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Excretion of 17-Ketosteroids in the Rhesus Monkey.* (23287)

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The excretion of 17-ketosteroids in the urine of the female Rhesus monkey was studied in order to determine whether this animal could be used in an experiment investigating adrenal-ovarian relationship and effect of cortisone on menstruation and 17-ketosteroid excretion. Marker isolated 4 mg of Androstanedione(1) from a 6 months pool of monkey urine. Dorfman and associates (2) reported amounts of 17-ketosteroids averaging 1.2 mg/24 hr for the female and 2.1 mg/24 hr for the male in the ketonic fraction of monkey urine, as determined by a Holtorff-Koch(3) modification of the Zimmermann reaction. Furthermore it was observed that Zimmermann chromogen was still present in the urinary extracts following removal of both gonads and adrenals. This led these investigators to the speculation that 17-ketosteroids might originate from a source other than the adrenals or the gonads.

Methods. Urine from Rhesus monkeys was collected into 2 pools over a period of several days and stored in the refrigerator. The pools measured 1080 ml (3 days) and 1500 ml (approximately 6 days) respectively. The total 17-ketosteroids were determined according to the method of Holtorff-Koch as 12 mg and 20 mg. The following procedures for fractionation and identification were then carried out on both urine pools. The urine was adjusted to pH 1.0 with 50% H_2SO_4 and extracted continuously for 48 hours with ether. The ether was washed with 5% NaOH until pigment-free, then with H_2O until neutral and the washings were added to the urine. Further hydrolysis was achieved by boiling for 30 minutes at pH 1.0. The urine was then extracted manually and washed as above. Both ether fractions were combined and dried over Na_2SO_4. After evaporation

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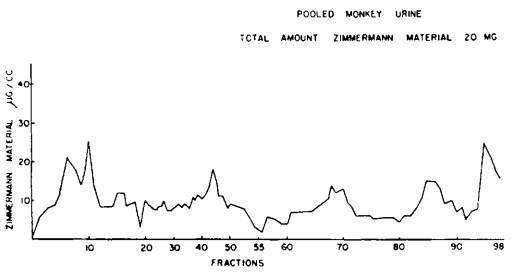

FIG. 1. Distribution of Zimmermann chromogen from monkey urine following chromatography on alumina.

of the ether, residual moisture was removed with benzene. The crude extract thus obtained was chromatographed on alumina in a gradient elution system (Lieberman and Lakshmanan(4)) in which 200 ml benzene and 6% ethanol was added slowly to 800 ml benzene during development of the chromatogram. Removal of more polar steroids from the column was assured by subsequent elution with 20 ml each of 5%, 10%, and 20% ethanol in benzene. The eluate was collected in 100 fractions. Aliquots of 1 ml were taken for Zimmermann reaction (Holtorff and Koch).

Results. Five distinct peaks were obtained (Fig. 1) throughout the chromatograms. Only 2.8 mg of purified Zimmermann reacting material were recovered from the crude extract of the small pool (total 12 mg), while the extract of the larger pool, containing 20 mg of crude material, yielded 5.8 mg. These values were within the range of Dorfman's findings. However, when identification of the material in the peak tubes was attempted by UV, sulfuric acid spectra and infrared spectrophotometry, none of the spectra resembled those of 17-ketosteroids or other steroids. Moreover, no carbonyl absorption was obtained at 1800-1580 cm^{-1} and no peaks were observed at 311 mμ in H_2SO_4. This led us to believe that the color obtained by the Zimmermann reaction was not specific. It is suggested that the technic used for determination of 17-ketosteroids in humans cannot be applied to monkey urine. This does not imply that the monkey does not excrete 17-ketosteroids, but merely that values given in the literature based on the Zimmermann reaction alone are misleading and have led to erroneous conclusions.

Summary. An attempt was made to study the excretion of 17-ketosteroids in Rhesus monkey urine. No 17-ketosteroids could be detected by ultraviolet adsorption, sulfuric acid and infra-red spectra following chromatography of Zimmermann-material. This suggests that values quoted in the literature, based on Zimmermann reaction alone have led to erroneous conclusions.

POSTSCRIPTUM: Since this paper was submitted a similar discrepancy between Zimmermann reactive material and true 17-ketosteroid content has been reported in pregnant goat's urine by Klyne *et al.* (Klyne, W., Wright, A. A., *Biochem. J.*, 1957, v66, 92.)

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Acetal Phosphatides in Adipose Tissue and Livers of Starved Rats. (23288)

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Previous work reported from this laboratory(1) showed that in the adipose tissue of newborn rats, where this tissue is rapidly filling with fat, the acetal phosphatides and phospholipids, initially elevated, decreased to only trace amounts by the fifth to eighth day of life. This finding posed the question: would phospholipid and the tissue lipid aldehyde fraction, presumably acetal phosphatides, in the reverse situation, as in starvation, also become elevated during the period of rapid mobilization of depot fat from adipose tissue.