for several weeks is certainly open to question.

Summary. Rats maintained in a normal environment showed a decreased thyroidal I^{131} uptake after 7 hours on a nitrate supplemented diet but were able to overcome this effect after two weeks. Initially the nitrate flushed I^{131} from the thyroid gland; however, this effect was overcome in one week. After 5 weeks, in the absence of tapazole, the nitrate-fed rats fixed more I^{131} as PBI^{131} than did the controls.

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Received July 2, 1962. P.S.E.B.M., 1962, v111.

Effect of Thyroxine on Maturation of the Testes and Prostate Gland of the Rat. (27770)

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Injection of thyroxine beginning at birth has been shown to accelerate such maturational processes as opening of the eyelids, hair growth, epiphyseal plate closure and tooth eruption in rats(1,2,3). Attempts to advance differentiation of the gametogenic cells of the testis or to induce earlier and more rapid growth of the male accessory reproductive glands by thyroid hormone administration have uniformly failed(4,5,6). In these latter studies, however, hormone administration was not begun until the animals were 2 to 3 weeks of age. Since spermatogenesis is well under way at this time(7,8) it seemed possible that the thyroid hormone had been administered at too late an age to significantly accelerate this process. For this reason in the present study thyroxine was administered from birth at which time the earliest Type A spermatogonia are not yet recognizable(8).

Methods and materials. Forty-seven rats from Wistar stock were raised in a room maintained at 76°F and 50% relative humidity. All animals used in this study were obtained from litters which had been reduced to 7 at birth and contained at least 3 males. Day-old males from each litter were divided into 3 groups. The animals in 2 of these groups were injected subcutaneously daily from birth to 36 days of age with DL thyroxine* in alkaline saline. The control group received alkaline saline only.

The thyroxine solutions were prepared at 2 concentrations: 6 μg/0.05 ml ("A" dose) and 2 μg/0.05 ml ("B" dose). Fresh solutions were prepared at 7-day intervals. The daily dose given to the animals in each experimental group was maintained at 6 μg and 2 μg until the rats were 10 days of age. At 11, 16, 21 and 28 days of age the 2 doses were increased so that the "A" dose was maintained at about 9 times the normal physiological level and the "B" dose at 3 times this level. Six micrograms per 100 g of body weight was considered normal.

Necropsy was performed on the day after the last injection. The testes and ventral prostate gland were weighed and the testes

* Thyroxine Crystals Squibb, E. R. Squibb and Sons, New York.

TABLE I. Effect of Thyroxine on Testes and Ventral Prostate Gland of Immature Rats.

Treatment	No. of animals	Body wt (g)	Testis wt (mg)	Tubule diam. (μ)	No. tubules with late-stage† spermatids	Prostate wt (mg)
"A" dose thyroxine	13	82 ± 3*	617 ± 53	178 ± 4‡	57 ± 6‡	29 ± 2‡
"B" dose "	15	93 ± 4	712 ± 48	178 ± 3‡	46 ± 6§	37 ± 3‡
Control (saline)	19	84 ± 5	899 ± 37	157 ± 4	21 ± 2	51 ± 3

* Stand. error of mean. † Spermatids have reached or passed Step 11 of spermiogenesis.
‡ p of difference from control group .01. § p of difference from control group .05.

fixed in 10% neutral formalin. Five micra transverse sections were stained with hematoxylin and eosin.

The degree of maturation of the seminiferous epithelium of the testes was determined by 2 methods: (a) Counting the number of sections of tubules which had reached or passed Step 11 of spermiogenesis, as defined by Leblond and Clermont(9), out of 150 such sections in one testis of each rat. This step and subsequent steps can be readily identified with hematoxylin and eosin staining by the presence of spermatids with clearly elongated nuclei(10). (b) Measuring the shortest diameter of 30 randomly selected transverse sections of seminiferous tubules in one testis of each rat.

Results. Seminiferous tubules of the animals receiving thyroxine were more mature than those found in the testes of the control animals (Table I). This was shown by the increased number of tubules which had reached or passed Step 11 of spermiogenesis. All the testes contained at least a few tubules which had reached Step 11 whereas none of the tubules in either the control or experimental groups contained spermatids with a clearly defined tail (Steps 16 to 19 of spermiogenesis). Further evidence of increase in rate of maturation of the seminiferous epithelium is shown by a highly significant greater diameter of the tubules in the testes of the thyroxine treated rats. The Leydig cells were not detectably different in quality or quantity in control and experimental animals.

Gross weights of testes and ventral prostate glands were significantly smaller in thyroxine treated animals. The smaller size of these organs was not related to a general reduction in rate of body growth since the body weight gain of the three groups of animals was comparable.

Both the "A" and "B" doses of thyroxine caused marked atrophy of the follicular epithelium and accumulation of colloid in the thyroid gland.

Discussion. It is not possible to determine from the data obtained in this study the exact extent of acceleration of maturation which was brought about by administration of thyroxine, but based upon the data of Clermont and Perey(8) it may be estimated that the amount of acceleration did not exceed 3 days.

Acceleration of gametogenesis in the testes of thyroxine treated rats was accompanied by an increase in the diameter of the seminiferous tubules. This increase occurred even though the weight of the testes was less in these hormone treated animals. Since the number of seminiferous tubules has been shown to be established prior to birth(9) and there is no apparent change in quantity of intertubular tissue one is led tentatively to conclude that the tubules did not increase in length as rapidly in thyroxine treated animals as in controls.

The smaller size of the testes and the ventral prostate gland shows that thyroid hormone does not accelerate all aspects of sexual maturation. The reason for the decreased rate of growth of these organs cannot be definitely determined from this study. However, since the Leydig cells did not appear to have been adversely affected by the thyroxine this study suggests that the decreased size of the ventral prostate is not due to a reduced secretion of androgen. Previous work by Smelser (6) has placed emphasis for this effect on a decreased responsiveness of the testes to gonadotrophin and the prostate gland to androgen in animals treated with thyroid hormone. Cohen(5), on the other hand, presented evidence that decreased growth of the accessory glands was due at least in part to

a lower level of circulating androgen in hyperthyroid rats.

Summary. Maintenance of elevated levels of thyroxine in rats from birth to 36 days of age accelerates the maturation of the seminiferous epithelium of the testis of the rat but at the same time reduces the rate of increase in weight of the testes and the ventral prostate gland.

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Received July 9, 1962. P.S.E.B.M., 1962, v111.

Some Properties of the Platelet-Connective Tissue Mixed Agglutination Reaction. (27771)

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Among the earliest events in hemostasis is adherence of platelets to the site of vascular injury. It has been suggested that the effective surface is damaged cells(1), or connective tissue fibers(2-4). Zucker and Borrelli (5) have shown that suspensions of connective tissue fragments from many sources and purified collagen agglutinate platelets of plasma anticoagulated with citrate. Platelets carry a strong electronegative charge(6), and the present study explores the possibility that platelet-connective tissue mixed agglutination represents reaction with a particle possibly carrying an opposite charge.

Materials and methods. Biological reagents were of human origin. Subcutaneous fat, found to be a convenient source of connective tissue(5), was obtained during surgery from the anterior abdominal wall,* and was kept frozen until ready for use. Preparation of suspended connective tissue fragments was modified from the method of Zucker and Borrelli(5). The fat was cut into small pieces, and homogenized for about 5 minutes in a Waring Blendor with about 5 volumes of isotonic saline. The resulting material was centrifuged for 30 minutes at about 3,000 rpm in an International Clinical Centrifuge, and the middle turbid layer was used. Unless otherwise noted, it was diluted to twice the lowest effective concentration for maximal agglutination. Blood was collected into siliconized containers and anticoagulated with one-tenth volume of 3.8% sodium citrate. Platelet-rich plasma was collected following centrifugation at about 600 rpm for 15 minutes. Washed platelets(7) were trypsinized as previously described for red cells(8) with crystalline trypsin (Armour) except that the trypsin solution was diluted to 250 NF units/ml. These trypsinized platelets still gave clot retraction when one-tenth volume containing about 500,000 platelets/ml[3] was added to platelet-poor recalcified plasma. Polybrene (hexadimethrine bromide polymers) with average molecular weights (MW) of 6,000 and 2,800 respectively were supplied by Abbott Laboratories.

Agglutination of platelets was estimated semiquantitatively in a Beckman DB spectrophotometer at wavelength 610. A mixture was made containing 1 cc each of platelet-rich plasma, the agglutinating reagent, and test inhibitor. A reading was taken immediately, against platelet-poor plasma as the reference

*Kindly provided by Dr. Arthur Aufses.