

terion but with greater concentrations this method is grossly inadequate. On the other hand, the chloruretic effect increased regularly over the entire range of concentrations of pituitrin studied. In 130 experiments using 2 control groups of 3 rats each or a total of 780 rats the chloride excretion in the urine never exceeded 90 microequivalents per 100 g of rat per 3 hours. Thus definite sensitivity is shown with 0.5 milliunits per 100 g of rat.

Summary. (1) Three different methods of

hydrating rats were explored and the diuresis produced was measured in 4 separate ways.

(2) The most reproducible results were obtained by hydrating with 2 doses of water and by recording diuresis as the area of the excretion curve.

(3) Pituitrin solutions, over a considerable range of concentrations, could be assayed more accurately by measuring their chloruretic effect than by measuring their anti-diuretic effect.

14255 P

Excretion of Androgens, 17 Ketosteroids and Estrogens in the Dog Following Administration of Androgens.*

K. E. PASCHKIS,[†] A. CANTAROW, A. E. RAKOFF, L. P. HANSEN, AND A. A. WALKLING.

From the Jefferson Medical College and Hospital, Philadelphia, Pa.

Injection of testosterone propionate in large therapeutic doses is followed in humans by excretion of considerable amounts of androgenic material in the urine.¹⁻³ Such treatment is also followed by increase of estrogenic potency of the urine.^{1,2,4,5} In contradistinction to the human, no estrogen and only traces of androgen were found in the urine of the dog after the injection of androgens.^{6,7}

Since we are conducting an extensive study of the metabolism of estrogens and androgens in the dog it was felt necessary to reexamine the urinary excretion of androgens and estrogens following the administration of androgens in this species.

Methods. Urine samples were collected in metabolism cages in 24-hour periods. In many instances four 24-hour samples were collected and pooled. Androgens and estrogens were extracted following hydrolysis with HCl at pH 1. Androgens were assayed on one-day-old cockerels by a modification of the method of Frank *et al.*⁸ The urine extracts were applied directly to the comb in ether solution as suggested by Koch.⁹ Estrogens were assayed on mice by vaginal smear and histological methods after Fluhman.¹⁰ 17 Ketosteroids were determined by the method of Holtorff and Koch.¹¹

Hormone excretion of the normal dog. Excretion of androgens, estrogens and 17 ketosteroids was studied in 3 female and 8 male dogs. Androgen excretion ranged from 150 to 350 γ per 24 hours, expressed as androsterone. One female dog excreted 1500 γ per 24 hours. 17 ketosteroid excretion ranged from 500 to 1500 γ per 24 hours,

* Aided by a grant from the Johnson Research Foundation, New Brunswick, N.J.

[†] J. E. Mears Fellow in Physiology and Medicine.

¹ Dorfman, R. J., and Hamilton, J. B., *J. Clin. Invest.*, 1939, **18**, 67.

² Hoskins, W. H., Coffman, J. R., Koch, F. C., and Kenyon, A. T., *Endocrinol.*, 1939, **24**, 702.

³ McCullagh, E. P., *J. Am. Med. Assn.*, 1939, **112**, 1037.

⁴ Steinach, E., and Kun, H., *Lancet*, 1937, **2**, 845.

⁵ Hamilton, J. B., and Dorfman, R. J., *J. Lab. Clin. Med.*, 1942, **27**, 917.

⁶ Kochakian, C. D., *Endocrinol.*, 1937, **21**, 60.

⁷ Kochakian, C. D., *Endocrinol.*, 1938, **23**, 463.

⁸ Frank, R. T., Klemperer, E., Hollander, F., and Kriss, B., *Endocrinol.*, 1942, **31**, 63.

⁹ Koch, F. C., *Assn. for Study of Internal Secretions*, 25th meeting, Atlantic City, 1941.

¹⁰ Fluhman, C. F., *Endocrinol.*, 1934, **18**, 705.

¹¹ Holtorff, A. F., and Koch, F. C., *J. Biol. Chem.*, 1940, **135**, 377.

expressed as androsterone. There was no difference in androgen or 17 ketosteroid excretion between male and female dogs. No estrogenic activity was detectable in the urine of dogs of either sex.

Excretion of androgen and estrogen following injection of testosterone propionate. Two male dogs received daily injections of 10 mg testosterone propionate[‡] in sesame oil for 12 days. Urine was collected (a) prior to injection, (b) during the last 4 days of treatment, and (c) one week after treatment had been discontinued. There was no increase in androgenic activity and no increase in 17 ketosteroid values in the urine. One of the two dogs excreted 10 i.u. per 24 hours of estrogenic material during the last 4 days of treatment. One week later no estrogenic activity was demonstrable. In experiments to be reported in another communication, large single doses of androgenic compounds (testosterone, androsterone, methyltestosterone) were given intravenously in alcoholic solution. Estrogens were found in the urine in 5 of 7 experiments (40, 40, 24, 6 and 100 i.u. per 24 hours respectively). In one experiment, intraduodenal administration of testosterone was followed by estrogen excretion in the urine. No increased excretion of androgens was observed in any of these experiments.

Androgen and estrogen excretion following the injection of testosterone propionate into castrate male dogs. The 2 male dogs in which sex hormone excretion following treatment with testosterone propionate had been studied were castrated. Several weeks later the dogs received another course of 12 in-

jections of 10 mg testosterone propionate. No androgen was excreted in the urine. The dog which had excreted estrogen during treatment with testosterone propionate prior to castration did so again after castration.

Androgen and estrogen excretion following injection of testosterone propionate into the adrenalectomized female dog. A female dog was adrenalectomized by the usual 2-stage operation and was maintained with desoxycorticosterone acetate. Androgen excretion prior to adrenalectomy was 150 γ per 24 hours. Following adrenalectomy no androgens were detectable. The dog was then given 12 daily injections of 10 mg testosterone propionate. During the last 4 days of treatment with testosterone, traces of androgens (8 γ per 24 hours) were excreted. At this time 10 i.u. of estrogens were excreted per 24 hours, while there had been no estrogen excretion either before or after adrenalectomy.

Discussion and Summary. In contradistinction to the human, urine androgens do not increase in the dog following administration of androgenic hormones. These findings confirm earlier observations by Kockakian.⁶ Administration of androgens also fails to increase the excretion of 17 ketosteroids. However, contrary to earlier findings by Kochakian,⁷ dogs were found to excrete estrogens following administration of androgens, as has been found in humans.^{1,2,4,5} This excreted estrogen may be either formed from the administered androgen or may be the result of stimulation of some source of estrogen production. The fact that estrogens are excreted in the castrated and in the adrenalectomized dog following testosterone treatment narrows considerably the possible sources of the estrogens. Conversion from the administered androgen seems more likely but is not yet definitely proven.

[‡] Testosterone, testosterone propionate, methyltestosterone and desoxycorticosterone acetate were generously supplied by Dr. E. Schwenk and Dr. M. Gilbert of the Schering Corp., androsterone by Mr. R. Mautner of the Ciba Corp.