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Authors

Parrott, Marshall W.
Johnston, Muriel E.
Durbin, Patricia W.

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Ernest O. Lawrence Radiation Laboratory

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Lawrence Radiation Laboratory and the Division of Medical Physics,
University of California, Berkeley, California

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ABSTRACT

Sexually mature female rats were made thyroid-deficient by surgery or by irradiation of the thyroid gland with I^{131} or At^{211}. Thyroid hormone was replaced with desiccated thyroid, l-thyroxine, or l-triiodothyronine. Supplemental calcium was given orally as calcium gluconate. Animals were mated to test reproductive capacity 2 to 3 months after thyroid removal thus assuring complete elimination of residual thyroid hormone.

Thyroid-deficient rats had smaller litters and fewer pregnancies, only 31% compared to normal, 79% pregnancies. All three forms of thyroid hormone supplement increased the number of conceptions to 84%, and litter sizes were within control limits.

Stillbirths, prolonged gestation, and maternal tetany were common in I^{131}-treated groups as well as in surgically thyroparathyroidectomized rats regardless of whether or not supplemental thyroid hormone was given. In 46% of the cases tested the serum calcium level of I^{131}-treated, thyroxine-supported rats was less than 6 mg% at parturition, whereas the average serum calcium of normal controls was 10.5 mg%.

Variable radiation damage to the parathyroid glands of I^{131}-treated animals was observed histologically. There was a rough correlation between the extent of structural parathyroid damage and a lower than normal serum calcium level.

Thyroid hormone was shown to be essential for normal fertility and pregnancy, but its role in parturition, if any could not be demonstrated. The difficult labor observed in the I^{131}-treated rats was considered to be due to parathyroid deficiency.

INTRODUCTION

It is generally believed that reproduction is impaired in the thyroid-deficient female mammal. However, experimental investigation in laboratory rodents of the apparent relationships among the thyroid gland, the anterior pituitary, and the ovary has not yet led to firm conclusions. The extensive experimental literature on this subject has been reviewed by Peterson et al. (1).

Disturbances in reproductive rhythm and structural alterations in the anterior pituitary and in the female reproductive organs are usually (but not invariably) found in chronically thyroid-deficient animals. The estrus cycle is prolonged in laboratory rodents following surgical thyroidectomy (2,3) or pharmacological suppression of the gland (3,4). Ross (2), Knude et al. (5), and Mazur (6) among others have reported a reduction in the number of maturing ovarian follicles in the hypothyroid rat. P'An (7) and Chu and You (8) reported ovarian and uterine atrophy in surgically thyroidectomized rats and rabbits.

Mating tests of thyroid-deficient laboratory rodents have produced the most contradictory results, and in a single species--the rat--observations range from sterility to no obvious reproductive impairment. The work of Ross (2), Barker (3), Chu (9), and Krohn and White (4) supports the view that hypothyroidism markedly interferes with reproduction in the female rat. Krichesky (10), Jones et al. (11), and Nelson and Tobin (12) found that litters were smaller, gestation was somewhat prolonged, and matings were occasionally sterile when rats were made thyroid-deficient surgically or with drugs shortly before or soon after conception. These latter investigators, however, did not regard their findings as clear-cut evidence for a specific effect of thyroid hormone on reproduction.

Watts (13) and Gemmill et al. (14), using the metabolic rate as an indicator, found that a severe hypothyroid condition does not exist in the rat until at least 40 days after thyroid gland removal. The time may be even longer between thyroid removal and the appearance of the histological and presumably functional anterior-pituitary changes associated with thyroid deficiency. The normal gestation period of the rat is 21 days. Therefore, a rat thyroidectomized or fed antithyroid drugs shortly before conception or early in pregnancy will have some residual thyroid hormone in its tissues during the gestation period. The presence of residual thyroid hormone with its supportive action on the ovary and pituitary gland may partially explain the inability of some investigators (10-12,15) to demonstrate the marked reproductive impairment which is seen in animals with long-standing hypothyroidism.

Calcium and--by inference--the parathyroid glands are also important factors in successful reproduction. Mazur (6) and Bodansky and his co-workers (16-19) concluded from their studies of the surgically thyroparathyroidectomized rat that parathyroid deficiency was the main source of such difficulties at term as prolongation of gestation, stillbirths, and maternal tetany. They found that tetany could be prevented and normal parturition promoted by feeding a high calcium diet or by injection of calcium salts near term.

These experiments were designed to test the apparently separate influences of the thyroid secretions on fertility and fetal maturation and viability and of the parathyroid secretions on parturition and maternal survival.

METHODS

The animals used were female rats of the Sprague-Dawley strain obtained from the parent colony when 70 days old. They were housed on wood shavings in metal cages, and were fed Purina Laboratory Chow[3] and tap water ad lib. The animals were sexually mature (22) when introduced into the experiment (75 days old in Experiment I and 110 days old in Experiments II and III). In each of three experiments 10 rats were set aside as controls. The remaining animals were thyroidectomized surgically or by irradiation with I^{131} or At^{211}.[4] Surgical thyroidectomy was carried out according to the method of Farris and Griffith (22). Radioiodine[5] was administered by intravenous injection at a level of 5 μC per gram body weight. This amount of I^{131} produces severe hypothyroidism with minimal structural damage to other organs (13). The At^{211} was prepared by methods described elsewhere (23) and was administered intravenously in isotonic saline at a level of 0.62 μC per gram body weight. This At^{211} dosage was found to produce maximal thyroid destruction with few acute radiation deaths (21).

Three essentially independent experiments were carried out. The experimental design, the ages of the animals, mode of treatment, and group sizes are shown in Table I.

Experiment I

Ten days after thyroidectomy one-half of each group was placed on a supplement of desiccated thyroid (Armour). A freshly prepared aqueous suspension of the thyroid substance (TH) was administered intragastrically three times weekly. The surgically thyroidectomized rats received 9 mg/week of active thyroid substance, and the I^{131} and At^{211}-treated rats received 12 mg/week. Standard metabolic rates (SMR) of the animals in each group were determined by measuring the time required for the consumption of a measured volume of oxygen in a closed system. The apparatus and calculations employed were essentially

Table I

Group size, mode of thyroid destruction, age at thyroidectomy, age at breeding, and experimental treatment. (TH=desiccated thyroid substance, T_4=l-thyroxine, T_3=triiodothyronine.)

Mode of thyroidectomy	Day of age at thyroidectomy	Treatment	Group size	Days of age at breeding
Experiment I				
Surgery	75		13	160
Surgery	75	TH[a]	12	160
I^{131}	75		13	160
I^{131}	75	TH	12	160
At^{211}	75		10	160
At^{211}	75	TH	12	160
Controls	75		10	160
Experiment II				
I^{131}	110		11	220
I^{131}	110	Calcium[b]	12	220
I^{131}	110	T_4	10	220
I^{131}	110	T_4 + calcium	10	220
Controls	110	Saline	9	220
Experiment III				
I^{131}	110	T_4	10	200
I^{131}	110	T_4 + calcium[c]	8	200
I^{131}	110	T_4 + T_3	9	200
I^{131}	110	T_3	9	200
Controls	110		10	200

[a] Desiccated thyroid substance (Armour).
[b] Veterinary calcium gluconate by intubation daily for last 5 days of pregnancy.
[c] Calcium gluconate, C.P., by intubation daily for last 5 days of pregnancy.

those described by Kleiber (24), Contopoulos et al. (25) and Evans et al.(26) as modified by Watts (13). Before any measurements were made, the animals were conditioned to the apparatus twice daily for 30 minutes for a period of 2 weeks. Repeated observations were made over a period of 3 months or until two successive observations for individual animals agreed within 5%.

When the animals were 160 days of age, breeding experiments were initiated. The animals were mated by exposing each cage of 5 female rats to 14 different males over a period of two weeks. At the end of this period the females were placed in individual cages. As the animals neared term, the cages were examined several times each day. A record was kept of the number of young born alive or dead.

Eleven months after the start of the experiment, all animals were given a tracer dose of 20 μC of I^{131} intramuscularly. Twenty-four hours later the animals were sacrificed with chloroform and the tracheas removed. The gamma-ray activity of the I^{131} in the residual peritracheal tissue was counted in a well-type NaI-TlI scintillating crystal counter. The ovaries and peritracheal tissue were dissected, fixed in Bouin's fluid, and prepared for microscopic examination.

Experiment II

All the animals were thyroidectomized with I^{131}. Five days later 20 of the 45 I^{131}-treated rats were placed on a schedule of daily subcutaneous injections of 0.1 cc of an alkaline solution of 60 μg/cc Na-l-thyroxine (Smith, Kline & French Laboratories). This dosage of l-thyroxine (T_4) has been found to sustain a normal SMR in the thyroidectomized rat (26,27). This more potent form of thyroid hormone was administered parenterally in this and the subsequent experiment. Hormone dosage could be more closely controlled (than when a slurry was given by mouth), and irritation of the upper respiratory tract and aspiration of intubated fluid were avoided. One-half the thyroxine-treated animals, and half of the remaining thyroidectomized animals received 1.5 grains of veterinary calcium gluconate (Haver-Lockhart Laboratories) intragastrically once each day during the last 5 days of pregnancy. The 10 intact control animals received daily sham injections of 0.1 cc isotonic saline subcutaneously. Beginning at 100 days post-thyroidectomy vaginal smears were examined to determine the estrus cycle (28). As epithelial cells appeared an adult male was placed in the cage with the female. The next morning the appearance of motile sperm or a sperm plug indicated conception had probably occurred. This was considered Day 1 of pregnancy (29). Sixty days after the last litter was delivered, the animals were autopsied as described above. The completeness of thyroidectomy was determined using a tracer dose of I^{131} as described in Experiment I.

Experiment III

Five groups of 10 rats each were used. Forty were given thyroid-destructive doses of I^{131}. Similar metabolic effects of 1-thyroxine (T_4) and 1-triiodothyronine (T_3) have been demonstrated repeatedly (26,30-34). However, it was considered possible that these two hormones might have some as yet unidentified independent actions and that either alone would not constitute adequate thyroid replacement during the many phases of reproduction. In this experiment T_4,T_3, and a mixture of the two hormones, T_3 and T_4, were used as supportive therapy. Thyroxine was given daily at a level of 7 µg per rat (26). Triiodothyronine was administered daily at a level of 1.2 µg per rat, an amount reported to sustain a normal BMR in the thyroidectomized rat (26,35-37). A third group received a daily injection of a mixture of 6.3 µg T_4 and 0.12 µg T_3--the proportions used were those found by Taurog (38) in the thyroid vein of the sheep. A fourth group on the T_4 regimen was given 65 mg calcium gluconate (C.P.) daily by gastric intubation during the last 5 days of pregnancy. After 60 days of hormone administration, metabolic rates were determined.

The animals were bred by placing a fertile male in the cage with a female for seven consecutive days. Lack of space restricted matings to 20 at a time. The females were examined daily for indications of pregnancy and later, onset of labor. The (T_3) and the ($T_4 \cdot T_3$) groups which were the first to be mated showed a high incidence of maternal tetany and stillbirths. These observations suggested a parathyroid deficiency evident only at term. Therefore, the remaining three groups of animals were sacrificed during labor or immediately following delivery for serum calcium measurement and parathyroid histology. With the animal under ether anaesthesia, blood was aspirated from the inferior vena cava into a heparinized syringe. Serum calcium was determined by the method of Baron and Bell (39). Peritracheal remnants were prepared for microscopic examination.

RESULTS

Thyroid destruction (in Experiment I) with either of the radioactive isotopes, I^{131} or At^{211}, caused a reduction of the SMR to 65% of the control mean, as shown in Table II. Watts (13) obtained similar results. Surgical removal of the thyroid gland resulted in an SMR only 15% below normal. Although the mean SMR of the surgically thyroidectomized group was within one standard deviation of the control mean, the t-test of Fisher (40) indicated that statistically, the two means were significantly different ($P < 0.01$).

The I^{131} tracer given 24 hours before sacrifice showed that surgical removal of the thyroid could be considered complete in five of nine cases;[5] the I^{131} uptake in peritracheal tissue was less than 0.1%. Radioiodine uptakes of the other four animals ranged from 0.8% to 2.2% of the administered tracer. There was, however, no detectable correlation between an uptake of the I^{131} tracer greater than 0.1% (indicating the presence of functional thyroid tissue) and a higher-than-average SMR or better-than-average reproductive capacity.

Thyroid destruction with I^{131} was judged complete in all cases; uptake of the I^{131} tracer ranged from 0.004% to 0.05% in Experiments I and II. Astatine, as reported previously, was not as efficient an agent for thyroid destruction as I^{131} (21). Uptake of the I^{131} tracer by the At^{211}-irradiated rats ranged from 0.01% to 1.4% of the dose. As in the surgically thyroidectomized group, there was no correlation between a higher uptake of the I^{131} tracer and an above-average SMR.

Desiccated thyroid substance at a level of 9 to 12 mg/wk brought the SMR of the surgically thyroidectomized animals and the At^{211}-irradiated animals up to low normal. However, 12 mg/wk of thyroid substance was not sufficient to bring the SMR of the I^{131}-irradiated animals even to the lower limit of normal. Evans <u>et al</u>. (41) points out that while 1 μg of l-thyroxine per day is not enough to maintain a normal SMR in the female rat, it is sufficient to promote

Table II

The effect of thyroparathyroid deficiency on the number of successful conceptions in the rat, and survival of young and mothers; the beneficial effect in these animals of thyroid and (or) calcium therapy.

Group treatment	S.M.R. ± Std.Dev. $Cal/m^2/hr.$	No. of Pregnancies	Avg.No.Young born alive[c]	Avg.No.Young born dead[d]	Mothers dying during labor[e]
Experiment I					
Surgery	44.0 ± 5.3	7/13	4.3	3.0	1
Surgery (TH)	47.8 ± 5.2	10/12	3.1	3.0	0
I^{131}	33.8 ± 5.4	3/13	2.7	1.0	0
I^{131} (TH)	35.4 ± 4.0	8/12	2.2	4.0	2
At^{211}	33.4 ± 5.3	0/10	-	-	0
At^{211} (TH)	45.2 ± 4.4	0/12	-	-	0
Controls	51.6 ± 3.8	7/10	7.4	0.03	0
Experiment II					
I^{131}	Not run	1/11	6.0	0.0	0
I^{131} (Ca)[a]	" "	4/12	3.0	3.2	0
I^{131} (T_4)	" "	9/10	1.4	5.1	2
I^{131} (T_4.Ca)[a]	" "	6/10	11.2	0.0	1[f]
Controls	" "	7/9	not recorded		0
Experiment III					
I^{131} (T_4)	41.4 ± 4.5	10/10	10.3	0.1	4
I^{131} (T_4.Ca)[b]	41.4 ± 4.5	7/8	8.8	2.0	4
I^{131} (T_4.T_3)	40.3 ± 5.6	8/9	7.7	2.7	3
I^{131} (T_3)	34.4 ± 3.0	9/9	6.7	3.3	5
Controls	36.3 ± 5.9	9/10	10.6	0.1	0

a Veterinary calcium gluconate by intubation daily for last 5 days of pregnancy.
b Calcium gluconate (C.P.) by intubation daily for last 5 days of pregnancy.
c Includes young delivered alive and young found alive in utero at necropsy.
d Includes young born dead and found dead in utero at necropsy.
e Includes animals in tetany sacrificed for serum calcium measurements.
f In tetany after only 2 days treatment with calcium.

normal skeletal growth and sexual development. The thyroid therapy was therefore considered adequate for the purpose of these experiments.

In Experiment III the use of T_4 (45.5 μg/wk), T_3 (8.4 μg/wk), or a mixture of 90% T_4 and 10% T_3 as thyroid replacement brought the metabolic rates well within the control limits. SMR's were not measured in Experiment II. The T_4 treatment was similar in Experiments II and III, therefore it seems reasonable to assume that the animals in Experiment II received adequate thyroid hormone replacement. We have no ready explanation for the discrepancy between the two values shown for normal SMR. The results for each experimental group were reproducible and internally consistent. Watts (13) in this laboratory obtained a mean SMR for normal rats of this strain and age of 43 ± 3 Cal/m^2/hr, almost midway between the control means for Experiment I and II.

Surgical thyroidectomy led to a reduction in the number of pregnancies; only 54% of the matings resulted in conception. In the I^{131}-irradiated groups, in Experiments I and II the highest percentage of pregnancies was 33%, and the lower limit was less than 10%. No pregnancies were observed in the At^{211}-treated group. The ovaries of this latter group of animals were atrophic on histological examination and had apparently been severely irradiated.

The reproductive capacity of all thyroid-deficient animals (except those whose thyroid tissue had been destroyed with At^{211}) was greatly enhanced by giving them some form of thyroid hormone. The number of pregnancies was increased from 31% in the thyroid-deficient animals (15 pregnant of 49) to 84% in thyroidectomized animals that received some form of supplemental thyroid hormone (67 pregnant of 80). In the three control groups 23 of 29 rats or 79% became pregnant after a similar single mating. The radiation damage in the ovaries of the At^{211}-treated rats was apparently not repaired to any significant extent by thyroid therapy.

As shown in Table II, the thyroid-deficient animals bore smaller litters--

4 to 7 pups--than did the normal controls--7 to 11 pups. Stillbirths were rare in control litters, but were common in the litters of animals whose thyroids had been removed surgically or with I^{131}, regardless of whether or not supplemental thyroid hormone was given. The number of stillbirths occasionally exceeded 50% of the litter.

When thyroid therapy was employed in Experiments I and II, the litters were slightly larger, usually 6 or more pups. In Experiment III litters were of normal size, averaging 10 pups. This larger average litter size may be due to increased amounts (or more potent forms) of thyroid hormone, to more frequent hormone administration (daily rather than three times a week), or to more precise observations, or to a combination of these factors.

The high proportion of surviving young in Experiment III is harder to explain, and may be somewhat misleading. As mentioned above, the thyroid supplement had no apparent effect on the proportion of stillbirths. From 30% to 75% of the pups born to animals whose thyroid tissue had been removed surgically or with I^{131} were delivered dead, died within a few minutes after delivery, or were found dead <u>in utero</u>. The mothers in Experiment III were under almost constant observation and most of them were killed just before or during delivery. A number of live pups was generally found <u>in utero</u>. Had these mothers particularly those in tetany not been watched so closely, many of their young might have died during or after delivery, or died <u>in utero</u>. In such cases they would have been recorded as stillbirths.

The length of gestation was observed accurately only in Experiment II. Day 1 of pregnancy was established by the method of Blandau (29). The gestation period of the control animals averaged 23 days (range 21-23 days), I^{131} (T_4) averaged 24 days (range 22 to 26 days), I^{131} (T_4.Ca) all delivered on day 23, I^{131} (Ca) averaged 23 days (22 to 24 days), and I^{131}, one animal only, delivered at 23 days.

By combining the information from all experiments, it was found that 11 of 37 untreated thyroid-deficient animals (no thyroid hormone or calcium) became pregnant (30%); one died in labor. Fifty-four of 82 rats that were given thyroid hormone (but no calcium) became pregnant (87%), and 16 of these animals (30%) either died in labor or were in tetany when sacrificed. No deaths during labor were recorded for the 23 control pregnancies.

In Experiments II and III, 17 pregnant rats were given either veterinary calcium gluconate or calcium gluconate (C.P.) by mouth daily during the last 5 days of pregnancy. Five of these animals (30%) died in labor or were in tetany when sacrificed. One animal included in the data had received calcium gluconate for only 2 days when labor began. Oral administration of (either veterinary or) calcium gluconate (C.P.) did not seem to improve maternal survival. There was, however, some indication that the veterinary preparation had some beneficial effect on length of gestation and the viability of the young. In Experiment II the six animals that received both thyroid hormone and the calcium supplement delivered large litters without incident, and no stillbirths were found. All six litters were successfully suckled until weaning. On the other hand, half of the young born to four thyroid-deficient rats -- I^{131} (Ca) -- were delivered dead. In Experiment III, maternal survival was no better, and the percentage of live young born to the group that received calcium gluconate (C.P.) was only slightly better than for those groups that did not receive supplemental calcium.

In the third experimental series it was necessary, because of the large number of animals involved, to breed 20 animals at a time. Although the number of pregnancies was larger and the litter sizes were close to normal for the (T_3) and ($T_4 \cdot T_3$) groups, it was obvious that these animals were delivering their litters with difficulty. On the average, one-third of the young were delivered dead, and 8 of the 17 pregnant rats in these two groups died in labor. The histological specimens from the two previous experiments were completed at about this time. In some of the sections of peritracheal tissues taken from I^{131}-irradiated animals, the parathyroid tissue, while still grossly intact, showed some indications of structural alteration. The structural changes of the parathyroid are discussed later in detail. Other investigators have commented on the absence of damage or have noted minimal damage of the parathyroid glands of rats whose thyroid glands had been destroyed with minimal doses of I^{131} (2,21, 42,43). We had previously reported nearly normal serum calcium levels in rats given thyroidectomizing doses of I^{131} (44). These previous observations apply only to animals under little stress. In the experiments reported herein it was considered likely that termination of pregnancy might produce sufficient stress to disclose a previously masked partial parathyroid impairment. The remaining experimental and control rats were bred and observed with great care so that serum calcium might be measured during or very soon after labor. Serum calcium levels and the stage of labor when the blood samples were taken are shown in Table III for 10 normal controls, 9 animals in the $I^{131}(T_4)$ group and 4 animals in the $I^{131}(T_4 \cdot Ca)$ group.[7] Animals are designated in the Table as "in normal labor"--contractions and(or) vaginal bleeding but no pups yet born; "delivering normally"--one pup had been delivered, but the mother was sacrificed before the placenta was eaten; or "post partum"--some or all of the pups were delivered and placentae eaten. Those animals showing convulsions or rigidity are designated as "in tetany." The range of control values was 9.95 mg% to 12.28 mg of calcium per 100 ml of serum. The range for the experimental animals was 7.11 mg% to 10.49 mg%, and for those in tetany the range was 3.12 mg% to 5.16 mg%.

Peritracheal tissue for histology was removed with the aid of dissecting

Table III

Serum calcium levels in iodine-131 thyroid-ablated rats at term.

Treatment and animal number	Condition of mother at necropsy	Serum calcium level mg%
Control		
2330	In normal labor	11.08
2342	Post-partum	9.95
2328	Delivering normally	10.16
2344	Post-partum	10.08
2333	" "	11.82
2334	" "	12.28
2353	In normal labor	10.20
2339	Delivering normally	10.70
2367	Post-partum	10.83
2329	" "	10.67
(T_4)		
2338	Delivering normally	8.74
2358	In normal labor	9.91
2337	Post-partum	10.49
2335	In tetany	3.89
2345	In normal labor	7.11
2363	Post-partum	10.24
2364	In tetany	5.16
2348	" "	3.83
2359	" "	3.12
(T_4 + Ca)		
2366	In normal labor	9.12
2369	" " "	8.85
2346	In tetany	4.08
2365	" "	4.46

microscope from the thyroid region of 18 I^{131}-irradiated animals in Experiment I, and from 15 I^{131}- irradiated rats in Experiment III for which a preautopsy serum calcium measurement was available. Both parathyroids were identified in only 8 of the 33 specimens examined; one gland was seen in an additional 17. The normal parathyroid gland as shown in Fig. 1 is highly vascular and densely cellular, and the cells are arranged in irregular cords and clumps supported by delicate reticular fibers. The gland is covered by a delicate connective-tissue capsule.

None of the parathyroid glands taken from I^{131}-irradiated rats could, upon careful examination, be classed as undamaged. In general, these glands were smaller than those of normal controls. They were surrounded by a thick band of fibrous connective tissue which was usually continuous with the scar tissue that had replaced the thyroid (Fig. 2). When the parathyroid appeared to have been originally located on the surface of the thyroid, the connective-tissue capsule was thinner on the outer surface. Only rarely were blood vessels seen to be penetrating this capsule. The reticular network of the irradiated glands was conspicuous in many specimens and comprised a large proportion of the total tissue in some of them. The capillary network was generally very much reduced or obscured by the connective tissue. Occasionally two or three large blood vessels were seen in a single section. The cellularity of the irradiated parathyroid glands varied from a few cells arranged in "nests" of two to four cells separated from one another by connective-tissue septa (Fig. 3) to densely cellular, deeply staining tissue with a structure more reminiscent of normal parathyroid (Fig. 4).

Correlation of structure with function was not clean-cut, particularly since only one parathyroid was usually seen. The two tiny pieces of peritracheal tissue from each animal were embedded in the same paraffin block. The blocks were not sectioned serially but were cut only until two pieces of tissue in rats whose thyroid tissue had been destroyed with I^{131} when triiodothyronine or thyroxine was given in amounts sufficient to maintain a normal standard metabolic rate. Judging from the number of pregnancies and the litter sizes of these animals, fertility did not seem to be impaired by the absence of the parathyroid glands, as suggested by Bodansky and Duff (19).

We were unable to demonstrate any beneficial effect of desiccated thyroid substance, thyroxine, or triiodothyronine on parturition. An increase in protein-bound iodine has been demonstrated in pregnant women near term (52). This substantial increase in thyroid hormone output at the end of gestation might have some effect on parturition, but no information on this point was available from these present experiments.

An acute need for serum calcium at term was not anticipated. Previous work gave no indication that severe parathyroid deficiency might be produced occasionally during radioiodine thyroid ablation. This lack of understanding of the difficulties encountered during parturition in the I^{131}-thyroidectomized rat was complicated by our own previous experimental work (44), as well as the observations of others (5,43,45,46). Following the "discovery" of very low serum calcium levels (less than 6 mg%), the parathyroid glands were specifically sought at necropsy, and histological preparations were made of peritracheal tissue.

The level of calcium in the Purina Laboratory Chow (1.35%) is apparently sufficient to maintain the parathyroid-deficient animals when not under stress. A large percentage of this calcium may be in an unutilizable form, since its source is fish meal and vegetable matter. This diet probably does not meet the standards for the high calcium diet recommended by Cox and Imboden (53) for support of parathyroidectomized rats. Augmenting this diet with additional calcium in the form of orally administered veterinary calcium gluconate appeared to have some beneficial effect (Experiment II) on the survival of the young.

could be seen in the paraffin ribbon. Consequently, identifiable parathyroid tissue was undoubtedly missed in many specimens. In spite of this technical error, it was still possible to make a rough correlation between the microscopic appearance of the parathyroid tissue and the condition of a particular animal at parturition. Even when the identification of each slide was concealed, it was possible to predict for 10 of 13 specimens from Experiment III that a "normal" or "very low" serum calcium level would be found. Parathyroid tissue was apparently missed during sectioning of one specimen from an animal with a serum calcium of 9.12 mg%. In the second case, only one parathyroid was seen, and this was a remnant encapsulated with fiber, and chiefly composed of fibrous tissue with a very few cells; the serum calcium was low normal, 8.74 mg%. In the third case, again only one parathyroid was found. In this specimen one-third of the specimen was fibrous replacement tissue containing a few cells with darkly staining cytoplasm at the periphery; serum calcium was 10.49 mg%.

A tetanic animal invariably had a serum calcium level of less than 6 mg%. The histologic preparations from six animals in tetany usually indicated that the parathyroids had originally been deeply embedded in the thyroid tissue. The glandular remnants were surrounded by a thick fibrous capsule and contained small tightly clumped pyknotic or darkly staining cells in an extensive fibrous network.

There were four animals that were delivering normally, whose serum calcium was greater than 8 mg%, and whose parathyroid histology fitted with their serum calcium level and general condition. Although fibrous invasion was prominent, the groups of cells retained their normal glandular structure; they had abundant cytoplasm, and the capillary bed was extensive. These four specimens had at least one parathyroid that appeared to have been on the surface of the thyroid gland before irradiation. This more favourable geometric location could account for the survival of much of the tissue which was only partially irradiated by the I^{131}. The presence or absence of structural damage could almost be judged on the basis of the spatial relationship of one or both parathyroids to the thyroid gland (embedded or on the surface)--at least insofar as this relationship could be reconstructed.

DISCUSSION

Our experiments support the view of many investigators that irradiation with I^{131} is a more effective means of thyroid destruction than surgical removal of the gland (43,45,46). However, this radiation procedure is not without side effects such as parathyroid damage and ovarian damage seen in I^{131} overdose (47, 48).

The 69% reduction in fertility in severely thyroid-deficient animals appears to be directly related to the absence of circulating thyroid hormone. Similar observations have been made by several workers (3,4,49,50), although Hammet (5) did not consider his results conclusive.

The long physiological life of thyroid hormone--more than 40 days in the rat, according to Watts (13)--seems to be the source of many of the contradictions in studies of the relationships between thyroid deficiency and reproduction (10-12, 15). The long life of this hormone may help to explain, for example, the negative results of Bruce and Sloviter (15). These investigators used mice and bred them for the first time only 21 days after thyroid destruction with I^{131}. Some of these thyroidless mice conceived and bore as many as six litters. Females were mated continuously except during parturition and the 19 days while a litter was being suckled. This breeding schedule suggests at least one explanation for the fertility of their presumably thyroid-deficient females. Taurog (51) found that l-thyroxine is normally excreted primarily in the feces of several species (rat, dog, sheep, rabbit). It passes from the bile through the gastrointestinal tract unchanged or as an enzymatically hydrolyzable conjugation product. Lactating rodents habitually consume the fecal matter of their nurslings; this would provide the thyroidless mothers with a ready source of l-thyroxine.

Fertility was nearly normal in our surgically thyroparathyroidectomized animals when thyroid supplements were given. Fertility was also nearly normal in rats whose thyroid tissue had been destroyed with I^{131} when triiodothyronine or thyroxine was given in amounts sufficient to maintain a normal standard metabolic rate. Judging from the number of pregnancies and the litter sizes of these animals, fertility did not seem to be impaired by the absence of the parathyroid glands, as suggested by Bodansky and Duff (19).

We were unable to demonstrate any beneficial effect of desiccated thyroid substance, thyroxine, or triiodothyronine on parturition. An increase in protein-bound iodine has been demonstrated in pregnant women near term (52). This substantial increase in thyroid hormone output at the end of gestation might have some effect on parturition, but no information on this point was available from these present experiments.

An acute need for serum calcium at term was not anticipated. Previous work gave no indication that severe parathyroid deficiency might be produced occassionally during radioiodine thyroid ablation. This lack of understanding of the difficulties encountered during parturition in the I^{131}-thyroidectomized rat was complicated by our own previous experimental work (44), as well as the observations of others (5,43,45,46). Following the "discovery" of very low serum calcium levels (less than 6 mg%), the parathyroid glands were specifically sought at necropsy, and histological preparations were made of peritracheal tissue.

The level of calcium in the Purina Laboratory Chow (1.35%) is apparently sufficient to maintain the parathyroid-deficient animals when not under stress. A large percentage of this calcium may be in a unutilizable form, since its source is fish meal and vegetable matter. This diet probably does not meet the standards for the high calcium diet recommended by Cox and Imboden (53) for support of parathyroidectomized rats. Augumenting this diet with additional calcium in the form of orally administered veterinary calcium gluconate appeared to have some beneficial effect (Experiment II) on the survival of the young.

Calcium gluconate, C.P., used in Experiment III, did not aid maternal survival or improve litter survival (4 of 7 animals in the (T_4.Ca) group were in tetany at parturition). These observations suggest that oral administration of calcium in the form of pure calcium gluconate is of little use in ameliorating the symptoms of acute parathyroid deficiency.

An interesting observation was made in the two groups of surgically thyroidectomized animals of Experiment I. Of a total of 46 pups that survived parturition, 24 died before they were more than 2 days old. The remaining 22 pups survived to weaning preceded by what appeared to be a 2-day period of malnutrition. This time period parallels some of the investigations of Munson (47), who found that parathyroidectomy in a lactating rat considerably reduced the calcium content of the milk for a period of 2 days. After this time the calcium concentration in the milk exceeded the concentration of calcium in the milk of normal lactating rats.

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FOOTNOTES

1. Work done under the auspices of the U. S. Atomic Energy Commission.
2. Derived from work submitted in partial fulfillment of the requirements for the M.A. degree, University of California.
3. Purina Lab. Chow contains 1.35% calcium by weight derived from skim milk, fish meal, and cereals. The Ca/P ratio is approximately 1.4 by weight.
4. At^{211} is a radioactive isotope of the heaviest halogen. It emits alpha particles with a mean energy of 6.8 Mev and decays with a half life of 7.5 hours. The thyroid gland selectively accumulates At^{211}, and the intense alpha-particle irradiation is capable of destroying sufficient thyroid tissue to produce permanent hypothyroidism (20,21).
5. Oak Ridge National Laboratory carrier-free processed I^{131}.
6. Four animals were lost to autopsy; one animal died on labor and three others died of pneumonia.
7. Four rats in these two groups either had died during labor or had successfully delivered their litters before serum samples could be obtained.

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FIGURE LEGENDS

1. Normal parathyroid tissue taken from a control animal in Experiment I. Note capsule of loose connective tissue and delicate reticular network. Cells in central region are arranged in cords; those at the periphery are arranged in clumps. Sectioned at 6 μ, H and E x 220.
2. Parathyroid from rat No. 2337; serum calcium 10.49 mg% during normal delivery. A thick fibrous replacement of the thyroid. Note the conspicuous reticulum and reduced cellularity in the region nearest the trachea. This gland had apparently been located on surface of thyroid. Sectioned at 6 μ, H and E x 220.
3. Parathyroid from rat No. 2348; serum calcium 3.83 mg%. Animal in tetany attempting delivery. Note thick fibrous capsule, and small clusters of cells separated from each other by connective tissue septa. This gland apparently had been embedded deep within the thyroid tissue. Sectioned at 6 μ, H and E x 220.
4. Parathyroid from rat No. 2369; serum calcium 10.40 mg% during normal delivery. Note conspicuous reticulum and variability of cellularity and grouping of cells--cords and sheets at the upper right and clusters at the lower left--reminiscent of normal parathyroid tissue. Sectioned at 6 μ, H and E x 220.

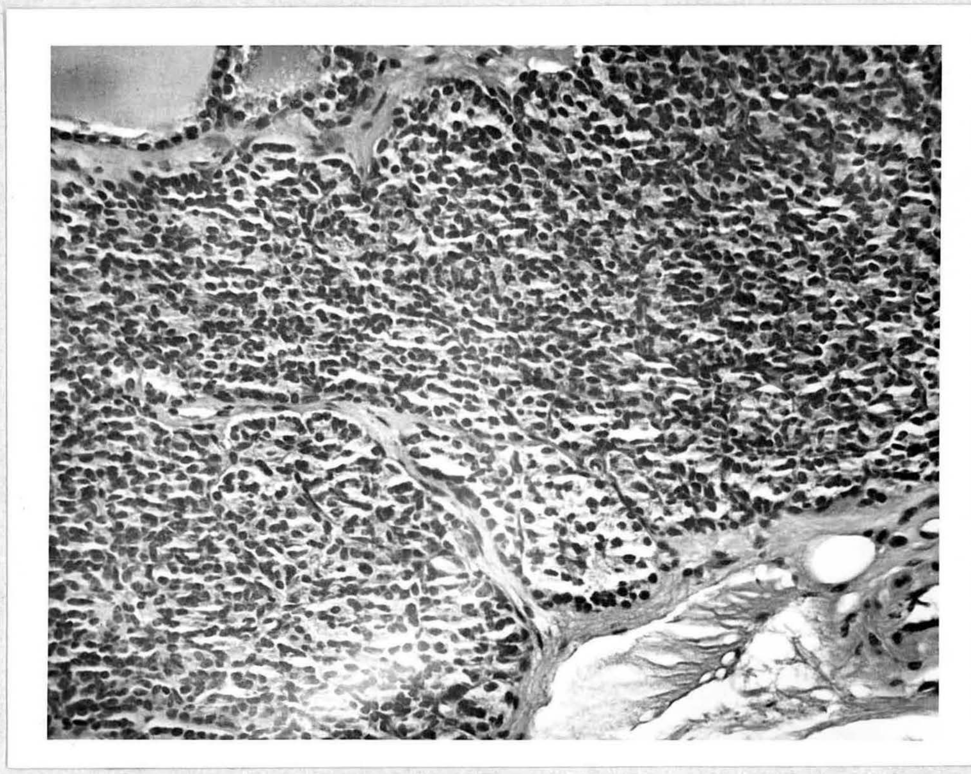

Fig 1

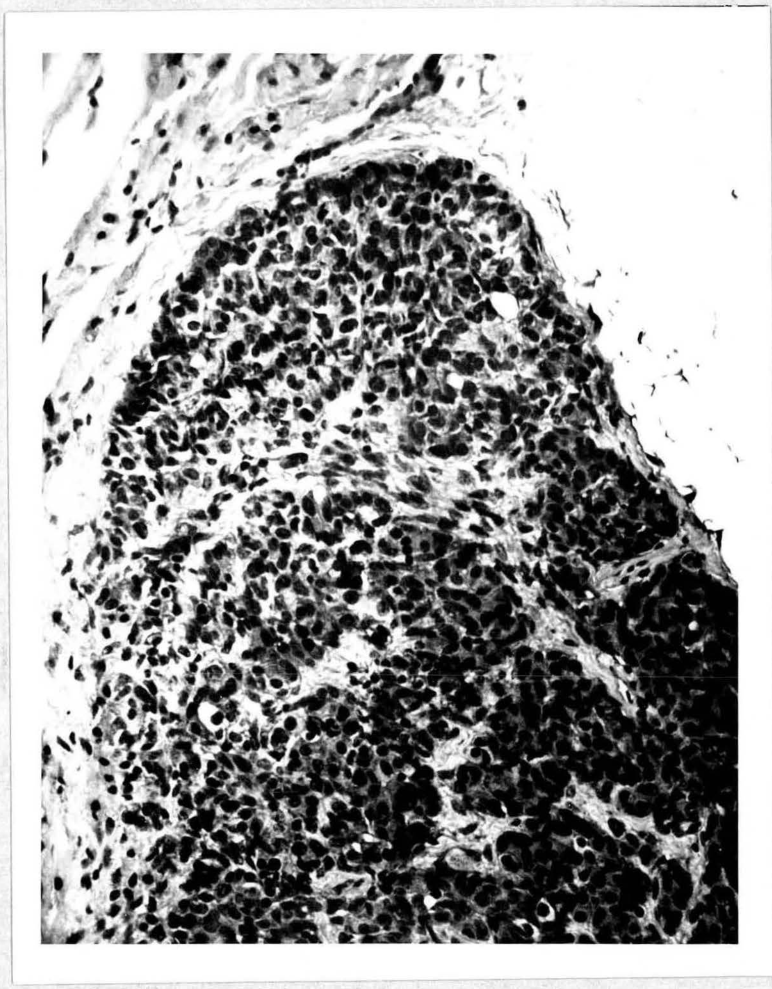

Fig 2

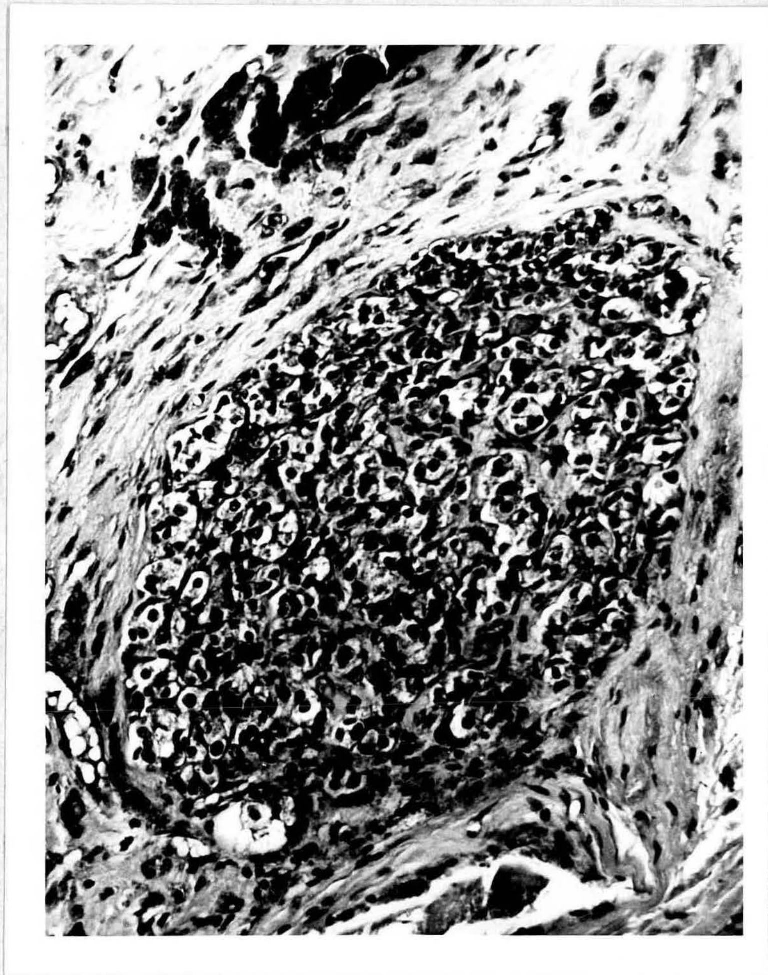

Fig 3

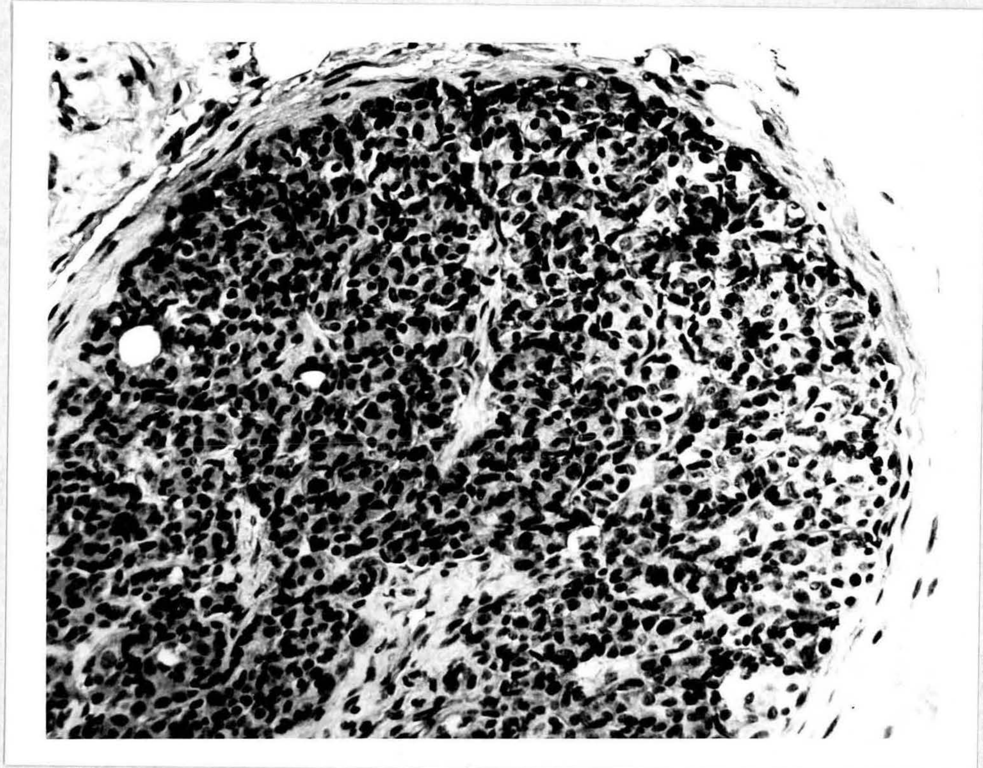

Fig 4