145
846
THS

INFLUENCE OF ENVIRONMENTAL TEMPERATURE AND THYROID STATUS ON THE REPRODUCTIVE ORGANS OF YOUNG FEMALE MICE

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Fouad Atalla Soliman

1950

This is to certify that the

thesis entitled

"Influence of Environmental Temperature and Thyroid Status on the Reproductive Organs of Young Female Mice"

presented by

Fouad Atalla Soliman

has been accepted towards fulfillment of the requirements for

M.S. degree in Physiology

E. P. Reineke
Major professor

Date May 19, 1950

O-169

INFLUENCE OF ENVIRONMENTAL TEMPERATURE AND THYROID STATUS ON THE REPRODUCTIVE ORGANS OF YOUNG FEMALE MICE

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

MASTER OF SCIENCE

Department of Physiology and Pharmacology

1950

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude for the help and guidance 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. Joseph Meites was also helpful in his valuable suggestions. My thanks are extended to Dr. Lois Calhoun, Head of the Department of Anatomy, who gave me many helpful suggestions, and also permitted me to use laboratory facilities for the histological work.

I appreciate the fine work done on the photographs by Mr. M. L. Gray of the Department of Animal Pathology. My appreciation, also, goes to Mr. J. Munroe for taking care of 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.

234587

TABLE OF CONTENTS

INTRODUCTION 1

REVIEW OF LITERATURE 1

The Effect of Environmental Temperature on Thyroid Activity 1

Thyroid Active Preparations 3

Goitrogens 4

The Effect of Mild Hyperthyroidism on Growth 5

The Relation Between the Thyroid Gland and the Female Reproductive System 6

Rat 7

Mouse 9

Rabbit 10

Guinea Pig 10

Cattle 11

MATERIALS AND METHODS 11

Feeding 12

Temperature and Light 12

Procedure 12

RESULTS 14

Effects of Thyroprotein Feeding on Growth 14

Effects of Thiouracil Treatment on Growth 15

Food and Water Consumption 15

Effects of Temperature 15

Effects of Hyperthyroidism 15

Effects of Hypothyroidism 16

Effects of Hyperthyroidism on Reproductive Organs 17

Effects of Hypothyroidism on Reproductive Organs 19

DISCUSSION 29

SUMMARY AND CONCLUSIONS 34

BIBLIOGRAPHY 36

INTRODUCTION

Seasonal variations accompanied by changes in such factors as temperature and light have been proved to influence the reproductive rhythm of female as well as male animals. The females permit copulation only during recurrent periods. On the other hand the evidence of such a relation between thyroid activity and the external environmental temperature and light has been proved. The thyroid gland is much more active in low temperatures and short days as those of the fall and winter than in high temperatures and long days as those of summer.

Such a sensitivity of both thyroid activity and reproduction to changes in temperature and light irradiation suggested an interrelationship between the thyroid and reproductive organs.

The purpose of this work is (1) to determine the effect of environmental temperatures on thyroid activity and reproductive processes of female immature albino mice, (2) to find out the effect of mild hyperthyroidism induced experimentally by giving the mice minute doses of thyroprotein and (3) to determine the effect of hypothyroidism induced by giving thiouracil, on the rate of growth and reproductive activity of these mice.

REVIEW OF LITERATURE

The Effect of Environmental Temperature on Thyroid Activity.

It is well known that the rate of thyroxine secret-

ion is partially under the influence of the environmental temperature. When the temperature is low (as in Winter) the rate of thyroxine secretion is high in order to increase the rate of heat production to compensate the lost heat. When the temperature is high (as in Summer) the rate of thyroxine secretion is low and the rate of heat production is low since less heat is lost from the body. Some of the work is cited here.

Dempsey and Astwood (1943) were able to determine the rate of thyroid hormone secretion in rats and found it to be high in cold environments (1° C.) and low in hot environments (25° C. and 35° C.).

Reineke and Turner (1945) determined the amount of thyroxine required to prevent an increase in the weight of the thyroid gland during the administration of thiouracil in White Plymouth Rock chicks at 2 weeks of age. Maximum secretion rate was observed during October and November. The thyroid secretion rate declined starting from the month of February and remained at a low level during the Summer.

Mills (1918) and Kenyon (1933) exposed rats to a cold temperature and found that it increased the vascular activity of the thyroid and caused cellular hypertrophy that was believed to be induced by the metabolic strain. Iodine in a dose of 10 mcg. daily diminished or prevented this change.

Further evidence was reported by Ring (1936) who measured the basal metabolic rates of rats kept at the temperature of 35° C. and their basal metabolic rates when kept at the temperature of 6-8° C. It was greatly elevated when the animals were kept at the low temperature. It seemed possible that the increase in the metabolism might be caused by the thyroid gland.

Brolin (1946) confirms that the thyroid activation in response to cold is associated with an increase in basophilic cells of the anterior pituitary and increased output of the thyrotropic hormone.

Thyroid Active Preparations.

Baumann (1895) discovered the presence of iodine in the normal thyroid gland. Kendall (1915) was able to isolate the active principle, thyroxine, from the thyroid gland and found that it contained a high percentage of iodine. Harington in 1927 produced the hormone synthetically. Crystalline thyroxine was recovered from iodinated proteins by Ludwig and Mutzenbacher (1939) and Harington and Pitt Rivers (1939) but the potency of the iodinated protein was low.

Reineke and Turner (1941, 1942) and Reineke (1949) prepared artificial thyroproteins by the iodination of proteins such as casein, soyabean, protein and egg albumin. Under optimal conditions of iodine input, temperature, and pH such preparations showed very high thyroidal activity. The actual hormone, thyroxine, was recovered from these preparations and its identity was confirmed by microscopic structure, iodine content and spectographic analysis.

They were highly active in stimulating metamorphosis of tadpoles. These preparations alleviated the symptoms of cretinism in thyroidectomized goats, elevated the metabolic rate of laboratory animals, and increased milk production when fed to lactating goats and dairy cattle.

Goitrogens.

Before the use of goitrogens in inducing hypothyroid conditions, the usual technique was thyroidectomy, either complete or partial.

Astwood et al. (1943) found that the administration of sulfonamide compounds and thiourea caused an enlargement in the size of the thyroid.

In 1944, Astwood and Bissell administered thiouracil to young rats and found a nearly complete disappearance of iodine from the thyroid gland in five days and a threefold increase in the size of the glands in 2 weeks. These effects were inhibited by hypophysectomy or by the injection of thyroxine, which proves that the thyroid enlargement occurs in response to excessive secretion of thyrotropic hormone resulting from decreased secretion of thyroxine (see also, Williams et al., 1944 and Paschkis et al., 1945).

Mixner et al. (1944) tried both thiouracil and thiourea in chicks. When 0.1 percent thiouracil was mixed with the feed they observed beginning enlargement of the thyroids by the fourth day and maximal enlargement by the twelfth day of thiouracil administration. By the injection of thyroxine to thiouracil-fed chicks the size of the thyroid was decreased. The amount of thyroxine that is necessary to maintain the normal size of the gland corresponds with the normal output of thyrox-

ine from the gland.

The Effect of Mild Hyperthyroidism on Growth.

The importance of the dose of thyroid employed in determining the results obtained was first emphasized by Parhon (1912) who suggested that small amounts of thyroid material are anabolic while a large dose is catabolic in effect.

Moussu (1899) and Dott (1923) found that small amounts of thyroid tissue given to immature dogs always resulted in accelerating the growth rate. By feeding 1.9 mg. of fresh thyroid to immature male and female mice Robertson (1928) noticed an increase in their rate of growth.

Koger, Reineke, and Turner (1943) reported that by giving 0.01 to 0.03 mg. of crystalline thyroxine-sodium daily or by feeding .04 to 0.32 percent thyroprotein in the ration of young growing mice their rate of growth was accelerated. Large amounts of thyroxine or thyroprotein were toxic and inhibited growth. The gain was a true one with a high percentage of water and protein and decreased percentage of fat.

In rats Koger and Turner (1943) found that with doses of 0.04 or 0.08 percent thyroprotein in their ration the growth rate was not affected or else was depressed. Chicks given mild doses of thyroprotein, from 0.025 to 0.2 percent in their ration were reported by Irwin, Reineke, and Turner (1943) and Parker (1943) to gain more than the control animals during early life.

Reineke et al. (1948) reported that in pigs fed thyroprotein at the level of 2.8 gm. per 100 pounds of feed there was a significant increase in the rate of gain.

Maqsood and Reineke (1950) showed that thyroprotein when fed to immature male mice kept at 24° C. for a period of 4 weeks at 0.025 and 0.05 percent in the ration caused a significant increase in the body weight gain when compared with the non-treated group. Similar growth stimulation of male mice was also reported by Novak (1950).

<u>The Relation Between the Thyroid and the Female Reproductive System.</u>

A relationship between the thyroid and the reproductive organs has been postulated for a long time, especially as regards the female generative organs.

The large size of the thyroid gland in women was noticed by the early anatomists, and the periodical enlargement of the gland during menstruation and pregnancy has long been known, as indicated in the report of Freund in 1883.

One indication of such a relationship is that during the sexual cycle the mitotic activity of the thyroid epithelium corresponds to the different phases of the sexual cycle.

Chouke et al. (1935) noted that in guinea pigs the minimum activity of the thyroid corresponds to the follicular phase, and the maximal activity to the luteal phase. A similar relationship was noted in the studies of Bing (1928) on the sexual cycle in women.

Hunt (1944) also noticed that in young female rats the mitotic activity of the thyroid gland increased somewhat in early estrus, but was maximal in late estrus; it declined in metestrus and attained its minimum activity during diestrus and proestrus.

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 in the skeletal muscle.

A survey of the literature shows little agreement on the relation of the thyroid status to the function of the genital system of the female. Much of the disagreement can probably be explained by difference in thyroid dosage and age, species or strain of animals employed. As pointed out by Meites and Chandrashaker (1949) rats appear to secrete more and mice appear to secrete less than an optimal amount of thyroid hormone not only as judged by their response to injected gonadotropin but also in their growth response.

<u>Rat:</u> Da Costa and Carlson (1933) gave immature rats daily doses of 0.5 to 1 mg. of desiccated thyroid and found that it slightly accelerated sexual maturity as determined by the opening of the vagina; large doses of 5 to 10 mg. definitely retarded sexual maturity.

Fluhmann (1934) reported that the feeding of 34 to 41 mg. of desiccated thyroid substance per 100 gm. body weight or

1.9 mg. thyroxine per 100 gm. body weight to immature rats injected with gonad stimulating extracts from either sheep pituitaries or human pregnancy blood resulted in markedly lessened increase of uterine and ovarian weight. He concluded that an overactive thyroid inhibits ovarian function.

In thyroidectomized rats Langham and Gustavson (1947) showed that subcutaneous injection of dosages of 1, 2, and 3 mcg. of dl-thyroxine per gm. body weight for 3,6, and 10 days decreased the estrus response of ovariectomized rats to 1.5 mcg. of estrone as determined by vaginal smears.

Hayashi (1929) and Weichert and Boyd (1933) reported that desiccated thyroid when given to mature rats caused prolongation of the diestrus periods to 13-24 days resulting in pseudopregnancy. Corpora lutea of large sizes were seen in sections of the ovaries. They suggested that hyperthyroidism stimulates the production of the luteinizing hormone of the anterior pituitary which is responsible for ovulation and the growth of the corpora lutea. These secrete progesteronewhich in turn inhibits the onset of estrus.

Ershoff (1945) found that female rats raised to maturity on diets containing 0.5 and 1.0 percent desiccated thyroid showed inhibition of ovarian development. Ovaries remained infantile both in weight and histologic appearance.

In regard to hypothyroidism, Lane (1935), Leonard (1936), Smelser and Levin (1941), and Stein and Lisle (1942) reported that thyroidectomy enhances the effect of the follicle

stimulating hormone in immature rats.

Mann (1945) found that by the administration of 0.1 percent thiouracil to white rats their estrous cycle was prolonged.

Riess and Pereny (1928) and Van Horn (1933) found that in castrate rats the amount of estrogenic substance necessary to induce estrus was increased 3-5 times when the animals were pre-treated for six days with thyroxine.

Langham and Gustavson (1947) found that thyroparathyroidectomy or the daily administration of thiourea resulted in an increase in the estrus response to 1.5 mg. of estrone.

Mouse: Cameron and Amies (1926) found that hyperthyroidism induced in mice by the administration of dried extracts of thyroid prolonged the estrous cycle as well as estrus itself.

Stein et al. (1947) found that the injection of 1 mg. of thyroxine into the ovarian capsule retarded mitosis in the germinal epithelium.

In male immature mice Butt (1949) showed that mild hyperthyroidism produced by giving 0.025 and 0.05 percent thyroprotein in their diet stimulated the spermatogenic activity in the testes and epithelial proliferation of the mucous lining of the seminal vesicles.

Meites and Chandrashaker (1949) concluded that mild hyperthyroidism induced by giving thyroprotein to young male mice increased the gonadotropic response, while in hypothyroidism caused by giving 0.1 percent thiouracil in the diet the response was reduced.

Krohn (1947) noticed that during the administration of 0.3 mg. of propyl thiouracil to mature mice, the estrous cycle was either lengthened or disappeared.

Mice fed thiourea (Dalton et al., 1945) or thiouracil (Morris et al., 1947) showed degenerated ovaries.

Rabbit: Hypothyroidism in rabbits, produced by thyroidectomy, seemed to increase the response of the females to gonadotropic hormone as noted by Barman (1940).

Hofmeister (1893-94) found that the ovaries of thyroidectomized rabbits contained very large follicles packed closely together.

Chu (1944) showed that extracts from pituitaries of normal animals when given to thyroidectomized animals showed more pronounced effect than when given to normal ones. Pituitary extracts from thyroidectomized rabbits induced follicular growth, but ovulation did not occur after coitus. Hyperthyroidism produced by thyroxine decreased the response to gonadotropic hormones in this species (Barman, 1940).

Kunde et al. (1929) induced severe hyperthyroidism by giving thyroid substance and he found that estrus, ovulation, fertilization, and implantation occurred but resorption of the embryos followed and no young were born.

Guinea Pig: The interrelationship between the thyroid and ovarian function was studied by Chouke et al. (1937) with the conclusion that the minimal activity of the thyroid gland corresponds to the follicular phase, and the maximum to the luteal phase.

Cameron and Amies (1926) found that feeding the animals dried thyroid materials at doses of 1/40,000 and 1/10,000 of body weight lengthened the estrous period without striking increases in the duration of the cycle.

Cattle: After thyroidectomy in cattle Brody and Frankenbach (1942), and Spielman et al. (1945) found that the normal physical signs of estrus and libido are absent.

Spielman et al. (1945) reported that fertile ova were produced in animals with marked myxedema as evidenced by the birth of three normal calves from these animals, and that incomplete thyroidectomy has no effect on either mature or immature animals.

In late pregnancy in a thyroidectomized goat, as described by Reineke and Turner (1941), and in cows by Spielman (1945) growth was restored due to the fetal production of thyroxine.

Reineke, Bergman and Turner (1941) reported that there is a decrease in the amount of gonadotropin in the pituitaries of thyroidectomized goats.

MATERIALS AND METHODS

Materials.

Immature female mice weighing 12-14 gm. were obtained from Rockland 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° C. for a period of one week to get acclimatized to the temperature and food.

Feeding.

Finely ground Purina Laboratory Chow manufactured by the Ralston Purina Company, St. Louis, Missouri, U.S.A. was offered ad libitum, in special trays to prevent its wastage, to get an accurate measure of food intake. Water was available at all times.

Temperature and Light.

In the first and fourth experiments the animals were kept in an air conditioned laboratory at the temperature of 24° C. ± 1. For the other two experiments two incubators with glass tops were used. The first maintained at the temperature of 30° C. ± 1, and the other at the temperature of 35° C. ± 1.

It was not possible to control the light, but in each experiment the control group was subjected to the same fluctuations in lighting as the experimental groups.

Procedure.

The animals were divided into groups of 10 mice having similar average body weights. They were numbered individually by coloring them in different areas of the body with picramic acid.

Thyroprotein containing 0.58 percent thyroxine (Reineke et al., 1950) and thiouracil were weighed to an accuracy of 0.1 mg. and mixed thoroughly into a weighed quantity of feed by means of a mechanical mixer.

Food and water were changed and weighed accurately every day. The animals were weighed once each week.

At the end of four weeks the animals were sacrificed by exposure to ether, then weighed. Their ovaries and uteri were dissected, cleaned carefully from surrounding tissue, and weighed accurately.

In addition to the macroscopic examination of these organs, histological sections were made for microscopic study.

The organs were fixed in Bouin's fixative, dehydrated in an alcohol series, and cleared with dioxane. Hematoxylin and eosin were used for staining.

Vaginal smears were taken daily from certain groups in experiment four, towards the end of the experiment. They were stained with methylene blue.

For statistical analysis of the data the following formulae were used:

1. For determining the standard error -

$$SE = \sqrt{\frac{\sum d^2}{n(n-1)}}$$

2. For determining the significance of differences between the means of different groups the "t" value was calculated -

$$t = \frac{M_1 - M_2}{\sqrt{SE_1^2 + SE_2^2}}$$

TABLE I

INFLUENCE OF THYROID STATUS AND ENVIRONMENTAL TEMPERATURE ON BODY WEIGHT GAINS OF YOUNG FEMALE MICE

Group No.	Dosage		Duration, Weeks	Number of Mice Initial	Number of Mice At End	Average Wt. (gm.) Initial	Average Wt. (gm.) At End	Mean Wt. Gain (gm.)
Experiment I At the Temperature of 24° C.								
1	Control		4	10	10	13.9 ± 0.14*	21.6 ± 0.92	7.8 ± 0.90
2	0.025%	TP	4	10	9	13.9 ± 0.14	26.1 ± 0.57	12.2 ± 0.93
3	0.05%	TP	4	10	9	14.3 ± 0.13	23.3 ± 0.9	9.0 ± 0.99
4	0.1%	TP	4	10	9	13.9 ± 0.14	23.7 ± 0.8	9.8 ± 0.51
5	0.2%	TP	4	10	10	13.9 ± 0.18	22.2 ± 1.3	8.3 ± 1.23
Experiment II At the Temperature of 30° C.								
6	Control		4	10	10	13.9 ± 0.14	21.8 ± 0.59	7.9 ± 0.58
7	0.005%	TP	4	10	9	13.9 ± 0.19	22.6 ± 0.99	8.7 ± 0.86
8	0.1%	TH	4	10	10	13.9 ± 0.16	20.0 ± 0.98	6.1 ± 0.66
Experiment III At the Temperature of 35° C.								
9	Control		4	10	10	13.9 ± 0.14	21.4 ± 0.93	7.5 ± 0.88
10	0.005%	TP	4	10	9	13.9 ± 0.18	23.1 ± 0.59	9.2 ± 0.59
11	0.1%	TH	4	10	10	13.9 ± 0.19	19.5 ± 0.93	5.6 ± 0.62
Experiment IV At the Temperature of 24° C.								
1	Control		4	10	9	15.6 ± 0.42	23.4 ± 1.0	7.8 ± 0.78
2	0.125%	TP	4	10	9	15.0 ± 0.88	23.6 ± 1.0	8.5 ± 0.77
3	0.025%	TP	4	10	8	15.2 ± 0.84	24.4 ± 0.91	9.2 ± 0.63
4	0.1%	TH	4	10	7	14.7 ± 0.81	18.1 ± 0.84	3.4 ± 0.42
5	0.125%	TP + 0.1% TH	4	10	9	14.6 ± 0.72	22.0 ± 0.77	7.9 ± 0.72

* - Standard Error

TABLE II INFLUENCE OF THYROID STATUS AND ENVIRONMENTAL TEMPERATURE ON FOOD AND WATER INTAKE

Group No.	Dosage		Food Intake	Water Intake
Experiment I				
At the Temperature of 24° C.				
1	Control		3.2	5.3
2	0.025%	TP	4.6	8.9
3	0.05%	TP	4.2	7.6
4	0.1%	TP	4.8	9.4
5	0.2%	TP	5.3	9.6
Experiment II				
At the Temperature of 30° C.				
6	Control		3.1	5.1
7	0.005%	TP	3.4	5.3
8	0.1%	TH	2.7	4.4
Experiment III				
At the Temperature of 35° C.				
9	Control		3.4	4.9
10	0.005%	TP	3.2	5.0
11	0.1%	TH	2.8	5.5

TABLE III

INFLUENCE OF THYROID STATUS AND ENVIRONMENTAL TEMPERATURE ON WEIGHT OF REPRODUCTIVE ORGANS OF YOUNG FEMALE MICE

Group No.	Dosage	Number of Mice		Average Ovarian Wt. in mg.	Ovary Wt. mg./100 gm. Body Wt.	Average Uterine Wt. in mg.	Uterus Wt. mg./100 gm. Body Wt.
Experiment I At the Temperature of 24° C.							
1	Control	10	10	7.7 ± .73*	37	21.2 ± 2.23	102
2	0.025% TP	10	9	7.8 ± .68	29	59.0 ± 8.75	226
3	0.05% TP	10	9	6.5 ± .81	27	38.6 ± 11.91	165
4	0.1% TP	10	9	6.0 ± .92	25	31.9 ± 7.06	134
5	0.2% TP	10	10	4.8 ± .59	21	36.2 ± 5.5	163
Experiment II At the Temperature of 30° C.							
6	Control	10	10	7.2 ± 1.0	33	19.0 ± .76	87
7	0.005% TP	10	9	7.3 ± 1.0	32	34.4 ± 4.00	152
8	0.1% TH	10	10	6.3 ± 1.0	31	15.8 ± 1.0	79
Experiment III At the Temperature of 35° C.							
9	Control	10	10	8.7 ± 1.0	40	29.9 ± 4.29	139
10	0.005% TP	10	9	6.9 ± .61	30	53.3 ± 11.21	230
11	0.1% TH	10	10	5.6 ± .69	27	19.3 ± 8.7	98
Experiment IV At the Temperature of 24° C.							
1	Control	10	9	4.7 ± .46	20	33.8 ± 4.73	144
2	0.0125% TP	10	9	5.8 ± .79	33	95.2 ± 14.81	403
3	0.025% TP	10	8	4.6 ± .36	19	122.5 ± 6.47	502
4	0.1% TH	10	7	3.4 ± .45	19	74.9 ± 5.82	413
5	0.025% TP + 0.1% - TH	10	9	5.3 ± .64	24	97.4 ± 6.12	442

* - Standard Error

RESULTS

Effects of Temperature on Growth.

The average gains in body weights of the three control groups kept at the three different temperatures of 24° C., 30° C., and 35° C. were 7.8, 7.9 and 7.5 gm., respectively. No significant differences were noticed between them.

Effects of Thyroprotein Feeding on Growth.

In Experiment I in which the mice were kept at the temperature of 24° C., group two which was given 0.025 percent thyroprotein in its food gave the maximal average gain in body weight of 12.2 gm. The other groups, given 0.05, 0.1, and 0.2 percent of thyroprotein in their feed, showed average gains in body weights of 9.0, 9.8, and 8.3 gm., respectively. The groups given 0.05 and 0.1 percent thyroprotein showed significantly increased rate of gain when compared to the control group.

In Experiment IV where the animals were kept at the temperature of 24° C. a lower dose of 0.0125 percent of thyroprotein was used. This group showed an average gain in body weight of 8.5 gm., while the group given 0.025 percent thyroprotein gave an increase in body weight of 9.2 gm. The gains in body weights of both these groups exceeded significantly the amount gained by the control group, which was 7.8 gm.

In Experiment II and III where the animals were kept at the temperature of 30° C. and 35° C. the animals given 0.005 percent of thyroprotein showed an increase in body weight of 8.7 and 9.2 gm., respectively, while the average gains in body weights of the respective control group were 7.9 and 7.5 gm.

Effects of Thiouracil Treatment on Growth.

In Experiment IV at the temperature of 24° C. feeding 0.1 percent of thiouracil gave an average gain in body weight of 3.4 gm. which is significantly less than the average amount gained by the control, at the same temperature and same conditions, which was 7.8 gm.

In Experiment II and III at the temperature of 30° C. and 35° C., respectively, groups fed 0.1 percent thiouracil gave the average gains in body weights of 6.1 and 5.6 gm. respectively which were significantly less than the average gains in body weights of the control group at the respective temperatures.

Food and Water Consumption.

Effects of Temperature: At the temperature of 24° C. the amount of food and water consumed per mouse per day was 3.2 gm. of food and 5.3 gm. of water, while the mice kept at the temperature of 30° C. consumed 3.1 gm. of food and 5.1 gm. of water, and the one kept at the temperature of 35° C. consumed 3.0 gm. of food and 4.9 gm. of water. From this it appears that the amount of food and water consumption decreases with rise of temperature.

Effects of Hyperthyroidism: In Experiment I the average daily food and water consumption of a mouse given 0.025 percent thyroprotein was 4.6 gm. of food and 8.9 gm. of water. The animals given 0.05 percent thyroprotein consumed 4.2 gm. of food and 7.6

gm. of water, while those given 0.1 percent thyroprotein consumed 4.8 gm. of food and 9.4 gm. of water, and those given 0.2 percent thyroprotein consumed 5.3 gm. of food and 9.6 gm. of water. In Experiment II at the temperature of 30° C. the average daily food and water consumption of an animal given 0.005 percent thyroprotein was 3.4 gm. of food and 5.3 gm. of water. In Experiment III at 35° C. the amount of daily food and water consumption per mouse was 3.2 gm. of food and 5.0 gm. of water.

From this it appears that the amount of food and water increases by giving thyroprotein; the higher the dose the greater was the amount of food and water consumed by the animals.

Effects of Hypothyroidism: While giving 0.1 percent thiouracil the amount of daily food and water consumption per mouse was 2.9 gm. of food and 4.6 gm. of water at 24° C., 2.7 gm. of food and 4.4 gm. of water at 30° C., and 2.8 gm. of food and 5.5 gm. of water at 35° C. This shows that a hypothyroid animal consumed less amount of food and water than a normal control animal.

Temperature and Reproductive Organs.

Ovary weights of the animals kept at the three temperatures of 24° C., 30° C., and 35° C. were 7.7, 7.2, and 8.7 mg., respectively (Table III). Uteri of the controls kept at 24° C. averaged 21.2 mg. as compared to 19.0 and 29.9 mg. for the uteri of the controls maintained at 30° C. and 35° C., respectively. In view of the relatively large standard errors none of the differences between groups were statistically significant.

Histological studies of the ovaries of nontreated animals kept at 24° C. showed a normal picture of an ovary with follicles at various stages of development ranging from primordial to mature and atretic follicles (Figure 1). Only one animal showed corpus luteum formation. The ovaries of the animals kept at 30° C. and 35° C. showed an infantile picture with follicles in an early developing stage, and poor blood supply (Figure 2).

The microscopic picture of the uteri of these three groups was that of an anestrus animal with poor blood supply and few small inactive uterine glands in their thin endometrium (Figure 5).

<u>Effects of Hyperthyroidism on Reproductive Organs</u>.

In Experiment I (24° C.) animals given thyroprotein at the levels of 0.025 percent, 0.05 percent, and 0.1 percent had ovary weights of 7.8, 6.5, and 6.0 mg., respectively, compared to the control weight of 7.7 mg. None of the differences between groups are significant. The group fed 0.2 percent thyroprotein showed an average ovary weight of 4.8 mg. which was significantly less than that of the control at the same temperature.

In Experiment IV, the ovary weight of the controls and those given 0.0125 percent and 0.025 percent thyroprotein were 4.7, 5.8, and 4.6 mg., respectively; no significant differences were noticed between them.

In Experiments II and III where the animals were kept at 30° C., and 35° C. the animals given 0.005 percent thyroprotein showed ovary weights of 7.3 and 6.9 mg., respectively. No significant differences in ovary weights were noticed between them and their respective controls.

The ovary weights expressed as mg. per 100 gm. body weight were less in the thyroprotein-treated animals than those of the controls. They showed a gradual decrease with increase in the dose of thyroprotein as shown in Table III.

In contradistinction to the effects produced on ovary weights all of the animals given small doses of thyroprotein showed an increase above their controls in uterus weights. In Experiment I at 24° C. the animals given thyroprotein at the levels of 0.025, 0.05, and 0.1 and 0.2 percent showed average uterus weights of 59.0, 38.6, 31.9 and 36.2 mg., respectively while that of the control was 21.2 mg.

In Experiment IV at 24° C. the average uterus weights of the mice given 0.0125 and 0.025 percent thyroprotein were 95.2 and 122.5 mg., respectively, while that of the control was 33.8 mg.

In Experiments II and III at 30° C. and 35° C. the average uterus weight of the animals given thyroprotein at the level of 0.005 percent were 34.4 and 53.3 mg., respectively in comparison with those of their respective controls of 19.0 and 29.9 mg.

The vagina of the thyroprotein-treated animals opened 10 days earlier than in the controls (Table IV). Vaginal smears taken from the groups given 0.0125 and 0.025 percent thyroprotein also showed that the animals were going into estrous cycles.

TABLE IV INFLUENCE OF THYROID STATUS ON DATE OF OPENING OF VAGINA OF YOUNG FEMALE MICE

Experiment IV

Dosage	Control		0.0125% TP		0.025% TP		0.1% TH		0.0125% TP -0.1% TH	
	Day	Hr.	Day	Hr.	Day	Hr.	Day	Hr.	Day	Hr.
Arithmetic Mean	18	-	9	-	8	2	10	-	12	-

Histological examination of the ovaries of these thyroprotein-treated animals showed corpora lutea, some with corpora lutea of two different ages as shown by their number and staining properties (Figure 3). This indicates that the mice had completed at least two ovulatory cycles. The ovaries had a very rich blood supply.

The microscopic picture of the uteri showed thick endometrium with numerous well developed uterine glands, rich blood supply and stromal cells with rounded nuclei (Figures 6 and 7).

Effects of Hypothyroidism on Reproductive Organs

Thiouracil administration caused a trend toward reduced ovary weights in all cases. The ovary weights of the thiouracil-treated animals kept at 30° C., and 35° C. averaged 6.3 and 5.6 mg., respectively, while those of their respective controls were 7.2 and 8.7 mg. In Experiment IV the ovary weights of the group given thiouracil averaged 3.4 mg. while their controls averaged 4.7 mg. In group III and IV the thiouracil-treated mice showed a significant decrease in their ovary weights when compared to those of their respective controls.

In Experiment IV the average uterus weight of the thiouracil-treated animals was 74.9 mg., a significant increase above the average control weight of 33.8 mg. At 30° C. and 35° C., however, the uterus weights of the thiouracil-treated animals were significantly reduced, as shown by the average values of 15.8 and 19.3 mg. for the thiouracil groups, compared to averages of 19.0 and 29.9 mg. for their respective controls.

The ovaries of all the thiouracil-treated animals had a poor blood supply and were packed with large follicles but no corpora lutea were seen (Figure 4). The uteri of the animals kept at 24° C. (Figure 8) showed a thick edematous endometrium with numerous leucocytes and tall epithelium. Vaginal smears showed that the animals were in continuous estrus (cornified cells).

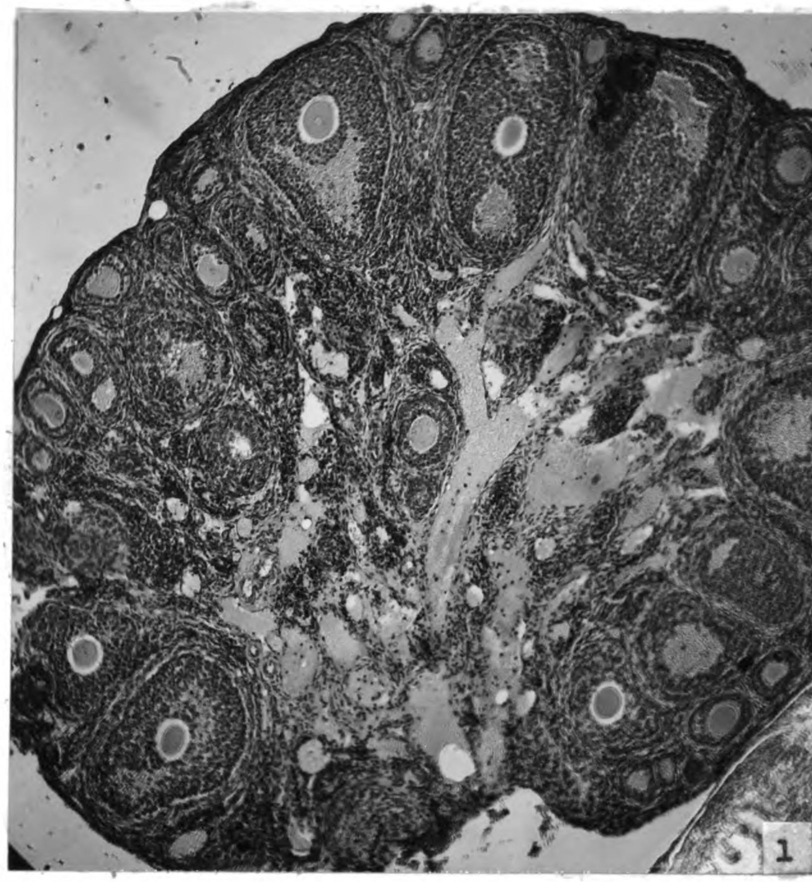
The histological picture in the thiouracil-treated animals kept at both the temperatures of 30° C. and 35° C. showed infantile thin-walled uteri, with thin endometrium.

Figure 1.

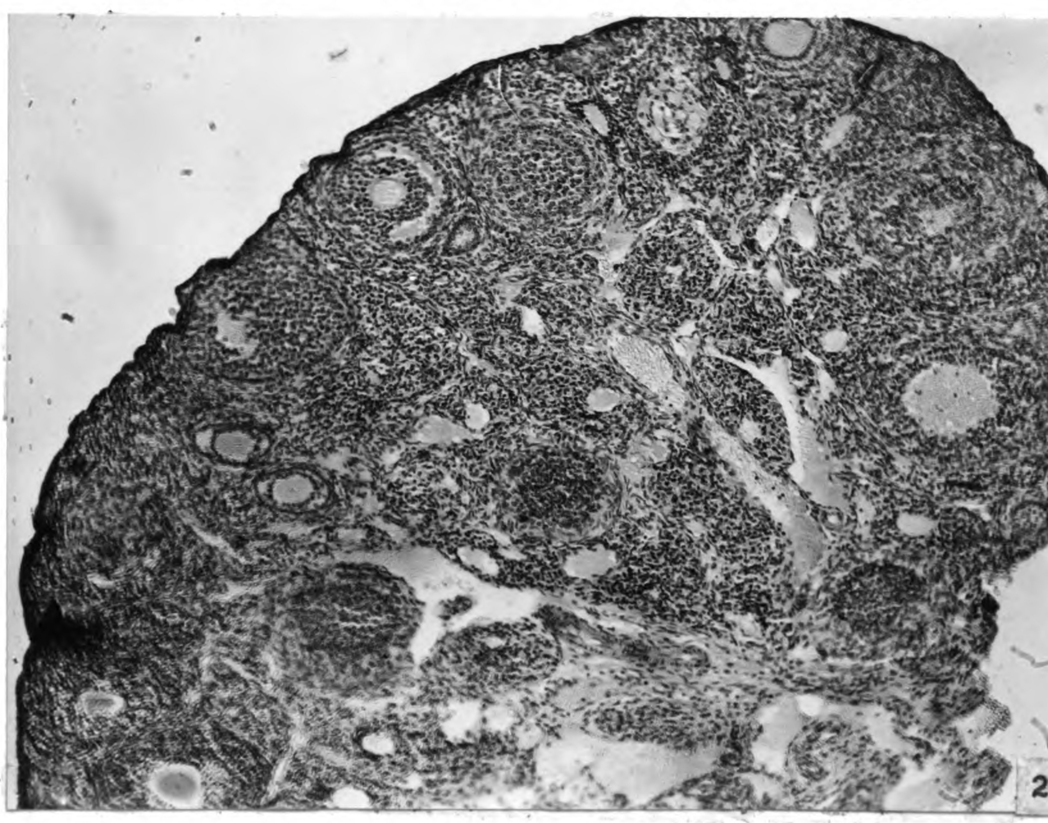
Ovary section of a normal mouse kept at 24° C. Note (1) Cortex with Graafian follicles at different stages of development. (2) Medulla with rich blood supply. (3) Lack of corpora lutea. (X100)

Figure 2.

Ovary section of a mouse kept at 30° C. Note the small size of the developing Graafian follicles. (X100)



1



2

Figure 3.

Ovary section of a mouse kept at 24° C. and given 0.05 percent thyroprotein in the ration. Note (1) Extensive development of corpora lutea. (2) The presence of growing follicles. (X100)

Figure 4.

Ovary sections of a mouse kept at 24° C. and given 0.1 percent thiouracil. Note the large number of Graafian follicles though they have not matured. (X120)


3

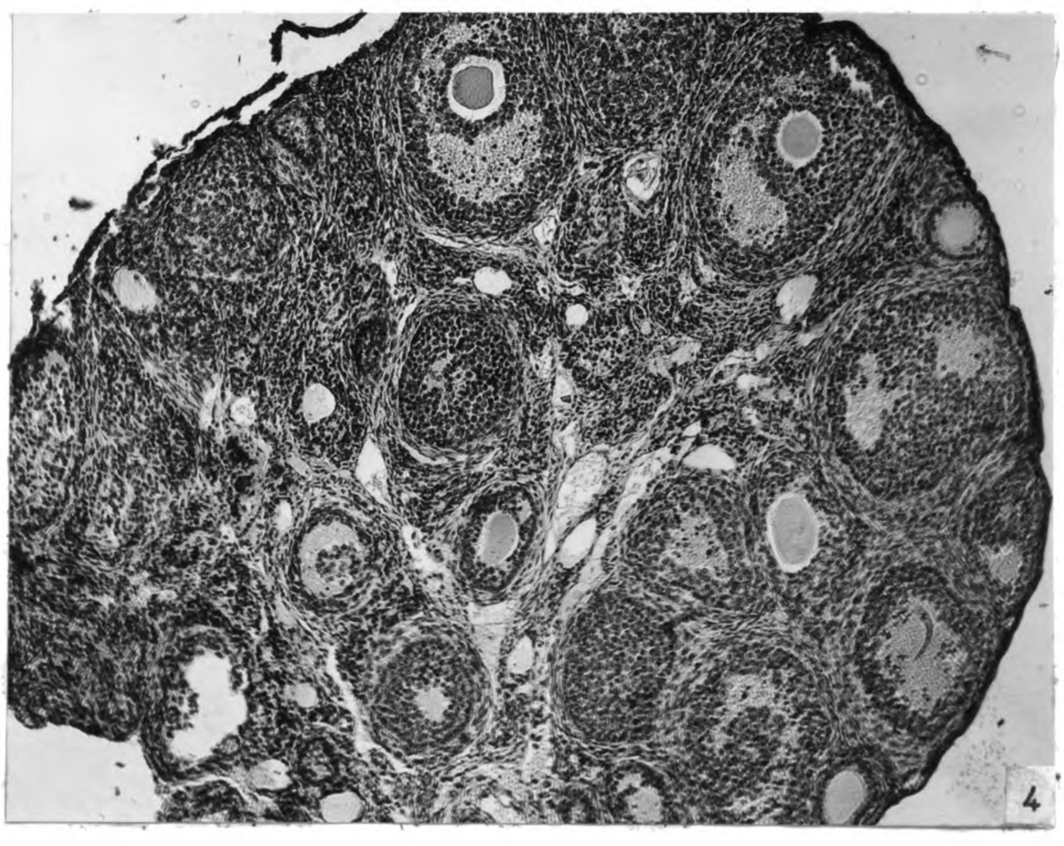
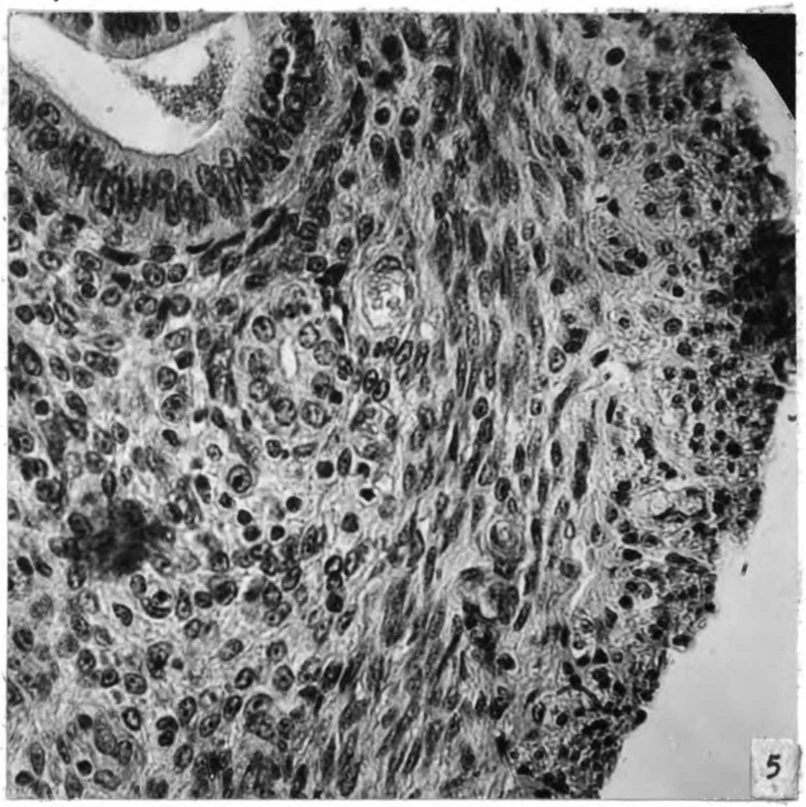

4

Figure 5.

Section of uterus of a control mouse kept at 24° C. Note (1) Thinness of the uterus wall. (2) Low epithelium. (X500)

Figure 6.

Sections from the endometrium of a mouse kept at 24° C. and fed 0.025 percent thyroprotein. Note the rounded stromal nuclei and distinct nucleoli in a thick endometrium. This is a typical progesterone response and is correlated with the luteinization observed in the ovary. (X500)


5

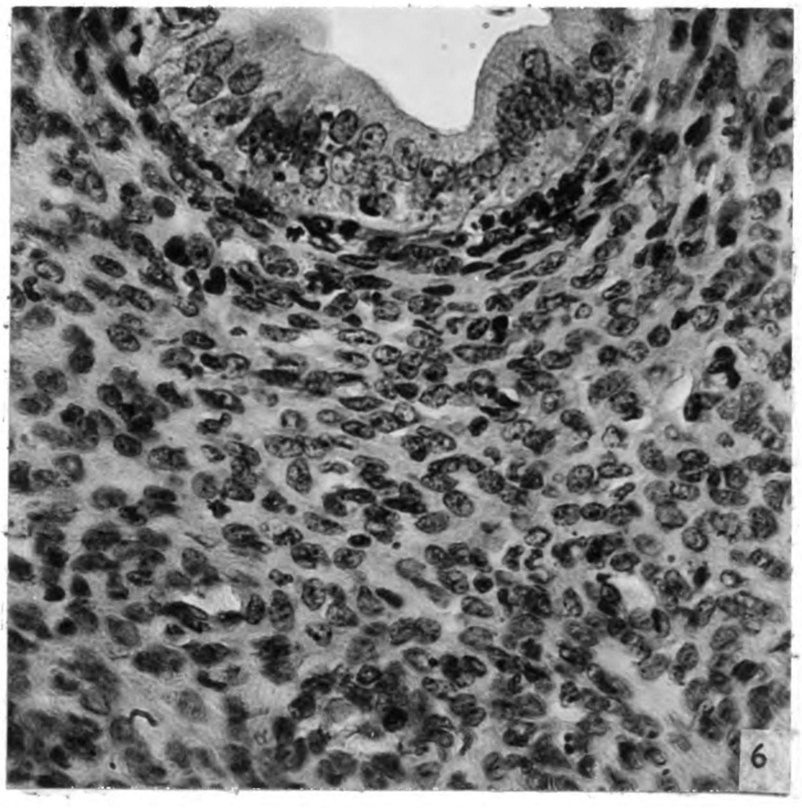
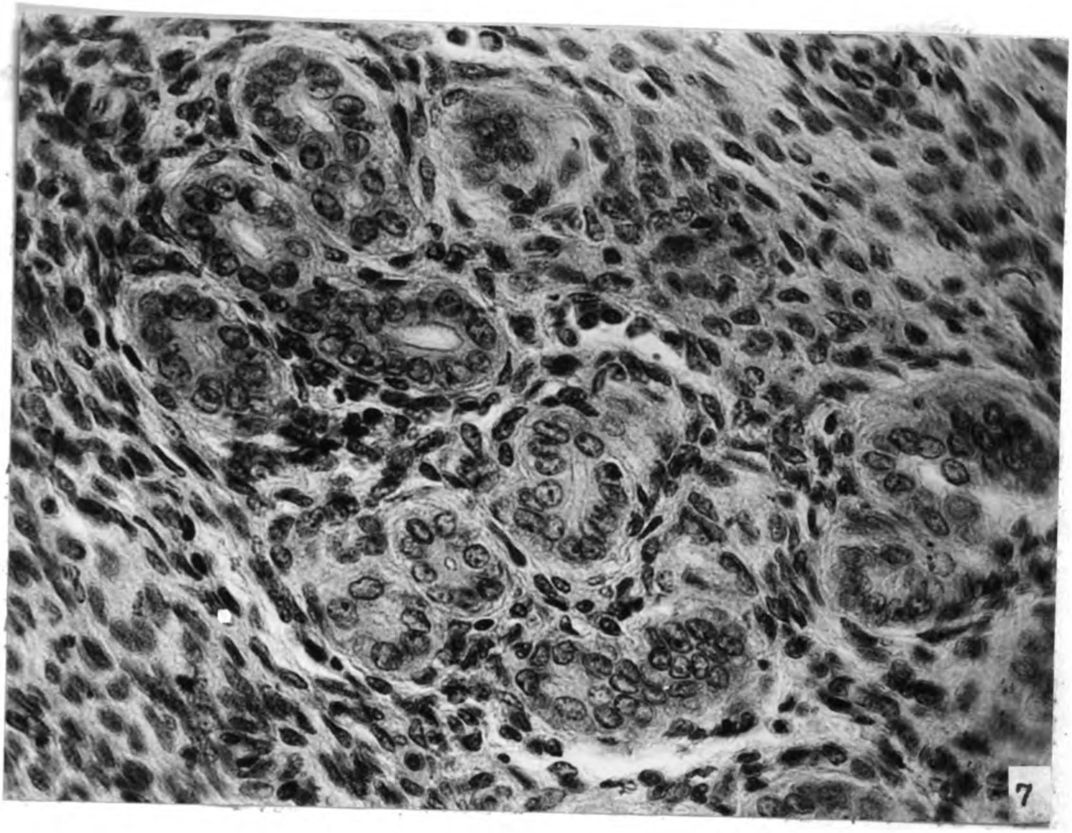

6

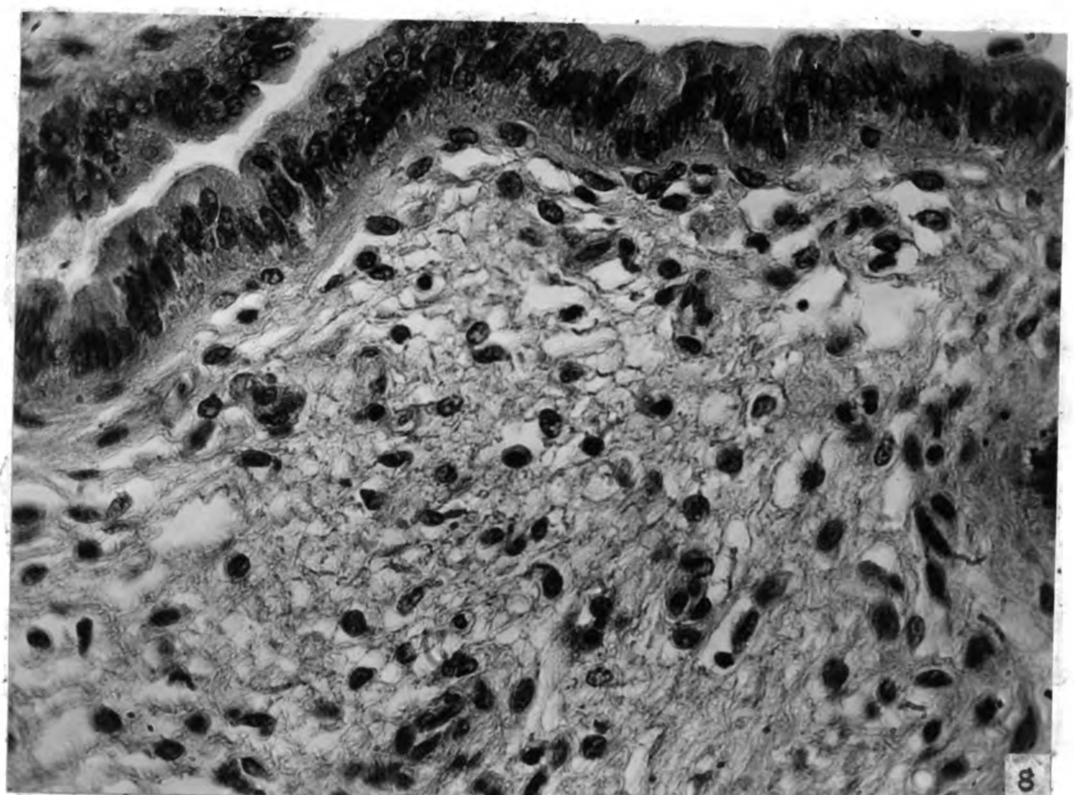
Figure 7.

Section from the endometrium of a mouse kept at 24° C. and given 0.025 percent thyroprotein. Note the well developed uterine glands. (X500)

Figure 8.

Section of the endometrium of a mouse kept at 24° C. and given 0.1 percent thiouracil in the ration. Note (1) The tall columnar epithelium with numerous granules. (2) The pronounced edema. (3) The shrunken elliptical nuclei of the stromal cells. (4) The presence of numerous leucocytes. (X500)


7


8

DISCUSSION

Growing female mice given mild doses of thyroprotein grew at a rate exceeding that of the nontreated animals. From this it appears that mice secrete thyroid hormone at a rate less than the optimal. Thiouracil further decreases the rate of growth and maturation. Food and water consumption were increased by giving thyroprotein, while they were decreased by giving thiouracil. They were decreased also at higher temperatures.

Hurst and Turner (1948) after estimation of the thyroid output of male and female mice found that by giving 20 to 60 times their own thyroid output they could increase their rates of growth. A dosage of 80 times their own thyroid secretion rate was found to be detrimental to growth.

It is generally agreed that the mouse is a relatively hypothyroid animal, as indicated in the work reported by Maqsood and Reineke (1950), Novak (1950) and Meites and Chandrashaker (1949). In this type a suitably regulated dosage of thyroidal substance will produce anabolic effects. In the work done by Butt (1949) on male mice, the optimal dosage of thyroprotein for growth was 0.05 percent of the ration while in our experiment it was 0.025 percent.

With regard to the effect of temperature on growth we did not observe significant differences between the nontreated female mice kept at the temperatures of 24° C., 30° C., and 35° C.

However, the animals kept at the high temperatures of 30° C. and 35° C. showed retarded maturation and their ovaries and ~~their~~ uteri were infantile.

Hurst and Turner (1948) found that by lowering the environmental temperature below thermoneutrality in male and female mice their thyroid secretion could be increased. They found that at 87° Fahrenheit the thyroid secretion rate in the female growing mouse was equivalent to 3.2 mcg. of d,l-thyroxine per 100 gm. body weight per day while it was equivalent to 5.5 mcg. of d,l-thyroxine per 100 gm. body weight per day when kept at 80° Fahrenheit. From such results it appears that the thyroid secretion rate increases when the animals are kept in cold environments and decreases when kept in hot environments. Similar findings have been reported by many authors including Ring (1936, 1939), Dempsey and Astwood (1943) and Reineke and Turner (1945).

There is no doubt that there is a close hormonal relationship between the thyroid and the ovary. It has been noticed that hypothyroidism induced by removal of the thyroid leads to certain regressive changes in the ovary. Ovariectomy also leads to increased activity of the thyroid gland (Franke and Ptaszek, 1933).

The mechanism by which the thyroid influences gonadal function is not yet known. However, there are three possible ways by which the thyroid can play a part in reproduction. The thyroid hormone is thought to be influencing the gonadal function by its effect on basal metabolic rate as suggested by Hammett (1926), Lee (1936) and Ross (1938). This suggestion agrees partially with the results that we obtained because there was a decrease in the ovarian weights following the decrease in rate of gain in the thiouracil-fed animals. On the other hand, though thyroprotein feeding increased the rate of growth, the ovarian weights did not vary from those of the control animals. On the contary, the high dose of 0.2 percent thyroprotein decreased the ovarian weight while the body weight gains were more than those of the control. Engle (1931) states that there is a very low correlation between body weight and ovarian weight in albino mice at any given life period.

The thyroid gland probably has a direct effect on the ovary. Variable amounts of iodine were recovered from the ovaries of different species of animals and humans (Ruff, 1933). Carter (1932) suggests that differences in iodine contents reported by different authors are results of variations of the iodine content during the ovarian cycle. In newborn babies the average amount of iodine in the ovaries was less than those of adults; it begins to drop with the appearance of menopause. Carter tried to determine the form in which iodine occurs in ripe ovaries of Echinus eschulentus. He could not detect thyroxine in any considerable proportion; but he found a substance which produced a marked influence on metamorphosis in tadpoles. Orr and Leitch (1929) found iodine of the cow's ovary to be concentrated principally in the

corpora lutea.

Tyndale (1937) observed that the thyroid hormone has an inhibiting action upon the mechanism of gonad stimulation of mature hypophysectomized rats and he claims that the thyroid effect is a direct one and not mediated through the pituitaries. However, Johnson (1949) found that tolerable doses of thyroprotein augmented the effect of gonadotropic hormone in female mice. From all of these observations it seems that the thyroid hormone at a certain critical point of secretion rate specific to every species favors the function of the gonadotropic hormone. If present in more or less than optimal amounts it will impair ovarian function.

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

The thyroid gland may exert its influence on the reproductive system through the pituitary gland. Cytological studies of the rat pituitary by Severinghaus et al., (1934), Zeckwer et al., (1935) and Goldberg and Chaikoff (1950) showed that after thyroidectomy there was an increase in the number of basophil cells while there was complete elimination of the acidophil cells. Similar results were proved to occur in the pituitary after ovariectomy.

Basophilic cells are believed to have a dual function, they secrete both thyrotropic hormone and follicle stimulating hormone, Severinghaus (1937). When either of their target glands (ovary or thyroid) is absent the basophil cells seem to become hyperactive and there is an increased output of both thyrotropic hormone and follicle stimulating hormone, Severinghaus (1934).

Under the influence of FSH the follicles will grow, but to reach maturity and ovulation they need the other fraction LH which is believed to be secreted by the acidophil cells. Severinghaus (1939) and Marine et al., (1935) confirmed the loss of acidophils after thyroidectomy. This may explain why ovulation did not occur in our thiouracil-treated animals since they were presumably only under the influence of FSH which would stimulate the growth of the follicles and continuous estrus, but would not influence ovulation.

Though there were large follicles in the ovaries of the thiouracil-treated animals kept at 30° C. and 35° C., the uteri were infantile. A good explanation for this may be that the temperature added to the thiouracil effect resulted in severe reduction of the thyroid hormone which seems to be essential for the gonadotropin to induce its effect. In the rat, highly purified preparations of follicle stimulating hormone produced follicular growth but the uteri and vagina remained infantile (Fevold, 1941). It was believed that these follicles did not liberate effective amounts of estrogen, while by the addition of minute amounts of LH estrus was induced.

The number of acidophil cells, which are believed to secrete the luteinizing hormone, increases in hyperthyroid animals as noted by Severinghaus (1939). Weichert and Boyd (1933) found that hyperthyroid rats showed persistent functional corpora lutea. Chouke et al., (1937) noticed that the maximal activity of the thyroid gland of the guinea pig corresponds to the luteal phase of the cycle. In our experiments we found thyroprotein given in mild doses to young female mice accelerated their rate of maturation as indicated by the early opening of their vagina and the onset of ovulation denoting greater ovarian activity than in either controls or thiouracil-treated animals.

SUMMARY AND CONCLUSIONS

Groups of young female mice were kept at an environmental temperature of 24° C., 30° C., or 35° C. for a period of four weeks. Certain groups received either thyroprotein or thiouracil as a percentage of their ration during this period.

1. Hyperthyroidism induced by giving mild doses of thyroprotein increased the rate of growth of these growing female mice. The animals kept at 24° C. and given thyroprotein at the levels of 0.025 percent showed the maximal gain in body weight.

2. Hypothyroidism induced by giving thiouracil at the level of 0.1 percent of their food retarded their rates of growth.

3. No significant differences were noticed between the rates of growth of the animals kept at the three different temperatures of

24° C., 30° C. and 35° C.

4. The animals kept at the temperatures of 30° C. and 35° C. did not reach maturity by the end of the experiments as was indicated histologically. The ovaries showed young immature graafian follicles. The uteri were infantile and thin walled with thin endometrium.

5. The ovary weights in mg. per 100 gm. body weight tended to decrease in animals receiving thyroprotein. They showed a gradual decrease with increasing dosage of thyroprotein.

6. Thyroprotein-treated animals reached maturity earlier than their controls as indicated by earlier vaginal opening and the onset of regular estrous cycles.

7. Histological examination of the ovaries revealed corpora lutea in the groups receiving thyroprotein with the most corpora present at the 0.025 percent level.

8. Thiouracil-treated mice either showed continuous estrus or anestrus.

9. Histological examination showed that the ovaries of the animals given thiouracil were packed with a great number of large graafian follicles though they did not reach a mature stage.

BIBLIOGRAPHY

Allen, E. 1939. "Sex and Internal Secretions." Williams and Wilkins, Baltimore.

Astwood, E. B., Sullivan J., Bissell, A., and Tyslowitz, R. 1943. Action of Certain Sulphonamides and of Thiourea Upon the Function of the Thyroid Gland of the Rat. Endocrinology. 32: 210-225.

Astwood, E. B., Bissell, A. 1944. Effect of Thiouracil on I_2 Content of the Thyroid Gland. Endocrinology. 34: 287-296.

Atkinson, W. B., and Hooker, C. W. 1945. The Day to Day Level of Estrogen and Progestin Throughout Pregnancy and Pseudopregnancy in the Mouse. Anat. Rec. 93: 75-95.

Barman, J. M. 1940. Factors Influencing the Sensitiveness of Rabbits to the Gonadotrophic Effect of Gravid Urine and Thyroid. Rev. Soc. Argentinia Biol. 16: 460-465.

Bauman, E. 1895. Über des normale Vorkommen Von Jod im. Thierkorper. Zeitschr. Physiol. Chem. 21: 319-330.

Bing, R. 1928. Über Periodische Hypothyreose. Endokrinologie. 2: 321-330.

Brody, S., and Frakenbach, R. F. 1942. Age Changes in Size, Energy, Metabolism, and Cardio-respiratory Activities of Thyroidectomized Cattle. Mo. Agri. Expt. Sta. Res. Bul. 349.

Brolin, S. E. 1946. A Study of the Structural and Hormonal Relations to the Pituitary Body of Rats Exposed to Cold. Acta. Anat. Suppl. 3: 1-165.

Butt, M. 1949. The Influence of Variations in Environmental Temperature and Thyroid Status on Growth and Sexual Development in the Male Mouse. Thesis Submitted to the School of Graduate Studies, Michigan State College.

Cameron, G. R., and Amies, A. B. 1926. The Effect of Thyroid Feeding on the Estrus of the Guinea Pig and Mouse. Aust. Jour. Expt. Biol. and Med. Sci. 3: 37-43.

Carter, G. S. 1932. Iodine Compounds and Fertilization. VI Physiological Properties of Extracts of the Ovaries and Testes of Echinus esculentus. Jour. Exp. Biol. 9: 253-263.

Chouke, K. S., H. Friedman, and Loeb, L. 1935. Proliferative Activity of the Thyroid Gland of the Female Guinea Pig During the Sexual Cycle. Anat. Rec. 63: 131-

Chu, J. P. 1944. Influence of the Thyroid Gland on Pituitary Gonadotrophic Hormone Activity in the Rabbit. Endocrinology. 34: 90-102.

Da Costa, E., and Carlson, A. J. 1933. The Effect of Feeding Desiccated Thyroid Upon the Sexual Maturity of the Albino Rat. Amer. Jour. Physiol. 104: 247-252.

Dalton, A. J., Morris, H. P., and Dubnik, C. S. 1945. Changes in the Organs of Female C_3H Mice Receiving Thiourea. Jour. National Cancer Inst. 5:451-454.

Dempsey, E. W., and Astwood, E. B. 1943. Determination of the Rate of Thyroid Hormone Secretion at Various Environmental Temperatures. Endocrinology. 32: 509-518.

Dott, N. M. 1923. An Investigation into the Functions of the Pituitary and Thyroid Glands. I. Technique of Their Experimental Surgery and a Summary of Their Results. Quart. Jour. Expt. Physiol. 13: 241.

Engle, E. T. 1931. Prepuberence Growth of the Ovarian Follicle in the Albino Mouse. Anat. Record. 48: 341-350.

Ershoff, B. H. 1948. Effect of Thyroid Feeding on Ovarian Development in the Rat. Endocrinology. 37:218

Fevold, H. L., and Hisaw, F. L. 1934. Interaction of Gonad Stimulating Hormones in Ovarian Development. Amer. Jour. Physiol. 109: 655-665.

Fluhmann, C. F. 1934. The Influence of the Thyroid on the Action of the Gonad Stimulating Hormones. Amer. Jour. Physiol. 108: 498-508.

Franke, M., et Ptaszek, L. 1933. Taux de L'iode dans le Sang Apres Castration chez les Chiennes. Compt. rend. Soc. Biol. 114: 1129-1130.

Freund, M. 1883. "Die Beziehungen der Schilddrüse zu den Weiblichen Geschlechts Organen." Deutsche Zeitsch. f. Chir. XVIII.

Goldberg, R. C., and Chaikoff, I. L. 1950. The Cytological Changes that Occur in the Anterior Pituitary Glands of Rats Injected with Various Doses of I^{131} and Their Significance in the Estimation of Thyroid Function. Jour. Endocrinology. 46: 91-104.

Hammett, F. S. 1926. The Relation of the Thyroid and Parathyroid to the Glands of Internal Secretion. Endocrinology. 10: 385-404.

Harington, C. R., and Pitt Rivers. 1939. Preparation of Thyroxine from Casein Treated with Iodine. Nature. 144: 205.

Hayashi, H. 1929. Action of Thyroid Substance on Female Generative Organs. Bul. Acad. Med. (Paris). 101: 115-118.

Hooker, C. W. 1945. A Criterion of Luteal Activity in the Mouse. Anat. Rec. 93: 333-347.

Hunt, T. E. 1944. Mitotic Activity in the Thyroid Gland of Female Rats. Anat. Rec. 90: 133-138.

Hurst, V., and Turner, C. W. 1948. The Thyroid Secretion Rate in the Mouse and Its Relation to Various Physiological Processes. Mo. Agr. Expt. Sta. Res. Bul. 417.

Irwin, M. R., Reineke, E. P., and Turner, C. W. 1943. Effect of Feeding Thyroactive Iodocasein on Growth, Feathering and Weights of Glands of Young Chicks. Poultry Sci. 22: 374-380.

Johnson, T. N. 1949. The Effect of Alteration in Thyroid Activity on the Response of the Ovaries of Immature Female Rats and Mice to Equine Gonadotrophin. Thesis Submitted to the School of Graduate Studies, Michigan State College.

Kendall, E. C. 1915. A Method of Decomposition of the Proteins of the Thyroid with a Description of Certain Constituents. Jour. Biol. Chem. 20: 501-509.

Kenyon, A. T. 1933. The Effect of Cold on the Thyroid Activity of Albino Rats. Amer. Jour. Path. 9: 347-368.

Koger, M., Reineke, E. P., and Turner, C. W. 1943. Influence Growth of Thyroactive Iodocasein. Proc. Soc. Expt. Biol. and Med. 52: 236-237.

Koger, M., Turner, C. W. 1943. The Effect of Mild Hyperthyroidism on Growing Animals of Four Species. Agr. Expt. Sta. Univ. Missouri. Res. Bul. 377.

Krohn, P. L. 1947. The Effect of Propyl- thiouracil on the Oestrus Cycle in Mice. Jour. Endocrinology. 5: XXXIII.

Kunde, M. M., Carlson, A. J., and Proud, T. 1929. The Ovary in Experimental Hypo- and Hyperthyroidism. Amer. Jour. Physiol. 88: 747-753.

Lane, C. E. 1935. The Follicular Apparatus of the Ovary of the Immature Rat and Some of the Factors which Influence It. Anat. Rec. 61: 141-153.

Langham, W., and Gustavson, R. G. 1947. Effect of Level of Thyroid Activity on Response of Ovariectomized Rats to Estrone. Amer. Jour. Physiol. 150: 760-767.

Lee, M. O. 1926. Studies on the Estrous Cycle in the Rat. The Effect of Thyroparathyroidectomy and Parathyroidectomy. Endocrinology. 10: 43-55.

Leonard, S. L. 1936. Hypophysis- Thyroid- Gonad Relationships. Proc. Soc. Expt. Biol. and Med. 34: 599-600.

Ludwig, W., und Mutzenbecher, P. von. 1939. Die Darstellung von Thyroxin, Monojodotyrosin und Dijodtyrosin aus Jodiertem Eiweiss. Z. Phsyiol. Chem. 258: 195.

Mann, W. C. 1945. The Effect of Thiouracil Upon Heart Rate, Estrous Cycle and Spontaneous Activity of the White Rat. Jour. Psych. 20: 91-99.

Maqsood, M., and Reineke, E. P. 1950. Influence of Variation in Environmental Temperature and Thyroid Status on Growth and Feed Consumption of the Male Mouse. Amer. Jour. Physiol. 160: 253-258.

Marine, D., Rosen, S. H., and Spark, C. H. 1935. Effect of Iodine and Desiccated Thyroid on Anterior Pituitary of Goitrous and Thyroidectomized Rabbits. Proc. Soc. Expt. Biol. and Med. 32: 803-810.

Meites, J., and Chandrashaker, B. 1949. The Effects of Induced Hyper- and Hypo-thyroidism on the Response to a Constant Dose of Pregnant Mare's Serum in Immature Male Rats and Mice. Endocrinology. 44: 368-377.

Mills, C. A. 1918. Effects of External Temperature, Morphine, Quinine and Strychnine on Thyroid Activity. Amer. Jour. Physiol. 46: 329-339.

Mixner, J. P., Reineke, E. P., and Turner, C. W. 1944. Effect of Thiouracil and Thiourea on the Thyroid Gland of the Chick. Endocrinology. 34: 168-174.

Morris, H. P., Dubnik, C. J., and Dalton, A. J. 1947. Action of Thiouracil on CH_3 Mice. Fourth International Cancer Research Congress. P. 152.

Moussu, M. G. 1899. Influence de L'Alimentation Thyroidienne sur la Croissance Reguliere. Compt. rend. Soc. Biol. 51: 241-249.

Novak, A. 1950. The Effect of Simultaneous Administration of Thyroprotein and Testosterone Propionate on Growth and on the Mechanism of Protein Metabolism. Ph.D. Thesis. School of Graduate Studies, Michigan State College.

Orr, J. B., and Leitch, I. 1929. Iodine Nutrition "A Review of Existing Information." Medical Research Council. Special Report Series No.123 (London).

Parhon, M. 1912. L'influence de la Thyroide sur le Metabolism du Calcium. Mem. Soc. de Biol. 72: 620.

Parker, J. E. 1943. Influence of Thyreactive Idocasein on Growth of Chicks. Proc. Soc. Expt. Biol. and Med. 52:234-236.

Paschkis, K. E., Cantarow, A., and Rakoff, A. E. 1945. Mitosis Stimulating in the Thyroid Gland Induced by Thiouracil. Endocrinology. 37: 133-135.

Reineke, E. P., and Turner, C. W. 1941. Effect of Thyroidectomy of Young Goats Upon Certain Anterior Pituitary Hormones. Endocrinology. 29: 306-312.

Reineke, E. P., and Turner, C. W. 1941. Growth Response of Thyroidectomized Goats to Artificially Formed Thyroprotein. Endocrinology. 29: 667-668.

Reineke, E. P., and Turner, C. W. 1942. Formation in Vitro of Highly Active Thyroproteins, Their Biological Assay and Practical Use. Agric. Expt. Sta. Univ. of Missouri Res. Bul. 355.

Reineke, E. P., and Turner, C. W. 1945. Seasonal Rhythm in the Thyroid Hormone Secretion of Chick. Poult. Sci. 24: 499-504.

Reineke, E. P., McMillen, W. W., and Bratzler, L. J. 1948. The Effect of Mild Thyroprotein Administration on Growth in Swine. Mich. Agr. Expt. Sta. Tech. Bul. 209.

Reineke, E. P. 1949. The Formation of Thyroxine in Iodinated Proteins. Annals of the New York Academy of Sciences. 50: 450-465.

Reineke, E. P., Wallach, D. P., and Wolterink, L. F. 1950. Thyroxine Content of Thyroactive Iodinated Proteins by an Isotope Dilution Method. Dept. of Physiology and Pharmacology, Michigan State College (unpublished).

Reiss, M., and Pereny, S. 1928. Thyroideahormon und Brunst. Endokrinology. 2: 181-186.

Ring, G. C. 1936. An Attempt to Stimulate the Thyroid Gland in Rats Exposed to Cold. Amer. Jour. Physiol. 116:129.

Ring, G. C. 1939. Thyroid Stimulation by Cold Including the Effect of Changes in Body Temperature Upon Basal Metabolism. Amer. Jour. Physiol. 125: 244-250.

Robertson, T. B. 1928. The Influence of the Thyroid Alone and of Thyroid Administration Together With Nucleic Acids Upon Growth and Longevity of the White Mouse. Anat. Jour. Expt. Biol. and Med. 5: 69-83.

Ross, R. 1938. Die Beziehungen der Schildrüse zur fortplansung. Wilhelm Roux Arch. Entwicklungsmech. Organ. 137: 733-803.

Ruff, S. 1933. Contenu d'Iode Dans la Thyroide et Autres Differents Organes. Folia. Biol. 1: 131-132.

Salter, W. T. 1949. The Metabolic Circuit of the Thyroid Hormone. Annals of the New York Academy of Sciences.

Severinghaus, A. E., Smelser, G. K., and Clark, H. M. 1934. Anterior Pituitary Changes in Adult Male Rats Following Thyroxine Injections or Thyroid Feeding. Proc. Soc. Expt. Biol. and Med. 31: 1125-1127.

Severinghaus, A. E., Smelser, G. K., and Clark, H. M. 1934. Anterior Pituitary Changes in the Adult Male Rat Following Thyroidectomy. Proc. Soc. Expt. Biol. and Med. 31: 1127-1129.

Severinghaus, A. E. 1937. Cellular Changes in the Anterior Hypophysis With Special Reference to Its Secretory Activities. Physiol. Reviews. 17: 556-588.

Severinghaus, A. E. 1939. Anterior Hypophysial Cytology in Relation to the Reproductive Hormones. "Sex and Internal Secretions." Chap. XIX: 1045-1087. Edited by E. Allen. Williams and Wilkins, Baltimore.

Smelser, G. K., Levin, L. 1941. The Phenomenon of Antagonism by Pituitary Extracts in the Absence of the Thyroid Gland. Endocrinology. 28: 233-236.

Spielman, A. A., Petersen, W. E., Fitch, J. B., and Pomeroy, B. S. 1945. General Appearance, Growth and Reproduction of the Thyroidectomized Bovine. Jour. Dairy Sci. 28: 329-337.

Stein, K. F., and Lisle, M. 1942. The Gonad Stimulating Potency of the Pituitary of Hypothyroid Young Male Rats. Endocrinology. 39: 16-24.

Stein, K. F., Quimby, J., and Moeller, A. 1947. Response of the Germinal Epithelium to Thyroid Tissue or Thyroxine in the Ovarian Capsule of the Mouse. Anat. Rec. 99: 249-264.

Tyndale, H. H., and Levin, L. 1937. Ovarian Weight Responses to Menopause Urine Injection in Normal, Hypophysectomized and Hypophysectomized Thyroxine Treated Immature Rats. Amer. Jour. Physiol. 120: 486-493.

Van Horn, H. H. 1933. The Relation of the Thyroid to the Hypophysis of Female Albino Rats. Physiol. Zool. 3: 461-466.

Weichert, C. K., and Boyd, R. W. 1933. Effect of Thyroid on the Corpus Luteum. Anat. Record. 58: 55-59.

Williams, R. H., Weinglass, A. R., Bissell, G. W., and Peters, J. B. 1944. Anatomical Effects of Thiouracil. Endocrinology. 34: 317-328.

Zeckwer, I. T., Davidson, L. W., Thomas, B. K., and Livergood, C. S. 1935. The Pituitary in Experimental Cretinism. Structural Changes in the Pituitary of Thyroidectomized Rats. Amer. Jour. Med. Sci. 190: 145.

ROOM USE ONLY

~~Oct 18 '50~~

USE ONLY