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THE EFFECT OF ALTERATIONS IN THYROID ACTIVITY ON THE RESPONSE OF THE OVARIES OF IMMATURE FEMALE RATS AND MICE TO EQUINE GONADOTROPIN

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THESIS

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THE EFFECT OF ALTERATIONS IN THYROID ACTIVITY ON THE RESPONSE OF THE OVARIES OF IMMATURE FEMALE RATS AND MICE TO EQUINE GONADOTROPIN

By

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INTRODUCTION

It has long been established that a functional relationship exists between the thyroid gland and the reproductive organs. Alterations in thyroidal activity have a definite influence on the function of the gonads. Clinical investigators have found that when reproductive disorders coexist with abnormal thyroid function, the reproductive system often returns to normal after the abnormal thyroid condition has been corrected.

Little is known regarding the physiological mechanisms of these clinical findings. Conceivably the thyroid could play a part in one or more of the following mechanisms:

1) It might have a direct effect on the reproductive organs.
2) It might be necessary for the elaboration of gonadotropins by the anterior pituitary.
3) It might act on the gonads indirectly through changing the metabolic rate of the body as a whole.

It was thought that a good experimental approach to one aspect of the problem would be to determine the influence of experimental alterations

of thyroid function in immature female rats and mice on the response of the gonads to a constant dose of exogenous gonadotropic hormone. Hyperthyroidism was induced by administering either thyroxine or Protamone, and hypothyroidism was induced by feeding thiouracil. The gonadotrophin used was pregnant mare's serum, which is predominantly a follicle stimulating hormone.

REVIEW OF THE LITERATURE

Functional Relationships between the Anterior Pituitary, Thyroid and Ovary

Thyroid-Ovarian Relations

Effects of Hypothyroidism: Menstrual irregularities have been associated with the symptoms of both hyperthyroidism and hypothyroidism. Foster *et al.* (1939) treated fifty patients who had dysmenorrhea, oligomenorrhea, amenorrhea, metrorrhagia or menorrhagia with desiccated thyroid. The average BMR preceding treatment was -15 per cent and all patients were otherwise healthy with normal pelvic findings. As a result of the thyroid treatment, 89 per cent of these patients recovered.

Lerman et al. (1942) reported that patients with myxedema due to primary dysfunction of the thyroid had amenorrhea that was not due to the menopause. The ovarian cycle returned to normal after the administration of thyroid.

Krohn (1947) observed that the normal estrous rhythm in mice was disturbed by daily subcutaneous injections of 0.3 mg. propyl-thiouracil. The cycles were lengthened and irregular or disappeared. Estrous rhythm returned 14 days after the cessation of injections. Single injections of 5 I.U. of chorionic gonadotropin into six mice showing prolonged anestrous during thiouracil treatment were followed in each case by a single estrus period. Vaginal sensitivity of spayed mice to estrogen was unaffected by propyl-thiouracil treatment.

Aranow, Engle, and Sperry (1946) administered increasing amounts of thiouracil up to 0.8 gm. daily for 14 months to 4 adult female Rhesus monkeys and observed menstrual irregularities with frequent periods of amenorrhea.

Scow and Simpson (1945) thyroidectomized 440 rats on the first day of life and reported that sexual infantilism occurred in these animals.

Jones, Delifs and Foote (1946) reported that hypothyroidism induced by thiouracil had no effect on the reproductive system in adult male rats as judged by their ability to sire litters. Sterility was not induced by thiouracil in females, but gestation was abnormal when the treatment was prolonged. Resorption of the fetus resulted in 100 per cent of the cases which received prolonged doses. When the treatment was administered during a period of less than 100 days, some of the females delivered litters that were normal in growth, development and reproduction.

Effects of Hyperthyroidism: Reineke and Turner (1943) used synthetic thyroprotein to influence some of the body processes of farm animals. It was pointed out that replacement therapy with thyroid material could restore the normal metabolic rate in the slightly hypothyroid animal and improve the reproductive system markedly. In males it was pointed out that normal sex life can be restored, and the quality of the semen improved, while in females the ovaries become active and normal estrous cycles ensue.

Ershoff (1945) found that female rats raised to maturity on diets containing 0.5 per cent and 1.0 per cent desiccated thyroid showed marked diminution in ovarian development. The ovaries remained infantile

in both weight and histological appearance.

Stein et al. (1947) noted that single thyroxine injections into mice invariably caused retardation of proliferation in the germinal epithelium. Retardation was greater in immature than in mature mice. It was also greater during diestrus than during estrus.

Langham and Gustavson (1947) administered d, l-thyroxine daily by subcutaneous injections at levels of 1, 2, and 3 Ug. per gram of body weight for 3, 6, and 10 days and thereby decreased the estrus response of ovariectomized rats to 1.5 gamma of estrone as determined by vaginal smears. Twenty-eight days after the 10-day period of thyroxine dosage the response to estrone returned to normal. The hormone secretion rate of rats is equivalent to approximately 3.5 micrograms of thyroxine per 100 grams body weight (Monroe and Turner, 1946). Since Langham et al. (1947) administered thyroxine at the rate of 100 micrograms or more per 100 grams body weight, their rats were probably stimulated to the thyrotoxic level.

Hill (1948) administered thyroxine within the physiologic range and reported that the estrous response of immature rats decreased with high doses of thyroxine; and conversely, increased with the

administration of thiouracil. Young rats given tolerable doses of thyroxine (7 mg. per 100 gm. rat), over a period of ten days, showed a decrease in response to estrone. Senescent rats given similar dosages showed a slightly increased response.

Hyperthyroidism appears to exert either a favorable or negative influence on reproductive processes in the rat but this does not appear to be true in other species.

Martinez (1947) fed thiouracil to the domestic fowl at a dosage of 0.1 per cent of the ration for a period of four weeks and reported little change in the semen volume, motility, or sperm concentration. Thyroprotein fed as 0.01 and 0.02 per cent of the ration did not influence semen production, but when fed at 0.04 per cent the total number of sperm per ejaculation increased by 65 per cent.

Meites and Chandrashaker (1949) reported that thyroprotein partially or completely inhibited the response of the seminal vesicles and coagulating glands of male rats when pregnant mares' serum was injected, while thyroprotein increased the gonadotropic response in the male mouse. These investigators also found that thiouracil increased the gonadotropic

response to pregnant mares' serum in rats and reduced the response in mice.

Effects of Altered Thyroid Status on Pituitary Gonadotropic Function: It is evident that there are complex interrelationships between the pituitary, thyroid and ovary, and it appears that a balance must be maintained between these glands for efficient reproductive function.

P'an (1940) reported that the gonadotropic potency of the anterior lobe of the pituitary was decreased following thyroidectomy in normal and castrate rats and normal rabbits. Evans and Simpson (1929) reported that the gonad-stimulating properties of the anterior pituitaries from hyperthyroid rats were increased, while the glands from hypothyroid rats were less effective than normal.

Chu (1944) found that in thyroidectomized rabbits the ovaries contained many more large follicles than normal controls, but ovulation did not take place after coitus. The animals that were operated on readily ovulated after the injection of pregnancy urine extract, and the ruptured follicles were more numerous than in normal estrus animals. Fresh saline extracts were prepared from the pituitaries of the normal and thyroidectomized

rabbits for the assay of their relative amounts of ovulating hormone. The pituitary extract from the normal animals caused 60 per cent ovulation in estrous rabbits, whereas pituitary extract from the thyroidectomized rabbits caused no ovulation and only induced growth of follicles.

Reineke, Bergman, and Turner (1941) reported on the effect of thyroidectomy in eight male kids. They were thyroidectomized between the ages of five and twenty-four days and killed at the end of four months, after growth stasis had appeared. Their pituitaries were assayed for gonadotropic hormone and compared with groups of normal controls of the same weight and age. The gonadotropic hormone was found to be present in low concentration and the testes showed lack of stimulation, weighing less than in the youngest group of normal controls.

It appears from the review cited that hyperthyroidism generally exerts unfavorable influences on reproductive processes in rats, while in other species a slight degree of hyperthyroidism is favorable to reproductive action.

It was thought that further light might be thrown on some of the endocrine interrelationships

involved by determining (1) the extent to which the response of the rat and mouse ovary to gonadotropin could be modified by alterations of thyroid function, and (2) whether there was any species or sex difference in the gonadal reactivity of the rat and mouse to these hormonal stimuli.

PROCEDURE

A total of 22 groups of immature female albino rats (averaging 6 animals per group) of the Michigan State College strain, and 14 groups of immature female albino mice of the Rockland strain were used for these studies. Various degrees of hyperthyroidism were induced in the first series of rats by the injection of thyroxine subcutaneously for a ten day period in dosages equivalent to 1.5 to 12.0 times the approximate normal thyroid secretion rate. Hyperthyroidism was induced in the second series of rats by incorporating Protamone* in the ration (Purina Laboratory Chow) in concentrations of .04 to 1.28 per

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* An iodinated casein product containing approximately 3 per cent thyroxine as determined by chemical assay. "Protamone was made available through the courtesy of the Cerophyl Laboratories, Inc., Kansas City, Mo.

cent and feeding each mixture for a ten-day period. Hyperthyroidism was induced in the mice by incorporating Protamone in the ration in concentrations of 0.2 to .32 per cent for a ten day period.

Varying degrees of hypothyroidism were induced in the rats by incorporating 0.1 per cent thiouracil in the ration and feeding for four to twenty day periods. Hypothyroidism was induced in the mice by including 0.1 per cent thiouracil in the drinking water for periods of from seven to twenty days.

Those groups of rats and mice which were to receive thiouracil for the longer periods were started at an earlier age in order to reach approximately the same body weights at the end of the experiment as the groups treated for shorter periods.

A constant dose of pregnant mare's serum (Gonadogen), one Cartland-Nelson unit, was always injected during the last four days of thyroxine, thyroprotein, or thiouracil administration in the rats.

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Kindly supplied by the Upjohn Company, Kalamazoo, Michigan.

One-half Cartland-Nelson unit was similarly injected into the mice.

Two control groups of rats, one untreated and the other injected with Gonadogen only, were used in the series of rat experiments. Two control groups of mice were similarly used in the thyroprotein-treated mouse series. A control group of mice, treated with Gonadogen only, was run with each group of mice which received thiouracil.

All animals were fed a balanced stock diet (Purina Laboratory Chow) and drinking water was available at all times. The animal groups were kept in individual cages, at a constant temperature of 75 degrees F.

Standard errors of the mean were determined for each group of animals. The significance of the differences between averages was determined by the "t" test as described by Snedecor (1946).

RESULTS

1. Effect of Thyroxine on Gonadogen Response in Young Female Rats.

For the purpose of estimating the thyroxine dosage, the thyroid secretion rate was assumed to be equivalent to 4.63 micrograms of d, l-thyroxine daily as reported by Monroe and Turner (1946).

The first series of rats received thyroxine injections subcutaneously in dosages ranging from 1.5 to 12 times their estimated normal secretion rate during a 10 day period, plus one Cartland-Nelson unit of pregnant mare's serum (Gonadogen) injected subcutaneously during the last four days.

In all the thyroxine treated groups, the response of the ovaries to the Gonadogen was reduced when compared to the controls which received Gonadogen only (Table 1, Fig. 1).

The rats which received the lowest thyroxine dose (1.5 times the normal) had ovaries weighing 71.36 mgs. per 100 gm. body weight, which represents a significant decrease below the control value of 99.0 mgs.

At thyroxine doses of 4 times normal and above, ovarian weight was depressed still further below that observed at 1.5 times the normal level. A statistical check (t test) shows these differences to be highly significant.

2. Effect of Protamone on Gonadogen Response in Young Female Rats.

The second series of rats were given Protamone in their feed in doses ranging from 0.04 to 1.28 per cent of the total diet during a period of ten days, plus one Cartland-Nelson unit of Gonadogen injected during the last four days (Table 2, Fig. 2).

The group of animals which received 0.04 per cent Protamone (lowest dose) had ovaries weighing 66.58 mg. per 100 gm. body weight, a highly significant decrease below the control value of 99.0 mg. There was a progressive decrease in the ovarian weights of all groups of rats which received doses of .08 to .32 per cent Protamone. However, the ovary weights of the rats that received 0.64 and 1.28 per cent Protamone were slightly above those that received 0.32 per cent Protamone.

A statistical check (t test) showed no significant differences between the groups which received .32, .64, and 1.28 per cent Protamone. On the whole, these results agree with the thyroxine injected series, since both thyroidal substances reduced the ovarian response to Gonadogen.

3. Effect of Thiouracil on Gonadogen Response in Young Female Rats.

The groups of rats which received 0.1 per cent thiouracil in their diet for periods of four and seven days, plus Gonadogen, showed a highly significant increase in their ovarian weights over the non-thiouracil treated controls.

The rats which received the thiouracil for the shortest period of time (4 days) had ovaries weighing 153.98 mg. per 100 gm. body weight (first group) and 161.74 mg. per 100 gm. body weight (second group), a highly significant increase above the control value of 99.0 mg. The two groups which received thiouracil for seven days showed a still greater increase in ovarian weight (Table 3, Fig. 3).

The rats which received thiouracil for 10 and 15 days had ovaries weighing more than the controls but

less than the groups that received the drug for 4 and 7 days.

The rats which received thiouracil for 20 days had ovaries weighing only 65.3 mg. per 100 gm. body weight (first group) and 66.19 mg. per 100 gm. body weight (second group), which represent significant decreases below the control value of 99.0 mg. Thus thiouracil increased the ovarian response to Gonadogen in all groups except when given for a 20 day period.

4. Effect of Protamone on Gonadogen Response in Young Female Mice.

Six groups of mice, consisting of seven animals each, received doses of Protamone varying from .02 to .16 per cent of their diet during a period of 10 days, plus one-half Cartland-Nelson unit of Gonadogen injected subcutaneously during the last four days.

The group of animals which received .02 per cent Protamone (lowest dose) had ovaries weighing 51.07 mg. per 100 gm. body weight, a highly significant increase above the control value of 41.14 mg.

In all the Protamone groups, the response of the ovaries to Gonadogen was increased when compared to the controls which received Gonadogen only (Table 4, Fig. 4).

There was a progressive increase in the weights of the ovaries in the groups that received .02 to .08 per cent Protamone. Although the group that received .16 per cent Protamone showed an increase in weights of the ovaries above the control value of 41.14 mg. per 100 gm. body weight, the ovaries of these mice weighed less than the ovaries of the mice which received the lowest dosage of Protamone.

A statistical check (t test) shows significant differences between the untreated control group of mice and the control group which received Gonadogen only. Highly significant differences were also found between the control group which received Gonadogen only and all the groups which received Protamone plus Gonadogen.

5. <u>Effect of Thiouracil on Gonadogen Response in Young Female Mice</u>.

It can be seen that the ovarian weights of

the four groups of mice which received thiouracil in their drinking water for periods of 7 to 20 days were not significantly different from the ovary weights of their corresponding control groups (Table 5, Fig. 5).

The group which received thiouracil for the shortest period of time (7 days), had ovaries weighing 68.94 ± 5.99 mg. per 100 gm. body weight, while the 7 day control group had ovaries weighing 62.34 ± 1.27. The 10, 15 and 20 day groups also did not differ significantly from their corresponding controls.

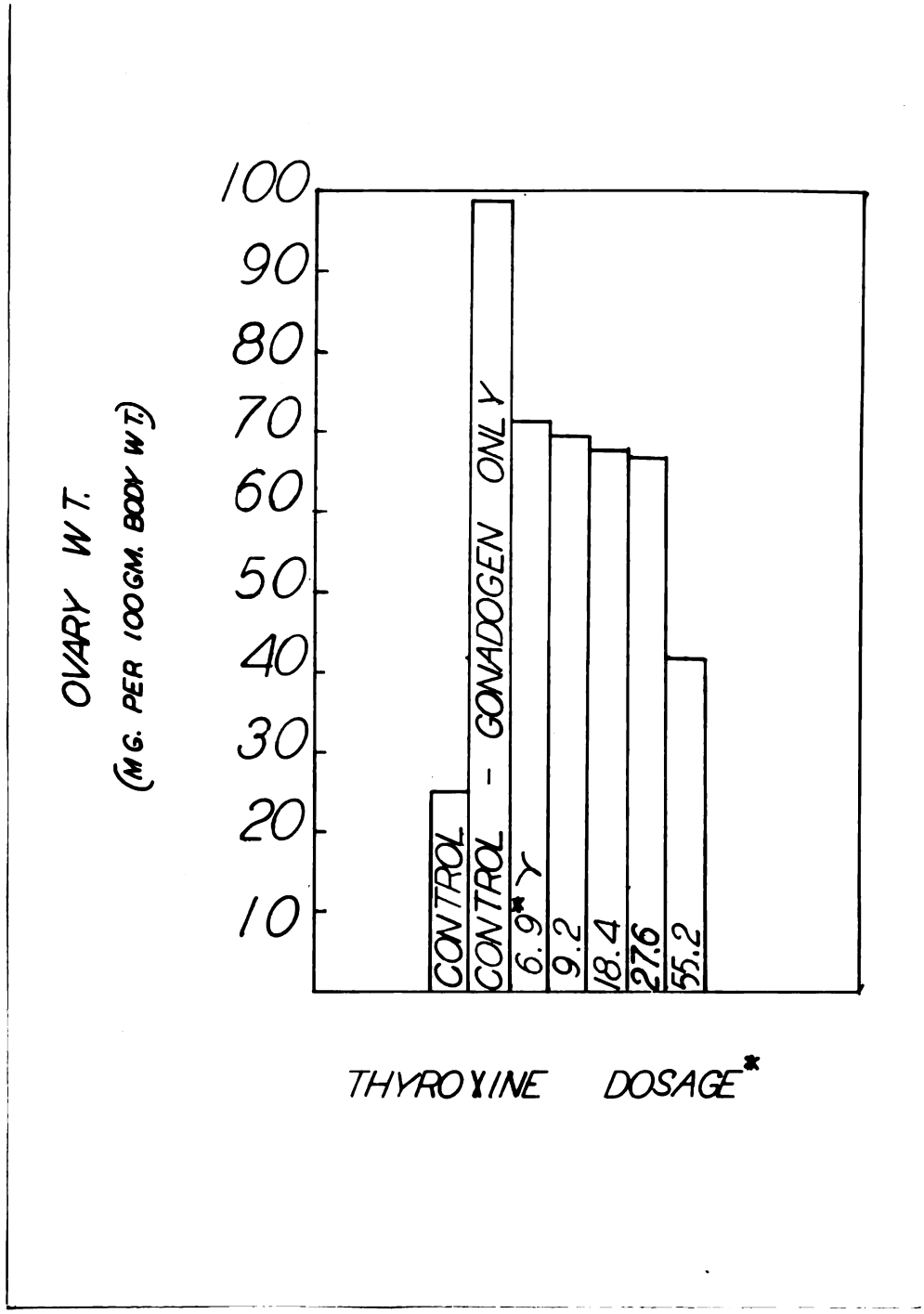

Figure 1

Effect of Thyroxine on Gonadogen Response in Young Female Rats.

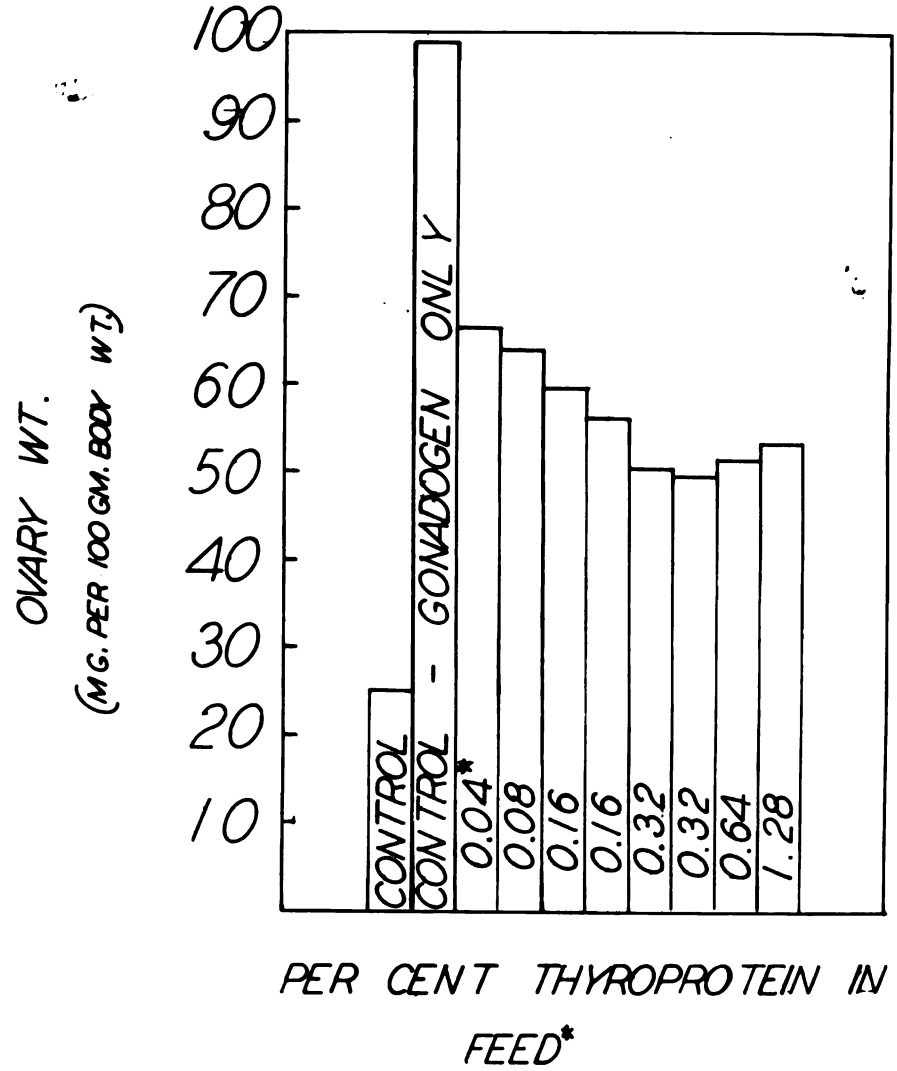

Figure 2

Effect of Protamone on Gonadogen Response in Young Female Rats.

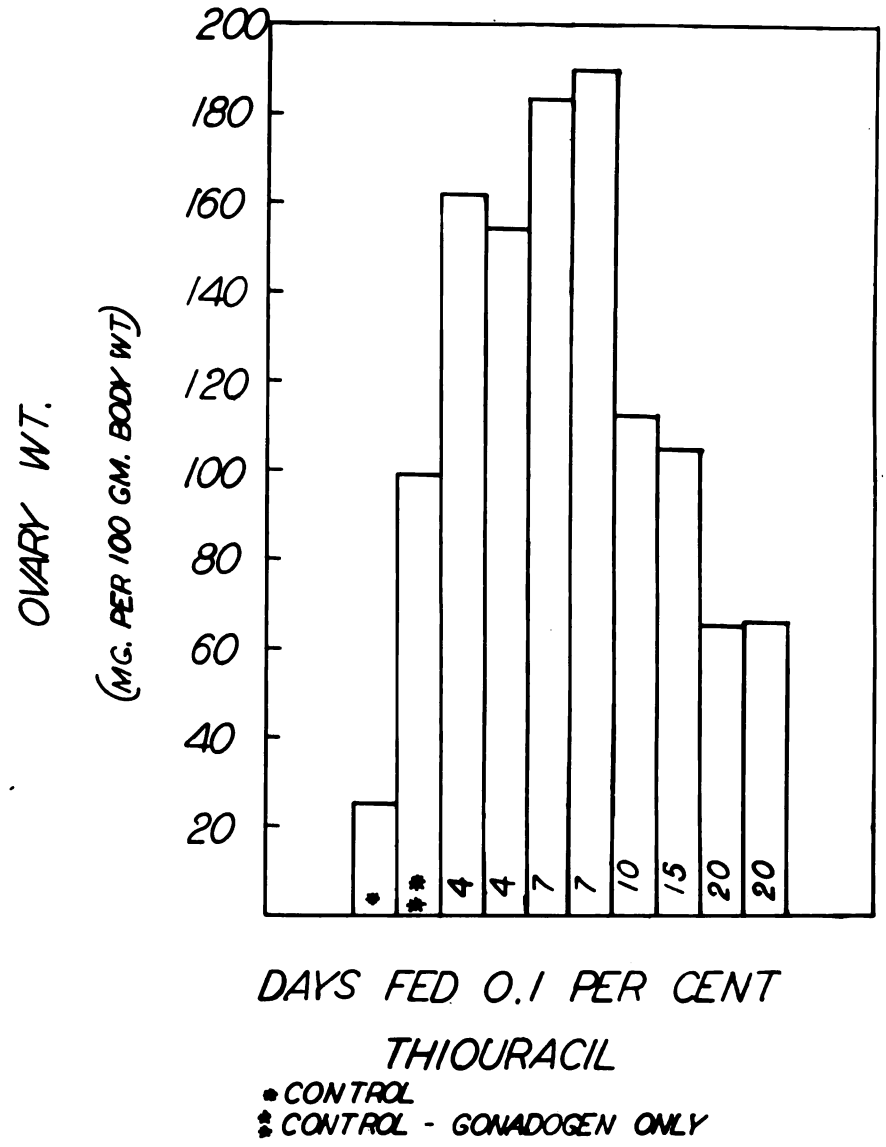

Figure 3

Effect of Thiouracil on Gonadogen Response in Young Female Rats.

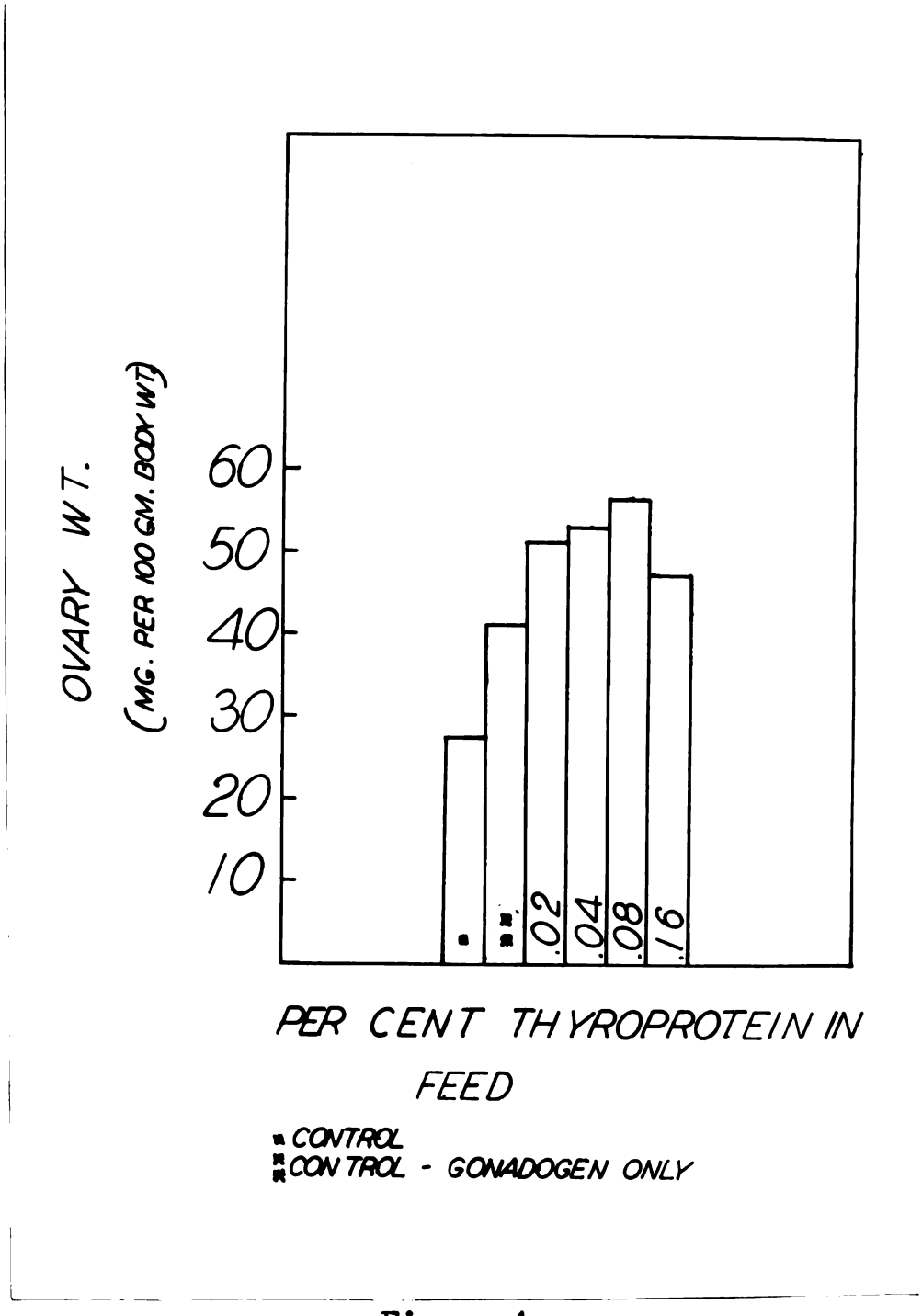

Figure 4

Effect of Protamone on Gonadogen Response in Young Female Mice.

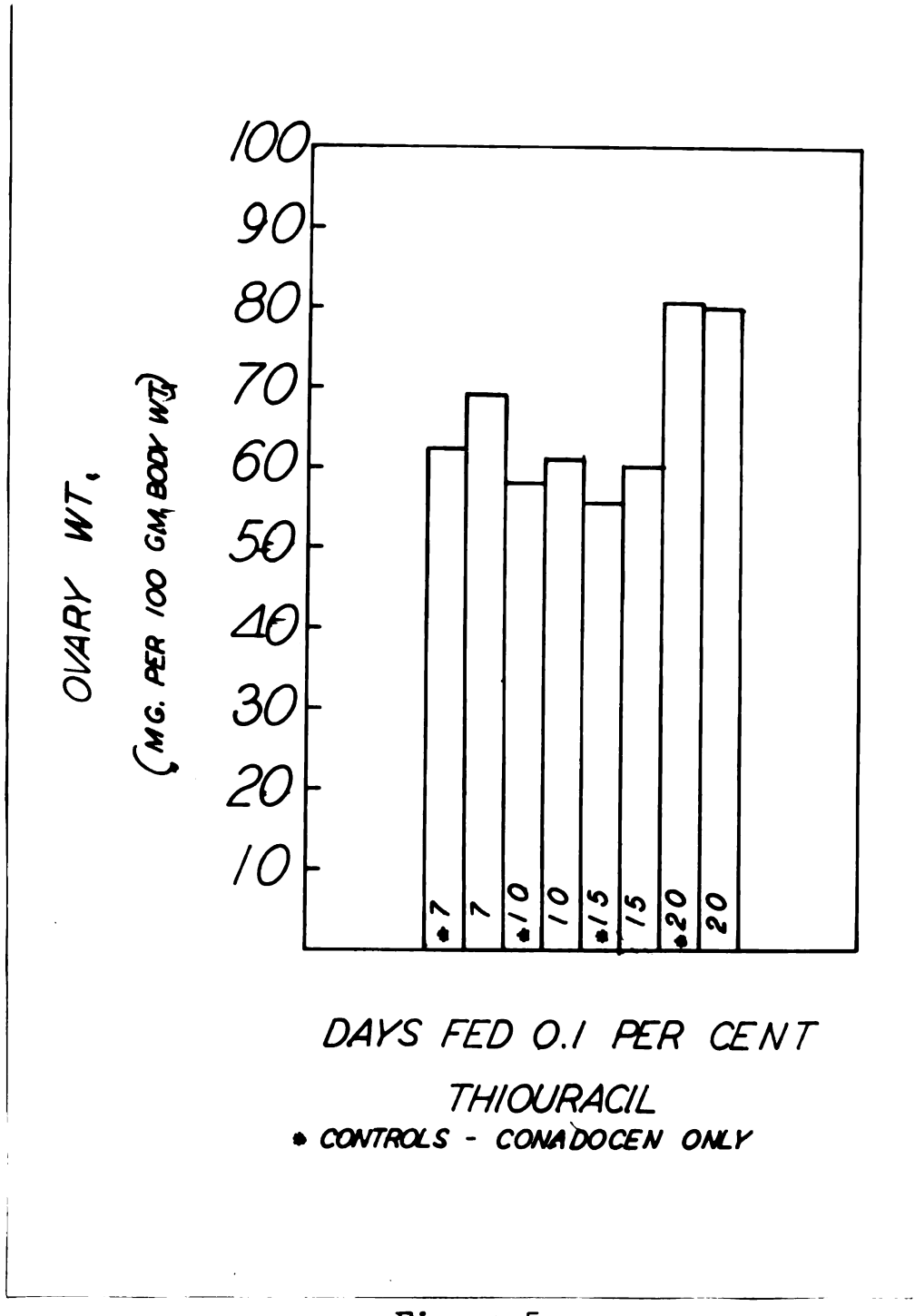

Figure 5

Effect of Thiouracil on Gonadogen Response in Young Female Mice.

DISCUSSION

The results of this work indicate that when thyroxine or Protamone is administered with the object of modifying gonadotropic function, both the species of animal and the hormone dosage levels are very important. The conflicting results of some of the earlier investigations were probably due either to a failure to take into account the very high dosage of thyroxine used, or to recognize that different species may react differently to the same hormonal stimuli.

Meites and Chandrashaker (1949) found that the administration of thiouracil or thyroprotein alone in male rats and mice had no effect on the weight of the seminal vesicles and coagulating glands. When pregnant mares' serum was injected, the growth responses of the seminal vesicles and coagulating glands were partially to completely inhibited by all except

the lowest levels of thyroprotein in the rat, while in the mouse all except the highest concentration of thyroprotein increased the gonadotropic response by 72 to 140 per cent. Thiouracil, particularly when fed for the longer periods, increased the gonadotropic response in rats by as much as 300 per cent, while the response in mice was reduced by 40 to 73 per cent.

The results reported in this thesis on female rats and mice are in agreement with the findings of Meites and Chandrashaker (1949) in male rats and mice, with the exception of the female mice which received thiouracil. A reduction in response to Gonadogen when given with thiouracil was not found in the female mice. A sex difference in the response of male and female mice to thiouracil plus Gonadogen is therefore indicated in these findings.

Species differences are evident in the fact that the female rats showed a decreased response to Gonadogen when thyroxine or Protamone was administered, while the female mice showed an increased response to

Gonadogen when Protamone was administered. Thiouracil increased the response to Gonadogen in the female rats, but had no effect on the response in female mice.

The question remains to be answered as to how alterations in thyroid function influenced the ovarian response to pregnant mare's serum in these animals. The possibility exists that altering thyroid function may change the secretion of gonadotropic hormone by the animal's own pituitary. However, previous work indicates that immature rats and mice of the age used in these experiments do not secrete gonadotropic hormones (Moore, 1939). Furthermore, it has been shown, (Meites and Chandrashaker, 1949), that in male immature rats and mice, the administration of Protamone or thiouracil does not alter the weights of the reproductive organs of these animals during short term treatments such as were used in these experiments.

It is possible that the thyroid hormone may exert a direct effect on the gonads, altering their reactivity to gonadotropic hormones. This would seem to be indicated from the recent observation of Warner and Meyer (1949) that the administration of thyroxine to ovariectomized female -- intact female parabiotic rats prevented ovarian hypertrophy in most of the intact partners.

The general metabolic changes induced in the body by altering thyroid function must also be taken into consideration, since changes in metabolic rate alone may affect gonadal function. However, no conclusive information is available on this point.

SUMMARY AND CONCLUSIONS

1. Immature female rats given tolerable doses of thyroxine (1.5 to 12 times the approximate thyroid secretion rate), over a period of ten days, showed a decrease in response to the gonadotropic hormone of pregnant mares' serum (Gonadogen).

2. Immature female rats given tolerable doses of Protamone (.04 to .32 per cent in the diet), over a period of ten days, showed a decrease in response to the gonadotropic hormone of pregnant mares' serum. Higher dosages of Protamone (.64 and 1.28 per cent in the diet), did not decrease the response of the ovaries more than .32 per cent Protamone.

3. Immature female rats given 0.1 per cent thiouracil for varying periods of time (4 to 15 days), showed an increase in response to the gonadotropic hormone of pregnant mares' serum. However, when these

ratswere given thiouracil for 20 days, the ovaries showed a decrease in response to Gonadogen.

4. Immature female mice given tolerable doses of Protamone (.02 to .08 per cent in the diet) over a period of ten days showed an increase in their response to Gonadogen. This response indicates a species thyroid difference between rats and mice. However, when the mice were given a higher dosage of Protamone (.64 per cent of the diet), the response of the ovaries to Gonadogen was less than with low dosages of Protamone.

5. Immature female mice given 0.1 per cent thiouracil for varying periods of time (4 to 20 days in the diet or drinking water), showed no significant changes in their ovarian response to Gonadogen when compared with controls which received Gonadogen only. This again indicates a species difference between the rat and mouse, since in the former thiouracil increases the response to Gonadogen while in the latter it decreases the response to Gonadogen.

APPENDIX

APPENDIX

Table 1. Effect of Thyroxine on Gonadogen Response in Young Female Rats.

Group	No. of Rats	Treatment: Gamma Thyroxine	Treatment: XN* Thyroxine	Ave. Orig. Body Wt. gm.	Ave. Final Body Wt. gm.	Ave. Wt. of Ovaries mg.	Ave. Wt. of Ovaries per 100 gm. Body Wt. mg.
1	6	Controls		45	69.7	17.55	25.17
2†	6	Controls Gonadogen only		40	77.1	76.68	99.0 ± 4.56**
3†	6	6.9	1.5	47	96.66	68.98	71.52 ± 2.71
4†	6	9.2	2	45	88.5	61.7	69.61 ± 1.75
5†	6	18.4	4	50	89.5	51.6	57.71 ± 0.82
6†	6	27.6	6	47	96.4	57.66	57.00 ± 0.87
7†	6	55.2	12	47	103.0	41.03	41.74 ± 1.31

* XN = Times normal thyroid secretion rate.

** Standard error of mean = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

† These groups received Gonadogen.

APPENDIX

Table 2. Effect of Protamone on Gonadogen Response in Young Female Rats.

Group	No. of Rats	Treatment Protamone %	Ave. Orig. Body Wt. gm.	Ave. Final Body Wt. gm.	Ave. Wt. of Ovaries mg.	Ave. Wt. of Ovaries per 100 gm. Body Wt. mg.
1 Controls	6	No Treatment	45	69.7	17.55	25.17
2 † Controls	6	Gonadogen Only	40	77.1	76.68	99.0 ± 4.56*
3 †	5	.04	45-50	90.2	59.86	66.58 ± 1.48
4 †	6	.08	45-50	85.41	54.6	63.9 ± 1.23
5 †	6	.16	40	76.66	45.8	59.72 ± 1.37
6 †	6	.16	45-50	102.83	55.38	56.33 ± 2.06
7 †	5	.32	50	107.8	54.48	50.56 ± 3.12
8 †	6	.32	45	91.0	45.0	49.68 ± 1.66
9 †	6	.64	45	95.6	50.2	52.49 ± 1.54
10 †	6	1.28	40-45	83.9	44.04	53.35 ± 1.19

* Standard error of mean $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

† These groups received Gonadogen.

APPENDIX

Table 3. Effect of Thiouracil on Gonadogen Response in Young Female Rats.

Group	No. of Rats	Days on Trial	Ave. Orig. Body Wt. gm.	Ave. Final Body Wt. gm.	Ave. Wt. of Ovaries mg.	Ave. Wt. of Ovaries per 100 gm. Body Wt. mg.
1 Controls	6	10	45	69.76	17.55	25.17
2 † Controls	6	10	40	77.1	76.68	99.0 ± 4.56*
3 †	6	4	40	72.3	111.3	153.98 ± 3.12
4 †	6	4	40-45	63.0	101.7	161.74 ± 2.37
5 †	6	7	40-45	78.16	143.28	183.31 ± 2.12
6 †	6	7	50	80.0	151.40	189.42 ± 2.58
7 †	10	10	45	86.5	97.2	112.41 ± 1.65
8 †	6	15	45	83.75	87.7	104.71 ± 1.85
9 †	6	20	45	93.0	60.6	65.3 ± 1.29
10 †	4	20	45	79.2	52.5	66.19 ± 2.5

* Standard error of mean $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

† These groups received Gonadogen.

APPENDIX

Table 4. Effect of Protamone on Gonadogen Response in Young Female Mice.

Group	No. of Mice	Treatment Protamone %	Ave. Orig. Body Wt. gm.	Ave. Final Body Wt. gm.	Ave. Wt. of Ovaries mg.	Ave. Wt. of Ovaries per 100 gm. Body Wt. mg.
1 Controls	7	No Treatment	8-10	16.4	4.5	27.8
2† Controls	7	Gonadogen Only	8-10	15.35	7.2	27.8 ± .65*
3†	7	.02	10	17.5	7.84	41.14 ± 1.27
4†	7	.04	10	16.5	8.71	51.07 ± 1.51
5†	7	.08	10-11	16.5	9.26	52.27 ± 2.82
6†	7	.16	11-12	15.6	7.34	55.74 ± 2.47
7**†	8	.32	10	----	----	47.49 ± 1.80

* Standard error of mean $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

** The animals in this group began to lose weight after the third day and died between the third and seventh days, possibly due to the toxicity of the high concentration of the drug.

† These groups received Gonadogen.

APPENDIX

Table 5. Effect of Thiouracil on Gonadogen Response in Young Female Mice.

Group	No. of Mice	Days on Trial	Ave. Orig. Body Wt. gm.	Ave. Final Body Wt. gm.	Ave. Wt. of Ovaries mg.	Ave. Wt. of Ovaries per 100 gm. Body Wt. mg.
Control	6	7	11-12	15.64	9.8	62.34 ± 1.29*
1	6	7	11-12	17.53	11.7	68.94 ± 5.99
Control	6	10	10-11	21.2	12.33	58.25 ± .60
2	6	10	10-11	17.3	10.5	61.35 ± 8.30
Control	6	15	8-10	21.7	12.1	55.42 ± .68
3	5	15	8-10	18.0	11.4	60.29 ± 4.11
Control	7	20	8-10	20.8	16.74	80.57 ± 1.35
4	6	20	8-10	21.4	17.1	79.81 ± 5.56

* Standard error of mean $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

One-half Cartland-Nelson unit Gonadogen injected during last 4 days of each experiment.

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