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THE EFFECT OF TESTOSTERONE DURING THE NEONATAL PERIOD ON THE THYROID GLAND OF MALE AND FEMALE RATS

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The essential features of the syndrome produced by androgenic hormone administration during the neonatal period in female rats—precocious vaginal opening, persistent vaginal cornification and failure of spontaneous ovulation which can, however, be produced by the administration of exogenous luteinizing hormone, were all described by Pfeiffer (1936). In his work, male hormone was received as a consequence of the grafting of testicular tissue. Later workers (see Takewaki, 1962 for references) have generally employed a single injection of an androgen, usually testosterone (17β-hydroxyandrost-4-en-3-one) to produce this syndrome experimentally at will. The normal adult female rat that is undergoing regular oestrus cycles shows a cyclic variation in the level of thyroid-gland activity in phase with the ovarian cycle, activity being greatest during the oestrus stage (Brown-Grant, 1962). The changes in the thyroid may be related to events in the hypothalamus and pituitary leading to a discharge of luteinizing hormone and hence ovulation (Brown-Grant, 1963*a*). The absence of ovulation in the androgen-treated female rat may be due to a failure of the hypothalamic mechanism involved in the cyclic release of LH (Barraclough, 1963) and a study of possible alterations in the level of the thyroid-gland activity in such animals is reported here. Some of the preliminary findings have been referred to in a short note published previously (Brown-Grant, 1963*a*).

Animals exhibiting the syndrome have been referred to by different workers as constant-oestrus rats, androgen-sterilized rats or 'anovulatory-persistent-oestrus' rats, but as these animals do not show normal female mating behaviour and the cornification of the vaginal smear is not constant, and ovulation can be produced by electrical stimulation of the brain, none of these names are satisfactory. The term testosterone propionate treated rats (TP rats) will be used here.

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MATERIAL AND METHODS

The animals were Wistar rats from a closed colony maintained under the same conditions as described previously (Brown-Grant, 1962), except that the pelletted diet fed *ad lib.* was diet 41*B* obtained from E. Dixon & Sons, Ltd., Ware. Testosterone propionate (TP), B.P. (1·25 mg in 0·05 ml.) was injected subcutaneously on Day 4 of post-natal life (Day 0 is the day of birth). In early experiments, half the control animals were injected with 0·05 ml. of oil at the same time, but no effect of oil injection on thyroid function was detected and in later experiments control animals were not injected. Some leakage of oil occurred from the site of skin puncture in some of these young animals though the main volume of oil deposited 2–3 cm from the point of skin puncture remained. In order to avoid any possible contamination of control animals, two litters born on the same day were each divided into control and injected animals and all injected animals were pooled and returned to one mother and all controls to the other.

Thyroid function was investigated by measurement of the 2½-hr uptake of ^{131}I by the gland, the thyroid-serum (T/S) concentration ratio for radio-iodide and the rate of release of ^{131}I from the gland *in vivo* by methods previously described (Brown-Grant, 1962). The only variation was that measurements of gland-^{131}I content *in vivo* were made daily in the afternoon with a scintillation counter instead of a Geiger–Muller tube and the percentage release of ^{131}I was calculated daily for each animal and the average of these daily values used for comparisons between groups of animals or of the rate of release before and after various treatments in the same group of animals.

Various organs were removed, trimmed and weighed to 0·1 mg when the animals were killed and organ weights were expressed as mg/100 g body weight. Tissues were fixed in formol-saline, embedded in paraffin, sectioned and stained with haematoxylin-eosin for microscopic examination. Values quoted in text are group means ± S.E. of the mean. Statistical comparisons were made by means of the *t* test or by analysis of variance using Snedecor's *F* ratio. Not significant (N.S.) indicates a value for *P* of $> 0{\cdot}05$.

RESULTS

Organ weights and ^{131}I data in control and TP-treated female rats

Control and TP-treated females were killed at various ages before and after puberty (spontaneous rupture of the vaginal membrane). Daily vaginal smears were obtained from normal adult animals for at least 14 days before the animals were killed at the oestrus or the dioestrus stage of the cycle after it had been established that they were showing regular vaginal cycles. All TP-treated animals had shown a cornified vaginal smear for at least 6 days before they were killed. The ovaries were examined macroscopically; all the post-pubertal TP-treated rats had the typical small pale ovary with large follicles but no corpora lutea. Organ weights are shown in Table 1. It is of interest that differences in ovarian weight are already established before puberty. No differences in adrenal weight were seen in these experiments. Submandibular gland weights were also measured (this gland is relatively heavier in male than in female rats and shows a marked sexual dimorphism in structure; see Brown-Grant, 1963*b*, for references). No consistent differences in weight, absolute or relative, be-

TABLE 1. Body and organ weights in control and TP-treated female rats of different ages and the thyroidal ^{131}I uptake (percentage injected dose) and T/S ratio for ^{131}I. In this and subsequent tables organ weights are given in mg/100 g body weight. Values given are mean ± S.E. of mean. Italic type indicates that the control value is significantly ($P < 0.05$) different from the value for TP-treated rats and bold type that the difference is highly significant ($P < 0.01$)

Age (days)	Status	No.	Body weight	Thyroid	Adrenal	Ovary	Uterus	% uptake (thyroid)	T/S ratio
26	Control	9	46 ± 2	11·7 ± 0·2	29·0 ± 1·3	**34·4 ± 1·3**	*56·4 ± 3·5*	4·99 ± 0·28	—
	TP treated	9	46 ± 3	12·2 ± 0·6	30·5 ± 1·1	21·2 ± 1·9	43·4 ± 3·3	5·13 ± 0·41	—
31	Control	6	62 ± 3	11·2 ± 0·5	32·8 ± 2·1	**29·7 ± 1·3**	55·4 ± 4·0	6·37 ± 0·57	—
	TP treated	7	65 ± 3	10·8 ± 0·5	31·0 ± 1·3	21·7 ± 1·9	77·6 ± 13·1	5·70 ± 0·46	—
120	Control	16	168 ± 2	8·6 ± 1·2	33·5 ± 1·2	**42·4 ± 2·1**	**231 ± 12**	8·87 ± 0·73	—
	TP treated	15	183 ± 4	8·0 ± 0·4	33·0 ± 0·7	15·4 ± 0·9	151 ± 7	6·36 ± 0·67	—
	Oestrus controls	9	—	—	—	—	—	**10·15 ± 0·83**	—
	Dioestrus controls	7	—	—	—	—	—	7·25 ± 1·10	—
130	Control	13	194 ± 4	7·2 ± 0·5	27·3 ± 0·7	**37·4 ± 1·7**	187 ± 10	—	78·4 ± 6·7
	TP treated	14	201 ± 7	7·4 ± 0·6	26·5 ± 0·9	16·7 ± 0·9	178 ± 9	—	63·3 ± 4·2
	Oestrus controls	8	—	—	—	—	—	—	**89·3 ± 8·2**
	Dioestrus controls	5	—	—	—	—	—	—	61·0 ± 5·8

TP, testosterone propionate.

tween control and TP-treated rats were observed and the glands could not be reliably assigned to one group or the other by microscopic examination.

The uptake of ^{131}I by the thyroid glands did not differ significantly before puberty. In adult animals, uptakes were lower in treated rats than in groups of control animals with a majority of animals killed during the oestrus stage of the cycle when uptake is highest. Values for TP-treated animals did not differ significantly from those obtained for animals killed in dioestrus but were much lower than the values from oestrus animals. The thyroid-serum ratio for ^{131}I in TP-treated rats was between the values obtained from animals in oestrus and those in dioestrus and was lower than, but does not differ significantly from, the mean value for control animals. The rate of release of ^{131}I (percentage gland content/day) was measured for 5 days in seven control and nine TP-treated rats giving respectively thirty-five and forty-five separate estimates of the release rate. The mean rate of release for treated rats was $8{\cdot}1 \pm 1{\cdot}0\%$/day which was not significantly different from the value $10{\cdot}4 \pm 1{\cdot}3\%$ for the control animals. When the values for the control animals were separated into those obtained during pro-oestrus and oestrus and those obtained during metoestrus and dioestrus, values of $14{\cdot}5 \pm 0{\cdot}8$ (mean of 18) and $8{\cdot}6 \pm 1{\cdot}1$ (mean of 17 measurements) were obtained. The release rate of the TP-treated rats was significantly lower ($P < 0{\cdot}001$) than the pro-oestrus plus oestrus values,but did not differ significantly from the metoestrus plus dioestrus value.

Organ weights and ^{131}I data in control and TP-treated male rats

Similar studies were carried out on control and TP-treated male rats at different ages and the results are given in Table 2. Growth of the testis appeared to be significantly retarded in the TP-treated rats as compared to controls and the level of androgenic stimulation was less, as judged by the weights of the seminal vesicles. The effects were marginal, however, and the differences were not seen in older animals. No significant difference in the absolute or relative weights of the submandibular gland were observed nor could glands from the two types of animals be distinguished by microscopical examination. The values for ^{131}I uptake showed no significant differences at any stage. The rate of release of ^{131}I from the gland was measured for 5 days in eight control and eight TP-treated male rats. Values (mean of 40) were $12{\cdot}0 \pm 1{\cdot}3$ for controls and $8{\cdot}8 \pm 1{\cdot}0$ for treated rats. The difference was just significant ($P = 0{\cdot}05$). The T/S ratio for five TP-treated rats was $46{\cdot}9 \pm 5{\cdot}0$ and for five control rats $49{\cdot}2 \pm 6{\cdot}2$ at 110 days of age.

TABLE 2. Organ weights and ^{131}I data from control and TP-treated male rats at different ages

Age (days)	Status	No.	Body weight (g)	Thyroid	Adrenal	Seminal vesicles	Testis	% uptake (thyroid)
25	Control	9	45 ± 2	11·9 ± 0·5	34·3 ± 1·4	21·5 ± 1·4	538 ± 12	3·95 ± 0·14
	TP treated	8	43 ± 1	12·4 ± 0·5	35·4 ± 1·7	22·2 ± 1·5	553 ± 14	3·69 ± 0·23
36	Control	5	**61** ± 5	12·3 ± 0·7	*34·0 ± 1·9*	18·8 ± 1·1	*595 ± 38*	3·27 ± 0·30
	TP treated	8	64 ± 2	10·0 ± 0·3	28·5 ± 0·7	19·7 ± 1·6	376 ± 60	3·16 ± 0·28
50	Control	9	142 ± 6	7·4 ± 0·2	18·7 ± 0·5	*44·4 ± 2·2*	**1372 ± 18**	5·86 ± 0·27
	TP treated	8	142 ± 9	7·3 ± 0·3	23·5 ± 2·2	32·3 ± 1·2	1127 ± 32	6·58 ± 0·56
68	Control	4	174 ± 9	7·2 ± 0·3	16·8 ± 0·5	*97·5 ± 10·0*	*1312 ± 46*	3·05 ± 0·24
	TP treated	7	164 ± 9	7·2 ± 0·2	19·3 ± 1·6	56·6 ± 7·7	972 ± 93	3·30 ± 0·24
110	Control	11	283 ± 6	5·2 ± 0·2	12·1 ± 0·2	161·0 ± 11·0	**1029 ± 27**	4·12 ± 0·19
	TP treated	11	299 ± 11	4·9 ± 0·2	13·4 ± 0·4	157·2 ± 7·5	867 ± 39	4·04 ± 0·24
170	Control	8	308 ± 7	6·2 ± 0·4	—	**163·1 ± 7·7**	864 ± 43	—
	TP treated	8	308 ± 11	5·8 ± 0·2	—	119·2 ± 10·6	822 ± 48	—
220	Control	8	**371** ± 13	5·4 ± 0·3	10·6 ± 0·4	141·8 ± 10·1	866 ± 27	5·78 ± 6·46
	TP treated	6	346 ± 10	5·7 ± 0·2	12·0 ± 0·4	130·7 ± 8·5	897 ± 43	5·70 ± 0·22

Effect of progesterone administration on thyroid function in TP-treated rats

The effect of progesterone (pregn-4-ene-3:20-dione, 2 mg in 0·2 ml. of oil injected subcutaneously) on the uptake of ^{131}I by the thyroid gland of TP-treated female rats was determined. All animals used had shown a fully cornified vaginal smear for at least six days before they were either injected with ^{131}I and killed as controls or injected with progesterone and killed 2–7 days later. In early experiments the effect of the injection of 0·2 ml. of oil on thyroidal ^{131}I uptake was also examined but no effect was observed and in later experiments control animals were not injected. The changes in the vaginal smear following progesterone were followed. About half the rats showed a dioestrus smear 24 hr after injection but the rest showed a very typical pro-oestrus smear composed of masses of nucleated epithelial cells, with no cornified cells or leucocytes. All injected animals had dioestrus smears at 48 hr which persisted for 2 or sometimes 3 days, followed by a day when the vaginal smear was typical of pro-oestrus and then a return to a persistently cornified vaginal smear in most animals. A few rats failed to show a typical pro-oestrus smear and returned directly to a continuously cornified smear. Values obtained on the first day of cornified smear for such animals were recorded as relating to Day 1 of oestrus. The results obtained from sixty-one rats are given in Table 3, where the animals are classified according to the type of smear on the day they were killed. Analysis of variance showed that there was no significant variation between experiments, but that the variation between stages is highly significant ($P < 0·001$). The uptake was highest as the rats again entered a period of continuous vaginal cornification. The data were also analysed with the animals grouped according to the number of days elapsed since the injection of progesterone. Analysis of variance showed a highly significant variation between days following the injection ($P < 0·001$). Values obtained on different days (mean $\pm$ S.E. of the mean) were (with the numbers of rats in brackets): controls (Day 0) 5·53 $\pm$ 0·30 (23); Day 2, 5·09 $\pm$ 0·41 (3); Day 3, 5·48 $\pm$ 0·38 (5); Day 4, 6·29 $\pm$ 0·48 (10); Day 5, 7·95 $\pm$ 0·80 (9); Day 6, 9·34 $\pm$ 1·11 (6); Day 7, 9·22 $\pm$ 1·57 (5). Thyroid gland weights, absolute and relative, were obtained for these sixty-one rats. No variations in weight with regard to the type of vaginal smear or days elapsing since progesterone injection were detected.

The effect of progesterone on the T/S ratio was determined in an experiment in which the values obtained in seven animals on the second day that they showed an oestrus smear after passing through a phase of vaginal dioestrus following the injection of 2 mg of progesterone were compared

TABLE 3. The uptake of ^{131}I by the thyroid gland of control TP-treated female rats showing persistent vaginal cornification and of TP-treated rats injected with 2 mg of progesterone. Figures in brackets after the thyroid values indicate the number of days elapsing after the injection of progesterone before the animals were killed. The animals are grouped according to the type of vaginal smear on the day ^{131}I uptake was measured

Experiment no.	Constant cornification	Dioestrus			Pro-oestrus	Oestrus			
		Day 1	Day 2	Day 3		Day 1	Day 2	Day 3	Day 4
1	4·58	—	—	—	8·35 (4)	7·01 (6)	—	—	—
	6·02	—	—	—	—	8·47 (6)	—	—	—
	4·55	—	—	—	—	—	—	—	—
	5·44	—	—	—	—	—	—	—	—
	6·33	—	—	—	—	—	—	—	—
2	6·91	—	5·84 (3)	—	4·47 (4)	12·15 (5)	—	—	—
	6·51	—	—	—	4·36 (4)	4·44 (4)	—	—	—
	7·27	—	—	—	—	8·92 (5)	—	—	—
						10·92 (5)	—	—	—
						6·65 (4)	—	—	—
3	3·39	—	5·15 (3)	—	—	9·81 (4)	—	—	—
	4·48	—	—	—	—	7·66 (5)	—	—	—
	6·65	—	—	—	—	—	—	—	—
	4·59	—	—	—	—	—	—	—	—
4	4·36	—	—	—	7·10 (4)	—	5·14 (5)	—	—
	4·85	—	—	—	4·94 (5)	—	—	—	—
	3·94	—	—	—	—	—	—	—	—
	4·50	—	—	—	—	—	—	—	—
	4·48	—	—	—	—	—	—	—	—
5	7·56	4·86 (2)	4·76 (3)	5·66 (4)	—	7·92 (5)	12·84 (6)	—	5·93 (7)
	5·20	—	—	5·39 (4)	—	—	11·36 (7)	—	—
	4·58	—	—	—	—	—	11·87 (6)	—	—
							6·79 (5)	—	—
6	7·46	5·90 (2)	—	6·60 (4)	5·60 (3)	7·18 (5)	—	10·63 (7)	13·09 (7)
	9·15	4·52 (2)	—	—	6·06 (3)	9·90 (6)	—	—	5·10 (7)
	4·49	—	—	—	—	—	—	—	5·94 (6)
Total no.	23		9		7	12	6		4
Mean ± S.E.M.	5·53 ± 0·30		5·41 ± 0·22		5·84 ± 0·55	8·42 ± 0·60	9·77 ± 1·26		7·51 ± 1·87

with values from six control animals showing constant vaginal cornification. The T/S ratio for the controls was 40·3 ± 8·7 and for rats on Day 2 of oestrus 71·1 ± 8·2 ($P < 0.05$). The effect on the rate of release of ^{131}I from the thyroid was studied in eight TP-treated rats. The percentage release/day was determined for five days and progesterone was then injected. Dioestrus smears were obtained for two days in five rats and three days in three rats. Five rats showed a pro-oestrus followed by an oestrus smear and three returned directly to a vaginal oestrus smear; values for pro-oestrus and Day 1 of oestrus were therefore combined. Values for Day 2 and for Days 3, 4 and 5 combined of vaginal oestrus, were also obtained. Results (mean ± S.E.M., number of determinations in brackets) were: control period, 9·9 ± 0·7 (40); Day 1 of dioestrus, 6·7 ± 1·0 (8); Days 2 and 3 of dioestrus, 4·3 ± 1·4 (13); pro-oestrus and Day 1 of oestrus, 13·4 ± 1·0 (13); Day 2 of oestrus, 9·2 ± 1·1 (8); Days 3,4 and 5 of oestrus, 3·9 ± 0·6 (24). The mean rate of release for Days 1, 2 and 3 of dioestrus combined (5·3 ± 0·9 (21)) was significantly different from the control rate ($P < 0.01$) as was the mean rate for pro-oestrus plus Day 1 of oestrus ($P < 0.01$). Day 2 values did not differ significantly but the mean rate for Days 3, 4 and 5 of oestrus was significantly lower ($P < 0.01$). The result of progesterone administration in TP-treated female rats appeared to be a transient depression of thyroid activity during the period of vaginal dioestrus with a marked increase at and about the time of return to continuous vaginal cornification. A possible parallel between this sequence and the changes during ovulation in the normal oestrus cycle will be discussed later. Ovulation did *not* occur in any of the TP-treated rats injected with progesterone; all these animals and also the control animals in this experiment had the characteristic small pale ovaries with large follicles and no corpora lutea when they were killed.

The possibility that the thyroid changes seen in TP-treated females injected with progesterone were not peculiar to this type of animal was examined. Normal and TP-treated adult male rats were injected with 2 mg of progesterone and the uptake of ^{131}I by the thyroid determined 1–7 days later in a series of experiments that are summarized in Table 4. Analysis of variance indicated no significant variation between experiments or between animals killed on different days after progesterone injection.

Effect of induced ovulation on thyroid function in TP-treated female rats

Ovulation was induced by injecting 15 I.U. of human chorionic gonadotrophin B.P. HCG (obtained from Organon Laboratories Ltd.) in 0·15 ml. of water subcutaneously between 11 a.m. and noon. Ovulation was confirmed by the recovery of tubal ova in animals killed 20–24 hr later or by microscopic identification of corpora lutea in the ovaries of rats killed

TABLE 4. The effect of 2 mg progesterone on 2½ hr ^{131}I uptake (percentage injected dose) by the thyroid gland of TP-treated and normal male rats at different times after injection

		Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
TP treated	Mean	6·00	7·01	6·98	6·54	6·97	6·65	6·45	5·52
	S.E.M.	0·20	0·60	0·29	0·62	0·39	0·46	0·45	0·45
	Number	7	7	7	7	7	7	7	8
Normal	Mean	4·30	—	4·74	3·73	4·22	4·18	4·07	4·24
	S.E.M.	0·21	—	0·74	0·39	0·42	0·48	0·48	0·36
	Number	7	—	7	7	7	7	7	7
Normal	Mean	5·46	5·54	—	—	—	—	—	—
	S.E.M.	0·39	0·46	—	—	—	—	—	—
	Number	14	14	—	—	—	—	—	—

TABLE 5. Changes in TP-treated female rats in which ovulation was induced by the injection of 15 I.U. of human chorionic gonadotrophin 20–24 hr before the animals were killed

Status	No.	Ovary	^{131}I uptake	T/S ratio
Control	7	14·9 ± 1·6	6·50 ± 0·52	—
Ovulated	7	**35·1 ± 1·8**	5·52 ± 0·36	—
Control	6	14·6 ± 1·4	—	40·3 ± 8·7
Ovulated	6	**25·5 ± 3·0**	—	**70·9 ± 5·3**
Control	6	15·6 ± 1·3	—	79·4 ± 8·6
Ovulated	6	**32·8 ± 3·4**	—	104·7 ± 12·1

some days after HCG injection. Vaginal-smear changes were not a reliable indication of whether ovulation had occurred or not the day after HCG injection. The commonest finding was a very typical pro-oestrus smear, but oestrus and metoestrus smears were also noted. Thyroid uptakes and T/S ratios were measured on the morning after HCG administration. The results obtained are shown in Table 5. There was a significant increase in ovarian weight following HCG injection. Ovulation induced in this way has no effect on the uptake of ^{131}I; the T/S ratio was significantly raised in one experiment but not the other. The effect on the rate of release from the thyroid was investigated in eight TP-treated females. Control rates were measured for four days before the injection of HCG and for seven days thereafter. The changes in vaginal smears were not uniform but all rats had dioestrus smears on at least four of the seven days after HCG injection. The ovaries of all animals contained corpora lutea when they were killed and mean ovarian weight (26·6 ± 1·2 mg/100 g) was much higher than is usual in adult TP-treated rats (Table 1). Release rates were 10·4 ± 0·6 %/day (mean of 32) during the control period and over the next seven days were 11·8 ± 1·8, 3·8 ± 0·8, 10·7 ± 1·9, 9·0 ± 0·6, 7·4 ± 1·0, 5·5 ± 1·4 and 7·5 ± 1·9. There was no significant increase in release rate following HCG injection, but rates on Days 2, 5 and 6 were significantly lower than control value ($P < 0·001$, $< 0·05$, $< 0·01$ respectively) and the mean of all values after ovulation (8·0 ± 0·6) was significantly lower than in the control period ($P < 0·02$).

Ovulation induced by injection of 15 I.U. of HCG did not result in an immediate increase in thyroid activity in TP-treated rats and the rate of release of ^{131}I from the gland over the seven days after ovulation was reduced.

DISCUSSION

The TP-treated rats described in this paper had all received 1·25 mg of testosterone propionate on the fourth day of post-natal life. This is a large dose by current standards; as little as 5 μg (Swanson & van der Werff ten Bosch, 1964) or 10 μg (Gorski & Barraclough, 1963) may be sufficient to produce the typical syndrome in adult life. These workers have also reported various differences between rats treated with small and large (about 1 mg) doses of testosterone. The results obtained in the present study may be of limited value as they may not be relevant to animals receiving lower doses of steroid. The high dose, however, ensured that all animals showed the characteristic syndrome; no single TP-treated female rat had an ovarian weight in the normal range or had corpora lutea in the ovaries in this study except after the injection of HCG.

The differences in organ weights shown in Tables 1 and 2 are in general

agreement with results of other workers and do not require further discussion. Thyroid weights did not differ in control and TP-treated male or female rats and the values for ^{131}I uptake, T/S ratio and ^{131}I release rates were not significantly different in male rats. These indices of thyroid gland activity were generally somewhat lower in TP-treated females than in normal females and consistently below the levels observed during the oestrus stage of the cycle in normal females. The fluctuations observed in these indices during the oestrus cycle in normal rats (Brown-Grant, 1962) make it difficult to assess the true mean value from measurements made over a few hours such as the ^{131}I uptake or T/S ratio and the deliberate choice of a majority of control animals in oestrus will also have tended to raise the mean values for the controls. Probably the best estimate of the average level throughout the cycle obtained in the present work was from the data on the rate of release of ^{131}I from the gland which included values from all stages of the cycle. The rates in TP-treated and normal females were not significantly different. There was no indication from the results obtained that the mean level of thyroid gland activity is altered in TP-treated female rats. More direct methods are available to study this question, such as the determination of the amount of thyroxine necessary to prevent goitre formation or to suppress the release of ^{131}I from the thyroid or the measurement of the rate of thyroxine degradation as described recently by Gregerman (1963), and their application would provide valuable confirmation of this conclusion. The methods chosen for this study did, however, enable an estimate to be made of the activity at different stages of the oestrus cycle and it was found that levels in TP-treated rats were consistently below the peak values observed during the oestrus stage. It could be argued that a similar fluctuation occurs in TP-treated rats but is not detectable. This cannot be refuted, but even when the highest values obtained for uptake and T/S ratio in TP-treated animals are selected they were not as high as the mean values observed in normal animals during oestrus. Where repeated determinations on the same TP-treated animals are available, from measurements of release rate, there was no obvious regularity in the distribution in time of the highest values obtained. Another relevant finding was that the slight differences between the mean uptake of ^{131}I in TP-treated and normal females were not seen in rats examined before puberty and the onset of regular ovarian cycles (Table 1). One possible explanation for these findings is that variations in TSH secretion which normally accompany the cyclic changes in gonadotrophin secretion in the female rat have been abolished in the TP-treated animals. The over-all effect has been a narrowing of the limits within which thyroid activity (and presumably pituitary TSH secretion) fluctuates without significant reduction in the mean level of activity.

The result of progesterone administration in TP-treated females was a transient fall in the level of thyroid activity during the phase of vaginal dioestrus followed by a rise above the preinjection level at and about the time of the return to vaginal cornification. Thyroid ^{131}I uptake, T/S ratio and release rates all showed this increase in response to progesterone. It appears reasonable, therefore, to attribute it to a rise in TSH secretion at this time. No such changes in ^{131}I uptake were seen in normal or TP-treated male rats; the presence of an ovary may be necessary for the response to occur. Progesterone administration appears to facilitate the induction of ovulation by electrical stimulation of the pre-optic area of the hypothalamus in TP-treated rats (Barraclough & Gorski, 1961). One possibility is that it acts through an increase in the LH content of the anterior pituitary (Gorski & Barraclough, 1962). However, in normal female rats progesterone at this dose level may cause a sufficient release of LH to induce premature ovulation (Everett, 1948). The changes in thyroid-gland function seen after progesterone administration are consistent with an increased release of TSH at the time of return to vaginal oestrus. It seems possible that this release of TSH may be analogous to that which has been postulated to occur during pro-oestrus in the normal cycle (Brown-Grant, 1963*a*) in association with the ovulatory 'surge' of LH release. The changes in TP-treated animals may represent an attempt at ovulation, the amount of LH released being inadequate in these, as in other experiments (Barraclough, 1963), to induce rupture of the follicles. This hypothesis would explain the facilitation of the ovarian response to hypothalamic stimulation at this time. Measurements of pituitary and serum levels of LH and TSH on the first day of vaginal oestrus *without* electrical stimulation of the pre-optic area might provide additional support for this hypothesis. A few of the TP-treated females studied in this work showed spontaneous changes in vaginal smears similar to those produced by progesterone administration. Several of these animals were killed on Days 2 or 4 of the return to vaginal oestrus. The ^{131}I uptakes were determined and were elevated compared with the values obtained in litter-mate controls killed while in persistent vaginal oestrus. Day 2 animals (3) had a mean uptake of $7{\cdot}95 \pm 0{\cdot}32\,\%$ and Day 4 animals (5) $7{\cdot}32 \pm 0{\cdot}76\,\%$. Both values are significantly ($P < 0{\cdot}05$) higher than the value of $5{\cdot}21 \pm 0{\cdot}54\,\%$ for control animals with constant vaginal cornification.

Ovulation induced by HCG administration had no significant effect on ^{131}I uptake and did not increase the rate of release of ^{131}I from the gland. In one experiment a significant rise in T/S ratio resulted, but this finding could not be reproduced. These results are consistent with the view that it may not be ovulation *per se* but rather the events leading to ovulation

that are responsible for the variations in thyroid activity seen during the normal cycle.

SUMMARY

1. Thyroid gland activity was determined in male and female rats receiving 1·25 mg of testosterone propionate on Day 4 of post-natal life by measuring ^{131}I uptake, the thyroid-serum concentration ratio for ^{131}I and the rate of release of ^{131}I from the gland.

2. No effects were detected in male rats; in female rats, the mean level of activity is the same or slightly less than the mean level in control animals, but is much below that seen during the oestrus stage of the cycle in the controls.

3. Progesterone (2 mg) may reduce the level of thyroid activity slightly during the period of vaginal dioestrus, but there is an increase in activity above pre-injection levels at the time of return to vaginal oestrus.

4. Ovulation induced by the injection of human chorionic gonadotrophin is not associated with any immediate increase in thyroid activity.

5. It is suggested that the effect of testosterone treatment in female rats is to abolish the fluctuations in TSH secretion and thyroid gland activity that normally occur during the oestrus cycle, but that it does not reduce the mean level of activity significantly.

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