Short Communications and Preliminary Notes

Conversion of cholesterol to corticosteroids in adrenal homogenates

Formation of corticosteroids from endogenous precursors in homogenates of adrenal cortex has been reported by VESTLING AND LATA[1] and since observed by others[2,3]. In this article data are presented demonstrating that cholesterol is a precursor of the corticosteroids formed in such homogenates.

Freshly excised chilled dog or beef adrenals were passed through a household meat grinder and the resulting mince homogenized gently in 1.5 volumes of BUCHER medium[4] at pH 7.4, either in a loose fitting Potter-Elvehjem homogenizer or in a Waring blender at reduced speed. The crude homogenate was centrifuged in the cold at 350 *g* for 6 min to remove connective tissue, unbroken cells, and nuclei. Incubation was carried out in 125 ml Erlenmeyer flasks at 37° with oxygen as gas phase. To 10.0 ml of homogenate were added 2 μmoles ATP, 4 μmoles DPN, 20 μmoles acetate, 30 μmoles fumarate, and varying amounts of adrenocorticotrophic hormone (ACTH) or KCl, to a total volume of 12.5 ml. The oxygen uptake of small aliquots was measured manometrically.

Following completion of the incubation, the corticosteroids were extracted by the method of MEYER[5] and washed with acid, alkali, and water. The residue remaining after removal of the ethyl acetate was subjected to benzene-water partition[6], and the aqueous phase extracted with chloroform. Quantitative estimation of corticosteroids separated in this manner was accomplished by the blue tetrazolium method of CHEN *et al.*[7] as modified by MADER AND BUCK[8], using desoxycorticosterone as standard, and by ultraviolet absorption at 240 mμ.

TABLE I

FORMATION OF CORTICOSTEROIDS

Time, 4 hours. Other details in text.

Exp.	*ACTH (0.3 I.U.)*	*Corticosteroids formed µg per mg N*
1	+	8.5
	−	8.5
	zero time	1.6
2	+	8.9
	−	8.3
	zero time	1.4

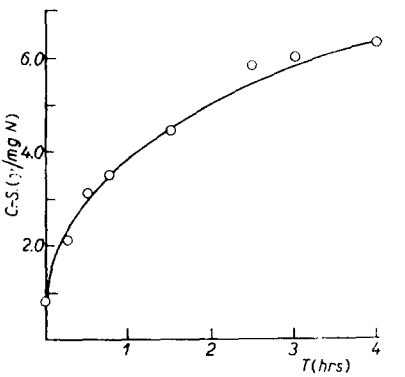

Fig. 1. Rate of corticosteroid formation in adrenal cortex homogenate.

The results of two typical experiments showing net formation of reducing corticosteroids are summarized in Table I. The presence of ACTH in the medium did not stimulate formation of corticosteroids, confirming the findings of others. The rate of corticosteroid production in the complete system is shown in Fig. 1. The initial rate of production is very high, corresponding to about 5.56 μ/mg N/h during the first 30 minutes, but decreases rapidly to almost zero at the end of 2.5 hours. The formation of corticosteroids did not require the addition of nicotinamide, Mg^{++}, ATP, DPN, or acetate although those components are known to be necessary for maximum rates of cholesterol biosynthesis from acetate in homogenates[4]. Omission of fumarate reduced corticosteroid production by about 60% and resulted in a very rapid drop in the respiratory rate, the initial rate never being maintained for more than 15 minutes. In the presence of fumarate, respiration remained linear for at least two and one-half hours. Omission of acetate decreased respiration, but did not affect corticosteroid production.

The corticosteroids formed were chromatographed on paper using the system described by PECHET[9], and were located by absorption of light at 240 mμ[10] and by reduction of triphenyl tetrazolium chloride (TPTZ)[11]. The R_F values of the corticosteroids formed were compared with those of authentic samples of known compounds. In two instances identification was verified by means of the fluorescence produced by spraying the papers with phosphoric acid[12]. In this way net formation of corticosterone and 17-hydroxycorticosterone in the homogenates was established, the former in

amounts greater than the latter. In addition, two as yet unidentified compounds, presumably steroids, were regularly produced by the homogenates. The first was a less polar substance than corticosterone, which absorbed at 240 mμ but did not reduce TPTZ; the second showed an R_F intermediate between those of corticosterone and 17-hydroxycorticosterone, absorbed at 240 mμ, and reduced TPTZ.

Uniformly labelled radio-cholesterol (specific activity, 6800 c.p.m./mg) was isolated from rat liver slices, following incubation with carboxyl-labelled ^{14}C-acetate. The labelled cholesterol was incubated with adrenal homogenate as follows: 9.5 mg and 4.5 mg were spread, in a thin layer, across the bottom of two flasks to which were added 10 ml and 5 ml of homogenate, respectively; for the third flask 5 mg of labeled cholesterol were dissolved in a few drops of acetone, and homogenized with the complete incubation medium. All three vessels were gassed with oxygen and the issuing carbon dioxide from the three flasks trapped in alkali and precipitated as $BaCO_3$, which was found to be radioactive.

Chromatography of the extracts showed radioactivity present in spots corresponding to corticosterone and 17-hydroxycorticosterone, as well as in the two other spots corresponding to the two unidentified steroids which possessed the characteristics described above. Elution of the spots visualized by absorption at 240 mμ and determination of the radioactivity contained therein accounted for about 2/3 of the activity present in the extract used for chromatography. Areas of the filter paper strip which showed no absorption at 240 mμ contained no radioactive substances. Neither the solvent front nor the spot of origin were radioactive. Table II represents a balance sheet for the utilization of the labeled cholesterol. It is seen that a large fraction of the degraded cholesterol was converted into $C_{21}O_4$, $C_{21}O_5$, and $C_{21}O_6$ steroids (*cf.*[13]).

TABLE II

BALANCE SHEET

Total radioactivity of cholesterol added to system	129,200/c.p.m.
Total radioactivity of respiratory CO_2	432/c.p.m.
Cholesterol equivalent of labeled CO_2 formed	2,592/c.p.m.
Total radioactivity of corticosteroids in aqueous phase (see text)	908/c.p.m.
Total radioactivity isolated from chromatogram	650/c.p.m.
Conversion of cholesterol utilized to $C_{21}O_4$, $C_{21}O_5$, $C_{21}O_6$ steroids	44%

It is concluded that homogenates of adrenal glands incubated as described are capable of converting cholesterol to corticosteroids, at least part of the side chain being oxidized to CO_2. When similar experiments were carried out with carboxyl-labeled ^{14}C-acetate in the medium instead of labeled cholesterol, no radioactivity could be detected on the chromatograms or in the cholesterol isolated from the medium. The presence of ACTH in the medium did not affect any metabolic pathways investigated.

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