Conversion of Pregnenolone to Progesterone by Primate Skin* (33532)

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Several investigators have demonstrated that mammalian skin and avian glands actively participate in the metabolism of steroid hormones (1–6). The present investigation is concerned with the continuation of the elucidation of the steroid metabolic pathway in skin of the squirrel monkey.

Materials and Methods. Radioactive pregnenolone was purchased from Nuclear-Chicago Corporation and purified by thin-layer chromatography with silica gel G as the support and chloroform–ethanol (98:2 v/v) as the developing solvent. Reference steroids were also purified by this procedure. Male squirrel monkeys, weighing 680–700 g were asphyxiated with ether and the skin over the outer hind legs, back, head, and abdominal regions were shaved and removed. The subcutaneous fat was scraped off with a dull scalpel. The skin was cut into slices approximately 5 cm long and 1 mm wide and then finely minced with surgical scissors.

Four hundred mg of the minced tissue were suspended in 10 ml of 0.1 *M* phosphate buffer (pH 7.2). A sample from each skin area was heated in boiling water for 20 min. Two mg each of TPN, DPN, citrate, isocitrate, 1000 units of penicillin G and 1 mg of streptomycin were added to all incubation mixtures. Immediately before incubation radioactive pregnenolone (2.12×10^5 cpm) was added to each mixture. Control mixtures, lacking either tissue or pregnenolone, and the experimental mixtures, prepared in duplicate, were incubated simultaneously in a Warner-Chilcott model 2156 water bath at 37° for 24 hr. After incubation, all mixtures were checked for the presence of bacteria by plating 1 ml on a blood agar plate. No bacteria were found in any of the incubation mixtures.

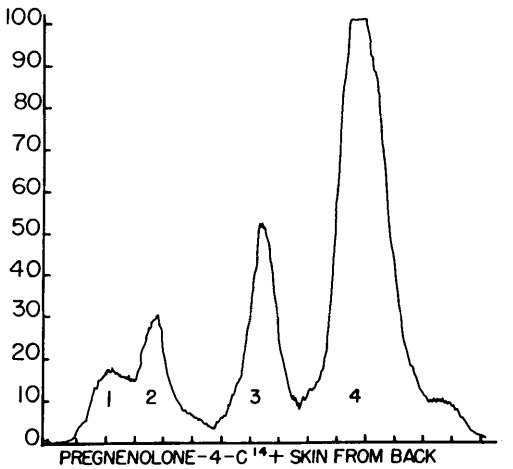

FIG. 1. A radiochromatogram scan of a residue mixture obtained from squirrel monkey skin (back) including penicillin, streptomycin, phosphate buffer, and pregnenolone-4-^{14}C.

Methods of extraction and identification of the steroid substrate and metabolites have been previously reported (4, 5). These include thin-layer chromatography, gas-liquid chromatography and crystallization to constant specific activity.

Results and Discussion. Radiochromatograms of the chloroform residues obtained from incubation mixtures of skin from the outer hind legs, back and abdominal regions were identical. Figure 1 is a typical thin-layer plate radiochromatogram scan of a chloroform residue obtained from an incubation mixture containing the dorsal skin of the squirrel monkey.

Figure 2 is a radiochromatogram scan of radioactive pregnenolone incubated in the absence of tissue slices. It is obvious from Fig. 2 that a small amount of pregnenolone break-

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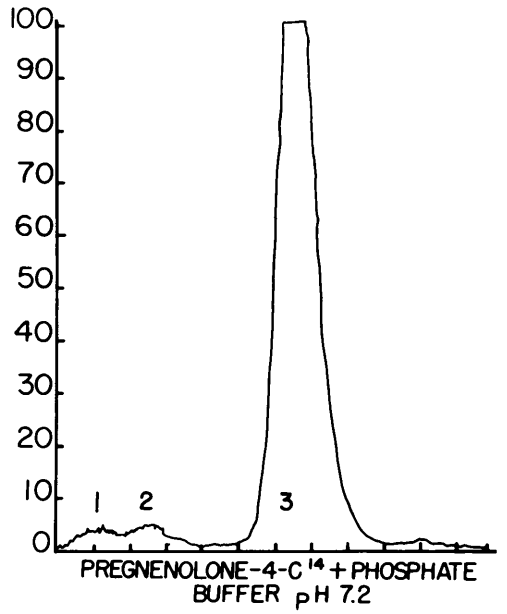

FIG. 2. A radiochromatogram scan of a residue obtained from an incubation mixture which contained phosphate buffer and pregnenolone-4-^{14}C only.

down occurred during the incubation and extraction procedures. The R_f value of peaks 1, 2, and 3 in Figs. 1 and 2 are identical to each other. Therefore, peaks 1 and 2 in Fig. 1 are not considered to be metabolites of this steroid while peak 3 is unchanged pregnenolone. Peak 4 in Fig. 1 has an R_f value identical to that of progesterone suggesting this substance as a possible metabolite.

Figure 3 is a radiochromatogram scan of an extract obtained from an incubation medium in which the tissue specimen was heated in boiling water for 20 min before the cofactors and substrate were added. It is readily seen that no enzymatic reaction took place since Figs. 2 and 3 are identical to each other.

Figure 4 is a radiochromatogram scan of the chloroform residue obtained from an incubation mixture in which skin from the scalp was used as the enzyme source. In comparing Figs. 1 and 4, it is readily seen that there are two additional minor metabolites, peaks 3 and 5, in Fig. 5. These may well be C-21 metabolites of pregnenolone, or metabolites of the newly formed metabolite progesterone. However, the possibility also exists that they may be C-19 metabolic products.

The silica gel areas corresponding to peak 3 in Figs. 1, 2, and 3; peak 4 in Fig. 1 and peak 6 in Fig. 4 were removed from their corresponding plates and the radioactive steroids eluted with absolute methanol. The methanol was evaporated in a stream of dry nitrogen and each residue rechromatographed as before. Then, the resulting radioactive areas were eluted again. This second chromatographic step resulted in obtaining what appeared to be a pure radioactive substrate and a metabolite.

Authentic pregnenolone was added to each of the eluates representing peak 3 in Figs. 1, 2, and 3. These mixtures were then chromatographed on thin-layer plates in a chloroform–ethanol system (98.5:1.5). Pregnenolone was located on the plates with iodine vapors and the radioactive steroid was localized by a radiochromatogram scan. The cold pregnenolone spot and the peak of radioactivity were at identical positions on the plate. The radioactive material (peak 3) was therefore considered to be unmetabolized pregnenolone. This material was also shown to be pregnenolone by using gas chromatographic

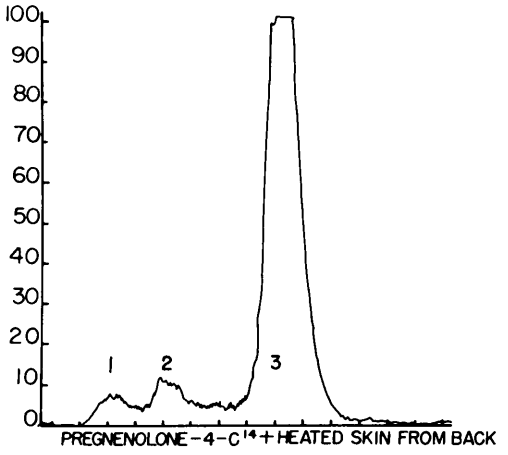

FIG. 3. A radiochromatogram scan of a residue mixture obtained from squirrel monkey skin (back) including penicillin, streptomycin, phosphate buffer, and pregnenolone-4-^{14}C. The skin tissue was heated in boiling water for 20 min before cofactors, antibiotics, and substrate were added to incubation medium.

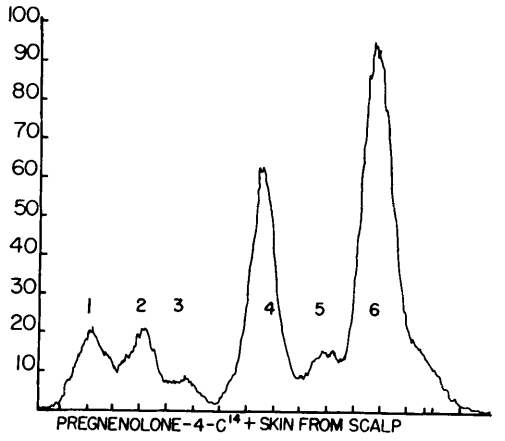

FIG. 4. A radiochromatogram scan of a residue mixture obtained from squirrel monkey skin (scalp) including penicillin, streptomycin, phosphate buffer, and pregnenolone-4-^{14}C.

techniques as previously described (5).

The radioactive compounds corresponding to peak 4 in Fig. 1 and peak 6 in Fig. 4 were identified in a similar manner. Aliquots of each peak were added to 1 mg of authentic progesterone and the mixtures chromatographed on thin-layer plates in a chloroform–ethanol system (99:1). The radioactive peak and iodine strains were at identical positions on the plate suggesting that the metabolite may be progesterone. Another aliquot of the radioactive metabolite was diluted with 2 mg of authentic progesterone and chromatographed in a Barber-Colman gas chromatography apparatus with their radioactive monitoring system. The two superimposed spectrums in Fig. 5, authentic progesterone and radioactive metabolite, shown by broken and solid

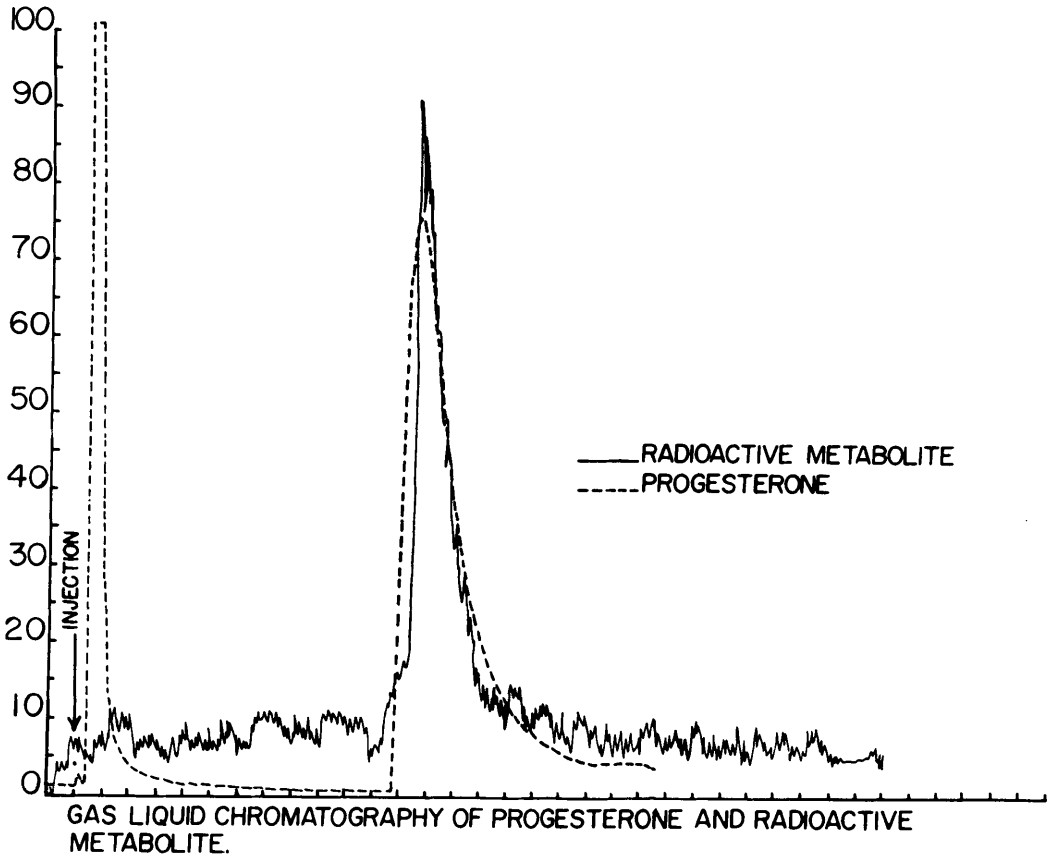

FIG. 5. Gas-liquid chromatography scan of authentic progesterone and on isolated radioactive metabolite from pregnenolone-4-^{14}C. Barber-Colman 5000 with a radioactive monitoring system; column 6 ft, 4% SE 30 on 8000/100 mesh anakrom; argon gas, flow rate 80 ml/min; column temp 230°, inj. 235°, flame 310°, oxidizer CuO at 650°.

TABLE I. Identification of Progesterone by Crystallization to Constant Specific Activity.

Steroid	Crystallizations (cpm/mg)			Source of radioactive compound
	1st	2nd	3rd	
Pregn-4-ene-3,20-dione	7362	7200	7250	Fig. 1, peak 4
Pregn-4-ene-3,20-dione	8620	8710	8740	Fig. 4, peak 6

lines, respectively, demonstrate that progesterone and metabolite have identical retention times and steroid numbers. The *O*-methyloxime derivatives of authentic progesterone and the radioactive metabolite were prepared by the method of Luukkainen (7). The *O*-methyloxime derivatives of authentic progesterone and the radioactive metabolite also had identical retention times and steroid numbers indicating that the metabolite isolated from the pregnenolone incubation mixture was progesterone. Further evidence for the identification of the metabolite was obtained by crystallization to constant specific activity. These results are shown in Table I.

Conclusion. The experimental data presented in this investigation demonstrate that primate skin is capable of converting pregnenolone to progesterone. It also demonstrates that skin obtained from the head appears to metabolize pregnenolone somewhat differently than skin from other areas of the body. This phenomenon was observed previously by the author (5). The appearance of the two as yet unidentified metabolites, peaks 3 and 5 in Fig. 4, indicates that the skin obtained from the head is capable of metabolizing pregnenolone to metabolites other than progesterone. However, progesterone itself may have been hydroxylated, reduced, or fission by the side-chain may have occurred.

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