Title

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ABSTRACT

An attempt is made to separate the endocrine and radiation effects on breeding. The thyroid was destroyed by surgery and by the radioactive halogens astatine and iodine. One-half the experimental animals were fed desiccated thyroid substance to produce a more nearly normal endocrine balance.

Standard metabolic rates and I^{131} thyroidal tracers for all groups are given, the SMR's for the surgically operated animals were 25% higher than those thryoidectomized with radioiodine.

Gross histological examinations showed a degradation in ovaries from normals through the astatine-treated group, which failed to respond to thyroid therapy on conception.

Surviving pups were few in number at parturition. Although many fetuses were expelled they were stillborn.

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It has been shown by Ross (1938) that breeding was markedly impaired in surgically thyroidectomized rats. In her opinion, littering could be correlated with incomplete thyroidectomy. Parrott et al. (1955) reported complete lack of breeding in a small group of rats "thyroidectomized" with 0.8 μC per g of body weight of At^{211}, an alpha-particle-emitting isotope of the heaviest halogen. Hamilton et al. (1953) investigated the distribution of small doses of At^{211} and radioactive iodine, I^{131}, in rats. During the first 24 hours after administration, ovarian retention of At^{211} was more than six times that of I^{131}; 4 hours postinjection the ovaries contained 0.18% per gram of wet tissue of I^{131}, as contrasted to 1.4% per gram for At^{211}.

With these methods of thyroid destruction at hand we thought it might be possible to determine the influence on breeding of (a) the lowered metabolic activity and endocrine inbalance, and (b) the direct action of ionizing radiation.

The following schedule was devised to study the effects of endocrine disturbance and radiation damage separately:

1. Normal controls
2. Surgical thyroidectomy: thyroid deficiency
3. Thyroid ablation with I^{131}: thyroid deficiency, moderate radiation effects
4. Thyroid ablation with At^{211}: thyroid deficiency and notable radiation damage to ovaries.
5. Surgical thryoidectomy plus TH (desiccated beef thyroid): repaired thyroid deficiency.
6. Thyroid ablation with I^{131} plus TH: repaired thyroid deficiency; only the radiation effects should be observed.

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[1] Deceased

7. Thyroid ablation with At^{211} plus TH: thyroid augmented; only radiation effects should be observed.

Method

The animals used in this study were female rats of the Sprague-Dawley strain, acquired from the original colony when 35 to 45 days of age. After rejection of the lightest and heaviest 10% of each shipment, the animals were housed on wood shavings in metal stock cages and were fed tap water and pelleted Purina Laboratory Chow ad lib. Respiratory infections were controlled by administering aureomycin (unrefined, Lederle) at a level of 2.5 grams per pint of drinking water for two days every other week. At the age of 58 to 73 days, the animals were weighed, earmarked, and divided at random into three groups of 20 to 30 animals each.

The animals in the first group were thyroidectomized surgically according to the method described by Farris and Griffith (1949), the second group was "thyroidectomized" with I^{131}, and the third group with At^{211}.

The carrier-free I^{131}, obtained from the Oak Ridge National Laboratory, was administered intravenously in isotonic saline at a level of 5 μC/g body weight, an amount determined by a pilot experiment to be sufficient to destroy the thyroid gland without producing notable radiation damage in other tissues. The At^{211} was prepared by methods described by Parrott et al, (1955), and was administered intravenously in isotonic saline at a level of 0.62 μC/g body weight. This level has been found to produce maximal thyroid destruction with few acute radiation deaths (Hamilton et al., 1954). Ten intact rats served as controls.

Ten days after either surgical or radiation thyroid ablation, one-half the animals in each treated group were placed on a supplement of TH (desiccated beef thyroid, Armour). A freshly prepared aqueous suspension of the thyroid substance was administered intragastically three times weekly. The surgically thyroidectomized rats received 9 mg/wk of active thyroid substance, and the "radio-thyroidectomized" rats received 12 mg/wk. Evans (1956) has shown that while 1 μg/day of thyroxine suffices to maintain skeletal growth and the structural integrity of the anterior pituitary, 5 μg/day is needed to support a normal basal metabolic rate in I^{131}-thyroidectomized rats.

The standard metabolic rates (SMR's)* of the animals in each experimental 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 (1940), and Contopoulos et al. (1954), as modified by Watts (1955). 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 on all the animals over a 3-month period, or until a pair of consecutive determinations agreed within 5%.

When the animals were 150 days of age (3 months postthyroidectomy), breeding experiments were initiated. A vigorous male rat was placed in each cage containing five females. On the following day each male was moved to the next cage. 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 breeding cages. As the animals commenced to litter, the cages were examined several times each day, and the dead young were removed. A record was kept of the approximate length of gestation, total number of young, and the number of surviving young, regardless of litter size. Those animals that appeared to be pregnant, but did not deliver at term, were kept separate for observation for an additional two weeks before being returned to the stock cages.

Eleven months after the start of the experiment, all animals were given 20 μC of I^{131} intramuscularly. The animals were sacrificed with chloroform, and their tracheas were removed to determine completeness of thyroid ablation. The I^{131} uptake in the thyroid glands (or thyroid remnants) and thymi was determined by measuring gamma activity with a NaI(-Tl) scintillating crystal, and a Tracerlab autoscaler. The ovaries, thymi, adrenals, and tumors (when present) were dissected, weighed, and fixed in 10% neutral formalin. Unfortunately, the histological preparations are not relevant to the etiology of the problem encountered, owing to the tremendous difference between age at conception and age at autopsy. This would obviously interfere with any conclusions drawn from a serious investigation of the tissues.

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*We prefer this expression to BMR, as the metabolic rates are rarely basal.

Results

Table I shows the standard metabolic rates (SMR's) of female rats thyroidectomized by surgery, I^{131}, or At^{211}, and three similarly thyroidectomized groups that received supplemental desiccated thyroid substance. The differences in SMR between normal controls and the radiothyroidectomized experimental animals coincided with those reported by Watts (1955); however the normal SMR's were slightly higher in these younger animals in accordance with the age changes in SMR shown by Kleiber (1956).

The results of the breeding experiments are presented in Table II. (The various groups are arranged in the table from top to bottom in the order of increasing deviation from normal littering.) It may be seen from this table that administration of TH somewhat improved the number of pregnancies and quality of litters in the surgically thyroidectomized group. The most notable improvement was seen in the I^{131}-thyroidectomized animals treated with TH, in which the number of pregnancies was greatly increased, although the litter quality remained poor. There was no correlation between either higher SMR or greater uptake of the I^{131} tracer in the three I^{131}-thyroidectomized animals that produced nearly normal litters.

Both groups of At^{211}-treated rats failed either to conceive (there were no false pregnancies observed in these groups) or to litter, which agrees with previous findings (Parrott et al., 1955). The SMR's were maintained at nearly normal levels by administration of thyroid substance, presumably creating a better endocrine balance.

A few false pregnancies did occur in the I^{131}- and surgically thyroidectomized rats. For the most part these animals returned to normal size within a few weeks, but autopsy at this late date failed to reveal any resorbing fetuses.

There was a surprisingly large number of pregnancies in these surgically or I^{131}-treated animals thyroidectomized long before breeding. A great number of these pregnancies however, terminated in abnormal litters. Many of the fetuses reached full term and were normal in gross appearance at birth, but did not survive even a few minutes.

Table I

Comparative Metabolic Rates in Thyroidectomized Rats

Treatment	No. of animals	Mean SMR ($cal/m^2/hr$)	Range of SMR (mean Std. dev) ($cal/m^2/hr$)	I^{131} uptake* (%)
Controls	10	51.6	47.8--55.4	7.700
Surgical thyroidectomy + thyroid administration	12	47.8	42.6--53.0	0.201
Surgical thyroidectomy	13	44.0	38.7--49.3	0.717
I^{131} thyroid ablation + thyroid administration	12	35.4	31.4--39.4	0.003
I^{131} thyroid ablation	13	33.8	28.4--39.2	0.009
At^{211} thyroid ablation + thyroid administration	12	45.2	40.8--49.6	0.156
At^{211} thyroid ablation	10	33.4	28.1--38.7	0.377

*In thyroid or peritracheal tissue.

Table II

Comparative Littering by Thyroidectomized Rats

Treatment	Numbers of animals					
	Total in test groups	Non-pregnant	Very abnormal	Abnormal	Normal	% Littering
Controls	10	3	0	1	6	70
Surgical Thyroidectomy + thyroid administration	12	2	5	2	3	84
I^{131} + thyroid administration	11	4	6	1	0	64
Surgical thyroidectomy	13	6	5	1	1	54
I^{131} thyroid ablation	13	10	0	2	1	23
At^{211} + thyroid administration	12	12	0	0	0	0
At^{211} thyroid abtation	10	10	0	0	0	0

Very abnormal: all offspring born dead, or female died attempting to deliver.
Abnormal: one or two offspring surviving, the remainder stillborn.
Normal: all except one or two offspring survived.

A summary of ovary weights and of gross autopsy findings for ovaries and uteri is shown in Table III. The ovaries and uteri of the two groups of surgically thryoidectomized animals were nearly indistinguishable from those of the normal controls. Most of these 25 animals had well-developed vascular uteri. The ovaries of all but three of these 25 rats contained many apparently viable follicles and numerous recent corpora lutea. The ovaries of the I^{131}-thyroidectomized rats that did not receive TH therapy were significantly smaller than those of normal rats. The TH supplement after thyroidectomy with I^{131} aided in the maintenance of the ovaries (as judged by weight). Examination of histological preparations from the surgically or I^{131}-thyroidectomized rats showed no correlation between apparently normal ovaries and normal littering, or hypoactive ovaries and abnormal littering. Seventy-five percent of the animals in both groups whose ovaries were judged normal or were only mildly hypoactive produced normal litters. None of the animals with ovaries that were diagnosed as inactive conceived. This condition, however, was restricted to the At^{211}-treated animals.

In contrast to the I^{131}-thyroidectomized-TH-treated group whose ovarian weights were maintained within the normal range, the TH supplement effected only a 25% increase in ovarian weight of the At^{211}-thyroidectomized rats (still 60% below that of intact controls). The TH supplement failed to effect repair of the ovaries, or to prevent ovarian atrophy following At^{211}. These results suggest that the ovaries were irreversibly damaged by At^{211} alpha irradiation.

Discussion

The SMR's of the surgically thyroidectomized rats were 25% greater than those of the animals "thyroidectomized" by either I^{131} or At^{211}. It is somewhat difficult to reconcile our results with those of Gemmill et al. (1956), who state that there is essentially no difference in basal metabolic rate between animals thyroidectomized surgically or with radioiodine. SMR data from the surgically thyroidectomized rats, with terminal thyroidal accumulations of more than 0.5% of the tracer dose of I^{131}, were examined to see if there were any correlation between high I^{131} uptake and an above-average SMR. All SMR's for these animals were well within two standard deviations of the means of their respective groups. Therefore all experimental animals

Table III

Comparison of Gross Autopsy Findings in Sex Organs of Thyroidectomized Rats

Treatment	Mean weight of one ovary	Gross condition of ovaries	Gross condition of uterus	Age at autopsy (days)
Control	26.0	Highly vascular many developing and ripening follicles. Many corpora lutea. (1 specimen encysted.)	Large and well vascularized	418
Surgical thyroidectomy + thyroid administration	30.8	Within normal limits. (2 specimens encysted.)	Fairly well vascularized, fluid-filled.	413
Surgical thyroidectomy	37.8	Low normal - mostly corpora lutea. (2 specimen encysted.)	Varied from well to moderately to poorly vascularized	413
I^{131} thyroid ablation + thyroid administration	32.4	Within normal limits.	Mostly well vascularized	268
I^{131} thyroid ablation	22.3	Low normal. (1 specimen atrophic.)	Mostly moderately vascular.	397
At^{211} + thyroid ablation + thyroid administration	10.4	One specimen low normal. Remainder atrophic.	Generally very poorly vascularized. One specimen with a small polyp.	237
At^{211} thyroid ablation	7.7	All specimens avascular and atrophic.	Moderate to very poor vasculature. Three Specimens with small polyps.	386

were included in the calculations. Microscopic examination of peritracheal remnants showed some regeneration of thyroid tissue in a few of the animals several months after the breeding tests.

The administration of desiccated thyroid substance enhanced, but did not entirely reproduce, a normal standard metabolic rate in rats thyroidectomized with I^{131}, with At^{211}, or by surgery, although an increase of as much as 30% was observed in those treated with At^{211}. The increase in the quantity of desiccated thyroid fed to the radiothyroidectomized groups was initiated after the demonstration of functional thyroid tissue in the thymus glands of rats of this strain [Asling et al. (1957)]. The higher percentage of pregnancies in the surgically thyroidectomized rats (than in the rats thyroidectomized with I^{131}) may be the result of the better endocrine balance in the presence of small amounts of thyroid hormone elaborated by such ectopic sites of thyroid tissue. These sites, if functional when the I^{131} was given, would have been destroyed along with the normal thyroid tissue.

Nelson and Tobin (1957) surgically thyroidectomized rats during pregnancy and obtained normal littering. Human beings thyroidectomized with I^{131} during pregnancy have experienced normal parturition, Russell et al. (1957). The I^{131}, however, concentrated in the fetal thyroid (as well as in that of the mother), causing cretinism. Friis and Hall (1957) also demonstrated normal delivery--but of cretin young--in the I^{131}-treated pregnant rabbit. Moreover, they demonstrated that exogenous I^{131}-labeled l-thyroxine did not pass through the placenta. Bruce and Sloviter (1957) showed that mice thyroidectomized with I^{131} had prolonged oestrus cycles and gestation periods, and bore fewer young than normal controls. Krohn and White (1950) also found that hypothyroid rats ovulated and conceived, but bore fewer young. Resorption of fetuses and stillbirths were common and accounted for the reduced litter size.

Bodansky and Cooke (1937) and Bodansky and Duff (1941) state that failure of the newborn to survive is related to parathyroid deficiency. Tetany was not apparent in any of the animals in our experiments. Gorbman (1950) describes the presence of parathyroid tissue in I^{131}-thyroidectomized mice, but he did not test its functional capacity. Goldberg et al. (1950) also reported the presence of apparently viable parathyroid tissue after a thyroidectomizing dose of I^{131}. Apparently functional parathyroid tissue

was demonstrated in histological sections of the tracheas of our I^{131}-thyroidectomized animals.

Our study and those cited above strongly suggest a relationship between severe hypothyroidism and abnormal parturition, i.e., increased resorption of fetuses and delivery of stillborn young. In our experiments supportive thyroid therapy seemed to augment the number of conceptions in the thyroidectomized animals, but it did not materially affect the viability of their offspring. Note particularly the difference between the I^{131}-thyroidectomized rats and their TH-treated counterparts, in numbers of pregnancies and in numbers of abnormal litters.

Summary

The surgical thyroidectomy procedure reduced the standard metabolic rate 14.7% below that of the normal animal, while radioiodine- and radio astatine-thyroidectomized animals had standard metabolic rates 34.4% below the control mean.

The thyroidectomized animals had fewer litters, and each litter contained fewer live young owing primarily to the large number of still births.

Desiccated thyroid substance raised the SMR to low normal, but it did not affect the ability of the animals to conceive.

Desiccated thyroid substance increased the percentage of animals littering but did not increase the number of surviving young.

The At^{211}-thyroidectomized rats were completely sterile, demonstrating irreparable ovarian damage.

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References

1. Asling, Durbin, Parrott, Johnston, Hamilton, Proc. Soc. Exptl. Biol. Med. 94, 200 (1957).
2. Bodansky, M., and W. R. Cooke: Proc. Soc. Exptl. Biol. Med 36, 188. 1937.
3. Bodansky, M., and V. B. Duff, J. Nutrition 21, 179 (1941).
4. Bruce, H., and H. A. Sloviter: J. Endocrinol. 15, 72. 1957.
5. Contopoulos, Evans, Ellis, and Simpson, Proc. Soc. Expt. Biol. Med. 86, 729 (1954).
6. Evans, E. S., Dissertation for Ph. D., University of Calif., 1956.
7. Farris, E. J., and J. Q. Griffith, Jr.: J. B. Lippincott Co. 2nd Ed. 1949.
8. Friis, T., and T. Hall, Acta Endocrinol. 24, 411 (1957).
9. Gemmill, C. L., K. R. Crispell, and K. M. Browning, Endocrinology 59, 522 (1956).
10. Goldberg, Chaikoff, Lindsay, and Feller, Endocrinology 46, 72 (1950).
11. Gorbman, A., J. Clin. Endocrinol. 10, 1177 (1950).
12. Hamilton, Asling, Garrison, and Scott, Univ. Calif. Publ. vol. 2, 283. (1953).
13. Hamilton, Durbin, and Parrott, J. Clin. Endocrinol. and Metabolism 14, 1161 (1954).
14. Kleiber, M. M., Univ. Calif. Publ. Physiol. 8, 207 (1940).
15. Kleiber, Smith, and Chernikoff, Am. J. Physiol. 186, 9 (1956).
16. Krohn, P. L., and H. C. White, J. Endocrinal. 6, 375 (1950).
17. Nelson, W. O., and C. E. Tobin, Endocrinology 21, 670 (1937).
18. Parrott, Durbin, and Hamilton, in Medical and Health Physics Quarterly Report, UCRL-3268, Jan. 1956.
19. Parrott, Garrison, Durbin, Johnston, Powell, and Hamilton; The Production and Isolation of Astatine-211 for Biological Studies, UCRL-3065, July 1955.
20. Ross, Renata, M., Dissertation for Ph. D. (University of Chicago Libraries, 1938, private ed) pp. 1-67.
21. Russell, Rose, and Starr, Surg. Gynecol. Obstet. 104, 560 (1957).
22. Watts, R. W. E., Proc. Soc. Exptl. Biol. Med. 89, 220 (1955).

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