

## 11319 P

**Effect of Testosterone Propionate Upon Thyroid and Parathyroid Glands of Intact Immature Female Rat.\***

IRA T. NATHANSON, AUSTIN M. BRUES AND RULON W. RAWSON.  
(Introduced by Joseph C. Aub.)

*From the Laboratories of the Collis P. Huntington Memorial Hospital, Harvard University.*

In previous communications it was shown that testosterone propionate acts directly upon the vagina and uterus of the immature female rat.<sup>1</sup> It was also determined that it stimulates the ovary indirectly by way of the anterior hypophysis<sup>1</sup> even in the absence of the adrenals.<sup>2</sup> It is the purpose of this paper to record the effect of testosterone propionate upon the histological appearance of the thyroid and parathyroid gland of the intact immature female rat.

*Method of Experiment.* Twenty-one immature rats of the Wistar strain weighing between 40 and 48 g and approximately 4½ weeks of age, were divided into 2 groups of 12 and 9 respectively. Five mg of testosterone propionate† in 0.2 cc of sesame oil were given in one dose to each of the first group (12) and 0.2 cc of sesame oil without the hormone were given to the second group (9) to serve as controls.

*Group I (Testosterone treated group).* Twelve hours after giving testosterone, colchicine (0.05 mg per 50 g of body weight) was given subcutaneously to one animal. This animal was sacrificed 12 hours later (24 hours after testosterone). Thereafter one animal was sacrificed every 24 hours, one dose of colchicine having been given 12 hours previously.

*Group II (Controls).* Three animals each were sacrificed, 72, 144 and 288 hours respectively after the onset of the experiment. Two of each group of 3 were given colchicine 12 hours before. The remaining animals received no other treatment.

---

\* This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.

<sup>1</sup> Nathanson, I. T., Franseen, C. C., and Sweeney, A. R., Jr., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 385.

<sup>2</sup> Nathanson, I. T., and Rawson, R. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 482.

† We are very grateful for the liberal quantities of testosterone propionate which were furnished us by Doctors Gregory Stragnell and Max Gilbert of the Schering Corporation, and by Dr. Ernst Oppenheimer of Ciba Co., under the trade names of Oreton and Perandren respectively.

The thyroid and parathyroid glands of each animal were fixed in Zenker's solution imbedded in paraffin, and representative sections were stained with phloxine methylene blue.

Measurements of height of the acinar cells of the thyroid were made according to the method of Rawson and Starr,<sup>3</sup> in which 100 cells were measured in each gland. Mitoses were counted, using the technic of Brues and Marble.<sup>4</sup> At least 4000 cells were counted in each gland, and at least one entire section of each gland was examined.

*Results.* Table I presents the results of this experiment.

It can be seen from the results presented in the table that testosterone propionate is capable of stimulating the thyroid and parathyroid glands of the intact immature female rat. The increase in mitotic activity especially in the first 96 hours seems particularly significant. This corresponds very closely with changes seen in the ovary during the same period of time. Increase in mitotic activity was noted even in the first 24 hours. After 96 hours the activity decreased, but in all instances the levels remained at the upper limits, or above those of the controls. In one animal (No. 109), which showed a high degree of activity, corpora lutea were noted in the ovaries. These were not seen in the ovaries of the other animals.

Increase in cell height could be demonstrated in the thyroid gland almost as soon as the increase in mitotic activity was noted. It can be seen that in the early stages of the experiment an increase in mitosis was associated with an increase in the size of the cell. However, there was no direct correlation between the number of mitoses and cell height.

In the control group no marked difference could be detected between the animals treated with colchicine and those that had none, although the mean cell height seemed to be slightly lower in the colchicine-treated group. One control animal (No. 110) deserves comment. In this rat there was an increase in mitosis and cell height in the thyroid, as well as stimulation of the follicles of the ovaries. Autopsy revealed a cyst of the pituitary gland.

It is noteworthy that in all groups mitotic activity in the thyroid and in the parathyroid gland of each animal was approximately equal.

The mechanism of this stimulation is not clear at present. Several possibilities seem tenable.

(a) Testosterone stimulates the thyroid and parathyroid glands directly.

---

<sup>3</sup> Rawson, R. W., and Starr, P., *Arch. Int. Med.*, 1938, **61**, 726.

<sup>4</sup> Brues, A. M., and Marble, B. B., *J. Exp. Med.*, 1937, **65**, 15.

TABLE I.  
Effect of Testosterone on Mitosis and Cell Height of Thyroid Gland.

No.	Wt. onset experiment	Wt. when sacrificed	Age when sacrificed in days	Hr after testosterone	Mitoses thyroid per 10,000 cells	Mitoses para-thyroid per 10,000 cells	Mean acinar cell height per 100 acini	Remarks
					Treated Group			
114	48	49	32	24	46	28	4.24 ± 0.004	Colchicine
103	46.5	48.5	33	48	41	—	3.82 ± 0.004	"
115	46	47	34	72	22	33	4.22 ± 0.004	"
105	45	57	35	96	118	77	4.19 ± 0.005	"
111	44	59.5	36	120	12	—	4.37 ± 0.004	"
101	43.5	63.5	37	144	12	10	4.15 ± 0.004	"
102	43.5	61	38	168	11	22	3.06 ± 0.004	"
109	43.5	69	40	216	75	—	4.29 ± 0.004	" corpora lutea
104	42	69	41	240	19	—	4.20 ± 0.004	"
107	42	85	42	264	18	15	4.05 ± 0.004	"
113	43	88	43	288	19	22	4.34 ± 0.004	"
					Control Group.			
116	40	52	35	—	8	11	3.85 ± 0.005	No colchicine
117	45	53	35	—	2	—	3.61 ± 0.004	Colchicine
110	46.5	54	34	—	36	—	4.17 ± 0.005	" cyst pituitary
108	45.5	68	38	—	6	—	3.58 ± 0.004	Colchicine
118	42	67	39	—	7	—	3.64 ± 0.004	"
119	41	66	39	—	2	9	3.74 ± 0.004	No colchicine
112	42	72	43	—	7	8	3.66 ± 0.004	Colchicine
120	40	82	44	—	6	4	4.03 ± 0.005	"
121	42	85	44	—	4	10	3.74 ± 0.004	No colchicine

(b) Testosterone stimulates the thyroid and parathyroid glands either by way of the anterior pituitary or through some other gland of internal secretion.

The second thesis seems more plausible, especially since the changes noted so closely parallel those in the ovary which we have shown are stimulated by way of the hypophysis. Experiments are under way at present to clarify these points.

*Conclusion.* Testosterone propionate is capable of stimulating the thyroid and parathyroid glands of the intact immature female rat. This stimulation is manifested by increased mitotic activity and by histological evidence of functional activity.

## 11320 P

### The Mechanism of Action of Heparin\*

MORRIS ZIFF AND ERWIN CHARGAFF.

*From the Departments of Biochemistry and Surgery, College of Physicians and Surgeons, Columbia University, New York.*

It is known that heparin does not prevent the coagulation of a fibrinogen solution by purified thrombin.<sup>1</sup> It likewise is unable to prevent the conversion of isolated prothrombin to thrombin.<sup>2, 3</sup> To exert its powerful anticoagulant action, heparin requires an additional factor which is known to be present in blood, plasma, and serum.<sup>1, 4, 5</sup> This factor has been shown by Quick<sup>4</sup> to be contained in the serum albumin fraction.

We have investigated the activity of various components of the albumin fraction in producing the anticoagulant effect of heparin on the fibrinogen-thrombin system.

The *thrombin* was obtained by the method of Eagle,<sup>6</sup> and the fibrinogen was prepared in the usual manner by repeated precipita-

---

\* This work has been supported by a grant from the John and Mary R. Markle Foundation.

<sup>1</sup> Howell, W. H., and Holt, E., *Am. J. Physiol.*, 1918-1919, **47**, 328.

<sup>2</sup> Mellanby, J., *Proc. Roy. Soc. (London)*, 1934, B **116**, 1.

<sup>3</sup> Quick, A. J., *Proc. Soc. Exp. Biol. and Med.*, 1936, **35**, 391.

<sup>4</sup> Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.

<sup>5</sup> Brinkhous, K. M., Smith, H. P., Warner, E. D., and Seegers, W. H., *Am. J. Physiol.*, 1939, **125**, 683.

<sup>6</sup> Eagle, H., *J. Gen. Physiol.*, 1935, **18**, 531.