^{-d})$.