TITLE CYCLIC VARIATIONS IN THYROID FUNCTION OF MATURE FEMALE RATS AND MICE

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UNIVERSITY MICH. STATE COLL. DATE 1952

DEGREE Ph. D. PUBLICATION NO. 4701

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By

FOUAD ATALLA SOLIMAN

A THESIS

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology and Pharmacology

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Approved E. P. Reineke

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ABSTRACT

A series of experiments was performed to determine some of the factors involved in the hormonal balance of the thyroid, pituitary, and ovary.

1. Determinations were made of oxygen consumption body temperature and body weights of rats under basal conditions during the four stages of the estrous cycle. It was found that there is a significantly increased oxygen consumption during estrus. There were no significant differences between basal temperatures or body weights at the four stages of the estrous cycle.

2. The study of radioiodine uptake by the thyroid glands of rats during the estrous cycle showed that there is a significantly increased uptake during estrus. The uptake gradually decreases at metestrus and attains its lowest level during diestrus and proestrus.

3. Similar studies of radioiodine uptake by the thyroid glands of mice during the estrous cycle showed a pattern of cyclic variation different from that of the rat. The maximal uptake of radioiodine is attained by the thyroids of proestrous mice.

4. A single injection of six or 300 rat-units of estradiol benzoate increased the six-hour uptake of radioiodine forty-eight hours after the estrogen injection. There were no differences between the effects of the two doses on thyroid uptake, with the conclusion that the dose of estrogen is not very critical.

5. The prolonged administration of a large dose of estrogen did not change the radioiodine uptake by the thyroids when compared to controls.

6. A single dose of 0.2 or 0.4 milligram of progesterone decreased the uptake of radioiodine by the thyroids while a dose of 0.8 milligram increased the uptake.

7. The injection of 0.4 milligram of progesterone counteracted the effect of estrogen on thyroid function with the net result of a decreased radioiodine uptake.

8. Experiments with hypophysectomized-ovariectomized rats showed that estrogen produces its stimulating effect on the thyroid gland via the pituitary gland since it did not change the radioiodine uptake in such animals.

9. Progesterone decreased the uptake of radioiodine by the thyroid glands of hypophysectomized rats showing that it does not induce its effect mainly via the pituitary.

10. The thyrotrophic hormone potency of the pituitaries of estrous rats is slightly higher than that of rats at the other stages of the estrous cycle. The thyrotrophic hormone content of the blood serum of estrous rats exceeds slightly that of rats at the other three stages of the cycle.

The results of the present investigations together with information reported earlier, established clearly the fact that there is a reciprocal balance between the hormones of the thyroid gland and the ovary. This balance is mediated in part via the pituitary and in part via other pathways.

In experimental animals hypothyroidism favors the production of follicle stimulating hormone while, hyperthyroidism favors the productions of lutenizing hormone. In the rat the thyroid gland is more active prior to and during ovulation. It becomes less active during the luteal development and also during the graafian follicle development.

Such cyclic variation of thyroid activity is controlled by the ovarian hormones. Estrogen activates it via the pituitary, while progesterone depresses it through other pathways than the pituitary.

To

my father,

Atalla Soliman

ACKNOWLEDGMENTS

I should like to express my sincere gratitude for the help, guidance, and encouragement given me by Dr. E. P. Reineke, Professor of Physiology, Michigan State College. Dr. B. V. Alfredson, Head of the Department of Physiology and Pharmacology, was very generous in providing the laboratory facilities of the department. Dr. L. F. Wolterink, Dr. Joseph Meites, and Dr. W. D. Collings were very helpful with their valuable suggestions. My thanks are extended to Mrs. Ellen F. Monkus, Miss Yu Sheng L. Feng, Mr. Wallace Friedberg, Mr. William P. Baker, and Mr. Robert L. Cornwall for their technical assistance and valuable suggestions.

I appreciate the assistance of Mr. J. Monroe in caring for the animals.

Finally, my deep appreciation is extended to the Egyptian Government for the opportunity afforded me through the scholarship that made this work possible.

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INTRODUCTION

The study of ductless glands and the significance of their secretions and functions have challenged the interests of a large number of investigators. It is a field in which the danger of inaccurately planned or controlled experiments is unusually great. This is true when studying the interrelationship between the thyroid and reproduction in the female.

Several factors, such as the dose of hormone, the species or environment, and differences in experimental conditions, might be responsible for disagreement between the several investigators in this field.

The early anatomists noticed that the thyroid gland is larger in women than in men. Freund (1883) noticed also that the thyroid enlarges in women during menstrual periods and pregnancy. Clinical investigations point out the importance of a normal thyroid for women to attain normal sex functions. Many clinical observations have been reported on menstrual disturbances during the course of thyroid diseases, usually amenorrhea, or irregularity of the onset of menses.

There is no doubt, then, that a hormonal interrelationship exists between the ovaries and the thyroid. So far, there is no evidence of the specific mechanisms involved.

Our investigations are aimed at presenting a clearer picture of thyroid-ovarian relationships in two species of animals: the rat and the mouse. The first step was (1) to find if there are differences in oxygen consumption and basal temperature of female rats during the four stages of the estrous cycle, and (2) to determine if there are differences in the uptake of radioactive iodine by the thyroid glands of mature female rats and mice at the four stages of the estrous cycle. Our aim also was to find if the cyclic variation in thyroid activity follows the same pattern in both species.

The results from these experiments led us to investigate also the effects of estrogen and progesterone given to spayed rats in physiological doses. In other words, we tried to duplicate as closely as possible the normal physiological conditions.

We were also interested in determining the role of both the adrenals and pituitary in controlling or modifying thyroid-ovarian responses.

The last step in our work was to assay the thyrotropic potency of the rat pituitaries and blood during the four stages of the cycle.

REVIEW OF LITERATURE

Thyroidal and Metabolic Rhythms in the Female

Thyroid Histology

Histological studies of the thyroid gland at the various stages of the cycle have shown that in young rats the mitotic activity of the thyroid and pituitary increased somewhat in early estrus and reached a maximal activity in both diestrus and proestrus, as reported by Hunt (1944). Since the greatest mitotic activity of the thyroid and hypophysis occurs in both glands during late estrus, it might be assumed that a single factor is responsible for their activity. Hunt stated that such stimulating effect appears first in the thyroid and not in the pituitary--that is, during early estrus. Therefore, the mitosis-stimulating factor is not necessarily the same for both glands.

Histological studies of the pigeon thyroid showed that this organ is in a state of hyperactivity during follicular growth as observed by Marza and Blinov (1936).

In another species, namely the guinea pig, Chouke *et al*. (1935) reported that the minimal mitotic activity of the thyroid corresponds to the follicular phase (estrus) and that maximal activity corresponds to the luteal phase (diestrus). A similar relationship was noticed in women where Bing (1928) reported maximal mitotic activity corresponding to the menstruation period.

Basal Metabolic Rate

Women. Several workers have measured the metabolic rate in the woman during the menstrual cycle. Their results vary considerably. Some investigators concluded that there were no demonstrable differences between the basal metabolic rate measurements either during the menstrual or intermenstrual periods (Gephart and Du Bois, 1916; Blunt and Dye, 1921; and Lanz, 1924).

Blunt and Dye found a decrease of 1.6 percent in the basal metabolic rate during menstruation, but they pointed out that variations of this magnitude may occur at any time without apparent causes.

However, there seems to be some agreement that there is a rise in the metabolic rate during the week preceding the onset of menstruation as indicated by Rowe and Eakin (1921), and Wakehams (1923).

Conklin and Clendon (1930) found that the basal metabolic rate tends to reach its lowest level following menstruation and its highest level preceding menstruation. Griffith et al. (1929) agreed with Conklin and Clendon. They added that there is no significant correlation between the intra-individual variations of body temperature and metabolism.

Kunde (1923) reported that there is a decrease in metabolic rate during the first or second day of menstruation. Hafkesbring and Collet (1924) reported that the basal metabolic rate is low on the first or second day of menstruation and often throughout the period. They also reported that there is frequently a premenstrual rise, and a second low point in the metabolic rate occurs at the middle of the intermenstrual period. The difference between high and low levels amounts to 5 percent.

Hitchcock and Wardwell (1929), from their study on the basal metabolic rate in twenty women, reported a strong tendency for the metabolic rate to be lowered during the menstrual period. They also found that there is another low point in the metabolic rate which occurs about the middle of the intermenstrual period.

Other investigators reporting similar results were Benedict and Finn (1928) and McClendon et al. (1929).

Dogs. Kunde et al. (1930) reported that there is a low normal basal metabolic rate in the dog during spontaneous estrus. The basal metabolic rate in normal or castrate dogs when injected subcutaneously with 100 or 200 rat-units of estrin during a period of ten to thirty days was only 2 percent below the average of the controls.

Rats. Lee (1927) determined the basal metabolic rate of female rats at the different stages of the estrous cycle. He found that heat production per square meter of surface area showed an average increase of 12 percent in the last ten hours of diestrus and at the beginning of proestrus. The R. Q. was constant at 0.75. Ovariectomized rats showed an average decrease in their heat production of 10 percent below the determinations made before the removal of the ovaries.

Wang (1923, 1924) and Slonaker (1925) noticed that in the rat the estrus phase is accompanied by a pronounced increase in locomotor activity and an increase in energy expenditure.

Brobeck *et al*. (1947) found that food intake during diestrus exceeded the intake during estrus by as much as 6 grams per day. He went further to say that food intake changes were more regular than the variations in activity; in fact, the magnitude of the changes in food intake and their regularity suggests that a record of food consumption might serve as an acceptable criterion of the sex cycle of the rat. This is true only if rats are kept at a constant environmental temperature. Estrous rats lost from 10 to 12 grams in the individual cycles due to decrease of food intake and increased expenditure of energy.

Basal Body Temperature

Women. The fluctuation in the basal temperature of women during the reproductive cycle has been reviewed by Tompkins (1944), and recently by Whitelaw (1952).

A review of the literature dealing with the determination of basal temperature of women shows a typical curve during the menstrual cycle. The temperature is relatively low during the first part of the cycle; it drops to a minimum about the time when ovulation occurs. It then rises sharply to a relatively high level, which is maintained until the next menses, when temperature drops abruptly (Van de Velde, 1904; Greulech and Morris, 1941; Martin, 1943; Rubenstein and Lindley, 1937, 1939, and 1940; Tompkins, 1944; and several other investigators).

Greulech and Morris (1941) reported that before maturity, after the menopause, and in men, similar temperature fluctuations are not found. They also found that if conception occurs, the temperature will remain at the high post-ovulation level. The important feature is the rapid rise in temperature at ovulation. Rubenstein and Lindley (1937) correlated a study of vaginal smears with a study of temperature and found that during the phase of follicular development the basal rectal temperature tends to drop progressively. The low point in the temperature curve is reached when the follicle matures. After ovulation and the beginning of production of progesterone, which counteracts in part the temperature-depressing action of estrone, the temperature begins to rise a few hours before ovulation. The temperature rise continues after ovulation and should exceed 0.5° Fahrenheit in the first twenty-four hours after ovulation, and 1° Fahrenheit the first week after ovulation. As soon as the corpus luteum has regressed, a few days before menstruation, new follicles begin to develop. Estrone begins to be produced again. Since there is now no functional corpus luteum, the temperature-depressing action of estrone is again apparent, and temperature begins to go down. During pregnancy the corpus luteum persists and temperature rise is maintained.

They added that pregnancy is indicated by a persistently high temperature curve.

Rothchild and Rapport (1952) reported that following a dose of 10 milligrams of progesterone administered daily for eleven days to cretins, or 25 to 50 milligrams of progesterone daily for nine days to feeble-minded male humans, they all responded with elevation in body temperature. Other investigations by Nieburg and Greenblatt (1948) showed that in castrated female rats the administration of progesterone increases the temperature above that of the castrated controls, while estrogen produces a decrease of temperature.

Whitelaw (1952) tended to believe that the use of the basal body temperature pattern as a reflection of the ovarian hormonal physiology may not rest on too sound a foundation. In a study of twelve normally menstruating women he found that in six of them, the temperature fell forty-eight hours before the flow; in four, the temperature dropped twenty-four hours before the flow; and in three, on the second day of menses; while in the remainder the temperature dropped after this time. In three of the ten cases whose temperature fell before menses, it rose sharply again twenty-four hours later. He also found that the administration of 125 milligrams progesterone plus 0.2 milligrams ethinyl estradiol starting on the twenty-second day of the cycle and continuing through the second day of bleeding did not prevent the onset of menstruation or the fall of the basal body temperature. He concluded that endogenous progesterone is not the hormone responsible for the rise and maintenance of the thermogenic effects.

Though Blunt and Dye (1921) reported that there was no constant change in pulse rate during menstruation and that there was no definite relation between pulse rate and level of metabolism, King (1914) found that the highest point of both basal temperature and heart rate are reached three to four days before the menses, and that the lowest point is attained three days after the cessation of menses. Kleitman and Ramsaroop (1948) recommended the use of both body temperature and heart rate curves to get a more exact determination of the occurrence of ovulation than could be obtained by body temperature alone. They found that an increase of ten to twenty heart beats per minute is approximately equal to a rise of 1° Fahrenheit in the basal temperature.

Rats. In rats, Brobek (1947) reported that low temperatures were consistently noted on the afternoon just preceding the night of hyperactivity associated with the onset of estrus. He also occasionally found it low a day before or a day after estrus. It was lower during estrus than during diestrus.

The Influence of Alterations in Thyroid Activity on Reproduction

Hypothyroidism

Menstrual cycle. Clinical patients with myxedema due to primary dysfunction of the thyroid had amenorrhea not attributable to menopause. In such cases, the normal ovarian cycle was resumed with the administration of thyroid materials. Lerman (1942) concluded that the most likely explanation is that the ovarian cycle in such cases is normal, but the flux of hormones is at a low level as a result of the low metabolism of the cells. Thyroid administration stimulates the ovary to increase the production of estrogen and progesterone, and the normal cycle returns.

Marine (1935) noticed that there was interstitial gonadal atrophy in a patient with myxedema. Engle (1946) showed that the hypothyroid monkey is amenorrheic to the extent of about one or two periods a year. But when treated with a very low dose of thyroid for ten days, the normal cycle always returned. The monkeys that were treated with thiouracil showed irregular cycles. Amenorrhea or extremely prolonged cycles of over fifty days were the rule.

Hertzler (1942), in his book "Diseases of the Thyroid Gland," pointed out that when the enlargement of thyroid of goitrus women disappeared following appropriate medications, the menstrual trouble also disappeared. He also reported that eye protrusion in toxic diffuse goiter is greatest at the menstrual period.

Estrous cycle. Thyroidectomy in the cow resulted in the disappearance of physiological estrus or libido. The administration of thyroid-active preparations restored normal breeding behavior, as reported by Brody and Frankenbach (1942) and Spielman _et_ _al_. (1945).

Several investigators seem to agree that hypothyroidism caused either by thyroidectomy or by the administration of goitrogenic substances will prolong the duration of the cycle, especially the estrus phase. Richter (1933) reported that vaginal smears taken from thyroidectomized rats with intact ovaries showed persistence of the cornified cells in the vaginal smears. The uteri of these animals were hypertrophied.

Pawick (1947) reported the observations made when mice were given 0.5 to 500 milligrams of methylthiouracil subcutaneously daily for three to five months. Regardless of the size of the dose, complete anestrus was produced in six to eight weeks. The ovaries showed marked congestion, with degeneration of the graafian follicles. Normal estrus reappeared fifteen to sixty days after the drug was discontinued.

Krohn (1947) showed that the normal estrous rhythm of mature albino mice was disturbed by the daily subcutaneous injection of 0.3 milligrams of propyl-thiouracil. The cycle was either lengthened or disappeared. The estrous rhythm returned to normal a fortnight after the cessation of thiouracil administration.

In immature female mice, Soliman and Reineke (1952) found that feeding thiouracil at the level of 0.1 percent of the food caused continuous estrus as judged by vaginal smears. They noticed also that ovulation did not occur in such treated animals as judged from a histological picture of ovaries with a large number of graafian follicles and the absence of corpora lutea. In thyroidectomized rabbits, histological examination has shown ovaries that contain a large number of closely packed follicles of a larger size than the normal controls. Ovulation does not take place after coitus (Hofmeister, 1894; Fredrikson, 1947; and Chu, 1944).

Tatum (1913) noticed that the ovaries of thyroidectomized rabbits showed a decrease in the number of primary follicles, an increased size of the follicles, and degenerative changes in the ova. These data were confirmed by Kunde _et al_. (1929).

This is in contrast to the report of Evans and Long (1921a) that thyroidectomy in rats affected neither the onset of puberty, nor the length of the estrous cycle.

Krohn (1951) found that pregnancy and parturition can follow a normal course in the thyroidectomized rabbit. Histological examination of the ovaries of thyroidectomized rabbits showed few large follicles, large masses of interstitial cells, and a few primary oocytes. There was no evidence that the number of follicles was increased. This confirms neither the observations of Chu (1944), who reported the impossibility of ovulation after coitus in thyroidectomized rabbits, nor the earlier report of Korenchevsky _et al_. (1943), who reported that the gestation period may become as twice the normal in thyroidectomized rabbits. No satisfactory explanation can be provided to explain the differences between the three sets of results, though there is always the possibility of incomplete thyroidectomy in any of the preceding experiments.

Response to gonadotrophins. In rats, hypothyroidism will enhance the effectiveness of the follicle-stimulating hormone as stated by Lane (1935), or gondatrophins as reported by Leonard (1936), Smelser and Levine (1941), and Johnson and Meites (1951).

Barman (1940) found that a hypothyroid condition produced by thyroidectomy increased the response of female rabbits to the the urine of pregnant women.

The mouse seems to be an exception. Johnson and Meites (1951) showed that hypothyroidism induced by thiouracil administration resulted in a reduction in their response to gonadotrophic hormone, as tested by ovarian weights.

Leonard and Leonard (1937) stated that while it is known that thyroidectomy enhances the effectiveness of injected follicle-stimulating hormone, the removal of the thyroid does not enhance the effectiveness of the follicle-stimulating hormone produced by the animals' own pituitary. They drew their conclusions from finding that, in thyroidectomized rats, after six to eight days, there was no significant change in the number of follicles when compared with the controls.

Pituitary gonadotrophin. Pituitary extracts from thyroidectomized rats (when injected into immature female mice) caused increased weight of ovaries and uteri in comparison to results with extracts from normal animals, as reported by Stein and Lisle (1942).

Pituitary extracts from thyroidectomized rabbits also induced the growth of the graafian follicles, but it did not cause ovulation. Chu (1944) continued to say that the hypophysis of thyroidectomized rabbits is apparently deficient in ovulating hormone, whereas the follicle-stimulating hormone may be considerably increased. Okans and Takana (1946) confirmed Chu's results.

Gestation. Hypothyroidism induced either by thyroidectomy (Ross, 1938) or administration of thiouracil (Seegar et al., 1946; and Krohn, 1950) did not cause sterility, but it interfered with the continuation of gestation. There was a decided increase of fetal resorption, though if delivered, the rats were normal and reproduced normally.

Korenchevsky et al. (1943) reported that the gestation period of thyroidectomized rabbits may become as long as twice the normal period. Krohn (1951) found that pregnancy and parturition can follow a normal course in the thyroidectomized rabbit.

Hyperthyroidism

DaCosta and Carlson (1933) showed that a daily dose of 0.5 to 1 milligram of desiccated thyroid slightly accelerated and relatively large doses of 5 to 10 milligrams definitely retarded sexual maturity in the female albino rat, as determined by the date of opening of the vagina.

Soliman and Reineke (1952) reported that mice rendered slightly hyperthyroid by feeding thyroprotein reached maturity earlier than the nontreated ones. The animals showed normal estrous cycles.

Though thyroid administration seems to hasten maturation, it also decreases the ovarian weights of the experimental animals. Fluhmann (1934) came to the conclusion that an overactive thyroid inhibits ovarian function. He found that feeding various amounts of desiccated thyroid substances or thyroxine administration to immature rats decreased the response of their uteri and ovaries to gonadotrophic extracts.

Ershoff (1945) reported that female rats raised to maturity on diets containing 0.5 to 1.0 percent desiccated thyroid showed a marked inhibition of ovarian development. The ovaries remained infantile both in weight and histological appearance. There was also a decrease in ovary weights of mice fed thyroprotein in their diet. The decrease in weight was proportional to the administered dose of thyroprotein (Soliman and Reineke, 1950). Stein et al. (1947) showed that the injection of 1 milligram of thyroxine into the ovarian capsule of the mouse retarded mitosis of its germinal epithelium.

In addition to this, there seems to be a general agreement that experimental hyperthyroidism induced by the administration of thyroid-active materials increases the size of the corpora lutea. It is thought that through its action on the corpus luteum the thyroid influences the estrous cycle (Hayashi, 1929; and Weichert, 1930). Weichert (1930) and Weichert and Boyd (1933) reported that feeding rats with desiccated thyroid prolongs the diestrus period from thirteen to twenty-four days. There were persistent functional corpora lutea suggesting increased output of the lutenizing hormone.

Evans and Long (1921b) did not notice any disturbance in the cycle of the rat after feeding fresh thyroid. One should always remember that the results will vary with the dose used.

In guinea pigs, Cameron and Amies (1926) reported that feeding of a dry extract of thyroid in doses of 1/10,000 and

1/40,000 of body weight produced a pronounced lengthening of estrus without a striking increase in the duration of the cycle. Mice treated the same way showed a lengthening of the whole estrous cycle, as well as the estrual phase itself. Soliman and Reineke (1952) reported that slightly hyperthyroid mice exhibited a regular estrous cycle.

Kunde _et al_. (1929) found that there was an apparent increase in the number of developing graafian follicles and primordial ova in the ovaries of rabbits converted into a condition of severe hyperthyroidism by the ingestion of thyroid material. Such animals came into estrus and ovulated.

Such results show very clearly that not only the dose of hormone given, but also the species response has an important influence on the results to be obtained by treatment with thyroid.

It is of interest to mention here, also, that Helpern and Hendryson (1935) reported that dinitrophenol, though producing an increased metabolic rate, did not completely inhibit estrus in the normal rat. However, the diestural period was prolonged considerably.

Gudernatsch (1915) reported that thyroid substances administered to female rats had a tendency to prevent pregnancy, and if pregnancy occurred, it usually caused abortion.

Hoskins (1910) observed that abortion was frequent in guinea pigs fed thyroid. Lintvareva (1929) found that feeding thyroid to guinea pigs resulted in sterility due to the impossibility of ovum implantation in the uterine wall because of degeneration of the corpora lutea. He added that this sterility was temporary; it disappeared with cessation of thyroid feeding.

Kunde et al. (1929) found that hyperthyroid rabbits came into estrus, ovulated, and the ova were implanted; but the young were never borne, due to fetal resorption.

Grumbrecht (1939) reported that the thyroid increases the weight of the ovaries of infantile rats receiving a constant dose of gonadotropic substances, the increase in weight being proportional to the dose of thyroid. This completely disagrees with the conclusions of Johnson and Meites (1950) that young female rats rendered slightly hyperthyroid by the administration of thyroprotein in the diet responded less to a constant dose of gonadotropin than the rats that were rendered hypothyroid by thiouracil administration. In the mouse, Soliman and Reineke (1952) showed that there is a significant decrease in ovary weights in mice rendered slightly hyperthyroid by giving thyroprotein at the levels of 0.0125, 0.025, 0.05, 0.1, and 0.2 percent in their diet. The decrease in weight was proportional to the dose of the thyroid.

Rogers (1910), in his report on the symptoms and treatment of exophthalmic goiter, said, "I have not found an exception in exophthalmic goiter when the female patient has not suffered with either amenorrhea, dysmenorrhea or menorrhagia. The ovaries are always large and tender." Hoskins (1911) reported that in the case of Basedow's disease, when the thyroid activities are abnormal, the sex-functions likewise are affected. Many clinical observations have shown menstrual disturbances during the course of this disease, usually amenorrhea or irregularity of the onset of menstruation.

Goldsmith et al. (1952) found that seventeen out of eighteen presumably premenopausal patients with thyrotoxicosis had oligomenorrhea or amenorrhea. Three of them with amenorrhea had evidence of ovulatory failure and hypoestrinism. Thirteen showed evidence that in spite of a scanty or irregular flow, ovulation was occurring and, therefore, there was no major interference with the pituitary-ovarian axis. The remaining sixteen patients resumed a normal menstrual pattern after appropriate treatment for thyrotoxicosis.

Seven out of ten premenopausal myxedematous female patients demonstrated ovulatory failure and an eighth showed an inadequate corpus luteum effect on the endometrium. The other two had normal ovulation and menses. All resumed a normal pattern after receiving desiccated thyroid.

The abnormalities were considered to be a result either of inadequate luteinizing hormone production or lowered effectiveness of the hormone produced.

Astwood (1951) reported that by treatment of nineteen women with hyperthyroid conditions with antithyroid drugs during or shortly before pregnancy, they completed their pregnancies and there were only three premature deliveries. Sixteen mothers remained well after the antithyroid therapy was stopped and three required further treatments.

Hormonal Interrelationships

Effect of Thyroid on Response to Estrogen

The thyroid level seems to be very critical for estrogen to induce its effects. Reiss and Pereny (1928), Van Horn (1933a, 1933b), Meyer and Wertz (1938), Langham and Gustavson (1947), and Hill (1948) all agree that in castrate rats the amount of estrogenic substance necessary to induce estrus was increased three to five times when the animals were pretreated with thyroxine.

Langham and Gustavson (1947) found also that thyroparathyroidectomy resulted in an increase in the estrus response to 1.3 micrograms of estrone. These results, the authors said, show that the level of thyroid function significantly affects the response to estrone. For normal animals, the rat unit was 1.33 micrograms as compared to 2.5 for the hyperthyroid group and 0.86 for the thyroparathyroidectomized group. Their hypothesis was that the excess of thyroxine may, by increasing metabolism, hasten the elimination of estrogenic compounds from the body.

The thyroid gland might also have a direct effect on the ovary. Lee (1925) reported that the thyroid exercises a stimulating effect on the ovary. He drew his conclusion from the fact that the removal of the thyroid resulted in the prolongation of the estrus cycle in the rat.

Iodine distribution. Iodine was recovered from the ovaries of different species of animals and humans as reported by Ruff (1933) and Carter (1932). There was a great variation in the amounts of iodine recovered from the ovaries by the several workers. Carter (1932) believed that such differences in iodine content might have been due to variation in the iodine content of the ovaries during the different stages of the cycle. He also reported that the iodine content of the ovaries of newborn babies was less than in those of adults; it begins to drop with the appearance of menopause. He tried to determine the form in which iodine occurs. He could not detect thyroxine in any considerable proportion, but he found a substance which induced metamorphasis to tadpoles.

Orr and Leitch (1929) found that iodine of the cow's ovary was concentrated principally in the corpus luteum.

Salter (1949) stated that thyroxine iodine is fixed in two general locations. The first is its accumulation in the pituitary and ovary. The second is its accumulation in the peripheral tissues, primarily the skeletal muscle. Tyndale and Levin (1937) concluded that the thyroid hormone exerts an inhibitory action on the gonads directly and not through the pituitary.

Thyroid-pituitary relationship. The thyroid gland may exert its influence on the reproductive system through the pituitary gland.

Evans and Simpson (1929) reported that the gonad-stimulating properties of the pituitaries of hyperthyroid rats were increased, while those from hypothyroid rats were less effective than the normal ones. On the contrary, Stein ahd Lisle (1942) reported that pituitary extracts from thyroidectomized rats stimulated development of heavier ovaries and uteri when injected into immature mice than did extracts from normal animals.

Reineke et al. (1941) reported that the pituitaries taken from thyroidectomized kids had a lower gonadotropic potency than those taken from controls of the same weight.

The administration of estrogenic materials to thyroidectomized rats (Janes, 1946; and Baker and Everett, 1947) or to rats with thyroid intact (Bradbury, 1947) resulted in the increase of pituitary weights. So did thyroidectomy alone (Janes, 1946). Baker and Everett (1947) came to the conclusion that this stimulating action on the pituitary is not mediated by the thyroid, and that it may be elicited in the adult as well as in immature rats.

Payne (1944) noticed that thiouracil treatment increases the thyroid weight and heightens the epithelium, which suggests a very marked increase in the thyroid-stimulating potency of the pituitary. However, the assay of pituitaries from thiouracil-treated chicks showed no significant difference in thyrotrophic potency when compared with the nontreated ones. Houssay et al. (1932) found that there was no change in the thyrotrophic potency of the rat pituitary following thyroidectomy. This was confirmed by Smelser (1934). These findings might suggest that there is an increased output of the thyrotrophic hormone which prevents any appreciable storage in the gland.

On the basis of cytophysiologic studies of the pituitary, several investigators came out with variable results regarding the site of formation of the various hormones of the pituitary. The hormones that are of interest to us here are the thyrotrophic and gonadotrophic hormones. There seems to be unanimity of opinion that the origin of the follicle-stimulating hormone is in the basophils. This is supported by the cytological work of Severinghaus (1937) with rats, Payne (1947) with chicks, also Breneman (1941), and Schooley and Riddle (1938) in the pigeon.

Severinghaus (1937, 1939) is of the idea that acidophils secrete thyrotrophic hormone. He also believes that the basophilic cells are the site for formation of follicle-stimulating hormone. He noticed that estrone activated the secretory cycle of the basophils to such an extent as to result in final exhaustion.

Morris (1952) showed that estrone administration inhibits the development of basophils and results in the presence of acidophils. There was also an increase in thyroid activity and thyrotrophic hormone potency of the pituitary which points to the acidophils as the active cell type in producing thyrotrophic hormone.

The effect of androgen treatment on the pituitary gave a picture similar to that of estrone, though it was not as great. The pituitaries of this group did not show a difference in their thyrotrophic potency over that of the untreated birds.

Zeckwer _et al_. (1935) found, on the other hand, that after thyroidectomy there was a vacuolation of the basophilic cells he suggested that these thyroidectomy cells secrete the thyrotrophic hormone.

Payne (1944) illustrated also the appearance of thyroidectomy cells in chick pituitaries and he agreed with previous investigators that the thyroid-stimulating principle is secreted by the basophils. He still thought, however, that they are not synonymous with the basophils of castration.

Other investigators as Romeris (1940), Gomori (1950), and Halmi (1950) believe that there are two types of basophils: beta and delta cells. Halmi (1950) concluded that the delta cells are the most likely source of both follicle-stimulating and thyrotrophic hormone.

Purves and Griesbach (1951a, 1951b, 1951c) were able to observe two types of cells containing glycoprotein granules in the anterior pituitary of the rat. These types of cells differ in their shape, affinity to the blood vessels, regional distribution, size, and most important, in their granule size and their staining properties and reaction to changes in the hormonal environment. The group of cells that is centrally situated responds only to thyroxine fluctuations, and is unaffected by changes in the amount of gonadal hormone. On the other hand, the cells situated somewhat medially beneath the lateral edge of the pars intermedia respond only to fluctuations in the gonadal hormones, namely estradiol benzoate, and are unaffected by variations in the amount of thyroxine. This specificity of response holds true only for moderate doses or doses close to the physiological level.

The authors also observed that large doses of either estradiol benzoate or thyroxine depress both types of cells. Excessive or prolonged administration of thyroxine resulted in an increase in the amount of glycoprotein in the gonadotrophic cells, giving a picture of a pituitary which is similar to that produced by gonadal insufficiency, though less extensive. This might possibly be due to an impairment of gonadal function (Severinghaus, 1937).

Thyroid Ovary Relationships

<u>The effects of castration of female animals on thyroid activity</u>. This is an area of investigation where an investigator might think by the first look at the literature that there is a disagreement between the several investigators on the effects of castration on thyroid anatomy and morphology. But a little reasoning and understanding might help in bringing out a single conclusion.

Loeser (1934, 1935) claims that eighteen days after castration, the thyroid gland of female guinea pigs is moderately stimulated. The production of T. S. H. was also increased. He found that the pituitary gland of a castrated guinea pig, when implanted into a normal animal, will cause an increased activity of the thyroid gland greater than that induced from normal noncastrated animals.

Kippen and Loeb (1936) reported that gonadectomy in guinea pigs during the early stages of sexual maturity increased the proliferative activity of the thyroid gland during the first week following the operation. They thought that this removal of follicular hormone produces its effect on the thyroid through the pituitary where it elicits an increased production of T. S. H. and F. S. H. The thyroid-stimulating hormone, they said, must be considered as distinct from F. S. H. This effect represents, therefore, a non-specific effect of gonadectomy on the anterior pituitary. In young immature guinea pigs, the authors reported that the changes were less definite than in older ones. Chouke (1930) found that castration in female guinea pigs causes no appreciable change in the proliferative activity, nor in the structural characteristics of the thyroid gland.

Starr and Bruner (1935) also reported that there were no gross histologic changes found in the thyroids of female guinea pigs eighteen to thirty-three days after castration. They also reported that castrated female guinea pigs responded to T. S. H. thirty days after castration with histologic changes in the thyroid not different from normal noncastrates. They added that the metabolic response of castrated guinea pigs to T. S. H. was of the same magnitude as was the response of the noncastrates.

The third group of investigators studied thyroid activity at a longer interval after castration. Andersen and Kennedy (1933) reported that there was a definite thyroid atrophy eight weeks after castration. But they reported that there were no changes in thyroid histology one week after the operation, and some animals showed atrophied thyroids three weeks after the operation.

Emge and Laqueur (1941) reported that withdrawal of ovarian hormones by castration of rats decreased their thyroid activities, as indicated morphologically by their follicular cells and the increased storage of colloid. In another report, Schilling and Laqueur (1941a) reported that in rats with hyperplastic thyroids, castration had no effect on thyroid weights.

From such reports, one would conclude that the effect of gonadectomy on thyroid activity varies with the interval after the operation and the age at the operation. It seems that about one to two weeks after castration, the thyroid activity is increased as indicated by histological studies. The activity of the thyroid glands then starts to decline with increasing time after the operation. Some authors examined it at a fairly long period and reported that there were no changes compared to the controls. Others studied it after longer periods and reported that the thyroid glands were atrophic. It was also reported that such changes do not occur in immature animals.

The effects of estrogenic compounds on thyroid activity. Here also, there are some factors that influence the response of the thyroid gland to estrogen treatment, such as the dose administered and the duration of its administration.

Pincus and Werthessen (1933) investigated the effects of various doses of estrin given for different periods. Their data on thyroid weights indicated an increase in weight as a result of estrin injection. Curiously enough, the authors said that their data indicated that this weight increase is at a maximum after five days of injection, and decreases fairly gradually to a minimum after forty days of injection as though there is an immediate effect of estrin injection which continued injection does not sustain. There was no indication of a dosage relation to this effect, with the possible exception of one-unit injection, which appears less effective than the higher dosages of 2, 4, 7, and 10 rat-units. Freudenberger and Clausen (1937) also reported that with prolonged administration of theelin at a dose of 200 international units every other day for seventy-five to seventy-nine days, they observed a decrease of 18 percent in the weights of the thyroids of such treated rats when compared to their controls.

Emge and Laqueur (1941), and Schilling and Laqueur (1941b) reported that estrone had no effect on thyroid weights of thyrohyperplastic rats.

Different results were reported by Kreitmair and Sieckmann (1945), who found in histological examination that the thyroid glands of infantile rats injected with diethylistilbestrol were in a resting condition and filled with colloid.

Koenig et al. (1945) reported that with the administration of 30 micrograms of estrone or 9.4 micrograms of diethylstilbestrol to young female rats daily for twelve weeks, they found that the iodine content of their thyroids was less than that of their controls.

Nakamura (1932) was the first investigator to study the effects of the hormones of the placenta, pituitary, and ovary on the iodine contents of the thyroid gland in rabbits. Placental extracts markedly accelerated the rate and slightly increased the amount of I_2 excretion in the urine. Injection of placental extracts in thyroidectomized rabbits produced no marked change in either the rates or amount of I_2 excreted. The author's conclusion was that iodine excretion is closely related to thyroid, which is one of the factors responsible for increasing the metabolic rate during pregnancy.

Desclin and Ermans (1950) reported that in rats estradiol benzoate was found to stimulate thyrotropic activity of the pituitary.

Few experiments have been conducted to investigate the effects of Estrogens on thyroid activity using I^{131} as an index.

The earliest paper found was that by Paschkis <u>et</u> <u>al</u>. (1948), who studied the four-hour uptake of I^{131} by thyroid glands in female rats, castrated female rats, and castrated rats given estradiol benzoate in doses of 50 micrograms per day for twelve days, eleven days, and thirteen days. Their data indicated that there were no differences in the uptake of a tracer dose of I^{131} in female rats sixty-four days after castration when compared to their controls with the ovaries intact; also, that I^{131} uptake by the thyroid glands of the animals treated with estradiol benzoate did not differ from that of the castrated controls.

Epstein and Wolterink (1949) showed that the output half-time of I^{131} from the thyroid glands of chicks given dianisylhexene was twice the half-time of controls.

In another experiment, Wolterink <u>et</u> <u>al</u>. (1950) and Wolterink (1952) demonstrated the effect of various dosages of dianisyl-hexene on the turnover rate of iodine in the thyroids of rats and mice. They found that a dose of 1 microgram of estrogen per day for three days increased the output of I^{131} by the thyroid in both rats and mice. They also found that 10 micrograms and 100 micrograms of estrogen given daily for three days, and also a massive single dose depressed I^{131} turnover. Money *et al*. (1950, 1951) reported that 50 micrograms of estrone injected daily into male rats for ten days caused a significant rise in I^{131} uptake without a change in thyroid weight. A dose of 1 milligram of estradiol benzoate daily for nine days or 50 milligrams daily for ten days did not influence either thyroid weight or I^{131} uptake.

Metabolic effects of estrogen. Lee (1927) reported that in ovariectomized rats, the mean heat production was about 10 percent below the determinations before the removal of the ovaries.

Sherwood *et al*. (1933) reported that they were able to produce from 13 to 42 percent drop in basal metabolism following injection of 10 to 800 rat units of amniotin.

Sherwood *et al*. (1936) and Sherwood (1940) found that when the basal metabolism of ovariectomized-thyroidectomized rats was increased by giving them a thyroid preparation, then followed immediately by injecting variable doses of amniotin from 200 to 300 rat-units daily for three or four days, the initial basal metabolic rate was reached in less than half the time necessary for the usual return to normal of the rats rendered hyperthyreotic only.

Stilbestrol given at the dose rate of 10 milligrams was apparently not as effective as amniotin in hastening the reduction of basal metabolism after the hyperthyroid level was attained (Sherwood, 1941). Zain (1937, 1939) reported that in hyperthyreotic female rats, the intramuscular injection of 1,000 rat-units of progynon B (Schering) daily for fourteen days lowered the metabolism and improved liver glycogen formation as compared to the rats rendered hyperthyreotic only. He concluded that progynon excited a weak antithyroid action.

The effects of progesterone on the thyroid. As has been stated before, it is well established that progesterone exercises a thermogenic effect. The physiologic mechanism through which progesterone exercises such effect has received little attention. Nieburg and Greenblatt (1948) reported that in castrated female rats the administration of progesterone increases the temperature above that of the castrated controls, while estrogen produces a decrease of temperature. Similar results, though less definitely, were obtained in thyroidectomized-ovariectomized animals. They also reported that in adrenalectomized-ovariectomized rats, estrogen increases the temperature above that of the controls and progesterone induces a further rise in the temperature. The authors concluded from such data that progesterone seems to influence body temperature and that the adrenal and thyroid glands change the effects of estrogen and progesterone on body temperature to a certain degree.

Rothchild and Rapport (1952) reported that a dose of 10 milligrams of progesterone administered daily for eleven days to cretins, or 25 to 50 milligrams of progesterone daily for nine days to feeble-minded male humans caused an elevation of body temperature. The determination of protein-bound iodine showed no correlation between it and the rise in temperature. They came to the conclusion that there is a lack of thyroid mediation or intervention in the physiologic processes by which progesterone exerts its thermogenic effect.

Histological studies of the hyperplastic thyroids of rats treated with progesterone showed that progesterone alone or progesterone plus estrogen administered parenterally were able to inhibit hyperplasia. Emge and Laqueur (1941) and Schilling and Laqueur (1941) also reported that although estrogen failed to change the thyroid weights of thyrohyperplastic rats progesterone or estrone and progesterone decreased the thyroid weight.

The only investigations where I^{131} was used to study the effect of progesterone on thyroid activity were those of Money _et al_. (1950, 1951). Their results were that a total dose of 50 milligrams progesterone injected into male rats during a period of ten days raised the percent of radioactive iodine collection by the thyroid. In this experiment progesterone did not influence thyroid weights.

Ovary-Adrenal Relationship

Stilling (1898) found that the adrenals of frogs hypertrophy during ovulation. Similar results were noticed in pigeons by Riddle (1923).

Foster (1934) found that in the ground squirrel the adrenals hypertrophy during estrus and pregnancy, but undergo involution after parturition.

Andersen and Kennedy (1932) reported that there is an increase in the weight of the adrenal of the rat at estrus. Andersen and Kennedy (1933) completed their study on the adrenal at the stages of the estrous cycle. Their histological studies showed hypertrophied and more active adrenals during estrus than during diestrus. They also reported that the histological appearance of the adrenals of spayed rats, a week after the operation, was similar to that of rats at estrus. Three weeks after the operation the adrenals atrophied.

Zuckerman _et_ _al_. (1938) found that the adrenals fluctuate in size in an approximately five-day rhythm in spayed rats injected daily with a threshold dose of estrone. In normal animals, they also found that the gland was larger at estrus than at diestrus, due to an increased size of the cells of the zona fasciculata.

Hunt (1940) found that in the rat mitosis in the adrenals was eight to sixteen times more frequent in the animals during the mid-leucocytic stage (diestrus) than in the cornified cell stage (estrus). He also found that 80 percent of the mitoses were in the zona fasciculata where the rhythmic activity was most marked. He also found that the zona granulosa exhibits the greatest number of mitoses during the cornified cell stage. Hunt concluded that a reasonable correlation may exist between the adrenal cortex, hypophysis, and corpora lutea.

Koets (1949) demonstrated a variation in the excretion of 17-Ketosteroids in women, with idiopathic hirsutism, a maximum excretion being obtained at the time of ovulation. Davis and Hulit (1949) reported a distinct eosinopenia in women at ovulation time of the cycle.

Zondek and Burstein (1952) reported that there is a cyclic variation in corticoid excretion in the urine of female guinea pigs. The maximal excretion rate was during estrus. Ovariectomy resulted in a low and acyclic output of corticoids. The pattern of corticoid excretion in the male guinea pig was the same as that of the ovariectomized female guinea pigs. They also found that the administration of estrogen causes an almost immediate increase of urinary corticoids in both intact and spayed female animals. They assumed that estrogens stimulate the anterior pituitary to discharge ACTH. Venning and Kazmin (1946) were not able to find any cyclic variation in the excretion of 17-ketosteroids in normal women.

It has been established by several investigators that large doses of estrogen enlarge the adrenals mainly due to hypertrophy of the cortex, as reported by Heskett and Hoffman (1943).

This area of endocrinology has been reviewed by Tepperman, Engel, and Long (1943); Hartman and Brownell (1949); and Zondek and Burstein (1952).

Hartman and Brownell (1949), in their book "The Adrenal Gland," reviewed the literature dealing with the effects of estrogens on the adrenals in several species, including the fowl, rat, mouse, hamster, and guinea pig. From this review, it can be concluded that in all these species, with the exception of the mouse, the administration of estrogenic compounds results in hypertrophy of the adrenals and an increase in their weight. This occurs in spayed as well as in normal animals.

Estrogens probably act through the pituitary because estrogenic substances fail to act on the adrenals after hypophysectomy (Bourne and Zuckerman, 1940).

In the mouse, Martin (1930) found that estrogen failed to change the picture of their x-zone when administered over a short period, but prolonged administration of estrogen to immature castrated and normal males and spayed and normal females resulted in the degeneration of this zone.

The effects of estrogen on the adrenal glands of the hamster has been reviewed by Wexler (1952). He found from his own work that a single intraperitoneal injection of 0.06 milligrams of stilbestrol given to castrated and spayed hamsters caused an increase in the ascorbic acid content of their adrenal glands to twice that of the normal values.

Regarding the effect of progesterone on the adrenals, Winter (1942) and Leonard (1944) showed that it failed to cause atrophy of the adrenals.

Thyroid-Adrenal Relationship

The effects of the Thyroid gland on the adrenals. The functional activity of the adrenal gland is influenced in some manner by varying thyroid activity.

When hypothyroidism is induced by the administration of goitrogenic substances such as sulfa drugs or thiouracil, as reported by Leblond and Hoff (1944), Baumann and Marine (1945), Freedman and Gordon (1950), there is a decrease in the adrenal weights, which is partly due to decreased secretion of ACTH. Zarrow and Zarrow (1951) thought that this might be due to the excessive production of TSH at the expense of ACTH.

The adrenal ascorbic acid content is also decreased, as shown by Freedman and Gordon (1950).

It has also been shown by Wallach and Reineke (1949) that following the administration of thyroidal substances to animals, the adrenals enlarge. The authors reported that the ascorbic acid content of the adrenals decreases to a minimum after four days of thyroxine administration. This is followed by progressive increases, both in adrenal weight and ascorbic acid content, that reach a maximum after four weeks of thyroxine administration.

Pekkarinen et al. (1951) investigated the effect of a thyroid preparation thyranine on the adrenals. They found that the administration of 100 milligrams of thyranine daily for a period of twenty days increased the adrenal weights of rats 61 percent above the control. This treatment also increased their adrenalin content by 88 percent, the ascorbic acid content by 330 percent, and cholesterol by 85 percent. With the administration of 20 to 30 milligrams of thiouracil for a period of twenty-six days, the adrenal weights were 23 percent less than the control weights, but adrenalin increased by 53 percent, ascorbic acid increased by 76 percent, and cholesteral decreased by 46 percent. The authors concluded that changes in the weight and chemistry of the adrenal glands suggest that after the injection of thyranine the medullary and cortical activity are increased, whilst after the administration of methyl-thiouracil and medullary activity increased but the cortical activity is decreased.

The effects of stress on thyroid activity. Williams et al. (1949) found that stress induced by fasting, exposure to cold (5° C.), to heat (38° C.), or typhoid vaccine, was associated with a subnormal concentration of iodine in the thyroid. They also reported that these various types of stress distinctly decreased the protein-bound iodine in the serum.

Paschkis et al. (1950) found that formalin injection in rats caused a decrease in thyroid function as indicated by the diminished uptake of I^{131}.

Mahorner (1930) found that dogs infected by injecting staphylcocci, or dogs which died from pneumonia showed hyperplasia of their thyroids. He also found that after injuring one or both adrenals by crushing, seven out of ten dogs showed no change, one showed retrogression, and two showed slight hyperplasia of their thyroids.

Soffer et al. (1949) and Gabrilove et al. (1950) found that the injection of epinephrine into intact rats resulted in a considerable decrease in I^{131} uptake. They also found that the administration of compound E inhibited the thyroid-stimulating effect of epinephrine. From these data, they concluded that the adrenal cortex exerts an inhibitory effect on the thyroid-stimulating action of epinephrine.

Gabrilove, et al. (1950) reported also that when 17-hydroxy-11-dehydrocorticosterone was administered together with epinephrine, the thyroid responded like in an intact rat given epinephrine alone.

Botkin and Jensen (1951) reported that epinephrine caused 56 percent depression of total I^{131} contents of the thyroids as compared to control values.

Williams et al. (1949) reported that adrenalin decreases the quantity of protein-bound iodine in the serum of adrenalectomized rats.

Botkin and Jensen (1952) found that there was an early decrease in the thyroid gland content of radioactive iodine after either epinephrine or thyrotropic hormone administration. The serum content of I^{131} after thyrotropic hormone injection was increased, particularly the protein-bound component, due to acceleration of the production and release of thyroxine. In the case of the epinephrine-treated rats, the serum content of protein-bound iodine was decreased. The authors' interpretation was that the increased utilization of thyroxine in the animals given epinephrine results in a decrease in the serum thyroxine. Such a lowering in the level of thyroxine in the blood causes an increased pituitary release of TSH, and therefore, epinephrine will produce the same effect on the thyroid gland as does TSH.

The effects of the adrenal cortex and ACTH on thyroid activity as studied by radioactive iodine. In the last few years, cortisone and ACTH have been used extensively for physiological and clinical investigations. Several investigators have been interested in studying the effect of these compounds on thyroid activity.

Albert (1952) reviewed the literature accumulated in the past three years dealing with this subject.

The first investigators who drew our attention to the effects of cortical hormones and ACTH on the thyroid, using I^{131}, were Soffer *et al*., (1949) and Williams *et al*. (1949).

Soffer *et al*. (1949) pointed out that the adrenal cortex exercises an inhibitory effect on the thyrotrophic effects of epinephrine, as mentioned before.

Williams *et al*. (1949) found that adrenalin or adrenal cortical extract decreased the concentration of I^{131} by the thyroid and that it also decreased the quantity of protein-bound iodine in the serum of adrenalectomized rats.

Gabrilove et al. (1950) found that the administration of 5 milligrams of ACTH daily for twelve days was successful in antagonizing the effect of propylthiouracil in increasing thyroid weight.

Hill et al. (1950) reported, however, that cortisone failed to alter iodine accumulation of hyperactive thyroid glands.

Money et al. (1950, 1951) studied the effects of a large number of steroid hormones on thyroid activity. The animals used were male rats injected daily with large doses of the steroids for a period of nine to ten days. Some of these results have been mentioned before while reviewing the effects of estrogen and progesterone on the thyroid. They found that cortisone and ACTH decreased the twenty-four hour uptake of I^{131} by the thyroid glands. They also found that desoxycorticosterone did not affect I^{131} uptake.

Soffer et al. (1951) reported that I^{131} uptake was reduced after the administration of 5 milligrams of ACTH daily for four days in both adrenalectomized and intact rats, and that there were no differences between the ACTH-treated adrenalectomized rats or rats with the adrenals intact. Jacobson et al. (1951) reported that out of six patients with active advanced Hodgkins's disease, treated with cortisone, five showed a reduced thyroid activity. The thyroid hypoactivity disappeared within a week following cortisone withdrawal.

Paschkis _et_ _al_. (1950) found on the other hand that both decoxycorticosterone and adrenal cortical extract (Upjohn) failed to influence significantly the four-hour uptake of I^{131} by the thyroid. It should be mentioned, also, that Money _et_ _al_. (1950, 1951) reported that both substances, namely desoxycorticosterone and adrenal cortical extract (Upjohn), failed to influence the twenty-four hour uptake of radioactive iodine by the thyroid.

Botkin and Jensen (1951) reported that adrenal cortex extract, cortisone, and ACTH all failed to give any significant alteration of the two-hour uptake of I^{131}.

Cortical hormones also have been reported to decrease the protein-bound I^{131} in the serum.

Hardy _et_ _al_. (1950) found that the administration of either ACTH or cortisone to patients with various collagen diseases resulted in a decrease in the serum protein-bound I^{131} of all patients.

Frederickson _et_ _al_. (1952) also found that massive doses of cortisone acetate, besides decreasing consistently the I^{131}

uptake of the thyroid glands, also resulted in a significant fall in the level of serum protein-bound iodine.

Hill et al. (1950) also found that ACTH and cortisone in normal subjects induced a depression of I^{131} uptake and serum protein-bound I^{131}.

It has also been reported by Williams et al. (1949) that adrenal cortical extracts decreased the quantity of protein-bound iodine in the serum of adrenalectomized rats.

With a differently planned experiment, Albert et al. (1952) reported that neither cortisone nor ACTH produced any significant effect on the secretion rate of the thyroid. The uptake of the tracer dose of I^{131} after twenty-four hours was, however, depressed by both substances. They injected ACTH or cortisone in rats after their thyroids had been labeled with I^{131} in order to avoid the diminished initial uptake of I^{131} that occurs if both hormones are injected first. If the hormone injection preceded the I^{131} administration the thyroids of the experimental rats given cortisone or ACTH would have less total I^{131} than the controls to start with. Thus, less I^{131} would be available for discharge from the thyroid, which in turn could lead to the erroneous conclusion that ACTH or cortisone interfere with the output of iodine from the gland.

Hill et al. (1950) reported that ACTH and cortisone resulted in a slight decrease in the basal metabolic rate in patients with a nephrotic syndrome. They also reported that ACTH seems to induce a slight temporary clinical improvement in both the so-called thyrocotropic and thyrotoxic varieties of exophthalmos.

Berson et al. (1952), in the discussion of their paper, said that it is possible that the hypofunction of the thyroid is responsible for the undesirable effects of cortisone therapy resulting in metabolic variations. They also suggested that such metabolic changes can be prevented by the administration of thyroid substances or thyroxine.

Kuhl and Ziff (1952) found that in ten patients receiving ACTH or cortisone, no significant changes were noted in the basal metabolic rate, or serum cholesterol levels after ten to sixteen days of ACTH administration.

Frederickson (1951) reported that in Addison's disease the occurrence of thyrotoxicosis is extremely high.

Albert et al. (1952) concluded from their results that have been cited earlier that cortisone or ACTH might decrease I^{131}

uptake due to the inhibition of thyrotrophin, or due to an increased renal clearance of iodide, or from a direct inhibitory action on the permeability of the thyroid cells, or on any of the mechanisms necessary for the selective accumulation of iodide by the thyroid cells, from an increased iodide space, or from other factors. They reported that the discharge of I^{131} from the thyroid was not affected by either ACTH or cortisone. They tried to explain such results by ruling out the possibility of inhibition of thyrotrophin. Their reasoning was that in the absence of thyotrophin (Randall _et al_., 1951) or its inhibition by thiouracil administration (Albert and Tenney, 1951), both the uptake and discharge of I^{131} are markedly lowered.

Woodbury _et al_. (1951) reported a decrease in thyroid uptake of I^{131} in hypophysectomized rats given both cortisone and TSH, when compared with similar rats given the same dose of TSH alone. This might indicate that cortisone inhibits the effect of TSH on the thyroid. They also reported that ACTH and cortisone given alone did not affect the thyroidal collection of I^{131} in hypophysectomized rats, but inhibited the action of thyrotrophin given concurrently.

Another possible explanation for the decreased uptake of I^{131} in rats treated with cortisone or ACTH is the increased rate of elimination of I^{131} in the urine. Berson et al. (1952) found that there was an elevation of the renal plasma I^{131} clearance rate.

Experiment I

Oxygen Consumption, Basal Temperatures, and Body Weights of Female Rats as Correlated with the Stages of the Estrous Cycle

Materials and Methods

A closed circuit apparatus was used for determining oxygen consumption of the rats (MacLagan and Sheahan, 1950, with modifications introduced by Reineke). The apparatus consists of twelve desiccators 6-1/4 inches in diameter. Each desiccator contained 200 grams of moist soda lime (Wilson), a thermometer which can be read from the outside, and a wire screen on which the animal can stand without touching the soda lime or disturbing the thermometer. The top of each desiccator was connected to a three-way stopcock. One branch led to both the desiccator and a mercury manometer by means of a T tube, another to a vacuum line, and the third branch to a bag filled with oxygen.

The volume of each desiccator was determined separately by weighing the amount of distilled water that it contained when filled completely, with the cover in place. From the weight and temperature of the water, the volume of the desiccator was computed. To this the volume of the tubing connections to the top of the mercury on the inner arm of the manometer was added. The volume of the soda lime, screen, and thermometer was subtracted to give the net volume of the unit.

The animal was weighed, placed in the desiccator and the cover was closed. With the three-way stopcock closed, the vacuum was turned on. The stopcock was cautiously opened to the vacuum line to draw a negative pressure of 200 millimeters of mercury. The stopcock was then turned to connect with the oxygen line and allow the manometer to come to zero pressure. The stopcock was then closed and the same procedure was repeated in all the twelve units.

The whole apparatus was kept in an air conditioned room at a temperature of 24° Centigrade ± 1°.

Readings were taken from the manometer at intervals of fifteen minutes. The readings from the first thirty minutes were usually omitted. This period was usually required for the apparatus to reach temperature equilibrium of 25° Centigrade.

It was necessary to tap the manometer before each reading to settle the mercury column.

Calculations

Oxygen consumption in milliliters per 100 grams body weight per hour:

$$[V - V_a]\,[P/760]\,[273/(273 + t)]\,[100/(\text{body weight})]$$

Where V = volume of unit in milliliters

Va = volume of animal (assuming that 1 gram = 1 milliliter)

P = pressure difference in millimeters of mercury in an hour period

t = temperature inside the desiccator

The daily oxygen consumption of thirty-six female rats was determined. Twelve rats were run simultaneously in individual units. Eight determinations were taken during a period of eight days for each rat in the first trial. Fifteen determinations were taken for each rat in the second group and ten determinations for the third group. Before starting the actual determinations the rats were put in the desiccator on two separate occasions and left for about an hour to get used to it. Before each determination they were fasted for a period of twelve to fifteen hours. Vaginal smears were taken daily. Rectal temperatures were taken only from the rats of the first group.

Results

The average length of the estrous cycles was 5.35 days (Table I).

Body weights. There were no significant differences between the weights of the same animals at the different stages of the estrous cycle in any of these experiments. When all the data from the three experiments were pooled together, the average body weights were 218.10 grams at proestrus, 218.85 grams at estrus, 219.22 grams at metestrus, and 219.46 grams at diestrus.

Basal temperature. Though there was a slight rise in the basal temperature during estrus, it was not statistically significant. The average basal temperatures were 100.23° Fahrenheit at proestrus, 100.37° Fahrenheit at estrus, 100.01° Fahrenheit at metestrus, and 100.12° Fahrenheit at diestrus.

Oxygen consumption. The oxygen consumption per 100 gram body weight per hour showed a significant rise during estrus above that recorded in any of the other three periods.

In experiment I(a), the average oxygen consumption in milliliters per 100 grams body weight per hour was 113.93 at proestrus,

TABLE I

O_2 CONSUMPTION AND BASAL TEMPERATURE OF FEMALE RATS AT THE VARIOUS STAGES OF THE ESTROUS CYCLE

	Pro-estrus	Estrus	Met-estrus	Di-estrus
	Experiment I(a)			
Body weight	205.88 ± 5.23*	203.75 ± 5.47	208.50 ± 7.53	203.60 ± 4.27
Basal temp.	100.23 ± 0.93	100.37 ± 0.18	100.01 ± 0.22	100.12 ± 0.10
O_2 consumption/100	113.93 ± 4.25	139.88 ± 5.29''	110.18 ± 4.36	114.78 ± 4.34
	Experiment I(b)			
Body weight	227.05 ± 3.54	226.23 ± 2.84	225.47 ± 2.08	224.20 ± 2.21
O_2 consumption/100 gm./hour	101.82 ± 1.65	115.58 ± 2.45''	99.37 ± 1.99	100.66 ± 1.32
	Experiment I(c)			
Body weight	212.54 ± 1.25	212.35 ± 1.12	211.43 ± 2.69	215.00 ± 1.16
O_2 consumption/100 gm./hour	101.83 ± 2.27	116.30 ± 1.89''	104.62 ± 2.87	100.68 ± 5.29
	Pooled Data			
Body weight	218.10 ± 2.42	218.85 ± 2.04	219.22 ± 2.56	219.46 ± 1.48
O_2 consumption/100 gm./hour	104.13 ± 1.49	120.62 ± 2.00''	102.61 ± 1.67	103.40 ± 1.17

* Standard error.

'' Significantly higher (1 percent level) than the other three periods.

The average length of the estrous cycle was 5.35 days ± 0.69.

Percent increase of O_2 consumption of the rats during estrus over that during diestrus is equal to 16.65 percent.

139.88 at estrus, 110.18 at metestrus, and 114.78 at diestrus. Oxygen consumption in Experiment I(b) was 101.82 at proestrus, 115.58 at estrus, 99.37 at metestrus, and 100.66 at diestrus. In Experiment I(c), the oxygen consumption was 101.83 at proestrus, 116.30 at estrus, 104.62 at metestrus, and 100.68 at distrus.

When the data accumulated from the preceding three experiments were pooled together, the average oxygen consumption was 104.13 milliliters of oxygen at proestrus, 120.62 at estrus, 102.61 at metestrus, and 103.40 at diestrus.

On the average, there was a rise in oxygen consumption of 16.65 percent during estrus above that during diestrus.

Experiment II

I^{131} Uptake by the Thyroid Gland of Female Rats at the Stages of the Estrous Cycle

In the preceding experiments it was found that oxygen consumption of adult female rats is significantly higher during estrus than during the other three stages of the estrous cycle. This led us to do the following two experiments using radioactive iodine uptake as an index of thyroid activity through the four stages of the estrous cycle expecting that there might be a cyclic variation in thyroid activity in a pattern that might correspond with results obtained from oxygen consumption determinations.

Materials and Methods

Thirty-five mature female rats weighing 250 to 270 grams were used in this experiment. They were obtained from Carworth Farms, New City, New York. At their arrival they were put in cages and kept in an air conditioned laboratory at the temperature of 24° Centigrade ± 1. They were fed a normal ration formulated by Dr. C. A. Hoppert from the Chemistry Department at Michigan State College. His formula for 4 kilograms is:

Yellow cornmeal	1,400 grams
Ground whole wheat	1,000 grams
Whole milk powder (Borden)	800 grams
Linseed oil meal	400 grams
Alfalfa leaf meal	240 grams
Brewer's yeast (Strain G, A. Busch)	120 grams
Table salt (0.01 percent NaI)	40 grams

Drinking water was available all of the time. Vaginal smears were obtained from the rats daily for three days, then every six hours for two days. Each animal was then injected intraperitonealy with 1 uc. of I^{131} contained in 1 milliliter of a normal saline solution. The animals were injected in groups of three with an interval of five minutes between each injection period. They were killed six hours after the time when injected with the radioactive iodine.

The animals were weighed. Their thyroid glands were dissected, cleaned carefully, and weighed with a torsion balance. Each thyroid gland was then dissolved in 10 milliliters of a 0.5 N NaOH solution containing 0.5 percent NaI to dissolve any liberated iodine. An allequot of 1 milliliter was obtained from such solutions by a pipette and transferred to a pyrex disc. These discs were put in an incubator having the temperature of 90° Centigrade for two hours, a period long enough for the samples to dry. The samples were then transferred to a desiccator containing anhydrous calcium sulfate and soda lime to absorb CO_2 and prevent the formation of Na_2CO_3. The samples were then counted by means of a Geiger counter. Since the dry weights of the thyroids were very similar, self-absorption corrections were not made. The pituitaries, adrenals, ovaries, and uteri were also dissected cleanly from the surrounding tissues and weighed. The ovaries were examined microscopically by the use of a lens to confirm the stage of the estrous cycle obtained from the vaginal smears.

It was necessary to kill all the animals in one day to be sure that they were all treated the same way and kept under the same conditions. This is the reason that uneven numbers of animals are included in each stage of the estrous cycle.

Results

I^{131} uptake. By looking at Table II, one will observe the highly significant increase in the uptake of radioactive iodine by the thyroid glands of the estrous animals. There was also a significant increase in the uptake of the thyroid glands during

TABLE II

SIX HOUR I^{131} UPTAKE BY THE THYROID GLAND OF FEMALE RATS AT THE STAGES OF THE ESTROUS CYCLE

	Pro-estrus	Estrus	Met-estrus	Di-estrus
Number of animals	7	16	6	6
Counts/sec./thyroid	145.80 ± 11.27*	230.51'' ± 4.01	171.52' ± 20.64	155.13 ± 12.12
Counts/mg./sec.	7.74 ± 0.53	11.00'' ± 0.63	7.79 ± 0.80	7.58 ± 0.23
Count/mg./sec./100 gm.	2.90 ± 0.30	4.39'' ± 0.20	2.93 ± 0.32	3.03 ± 0.39
Percent of dose	11.16 ± 0.86	17.48'' ± 0.73	12.94 ± 1.64	11.76 ± 0.95

* Standard error.

' Significant at 5 percent level.

'' Significant at 1 percent level.

metestrus over that during proestrus or diestrus, though it was significantly less than that taken up during estrus.

The average counts of the thyroid glands per second were 145.80 in the proestrous animals, 230.51 in the estrous animals, 171.52 in the metestrous animals, and 155.13 in the diestrous animals.

When thyroid uptake was expressed as counts per milligram thyroid per second or percent of injected dose, it was also found that the thyroids of estrus rats had a higher capacity to concentrate radioactive iodine. The thyroid weights did not vary during the estrous cycle, which indicates that the increased uptake during estrus was not due to an increased weight of the thyroid, but to an increased capacity to concentrate radioactive iodine.

The weights of organs. The average body weight of the estrous animals was significantly smaller than the rest because, as mentioned before, the animals were picked at random. The data of Table I have already shown that there is no significant change in body weight with stage of the estrous cycle.

There were no significant differences between the weights of either the thyroids or pituitaries of the four groups. The thyroid weights expressed in milligrams per 100 grams body weight were 7.11 in the case of proestrous animals, 8.43 in estrous animals, 8.20 in metestrus, and 8.14 in diestrus.

The adrenal weights of the estrous animals, when expressed in milligrams per 100 grams of body weight, were significantly higher than those of the proestrous animals. In this experiment also, the ovary weights, when expressed in milligrams per 100 grams of body weight, showed a significant increase in estrus, metestrus, and diestrus over that of proestrus.

There was also a highly significant increase in uterus weights during estrus over those of the uteri of the other three stages.

TABLE III

ORGAN WEIGHTS AT THE STAGES OF THE ESTROUS CYCLE

	Pro-estrus	Estrus	Met-estrus	Di-estrus
Body weight	267.57 ± 11.14	251.56'' ± 4.21	270.40 ± 13.96	257.33 ± 15.91
Thyroid mg./100 gm.	7.11 ± 1.11	8.43 ± 0.64	8.20 ± 0.70	8.14 ± 0.54
Pituitary mg./100 gm.	6.35 ± 0.48	6.81 ± 0.20	5.89 ± 0.47	6.60 ± 2.05
Adrenal mg./100 gm.	17.30 ± 1.17	21.80'' ± 1.32	18.79 ± 1.73	18.77 ± 1.68
Ovary mg./100 gm.	9.45 ± 0.45	13.55'' ± 0.80	15.13'' ± 2.31	15.44'' ± 1.46
Uterus mg./100 gm.	175.86 ±11.58	218.55'' ± 3.10	180.05 ± 7.14	173.81 ± 10.91

'' Significant at 1 percent level.

Experiment III

I^{131} Uptake by the Thyroid Gland of Female Rats Getting Standard NaI Intake at the Stages of the Estrous Cycle

As demonstrated in the preceding experiment, we found that the maximal uptake of radioactive iodine was attained by rats during estrus. In order to exclude the possibility that such variations in thyroid activity might be simply due to the variation in food intake during the estrous cycle as reported by Brobeck (1947), and consequently in the iodine intake, the same experiment was repeated with the exception that the iodine intake of all the animals was standardized.

Materials and Methods

Forty-three mature female rats weighing 180 grams on the average were used in this experiment. These rats were obtained from Carworth Farms, New City, New York. They were put in cages and kept in an air conditioned laboratory having temperature of 24 ± 1° Centigrade.

They were fed an Iodine low test diet (Remington, 1937) and drank double distilled water for fifteen days. The animals were injected intraperitonealy daily with 5 micrograms of NaI contained in 0.2 milliliters of double distilled water. Vaginal smears were obtained daily for three days starting on the tenth day of the experiment; then the smears were taken every twelve hours for the last two days, and immediately after they had been killed. On the last day of the experiment, they were injected intraperitoneally with 1 microcurie of I^{131} and 5 micrograms of NaI contained in 1 milliliter of a normal saline solution. The animals were injected in groups of four with a five-minute interval between each injection period. They were killed six hours after the time when injected with the radioactive iodine.

The thyroid glands were dissected, cleaned carefully, weighed with a torsion balance, and placed at the center of small metal discs. The thyroids were dried in an oven adjusted at the temperature of 90° Centigrade for twelve hours, then counted on the third shelf of a Geiger Counter. The following organs were also dissected out, cleaned and weighed: pituitaries, adrenals, ovaries, and uteri. The ovaries were examined macroscopically to confirm the vaginal smear readings.

Results

I^{131} uptake. Just as in the preceding experiment, there was a significant increase in the uptake of radioactive iodine by the thyroid glands of both estrous and metestrous animals over that of proestrous or diestrous animals. The counts of the whole thyroids were 22.48 during proestrus, 44.62 during estrus, 29.63 during metestrus, and 24.73 during diestrus. It will be noticed also that though the uptake during metestrus was high, it was significantly less than that of estrous animals.

The same trends in I^{131} uptake were found also when the values were expressed as counts per milligram of thyroid per second, counts per milligrams of thyroid per 100 grams body weight or percent of injected dose.

The weights of organs. There were no significant differences between the body weights, thyroid weights, adrenal weights, or pituitary weights of the animals at the four stages of the cycle.

The ovary weights of the animals killed when in diestrus were significantly heavier than those of animals killed during proestrus. The uterus weights of the animals killed during estrus

TABLE IV

SIX HOUR I^{131} UPTAKE BY THE THYROID GLAND OF FEMALE RATS AT THE STAGES OF THE ESTROUS CYCLE

	Proestrus	Estrus	Metestrus	Diestrus
Number of animals	13	8	14	8
Counts/sec.	22.48 ± 1.60*	44.62'' ± 1.80	29.63'' ± 1.69	24.73 ± 2.16
Counts/mg./sec.	1.55 ± 0.14	2.61' ± 0.18	1.85 ± 0.14	1.68 ± 0.17
Counts/mg./sec./100 gm.	0.85 ± 0.10	1.46 ± 0.14	1.03 ± 0.10	0.91 ± 0.10
Percent of dose	8.17 ± 0.64	16.23'' ± 0.65	10.79' ± 0.67	8.99 ± 0.78

* Standard error.

' Significant at 5 percent level.

'' Significant at 1 percent level.

TABLE V

ORGAN WEIGHTS AT THE STAGES OF THE ESTROUS CYCLE

	Pro-estrus	Estrus	Met-estrus	Di-estrus
Body weight	182.92 ± 6.60	182.25 ± 6.64	180.64 ± 2.50	184.00 ± 2.86
Thyroid wt. in mg./100 gm.	8.15 ± 0.45	9.48 ± 0.33	9.00 ± 0.41	8.12 ± 0.43
Thyroid dry wt.	2.67 ± 0.14	2.98 ± 0.14	2.79 ± 0.41	2.81 ± 0.26
Pituitary mg./100 gm.	5.13 ± 0.32	5.75 ± 0.20	6.11 ± 0.36	5.48 ± 0.33
Adrenal mg./100 gm.	22.26 ± 0.99	24.31 ± 2.50	20.87 ± 0.87	20.64 ± 1.19
Ovary mg./100 gm.	21.08 ± 1.20	24.13 ± 3.32	23.80 ± 2.05	26.46' ± 3.18
Uterus mg./100 gm.	138.85 ± 2.63	230.62'' ± 19.47	155.71'' ± 13.49	156.38'' ± 8.72

' Significant at 5 percent level.

'' Significant at 1 percent level.

were significantly heavier than those of the animals killed during the other three stages.

Experiments IV (A) and (B)

I^{131} Uptake by the Thyroid Gland of Female Mice at the Stages of the Estrous Cycle

It has been pointed out in our review of the literature that one of the reasons why investigations of thyroid-ovarian relationship are contradictory is the species difference. The following two experiments were done to compare the pattern of thyroid activity during the estrous cycle of mice with that obtained in rats.

Materials and Methods

Fifty mature female mice weighing 20 to 25 grams were used in this experiment. The mice were obtained from Carworth Farms, New City, New York. They were put in cages and kept in an air conditioned laboratory having the temperature of 24 ± 1° Centigrade. The animals were maintained on low iodine test diet (Remington), and distilled water containing 1 microgram NaI in every 5 milliliters daily. Vaginal smears were obtained daily for three days starting on the tenth day of the experiment. The smears were then taken every twelve hours and at the time of sacrificing the animals.

On the last day of the experiment the mice were injected intraperitoneally with I^{131} contained in 0.5 milliliters of saline solution. The animals were injected in groups of four with a five-minute interval between each injection period. They were killed six hours after being injected with the radioactive iodine.

The thyroid glands were dissected, cleaned carefully, weighed with a torsion ballance, and placed at the center of small metal discs. The thyroids were dried in an oven adjusted at the temperature of 90° Centigrade for twelve hours, then counted with a Geiger Counter at a fixed geometry. The ovaries were examined with a binocular microscope to confirm the vaginal smear reading. The same experiment was repeated using the same number of animals. The mice in this Experiment IV (B) were injected with 1 microcurie of I^{131} instead of 0.5 microcurie as in the preceding experiment.

Results

I^{131} uptake. In the case of mice, I^{131} uptake by the thyroid glands had a different pattern from that of the rats. The mice that were sacrificed while in proestrus showed a statistically significant increase in I^{131} uptake compared to the other stages in both experiments. In experiment IV (A) the diestrous mice also showed a significant increase in I^{131} uptake over that of estrous and metestrous animals.

The average counts of the thyroid glands per second in experiment IV (A) were 67.69 in the proestrous animals, 39.29 in the estrous animals, 39.96 in the metestrous animals and 48.52 in the diestrous animals.

The thyroid counts per second in Experiment IV (B) were 26.24 in the proestrous animals, 15.49 in the estrous animals, 15.64 in the metestrous animals, and 16.76 in the diestrous animals.

When thyroid activity was expressed as counts per milligram of thyroid per second or as percent uptake of the injected dose, the data showed a highly significant increase in radioactive iodine uptake only during proestrus, in both experiments.

Body weights and thyroid weights. There were no significant differences between either the body weights or thyroid weights of the different groups in either experiment.

TABLE VI (A)

I^{131} UPTAKE BY THE THYROID GLANDS OF MICE AT THE STAGE OF ESTROUS CYCLE

Stage of the Cycle	No. of Animals	Body Wt.	Cts./ Sec.	Cts./ mg./ sec./ 100 gm.	Pct. Dose	Thyroid mg./100 gm. Body Wt.
Pro-estrus	12	22.42 ± 0.40*	67.69'' ± 4.85	122.04'' ± 13.39	15.74'' ± 1.13	11.42 ± 0.30
Estrus	8	22.88 ± 0.42	39.29 ± 3.45	65.43 ± 6.08	9.14 ± 0.81	11.79 ± 0.45
Met-estrus	13	23.00 ± 0.41	39.96 ± 2.43	66.03 ± 5.00	9.29 ± 0.56	11.84 ± 0.68
Di-estrus	17	22.82 ± 0.40	48.52' ± 2.98	74.75 ± 8.50	10.62 ± 0.66	11.31 ± 0.62

* Standard error.

' Significant at 5 percent level.

'' Significant at 1 percent level.

TABLE VI (B)

I^{131} UPTAKE BY THE THYROID GLANDS OF MICE AT THE STAGE OF ESTROUS CYCLE

Stage of the Cycle	No. of Animals	Body Wt.	Cts./ Sec.	Cts./ mg./ sec./ 100 gm.	Pct. Dose	Thyroid mg./100 gm. Body Wt.
Pro-estrus	16	21.69* ± 0.67	26.24'' ± 1.37	49.12'' ± 6.73	23.85'' ± 1.24	13.47 ± 0.69
Estrus	7	23.00 ± 0.57	15.49 ± 1.77	21.03 ± 3.72	14.08 ± 1.61	14.81 ± 1.29
Met-estrus	8	22.25 ± 0.61	15.64 ± 1.62	23.90 ± 3.69	14.22 ± 1.47	14.30 ± 1.16
Di-estrus	19	22.10 ± 1.27	16.76 ± 1.13	22.84 ± 1.99	15.00 ± 1.06	15.83 ± 0.88

* Standard Error.

'' Significant at 1 percent level.

Experiment V

The Effects of Estrogen and Progesterone on I^{131} Uptake by the Thyroid Gland of Ovariectomized Rats

The following four experiments were planned to study the mechanisms involved in inducing and controlling the cyclic variations of thyroid activity in the rat.

Experiment V was planned to duplicate as nearly as possible the changes in the steriod hormone balance occurring during the four stages of the estrous cycle. Physiological doses of estrogen, progesterone, and combinations of them were injected into ovariectomized rats to determine their influence on the uptake of radioactive iodine by the thyroid. Under these conditions it was impractical to use normal control animals because of the cyclic variation of their thyroid activity.

Materials and Methods

Forty female rats weighing 150 to 180 grams were obtained from Carworth Farms, New City, New York. At their arrival, they were put in cages in an air conditioned laboratory having the temperature of 24 ± 1° Centigrade. They were offered food (Hoppert ration) and water *ad libitum*. The animals were ovariectomized by using the dorsal approach. They were then divided into six groups of six or seven rats in each. They were numbered individually by coloring them in different areas of the body with picramic acid.

Fifteen days after the operation, the animals were maintained on a low iodine test diet (Remington), and double distilled water. They were injected intraperitoneally daily with 5 micrograms NaI contained in 0.2 milliliters of double distilled water for ten days.

The estrogen used was estradiol benzoate (Schering) dissolved in peanut oil. It was diluted in corn oil so that 6 rat-units were contained in 0.1 milliliter of corn oil. Progesterone was dissolved in ether, then mixed with corn oil and ether was evaporated. Both injected doses of 0.4 or 0.8 milligrams were contained in 0.1 milliliter of corn oil.

Twelve days after standardizing the NaI uptake the animals were treated as follows: Group I was kept as control. Groups II, III, and IV were primed by injecting intraperitoneally 2 rat-units of estradiol benzoate one day before injecting the 6 rat-units.

The animals in Group II were injected intraperiotoneally with 6 rat-units of estradiol benzoate forty-eight hours before autopsy.

Groups III and IV were injected first with 6 rat-units of estradiol benzoate seventy-two hours before being sacrificed. They were also injected on the next day with 0.4 and 0.8 milligrams progesterone respectively.

The animals in Groups V and VI were injected with 0.4 and 0.8 milligrams progesterone, respectively, forty-eight hours before autopsy.

All animals were injected intraperitoneally with 1 microcurie of I^{131} contained in 1 milliliter of saline solution six hours before being sacrificed.

The animals were then sacrificed, their thyroid glands were dissected, cleaned carefully, weighed on a torsion balance, and placed in the center of metal discs. The thyroid glands were then dried for twelve hours in an oven adjusted at the temperature of 90° Centigrade. The thyroid uptake was measured by counting the dry thyroids by the use of a Geiger Counter.

Results

I^{131} uptake. Forty-eight hours after injecting 6 rat-units of estradiol benzoate the thyroid uptake of I^{131} was significantly higher than that of the control. When 0.4 milligrams of progesterone was injected, either alone or twenty-four hours after 6 rat-units of estradiol benzoate the forty-eight hour uptake of I^{131} was decreased significantly when compared to that of the control group.

In rats injected with 6 units of estradiol followed by 0.8 milligrams of progesterone twenty-four hours later, the six-hour uptake of I^{131} did not differ significantly from that of the control.

Forty-eight hours after injecting 0.8 milligrams of progesterone the I^{131} uptake was increased significantly.

The counts per thyroid per second of the control group was 56.07 compared to 67.61 of the group injected with 6 rat-units of estradiol benzoate. The thyroid glands of the groups injected with 6 rat-units estradiol benzoate followed by 0.4 or 0.8 milligrams progesterone registered 46.22 and 53.08 counts per second, respectively. The thyroid glands of the groups injected with 0.4 and 0.8 milligrams progesterone showed 41.55 and 61.21 counts per second, respectively.

Similar differences were found when the values were computed as counts per milligram of thyroid per 100 grams body weight or as percent of injected dose.

TABLE VII

THE EFFECTS OF ESTROGEN AND PROGESTERONE ON I^{131} UPTAKE BY THE THYROID GLAND OF OVARIECTOMIZED RATS

Group No.	Treatment	No. of Animals	Body Wt.
I	Spayed controls	6	220.33 ± 5.41*
II	Spayed + 6 rat-units Estradiol Benzoate	6	214.33 ± 9.84
III	Spayed + 6 rat-units Estradiol Benzoate + 0.4 mg. progesterone	7	223.86 ± 7.04
IV	Spayed + 6 rat-units Estradiol Benzoate + 0.8 mg. progesterone	6	209.17' ± 4.59
V	Spayed + 0.4 mg. progesterone	7	221.11 ± 7.98
VI	Spayed ± 0.8 mg. progesterone	7	229.86 ± 8.37

* Standard error.

' Significant at 5 percent level.

'' Significant at 1 percent level.

TABLE VII (Continued)

Cts./Sec./ Thyroid	Cts./mg./ 100/Sec.	Pct. Dose	Thyroid/ 100	Thyroid Wt. (dry)
56.07 ± 5.96	1.75 ± 0.20	13.91 ± 0.47	6.60 ± 0.46	3.27 ± 0.17
67.61' ± 9.93	2.25' ± 0.40	16.78' ± 0.87	6.96 ± 0.36	3.07 ± 0.45
46.22'' ± 2.22	1.07 ± 0.24	11.47 ± 0.56	7.63 ± 0.62	3.23 ± 0.77
53.04 ± 3.99	1.81 ± 0.25	13.16 ± 0.98	6.87 ± 0.33	2.97 ± 0.14
41.55'' ± 3.05	1.63 ± 0.25	10.31 ± 0.75	5.59 ± 0.41	2.68 ± 0.25
61.21' ± 3.94	1.88 ± 0.17	15.19' ± 0.68	6.17 ± 0.39	3.28 ± 0.89

Body and thyroid weights. There were no significant differences between the body weights or thyroid weights of any of the groups.

Experiment VI

The Effect of Estrogen on I^{131} Uptake by the Thyroid Glands of Ovariectomized and Ovariectomized, Adrenalectomized Rats

Materials and Methods

Forty-eight adult female rats weighing 210 to 250 grams were used in this experiment. They were supplied by Carworth Farms, New City, New York. Twenty-four of these rats were ovariectomized, then divided into three groups--I, II, and III--with eight animals in each group.

The other twenty-four rats were ovariectomized and adrenalectomized, then divided into three groups--IV, V, and VI. Both operations were performed by the dorsal approach.

The rats were allotted to groups four days after the operation and maintained on their specified experimental treatment for twenty-one days.

To permit standardizing the iodine intake, all groups were fed an iodine deficient diet (Remington diet). The adrenalectomized rats were maintained on 1 percent NaCl in double distilled water, while the rest were maintained on double distilled water.

The sodium chloride was analyzed for iodine content by the method of McCullagh (1934). The lot of salt used (Baker C. P.) contained 0.36 micrograms of I_2 per gram of salt, which was equivalent to 0.85 micrograms NaI. The average daily salt water consumption of the adrenalectomized rat was about 25 milliliters, which contained 0.21 micrograms NaI. It was then necessary to add NaI to the water of the nonadrenalectomized rats so that they ingested the same amount of NaI in their drinking water.

The animals were also injected intraperitoneally daily with 5 micrograms of NaI contained in 0.2 milliliters of double distilled water.

Estradiol benzoate (Schering) was dissolved in peanut oil. It was diluted with corn oil so that either 6 rat-units or 300 rat-units of it were contained in 0.1 milliliter of corn oil.

The rats in groups I and IV were used as controls and were injected intraperitoneally with 0.1 milliliter of corn oil. Groups II and V were injected intraperitoneally with 4 rat-units of estradiol benzoate at 6-day intervals to simulate a spontaneous estrous cycle. Forty-eight hours before ending the experiment they were injected intraperitoneally with 6 rat-units of estradiol benzoate.

The animals in Groups III and VI were injected intraperitoneally with 300 rat-units of estradiol benzoate every other day for twenty days. The last injection was given forty-eight hours before ending the experiment.

All rats were then injected with 0.5 microcurie of I^{131} six hours before autopsy. Four animals were injected at a time with a five-minute interval between injection times. The animals were then killed with ether, their thyroid glands dissected, cleaned carefully, weighed on a torsion balance, and placed in the center of metal discs. The thyroids were then dried by leaving them for twelve hours in an oven having the temperature of 90° Centigrade. They were counted by the use of a Geiger Counter at a fixed geometry to its end-window. The pituitary glands were weighed on a torsion balance. The adrenals of the rats that had their adrenals intact were also weighed.

Results

I^{131} uptake. The spayed, adrenalectomized rats given estradial benzoate showed a significant increase in the uptake of I^{131} by their glands when compared to their control. The thyroid uptake of I^{131} of the spayed adrenalectomized rats was significantly less than that of the rats that were spayed only. There is also a statistically significant increase in the uptake of the thyroid of adrenalectomized rats given 6 rat-units of estradiol benzoate, when compared to the uptake of the rats that were spayed only, and received the same treatment.

Though the increase in the uptake of the spayed, adrenalectomized rats treated with ten doses of 300 rat-units of estradiol benzoate injected every other day was significantly less than the uptake by the thyroids of spayed rats treated the same way, yet it was significantly higher than its own control. This was not true in the case of spayed rats. The counts per second per thyroid of the spayed group which was injected with 6 rat-units and the other group that was given ten injections of 300 rat-units estradiol benzoate, were 45.99 and 42.49, respectively, compared to their control which was 39.98 (Table VIII). Groups II which was injected with 6 rat-units of estradiol benzoate had a significantly higher uptake than the spayed control group.

The counts per second per thyroid of the spayed and adrenalectomized rats, given 6 rat-units of estradiol benzoate, and those given ten injections of 300 rat-units of estradiol benzoate

TABLE VIII

THE EFFECT OF ESTROGEN ON I^{131} UPTAKE BY THE THY- ROID GLANDS OF OVARIECTOMIZED AND OVARIECTOMIZED ADRENALEXTOMIZED RATS

Groups	Treatment ..	Cts./sec. Thyroid	Cts./mg. 100 gm.
I	Spayed Control	39.98' ± 1.77	1.02 ± 0.12
II	Spayed ± 6 rat-units estradiol benzoate killed after 48 hours	45.99' ± 1.81	1.00 ± 0.20
III	Spayed ± 300 rat-units estradiol benzoate injected every other day (10 doses)	42.49 ± 1.98	1.10 ± 0.14
IV	Spayed and Adrenalectomized control	29.96'' ± 1.61	0.80 ± 0.26
V	Spayed and Adrenalectomized + 6 rat-units estradiol benzoate	54.47'' ± 1.89	1.11 ± 0.14
VI	Spayed and Adrenalectomized ± 300 rat-units estradiol benzoate (10 doses)	35.69' ± 3.22	0.86 ± 0.10

* Standard error.

' Significant at 5 percent level when compared to its co trol.

'' Significant at 1 percent level when compared to its cc trol.

TABLE VIII (Continued)

Pct. Dose	Thyroid/100 Wt. mg./gm. Body Wt.	Thyroid Wt. (dry)	Pituitary/ 100	Adrenal/ 100
15.03 ± 0.66	5.85 ± 0.21	2.88 ± 0.24	4.77 ± 0.40	15.82 ± 1.70
17.69' ± 0.68	6.42 ± 0.39	3.25 ± 0.20	5.42 ± 0.30	20.17' ± 0.94
15.97 ± 0.75	7.37' ± 0.16	3.21 ± 0.20	5.83 ± 0.10	24.45'' ± 2.09
11.26'' ± 0.61	7.17 ± 0.50	3.15 ± 0.24	4.37 ± 0.33	-- --
20.48'' ± 0.96	8.05' ± 0.45	4.03 ± 0.32	5.16 ± 0.20	-- --
13.42 ± 1.22	8.13' ± 0.50	3.20 ± 0.24	5.97' ± 0.14	-- --

were 54.47 and 35.69 compared to their adrenalectomized control which was 29.96.

When thyroid activity was expressed as counts per milligram of thyroid per 100 grams body weight, no significant differences between the various groups was found.

The counts per milligram thyroid per 100 grams body weight of the spayed rats given 6 rat-units estradiol benzoate and those given ten doses of 300 rat-units of estradiol benzoate were 1.00 and 1.10, respectively, compared with their control which was 1.02. The spayed adrenalectomized rats given 6 rat-units and those given ten doses of 300 rat-units estradiol benzoate counted 1.11 and 0.86, compared to their own control which averaged 0.80 counts per milligram thyroid per 100 grams body weight.

When the thyroid activity was expressed in terms of percent uptake of the injected dose of I^{131}, the thyroids of the spayed controls fixed 15.03 percent of the dose, the spayed rats given 6 rat-units fixed 17.96 percent of the dose, the spayed rats given 300 rat-units of estradiol benzoate every other day for twenty days fixed 15.97 percent. The spayed, adrenalectomized controls fixed 11.26 percent of the dose, while the spayed and adrenalectomized rats that were given 6 rat-units of estradiol benzoate fixed 20.48

percent of the dose and the spayed and adrenalectomized rats that were given 300 rat-units every other day for twenty days fixed 13.42 percent of the dose. The thyroids of the spayed rats given 6 rat-units of estradiol benzoate fixed a higher percentage of the injected I^{131} than their control. This increase in the uptake was significant. Still the percent uptake by the thyroid of the spayed, adrenalectomized rats given 6 rat-units of estradiol benzoate was significantly higher than both the control groups and also significantly higher than the spayed rats treated the same way.

Body weights. The gain by the spayed rats given 6 rat-units of estradiol was significantly higher than that of their controls, while the gain of those given the larger dose of estrogen was significantly less than that of their controls.

The adrenalectomized rats given the large dose of estrogen lost weight. The average body weights at the end of the experiment were 269.6 grams in the spayed controls, 264.5 grams in spayed rats given 6 rat-units of estradiol benzoate, 233.0 grams in the case of spayed rats given ten doses of 300 rat-units of estradiol benzoate every other day, 226.5 grams for spayed adrenalectomized controls, 237.25 grams in the case of the spayed, adrenalectomized rats given 6 rat-units of estradiol benzoate and

TABLE IX

INFLUENCT OF ESTROGEN ON BODY WEIGHT GAINS AND FOOD AND WATER CONSUMPTION OF OVARIECTOMIZED AND OVARIECTOMIZED, ADRENALECTOMIZED RATS

Group No.	Treatment	Number of Animals
I	Spayed control	8
II	Spayed + 6 rat-units estradiol benzoate killed after 48 hours	
III	Spayed + 300 rat-units estradiol benzoate every other day (10 doses)	8
IV	Spayed and adrenalectomized control	8
V	Spayed and adrenalectomized + 6 rat-units estradiol benzoate	8
VI	Spayed and adrenalectomized + 300 rat-units estradiol benzoate every other day (10 doses)	7

* Standard error.

' Significant at 5 percent level when compared to its cont

'' Significant at 1 percent level when compared to its con

TABLE IX (Continued)

Initial Body Wt.	Final Body Wt.	Changes in Body Wt.	Food Consumption per Animal per Day	Water Consumption per Animal per Day
233.62 *± 9.73	269.62 ± 3.91	23.50 ± 3.92	13.17	28.17
233.00 ± 7.42	264.50 ± 3.23	31.00' ± 3.52	15.17	16.83
217.75 ± 6.49	233.00 ± 6.15	16.00' ± 5.83	12.00	15.88
216.00 ± 4.80	226.50 ± 2.90	10.50 ± 6.76	11.67	27.83
226.50 ± 7.14	237.25 ± 2.47	10.75 ± 3.37	11.88	22.50
230.00 ± 7.97	221.28 ± 4.66	-6.28'' ± 5.50	10.90	16.83

221.28 grams in the case of spayed, adrenalectomized rats given ten doses of 300 rat-units of estradiol benzoate every other day.

The spayed controls gained 23.5 grams, while the spayed rats treated with 6 rat-units estradiol benzoate gained 31.0 grams and the spayed rats given ten doses of 300 rat-units every other day gained 16.0 grams. The spayed, adrenalectomized controls gained 10.5 grams, while the spayed, adrenalectomized rats given 6 rat-units of estradiol benzoate gained 10.7 grams and the spayed, adrenalectomized rats given ten doses of 300 rat-units of estradiol benzoate lost 6.3 grams.

Thyroid weights. When expressed in milligrams per 100 grams body weight the thyroids of the spayed controls, the spayed rats given 6 rat-units of estradiol benzoate and those given ten doses of 300 rat-units every other day were 5.85 milligrams, 6.42 milligrams, and 7.37 milligrams, respectively. The thyroids of the group treated with the large dose of estrogen showed a significant increase in weight above their control group, due to the lighter weight of the control animals. The thyroid weights when expressed in the same way were 7.17 milligrams for the spayed adrenalectomized rats, 8.05 milligrams for the spayed adrenalectomized rats given 6 rat-units of estrogen and 8.13 milligrams for the spayed adrenalectomized rats given ten doses of 300 rat-units of estrogen every other day.

Pituitary weights. There were no significant differences between the pituitary weights. The pituitaries of the ovariectomized animals given 6 rat-units, and those given 300 rat-units of estradiol benzoate weighed 5.42 and 5.83 milligrams per 100 grams body weight, respectively, compared to their control which weighed 4.77 milligrams. There were no significant differences between them. The pituitaries of ovariectomized, adrenalectomized animals given 6 rat-units and those given 300 rat-units of estradiol benzoate weighed 5.16 and 5.97 milligrams per 100 grams body weight compared to their control weight of 4.37.

Adrenal weights. The adrenal weights of both groups treated with estradiol benzoate were significantly heavier than those of their controls. The adrenal weights of the group treated with ten doses of 300 rat-units on alternate days were significantly heavier than those of the group treated with the single injection of 6 rat-units of estradiol benzoate.

The adrenal weights of the ovariectomized animals given a single injection of 6 rat-units, and those given ten injections of 300 rat-units of estradiol benzoate were 20.17 and 24.45 milligrams per 100 grams body weight compared to their control's weight of 15.82.

The following significant facts are brought to light by this experiment:

1. The iodine uptake of the thyroids of spayed rats was significantly increased by a small dose of estrogen given for a short period, but not by a large dose given continuously.

2. Thyroid iodine uptake of the spayed rat is depressed by adrenalectomy. However, removal of the adrenal increases the response of the thyroid of spayed rats to estrogen. The increase in iodine uptake is greater with a small, short-term dosage estrogen than with a large chronic dosage.

3. Adrenal weights of spayed rats were significantly increased with either a large or a small dose of estrogen.

Experiment VII

The Effects of Estrogen and Progesterone on I^{131} Uptake by the Thyroid Glands of Hypophysectomized-Ovariectomized and Ovariectomized Rats

Materials and Methods

Forty hypophysectomized-ovariectomized rats, and forty female rats with an average body weight of 150 grams were purchased from the Hormone Assay Laboratories, Chicago. On the day after their arrival the normal rats were ovariectomized, using the dorsal approach. The animals were divided into ten groups with eight rats in each. They were maintained on a low iodine test diet (Remington), and distilled water. The rats were injected daily during the fourteen days of the experiment with 5 micrograms of NaI.

Estradiol benzoate (Schering) was supplied dissolved in peanut oil. It was diluted by using corn oil so that the injected amount was always contained in 0.1 milliliters.

Progesterone was first dissolved in ether, then mixed with corn oil and the ether was evaporated by an air current. The injected dose of progesterone was always contained in 0.1 milliliter of corn oil.

All injection materials were administered forty-eight hours before autopsy.

Groups I, II, III, IV, and V were the hypophysectomized-ovariectomized rats. Groups VI, VII, VIII, IX, and X were ovariectomized only.

Twelve days after hypophysectomy operations the rats were treated in the following way. Groups I and VI were used as controls; they were injected intraperitoneally with 0.1 milliliter of corn oil.

Groups II and VII were injected with 6 rat-units of estradiol benzoate. Groups III and VIII were injected with 300 rat-units of estradiol benzoate. Groups IV and IX were injected with 0.2 milligrams of progesterone. Groups V and X were injected with 0.4 milligrams of progesterone.

The rats were then injected with 3 microcurie of I^{131}. Four animals were injected at a time with five-minute intervals between injections.

Six hours after injecting I^{131}, which was also forty-eight hours after injecting with estrogen or progesterone, the animals were sacrificed. The thyroid glands were dissected, cleaned carefully, weighed on a torsion balance and placed in the center of metal discs. They were dried by leaving them for twelve hours in an oven adjusted at the temperature of 90° Centigrade. The thyroids were counted at a fixed geometry from the end window of a Geiger Counter. It was necessary to use an aluminum absorber of 59.2 milligrams per cm^2 to make it possible to count the highly active thyroids of the groups that had their hypophysis intact. With the use of this absorber only 13.37 percent of the original counts were detected. This made it necessary to correct the counts back to 100 percent in order to compare them with the hypophysectomized rats having thyroids of low activity.

The pituitaries of the nonhypophysectomized rats and all adrenals were weighed.

Results

I^{131} uptake. There were no significant differences between the I^{131} uptakes by the thyroid glands of the hypophysectomized-ovariectomized rats, except in Group V, which was given a dose of 0.4 milligrams progesterone. This group showed a statistically significant decrease in its uptake of I^{131} when compared to its own control. Such differences were significant only when expressed as total thyroid counts, but they were not significant either when the thyroid uptake was expressed in terms of counts per milligram thyroid per 100 grams body weight or when expressed in terms of percent uptake.

The ovariectomized rats with their pituitaries intact showed a statistically significant increase in the uptake of I^{131} by the thyroids of the animals injected with either 6 rat-units or 300 rat-units of estradiol benzoate when compared to their control. Injections of either 0.2 or 0.4 milligrams of progesterone significantly decreased thyroid uptake of I^{131}. The same trends in I^{131} uptake were found also when the values were expressed in counts per milligram of thyroid per second or percent of injected dose.

The thyroid glands of the ovariectomized-hypophysectomized rats collected 8.04 percent of the I^{131} collected by the ovariectomized controls.

Body weights. The body weights of the ovariectomized-hypophysectomized animals were lighter than the animals with their hypophysis intact. The body weights of the hypophysectomized rats given 6 and 300 rat-units of estradiol benzoate and the hypophysectomized rats given 0.4 milligrams of progesterone

TABLE X

THE EFFECTS OF ESTROGEN AND PROGESTERONE ON I^{131} UPTAKE BY THE THYROID GLANDS OF HYPOPHYSECTOMIZED-OVARIECTOMIZED AND OVARIECTOMIZED RATS

	Body Weight	Cts./sec.	Cts./mg. wet/100 sec.
1 Ovariectomized-hypophysectomized control	137.75 ± 2.18	15.87 ± 2.87	1.28 ± 0.14
2 Ovariectomized-hypophysectomized + 6 rat-units estradiol benzoate	132.75' ± 2.60	16.68 ± 2.63	1.53 ± 0.24
3 Ovariectomized-hypophysectomized + 300 rat units estradiol benzoate	130.62' ± 3.97	13.82 ± 1.60	1.23 ± 0.12
4 Ovariectomized-hypophysectomized + 0.2 mg. progesterone	138.50 ± 2.59	14.18 ± 2.03	1.19 ± 0.10
5 Ovariectomized-hypophysectomized + 0.4 mg. progesterone	130.50'' ± 2.89	10.14' ± 1.57	1.01 ± 0.10
6 Ovariectomized control	173.75 ± 3.00	197.36 ±28.78	9.38 ± 1.25
7 Ovariectomized + 6 rat-units estradiol benzoate	172.63 ± 1.66	363.23'' ±28.55	16.83'' ± 1.25
8 Ovariectomized + 300 rat-units estradiol benzoate	167.67 ± 2.07	324.71'' ±22.49	16.31'' ± 0.94
9 Ovariectomized + 0.2 mg. progesterone	170.11 ± 2.12	180.34' ±18.30	8.99 ± 0.89
10 Ovariectomized + 0.4 mg. progesterone	178.25 ± 1.89	140.04'' ±15.10	6.10'' ± 0.17

* Standard error.

' Significant at 5 percent level when compared to its control.

'' Significant at 1 percent level when compared to its control.

TABLE X (Continued)

Pct. Dose	Thyroid Wt. in mg./100 gm./body wt.	Thyroid Wt. (dry)	Pituitary Wt. in mg./100 gm. body wt.	Adrenal Wt. in mg./100 gm. body wt.
0.80 ± 0.14	6.29 ± 0.49	1.71 ± 0.27	---	16.10 ± 0.50
0.84 ± 0.14	6.18 ± 0.87	1.42 ± 0.17	---	15.97 ± 0.52
0.70 ± 0.25	6.47 ± 0.26	1.25 ± 0.30	---	16.06 ± 0.94
0.72 ± 0.10	6.07 ± 0.39	1.31 ± 0.17	---	16.41 ± 0.71
0.51 ± 0.25	5.88 ± 0.35	1.16 ± 0.10	---	17.14 ± 0.44
9.95 ± 1.45	6.78 ± 0.32	1.85 ± 0.66	5.79 ± 0.14	26.83 ± 0.94
18.31'' ± 1.44	6.95 ± 0.36	2.02 ± 0.14	6.03 ± 0.26	30.48' ± 0.73
16.36'' ± 1.14	7.15 ± 0.47	1.98 ± 0.14	6.12 ± 0.17	31.97'' ± 0.98
9.09 ± 0.29	6.96 ± 0.32	1.70 ± 0.14	5.84 ± 0.20	31.49'' ± 1.11
7.06 ± 0.76	7.18 ± 0.25	1.65 ± 0.17	5.81 ± 0.22	31.18'' ± 0.41

were significantly lighter than that of their control. There were no significant differences between the body weights of any of the groups which had their hypophysis intact.

Thyroid weights. There were no significant differences between the thyroid weights of all groups when expressed in milligrams per 100 grams body weight.

Pituitary weights. There were no significant differences between the pituitary weights of the estrogen or progesterone treated animals and their controls.

Adrenal weights. There were no significant differences between the adrenal weights of the ovariectomized-hypophysectomized rats treated with either estrogen or progesterone when compared to their control.

The ovariectomized rats treated with either estrogen or progesterone showed a statistically significant increase in their adrenal weights compared to their control.

Experiment VIII

Thyrotrophic Hormone Potency of the Pituitaries and Plasma of Rats at the Stages of the Estrous Cycle

After finding that the thyroids of estrous rats showed an increase in the uptake of I^{131} above that collected during other stages of the cycle, it seemed desirable to determine whether there were comparable differences in the thyrotrophic potency of their pituitaries and blood serum.

As the differences between the thyrotrophic hormone contents of both pituitary and blood were expected to be too small to be detected by the regular methods, a new technique was used. This method was reported by Besford _et al_. (1952). It employs the uptake of P^{32} by the thyroids of two-day-old chicks as an index of thyrotrophic hormone potency. The authors reported that this method was sensitive to approximately 0.02 Junkmann-Schoeller unit.

Materials and Methods

Four-day-old unsexed chicks were used for the assay. The pituitaries of the rats of each stage of the cycle were pooled and suspended in a saline solution. The sera of the rats of each stage of the cycle were also pooled. A dose of 2 milligrams of fresh pituitary contained in 0.5 milliliter of a saline solution was injected per chick. One and one-half milliliters of plasma were injected per chick. Four hours after the injection of either the pituitary suspension or serum the chicks received 20 microcuries of P^{32} in 1 milliliter of a saline solution. Both injections were given intraperitoneally. Two hours later the chicks were killed, their thyroids dissected out and weighed.

The thyroids were dried by leaving them for two hours in an oven adjusted to the temperature of 90° Centigrade. They were counted by a Geiger Counter.

Results

The analysis of variance showed that there were no significant differences between the body weights or thyroid weights of all groups of chicks.

The chicks given 2 milligrams of rat pituitaries showed a statistically significant increase in their radioactive phosphorus content when compared to the controls.

The radioactive phosphorus content of the thyroids of the chicks given the pituitary from estrous rats showed a slight but

TABLE XI

CHICK ASSAY FOR THYROTROPHIC HORMONE POTENCY OF PITUITARIES OF FEMALE RATS AT THE FOUR STAGES OF THE ESTROUS CYCLE, USING P^{32}

Treatment	Sex	Number	Body Wt.	Thyroid Wt. mg./ 100 gm.	Cts./ Sec./ Thyroid
Control	Males	6	51.62 ± 1.59*	9.52 ± 0.89	1.94 ± 9.29
	Females	8	53.41 ± 1.11	10.80 ± 0.92	2.38 ± 0.22
Proestrus	Males	10	51.88 ± 0.89	9.34 ± 0.82	3.42 ± 0.34
	Females	17	51.92 ± 1.39	11.74 ± 0.80	4.10 ± 0.41
Estrus	Males	10	51.53 ± 1.64	11.33 ± 0.80	3.75 ± 0.41
	Females	11	50.18 ± 1.05	11.75 ± 0.73	4.55 ± 0.42
Metestrus	Males	20	50.23 ± 1.09	10.66 ± 0.62	3.97 ± 0.94
	Females	10	51.46 ± 2.21	10.93 ± 0.84	4.08 ± 0.33
Diestrus	Males	13	53.63 ± 1.17	11.14 ± 0.73	3.65 ± 0.31
	Females	15	51.73 ± 1.26	11.27 ± 1.01	3.43 ± 0.34

* Standard error.

TABLE XII

CHICK ASSAY FOR THYROTROPHIC HORMONE POTENCY SERUM OF FEMALE RATS AT THE FOUR STAGES OF THE ESTROUS CYCLE

Treatment	Sex	Number	Body Wt.	Thyroid Wt. mg./ 100 gm.	Cts./ Sec./ Thyroid
Control	Males	6	51.62 ± 1.59*	9.52 ± 0.89	2.19 ± 0.16
	Females	8	53.41 ± 1.11	10.80 ± 0.92	
Proestrus	Males	0	50.66 ± 1.30	11.90 ± 0.37	2.66 ± 0.48
	Females	8			
Estrus	Males	2	48.98 ± 1.82	10.33 ± 1.05	3.05 ± 0.39
	Females	4			
Metestrus	Males	2	48.08 ± 1.62	11.59 ± 0.46	2.41 ± 0.10
	Females	4			
Diestrus	Males	3	53.08 ± 1.47	9.77 ± 0.77	2.38 ± 0.08
	Females	3			

* Standard error.

nonsignificant increase when compared to that of the thyroids of chicks injected with pituitaries of the rats killed at the other three stages of the estrous cycle.

When injected with rat serum, the only group of chicks which showed a significant increase in the radioactive phosphorus content of their thyroids when compared to the male controls or the average of both males and females together, were those injected with serum from estrus rats. There were no significant differences between the radioactive phosphorus content of the thyroids of chicks treated with serum of rats at the four stages of the cycle.

One could conclude, though not definitely, that the thyrotrophic potency of the pituitaries of estrus rats have a slightly higher potency when compared to those of rats at the other three stages of the estrous cycle. From this experiment also, it seems that the thyrotrophic hormone content of the serum of estrus rats exceeds that of rats at the other three stages of the cycle.

As this method of assaying thyrotrophic hormone is new, it seems probable that with further improvement of the techniques one could obtain more conclusive results. By using larger doses of radioactive phosphorus, pituitary and serum larger absolute differences between groups should be found.

DISCUSSION

The data presented in experiment I showed an increased oxygen consumption of rats during estrus. On the average there was an increase of 16.65 percent during estrus over that during diestrus. This is in agreement with the findings of Laqueur et al. (1926), who found that the basal metabolism rose 20 percent in normal and ovariectomized rats within three days during the injection of estrogen.

Slonaker et al. (1927) and Wang (1932) noticed that in the rat the estrus phase is accompanied with a pronounced increase in locomotor activity and an increase in energy expenditure.

Brobeck et al. (1947) also found that estrous rats lost from 10 to 12 grams in the individual cycle due to decreased food intake and increased expenditure of energy. They thought that the periodic changes in the rates of energy intake and expenditure appeared to be derived from either hypophysial or ovarian cycles which they stated is compatible with the hypothesis that processes of energy exchange are regulated and integrated by specialized cells, possibly by cells which lie within the hypothalamus. We also believe that the variation in energy exchange as indicated by increased oxygen consumption during estrus is most probably controlled by hypophysial ovarian and thyroid hormones, even though there was no loss in body weight.

Estrogen predominating during estrus might have direct metabolic effects, increasing oxygen consumption or possibly acting indirectly via the pituitary, thyroid and adrenal glands.

Under our condition of studying the rats under basal conditions there were no significant differences between the body weights of the animals at the different stages of the estrous cycle.

When the rats were fed ad libitum Brobeck et al. (1947) found that estrous rats lost from 10 to 12 grams in the individual cycle. They also found that food intake during estrus was always less than the intake during diestrus, by as much as 6 grams per day. This, together with the increased energy expenditure during estrus, might partly explain the decrease in body weights of such rats. Thorn et al. (1938) reported that normal women in whom dietary restrictions were imposed were observed to gain weight during the intermenstrual as well as during the premenstrual phase of the cycle. An increase in appepite and thirst was a striking symptom noted during the premenstrual period. They concluded that the increase in the secretion of sex hormones both estrogen and progesterone (which induce retention of sodium, chloride and water during the intermenstrual as well as the premenstrual phase of the cycle) together with the increase in appetite and thirst appear to be contributing factors in body weight gain, that occurs during the menstrual cycle. They also found that the onset of menstruation was associated with an increase of renal excretion of sodium, chloride and water.

Our data did not show a distinct variation in the basal temperatures of rats during the estrous cycle. Brobeck _et_ _al_. (1947) reported that low body temperatures were consistently noticed in rats on the afternoon just preceding the night of estrus. The temperatures were also occasionally found to be low a day before or after estrus. It was definitely lower during estrus than during diestrus. It should be noticed that these were not basal temperatures. Nieburg and Greenblatt (1948) showed that in castrated female rats, the administration of progesterone increases the temperature above that of the castrated controls while estrogen produces a decrease of body temperature. Rothchild and Rapport (1952) reported also that the administration of progesterone to male cretins or feeble-minded humans caused an elevation of body temperature, and that such an effect was not mediated through the thyroid gland.

Whitelaw (1952) on the other hand concluded that endogenous progesterone is not the hormone responsible for either the rise or maintenance of the thermogenic effects occurring during the menstrual cycle. As we will point out later one should keep in mind the dosage rate and period of administration, since these factors can considerably vary the results. Griffith et al. (1929) found that there is no significant correlation between the intraindividual variations of temperature and metabolism.

The results from experiments II and III showed that in rats there is a statistically significant increase in the uptake of radioactive iodine by the thyroids during estrus. It was also found that the uptake of metestrous rats, though significantly less than that of estrous rats, was significantly higher than that of rats in diestrus and proestrus.

In experiment II the rats were fed a regular diet. Brobeck (1947) reported, as mentioned before, that estrous rats eat 6 grams less food daily than diestrous rats. Consequently, the iodine intake would also be less during this stage.

Another fact is that the uptake of I^{131} is inversely proportional to the iodide intake as reported by Vanderlean and Greer (1950). They also added that the iodine concentrating mechanism appears to be so regulated that under conditions of varying iodine supply the efficiency of iodine accumulation can be greatly altered; for example when iodine is deficient, there is a compensatory alteration of thyroid function so that the available supply of iodine can be trapped for more efficiency.

These two factors suggested the possibility that the variations in thyroid uptake observed in our first experiment might simply be due to the variation in food intake and the consequent variation in NaI uptake. It was then decided to standardize the uptake of NaI.

In experiment III the rats were fed an iodine deficient diet and drank double distilled water. They were injected intraperitoneally daily for fifteen days with 5 micrograms of NaI. Under these conditions we observed an exactly similar pattern of thyroid uptake to that found in the preceding experiment where the animals were fed a regular diet and drank tap water.

The results from these two experiments pointed out clearly that such cyclic variation in thyroid activity is not due simply to variability in the amount of ingested iodine, but it is definitely a hormonal mechanism which is closely related to ovarian activity.

Another point we would like to defend before we go any further is: how reliably can the radioactive iodine uptake by the thyroid be used as an index of thyroid activity? There are many investigators who look with suspicion upon data where I^{131} uptake is used as an index of thyroid activity. It is necessary at this time to report here the results of the investigations of Dougherty et al. (1951). These investigators concluded from their experiments that the uptake of iodine and the release of iodine-containing hormone occur simultaneously, continuously and at an approximately constant rate. In their experiments the rate of uptake of iodine was determined by the amount of radioiodine found in the gland four hours after the injection. This represented the amount entering the gland minus that leaving the thyroid over the four-hour period. However, they say that the amount of radioactive PBI in the blood was only about 2 percent of the amount of I^{131} in the two thyroid lobes. Therefore, the release of the amount organically bound from the thyroid must have been small enough to make it possible to consider the thyroid counts as a good index of the amount of radioiodine entering the gland since the time of injection.

Examination of individual data indicated that the PBI activity was high when the thyroid radioactivity was high and vice versa.

In the mouse the case was different from that observed in rats. Though there was a cyclic variation in thyroid activity when correlated to the estrous cycle, the highest uptake of radioactive iodine was attained by the mice in proestrus, as shown by the data of experiments IV (A) and (B). Here is a good illustration of two species of animals with different patterns of thyroid activity. It is true that both species exhibited a cyclic variation through the estrous cycle but the rat showed maximal activity during estrus, while the mouse thyroid had its highest activity during proestrus.

It is generally agreed that the mouse is a relatively hypothyroid animal and that the rat is a slightly hyperthyroid animal as indicated in the work reported by Maqsood and Reineke (1950), Novak (1950), Meites and Chandrashaker (1949), and Johnson and Meites (1951).

In the mouse it seems that estrogen at a very low level has a target effect on the thyroid giving a spurt of increased activity followed by a depression. Perhaps the thyroid gland of the mouse responds to estrogen at a low threshold then declines, possibly a time factor is involved also, after which the thyroid gland becomes refractory to estrogen, or possibly it becomes refractory to high levels of estrogen. Another explanation may be that the thyrotrophic hormone depot is exhausted quickly.

So far we pointed out that oxygen consumption of the female rat is increased during estrus. We also found that in the rat the uptake of radioactive iodine reaches its highest peak during estrus. It then gradually falls, starting at metestrus, and attains its lowest level during diestrus and proestrus.

The organs involved in such a cyclic variation of thyroid activity are most probably the ovaries, adrenals and pituitary. The relationship between the thyroid and these glands has been studied in experiments V, VI, and VII.

A single injection of six rat-units (1 microgram) of estradiol benzoate, a dose large enough to elicit an estrus smear in ovariectomized rats within forty-eight hours after injection, succeeded in increasing significantly the uptake of radioactive iodine.

Studies of the effect of estrogen on the turnover rate of I^{131} by the thyroids of chicks, Epstein and Wolterink (1949), rats and mice, Wolterink et al. (1950), and Wolterink (1952) showed that small doses of estrogen (1 microgram) daily for three days increased the output of I^{131} by the thyroid, while large doses of

10 or 100 micrograms of estrogen given daily for three days and also a massive single dose of estrogen depressed I^{131} turnover. In other words, a dose of estrogen large enough to bring a rat into estrus is able to increase the iodine concentrating capacity of its thyroid. It also increases the rate of release of thyroid hormone.

These two effects are characteristic of the effect of thyrotrophic hormone. The results from experiments VII and VIII help to prove this new point. In hypophysectomized animals the same dose of estrogen failed to exercise the usual stimulating effect on the thyroid. It was unable to increase the I^{131} uptake by the thyroid as it did in the animals with the hypophysis intact.

The assay of thyrotrophic hormone potency of the pituitary and serum of the rats gave an indication, though not a significant one, that there is a higher thyrotrophic hormone content in the serum of estrus rats.

Saxton and Green (1942) found that thyrotrophic hormone is released from the female rabbit hypophysis after coitus together with FSH and LH and probably ACTH. Ovulation in the rabbit occurs only after coitus, while in the rat it is spontaneous. In other words, there is an increased secretion of TSH prior to and at ovulation in both species.

The presence of thyrotrophic hormone is necessary for the thyroid gland to concentrate the iodide circulating in the blood. Following hypophysectomy this capacity is lost although not entirely. Randall and Albert (1951) found that following hypophysectomy the rat thyroid collected about 12 percent as much iodine as before hypophysectomy. They also found that the defect in collection of iodine occurs soon after the operation and becomes maximal between five and six days after the operation. Five or six days after hypophysectomy the thyroidal uptake of I^{131} reached a minimal value of about 8 percent of normal and remained at this level throughout the subsequent period of observations. The data obtained from experiment VII showed that the ovariectomized-hypophysectomized rats thirteen days after the operation collected 8.04 percent of the I^{131} collected by the ovariectomized controls. These values are similar to those obtained by Randall and Albert.

Prolonged administration of a large dose of estradiol benzoate at the dosage rate of 300 rat-units (50 micrograms) 10 times in a period of twenty days had no effect on the thyroid uptake of radioactive iodine.

Our data in this case agree with those of Paschkis _et al._ (1948), who reported that 50 micrograms of estradiol benzoate for twelve, eleven, and thirteen days did not have any effect on I^{131} uptake when compared to the castrated control. Money _et al._ (1950, 1951) reported similar results with estradiol benzoate. However, 5 micrograms of estrone given daily for ten days caused a significant rise in I^{131} uptake with no increase in thyroid weight.

While prolonged administration of a large dose of estradiol benzoate failed to show a detectable change in thyroid activity, a single injection of 300 rat-units (50 micrograms) of estradiol benzoate resulted in an increased uptake of I^{131} not significantly different from the increased uptake induced by a single injection of six rat-units. This shows clearly that it was not the dose but the period of administration which resulted in our failure to detect any differences between the rats treated with estrogen for a long period and the controls. The most probable explanation for such a phenomenon is that the first few injections of estrogen will stimulate the thyroid via the pituitary gland which secretes thyrotrophic hormone. With prolonged administration of estrogen the thyrotrophic hormone depots are exhausted and consequently the thyroid gland will be unable to concentrate iodine as before.

Pincus and Werthessin (1933) showed that there was no indication of a dosage relation to the effect of estrin injections on increasing thyroid weight, and that it is only a matter of the period of administration. They got a maximum increase in thyroid weight after five days of injecting estrin, and this decreased fairly gradually to a minimum after forty days of injection, indicating that the effect of estrin on the thyroid is not sustained with prolonged injection. Koenig *et al*. (1945) also found that prolonged administration of estrone resulted in a decrease in the iodine content of the thyroids.

Progesterone given at the dose levels of 0.2 and 0.4 milligram succeeded in decreasing the six-hour I^{131} uptake by the thyroids of ovariectomized rats forty-eight hours after progesterone administration, as shown in experiments V and VII. It was also demonstrated that a dose of 0.4 milligram of progesterone was able to counteract the effect of six rat-units of estrodiol benzoate with a resultant decrease in I^{131} uptake.

The dose of 0.8 milligram progesterone alone increased the I^{131} uptake, while the same dose of progesterone antagonized the stimulating effect of estrogen on the thyroid.

The only prior investigations where I^{131} has been used for the study of the effect of progesterone on thyroid activity were those of Money et al. (1950, 1951). They reported that a total dose of 50 milligrams progesterone injected into male rats in a period of ten days increased the uptake of I^{131} by the thyroid without influencing the thyroid weights.

Histological studies of hyperplastic thyroids of rats have shown that the parenteral administration of progesterone alone or progesterone plus estrogen were able to inhibit hyperplasia as reported by Emge and Laqueur (1941) and Schilling and Laqueur (1941). They also reported that although estrogen failed to change the thyroid weights of thyrohyperplastic rats, progesterone or estrone and progesterone together decreased the thyroid weights.

The possibility that progesterone might exert its inhibitory action on the thyroid mainly via the pituitary was ruled out. It was found that a dose of 0.4 milligram progesterone administered to ovariectomized-hypophysectomized rats caused a statistically significant decrease in the uptake of I^{131} when compared to that of control ovariectomized-hypophysectomized rats.

Cutuly (1941) found that the administration of 0.5 to 3.0 milligrams daily of progesterone to castrated adult male and female rats did not correct histological castration changes of the anterior pituitary. The weight changes of pituitaries of females were irregular. They also reported that progesterone did not have a selective inhibitory action on the different components of gonadotrophins.

By appropriate hormone injections in spayed rats it was possible to imitate closely the changes in thyroid iodine uptake occurring during a normal estrous cycle. The data at the top of the following page, collected from Tables II, IV, and IX, illustrate that this was accomplished.

A stage similar to estrus was produced by injecting six rat-units of estradiol benzoate. This was accompanied by an increased uptake of I^{131}. A stage similar to metestrus was observed in rats that were injected with six rat-units of estradiol benzoate followed after twenty-four hours by 0.4 milligram progesterone. This was accompanied by a decrease in the uptake of I^{131}. When ovariectomized rats were injected with a dose of 0.4 milligram progesterone only there was a still further decrease in I^{131} uptake. Such animals may be considered similar to rats

PERCENT OF INJECTED DOSE OF I^{131}

	Proestrus	Estrus	Metestrus	Diestrus
Hoppert diet	11.2 ±0.9	16.2 ±0.7	12.9 ±1.6	11.8 ±1.0
Remington diet . . .	8.2 ±0.6	16.2 ±0.7	10.8 ±0.7	9.0 ±0.8

	Spayed Plus			
	Estradiol Benzoate (6 r.-u.)	Estradiol Benzoate + 0.4 mg. Progesterone	0.4 mg. Progesterone	Control (spayed)
Remington diet	16.8 ±0.9	11.5 ±0.6	10.3 ±0.8	13.9 ±0.5

in diestrus.

In each case the I^{131} uptake in the treated group was not significantly different from that in normal rats in a comparable stage of the estrous cycle. When ovariectomized rats were injected with 0.8 milligram progesterone, the uptake of I^{131} was increased. Money et al. (1950) also reported that a high dose of progesterone increased the I^{131} uptake.

There were no significant differences between the thyroid weights of the rats during the different stages of the estrous cycle. The thyroid weights also were not affected by the administration of either estrogen or progesterone alone or together. Money et al. (1950, 1951) also reported that neither estrogenic compounds nor progesterone changed the thyroid weights. With prolonged administration of large doses of estradiol benzoate the result was an increase in thyroid weight when expressed in terms of milligrams per 100 grams body weight. Such an increase in thyroid weight is only a reflection of the smaller body size of this group of rats.

Actually then there is no relation between the size of the thyroid gland and its hormonal activity. We would like here to quote Vanderlean and Greer (1950): ''Although hyperplasia of the thyroid gland ordinarily attends an increased efficiency for accumulating iodine, and hypoplasia a decreased efficiency, from such an experiment such a relationship is divorced because, the animals that were fed thiouracil before hypophysectomy attained the largest size of cells and possessed a very low capacity for iodine accumulation.''

The data concerned with the cyclic variation of the adrenal gland activity as correlated to the estrous cycle showed (1) that the adrenal glands weigh more during estrus than during the other three stages, (2) a single injection of as small a dose as six rat-units (1 microgram) of estradiol benzoate increased the adrenal weight, (3) a dose of 0.2 and 0.4 milligram progesterone also increased adrenal weights. Zondek and Burstein (1952) reported that the maximal excretion rate of corticoids in guinea pigs was during estrus. (4) In the absence of the pituitary neither estrogen nor progesterone failed to exercise its effect on increasing the adrenal weight. It is important to stress the fact that our data add another proof to the fact that estrogen induces its effect on the adrenals through the pituitary, as reported by Bourne and Zuckerman (1940).

Although it has been reported by several investigators that the adrenal glands tend to exercise an inhibitory effect on the thyroid capacity to accumulate I^{131}, from our experiments it appears that the adrenal is also essential for normal thyroid function. The I^{131} uptake of the ovariectomized-adrenalectomized rats was significantly lower than that of rats that were ovariectomized only.

In the absence of the adrenals the thyroid glands of ovariectomized rats responded with a higher uptake of I^{131} to the stimulating effect of a physiological dose of estrogen than that of the ovariectomized rats with the adrenals intact that were given the same dose of estrogen.

In conclusion it seems that the adrenal glands are essential for the normal function of the thyroid. It can also work as a secondary safety control on thyroid activity especially when it reaches its maximal stage.

Cortical hormones induce their effect on the thyroid gland either by inhibiting the effect of TSH as reported by Woodbury et al. (1951) or by increased rate of elimination of iodine in the urine as reported by Berson et al. (1952) or probably through expanding the iodide space or any other mechanisms necessary for the selective accumulation of iodide by the thyroid cells.

In the light of all of these results we can summarize our hypothesis of the interrelationship between thyroid, ovary, adrenal and pituitary of normal mature female rats as follows:

1. During proestrus the thyroid gland is in a relatively hypoactive condition. Under this condition FSH is produced and released resulting in the gradual growth of graafian follicles, as stated by Richter (1933) in the rat, Soliman and Reineke (1952) in the mouse, Hofmeister (1893) and Chu (1944) in the rabbit. In rats hypothyroidism will enhance the effectiveness of the follicle stimulating hormone as stated by Lane (1935) or gonadotrophins as reported by Leonard (1936), Smelser and Levine (1941), and Johnson and Meites (1951). Pituitary extracts from thyroidectomized rats when injected into immature female mice caused increased weight of the ovaries and uteri when compared to the results with extracts from normal animals, as reported by Stein and Lisle (1942).

2. During estrus estrogen is produced in amounts large enough to stimulate thyroid activity via the pituitary. Increased thyroid activity is believed to favor the production of LH. Weichert (1930) and Weichert and Boyd (1933) reported that feeding rats

with desiccated thyroid prolongs the diestrus period from thirteen to twenty-four days. There were persistent functional corpora lutea suggesting increased output of the luteinizing hormone. The thyroid hormone also might have a direct effect on the ovary (Lee, 1925; Tyndale and Levin, 1937). Salter (1949) stated that the thyroxine iodine is fixed in two general locations. The first is its accumulation in the pituitary and ovary. The second is its accumulation in the peripheral tissues, primarily the skeletal muscle.

Allen (1939) suggested that changes in the follicle are initiated by thyroxine as well as by the maturation hormone of the anterior pituitary but that the latter only produces rupture.

There is also a safety mechanism induced by the stimulated adrenal at this stage to prevent or limit the overactivation of the thyroid gland, such an effect is also induced via the same highway, the pituitary.

3. During metestrus the rate of secretion of estrogen is slowed down and progesterone is produced in gradually increasing amounts. Under these conditions thyroid activity gradually decreases.

4. During diestrus progesterone predominates over estrogen resulting in decreased thyroid activity. This mechanism is not mediated via the pituitary. It may be due to a direct effect of progesterone on the thyroid or most probably by expanding the iodide space. Thorne et al. (1938) reported that the injection of crystalline preparations of estrone and progesterone induced the retention of sodium, chloride and water in normal dogs. Therefore, it seems that progesterone and also estrone could expand the extracellular space of the body, and consequently the iodide space.

5. Again, in proestrus, neither estrogen nor progesterone are produced at high levels, resulting in a hypoactive thyroid gland, and the cyclic variations start over.

6. Large amounts of progesterone increase thyroid activity as pointed out from our experiments and from the reports of Money et al. (1950 and 1951). These conditions may be similar to what occurs during pregnancy.

SUMMARY AND CONCLUSIONS

1. Determinations were made of oxygen consumption, basal temperature and body weights of rats under basal conditions during the four stages of the estrous cycle. It was found that there is significantly increased oxygen consumption during estrus. There were no significant differences between basal temperatures or body weights at the four stages of the estrous cycle.

2. The study of I^{131} uptake by the thyroid glands of rats during the estrous cycle showed that there is an increased uptake during estrus. The uptake gradually decreases at metestrus and attains its lowest level during diestrus and proestrus.

3. Similar studies of I^{131} uptake by the thyroid glands of mice during the estrous cycle showed a pattern of cyclic variation different from that of the rat. The maximal uptake of I^{131} is attained by the thyroids of proestrous mice.

4. A single injection of 6 or 300 rat-units of estradiol benzoate increased the six-hour uptake of I^{131} forty-eight hours after the estrogen injection. There were no differences between

the effects of the two doses on thyroid uptake, with the conclusion that the dose of estrogen is not very critical.

5. The administration of ten doses of 300 rat-units of estradiol benzoate on alternative days did not change the I^{131} uptake by the thyroids when compared to controls.

6. A single dose of 0.2 or 0.4 milligram of progesterone decreased the uptake of I^{131} by the thyroids while a dose of 0.8 milligram increased the uptake.

7. The injection of 0.4 milligram of progesterone counteracted the effect of estrogen on thyroid function with the net result of a decreased I^{131} uptake.

8. Experiments on hypophysectomized-ovariectomized rats showed that estrogen produces its stimulating effect on the thyroid gland via the pituitary gland since estrogen did not change the I^{131} uptake in hypophysectomized rats.

9. Progesterone decreased the uptake of I^{131} by the thyroid glands of hypophysectomized rats, showing that it does not induce its effect mainly via the pituitary. It might have a direct effect on the thyroid or act by simply expanding the iodide space.

10. An experiment with ovariectomized-adrenalectomized rats showed that the adrenal glands seem to be essential for normal thyroid function as indicated by the decreased I^{131} uptake of the ovariectomized-adrenalectomized rats when compared to ovariectomized rats.

11. The I^{131} uptake by the thyroid glands of ovariectomized-adrenalectomized rats treated with estrogen was higher than that of rats with their adrenals intact but given the same dose of estrogen. The adrenal glands of the estrogen-treated animals were heavier than controls, giving the impression that when the adrenal glands are stimulated it decreases the concentrating capacity of the thyroid for iodine more than if the adrenal were absent.

12. The thyrotrophic hormone content of the pituitaries and blood sera of the rats killed at the four stages of the estrous cycle, were assayed using the uptake of P^{32} by chick thyroids as an index of thyroid response to the thyrotrophic hormone potency. The thyrotrophic hormone content of the serum of estrus animals seemed to be higher than that of the sera from rats at the other three stages of the cycle.

13. There were no significant differences between the thyroid weights of rats or mice at the four stages of the estrous cycle.

14. A single dose of either 6 or 300 rat-units of estradiol benzoate did not have an affect on the thyroid weights.

15. Prolonged administration of 300 rat-units of estradiol benzoate increased thyroid weights only when expressed in terms of milligrams per 100 grams body weight, due to the lighter body weight of the rats. The adrenal weights were heavier during estrus than during all other stages of the cycle.

16. There were no significant differences between the pituitary weights of rats at the four stages of the cycle.

17. In rats the ovary weights of the animals killed when in diestrus were significantly heavier than those of animals killed during proestrus.

18. In rats the uterus weights of the animals killed during estrus were significantly heavier than those of the animals killed during the other three stages of the cycle.

19. In the light of all the aforementioned results, a hypothesis was presented to explain the possible relationship between the thyroid, ovary, adrenal, and pituitary.

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