anterior pituitary and equine chorionic gonadotrophins.[10] Under comparable conditions the interval from progesterone injection to forced ovulation of C_8 follicles falls between 8 and 11 hours. The details of these and other tests now in progress will be reported elsewhere.

Summary. The administration of crystalline progesterone to the common domestic hen is highly effective, under stated conditions, in causing premature ovulation of normally developing ovarian follicles. Results differ with route of injection, time from injection to expected normal ovulation, injection level, and place of the follicle in the clutch sequence. First follicles of clutch sequences are ovulable at least 6 hours prematurely in 90 to 95% of hens receiving 0.5 and 1.0 mg progesterone intravenously, or 1.0 to 10.0 mg subcutaneously. Follicles other than the first of clutch sequences are ovulable by at least 7 hours prematurity in 70 to 75% of subcutaneously injected hens at dosage levels of 0.5 to 5.0 mg progesterone. Under identical conditions the intravenous administration of progesterone is relatively ineffective. Observed intervals from injection to ovulation varied from 7 to 11 hours.

10 Fraps, R. M., Riley, G. M., and Olsen, M. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 313.

14152

Effects of Adrenal and Thyroid Hormones on Water Exchange in Hypophysectomized Rats.*†

MALVINA SCHWEIZER, ALEXANDER EHRENBERG AND ROBERT GAUNT.

From the Department of Biology, Washington Square College of Arts and Science, New York University.

After hypophysectomy in the rat there is a transient high diabetes insipidus (d. i.) which subsides fairly rapidly and is followed by a mild chronic diuresis. If only the neurohypophysis is removed or inactivated a permanent d. i. results.[1,2,3]

It has been found that under certain circumstances anterior pituitary extract could reestablish a latent d. i. in hypophysectomized rats,[1,4‡] as it does in other species, whereas neither pituitary preparations containing primarily adrenotropic hormone, nor whole adrenal cortical extract, would do so.[4] In view of reports[5,6] that desoxycorticosterone acetate (DCA) can reinduce a polyuria in hypophysectomized rats, further observations on adrenal cortical materials are reported here,

* This work was aided by a grant from the Committee on Research in Endocrinology, National Research Council.

† We are indebted to Dr. Erwin Schwenk, Schering Corporation, for the desoxycorticosterone acetate (Cortate) used here.

We wish to acknowledge the assistance of Mr. Bennett H. Young during part of this work.

1 Richter, C. P., *Res. Publ. Assn. Nerv. Ment. Dis.*, 1938, **17**, 392.

2 Fisher, C., Ingram, W. R., and Ranson, S. W., *Diabetes Insipidus and the Neuro-hormonal Control of Water Balance*, Edwards Bros., Ann Arbor, 1938.

3 Gersh, I., *Res. Publ. Assn. Nerv. Ment. Dis.*, 1939, **18**, 436.

4 Schweizer, M., Gaunt, R., Zinken, N., and Nelson, W. O., *Am. J. Physiol.*, 1941, **132**, 141.

‡ It has been more difficult to maintain d. i. with anterior pituitary preparations in hypophysectomized rats than in other species. We have found that different lots of crude beef anterior pituitary extract, apparently prepared in a similar manner, and equally effective as stimulants of growth and appetite, do not all have the marked diuretic effect previously reported in hypophysectomized rats.[4] This is perhaps due to variation in the amounts of antidiuretic substances contained in the extracts, although quantitative studies have not been made.

5 Selye, H., and Bassett, L., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 272.

6 Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1941, **133**, 511.

together with studies on the effects of thyroid substances.

Methods. Routine technics were the same as in earlier experiments.[4] Figures quoted here are all from rats at least 4 weeks after hypophysectomy. Complete pre- and post-operative figures are available but essentially duplicate earlier findings.[4] Food intake figures are not detailed since the only change brought about by the various treatments was a 27% increase with thyroid-feeding. Oxygen-consumption was measured at weekly intervals by the method of Tainter and Rytand.[7] Urine specific gravity was determined generally twice weekly with a falling drop apparatus (Fisher) on samples specially collected to prevent contamination.

Desoxycorticosterone acetate. This material, given in 1 mg daily doses, elevated the water exchange but the responsiveness of animals varied widely. The effect was sometimes not noticeable until after one week (*cf.* [4]) and reached its maximum after 2 weeks of treatment. These results are in general agreement with those of others who obtained a more rapid and extensive response by using larger doses. The diuresis occurs in the absence of any significant change in the other factors measured, viz., food intake, body weight, specific gravity of the urine, and oxygen-consumption. These and other results are summarized in Table I.

Whole Extract of the Adrenal Cortex. The material given was a concentrate prepared in sesame oil at Princeton University. Quantitative studies of a similar preparation have been reported in other connections.[8] It was given in 0.5 cc daily doses (equivalent to 125 g fresh tissue), and treatment was continued for 3 weeks, in contrast to the earlier 7-day observations with aqueous cortical extract.[4] Again, however, no effects were obtained on water exchange or on the other variables measured here.

Thyroid-feeding. When thyroid powder was fed in doses regulated to restore the consumption of oxygen to normal (approximately 75 mg per day) there was a small but consistent rise in water intake, without an accompanying rise in urine volume. In half of the cases the urine output was if anything reduced. The absence of the expected diuresis was associated with an increase in the specific gravity of the urine. Food intake was increased. The weight losses characteristic of untreated hypophysectomized rats were exaggerated by the thyroid-treatment. Toxic symptoms were noted and several animals, not included in the tables, died during the course of the treatment.

When 0.02 mg of thyroxin was injected daily the results were similar to those obtained above, but are not included in the tables.

Since these results were possibly complicated by the toxicity of the treatments,[9] a dose of thyroxin was sought which would give some stimulation to oxygen-consumption but which would not cause serious weight losses or increase mortality. Thyroxin in 0.005 mg daily doses was found to meet these requirements. In such non-toxic doses, however, it had no effect on water exchange.

Thyroid-feeding and DCA Combined. When thyroid powder in doses which restored oxygen-consumption to normal was fed together with daily injections of 1 mg of DCA, no toxic action of the former was apparent. A water intake greater than that seen with either treatment alone occurred while the urine volume increased some but not proportionately to the water intake. The relative absence of diuresis again was associated with an elevation of the specific gravity of the urine, and under these circumstances indicates that this effect of thyroid-feeding was not due solely to the toxicity of the treatment.

Discussion. In hypophysectomized dogs several investigators have found a diuretic action of thyroid treatment,[9,10] but not in the rat.[11] Thyroxin will, however, augment the already high d. i. which follows hypothalmic lesions in the rat,[12] as in other species. Our results would imply that in the rat the

[7] Tainter, M. L., and Rytand, D. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 361.

[8] Eversole, W. J., Gaunt, R., and Kendall, E. C., *Am. J. Physiol.*, 1942, **135**, 378.

[9] Keller, A. D., *Endocrinology*, 1942, **30**, 408.

[10] Bruhn, J. M., *Am. J. Physiol.*, 1942, **135**, 572.

[11] White, H. L., *Am. J. Physiol.*, 1937, **119**, 5.

[12] Clark, G., *Quart. Bull. Northwestern Univ. Med. School*, 1940, **14**, 96.

TABLE I.
Water Exchange, Oxygen Consumption, and Urine Specific Gravity in Hypophysectomized Rats Receiving Various Treatments.

	Water intake (cc)						Urine volume (cc)						O_2 consumed (cc/cm²/hr)		
	Before treatment	During treatment			After treatment		Before treatment	During treatment			After treatment		Before treatment	During 2nd and 3rd wks	After treatment
Treatment	7 days	1st wk	2nd wk	3rd wk	1st wk	2nd wk	7 days	1st wk	2nd wk	3rd wk	1st wk	2nd wk			
DCA	37*	52	73	72	47	33	32	43	63	68	42	27			
	16	20	20		18	12	9	10	13		12	9			
	15	18	24		15	13	11	16	24		12	12			
	17	19	23		23	16	14	16	21		20	15			
	21	20	27		24	22	15	17	24		20	20			
	23	29	36	34	31	21	17	26	31	32	24	21	.50	.45	.35
	15	15	18	19	13	12	13	13	15	15	11	10	.47	.48	.52
	20	29	37	30	22	19	13	23	25	19	16	14	.39	.54	.45
	18	27	30	33	23	19	13	19	23	25	19	14	.48	.55	.54
	—	—	—		—	—	—	—	—		—	—	—	—	—
Avg	18.	22.	27.		21.	17.	13.	18.	22.		17.	14.	.46	.51	.47
Specific gravity of urine (avg)							1.018	1.020	1.016	1.015	1.014	1.016			
Adrenal	24	20	20	22	19	19	16	15	15	15	15	16	.50	.58	
Cortical	16	13	14	12	14	14	12	12	9	7	8	11	.46	.50	
Extract	14	13	12	11	12	16	12	10	8	8	8	9	.47	.54	
	16	21	21	19	20	23	11	14	14	14	14	17	.46	.42	
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Avg	18.	17.	16.	16.	16.	18.	13.	13.	12.	11.	11.	13.	.47	.51	
Specific gravity of urine (avg)							1.020	—	1.018	1.019	1.020	1.021			
Thyroid-fed	16	15	22	24	25	18	10	8	10	10	10	12		.97	
	14	15	16	17	17	10	11	9	8	8	4	7		.82	
	11	12	15	17	17	10	7	6	6	6	7	6		.99	
	19	15	16	14	14	12	15	11	6	6	6	9		.80	
	14	16	16	18	24	19	10	9	7	6	12	12		1.07	
	17	23	22	23	26	23	11	14	9	10	15	15		1.08	
	18	29	26	29	32	24	15	19	14	15	20	17		1.28	
	—	—	—	—	—	—	—	—	—	—	—	—		—	
Avg	16.	18.	19.	20.	22.	12.	11.	11.	9.	9.	11.	11.		1.00	
Specific gravity of urine (avg)							1.020	1.034	1.031	1.037	1.031	1.022			
DCA and	17	28	40	47			13	16	19	26			.54	1.18	
Thyroid-fed	22	20	25	38	38	24	14	16	14	23	21	15	.50	1.07	.60
	18	26	33	37	20	14	17	17	20	21	15	10	.47	1.04	.48
	17	21	35	38	22	18	14	17	22	23	12	12	.50	1.10	.47
	20	28	45	54	47	36	15	19	27	34	32	21	.55	.96	.68
	19	26	31	37	18	17	15	16	20	25	12	13	.49	1.04	
	22	35	42	51	30	24	18	31	36	43	23	19	.39	.91	
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Avg	20.	26.	35.	43.	29.	22.	16.	19.	23.	28.	19.	15.	.49	1.04	.56
Specific gravity of urine (avg)							1.018	1.029	1.031	1.030	1.025	1.021			
Thyroxin-	23	22	21				15	13	15					.78	
injected	21	24	21				14	15	15					1.00	
(small	28	30	27				17	17	16					.72	
dose)	19	20	24				9	9	10					—	
	24	29	23				11	12	12					.97	
	33	31	27				22	16	16					.89	
	19	26	24				11	14	13					.71	
	—	—	—				—	—	—					—	
Avg	24.	26.	24.				14.	14.	14.					.84	

Average Figures on Normal, Intact Animals.
Daily water intake, 21 cc; Specific gravity of urine, 1.06.
Daily urine volume, 8 cc; Oxygen consumption cc/cm2/hr, 1.00 ± 0.024 (St. Er.).
* This case is not included in the average because it is obviously atypical.

diuretic action of thyroid hormone is dependent upon the presence of a functional anterior pituitary.

Silvette and Britton[13] developed the hypothesis that the diuretic action of the anterior pituitary was elaborated through the adrenal cortex, and Corey and Britton demonstrated the diuretic action of DCA in short (12-hour) tests in hypophysectomized rats.[6] In experiments of longer duration Selye and Bassett[5] restored a high d. i. in hypophysectomized rats with DCA. Both groups of investigators use large doses. Ragan *et al.*[14] showed at about the same time that a d. i.-like syndrome could be induced in intact dogs with prolonged treatment with DCA—a study supplemented by Mulinos *et al.*[15]

The 1 mg doses of DCA used here were selected to see if amounts that might be considered physiological would also restore d. i. in hypophysectomized rats. The selection of a "physiological" dose was admittedly arbitrary. The 1 mg dose used was about 20 times the minimal life-maintaining dose for adrenalectomized rats in our colony. Its effects in increasing water exchange were definite but mild, and it did not produce anything like a maximal d. i. No indication of an action of this type was seen, however, when animals were treated with a whole extract of the adrenal cortex in large dosage, suggesting another difference[16] in the biological effects of the various cortical steroids.

Since the amounts of DCA needed to produce a real d.i. after hypophysectomy are large, and as the amount of this substance in cortical extracts or tissue is small,[17] the possibility cannot be ignored that its action in this respect is a pharmacological overdosage phenomenon. In any case it hardly seems likely that the adrenal cortex is the sole route through which the anterior pituitary effects its diuretic action.

Desoxycorticosterone, or related adrenal steroids, may constitute one of a complex of influences under anterior pituitary control which act together to maintain d. i. when the posterior pituitary is absent. The thyroid is probably involved in this complex, as are the pituitary factors which maintain a normal growth, activity, and appetite[18,1] and hence normal salt intake,[19] nitrogen excretion,[20] etc.

Summary. The effects of adrenal cortical and thyroid substances were studied on the water exchange and related phenomena in hypophysectomized rats during periods after the initial high postoperative d. i. had subsided. 1. Desoxycorticosterone acetate in 1 mg daily doses elevated water exchange, but not to levels characteristic of d. i., whereas whole extract of the adrenal cortex was without effect. 2. Thyroid-feeding in doses that restored oxygen consumption at least to normal elevated the water intake slightly but not the urine output. There was an associated rise in urine specific gravity. The diuretic effects of thyroid hormone under other circumstances in the rat must depend upon the presence of an anterior pituitary. 3. Non-toxic doses of thyroxin, although affecting oxygen consumption, had no effect on water exchange. 4. When desoxycorticosterone and the larger doses of thyroid were given in combination, the toxicity of the latter was overcome. Water intake was doubled, but urine volume did not increase proportionately due to the opposing action of the thyroid substance.

13 Silvette, H., and Britton, S. W., *Science*, 1938, **88**, 150.

14 Ragan, C. J., Ferrabee, J. W., Phyfe, P., Atchley, D. W., and Loeb, R. F., *Am. J. Physiol.*, 1940, **131**, 73.

15 Mulinos, M. G., Spingarn, C. L., and Lojkin, M. E., *Am. J. Physiol.*, 1941, **135**, 102.

16 Kendall, E. C., *Arch. Path.*, 1941, **32**, 474.

17 Pfiffner, J. J., *Advances in Enzymology*, 1942, **2**, 325.

18 Richter, C. P., *Am. J. Physiol.*, 1934, **110**, 439.

19 Swann, H. G., and Penner, B. J., *Endocrinology*, 1939, **24**, 253.

20 Winter, C. A., and Ingram, W. R., *Am. J. Physiol.*, 1941, **133**, P495.