of the 3 experiments on the excretion of insulin by the rabbit kidney rendered pathologic with uranium nitrate. These protocols show (1) insulin is not a constituent of the urine of normal rabbits or of the urine excreted by the rabbit kidney rendered pathologic with uranium nitrate, at least in the quantities employed. (2) Following the intravenous administration of insulin, insulin or an insulin-like substance† is excreted readily by the rabbit kidney. The greatest concentration of insulin in the urine occurs directly after its administration, the rate of excretion tapering off until 3 to 3½ hours later when insulin no longer could be detected in the urine. (3) After the intravenous administration of insulin, this hormone is not excreted in greater amounts by the pathologic kidney than by the normal rabbit kidney.‡

10035

Influence of Thyroid Hormone on Estrin Action.

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The interference of hyperthyroidism with the response of castrate female rats to estrogenic hormones was first reported by Reiss and Perény.[1] These authors came to the conclusion that there is

† Although no definite proof can be offered to substantiate the claim that this insulin-like substance is insulin, the following will be of interest. 148 cc of urine was obtained from 1 rabbit within a period of 4 hours after the intravenous injection of 200 units of insulin. A modified Dudley's procedure (picric acid precipitation) was then employed for the precipitation of insulin from this urine. The final precipitate thus obtained, though very small in quantity, was dissolved in distilled water and the solution injected subcutaneously into a fasting rabbit. In 2 hours, the blood sugar decreased from 132 mg to 66 mg %. Evidently, this hypoglycemic principle present in rabbit urine after the administration of insulin, either is insulin or a substance having identical properties.

‡ Since blood was present frequently in the urines collected after the injection of insulin (due to trauma or to a nephrotoxic action of the insulin protein), it is possible that the insulin detected in the urine was not eliminated by the kidney but represents contamination with blood containing insulin. For this reason, the amount of blood present in the urine was quantitated chemically during those times when this blood was known to contain insulin; values of 1 part of blood in 2000 parts of urine to 1 in 5000 were obtained. Obviously, the quantity of blood present in the urine could not have accounted for its content of insulin.

[1] Reiss, M., and Perény, S., *Endokrinologie*, 1928, **2**, 181.

an antagonism between the two hormones. Their observation has been confirmed later by Weichert and Boyd[2] and Van Horn.[3] The latter advanced the explanation that the increased metabolism obtained by thyroid feeding brings about a more rapid elimination of the estrogenic substance.

In all these experiments, a rather large dose of thyroid has been employed over a relatively long period, producing a considerable and sustained increase of metabolism. The present investigation was suggested by the idea that quantities of thyroid, too small to produce an appreciable increase of metabolism, might have a reversed action on the estrin effect, sensitizing the animal to estrogens. This expectation was admissible in view of the fact that thyroid at low dosage exerts an anabolic effect in contrast to the catabolic action at medication with larger quantities. This latter contention is based on the clinical observation that emaciated patients frequently gain weight when small doses of thyroid are given. Our own observation in thyroidectomized rats is also in agreement with this view. The repair of loss in weight suffered through the operation is extremely slow for a long time, but rapid gain follows medication with minute doses of thyroid. Whether or not the non-thyroxine fraction of the thyroid gland might act differently than the thyroxine fraction in this respect, was a second question to be investigated.

Female rats 80 days of age and of established estrous cycle were castrated and after allowance of a reasonable time for recovery tested for absence of estrus. For priming, a safe excess of urinary estrin was used and thereafter the minimum effective dose was determined. The total quantity of estrin given to induce estrus in a group of rats was termed a unit. Injections were given 6 times, 3 each on 2 consecutive days. Vaginal smears were taken repeatedly on the fourth and fifth day. Only perfect estrus was counted as positive response; proestrus reaction was not counted. Rats not showing full estrus after treatment with the dose that produced the effect in the majority of animals of the group (with 1 unit), were discarded. With consideration of the possibility that treatment with thyroid might have an indefinite influence over a prolonged time and complicate subsequent medication, each animal was used only for a single experiment.

The thyroid preparations were given by mouth; in the case of thyroxine, a stomach tube was used, in order to avoid any possible loss of substance. Perfect ingestion of the accurately determined quantity was secured in every instance.

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2 Weichert, C. K., and Boyd, R. W., *Anat. Rec.*, 1933, **58**, 55.

3 Van Horn, W. M., *Endocrinology*, 1933, **17**, 152.

The following preparations were used in these experiments:

1. Crystalline racemic thyroxine.

2. Dry thyroid substance of 0.46% iodine content, *i. e.*, more than twice U.S.P. strength.

3. Thyroid globulin, prepared by extraction of fresh pig's thyroid; it contained 0.565% iodine, 0.14% of which was in form of thyroxine iodine as determined by the method of Leland and Foster.[4] In consideration of a probable error of 15%, as calculated by these authors, the figure would have to be increased to about 0.165%, corresponding to 0.25% thyroxine.

4. An acid soluble fraction, prepared by alkaline hydrolysis of the previous preparation. This preparation had an iodine content of 0.26%; although supposed to contain only the diiodotyrosine fraction, its chemical assay still showed 0.0085% of thyroxine iodine or 0.01% corrected.

The approximate calorigenic effect of the thyroid preparations, as determined in preliminary metabolic experiments, was estimated to be at least 3 times that of the corresponding thyroxine content. The metabolic effect in castrate females, however, was not determined. The experiments performed are compiled in Tables I-III.

The results seem to permit the following conclusions:

Table I. Treatment with thyroid material at moderate but efficacious doses for 3 days does not interfere markedly with the action

TABLE I.

Single Dose		Times	Days of medication	Units Estrin	Days of injection	Reaction pos.	Reaction neg.	Remarks
1. Thyroxine	.5 mg	1	1	1	3 and 4	6	0	
2. "	.33 "	3	1-3	1	5 and 6	6	0	continued
Thyroid*	30 "	10	11-20	1	19 and 20	0	8	test
3. Thyroxine	.6 "	3	1-3	1.5	5 and 6	6	1	
4. Thyroglobulin†	30 "	5	1-5	1.4	7 and 8	4	2	continued
"	30 "	11	11-22	1.4	21 and 22	0	6	test

*This dose is equivalent in metabolic effect to 0.2 mg thyroxine, by estimation.
†Equivalent to 0.225 thyroxine.

TABLE II.

Single Dose	Times	Days of medication	Units Estrin	Days of injection	Reaction pos.	Reaction neg.	Remarks
Thyroglobulin	4 mg 5	1-5	0.8	7 and 8		8	continued
"	4 mg 11	11-22	0.8	21 and 22		8	test

[4] Leland, J. P., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 165.

TABLE III.

Acid soluble thyroid fraction, 100 mg, equivalent in activity to approximately 0.12 thyroxine.

Single Dose	Times	Days of medication	Units Estrin	Days of injection	Reaction pos.	Reaction neg.	Remarks
1. 250 mg	1	1	0.6	2 and 3		9	continued
200 ''	3	10-12	0.6	14 and 15		9	test
200 ''	11	21-32	1.0	29 and 30	2	7	
			1.0	39 and 40		9	
			1.50	49 and 50	9		
2. 250 ''	8	1-6, 8, 9	1	8 and 9	7	1	
After 3 weeks	without	medication	1		1	7	
'' 4 ''	''	''	1.2		6	2	
100 ''	5	1-5	1	7 and 8	5	2	continued
100 ''	11	11-22	1	21 and 22		7	test
100 ''	5	1-5	1.5	7 and 8	7		continued
100 ''	11	11-22	1.5	21 and 22	7		test

of estrin; treatment for more than 5 days increases the threshold for estrin.

Table II. Thyroid globulin at subminimal doses does not sensitize the animals to the action of estrin.

Table III. A product low in thyroxine, but high in the diiodotyrosine fraction, given at a comparatively low, but on prolonged administration efficacious, dosage does not sensitize to estrin during a few days of medication. A calorigenic effect cannot yet be supposed after this short treatment. Longer treatment causes interference. This effect continues increasing for some time after cessation of treatment. At the doses used, the estrin requirement for induction of estrus is not increased beyond 50%.

The impression is gained that the need for increased estrin after thyroid treatment is dependent on the increase in metabolism, be it that estrin is metabolized at a proportionately augmented rate or that the tissues in stimulated activity require larger quantities of estrin to respond.