cause typical signs of IBR and a definite antibody response(3). In spite of this decrease of infectivity, however, the antigenicity of the virus when inoculated intramuscularly, regardless of passage level or tissue culture employed, was not significantly changed as shown by serum neutralization titers of experimental cattle. Thus, the PKTC-propagated virus could be used as a vaccine with the additional advantage that such a product would be free of cattle viruses which might be present in bovine kidney.

Summary. 1. IBR virus was isolated in porcine kidney tissue culture from infected respiratory tissues of cattle, and produced a typical CPE on these cells. The virus was maintained for 10 passages and, when inoculated intranasally into susceptible cattle, produced signs of illness characteristic of IBR. 2. IBR virus passed previously through 7 passages in BKTC could be successfully propagated in PKTC in 2 separate attempts, and was passed 60 times (P-60) and 100 times (RP-100), respectively, in PKTC. 3. Inoculation of P-60 and RP-100 did not cause disease when inoculated either intranasally or intramuscularly into susceptible animals. Intramuscular inoculation of these viruses resulted in a specific antibody response.

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Received January 22, 1958. P.S.E.B.M., 1958, v97.

Conversion of Progesterone-4-C^{14} to Aldosterone by Perfused Calf Adrenals. (23845)

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Previous studies with isolated calf adrenals have demonstrated production of sodium retaining activity following perfusion with an artificial medium, both with(1) and without (2) added progesterone. The zone containing this activity migrated with cortisone in the toluene-propylene glycol(3) paper chromatographic system and with cortisol in the Bush C system(4), thus resembling aldosterone. Because of low yield of this bio-conversion, chemical identification of the aldosterone has not thus far been practicable. With isotopic technics, we have been able to show definitively that perfused calf adrenals can produce aldosterone from progesterone. Tritiated aldosterone was added to aldosterone-4-C^{14} isolated from a perfusate containing progesterone-4-C^{14}, and the mixture shown to have a constant C^{14} to H^3 ratio through 3 chromatographic systems before, and 2 after, acetylation to aldosterone diacetate.

Methods. Seven left calf adrenals were perfused for 2 hours with one liter of artificial perfusion medium(5) containing 101 μc/40 mg progesterone-4-C^{14}.† The progesterone was added to the medium in 5 ml of propylene glycol. The perfusion fluid was extracted 5 times with 500 ml of dichloromethane, and the wash fluid from the apparatus (800 ml) extracted 3 times with 400 ml dichloromethane. These extracts were dried over sodium sulfate, combined, and evaporated *in*

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† Progesterone-4-C^{14} was obtained from Tracerlab, Boston, Mass., (sp. act. of 1.71 mc/mM). The 20.5 mg obtained was diluted with 19.5 mg of non-radioactive progesterone.

TABLE I. Chromatographic and Isotopic Characterization of Aldosterone.

System No.	Composition		Ref.	Aldosterone mobility, cm / hr	Cortisol®	% counted	C^{14} cpm	H^3 cpm	Ratio, tritium /carbon
I	E_2B		7	24.5/20	.71	5	302	7954	26.3
II	Benzene	4	4, 8	43.0/15	1.4	10	387	9782	25.3
	Methanol	2							
	Water	1							
III	Bush C		4	16.7/ 5	1.0	15	416	10276	24.7
IV	Cyclohexane	4	8	27.4/15		20	441	11461	26.0
	Dioxane	4							
	Methanol	2							
	Water	1							
V	Cyclohexane	4	8	31.5/12		25	326	8674	26.6
	Benzene	2							
	Methanol	4							
	Water	1							

C^{14} counts at Tap 3, 10-25 V. H^3 counts at Tap 10, 10-∞ V. Bkgd: 2.9 cpm, Tap 3; 91 cpm, Tap 10.

vacuo. Isolation of aldosterone was performed on the dried extract. An aliquot was run in the toluene-propylene glycol system(3) for 96 hr. and the zone containing cortisone was eluted with ethanol. Cortisone was then separated from aldosterone by running in a toluene-ethyl acetate-methanol-water system(4) for 5 hours. in which aldosterone migrates in the cortisol zone. This zone was located by its ultra-violet absorption. and eluted. Approximately 0.5 μg of tritium labelled aldosterone of high specific activity‡ and 52 μg of authentic aldosterone§ were added to the radioactive carbon material from the Bush C system. Non-radioactive aldosterone was added to facilitate location of the aldosterone zones by ultra-violet absorption. The steroid mixture was chromatographed in 3 successive systems (Table I). Regions containing aldosterone were located each time by their ultra-violet absorption, with reference to that of cortisol and cortisone standards. Each paper chromatogram was scanned for carbon activity using an automatic paper chromatogram scanner (Eisenberg, F. unpublished) fitted with an 0.8 mg/cm^2 cellophane window. In each instance, carbon activity corresponded exactly with ultra-violet absorption. Regions containing aldosterone were eluted with ethanol, and an aliquot taken for double isotope assay, the remainder dried and used for the succeeding system. Following third chromatography of free steroids, the mixture was acetylated overnight to aldosterone diacetate with 0.1 ml acetic anhydride and 0.2 ml pyridine. Aldosterone-21-monoacetate (Ciba) was acetylated in the same manner and then run in parallel as a chromatographic standard. The diacetates were run in 2 different systems (Table I). Aldosterone diacetate migrates with Δ 4-androstene-11β ol, 3, 17-dione in System IV and with adrenosterone in System V and these steroids were accordingly run as standards. Scanning and isotope assays were carried out exactly as for free steroids. Tritium and carbon were counted in the Packard Tri-Carb Model 314-DC Liquid Scintillation Spectrometer. Toluene containing 0.3% 2,5-diphenyloxazole (PPO) and 0.01% 1,4-di [2-(5-phenyloxazolyl)] benzene (POPOP) was utilized as counting medium. Carbon activity was obtained at photomultiplier tube voltage tap 3 (810 V.) using a 10-25 volt window, and carbon-plus-tritium activity was counted at tap 10 (1380 V.) using a 10-∞ volt window. The ratio of carbon counts at tap 10 to those at tap 3 was 2.78. Counting error was less than ± 1.5% in each instance.

‡ Tritiated aldosterone (sp. act. approx. 1.1 μc/μg) was kindly supplied by Drs. Ralph Peterson and Bernard Kliman of NIAMD, NIH, Bethesda, Md. It was obtained by tritiation of aldosterone-21-monoacetate (Ciba) by the Wilzbach(6) technic. Free aldosterone was recovered by enzymatic hydrolysis of the acetate and purified by several successive chromatographic separations.

§ Authentic non-radioactive aldosterone was obtained from Dr. H. L. Mason, Mayo Foundation.

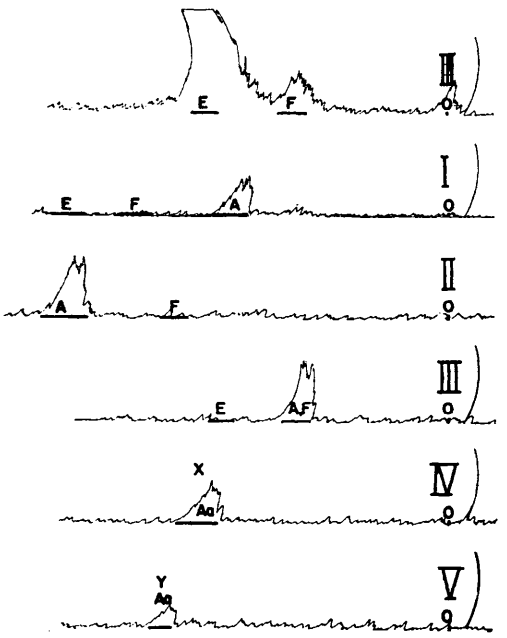

FIG. 1. Carbon radioactivity scan of paper chromatograms. Roman numerals refer to paper chromatographic systems listed in Table I. The top 2 chromatograms were recorded at 1500 cpm full scale deviation and the remaining 4 chromatograms at 500 cpm full scale. Horizontal bars indicate positions of standards. O, origin; E, cortisone; F, cortisol; A, aldosterone; Aa, aldosterone-di-acetate; X, Δ^4-androstene 11β ol, 3,17-dione; Y, adrenosterone.

Results. Fig. 1 (III, top curve) shows the C^{14} radioactivity scan of the Bush C chromatogram which followed initial separation of aldosterone from cortisol in the Zaffaroni system. To the material eluted from the cortisol zone of this Bush C chromatogram was added tritiated and non-radioactive aldosterone.

In each of the 5 chromatographic systems (Fig. 1, I-V) in which the mixed isotopes were run with authentic aldosterone, the ultraviolet absorption corresponded exactly with the zone containing C^{14} radioactivity as measured on the automatic scanner. Fig. 1 shows also location of standard run in parallel with aldosterone and its diacetate in each system: in all cases it was identical with that of radioactive aldosterone. Ultra-violet absorption was easily detected even after final chromatography in System V.

The tritium:carbon isotope ratio remained constant (Range 24.7-26.6) throughout the 3 chromatographic separations as free steroid and the 2 after acetylation, as shown in Table I. This constitutes excellent evidence for presence of radioactive aldosterone in the adrenal perfusate. It is concluded that radioactive progesterone was converted to radioactive aldosterone by the perfused calf adrenal.

No precise estimate of yield could be made. However, the activity of the purified radioactive aldosterone indicated about 0.1% conversion of progesterone-4-C^{14} to aldosterone. This can be compared with an estimated 0.5% yield based on sodium retaining activity of a calf adrenal perfusate(1).

Although there was no C^{14} activity in cortisol or cortisone zones of the E_2B (I) system, indicating complete separation of aldosterone from these steroids, a small amount of activity running slower than aldosterone was detected.

Summary. Calf adrenal glands were perfused with progesterone-4-C^{14} and the presence of radioactive aldosterone in the perfusate was established by isotopic and paper chromatographic technics.

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Received January 23, 1958. P.S.E.B.M., 1958, v97.