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Conversion of Corticosterone-4-C^{14} to Aldosterone by Human Adrenal Slices.* (25076)

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Investigations of the biosynthetic pathways of aldosterone have yielded conflicting results. Kahnt(1) demonstrated incorporation of desoxycorticosterone-21-C^{14} into aldosterone using beef adrenal homogenates. Addition of progesterone to the incubation medium did not increase aldosterone production. Rosemberg(2) has shown that perfusion of isolated calf adrenals with progesterone increased production of aldosterone while perfusion with corticosterone or desoxycorticosterone had little or no effect. Incubation of C^{14}-labelled progesterone, desoxycorticosterone or corticosterone with beef capsule strippings by Ayres(3) and later by Travis(4) demonstrated the incorporation of each into aldosterone. The data are conflicting in regard to the most efficient precursor. Little is known of the pathways of aldosterone biosynthesis in man. Seltzer(5) demonstrated that infusion of large quantities of corticosterone increased urinary excretion of aldosterone. However, it was not determined whether this increased aldosterone excretion resulted from a direct incorporation of corticosterone or by indirect effect of corticosterone upon adrenal cortical function. Moreover, despite continuous infusion of corticosterone, aldosterone excretion diminished. The present report describes incorporation of corticosterone-4-C^{14} into aldosterone by human adrenal slices.

Methods. A patient with metastatic cancer of the breast underwent a bilateral adrenalectomy. The right adrenal was immediately immersed in cold isotonic saline, then freed of fat, weighed, and sliced with a Stadie-Riggs microtome. Approximately 2.22 x 10^5 DPM (1.4 x 10^5 CPM)/24 μg of corticosterone-4-C^{14}§

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TABLE I. Chromatographic and Isotopic Characteristics of Aldosterone.

System	Exp.	Mobility, cm/hr	$R_{cortisol}$*	Amt, μg	C^{14}, cpm	S.A., μc/mg
I Toluene propylene glycol	A	4.5/13	2.1			
	B	4.8/13	2.0			
II Bush C	A	11 / 3	1.0	3.2	4720	1.0
	B	11 / 3	1.0	2.9	8800	2.1
III E_2B	A	19 / 8	.79		1904†	
	B	18 / 8	.78		3084†	

* $R_{cortisol}$ refers to mobility of unknown compound to standard cortisol. Mobilities shown for compound isolated are similar to those of authentic aldosterone in these systems.

† 86 and 87% recovery respectively of the aliquot of aldosterone from the Bush C chromatogram which was applied to the E_2B system.

in ethanol was added to each of 4 flasks (A-1, A-2, B-1, B-2) and the ethanol evaporated. Approximately 500 mg of adrenal slices and 20 cc of Krebs-Ringer bicarbonate buffer with 200 mg % glucose were added to each flask. Incubation was carried out at 37°C in a Dubnoff incubator in an atmosphere of 95% O_2, 5% CO_2 for 2 hours. The incubation began approximately 45 minutes after surgical removal of the gland. After incubation the media of flasks A-1 and A-2 were pooled to form flask A; B-1 and B-2 to form flask B. The media were extracted twice with 3 volumes of redistilled chloroform. The chloroform extracts were washed once with 1/10 volume 0.05 NaOH, twice with 1/10 volume distilled water, dried with anhydrous sodium sulfate and evaporated *in vacuo*. The residues were applied to paper chromatogram in the toluene-propylene glycol system(6) along with appropriate standards. The steroids were located by ultraviolet scanning method. In this system, aldosterone has the same mobility as cortisone. The cortisone area was eluted with ethanol and rechromatographed in the Bush C system(7). The amount of aldosterone eluted from the Bush C system was estimated by alkaline fluorescence(8) using cortisol as the standard. An aliquot of the eluted aldosterone was rechromatographed on the E_2B system(9). The aldosterone area from Exp. B was eluted and the eluate mixed with 25 μg of aldosterone monoacetate. Acetylation was performed with 25 lambda of 15% acetic anhydride in anhydrous benzene, and 20 lambda of absolute pyridine for 48 hours at room temperature. The diacetate derivative was extracted with methylene chloride and chromatographed as indicated in Table II. Aldosterone diacetate migrates with androsterone in system IV and with Δ-4-androstene-11 β ol, 3, 17-dione in System V as reported by Kliman(10) and confirmed in this laboratory. After elution from the second diacetate system, chromic acid oxidation of radioactive aldosterone diacetate was performed. The monoacetate oxidation product was chromatographed in the cyclohexane-benzene-methanol-water system where it had the same mobility as 11 α-hydroxy progesterone.‖ Radioactivity was counted to a standard error $\pm$ 2% in a liquid phosphor scintillation counter with a counting efficiency of 64% for C^{14} and a background of approximately 20 CPM.

Results. Chromatographic mobilities from successive paper chromatographies of the "aldosterone" from Exp. A and B are listed in Table I. These mobilities are similar to those of authentic aldosterone. After elution from the Bush C chromatogram (System II), the quantity of aldosterone was approximately equal in each experiment. However, incorporation of corticosterone-4-C^{14} was appreciably greater in Exp. B. Radioactivity in the aldosterone areas of A and B represented approximately 2% and 3% respectively of the added C^{14} precursor. When aliquots of the aldosterone were rechromatographed in the E_2B system (System III) 86% and 87% of radioactivity was located in the aldosterone area

§ Corticosterone-4-C^{14} was obtained through courtesy of Endocrinology Study Section, Nat. Inst. Health (sp. act. 4.16 μC/mg.)

‖ Personal communication of Peterson, R. E., and confirmed by authors with chromic acid oxidation of authentic aldosterone diacetate-C^{14}.

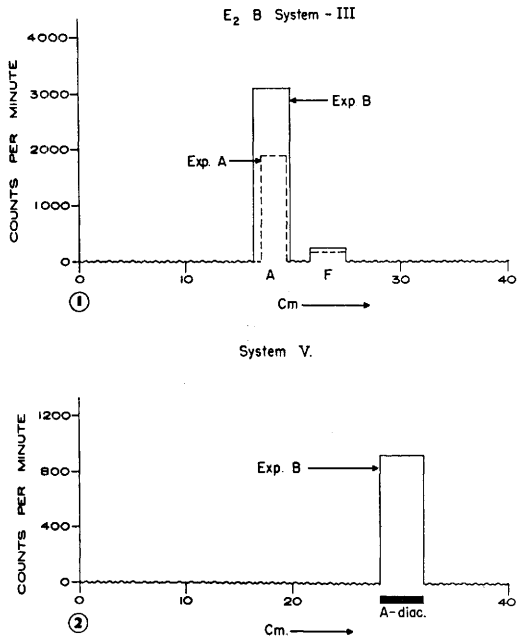

FIG. 1. Location of carbon radioactivity on paper chromatogram. After aldosterone (A) and cortisol (F) areas were cut out, remainder of chromatogram was cut into 4-5 cm strips, eluted and counted. The irregular baseline indicates background activity. 0 denotes origin.

FIG. 2. Location of carbon radioactivity on paper chromatogram. Roman numeral refers to paper chromatogram system listed in Table II. After aldosterone diacetate (A-diac.) area was cut out, remainder of chromatogram was cut into 4-5 cm strips, eluted and counted. The irregular baseline indicates background activity. 0 denotes origin.

(Fig. 1). After the aldosterone area was eluted the remainder of the chromatogram was cut up into 4-5 cm strips, eluted and counted.

An aliquot of aldosterone from Exp. B eluted from the E_2B system was mixed with 25 μg of authentic aldosterone monoacetate, acetylated, and the diacetate derivative chromatographed as indicated in Table II. After the second chromatogram (System V), 61% of radioactivity corresponded with the ultraviolet absorbing area of aldosterone diacetate. No other area contained a significant amount of radioactivity (Fig. 2).

An aliquot of the aldosterone diacetate was oxidized with chromic acid and chromatographed in System IV (Table II). Seventy % of radioactivity was located in the aldosterone monoacetate oxidation product area. The percentage recovery represents the limitation

TABLE II. Chromatographic Characteristics of Aldosterone Diacetate. 1541 cpm* 25 μg aldosterone monoacetate: acetylated.

System		Mobility, cm/hr	Rf†	C^{14}, cpm	Recovery, %
IV	Cyclohexane 4				
	Benzene 2				
	Methanol 4				
	Water 1	35/12	1.0		
V	Cyclohexane 4				
	Dioxane 4				
	Methanol 2				
	Water 1	30/10	1.0	940	61
	470 cpm: Chromic acid oxidation				
IV	Cyclohexane 4				
	Benzene 2				
	Methanol 4				
	Water 1	19/12	1.0‡	328	70

* Exp. B—aldosterone from E_2B system.

† Rf aldosterone diacetate refers to mobility of radioactive "aldosterone diacetate" to authentic aldosterone diacetate standard.

‡ 11α-OH progesterone was used as the reference standard.

of the oxidation method rather than impurities in the aldosterone diacetate since in the authors' hands the best recoveries with authentic aldosterone diacetate-C^{14} have been about 80%.

Discussion. Human adrenal tissue slices synthesized aldosterone from corticosterone C^{14} *in vitro*. Although the amount of aldosterone produced was small, the chemical evidence indicates the compound isolated was aldosterone. The radioactive compound had the same mobility as aldosterone in 3 successive chromatographic systems; after acetylation as aldosterone diacetate in 2 systems; and as the aldosterone monoacetate oxidation product after chromic acid oxidation.

This evidence directly demonstrates that corticosterone may be a precursor for aldosterone formation. It is supported by the indirect evidence of Seltzer(5) who showed that increased quantities of aldosterone were excreted in the urine after infusions of corticosterone. The present experiments do not exclude other pathways or imply that corticosterone is the most efficient precursor of aldosterone.

The variation of incorporation of corticosterone-4-C^{14} between Exp. A and B indicates the dangers of quantitatively comparing different precursor incorporation when tissue

slices are used. Despite the fact that approximately equal weights of adrenal tissue from the same adrenal and equal quantities of radioactive corticosterone were present, Experiment B had twice the specific activity of Exp. A, but similar microgram amounts of aldosterone. It is difficult to interpret this quantitative discrepancy.

Cell permeability, precursor pool size, and state of enzyme activity are important variables in *in vitro* steroid synthesis. For ideal comparisons of different precursors, the same adrenal tissue should be studied simultaneously, using equimolar tracer quantities of precursors with identical specific activities; and rates of synthesis of aldosterone with its immediate precursors determined.

Conclusions. Synthesis of aldosterone by human adrenal slices from corticosterone-4-C^{14} has been demonstrated *in vitro*.

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Analysis of Serum Magnesium in Presence of Calcium with Chrome Fast Blue BG. (25077)

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Colorimetric methods for determination of magnesium have utilized principally a series of yellow dyes(1) (Titan yellow, brilliant yellow, thiazol yellow, Clayton yellow) and Eriochrome Black T(2). Of the yellow dyes, Titan yellow has been widely accepted, but, as with all yellow dyes, a stabilizer such as gum ghatti, or polyvinyl alcohol must be used and pH values of 12.8 or more maintained. Methods using other dyes require removal of calcium and/or elapses of time for color development. Use of azo dye, 1, 8-dihydroxy-2-(3'-chloro-6'-hydroxybenzene azo) naphthalene-3-6-disulfonic acid, or Chrome Fast Blue BG, requires no removal of calcium, no waiting for color development, and no stabilizers. It is sensitive to quantities as small as 0.01 microequivalents of magnesium/ml of dye solution. This dye was first used by Japanese workers and was introduced in this country by Carson (3). Its application to analysis of magnesium was initiated in this laboratory by Lewis and Kerwin(4) and has now been refined.

Methods. Chrome Fast Blue BG was obtained from American Cyanamid Co., Bound Brook, N. J., and used after further purification.* An aqueous solution of 0.001 M (molecular weight of Na salt = 518.5) was prepared and can be stored several months. To 7 ml of 0.001 M Chrome Fast Blue BG was added 10 ml of 1 M ethylamine buffer (adjusted to pH 11.1 with HCl) and 83 ml of water. To 3 ml of this buffered dye solution in a Corex cuvette was added solutions of 0.2% trichloroacetic acid (TCA) for blanks, of $MgCl_2$ in 0.2% TCA for standardization (Fig. 1), and of the deproteinized serum (see below) for determination of Mg-content of serum. All optical densities were obtained with Beckman DU spectrophotometer. A standard

* Procedure for purification is available from Am. Cyanamid Co.