TABLE I.
Influence of Pregnant Mare Serum on Hypophysectomized Immature Male Rats.

Total dose	No. of rats	Avg organ wt (mg ± E_m) Testes	Sem. ves.
0	7	231	7.6 ± 0.6
2.5	10	443	14.9 ± 2.1
5.0	13	335	17.9 ± 2.9
12.5	7	273	19.3 ± 3.0
25.0	9	409	30.7 ± 2.8
50.0	11	366	29.9 ± 4.0
100.0	10	451	41.8 ± 4.5
Control rats 5 days after hypophysectomy		365	7.5
Unoperated control rats 35 days of age		718	13.2

E_m = mean deviation of the mean.

dosage injected.[6] A low dose (10 r. u.) causes marked thecal luteinization with little growth of follicles. A larger dose (25 r. u.) results in the development of large vesicle follicles and slight thecal luteinization whereas further dosage increases (50 r. u.) causes the formation of corpora lutea. The lot of hormone used in these experiments produced the same effect in the hypophysectomized female rats as those just described. It was of interest to find that a 25 r. u. dosage which produces a 10-fold ovarian weight increase and primarily a follicle stimulating effect also causes a 4-fold increase in seminal vesicle weight in hypophysectomized male rats. These results indicate that the potency of a dosage of pregnant mare serum as determined by its action on the ovary does not indicate its ability to increase seminal vesicle weight.

Summary. Pregnant mare serum maintains testis weight and stimulates seminal vesicle weight in hypophysectomized male rats treated for a 5-day period. These data are compared with those from similarly treated female rats.

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Reproducible Diuresis and Chloruresis for Bioassay of Antidiuretic Activity.

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For the bioassay of antidiuretic activity it is essential that control observations shall provide a diuresis which is both reproducible and quantitative. In a previous communication[1] certain results obtained by a satisfactory method were given. It is the purpose of this paper to describe in greater detail a comparison of the various procedures explored. In agreement with the prediction of Silvette[2] it appears that extracts of the posterior lobe of the pituitary gland can be assayed more accurately by measuring urinary chloride excretion than by measuring the inhibition of water excretion.

The rat method of assaying the antidiuretic activity of extracts of the posterior lobe of the pituitary gland was introduced by Burn.[3] Subsequently many modifications have been described both in the manner of producing hydration and in the calculation of results. These procedures have been employed to assay the antidiuretic activity of urine, blood serum and pituitary extracts.

Three different methods of hydration were chosen for comparison. (A) Gilman and Goodman[4] administered a preliminary "hy-

* This work done during tenure of a Commonwealth Fund Fellowship, 1940-41.

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3 Burn, J. H., *Quart. J. Pharm. and Pharmacol.*, 1931, **4**, 517.

4 Gilman, A., and Goodman, L., *J. Physiol.*, 1937, **90**, 113.

drating" dose of water amounting to 2.5% of the rat's body weight by gavage 3 hours before beginning the assay. Then at the beginning of the assay period a second dose of water amounting to 5% of the body weight was administered. (B) Silvette[5] administered a total of 10 cc of 0.2% sodium chloride solution intraperitoneally per 100 g of rat. (C) Kreiger and Kilvington,[6] using rats weighing approximately 200 g, administered to each 12 cc of water by gavage.

The control diuresis produced by each of the 3 hydrating procedures was in turn measured and calculated by 4 different methods. (a) Burn[3] determined the average time required for the rat to excrete 50% of the total fluid administered. (b) Robinson and Farr[7] recorded the average time required for the rat to excrete a volume of urine equaling 2.5% of the initial body weight. (c) Silvette[5] determined the total volume of urine in cc excreted per 100 g of rat in the 6 hours elapsing after the fluid was administered. (d) Kreiger and Kilvington[6] plotted the amount of urine against time at 15-minute intervals for 5 hours and measured the area subtended by this curve. Antidiuretic agents prolonged the time required to reach the chosen endpoints in methods a and b, reduced the volume of urine excreted in 6 hours in method c, and reduced the area of the curve in method d.

In the comparative study here described new rats maintained under carefully controlled conditions of feeding, housing and hydration as described previously[1] were used in each experiment. The 3 methods of hydration (A, B, C, above) and 4 methods of analysis (a, b, c, d, above) were performed 3 times, using 2 groups of 6 rats in each experiment. The results of each group were averaged and then a grand average was computed for all 6 groups. Each figure therefore includes results on 36 rats or six groups of

TABLE I. Results of the 3 Methods of Hydration and the 4 Methods of Measurement and Calculation of the Diuresis Produced.

	a. 50% excretion time in minutes			b. Time in min. to excrete volume of urine equal to 2.5% of body wt			c. Total cc urine excreted in 6 hr			d. Area in square inches					
										6 hr			3 hr		
	Avg Min	Max. deviation %	Mean deviation %	Avg Min	Max. deviation %	Mean deviation %	Avg cc	Max. deviation %	Mean deviation %	Avg sq. in.	Max. deviation %	Mean deviation %	Avg sq. in.	Max. deviation %	Mean deviation %
A. Gilman and Goodman. Water equal to 2.5% and 5.0% of body wt by gavage.	73	29	9.6	64.6	27.8	11.3	5.73	7.8	4.7	24.5	4.9	3.3	18.61	10.7	5.5
B. Silvette. 10 cc 0.2% NaCl sol. intraper. per 100 g wt	196	22	14.3	120	36	10.8	7.01	22	9	24.2	26.8	10	11.43	34.9	11.6
C. Kreiger and Kilvington. 12 cc water by gavage.	124	21	12	82	19.5	10.4	8.8	17	6.6	32.7	18.3	6.9	18.64	23.7	12.8

[5] Silvette, H., *Am. J. Physiol.*, 1940, **128**, 747.

[6] Kreiger, V. I., and Kilvington, T. B., *M. J. Australia*, 1940, **1**, 575.

[7] Robinson, F. H., Jr., and Farr, L. E., *Ann. Int. Med.*, 1940, **14**, 42.

6 each. The mean deviation and maximum deviation from the final average were determined for all 6 groups and are shown in Table I.

These results showed that the method of double hydration described by Gilman and Goodman (A) and the area method of measurement of the resulting diuresis described by Kreiger and Kilvington (d) yielded the most reproducible results. In order to shorten the 6-hour period of observation required by this method so that 2 runs could be completed in one day the data were recalculated on the basis of the areas subtended by a curve plotted for 3 hours. The maximum and mean deviation for this shorter period are shown for each method of hydration in Table I. Despite the somewhat larger variation, the 2-dose technic was superior and for practical reasons was chosen for routine use in subsequent studies on the antidiuretic activity of human urine already reported.[1]

In preliminary observations, using known amounts of pituitrin,† it was found that the 3-hour area method described was quite accurate with doses of 0.5 to 10 milliunits of pituitrin per 100 g of rat. Doses larger than this produced curves with areas slightly greater than those for 10 milliunits and hence paradoxically smaller numerical values for antidiuresis. Examination of these curves revealed that doses exceeding 10 milliunits per 100 g of rat produced a brief diuresis and then secondarily, a profound antidiuresis, whereas the smaller doses produced antidiuresis only. This effect has been described before with larger doses and may be related to the temporary rise in blood pressure produced by pituitrin. Since the expression of results in terms of area includes the total effect, it is essential, with pituitrin at least, to dilute the sample of unknown potency to the proper range to obtain accurate quantitative results.

Although the larger doses of pituitrin had a paradoxically smaller antidiuretic activity over the three-hour period chosen their effect on the excretion of chloride in the urine, or their "chloruretic"‡ activity, increased steadily as the dose of pituitrin increased over a range of 0.1 to 100 milliunits per 100 g of rat. In fact comparison showed that pituitrin solutions may be assayed more accurately by measuring their effect on chloride excretion, than by measuring their antidiuretic activity alone. Table II shows both the antidiuretic effect and the chloruretic effect of solutions containing 0.1 to 100 milliunits of pituitrin per 100 g of rat when administered intraperitoneally in a total volume of 1 cc of physiological saline per 100 g of rat. Six groups of 3 rats were used in each assay; 2 groups as saline controls and 2 groups for each concentration of pituitrin. As previously described[1] the antidiuretic effect is expressed as the difference in square inches between the areas subtended by the curves of the test substance and the saline controls. The total chloride excretion of each group, per 100 g, during the 3-hour period of observation, was measured by the open Carius method of the Volhard titration as described by Van Slyke and Sendroy.[8]

TABLE II.
Antidiuretic and Chloruretic Effects of Pituitrin Solutions Ranging from 0.1 milliunits to 100 milliunits per 100 g of Rat.

Pituitrin in milliunits per 100 g of rat	Antidiuretic effect—difference in square inches from controls	Total chloride excreted—microequivalents per 100 g of rat per 3 hr
780 saline controls	± 0.78	Less than 90.0
0.1	+ 1.2	74.25
0.5	— 1.5	109.0
1.0	— 3.2	121.0
2.5	— 5.3	219.0
5.0	— 9.2	252.0
10.0	—10.3	273.0
25.0	— 4.9	362
50.0	— 5.3	436
75.0	— 5.0	512
100.0	— 4.7	653

Assay of pituitrin solutions containing from 0.5 to 10 milliunits per 100 g of rat is quite accurate using antidiuresis as the cri-

† Obstetrical pituitrin, Parke Davis, Lot No. 3260960, 1 cc containing 10 international units.

‡ Chloruretic: Gr. chloros, yellowish green; plus Gr. ouresis, urination; urination of chlorides.

8 Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry. Methods*, Williams & Wilkins, Baltimore, 1932, p. 835.

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 antidiuretic effect.

14255 P

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

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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 reëxamine 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,

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

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