Conversion of pregnenolone to dehydroepiandrosterone

Pregnenolone (3β-hydroxypregn-5-en-20-one) as well as cholesterol have been demonstrated, in this report, to be precursors of dehydroepiandrosterone. This was accomplished by incubating [^{3}H]pregnenolone and [4-^{14}C]cholesterol with a homogenate prepared from a human adrenal-cortical adenoma* (Cushing's syndrome), adding dehydroepiandrosterone and isolating [^{3}H, ^{14}C]dehydroepiandrosterone which, after chromatography, crystallization, acetate formation, and recrystallization, was shown to be radiochemically pure.

The adrenal tumor tissue (7.5 g) was homogenized in 15 ml phosphate buffer, pH 7.4, containing DPN ($5 \cdot 10^{-4}$ *M*) and the following constituents at $5 \cdot 10^{-3}$ *M*: ATP, nicotinamide, $MgSO_4$ and sodium fumarate. In addition, 40 I.U. ACTH were added together with 0.5 mg [4-^{14}C]cholesterol ($2.2 \cdot 10^{6}$ counts/min/mg) and 5.0 mg [^{3}H]pregnenolone ($1.9 \cdot 10^{6}$ counts/min/mg). The mixture was incubated in air for 3 h at 37°, then precipitated with acetone and the insoluble material extracted successively with acetone, ethyl acetate, and chloroform. The acetone fractions together with the ethyl acetate and chloroform extracts were concentrated to dryness *in vacuo* and the dry residue partitioned between heptane and 90% methanol. The methanol fraction was subjected to silica gel column adsorption chromatography and paper chromatography in a toluene-propylene glycol system and 3 mg of non-radioactive dehydroepiandrosterone were added to the material eluted from the dehydroepiandrosterone zone. This material was rechromatographed successively on paper in the heptane-propylene, cyclohexane-formamide, and the Bush B systems. The material eluted from the dehydroepiandrosterone zone had specific activities

TABLE I

THE SPECIFIC ACTIVITY OF DEHYDROEPIANDROSTERONE AT SUCCESSIVE STAGES OF PURIFICATION

Procedure	*Specific Activity Counts/min/mg*	
	^{14}C	^{3}H
Free compound:		
first crystallization	100	1320
second crystallization	120	1250
third crystallization	140	1280
Acetate:		
first crystallization	120	1250
second crystallization	140	1230

of 110 and 1450 counts/min/mg respectively for ^{14}C and ^{3}H. The compound was recrystallized three times, the acetate formed in the conventional manner and recrystallized twice. As indicated in Table I, no significant changes were detected in the specific activities of the compound with respect to the free compound or the acetate.

On the basis of these data we can conclude that both cholesterol and pregnenolone may be considered to be precursors of dehydroepiandrosterone. That cholesterol is a

Abbreviation: ATP, adenosine triphosphate; ACTH, adrenocorticotropic hormone.

* This tissue was kindly made available to us by Dr. Louis J. Soffer and J. Lester Gabrilove, Mount Sinai Hospital, New York City.

precursor has been demonstrated previously[1]. The possibility of a conversion of pregnenolone to dehydroepiandrosterone has been suggested by LIEBERMAN[2] but this reaction has not been demonstrated previously. A likely pathway for the conversion might involve 17α-hydroxypregnenolone.

This work was supported in part by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

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Received December 10th, 1959

Biochim. Biophys. Acta, 38 (1960) 190–191

Fractionation of calf-lens protein

Since 1894 when MÖRNER[1] reported that lens contains three soluble proteins, α-, β- and γ-crystallins, attempts have been made to obtain a definitive fractionation of soluble lens protein. Different results have been obtained by different methods of fractionation. Thus conventional electrophoretic techniques[2,3] have indicated the presence of two to three components; continuous-flow electrophoresis[4], 5 components; immunological studies[5,6] from five to eight components. Furthermore FRANÇOIS[7] has reported the fractionation of embryonic lens into ten fractions by means of ultramicro electrophoresis in agar gel.

A method has now been developed for the chromatographic fractionation on DEAE-cellulose* of the soluble protein of calf lens. By this procedure, 10 components have been isolated.

DEAE-cellulose (capacity, 0.8 mequiv./g) was prepared by washing with 0.5 *M* NaOH and water. The fine particles were decanted and the cellulose was suspended in 0.001 *M* phosphate buffer, pH 7.0. All further operations were performed in a cold room at 4°. The DEAE-cellulose column, prepared by the procedure of PETERSON AND SOBER[8], contained 16 g of adsorbant.

In a Potter-Elvehjem homogenizer, 1 g of calf lens was homogenized in 10 ml water. The lens homogenate was then centrifuged for 2 min at 11,000 × *g* and the insoluble material was discarded. The soluble lens protein was dialyzed against distilled water for 18 h, after which an equivalent of 250 mg (dry wt.) was added to the column.

The components were fractionated by stepwise elution. All buffers were prepared by appropriate dilution of 0.500 *M* (NaH_2PO_4, K_2HPO_4), pH 6.80 ± 0.01. Fractions were collected in 10-ml aliquots at the average rate of 40 ml/h. Only slight modification of the elution pattern was found when the flow rate was 100 ml/h. Regeneration was accomplished by washing the column with the following solutions: 180 ml

* Purchased from Brown Co., Berlin, New Hampshire.
Abbreviation, DEAE-, diethylaminoethyl-.