change is an intramolecular process. Formation of the transition state may involve the breaking of one C-Al bond, or perhaps a deformation of the molecule in which no bonds are broken, leading to a structure having four bridging methyl groups at the corners of a square, *i.e.*



The 3d orbitals of the aluminum atom then would be expected to contribute to the bonding, involving four electron pairs in six-center molecular orbitals.

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(6) Lubrizol Corporation Predoctoral Fellow.

DEPARTMENT OF CHEMISTRY PURDUE UNIVERSITY NORBERT MULLER LAFAYETTE, INDIANA DONALD E. PRITCHARD⁶ RECEIVED NOVEMBER 23, 1959

THE SYNTHESIS OF ALDOSTERONE FROM PROGESTERONE BY THE AMPHIBIAN ADRENAL¹ Sir:

The mammalian adrenal cortex forms both glucocorticoids^{2,3} and mineralocorticoids^{4,5} from progesterone. In the lower vertebrates, where the major function of the adrenal cortex may be electrolyte regulation, the pathways of corticosteroid biosynthesis are not known. Recent evidence⁶ indicates that aldosterone is the most abundant corticosteroid produced by the adrenals of the American bullfrog. This communication reports that the adrenals of the same species synthesize aldosterone from C¹⁴-labeled progesterone.

Minced adrenal tissue (0.5 g.) from 6 bullfrogs (*Rana catesbeiana*) was incubated as described,⁶ in an atmosphere of 95% O_2 -5% CO_2 at 26° for 3 hr. in 5 ml. of isotonic Krebs-Ringer bicarbonate solution (*p*H 7.4) containing 0.2% glucose, in the presence of 5 units of bovine adrenocorticotrophic hormone and 0.15 mg. of progesterone-4-C¹⁴ (7.40 × 10⁶ c.p.m.). After incubation, 1.70 × 10⁶ c.p.m. of tritium-labeled aldosterone⁷ was

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(7) Randomly-labeled aldosterone-H⁴ was prepared by irradiating dl-aldosterone with tritium (K. E. Wilzbach, *ibid.*, **79**, 1013 (1957)), then exchanging with methanol and a chromatographic purification to a constant specific activity of 1.3 microcuries per microgram.

added as an internal standard and 0.5 mg. of nonradioactive dl-aldosterone as carrier. The incubation mixture was extracted with ethyl acetate and the extract washed with water and evaporated. The sequence of paper chromatographic separations shown in Table I next was carried out. After the completion of each chromatogram, the aldosterone zone was located by scanning the paper with ultraviolet light $(254 \text{ m}\mu)$, then eluted, and an aliquot was counted in a Tricarb liquid scintillation spectrometer to determine the H³/C¹⁴. Tritium and C^{14} were measured simultaneously by the discriminator-ratio method⁸ at a photomultiplier voltage of 1230 and discriminator settings of 10-100 for channel 1 and 100 to infinity for channel 2. After the completion of the second chromatogram (Table I), the eluate containing aldosterone was acetylated with acetic anhydride in pyridine and the third and fourth chromatographic separations were carried out on the 18,21-diacetoxy derivative of aldosterone.

TABLE I

	PURIFICATION OF ISOLATED C ¹⁴ -ALDOSTERONE		
	Chromatographic System	Steroid	H3/C14
1	Toluene/propylene glycol	Aldosterone	4.4
2	Toluene:ethyl acetate 9:1/	Aldosterone	5.1
	methanol:water 1:1		
3	Methylcyclohexane:toluene	18,21-diacetoxy-	5.3
	4:1/methanol:water 4:1	aldosterone	
4	Methylcyclohexane:toluene	18,21-diacetoxy-	5.2
	4:1/formamide	aldosterone	

The agreement in the ratio of tritium to C¹⁴ after the second, third and fourth chromatograms was taken as evidence that the C¹⁴ containing moiety was chemically indistinguishable from the aldosterone-H³ which had been added to the incubation mixture. The ratio of cpm. aldosterone-H³ to c.p.m. purified aldosterone-C¹⁴ was 5.2. The c.p.m. of aldosterone-C¹⁴ which was present after incubation was therefore 3.3×10^5 (1.70 $\times 10^6/$ 5.2).

The conversion of progesterone- C^{14} to aldosterone to the extent of 4.4% ($3.3 \times 10^5/7.40 \times 10^6$) indicated that a biosynthetic pathway characteristic of the mammalian adrenal cortex was present to a significant degree in a lower vertebrate. The yield of aldosterone was comparable to that reported^{5,9} for capsule strippings of bovine adrenal glands. The results suggest a simple biosynthetic method for the preparation of labeled aldosterone.

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