PN 1236

Formation of steroid sulphates by extracts of human adrenals

Enzymes which can convert steroids to their water-soluble sulphate esters have to date been found only in mammalian liver[1]. Conjugation of steroids to form the glucuronides also takes place in the liver but the kidney of the mouse has recently been shown to be capable of forming glucuronides with certain steroids[2]. By contrast, kidney does not contain steroid sulphokinases[2,3]. Results described below show that cell-free extracts of human adrenals form steroid sulphates from adenosine 3′-phosphate 5′-phosphosulphate and estrogens and androgens but not with the corticosteroids tested under the experimental conditions employed.

Fresh human female adrenal tissue was obtained from the operating theatre and immediately homogenised in the cold with 2 vol. of 0.01 M phosphate in normal saline (pH 7.4). The homogenate was then centrifuged at 100000 *g* for 1 h and the supernatant stored at −40° until used. Steroid sulphokinase activity was retained for at least 2 months at this temperature.

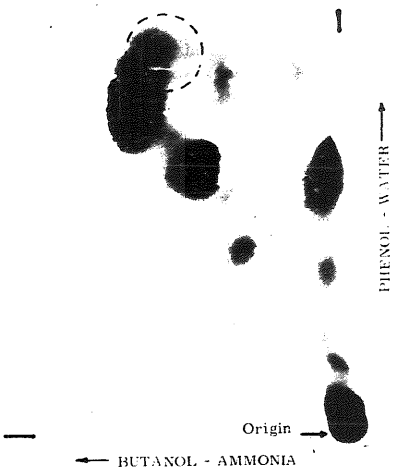

Fig. 1. Two-dimensional chromatogram of an aliquot of an incubation mixture comprising: 0.05 ml of 0.2 M phosphate buffer (pH 7.0), adenosine 3′-phosphate 5′-phospho-^{35}S sulphate (100000 counts/min) in 0.01 ml, and 0.05 ml high-speed human adrenal extract. The area outlined indicates the position of a new radioactive zone formed when a steroid (0.05 μmole) was added to the tube as an alcoholic solution and the solvent removed prior to addition of the other components. The time of incubation was 1.5 h at 37°. Autoradiographs were developed after a period of 2 weeks in contact with Kodak Blue-Brand X-ray film.

Fig. 1 demonstrates the complex pattern of ester sulphates formed by sulphokinases acting on endogenous acceptors present in the extract. When incubated in the presence of added steroid, a new zone appearing in the position outlined was

apparent with estrone, dehydroepiandrosterone, androsterone, pregnan-3α,20α-diol, testosterone and aetiocholan-3α-ol-17-one but not with cortisone, hydrocortisone or aldosterone. The position outlined in Fig. 1 is the one to which most authentic steroid sulphates, and the ^{35}S-labelled steroid sulphates formed by extracts of rat liver and exogenous steroids, migrate in the solvent system used[4]. When sulphation of exogenous steroids occurred by the adrenal extracts, some of the ester sulphates in Fig. 1 were either considerably reduced or completely missing.

In the case of dehydroepiandrosterone, proof that the corresponding 3-sulphate ester was formed is shown in Table I. The specific activity of the enzymically synthesised species eluted from two-dimensional paper chromatograms and mixed with authentic material was constant after three recrystallisations. This procedure could not be applied to estrone sulphate due to its marked instability in hot organic solvents[5]; indeed complete rupture of the ester sulphate group occurred on attempted recrystallisation of the potassium salt from hot isopropanol. However, when the enzymically synthesised [^{35}S]sulphate esters, formed from dehydroepiandrosterone and estrone, were isolated from chromatograms and co-chromatographed in two dimensions with potassium dehydroepiandrosterone-3-sulphate and potassium estrone-3-sulphate, respectively, the zones revealed by autoradiography were "fingerprints" of zones demonstrated by chemical staining with Zimmerman reagent. Furthermore, in these two cases the enzymically synthesised steroid sulphates had identical R_F values in three different solvent systems (designed for separating steroid sulphates[6]) to the authentic substances.

TABLE I

SPECIFIC ACTIVITY OF DEHYDROEPIANDROSTERONE [^{35}S]SULPHATE ISOLATED FROM TWO-DIMENSIONAL CHROMATOGRAMS AND MIXED WITH AUTHENTIC MATERIAL

The incubation in this case contained 1.5 μmoles ATP, 3 μmoles $MgCl_2$, 20 μmoles Tris buffer (pH 8.3), 40 μC carrier-free $Na_2{}^{35}SO_4$, 0.1 μmole dehydroepiandrosterone and 0.17 ml adrenal extract in a total volume of 0.35 ml. Chromatography as in Fig. 1 and after locating the steroid [^{35}S]sulphate by autoradiography it was eluted from the paper with water.

No. of crystallisations from water	*Counts/min at infinite thickness*
1	18 100
2	16 400
3	18 200

It is apparent then that the cell-free adrenal extracts can sulphate 3α- and 3β-hydroxyl groups and also secondary 17α-hydroxyl groups (testosterone) but not *tert.* α-hydroxyl (cortisone) nor 11β-hydroxyl groups (hydrocortisone and aldosterone). These results closely parallel those described for the conjugation of steroids by soluble enzymes of rabbit liver[6]. It should be emphasised, however, that corticosteroids normally undergo transformation in the liver to the "tetrahydro" derivatives (of Ring A) followed by subsequent conjugation. Such transformations evidently do not occur in unfortified cell-free extracts of liver[7].

In the vicinity of the area outlined in Fig. 1, a number of weak zones are present

which are probably formed by sulphation of endogenous steroids. Phenyl sulphates, and other sulphates formed from sulphate acceptors of low molecular weight, do not migrate into this region on chromatography[4]. The presence of steroid sulphokinases in a tissue responsible for steroid biosynthesis poses the question as to their biological significance. Conjugates may be the transport form of steroids, or possibly because of an increased polarity, this form of the steroid may play a specific, and as yet unknown role, in their mode of action.

The author wishes to thank Dr. E. GOULSTON and Dr. R. MELVILLE for their generous help in providing surgical material and to Dr. K. STARR for his encouragement and support.

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Received January 28th, 1963