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THE EFFECT OF EXOGENOUS CALCIUM AND ℓ-THYROXINE ON PARTURITION IN THE RADIOIODINE-THYROIDECTOMIZED RAT

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Marshall Ward Parrott

(Master's Thesis)

December 28, 1959

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Contents

THE EFFECT OF EXOGENOUS CALCIUM AND ℓ-THYROXINE ON PARTURITION IN THE RADIOIODINE-THYROIDECTOMIZED RAT

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ABSTRACT

The effects of hypothyroidism and hypoparathyroidism and replacement therapy on reproduction have been studied in the rat.

Hypothyroidism was produced by surgery or by irradiation with I^{131} or At^{211}. Replacement therapy was attempted with calcium gluconate, desiccated thyroid substance, thyroxine, triiodothyronine, and a mixture of 90% thyroxine and 10% triiodothyronine. Radioisotopes were administered in sufficient quantity to produce highly significant depressions in metabolic rate and thyroidal uptakes of a tracer dose of I^{131}. Enough time was allowed to elapse to permit complete metabolism of residual thyroid hormone before any attempt was made to breed the animals.

The number of pregnancies was drastically reduced in the hypothyroid animals. Thyroid supplement brought the number of conceptions to well within the normal limits. No difference in fertility was observed when TH, T_4, or T_3 were used as the thyroid replacement.

Still births and prolonged gestation were common in surgically thyroparathyroidectomized animals and in animals whose thyroids had been destroyed with I^{131}. The number of fetal deaths was reduced, and the length of gestation was reduced to the normal 23 days by intragastric administration of veterinary calcium gluconate daily for at least 5 days before term in the I^{131}-irradiated, thyroxine-treated animals. Calcium gluconate (C. P.) was apparently ineffective when given by mouth.

Serum calcium levels were depressed to less than 6 mg% during parturition in I^{131}-thyroidectomized, thyroxine-supported animals (46% of the cases tested).

It is concluded that the thyroid gland is essential for the maintenance of the reproductive system in order to permit normal conception and pregnancy. However, its exact role, if any, in parturition could not be determined. It must be assumed that destruction of, or damage to the parathyroid glands was a major contributor to the difficulties observed in parturition.

INTRODUCTION

The purpose of this series of studies is to test the apparently separate influences of the thyroid and parathyroid secretions on conception, gestation, and parturition in rats.

Disturbances in reproductive rhythm of rats have been observed by Ross[1] following surgical thyroidectomy, by Barker[2] following suppression of the gland with thiouracil, andby Krohn and White[3] following either surgical thyroidectomy of pharmacological suppression of the gland. Aranow et al have observed sterility in monkeys and women with chronic hypothyroidism or following preconception thyroidectomy.[4]

Structural changes in the female reproductive organs of the rat following thyroidectomy have been observed by many investigators. Reduction in the number of follicles present in the ovaries of rats were seen by Ross[1], Knude et al.[5], and Mazur[6], while uterine and ovarian atrophy and rare evidence of ovulation were observed by Gorbman after thyroidectomizing mice with I^{131},[7] by P' An after surgical thyroidectomy of the rat and rabbit,[8] and by Chu and You following surgical thyroidectomy of the rabbit.[9] The direct influence of thyroid hormone on the hypophyseal secretion of luteinizing and follicle-stimulating hormones has been suggested by P'An using the thyroidectomized rat and rabbit[8], by Chu and You[9] and by Chu[10] using thyroidectomized rabbits, and by Krohn and White in the thyroidectomy of the rat.[3] This influence could not be demonstrated by Smith, using the procedure of pituitary implants in immature mice from thyroidectomized rats[11], or by Krichesky[12] and Krohns[13] who thyroidectomized rabbits during pregnancy. The last three investigators base their conclusions on a thyroidless state of rather short duration.

The observations on a single species, the rat, range from failure of conception[1,2] to no obvious impairment of reproductive capacity.[12,14,15] In the latter case, the investigators found normal gestation when thyroidectomy or an antithyroid drug was used near the time of conception.

Data presented by Watts[16] and Gemmill et al.[17] on the metabolic rate of rats following I^{131} thyroidectomy indicate a hypothyroid condition does not exist in the rat until it has been thyroidectomized for at least 40 days, almost twice the normal gestation period for this animal. It is evident that a rat, thyroidectomized near or during pregnancy, will have considerable residual thyroid hormone in the tissues during gestation, indicating one of the possible sources of error in the interpretation of data.

Calcium and, by inference, the parathyroid glands are also considered by many workers to be important factors in reproduction. Calcium metabolism in thyroidectomized animals has been studied during parturition and lactation but not as extensively as the thyroid itself. Mazur[6] and Bodansky and co-workers[18, 19, 20, 21], in their studies on the surgically thyroidectomized rat considered parathyroid deficiency as the main source of such difficulties at term as prolongation of gestation, still births, and maternal tetany. These investigators found that tetany could be prevented and normal parturition promoted by feeding a diet high in calcium or by injection of calcium salts near term.

In reality, there is uniformity in the available data if one considers such important factors as (a) the rather long lifetime of thyroid hormone, (b) the mode of thyroidectomy, (c) the concomitant removal of the parathyroid glands during thyroid surgery, and (d) the dietary level of calcium and phosphorus. Rather, the issue is the differing interpretation of data by many authors, some of whom fail to consider one or more of the above factors in drawing their conclusions.

We first became interested in the reproductive capacity of the hypothyroid rat when it was found that a thyroid-destructive dose of radioactive At^{211} [*22, 23] rendered the young female rat sterile.[24]

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* At^{211} is a radioactive isotope of the heaviest halogen. It emits alpha particle with a mean energy of 6.8 Mev and decays with a half life of 7.5 hr. The thyroid gland selectively accumulates At^{211}; and the intense alpha-particles irradiation is capable of destroying sufficient thyroid tissue to produce permanent hypothyroidism. Hamilton _et al_.[22, 23]

In an initial attempt to examine both endocrine and radiation effects on reproduction, rats were made hypothyroid by surgery or by treatment with At^{211} or I^{131}. For several weeks before breeding was initiated, desiccated thyroid substance (TH) was given regularly to half of the rats in each group to re-establish normal standard metabolic rates (SMR). Very few pregnancies were observed in the rats thyroidectomized surgically or with I^{131}. These findings agreed with those of other workers who studied similar time intervals between thyroidectomy and breeding.[1,2] A significantly larger number of the TH-fed rats conceived, but several of these rats died during delivery or delivered still-born young. Gestation was prolonged in all but the normal control group. The possibility of parathyroid deficiency was considered, although thyroidectomizing doses of either At^{211} or I^{131} are generally reported to produce little structural damage in the parathyroid glands.[7,23,25,26] Serum calcium was determined on groups of rats thyroidectomized surgically or with I^{131}. Serum calcium was low in the surgically thyroidectomized animals corresponding to the levels reported by Bodansky and Duff[19], but was within normal limits in the I^{131}-treated rats.[27]

Because of the difficulties of administering accurately known amounts of a slurry of desiccated thyroid substance, pure ℓ-thyroxine (Smith, Kline, and French Laboratories) was used in further experiments. It was also considered that adequate thyroid replacement might not be achieved using pure ℓ-thyroxine alone, since it was conceivable that the two active thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3) working together not only influenced the integrity of the reproductive organs, but affected calcium metabolism as well. The participation of thyroid hormones in skeletal maturation and other phases of calcium metabolism has been established.[28-30] The processes involved in parturition and their regulating forces are poorly understood, but the need for oxytocin, relaxin, and calcium has been demonstrated.[10,31-34] Thyroid hormones have been shown to affect the production of a number of the secretions of the anterior pituitary.[8,10,11] The high concentration of T_4 and T_3 in the hypothalamus[35-38], which was probably the phenomenon of hormonal concentration, suggested the possibility that T_3

might have some independent influence on the production of posterior-pituitary secretions such as oxytocin. Many investigators have demonstrated similar metabolic actions for T_4 and T_3.[39-44]

In the following experiments, female rats were made hypothyroid by surgery or by irradiation with At^{211} or I^{131}. Thyroid hormones was replaced with desiccated thyroid substance (TH), ℓ-thyroxine (T_4), ℓ-triiodothyronine (T_3), or a 10% T_3-90% T_4 mixture. Sufficient thyroid replacement was given to achieve standard metabolic rates within normal limits. The time interval between thyroid removal and breeding was long enough to allow for complete utilization of the thyroid hormone present in the tissues at thyroidectomy. Veterinary or C. P. calcium gluconate was administered by mouth to some of the animals for the last five days of pregnancy to test the ability of calcium compounds to promote normal parturition in thyroidectomized rats. The functional state of the parathyroids at parturition was studied by measuring the level of serum calcium in rats whose thyroids had been destroyed with I^{131}.

METHODS

The animals used in these experiments 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* and tap water ad lib. When the animals were 110 days old (75 days old in Experiment I) they were ear-marked, weighed, and the lightest and heaviest 10% of the group were discarded. 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}. Surgical thyroidectomy was carried out according to the method described by Farris and Griffith.[45] Thyroid ablation with radioiodine was accomplished by an intravenous injection of 5 microcuries (μC) of I^{131}† per gram of body weight, an amount found to be sufficient to produce severe hypothyroidism without any apparent damage to other organs.[16] Thyroid destruction with I^{131} is essentially complete, whereas the occasional small fragments remaining after surgical thyroidectomy can become quite active through compensatory hypertrophy.[46] Active extrathyroidal tissue which has been found to occur in the rat is most likely also destroyed by I^{131}.[47] The At^{211} was prepared by methods described elsewhere[48] and was administered intravenously in isotonic saline at a level of 0.62 μC per gram of body weight. This level has been found to produce maximal thyroid destruction with few acute radiation deaths.[23] Three essentially independent experiments were carried out. The general design of these three experiments, the ages of the animals, mode of treatment, and group sizes are shown in Table I.

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*Purina Laboratory Chow contains 1.3% calcium by weight derived from skim milk, fish meal, and cereals. The Ca/P ratio is approximately 1.4 on a weight basis.

† Carrier-free I^{131} was obtained from Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Table I

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

Mode of thyroidectomy	Age at thyroidectomy (days)	treatment	Group size	Average age at breeding (days)
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[b]	10	220
Controls	110		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]Chemically pure calcium gluconate by intubation daily for last 5 days of pregnancy.

Experiment I

Ten days after thyroidectomy one-half of the animals in each thyroidectomized group were placed on a supplement of desiccated beef thyroid (TH)(Armour). A freshly prepared aqueous suspension of the thyroid substance 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} thyroidectomized 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 those described by Kleiber[49] and by Contopoulos _et al._[50] as modified by Watts.[17] Before any measurements were made, the animals were conditioned to the apparatus twice daily for 30 minutes for a period of two weeks. Repeated observations were made over a period of three months or until the observations agreed within 5%.

When the animals were 160 days of age, breeding experiments were initiated. A vigorous male rat of the same strain was placed in a cage containing five females. On the following day each male was moved to the next cage to avoid sterile matings. This procedure was repeated for 14 days; then the males were removed. The females were examined daily for indications of pregnancy, and obviously pregnant rats were placed in individual cages. As the animals commenced to litter, the cages were examined several times each day, and any dead pups were removed. A record was kept of the number of young that were born alive or dead. The pertiment results of Experiment I appear in Table II.

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

Table II

The effect of thyro-parathyroid 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 replacement therapy.

Group treatment	S.M.R. ±Std. Dev. $(Cal/m^2\ hr^{-1})$	No. of Pregnancies	Avg. No. young born alive[c]	Avg. No. young born dead[d]	No. mothers not surviving 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	0.0	0.0	0
At^{211} + TH	45.2 ± 4.4	0/12	0.0	0.0	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	" "		not recorded		
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 yound 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.

Experiment II

All of the animals except the controls were thyroidectomized with I^{131} as previously described. Five days after thyroidectomy, daily subcutaneous injections of 0.1 cc of a solution of 60 µg/cc of Na-ℓ-thyroxine (Smith, Kline, and French Laboratories) were started on 20 of the 45 thyroidectomized animals. This dosage of ℓ-thyroxine has been found to sustain a normal SMR in the thyroidectomized rat.[51, 43] One-half of these animals and half of the remaining thyroidectomized animals received 1.5 grains of veterinary calcium gluconate (Haver-Lockhart Laboratories) intragastrically daily during the last 5 days of pregnancy. Ten intact untreated animals received daily sham injections of 0.1 c c isotonic saline subcutaneously and served as controls.

Two of the I^{131} thyroidectomized animals, and one of the control animals died of pneumonia during the course of the experiment and are not included in the data.

At 100 days post-thyroidectomy the animals were examined by vaginal smear to determine the estrus cycle.[52] With the appearance of epithelial cells, a normal fertile adult male of the same strain was placed in the cage with the female. The following morning the appearance of motile sperm or a sperm plug indicated conception had probably occurred, and the male was removed. This was then considered day 1 of pregnancy.[53] The results of the breeding appear in Table II. At the conclusion of all pregnancies the thyroxine was withdrawn. All surviving animals were sacrificed 60 days later. Withdrawal of the thyroxine was necessary to avoid thyroidal blocking of a tracer dose of I^{131} used to determine the completeness of thyroidectomy as previously described.[54, 55]

Experiment III

Five groups of animals, 10 animals per group, were used. Forty of them were thyroidectomized with I^{131} by previously described methods and dosages. The remaining 10 animals served as controls. The first group received 1.2 µg of triiodothyronine (T_3), an amount reported to sustain a normal SMR in thyroidectomized rats.[43, 56-58]

The second group received a mixture of 6.3 µg of thyroxine and 0.12 µg of triiodothyronine (T_4+T_3) -- the proportions of these two hormones being those found by Taurog in the thyroid vein of the sheep.[59] The third (T_4) and fourth groups (T_4+Ca) were given 7 µg of ℓ-thyroxine.[43] The hormones were administered daily by subcutaneous injection. In addition, the last group (T_4+Ca) was given 65 mg of calcium gluconate (C. P.) intragastrically daily during the last 5 days of pregnancy.

After 60 days of administration of the hormones, metabolic-rate determinations were made as previously described. The results appear in Table II.

The animals were bred by placing a male of proven fertility in the cage with the female for seven consecutive days. Lack of space restricted breeding to only twenty at a time. The females were examined daily for signs of pregnancy and time of onset of labor. Abnormal parturition was obvious soon after the breeding schedule was underway; therefore, as many of the remaining animals as possible were sacrificed as soon as they went into labor and serum calcium was measured. The blood was taken during labor, immediately following delivery or during the final stages of tetany. The animals were anesthetized with ether and the abdomen opened. Ten cc of blood were aspirated from the inferior vena cava into a heparinized syringe. The animals were then sacrificed by an overdose of ether. Red cells were separated by centrifugation, and serum calcium was determined by the method of Baron and Bell.[60] The total number of fetuses and the number of dead fetuses were recorded. The results appear in Table III.

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	Post-partum	11.82
2334	Post-partum	12.28
2353	In normal labor	10.20
2339	Delivering normally	10.70
2367	Post-partum	10.83
2329	Post-partum	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	In tetany	3.83
2359	In tetany	3.12
T_4 + Ca		
2366	In normal labor	9.12
2369	In normal labor	8.85
2346	In tetany	4.08
2365	In tetany	4.46

RESULTS

Completeness of Thyroid Removal

In Experiment I, thyroid destruction with either of the radioactive isotopes, I^{131} or At^{211}, lowered the standard metabolic rate to 65% of the control value (Table II), an observation in agreement with the findings of Watts.[16] Surgical removal of the thyroid lowered the metabolic rate to only 85% of the normal mean. The range of SMR values of the surgical group overlapped the normal range; however, the t test of Fisher indicates that these two means are significantly different ($p<01$).[61] Surgical removal of the thyroid gland was considered complete in five of the nine rats given an I^{131} tracer 24 hr before sacrifice;* I^{131} uptake in peritracheal tissue was less than 0.1%. The I^{131} uptakes of the other four animals ranged from 0.83% to 2.18% of the administered dose; however, there was no correlation between high uptake of the I^{131} tracer (indicating the presence of functional thyroid tissue) and a high SMR or better-than-average ability to conceive.

Thyroid destruction with I^{131} was judged complete in all cases, as indicated by the average uptake of the I^{131} tracer of less than 0.1% (range 0.004% to 0.05%) in Experiments I and II.

As reported previously, At^{211} was not as efficient an agent for thyroid destruction as I^{131}.[23] The mean uptake of the I^{131} tracer by the At^{211} irradiated animals was 0.4% (range 0.01% to 1.4%). As in the group whose thyroids were removed surgically, the At^{211}-treated rats showed no correlation between a higher-than-average uptake of the I^{131} tracer and a higher-than-average SMR.

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* One animal died during labor, and three others succumbed to pneumonia before they could be autopsied.

Adequacy of Thyroid Replacement Therapy

Desiccated thyroid substance at a level of 9 mg per week in the surgically thyroidectomized animals, and 12 mg per week in the At^{211}-thyroidectomized animals brought the standard metabolic rates within the normal range, while 12 mg per week was not sufficient to bring the I^{131}-thyroidectomized animals to this level. Evans points out that 1 μg of thyroxine per day is sufficient to maintain the integrity of the pituitary and to promote normal growth and development.[30] The thyroid therapy was therefore considered adequate for the purpose of these experiments. The number of pregnancies in this group, 8 of 12 rats, suggests that the amount of thyroid hormone needed for normal reproduction is less than that required to support a normal standard metabolic rate.

In Experiment III, the use of T_4 (45.5 μg per week), T_3 (8.4 μg per week), or a mixture of 90% T_4 and 10% T_3 as thyroid replacement brought the metabolic rates well within the control limits. Standard metabolic rates were not measured in Experiment II; however, the T_4 treatment was essentially the same as in Experiment III, and for the purposes of this paper, the animals in Experiment II were considered to be receiving adequate thyroid-hormone replacement.

Reproduction Following Thyroid Destruction or Removal

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

Reproduction Following Thyroid Replacement Therapy

The reproductive capacity in all thyroid deficient animals (except those thyroidectomized with At^{211}) was considerably enhanced by giving them some form of replacement thyroid hormone. The number of pregnancies was increased 31% (15 pregnancies in 49 thyroid-deficient animals) to 84% (67 pregnancies in 80 thyroidectomized animals that received supplemental thyroid hormone). There were 23 pregnancies in 29 rats (79%) in the three control groups. The radiation damage to the ovaries of the At^{211}-treated rats was apparently not repaired to any significant extent by thyroid therapy.

Average Litter Size and Viability of Offspring Following Thyroid Destruction or Removal

Litters of the thyroid-deficient animals were somewhat smaller--four to seven animals per litter--than those of the control animals--six to thirteen pups per litter. Very frequently, stillbirths were delivered by animals whose thyroids had been removed surgically or irradiated with I^{131}. In most of these cases the number of young delivered alive was only 50% of the total litter. Still births were rare in the control groups, as shown in Table II.

Viability of Offspring and Average Litter Size Following Thyroid Therapy

When thyroid therapy was employed, the litter sizes were closer to normal, averaging six or more pups per litter in Experiments I and II. Daily thyroid treatment in Experiment III increased the number of pups per litter to the control average. The average number of still births per litter was very high even when animals were maintained on a thyroid supplement. Still births ranged from approximately 30% of the pups delivered by the $I^{131} + T_4 + T_3$ group in Experiment III to 75% in $I^{131} + T_4$ group of Experiment II. The higher proportion of live "births" in the I^{131} T_4 group of Experiment III is somewhat misleading. Most of these latter animals were sacrificed during parturition for serum calcium determinations, and only total litter size should be considered. The numbers of surviving pups in the $I^{131} + T_4 + T_3$ and $I^{131} + T_3$ groups are higher than for the other thyroid-therapy animals in the two earlier experiments. When the animals in Experiment III were found to be going into tetany (a constant vigil being kept on their progress), they were autopsied, and a number of live pups were generally found in utero. Had these animals not been watched so closely, the fetuses would most likely have died shortly after the death of the mother, and they would have been recorded as dead in utero.

Length of Gestation

The length of gestation was observed accurately and recorded only in Experiment II. Day 1 of pregnancy was established by the method of Blandau.[53] The gestation period of the control animals averaged 23 days (range 21 to 23 days); the $I^{131} + T_4$ group 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) was 23 days.

Maternal Survival

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

Effect of Exogenous Calcium on Viability of Young and Survival of Mothers

In Experiments II and III, 17 pregnant rats were given either veterinary or C. P. calcium gluconate by mouth daily during the last five days of pregnancy. Five of these animals (30%) died in labor or were in active tetany when sacrificed. Included in the data is one animal that received calcium gluconate for only 2 days when labor began. Oral administration of either veterinary or C. P. calcium gluconate did not 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 animals that received both thyroid hormone and the calcium supplement delivered large litters without incident, and no still borns were found. All six of these litters were successfully suckled until weaning. On the other hand, half of the young born to the 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.

Serum Calcium Levels at Term

In the third experimental series, it was necessary, because of the large number of animals involved, to breed them one group at a time. Although the number of pregnancies was high, and the litter sizes were close to normal for the T_3 and T_4 + T_3 groups, it was immediately obvious that these animals were having considerable difficulty in delivering their litters. One-third of their young, on the average, were delivered dead, and eight 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 tissue taken from I^{131}-irradiated animals, the parathyroid tissue, while still grossly intact, showed some indications of structural alteration. The parathyroid structural changes are discussed in detail in the next section. The observations of others concerning absence of damage or minimal damage to the parathyroids, as well as our own previous measurement of nearly normal serum-calcium levels in rats whose thyroid glands had been destroyed with I^{131}, applied strictly to animals under little if any stress. It was considered quite likely that termination of pregnancy might produce sufficient stress to disclose a partial parathyroid impairment. The remaining experimental and control rats were bred and observed with great care so that blood samples for serum-calcium measurement might be taken during or very close to labor. Serum-calcium levels, and the stage of labor when the blood samples were taken are shown in Table III for ten normal controls, nine animals in the I^{131} + T_4 group, and four animals in the I^{131} + T_4 + Ca group.*

*Four rats in these two groups either had died or successfully delivered their young before blood samples could be obtained.

Animals are designated in the Table as "in normal labor"--contractions and (or) vaginal bleeding but no pups born; "delivering normally"--one pup delivered, but mother was sacrificed before the placenta was eaten; or "post partum"--some or all of the pups delivered and placentae eaten. Some animals showed convulsions or rigidity--these are designated as "in tetany". The range of control values was 9.95 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 from 3.12 mg% to 5.16 mg%.

Histology of the Parathyroid

Tissue remnants were taken from the thyroid region of 18 I^{131} -irradiated animals in Experiment I, and from 13 I^{131} -irradiated rats in Experiment III for which pre-autopsy serum calcium measurements were also available. Both parathyroids were identified in only nine of the 31 specimens examined. 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 parathyroids taken from I^{131} -irradiated rats could, upon careful examination, be classed as undamaged. In general, these glands were smaller than in the 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 surface. Only rarely were blood vessels seen to be penetrating this capsule. The reticular network of the irradiated glands was very 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 each other by

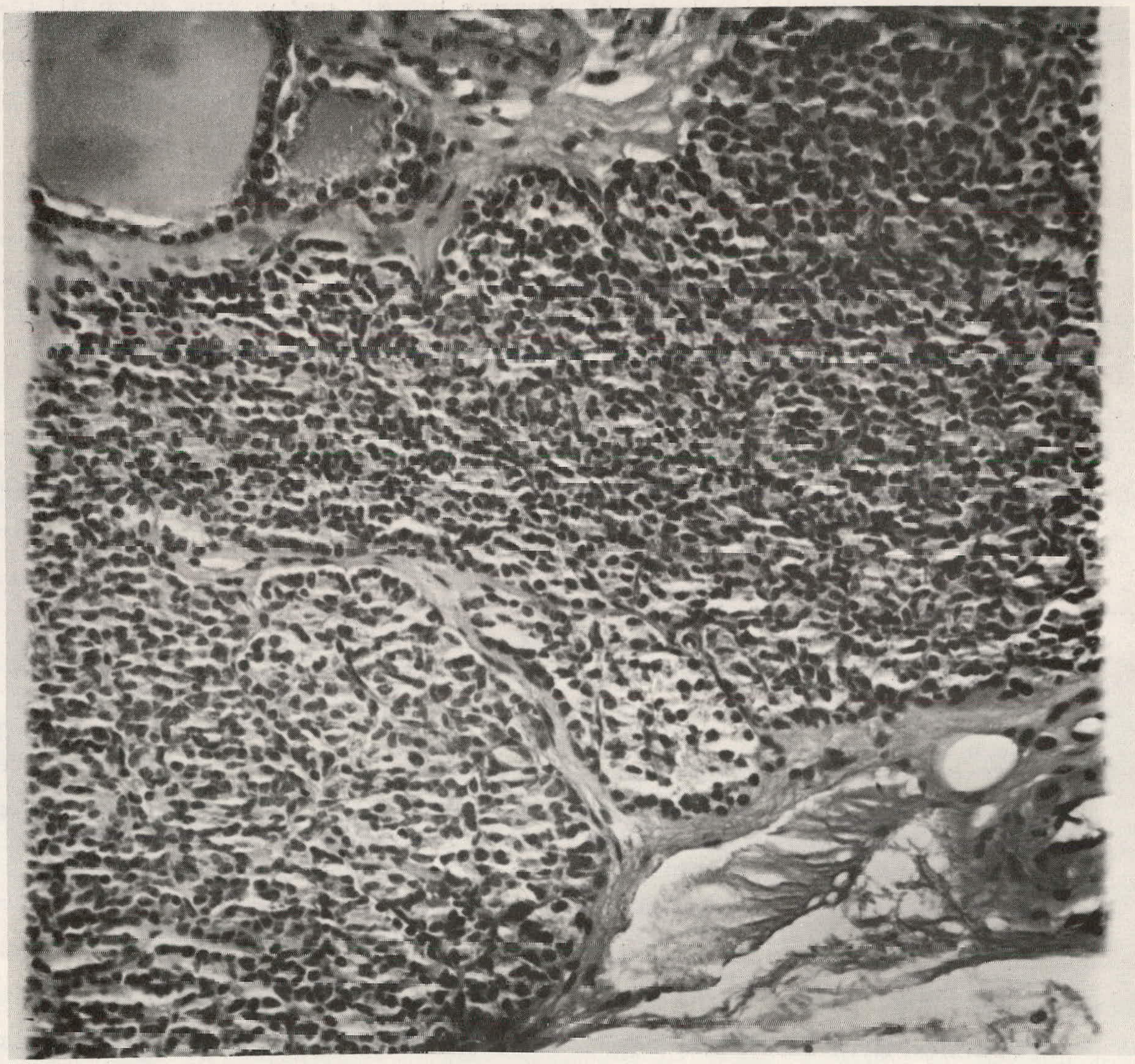

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Fig. 1. Normal parathyroid tissue taken from a control animal in Experiment I. Note capsule of loose connective tissue and delicate reticular network. Cells in central region are arranged in cords; those at the periphery are arranged in clumps. Sectioned at 6 μ, H and E, x 220.

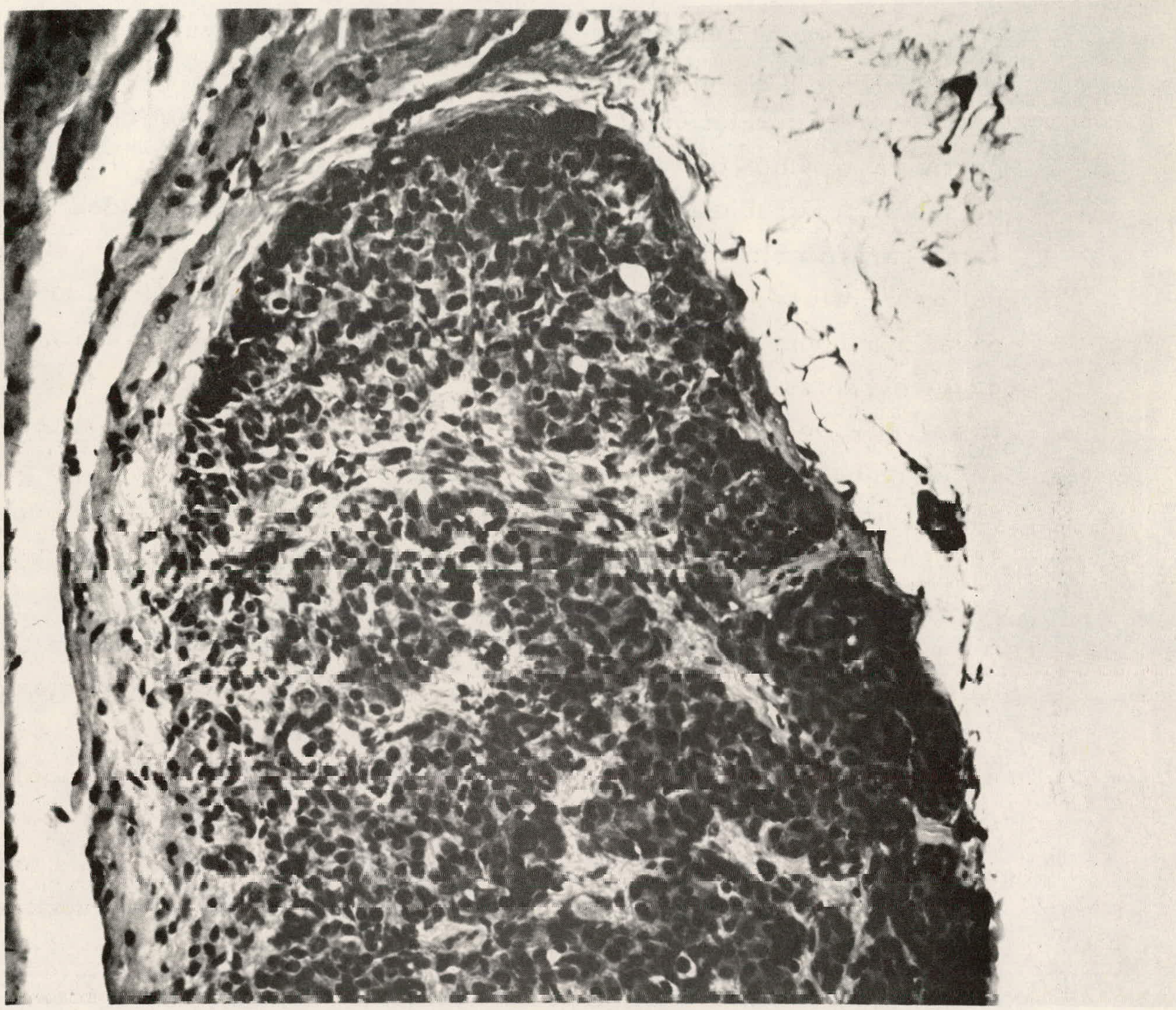

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Fig. 2. Parathyroid from rat No. 2337; serum calcium 10.49 mg% during normal delivery. There is a thick fibrous replacement of the thyroid. Note the conspicuous reticulum and reduced cellularity in the region nearest the trachea. This gland had apparently been located on surface of thyroid. Sectioned at 6 μ, H and E, x 220.

connective tissue septa (Fig. 3) to densely cellular, deeply staining tissue with a structure 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 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 could be seen in the section of 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. When the identification of the slides was covered, 10 of 13 specimens from Experiment III were correctly diagnosed as probably having a "normal" or "very low" serum calcium level. In those for which no correlation could be established, one animal with a serum calcium of 9.12 mg%, the parathyroid tissue was apparently missed during sectioning. 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 at 8.74 mg%. In the third case, again only one parathyroid was found. One-third of the tissue was fibrous replacement. There were a few peripheral cells with darkly staining cytoplasm. A normal serum calcium of 10.49 mg% was found. 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 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 fit with their serum calcium level and general condition. Although considerable fibrous invasion was seen, the groups of cells retained their normal glandular structure. They had abundant cytoplasm, and the capillary bed was extensive. These four had at least one parathyroid that appeared to have been on the surface of the thyroid gland before irradiation. This favorable geometric location could account for the survival of much of the tissue which was only

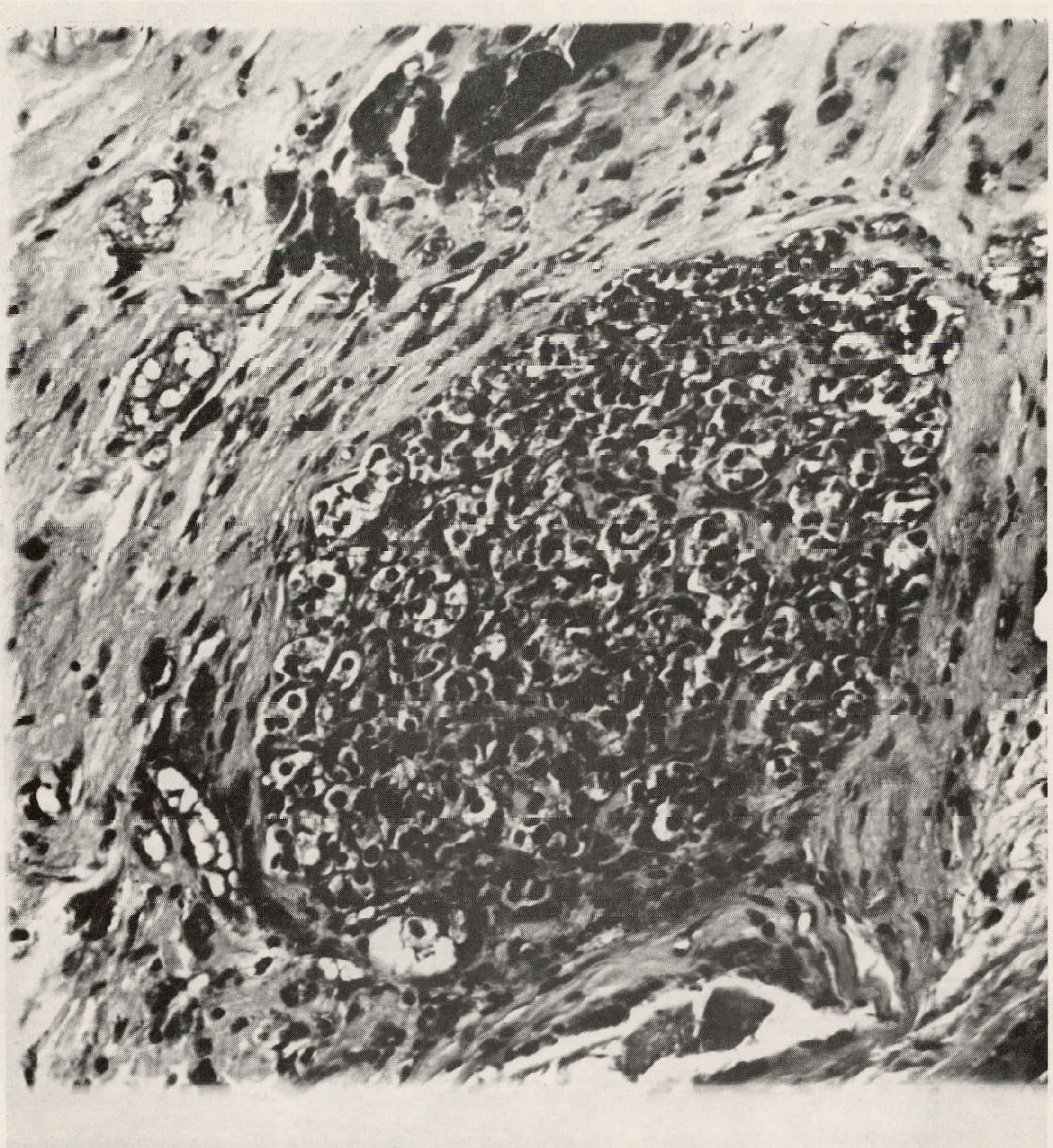

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Fig. 3. Parathyroid from rat No. 2348; serum calcium 3.83 mg%. Animal in tetany attempting delivery. Note thick fibrous capsule, and small clusters of cells separated from each other by connective-tissue septa. This gland apparently had been embedded deep within the thyroid tissue. Sectioned at 6 μ, H and E, x 220.

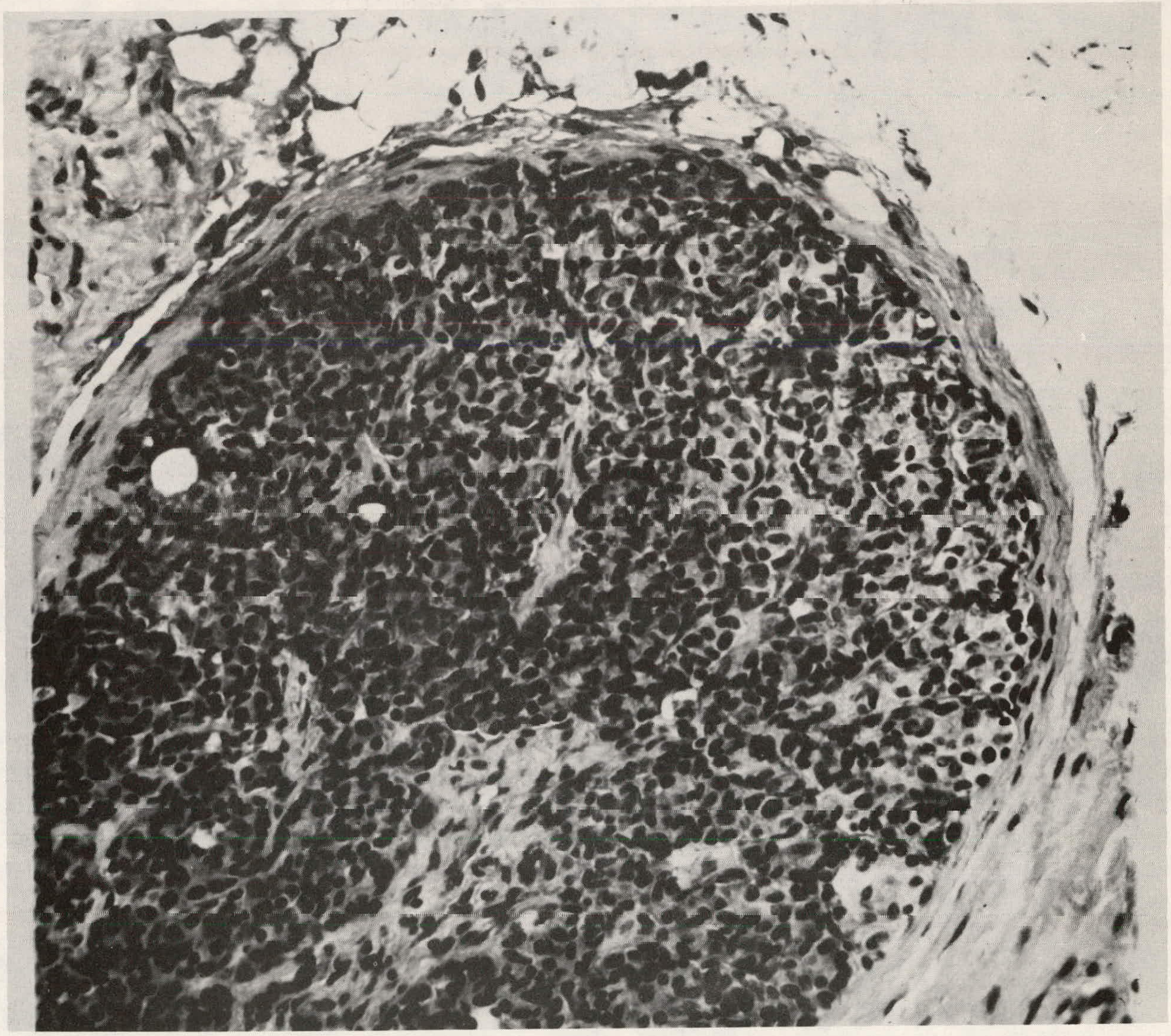

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Fig. 4. Parathyroid from rat No. 2369; serum calcium 10.40 mg% during normal delivery. Note conspicuous reticulum and variability of cellularity and grouping of cells--cords and sheets at the upper right and clusters at the lower left--reminiscent of normal parathyroid tissue. Sectioned at 6 μ, H and E, x 220.

partially irradiated by the I^{131}. The presence or absence of considerable damage could almost be judged on the basis of the spatial relationship of one or both parathyroids to the thyroid gland, at least insofar as this relationship could be reconstructed.

DISCUSSION

Our experiments support the results obtained by many investigators--thyroid ablation with I^{131} is a far more effective means of thyroid destruction than surgical removal of the gland.[25, 26, 62] However, this procedure does not necessarily preclude parathyroid damage,[7] as will be discussed later.

The long life of thyroid hormone in the animals--greater than 40 days according to Watts[16]--has lead to many of the contradictions in studies of the relationships between hypothyroidism and reproduction. The fallacy in neglecting the long life of this hormone is demonstrated by the experiments of Bruce and Sloviter on mice.[63] They state: "In the present study, the complete loss of thyroid tissue had no significant effect on the ability of the mice to mate and conceive young." They also state: "Gorbman found reproduction 'is virtually halted' in female mice given enough I^{131} to destroy all thyroid tissue", and further, "he describes the ovaries as being 'devoid of ova'", which is contrary to their findings. "There is no obvious explanation for the differences in results except that different strains of mice and different basal diets were used." The apparent error arises in their statement: "The observations on reproductive function in the present investigation were made relatively soon after induction of the thyroid-deficient state". This statement is quite obviously true, since their results show the animals were bred only 21 days after thyroidectomy. The hypo-or, preferably, athyroid condition thus would not be established until the 19-day gestation period had already been completed.

The obvious reduction in fertility shown in our experiments appears to be directly related to the absence of circulating thyroid hormone. Similar observations have been made by several workers[2, 3, 5, 64] and not admitted by another.[65]

Fertility was nearly normal in surgically thyro-parathyroidectomized animals when given thyroid supplements and did not seem to be impaired by the absence of the parathyroid glands as suggested by Bodandky and Duff.[21] Fertility was also close to normal in I^{131}-thyroidectomized rats when triiodothyronine or thyroxine were given in sufficient amounts to maintain a normal standard metabolic rate.

We were unable to demonstrate any beneficial effect of desiccated thyroid substance, thyroxine, or triiodothyronine therapy on parturition. An increase in protein-bound iodine has been demonstrated in pregnant women near term.[66] This substantial increase in thyroid hormone output at the end of gestation might have some effect on parturition, but it was not seen in these experiments.

The lack of understanding of the difficulties encountered during parturition in the I^{131}-thyroidectomized rat was apparently complicated by our own experimental work[27] as well as that of other investigators.[5, 25, 26, 62] An acute need for additional serum calcium at term was not anticipated. Previous work gave no indication that severe parathyroid deficiency might be produced occasionally during radioiodine thyroid ablation. Following the "discovery" of very low serum-calcium levels (less than 6 mg%), the parathyroid glands were specifically sought at necropsy, and histological preparation 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 any kind of stress. A large percentage of this calc ium may be in a nonutilizable form since its source is fish meal or vegetable matter. Thus, it would not meet the standards of the high-calcium diet recommended by Cox and Imboden for support of parathyroidectomized rats.[31] Augmenting this diet with additional calcium in the form of 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 (T_4 + Ca in which four of seven animals were in tetany) did not aid maternal survival or markedly improve litter survival. These observations suggest that oral administration of calcium in the form of 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 who found that parathyroidectomy in a lactating rat reduced considerably the calcium content of the milk for a period of two days.[67] At this time, the calcium returned to a concentration that exceeded that of the milk of normal lactating rats.

Some mechanism appears to be operative during lactation that comes into play in the absence of the parathyroid glands. This poses an interesting problem: Is there a mechanism associated with lactation responsible for an absolute increase in absorption of dietary calcium, or an increase in dietary intake of calcium by stimulation of food intake while the absolute absorption level remains constant?

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