THE EFFECT OF AGE, AND THYROID STATUS ON THE RESPONSE OF OVARIECTOMIZED RATS TO ESTRONE

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E. P. Reineke
Major professor

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THE EFFECT OF AGE, AND THYROID STATUS ON THE RESPONSE OF OVARIECTOMIZED RATS TO ESTRONE

By

MARION HANLEY HILL

A THESIS

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DEDICATIONS

To my husband, Professor John D. Hill, this Thesis is affectionately dedicated. His constant understanding and co-operation in caring for our children, Joan Patricia, John Dillard and Mary Elizabeth have made this work possible. It is also dedicated to my parents, Mr. and Mrs. Edmund C. Hanley, who have provided a continual source of inspiration.

Marion Hanley Hill

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TABLE OF CONTENTS

TABLE OF CONTENTS (Con't.)

INTRODUCTION

The number of investigations providing quantitative data on the effects of both hyper- and hypothyroidism on the response of spayed rats to estrone is rather limited. In general, it is thought that thyroid at low dosage exerts an anabolic effect in contrast to the catabolic action of medication with larger quantities.

Meyer and Wertz (1938) used desiccated thyroid powder, thyroid globulin and d,l-thyroxine, and found that treatment with these materials at moderate doses for three days did not interfere markedly with the action of estrone; however, the demand for estrogen rises when thyroid is administered for more than a five day period. These experiments were carried on with eighty-day-old rats.

Much of the literature indicates that results obtained are negative, with doses outside the physiological range. By physiological range, is meant a dosage level the same as, or very close to the normal secretion rate of the animal; a rate at which no undesirable side effects can be determined by observation.

It is believed that young rats are normally hyperthyroid. Experiments have shown that their estrus response decreases with high doses of thyroxine; and conversely, increases with the admin-

istration of thiouracil, a thyroid-inhibiting drug. Normally, with increasing age, thyroid secretion declines. It is the purpose of this paper to describe experiments showing that the estrus response is increased in old, presumably hypothyroid rats, which have been administered a tolerable dosage of thyroxine for a period of time. It will also be observed that somewhere between twelve and fourteen months, the sensitivity to estrogen decreases and the sensitivity to thyroxine increases.

REVIEW OF THE LITERATURE

There is evidence that thyroxine, in some way, plays a role in reproduction in the female. The evidence of a specific relationship between the thyroid, ovaries or testes, and the anterior hypophysis is conflicting. As Francois Rabelais so appropriately put it, (Pantagruel, Book III, Chapter 4) "The hierarchy (of the organs) is such that incessantly one borrows from another, one lends to the other, one is the other's debtor.... each member prepares itself and strives anew to purify and refine this treasure.... to preserve and perpetuate the Human Race. All this is done by Loans and Debts one to the other." It is well to recall then, while considering the following experiments, that one primary function of an organism is to maintain a normal endocrine balance.

1. Functional Relationship between the Thyroid Gland and the Ovaries

The inter-relation between the thyroid and the ovary has been appreciated clinically for centuries, on account of the increased size of the thyroid at menstruation and pregnancy.

Clinical cases have been cited by Lerman (1942), in which patients with myxedema due to primary dysfunction of the thyroid had amenorrhea not attributable to the menopause. On administration of thyroid the ovarian cycles of these patients became normal. This suggests that the thyroid, by stimulating the ovary - either directly or indirectly, increases the production of estrogen and/or progestin, and the normal cycle returns. This conception fits with the observations of Grumbrecht (1939) that thyroid increases the weight of the ovaries of infantile rats receiving a constant dose of gonadotropic substance, the increase in the weight being proportional to the dose of the thyroid.

Engle (1946) reported that the hypothyroid monkey is amenorrheic to the extent of about one or two periods a year. But when a hypothyroid monkey is put on a very low thyroid dosage for a period of ten days, the menstrual function always returns. A single treatment will give either two or three successive normal menstrual cycles.

In the hypothyroid monkey the amount of estrogen needed to induce estrogen withdrawal bleeding is greatly increased. In monkeys treated with thiouracil, the menstrual cycles will again become very irregular. Amenorrhea or extremely prolonged cycles of over fifty days are the rule.

2. Response to Estrogen as Observed by Vaginal Smears

Lee (1925), in his studies on the estrus cycle in the rat, found that following thyroidectomy there was, on the average, a one day lengthening of the cycle. His results also indicate that pre-pubertal thyroidectomy did not materially change the period required to reach sexual maturity. He offers two alternative explanations: (a) Normally, the thyroid exercises a stimulating influence on the ovary, and the removal of this stimulus causes a lengthening of the cycle; (b) Ovarian activity varies with general body activity, and thyroidectomy affects the estrus cycle through its effect on metabolic processes. Conflicting evidence was presented by Reiss and Perény (1928) when they reported that the thyroid hormone was an antagonist of the ovarian hormone (theelin) as evidenced by the fact that subcutaneous injections of thyroid autocoid not only prevented heat in normal female rats and mice, but also inhibited the action of the ovarian hormone in castrate animals. A subsequent experiment by Van Horn (1933) confirmed these findings. He fed desiccated thyroid powder to ovariectomized rats and observed their response to estrone by noting the cellular composition of vaginal smears. He found that approximately three times as much estrogen was required to produce positive estrus smears as was necessary before thyroid feeding.

Richter (1933), reported that vaginal smears taken on thyroidectomized animals with intact ovaries showed a persistence of cornified cells in the vaginal smears at all times, as if there was a

tendency toward a prolonged estrual phase. At autopsy, the animals showed hypertrophied uteri.

Contrary to what one might expect, that is, that hypothyroidism would have the opposite effect to hyperthyroidism, Müller (1938) reported that thyroidectomy was without effect on the response of ovariectomized rats to estradiol benzoate.

Evans and Long (1921a) reported that thyroidectomy in rats affected neither the onset of puberty, nor the length of estrual cycles. They also reported (1921b) that feeding of fresh thyroid gland substance had no disturbing effect upon the estrual cycles.

Da Costa and Carlson (1933) reported that female albino rats fed 0.5 to 1 mgm. of desiccated thyroid daily showed slight acceleration in sexual maturation, while those fed 5 to 10 mgm. of thyroid daily showed a definite retardation in pubescence.

Langham and Gustavson (1947) reported on the effect of the level of thyroid activity on response of ovariectomized rats to estrone. They found that daily administration of d,l-thyroxine by subcutaneous injection at dosage levels of 1, 2 and 3 mcg. per gram of body weight for 3, 6 and 10 days decreased the estrus response of ovariectomized rats to 1.5 mcg. of estrone, as determined by the method of vaginal smears. Both the weight loss and the decrease in sensitivity to estrone were found to be logarithmic functions of the thyroxine dosage.

The authors also reported that the daily administration of thiourea at levels of 0.05, 0.1 and 0.3 per cent in the drinking

water of ovariectomized rats over a period of seventy days inhibited growth and produced marked hyperemia and enlargement of the thyroid gland. The estrus response of spayed rats to 1.3 mcg. of estrone was first reduced and then increased as dosage with thiourea continued.

3. Relation of Thyroid to Pregnancy

In the ovaries of cretin rabbits Tatum (1915) found histologically a decrease in the number of primary follicles, an increased size of the follicular space and degenerative changes in the ova. These degenerative changes of the ovary were confirmed by Kunde, Carlson and Proud (1929). When 60 to 140 mgm. of desiccated thyroid were fed daily to cretin rabbits for a period of four to eight weeks, or until symptoms of hyperthyroidism became definitely established, Kunde observed a decided increase in the number of developing Graafian follicles and primordial ova. In rabbits with severe hyperthyroidism, experimentally induced, the process of estrus, ovulation, fertilization, migration and implantation take place, but in most instances the young are never born, resorption occurring during the latter two-thirds of the gestation period. Similarly, Weichert (1930) found that feeding 0.25 gm. to 0.5 gm. of desiccated thyroid daily to pregnant rats caused resorption of young in some animals, abortion in others, and in some cases a prolonged period of gestation with the ultimate delivery of dead young.

Hoskins (1910) subjected pregnant guinea pigs to thyroid feeding with the intent of influencing the gland weight of their offspring. The variations in susceptibility to the ingested thyroid were demonstrated by the following results: One animal died of hyperthyroidism after eight days of feeding 0.01 gram per day, whereas another animal survived for the same period of time on a dose twenty-five times as great. The author incidentally noted that abortions were frequent in the surviving animals.

Gudernatsch (1915) reported that thyroid substance administered to male or female rats, had a tendency to prevent pregnancy, and when pregnancy occurred to produce abortion.

4. Relation of Thyroid to Sperm Production

Many factors enter into the picture of sperm production versus the thyroid gland. Observations show a rather distinct seasonal variation in sperm production. In sheep thyroidectomy of rams has been reported to depress sperm production, whereas in bulls normal semen and sperm were observed. Recent reports present definite qualitative data on the relationship of the thyroid to the production of sperm.

Berliner and Warbritton (1937) observed twenty-four rams during a two year period, and found a seasonal variation in sperm production, summer being the low season. All Shropshires produced

semen of poor quality during the hot months but only the poorer Hampshires declined noticeably during the summer. It was suggested that poor summer sperm production was due to the decline in thyroid hormone secretion due to the high temperatures prevailing during the summer.

An intact Shropshire ram treated with thyroxine produced more normal sperm than usual, though in low concentration, during the summer. Two intact and one thyroidectomized Hampshire rams with high abnomality counts returned to normal after two doses (2 mg.) of thyroxine in August. They conclude that thyroxine therapy in a relatively normal animal with a less active thyroid can prevent the summer decline in normal sperm production, or can restore sperm production early in the fall.

A study was made by Petersen, Spielman, Pomeroy and Boyd (1941) on a male Jersey, which was thyroidectomized at four months of age. They reported that thyroidectomy of the male bovine causes a complete inhibition of libido, but had no effect upon spermogenesis or normality of the sperm. Oral administration of 25 grams of desiccated thyroid over a period of three days restored normal activity and sexual behavior.

Reineke (1946) reported on the effect of synthetic thyroprotein on sterility in bulls. Fourteen bulls which had unsatisfactory breeding performance records, were fed thyroprotein. Of this number improvement in vigor and libido was observed in ten.

The time required for an observable effect to occur ranged from seven to forty days.

Definite evidence of improvement in the conception record was obtained in only four cases. The limited conception records are suggestive, however, of an improvement in spermatogenesis. The thyroprotein was fed at the rate of about 0.5 to 1.0 gram per 100 pounds body weight.

Da Costa and Carlson (1933) found in male albino rats fed 0.5 to 10 mgm. of desiccated thyroid daily, a decidedly lower relative weight of the testes than in the controls. In most cases the sexual maturation of the gonads of the male rat was depressed by thyroid feeding (0.5 - 10 mgm. per day).

Martinez (1947) reported on the influence of varying levels of thyroid activity on semen production in the domestic fowl. The results showed that thyroprotein fed as 0.04 per cent of the ration causes a definite stimulation of spermatogenesis. Both the semen volume and the sperm concentration are increased markedly; consequently the total number of sperm per ejaculation is also increased.

Wilwerth (1948) found that thyroprotein fed as 0.04 per cent of the ration causes a definite stimulation of spermatogenesis, in the domestic fowl. As compared to the control birds both semen volume and sperm concentration increased to some extent with the result that the total sperm per ejaculate increased rather markedly. In older roosters, similar but less pronounced trends were observed.

Thyroprotein when given at higher levels of 0.08 and 0.16 per cent of the ration, depressed semen production and the total number of sperm decreased markedly.

Hoskins (1916) reported only a very slight increase in weight of testes of rats fed desiccated thyroid.

5. Gonad, Thyroid, Anterior Hypophysis Relationship

There is the possibility that the thyroid has an influence on the normal function of the hypophysis.

Evans and Simpson (1930) observed 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. They reported a qualitative relationship only.

Reineke, Bergman and Turner (1941) reported on a group of eight male kids that were thyroidectomized between the ages of five and twenty-four days, then killed at about four months of age, after growth stasis had appeared. Their pituitaries were then assayed for gonadotropic hormone in comparison with groups of normals of the same weight (but younger) and of the same age (but normal weight).

The gonadotrophic hormone was found to be present in low concentration and the testes showed lack of stimulation, averaging lighter than the youngest group of normal controls.

Bradbury (1947) reported that when estrogen is given to the immature female rat there is an increase in ovarian and pituitary weight in seventy-two to one hundred and twenty hours. The pituitary decreases in gonadotrophic hormone content in seventy-two to ninety-six hours. The loss of pituitary potency precedes the increase in ovarian weight, indicating that effective quantities of gonadotrophin are released into the blood stream. Small doses of estrogen, 1 to 2 mcg. are sufficient to produce these effects.

If rats are spayed the day before the initiation of estrogen treatment, the pituitary does not decrease in potency in ninety-six hours even though the dose of estrogen is increased up to 200 mcg.

The ovary accelerates or augments the estrogen stimulation of the pituitary of the infantile female rat.

Baker and Everett (1947) reported that the injection of 2 mcg. daily of diethylstilbestrol into immature thyroidectomized rats for four days or into adult ovariectomized - thyroidectomized rats for ten days caused an increase in weight of the hypophysis, cellular enlargement, an accelerated mitotic activity among the acidophiles and an increase in their number. Thus, it was demonstrated that this stimulating action of estrogen on the hypophysis is not mediated by the thyroid gland and that it may be elicited in the adult, as well as in immature rats.

It may be well for the author to mention, in closing the review of literature, that as reported by Sadhu (1948), thyrotropic hormone has been assayed by the response in day-old chicks and confirmed histologically by measuring the height of the epithelia in the thyroid acini.

From the foregoing review it is evident that the thyroid secretion plays a part in the complex of hormones involved in reproduction in both the male and female. A report made by Schneider (1939), covering a review of the literature up to that time, on the effects of feeding thyroid substance, summarizes the subject somewhat in the following manner: Thyroid feeding of the vertebrates shows that the thyroid which normally is an organ of internal secretion, may, if given orally, induce definite and considerable changes in the organism both immediately and over considerable periods of time. These changes, which are seen as accelerations of metamorphosis in amphibians, or of either the moulting cycle or feather production in birds, or of growth, or of milk or milk fat secretion in mammals, are all manifestations or expressions of the metabolic regulator function of the gland which is its basic job. Thyroid undoubtedly exerts its metabolic regulator activity on all protoplasm irrespective of the organism in which it occurs.

The abundance of controversial evidence in the literature on thyroid feeding, or medication, prevents the drawing of any specific generalizations on the subject; hence there is need for

carefully controlled experimentation in this field, with particular emphasis placed on the importance of dosage in the physiological range of the animal.

PROCEDURE

The animals used were from a colony of albino rats that have been kept in this laboratory for several years, designated as the Michigan State College strain.

Two groups of approximately thirty-six and sixty castrate female rats, ranged in age from nine to fourteen months, and twelve to fourteen months respectively. Also one group of forty-two castrate female rats, three to four months old were used for a series of experiments, which were conducted to study the effects of age, and size of thyroxine dosage on the response of ovariectomized rats to estrone.

The rats were fed a balanced stock diet, and drinking water was available at all times. They were kept in individual cages, under constant temperature of 75 degrees F.

For the first four experiments the animals were divided into four groups of nine animals each. For experiments five, six and seven, a total of sixty rats were divided into groups of fifteen each. In all cases, each of the four groups were designated as:

Group A, which received the lowest dosage of estrone; Group B, which received the middle dosage of estrone, and served as a control group for Group D; Group C, which was treated continuously with the highest dosage of estrone; Group D, which received the same middle dosage of estrone as did Group B, and was injected daily with d,l-thyroxine for ten days prior to the estrone injection. The last dose of thyroxine was given at the same time as the rats received the various doses of estrone. Forty-eight hours later the response to estrone was determined by the method of vaginal smears (Allen and Doisy, 1923).

All D Groups in experiments one to six received daily for ten days prior to the estrone injection, 7 mcg. of d,l-thyroxine per 100 gm. body weight. This thyroxine dosage is about twice the normal secretion rate (Reineke, Unpublished). It will be noted that these animals were being given this dose of thyroxine daily, almost continuously from November 8, 1947 to April 27, 1948; or for approximately six months. Group D in experiment number seven, had ten times this thyroxine dosage, or 70 mcg. per 100 gm. rat. This amounts to twenty times the normal thyroid secretion rate.

The estrone dosage was .1, .2, .4 and .8 mcg. per 100 gm. rat in the first four experiments, and continued at .4, .8 and 1.6 mcg. per 100 gm. rat in experiments five, six and seven. Except in experiments one through four, all rats were primed on the fifth day of each experiment with 1.6 mcg. per 100 gm. rat.

The animals were weighed before each injection of estrone, and the rats in Group D were weighed at the beginning of each experiment, to have a basis for the thyroxine dosage.

In experiment eight, two groups of twenty-one rats were set up. One was treated for ten days with d,l-thyroxine at the rate of 7 mcg. per 100 gm. rat. The other group served as a control. At the end of the fifth day all forty-two rats were primed with .4 mcg. estrone per 100 gm. rat. The tenth day all of the rats were again injected with .4 mcg. estrone per 100 gm. rat.

Vaginal smears were made previous to experiment one and experiment five, to determine that the animals were in the diestrus stage. Smears were taken again forty-eight hours after the priming dose to determine the percentage of the animals in the positive stage; that is, proestrus, or estrus. Five days after the priming dose smears were made to determine that the rats were again in the negative condition. Smears were made to determine the final results of the experiments thirty-six and forty-eight hours after the terminal injections of thyroxine and estrone.

For individual smear classification, see Tables 1 to 8 in the Appendix.

RESULTS

Normally, according to Lee (1925) any infection, inanition or other abnormal condition is quickly shown by a lengthened diestrum.

If the rats are accustomed to being handled, the procedure of withdrawing samples of vaginal fluid does not affect the estrus rhythm.

1. Response of Old Rats to Estrone and Effect of Thyroxine

Rats in the first seven experiments, having been spayed for about a year, were so depleted of estrogen that the first four experiments are offered merely as proof that priming was absolutely necessary. Priming with 1.6 mcg. per 100 gm. rat, and final doses of .4, .8 and 1.6 mcg. per 100 gm. rat, for various groups worked out most satisfactorily. It was found necessary to give all dosages of both thyroxine and estrone, in proportion to the body weight of the rat, in order to obtain consistent results.

In experiments one and two the rats were not primed and there was no response whatsoever, to .2 mcg. of estrone. The rats in experiments three and four were primed with .6 mcg. of estrone per rat, and the response to .2 mcg. of estrone was in each case 11 per cent, or one rat out of nine was in estrus. These results may be checked for individual response to estrone in Tables 1 to 4 in the Appendix. Since all response in the first four experiments was very low, or negative, the dosage was increased for experiments five, six and seven and continued in a uniform manner for comparative purposes.

When both estrone and thyroxine were given at physiologically effective levels, the per cent of rats showing a positive vaginal response was increased above that observed with the same dose of estrone given alone (Figure 1). The thyroxine treated group showed an average

response of nine per cent above the controls when both received .8 mcg. of estrone per 100 gm. rat. There is also evidence that the sensitivity to estrone is decreased in rats between eleven and fourteen months of age.

Ten days previous to experiment seven, rats were injected daily with excessive doses of thyroxine (70 mcg. per 100 gm. rat). All doses of estrone remained the same in experiments five, six and seven. That is, Group A received .4 mcg., Group B .8 mcg., Group C 1.6 mcg. and Group D .8 mcg. per 100 gm. rat. However, in experiment seven, the response in Groups B, C and D were lower than in the two previous experiments; in experiments five and six Group C had a forty per cent response, and in experiment seven this dropped to thirty-three per cent. Group D in the last experiment showed a twenty-one per cent response, and in the two previous experiments thirty-three per cent. Group B in experiments five and six showed a positive response to estrone of twenty-six and twenty-one per cent respectively, which was less in all cases than the response in thyroxine - treated Group D. Group A showed a twenty per cent response in experiment five, a fourteen per cent response in experiment six and in experiment seven the response was fifteen per cent, or two per cent above the results in Group B of the same experiment.

2. Response of Young Rats to Estrone

It is interesting to note that in this particular strain of rats, approximately four times as much estrone is required by

year-old rats, as by rats three months of age, for the same estrus response. This is shown in the following comparison:

OLD RATS			YOUNG RATS		
Dose	Age	Per Cent Response	Dose	Age	Per Cent Response
.8 mcg.	14 mo.	16	.1 mcg.	3 mo.	7
.8 mcg.	12½ mo.	21	.2 mcg.	3 mo.	21
1.6 mcg.	14 mo.	36	.4 mcg.	3 mo.	35
Primed 1.6 mcg. per 100 gm rat.			Primed .4 mcg. per 100 gm. rat.		

3. Effect of Thyroxine on Estrone Response in Young Rats

In experiment eight, the control group of twenty-one rats showed a response of fourteen per cent above the thyroxine treated group (7 mcg. per 100 gm. rat, daily for ten days). This is in agreement with the work done by Meyer and Wertz (1938), and corroborates their contention that the demand for estrogen rises when thyroid is administered for more than a five day period, in three-month-old rats. Detailed data concerning this experiment may be found in Table 8 of the Appendix.

4. Effect of Estrone, Thyroxine and Age, on Weight of Old Rats

A constant weight was maintained in rats ranging in age from nine to eleven and one-half months, during the first three experiments. This is readily observed in the graph (Figure 2),

which shows the effect of estrone and estrone plus d,l-thyroxine administration, and age on the weight of ovariectomized rats. This is taken to indicate that a tolerable dose of thyroxine was being given. However, somewhere between eleven and one-half and fourteen months, the weight decreased noticeably with the same dosage of thyroxine. It is therefore concluded, that at approximately the same time that sensitivity to estrone decreases, sensitivity to thyroxine increases. When an excessive dose of thyroxine (70 mcg. per 100 gm. rat) was injected daily for ten days previous to the experiment, there was also a marked decrease in the weight. The animals at this time were thirteen and one-half to fourteen months old. For detailed data on individual weight see Tables 1 - 7 in the Appendix.

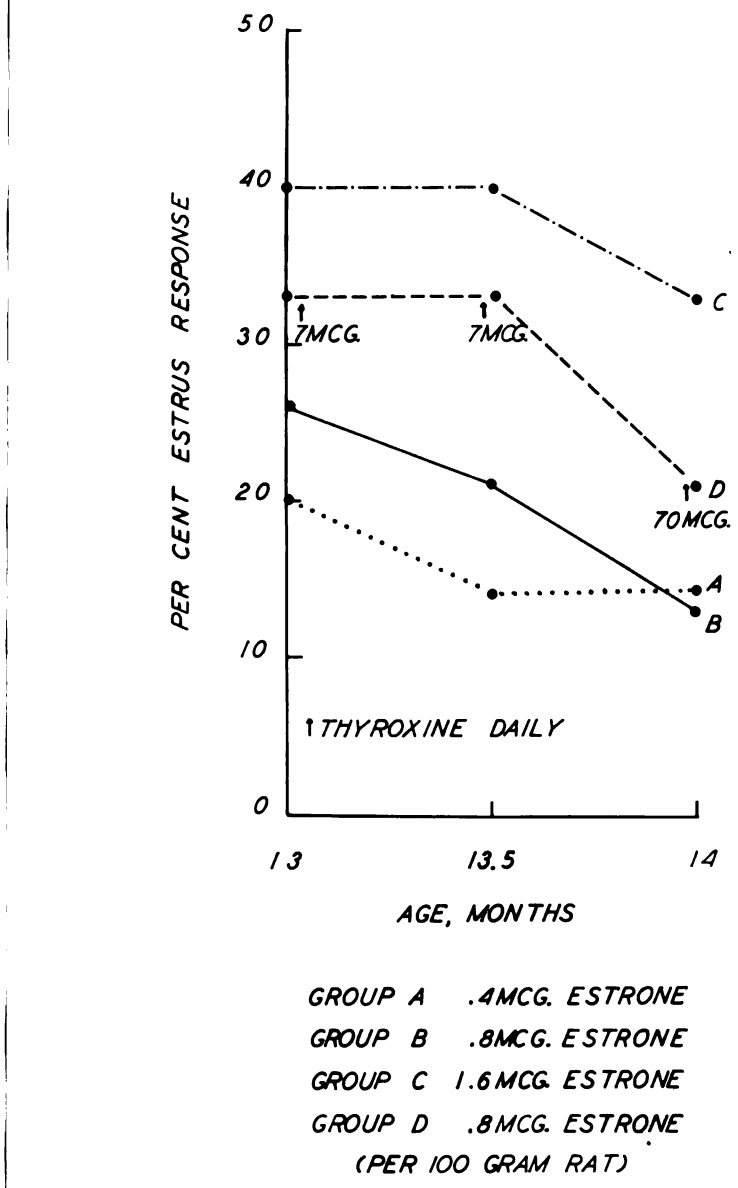

Figure 1

The Effect of Estrone and Estrone Plus d,l-thyroxine Administration, in Relationship to Age, on Per Cent Response of Ovariectomized Rats.

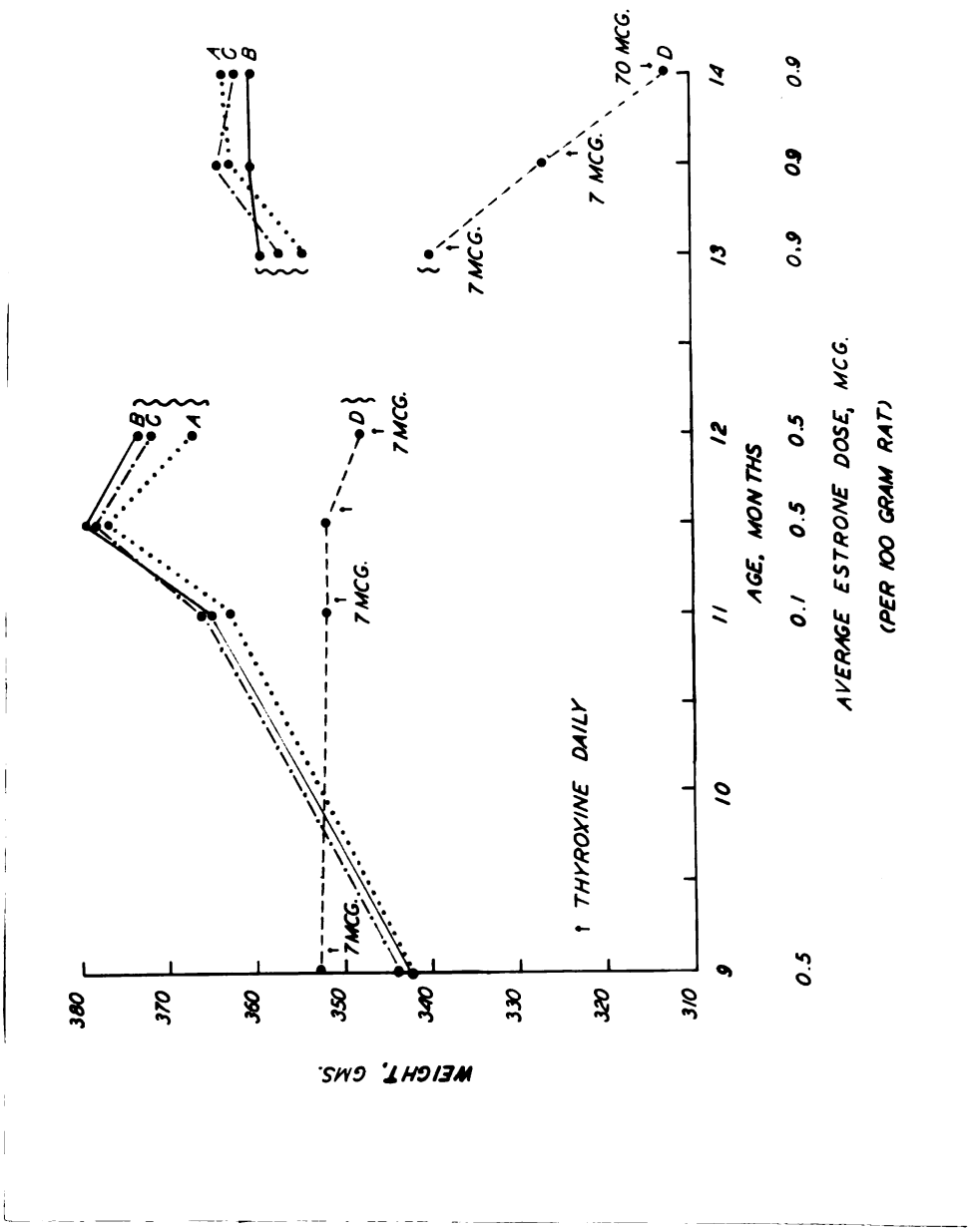

Figure 2

The Effect of Estrone and Estrone Plus d,l-thyroxine Administration and Age on Weight in Ovariectomized Rats.

DISCUSSION

It is evident from the results of these experiments that much of the apparently contradictory evidence in the literature, with regard to the influence of thyroid function on reproduction, can be explained if the critical nature of the thyroid dosage level in determining the physiologic effect, is kept in mind.

It was not realized that the dose of thyroxine must be near the animals own secretion rate, until Dempsey and Astwood (1943) reported on the determination of the rate of thyroid hormone secretion at various environmental temperatures. This paper was followed by a report made by Reineke, Mixner and Turner (1945) on the effect of graded doses of thyroxine on metabolism and thyroid weight of rats treated with thiouracil. It is evident that the importance of a tolerable dosage of thyroxine was not taken into account, by the excessive amounts of thyroxine which were administered by the early investigators. As a matter of fact, it is impossible to determine just what relation these dosages of thyroid substance actually bear in relation to the animal's own thyroid secretion rate, or tolerance level, because their thyroidal potency was not reported.

The results of the present investigation show that there is a difference in the response to estrone of old and young ovariectomized rats, put on a tolerable dosage of thyroxine for ten days. The mechanism of thyroid action in this relation is not thoroughly established. Evidence of a relationship between the anterior pituitary, the thyroid

and the gonads was reported by Evans and Simpson (1930). They observed 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. Reineke, Bergman and Turner (1941) reported that thyroidectomized animals show a decrease in the gonadotrophic hormone of the hypophysis.

There is also evidence of a direct effect of thyroxine upon the gonads. Richter (1933) found a direct effect upon the length of the estrus cycle; while DaCosta and Carlson (1933) reported an acceleration in sexual maturation. Reineke (1946), Martinez (1947) and Wilwerth (1948) reported on a direct relationship between thyroxine and an improvement in spermatogenesis. This paper presents results which indicate that the sensitivity to estrone of the rat, a year or more old, decreases with age. This is presumably correlated with a decline in thyroid function. Rats nine months to a year, show a slight increase in estrus response when given a tolerable dosage of thyroxine for ten days, but never reach the per cent of response obtained in young rats (three to six months of age); so evidently, thyroid is only one factor in the changes of senescence.

The estrus response of both young and old rats treated with thyroxine at a level equivalent to twice the normal secretion rate remained the same, while the weight of rats given an excessive dose decreased. Although the mechanism by which this is accomplished is not clearly understood, it has been observed that during the senescent period, that is, somewhere between eleven and fourteen months, the

sensitivity of the rat to estrogen decreases, and the sensitivity to thyroxine increases.

The results mentioned in this paper seem to indicate that if the anterior pituitary, which nature devised to keep in perfect check the entire endocrine system, is not over influenced by one or more of the glandular secretions, the glands continue to function in a favorable, or normal manner.

SUMMARY AND CONCLUSIONS

1. Young rats given tolerable doses of thyroxine (7 mcg. per 100 gm. rat), over a period of ten days, show a decrease in response to estrone.

2. Normally, with increasing age, thyroid secretion declines. The estrus response is increased in old rats (age thirteen to fourteen months) when given a tolerable thyroxine dosage (7 mcg. per 100 gm. rat), for a period of ten days.

3. The estrus response and body weight are both decreased in old rats, when excessive doses of thyroxine (70 mcg. per 100 gm. rat) are administered daily for a ten day period.

4. It is observed that somewhere between eleven and one-half and fourteen months, the sensitivity to estrogen decreases, and the sensitivity to thyroxine increases.

APPENDIX

APPENDIX

Table 1. The vaginal smear ratings of rats in experiment 1 taken 48 hours after injection with estrone. These rats were not "primed" prior to the trial. The body weights were taken in grams immediately before the estrone injection.

GROUP	A		C		B		D *	
Estrone Dose mcg.	0.2		0.8		0.4		0.4	
Rat No.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.
1	D	410	D	400	D	410	E	400
2	D	390	D	380	D	390	P	380
3	D	370	D	360	D	360	P	360
4	D	350	E	350	D	350	D	350
5	D	340	D	340	D	340	D	340
6	D	320	D	330	D	340	D	340
7	D	320	D	310	D	320	D	420
8	D	300	D	300	D	300	M	300
9	D	280	D	320	D	280	D	290
Av. Body Wt. and Per Cent Estrus	0	344.4	11	343.3	0	343.3	33	353.3

* Injected subcutaneously with 7 mcg. per 100 gm. rat, d,l- thyroxine, for 10 days prior to the estrone injection.

Rats born February 1947; spayed May 1947.

Experiment date: 11-18-47.

APPENDIX

Table 2. The vaginal smear ratings of rats in experiment 2 taken 48 hours after injection with estrone. These rats were not "primed" prior to the trial. The body weights were taken in grams immediately before the estrone injection.

GROUP	A		C		B		D *	
Estrone Dose mcg.	0.05		0.2		0.1		0.1	
Rat No.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.
1	D	415	D	420	D	435	D	390
2	D	410	D	400	D	400	D	360
3	D	400	D	385	D	380	D	360
4	D	355	D	370	D	375	D	350
5	D	360	D	350	D	365	D	345
6	D	370	D	345	D	360	D	340
7	D	365	D	330	D	330	D	420
8	D	330	D	330	D	315	D	310
9	D	290	D	340	D	325	D	300
Per Cent Estrus and Av. Body Wt.	0	366.1	0	363.3	0	365.0	0	352.8

* Injected subcutaneously with 7 mcg. per 100 gm. rat, d,l-thyroxine, for ten days prior to the estrone injection.

Rats born February 1947; spayed May 1947.

Experiment date: 1-18-48

APPENDIX

Table 3. The vaginal smear ratings of rats in experiment 3 taken 48 hours after injection with estrone. These rats were "primed" with .6 mcg. of estrone per rat five days prior to the trial. The body weights were taken in grams immediately before the final estrone injection.

GROUP	A		C		B		D *	
Estrone Dose mcg.	0.2		0.8		0.4		0.4	
Rat No.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.
1	D	420	P	430	D	445	M	380
2	E	420	P	415	D	410	P	340
3	M	420	M	400	D	400	M	365
4	M	360	M	380	D	390	P	355
5	D	380	M	360	D	380	M	350
6	D	380	M	360	D	380	M	340
7	D	380	M	345	D	335	P	420
8	M	340	E	350	D	325	M	310
9	D	310	M	355	D	350	M	310
Per Cent Extrus and Av. Body Wt.	11	378.8	33	377.2	0	379.4	33	352.2

* Injected subcutaneously with 7 mcg. per 100 gm. rat, d,l-thyroxine, for ten days prior to the final estrone injection.

Rats born February 1947; spayed May 1947.

Experiment date: 1-30-48.

APPENDIX

Table 4. The vaginal smear ratings of rats in experiment 4 taken 48 hours after injection with estrone. These rats were "primed" with .6 mcg. of estrone per rat five days prior to the trial. The body weights were taken in grams immediately before the final estrone injection.

GROUP	A		C		B		D *	
Estrone Dose mcg.	0.2		0.8		0.4		0.4	
Rat No.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.
1	D	410	M	420	D	430	D	380
2	D	410	M	400	M	410	M	350
3	E	400	D	390	P	390	D	365
4	M	360	M	360	D	375	D	345
5	D	370	M	350	M	370	D	350
6	D	380	D	360	D	380	E	320
7	D	360	D	350	D	340	M	410
8	D	320	E	330	D	325	M	320
9	D	300	M	360	D	350	D	300
Per Cent Estrus and Av. Body Wt.	11	367.7	11	368.8	11	374.5	11	348.8

* Injected subcutaneously with 7 mcg. per 100 gm. rat, d,l-thyroxine, for ten days prior to the final estrone injection.

Rats born February 1947; spayed May 1947.

Experiment date: 2-16-48.

APPENDIX

Table 5. The vaginal smear ratings of rats in experiment 5 taken 48 hours after injection with estrone. These rats were "primed" with 1.6 mcg. per 100 gm. rat five days prior to the trial. The body weights were taken in grams immediately before the final estrone injection.

GROUP	A		C		B		D *	
Estrone Dose mcg.	0.4		1.6		0.8		0.8	
Rat No.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.
1	D	420	E	440	E	445	E	385
2	dead	dead	D	420	D	430	P	360
3	E	390	D	370	D	420	dead	dead
4	M	370	D	380	D	360	D	345
5	M	365	P	360	P	365	D	360
6	D	380	D	360	D	380	E	320
7	E	375	D	340	D	340	M	420
8	E	325	E	320	P	320	D	340
9	D	300	D	360	D	360	E	300
10	D	390	M	370	D	365	D	360
11	D	340	D	320	P	360	D	350
12	D	340	P	320	M	340	P	340
13	D	320	E	340	D	320	M	320
14	D	320	P	325	D	300	M	285
15	D	325	D	340	D	290	D	280
Per Cent Estrus and Av. Body Wt.	20	354.3	40	357.7	26	359.7	33	340.4

* Injected subcutaneously with 7 mcg. per 100 gm. rat, d,l-thyroxine, for ten days prior to the final estrone injection.

Rats Nos. 1 - 9 born February 1947; spayed May 1947.
Rats Nos. 10 - 15 born February 1947; spayed February 1948.

Experiment date: 3-12-48.

APPENDIX

Table 6. The vaginal smear ratings of rats in experiment 6 taken 48 hours after injection with estrone. These rats were "primed" with 1.6 mcg. per 100 gm. rat five days prior to the trial. The body weights were taken in grams immediately before the final estrone injection.

GROUP	A		C		B		D *	
Estrone Dose mcg.	0.4		1.6		0.8		0.8	
Rat No.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.
1	D	440	E	440	P	440	D	360
2	dead	dead	P	430	D	440	M	340
3	E	390	D	360	nothing on smear	425	dead	dead
4	D	380	D	380	D	340	M	340
5	M	380	P	370	D	370	P	350
6	M	390	D	370	D	400	D	300
7	M	380	D	350	M	340	P	410
8	E	330	P	320	D	330	E	320
9	D	310	M	360	P	360	D	280
10	M	400	M	370	D	350	D	350
11	M	350	D	340	D	340	D	350
12	M	350	P	340	M	340	M	310
13	D	330	M	360	M	330	P	330
14	D	320	D	330	P	310	E	280
15	M	320	P	340	D	290	D	270
Per Cent Estrus and Av. Body Wt.	14	362.1	40	364.0	21	360.0	33	327.9

* Injected subcutaneously with 7 mcg. per 100 gm. rat, d,l-thyroxine, for ten days prior to the final estrone injection.

Rats Nos. 1 - 9 born February 1947; spayed May 1947.
Rats Nos. 10 - 15 born February 1947; spayed February 1948.

Experiment date: 4-3-48.

APPENDIX

Table 7. The vaginal smear ratings of rats in experiment 7 taken 48 hours after injection with estrone. These rats were "primed" with 1.6 mcg. per 100 gm. rat five days prior to the trial. The body weights were taken in grams immediately before the final estrone injection.

GROUP	A		C		B		D *	
Estrone Dose mcg.	0.4		1.6		0.8		0.8	
Rat No.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.	Vag. Smear	Body Wt.
1	D	440	D	440	E	440	M	350
2	dead	dead	E	440	D	450	E	320
3	D	395	D	370	D	425	dead	dead
4	P	380	D	380	D	340	D	340
5	D	380	E	370	D	360	E	320
6	D	385	D	360	D	390	D	260
7	D	385	E	340	D	350	P	400
8	E	330	P	310	D	340	M	310
9	D	310	E	360	P	370	D	270
10	D	400	D	370	D	360	D	340
11	D	350	D	320	D	345	D	330
12	D	360	D	330	D	350	D	300
13	D	330	D	360	D	320	D	320
14	D	320	D	340	D	310	D	270
15	D	320	D	340	D	280	D	260
Per Cent Estrus and Av. Body Wt.	15	363.2	33	362.0	13	360.3	21	313.6

* Injected subcutaneously with 70 mcg. per 100 gm. rat, d,l-thyroxine, for ten days prior to the final estrone injection.

Rats Nos. 1 - 9 born February 1947; spayed May 1947.
Rats Nos. 10 - 15 born February 1947; spayed February 1948.

Experiment date: 4-27-48.

APPENDIX

Table 8. The vaginal smear ratings of rats in experiment 8 taken 48 hours after injection with estrone. These rats were "primed" with estrone, .4 mcg. per 100 gm. rat five days prior to the trial.

Group	Control	Thyroid Treated *
Estrone Dose mcg.	0.4	0.4
Rat No.	Vaginal Smear	Vaginal Smear
1	P	D
2	D	P
3	P	D
4	P	P
5	P	D
6	P	D
7	D	D
8	P	P
9	P	D
10	P	D
11	D	D
12	D	P
13	P	P
14	D	D
15	D	D
16	D	D
17	D	D
18	P	P
19	D	D
20	D	P
21	D	D
Per Cent Estrus	47.1	33.3

* Injected subcutaneously with 7 mcg. per 100 gm. rat, d,l-thyroxine, for ten days prior to the final estrone injection.

Age of rats - 3 months; spayed at 2 months of age.

Experiment date: 6-14-48.

APPENDIX

Table 9. Procedure for making up solutions of estrone and d,l-thyroxine used in injecting the rats.

1. Solution of Estrone* in Corn Oil

Step No.	Dilution
1	Stock Solution (original made up 11-14-47) 1000 mcg. per 2 cc. 500 mcg. per 1 cc.
2	Stock Solution 500 mcg. per 1 cc. original stock solution 1 cc. of No. 1 and 9 cc. of corn oil 50 mcg. per 1 cc.
3	0.1 mcg. per .05 cc., take 1 cc. of No. 2 and 24 cc. of corn oil
4	0.2 mcg. per .05 cc., take 1 cc. of No. 2 and 11.5 cc. of corn oil
5	0.4 mcg. per .05 cc., take 1 cc. of No. 2 and 5.25 cc. of corn oil
6	0.05 mcg. per.05 cc., take 1½ cc. of No. 5 and 10½ cc. of corn oil
7	1.6 mcg. per .05 cc., take 12.8 cc. of No. 2 and 7.2 cc. of corn oil

* Estrone obtained from Upjohn Company, Kalamazoo, Michigan

2. Solution of d,l-thyroxine

Step No.	
1	Weigh out X mcg. d,l-thyroxine
2	Wash off coverglass with small amount of 1 Normal NaOH (1N sol. 1 gm. per liter)
3	Shake until thyroxine dissolves and add several drops HCL (to neutralize) until slightly cloudy

4	Add about one drop NaOH to redissolve
5	100 ml. flask

Strength of solutions used in foregoing experiments:

7 mcg. per 100 gm. rat	10 times as much d,l-thy-
.01 cc. per 10 gm.	roxine to make 70 mcg. per
1 cc. per 100 gm. rat	100 gm. rat

APPENDIX

Table 10. Standards used for classification of vaginal smears in the rat, as reported by Asdell (1947).

Stage I

Small, round nucleated cells only.
Duration about 12 hours.

PROESTRUM (P)

Stage II

Cornified cells only. Mating mostly in this stage.

ESTRUS (E)

Stage III

Late cornified stage, abundant cheesy smear.
Mating no longer allowed, as a rule.
Stages II and III last about 27 hours.

Stage IV

Cornified cells and leucocytes.
Duration about 6 hours.

METESTRUM (M)

Stage V

Epithelial cells and leucocytes.
Duration about 57 hours.

DIESTRUM (D)

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