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Comparative Effects of Dinitrophenol and Thyroid on Pituitary-Gonadal Complex of Female Rats.

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Van Horn[1] advanced the view that thyroid feeding in female rats, by raising the metabolic rate, increased the elimination of estrin thereby keeping the estrin level below normal and the animal in a continual state of diestrus. In this way Van Horn believed the pituitary-gonadal complex was affected. The present investigation was undertaken to test this hypothesis using some substance, other than thyroid, which would raise the metabolic rate. Accordingly, dinitrophenol was used. The appearance of the vaginal smear was used as the measure of the estrin level. In addition, the ovaries and the pituitaries were examined microscopically.

Thirty-six normal, adult females were used in this study. A preliminary period of observation for 15 days, during which daily vaginal smears were made, served as a control. Only those animals exhibiting regular 4 to 5 day cycles were chosen. Twelve rats were injected subcutaneously, twice a day, with 25 mg. of dinitrophenol per kilo of body weight. An aqueous solution of 0.5% Eastman's 2-4 dinitrophenol was used, a half gram of sodium bicarbonate being added to each gram of dinitrophenol to facilitate the solution of the latter. With this large dose the rats showed no very marked ill effects; only one rat died. Sixteen rats were divided into 2 equal groups, A and B, and fed desiccated thyroid for varying periods of time, 20 to 42 days. Group A received 0.25 gm. daily and Group B 0.5 gm. The ovaries and pituitaries of the remaining 8 rats, together with glands of 42 rats from another experiment, were studied as controls.

I. *Effect on the Estrous Cycle.* A. Dinitrophenol. (Fig. 1.) An almost constant response is the lengthening of the cycle with an increased period in diestrus. In rats D12 and D16 blood was present in the vagina and it was difficult to secure sufficient material for examination. They appeared to be in diestrus during this time. However, in not one instance did any of the rats receiving dinitrophenol remain permanently in diestrus as did some of the thyroid-fed rats.

[1] Van Horn, W. M., *Endocrinology*, 1933, **17**, 152.

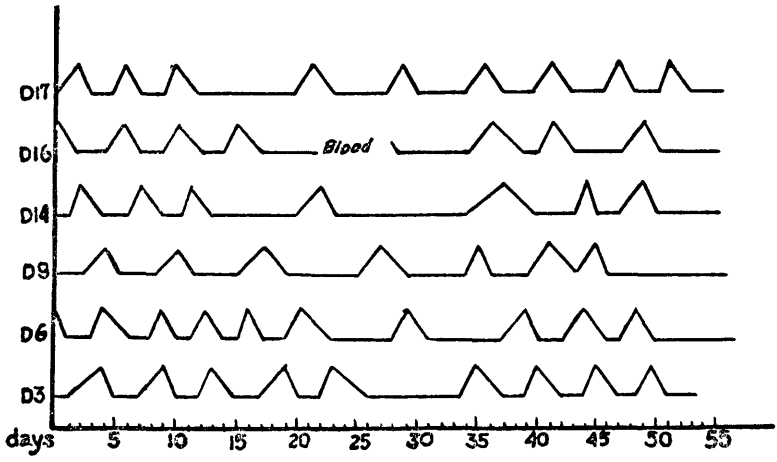

FIG. 1.

Representative estrous curves of 6 normal adult females receiving 0.25 mg. per kg. body weight of 2-4 dinitrophenol, twice daily. Injections began on the fifteenth day. The base corresponds to diestrus, and the peak to estrus (stage 2-3).

B. Thyroid. Following the commencement of thyroid feeding the rats exhibited one or two normal cycles and then went into a continuous diestrus. Rat T34 proved the exception. Although fed 0.5 gm. of thyroid daily for 42 days she maintained normal cycles throughout the course of the experiment.

II. *Effect on the Ovaries.* A. Dinitrophenol. The ovaries were weighed, fixed in Zenker's, and stained with hematoxylin and eosin. The weight and histology were essentially normal, with a tendency for some of the ovaries to have an increased amount of lutein tissue. Rats D12 and D16 received dinitrophenol for 39 days and 42 days, respectively. Blood was present in the vagina of these rats for several weeks. Their ovaries were heavier than normal. In each instance the increase in weight was due to a large hard mass which on cutting exuded a green purulent fluid. Microscopically leucocytes, red blood cells, and cellular debris were noted.

B. Thyroid. In rats receiving 0.25 gm. of thyroid and in those receiving 0.5 gm. for a short period of time the ovaries were heavier and contained numerous corpora lutea, as previously reported by Weichert and Boyd.[2] In rats fed 0.5 gm. thyroid for long periods of time there was a slight loss in weight and a decrease in the number of corpora lutea. Moreover, in this latter group several ovaries showed gross and microscopic signs of cysts. These observations are similar to those reported by Hayashi.[3]

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

3 Hayashi, H., *Bull. Acad. de Med.*, 1929, **101**, 115.

III. *Effect on the Pituitary.* A. Dinitrophenol. The glands were cut in serial sections and stained with either Martin's[4] acid fuchsin-methyl blue, or with Severinghaus'[5] modification of the Champy-Kull-Nassanov method. The pituitaries appeared normal in all respects.

B. Thyroid. Our observations are in agreement with Campbell, Wolfe, and Phelps[6] who have already described the anterior pituitaries of hyperthyroid female rats. Since we used a cytological technique to demonstrate the Golgi apparatus and mitochondria we will briefly describe these additional findings. The basophils, which were full of deeply blue stained granules, contained an hypertrophied Golgi apparatus and an increased number of mitochondria. A similar condition of the Golgi apparatus and the mitochondria was observed in many of the enlarged chromophobes. The acidophiles appeared to be diminished in number. Campbell, Wolfe, and Phelps making cell counts found this to be true.

In some respects the pituitaries of thyroid-fed rats resembled the glands of short-time estrin-injected rats (Halpern and D'Amour[7]) in that there was some hyperplasia and hypertrophy of the chromophobes with an increase in the number of mitochondria and the size of the Golgi apparatus. Inasmuch as the ovaries of the hyperthyroid and estrin-injected rats had a predominance of lutein tissue over follicular tissue it seemed quite probable that in both instances the lutein-stimulating hormone of the pituitary had been activated.

Conclusion. Although dinitrophenol apparently did raise the metabolic rate it had scarcely any effect on the pituitary-gonadal complex. The pathology in several ovaries and the slightly increased length of the estrous cycles appeared to be due to the toxicity of the drug. This strongly suggests that heightened metabolism *per se* does not lead to an increased rate of estrin elimination, and is, therefore, not sufficient explanation for the marked changes in the estrous cycles, ovaries, and pituitaries of thyroid-fed rats. While this experiment does not completely rule out the effect of increased metabolism on the pituitary-gonadal complex, it does seem to indicate that the thyroid hormone has a specific action on the pituitary and gonads as reflected by the morphological changes observed in these glands.

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[4] Martin, T., *Compt. rend. Soc. de Biol.*, 1933, **113**, 1275.

[5] Severinghaus, A. E., *Anat. Rec.*, 1932, **53**, 1.

[6] Campbell, M., Wolfe, J. M., and Phelps, D., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 205.

[7] Halpern, S. R., and D'Amour, F. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 108.