Conversion of C^{14}-Arterenol to Epinephrine *in vivo*.* (22645)

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Considerable progress has been made in elucidating the pathway of epinephrine biosynthesis by tracer studies. It has been demonstrated that phenylalanine and tyrosine are precursors of epinephrine *in vivo*(1,2) and *in vitro*(3). Adrenal epinephrine has been obtained after injection of C^{14}-3,4-dihydroxyphenylalanine and C^{14}-3,4-dihydroxyphenethylamine into rats(4). Injection into rats of C^{14}-methionine labelled in the methyl group produced C^{14}-epinephrine in the adrenal gland(5). Although indirect evidence has been offered(6,7,8), the main evidence for the conversion of arterenol to epinephrine is based on the experiments of Bülbring(9), who used bioassays to measure the increase of epinephrine in adrenal preparations incubated with arterenol and methyl donor *in vitro*. Several attempts to repeat Bülbring's experiments in this laboratory gave negative results. The present paper reports a search for C^{14}-epinephrine in the adrenals of rats after the intraperitoneal injection of C^{14}-arterenol.

Materials and methods. The α-C^{14}-dl-arterenol hydrochloride was synthesized by Howton, Mead, and Clark(10). Its activity was 16 mc per millimole. The sample had deteriorated somewhat on storage and in some experiments was purified just before use by ion exchange chromatography. A solution of 8 mg of the impure C^{14}-arterenol in 8 ml water was passed onto a column (24 cm long, 2.4 cm O.D.) of Dowex 50 resin (200-400 mesh, 8% crosslinked) in the hydrogen form in 0.5 *N* HCl. The column was developed with 0.5 *N* HCl, and fractions of approximately 20 ml were collected. The arterenol fractions (#83-107) were combined, passed through a column of 250 g Dowex 1 resin in the acetate form (1.5 times the theoretical amount) to remove the chloride ions, and lyophilized. Its purity was determined by chromatography on Whatman #1 paper using as developing solvent phenol-0.1 *N* HCl (85:15 (wt./vol.)) saturated with SO_2. The chromatogram was cut into segments and the radioactivity measured in a windowless flow counter (Fig. 1).

Four adult male Long-Evans rats were injected intraperitoneally with α-C^{14}-dl-arterenol hydrochloride twice a day for 3 days and sacrificed under nembutal anesthesia 24 hours after the last injection (Exp. 1 and 2; 2 rats per experiment). Six other rats were given 6 injections at hourly intervals and sacrificed one hour after the last injection (Exp. 3, 4 and 5; 2 rats per experiment). The adrenal glands were removed and kept frozen until used. Adrenals of 2 rats were ground in a glass homogenizer with 2 *N* acetic acid. This suspension was divided into 2 equal parts. One part was used for paper chromatography. It was evaporated *in vacuo* at 45°-50° C., and the residue extracted with several portions of ethanol (total volume, 1.0 ml.) and centrifuged. The supernatant was evaporated to less than 0.5 ml with a stream of nitrogen and the entire solution spotted on Whatman #1 paper. The chromatogram was developed overnight in the phenol solvent, washed with benzene, dried, sprayed with $K_3Fe(CN)_6$ solution(11), and counted as above.

The other half of the suspension was used for the determination of specific activity after clarifying by adjusting to pH 6.5 with 2 *N* NH_4OH and centrifuging. An aliquot of the supernatant was assayed for epinephrine by a modified Weil-Malherbe procedure‡(12). To the rest of the extract 15.0 mg of unlabelled l-epinephrine (as 27.3 mg of the syn-

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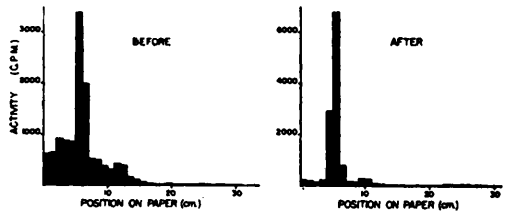

FIG. 1. Distribution of radioactivity on paper chromatogram of α-C^{14}-dl-arterenol before and after ion exchange resin treatment.

thetic bitartrate) was added as carrier. The l-epinephrine was crystallized by adjusting the solution to pH 8.8 with 2 *N* NH_4OH. The precipitate was filtered with suction, washed 3 times with cold water and once with ethanol, plated out, and counted. It was found impossible to separate epinephrine from contaminating radioactive arterenol in control experiments by a few crystallizations (also reported by Udenfriend and Wyngaarden(4)), so a method was devised which removed arterenol by virtue of its reactivity with pyridoxal-5-phosphate(13) (Table I). Sodium pyridoxal-

TABLE I. Separation of Labelled Arterenol from Carrier Epinephrine in Control Experiments.

		c.p.m./mg	% arterenol in epinephrine
A. Original mixture		8100	.40
Crystallized	1×	6800	.34
"	2×	5500	.27
"	3×	4800	.24
Pyridoxal-5-phosphate	1×	325	.016
B. Original mixture		67	.033
Crystallized	1×	42	.021
Pyridoxal-5-phosphate	1×	3.4	.0017
"	2×	1.1	.0006

5-phosphate solution was prepared from 1 mg of the calcium salt§ (approximately 60% pure) in 0.1 ml of 0.5 *M* phosphate buffer at pH 6.8. The epinephrine sample, which had been redissolved in acetic acid and adjusted to pH 6.8, was allowed to react with the sodium pyridoxal-5-phosphate solution for 30 minutes at 45°C. Then the epinephrine was crystallized, filtered, washed, plated, and counted as before. A second incubation and crystallization was usually sufficient to obtain constant activity.

§ Obtained from the Merck Institute for Therapeutic Research, Rahway, N. J.

TABLE II. Conversion of C^{14}-Arterenol to Epinephrine.

Exp. No.	Hr	Total counts injected	Adrenal epinephrine + carrier (total c.p.m.)	Specific activity of adrenal epinephrine (c.p.m./μM)
1	72	6×10^7	683	
2	72†	6×10^7	955	
3	6	2×10^7	239	4,994
4*	6	2×10^7	383	23,728
5*	6	2×10^7	285	11,463

* Arterenol purified by ion exchange resin treatment.

† Marsilid, 100 mg/kg inj. daily.

Results. It can be seen from Table II that radioactive arterenol injected into rats was converted to adrenal epinephrine. Significant labelling of epinephrine occurred even during the 6-hour experiments. The chromatographic evidence for the presence of radioactive epinephrine is illustrated in Fig. 2, which shows the distribution of radioactivity in a typical paper chromatogram of the adrenal extract (Exp. 5). The chromatogram had been sprayed before counting and the positions of highest activity were found to correspond exactly with the colored spots due to endogenous amines.

As described by Howton, *et al.*(10), a contaminant in the radioactive arterenol has an R_F corresponding to 3, 4-dihydroxyphenethylamine. The possibility that this contaminant may have been responsible for the radioactive epinephrine in the adrenal gland does not appear to be likely, since the specific activities of adrenal epinephrine in Exp. 4 and

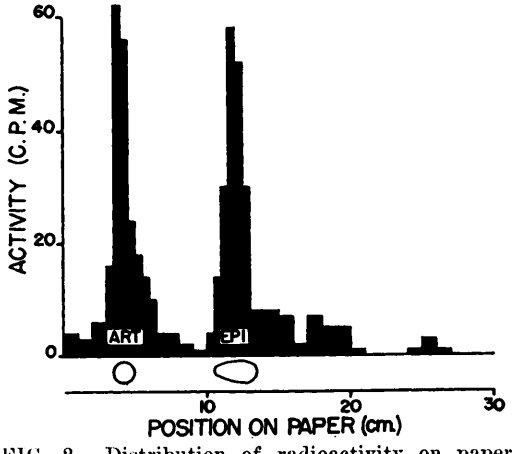

FIG. 2. Distribution of radioactivity on paper chromatogram of adrenal extract from rat inj. intraper. with α-C^{14}-dl-arterenol.

5 are higher than in Exp. 3 even though the contaminant was almost completely removed in the purification of the arterenol.‖

These experiments represent the first direct evidence that arterenol is converted to epinephrine and completes the reaction sequence postulated to lead from phenylalanine to epinephrine.

Summary. C^{14}-epinephrine has been found in the adrenal glands of rats administered α-C^{14}-arterenol intraperitoneally. Its presence has been demonstrated by paper chromatography and by isotope dilution using a new method, involving reaction with pyridoxal-5-phosphate, for separating these catecholamines.

‖ We wish to thank Dr. William Drell who developed this method and demonstrated that it completely separated arterenol from epinephrine and 3,4-dihydroxyphenethylamine. Furthermore, a paper chromatogram of carrier 3,4-dihydroxyphenethylamine hydrochloride which had been recrystallized from a solution containing the purified C^{14}-arterenol hydrochloride showed no radioactivity at the R_F of the carrier.

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Investigations on Polyvinylpyrrolidone in Mice Given Brucella Endotoxin. (22646)

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Studies in these laboratories with endotoxins prepared from various species of Gram-negative bacteria, including brucellae, have confirmed the observations that these endotoxins cause profound vascular disturbances in animals, terminating in collapse and death (1,2). More precise measurements with non-lethal doses of brucella endotoxin have shown that the daily temperature rhythm of mice is affected by small amounts of the endotoxin (3). The lethal effects of endotoxin in mice can be prevented by administration of adrenal corticosteroids or chlorpromazine, and tolerance to lethal doses of endotoxin can be produced by administration of sublethal amounts of heterologous endotoxins(1,4,5,6). The hypothermia of mice given brucella endotoxin also can be counteracted with added corticosteroid(7).

The present studies were undertaken to determine if the polymer, polyvinylpyrrolidone (PVP), would protect mice against brucella endotoxin. Originally introduced as a plasma expander by Hecht and Weese(8), interest turned to using PVP as a "detoxifying" agent. Toxic substances not usually excreted in the urine were reported to be "adsorbed" to PVP and excreted as such(9,10,11). PVP not only protected animals, but it was described as also effective in the treatment of human cases of tetanus and of diphtheria(12,13,14). According to these investigators, the most effective form of PVP was incorporated in the preparation known as Periston N (PVPN), having a smaller molecular weight than the original PVP (molecular weights: PVP~100,000, PVPN~12,600). More recently in a therapeutic trial on 10 patients with severe tetanus, no benefit attributable to PVPN was noted(15). Likewise, in mice PVPN given 3 hours after tetanus toxin was ineffective, but it protected when given within one hour after