THE EFFECTS OF ESTROGEN ON MAMMARY STRUCTURE OF ADRENALECTOMIZED AND THIOURACIL TREATED CASTRATE RATS

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Raymond Frank Johnston

1948

This is to certify that the

thesis entitled

"The Effects of Estrogen on Mammary Structure of Adrenalectomized and Thiouracil Treated Castrate Rats"

presented by

Raymond F. Johnston

has been accepted towards fulfillment of the requirements for

M.S. degree in Anatomy

H. C. Smithcors
Major professor

Date May 21, 1948

M-795

THE EFFECTS OF ESTROGEN ON MAMMARY STRUCTURE OF ADRENALECTOMIZED AND THIOURACIL TREATED CASTRATE RATS

By

RAYMOND FRANK JOHNSTON

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 Anatomy

1948

ACKNOWLEDGEMENTS

The writer wishes to express his appreciation to Dr. J. F. Smithcors, Department of Anatomy, and Dr. E. P. Reineke, Department of Physiology and Pharmacology, for guidance during preliminary studies, and advice and criticism in preparation of the manuscript. Special thanks are due Dr. B. V. Alfredson, Department of Physiology and Pharmacology, for supplying and housing the experimental animals, and to Mr. John Monroe for his help in caring for them. The diethylstilbestrol was supplied through the courtesy of Dr. D. F. Green, Merck and Company, Rahway, New Jersey; the thiouracil was obtained from Lederle Laboratories, Pearl River, New York.

INTRODUCTION

The present work was suggested by certain studies made by Leonard and two of his students, Smithcors and Reeder, on the relation of the thyroid and adrenal glands to mammary structure. This study was planned under the direction of Dr. J. F. Smithcors, with a view of studying the combined effects of these two glands on mammary structure. The advent of the thyroid inhibiting drug, thiouracil, made it unnecessary to subject the animals to two major operations. Since in both of the studies mentioned above estrogenic substances were used as growth stimulating factors, it was decided to follow a similar technique in the present study.

Dedicated to My Family

ANNA BELLE and

SHARON ANN

TABLE OF CONTENTS

REVIEW OF LITERATURE

Normal growth of the mammary gland

Turner and Schultze (1931) and Turner (1939) have described the normal structure of the mammary gland of the rat. Normally there are six pairs of mammary glands in the rat; three pairs of thoracic, one pair of abdominal, and two pairs of inguinal glands. The abdominal glands are especially convenient for study. The mammary gland is a compound tubular-alveolar gland, composed of a number of primary, secondary and tertiary ducts, the latter with several terminal branches. Except for terminal enlargement of ducts and a few lateral duct buds, only a bare duct system is present in the immature rat. The duct system of the male is much less extensive than that of the female at this age. With recurring estrous cycles in the female, the lateral and terminal duct buds increase in complexity, resulting in the development of a lobule-alveolar system. The extension of the duct system and its increase in complexity have been shown to be due largely to the action of the female sex hormones in conjunction with the mammogenic hormone of the pituitary gland (Trentin and Turner, 1948). During pregnancy clusters of alveoli form and coalesce to form lobules. This has been shown to be due largely to the action of the hormone of the corpus luteum, progesterone, in conjunction with the pituitary mammogen

(Mixner and Turner, 1942). In the mature male rat lesser extension of the duct system, but marked lobule formation is found. This is evidently due to action of the male hormone (Turner, 1939).

The effects of castration and estrogen on mammary structure

Castration of young rats of either sex results in arrested development of the gland structure of the immature rat resulting in a moderately extensive duct system; for this reason young castrate rats of either sex may be used in experimental studies. In the rat, according to Weichert, Boyd and Cohen (1934), the development of the mammary gland cannot progress beyond the duct stage unless the ovaries are intact or properly proportioned doses of ovarian hormones are administered. Small amounts of estrogenic substances, administered to castrate immature male and female rats result in extension of the duct system, while large amounts cause limited lobule-alveolar development. (Turner and Schultze, 1931; Turner, Frank, Gardner, Schultze and Gomez, 1932; Weichert, Boyd and Cohen, 1934; Halpern and D'Amour, 1934; Nelson, 1935; Astwood, Geschickter and Rausch, 1937; Turner, 1939). The synthetic estrogen, diethylstilbestrol, has been shown to affect mammary gland structure in much the same manner as natural estrogenic compounds (Lewis and Turner, 1940). Large doses of estrogen interfere with normal development and result in stunted abnormal mammary glands (Gardner, Smith and Strong, 1935; Astwood, et. al., 1937).

The effects of thyroidectomy and thiouracil on mammary structure

The general effects of hypothyroidism whether induced by thyroidectomy or administration of thyroid inhibiting substances, thiouracil or thiourea, are lowered metabolism, decreased food intake and retarded growth; the latter substances have been shown to block the synthesis of thyroxine by the thyroid gland. (Astwood, Sullivan, Bissell, and Tyslowitz, 1943; MacKenzie and MacKenzie, 1943; Dempsey and Astwood, 1943; Hughes, 1944; Williams, Weinglass, Bissell and Peters, 1944; Leblond and Hoff, 1944). That growth of the mammary gland can take place in the hypothyroid state was reported by Nelson and Hickman (1937). They reported marked mammary development following estrogen treatment in either thyroidectomized or thyroidectomized castrate rats, but no comparison was made with adequate controls. In a more extensive study by Leonard and Reece (1941), thyroidectomy resulted in stimulation of the alveolar system and thickening of the ducts in normal castrate and estrogen treated castrate female rats.

Smithcors and Leonard (1942), reported that thyroidectomy in normal immature male rats resulted in inhibition of duct growth and stimulation of alveolar development. The same changes, but to a

lesser degree, were found in castrate males. Injection of estrogenic substances in thyroidectomized castrate males resulted in mammary glands with a marked lobule-alveolar development and inhibition of duct growth. The general effects of thyroidectomy on mammary growth were inhibition of duct extension and marked lobule-alveolar development. Smithcors (1945), reported that thiouracil administered to normal or castrate male and female rats failed to induce an alteration of mammary structure similar to that obtained following thyroidectomy. Administration of estrogen resulted in marked lobule-alveolar development similar to that found in estrogen treated thyroidectomized rats.

Apparently rats differ from mice inasmuch as Mixner and Turner (1942), reported that thyroidectomy decreased the ability of mice to respond to injection of estrogen. They also were able to demonstrate that thyroxine in an optimal dose increased significantly the percentage of castrate female mice which responded with mammary lobule-alveolar development to minimal doses of progesterone and estrone. Mixner (1947), in work with mice concluded that thiouracil acts in a manner similar to thyroidectomy in decreasing the responsiveness of the mammary gland to exogenous mammary growth stimulating substances.

The effects of adrenalectomy on mammary structure

One of the main functions of the adrenals is to regulate sodium chloride and water concentration of the blood and tissues; upon removal of the adrenals, water is withdrawn from the tissues. The rat demonstrates classical symptoms of adrenal insufficiency characterized by muscular weakness, loss of body weight, and lowering of body temperature (Gaunt, 1933). The fact, first clearly established by Stewart and Rogoff (1925), that administration of large quantities of salt solution to adrenalectomized animals delayed the appearance of adrenal insufficiency symptoms, has been confirmed by several groups of workers, (Banting and Gairns, 1926; Corey, 1927; Marine and Baumann, 1927; Swingle, Pfiffner, Vars, and Parkins, 1934).

Reeder and Leonard (1944), reported that adrenalectomy in normal or castrate immature male rats resulted in an increased number of lateral buds on the duct system of the mammary tree, and in some cases, increased end bud growth. Administration of estrogen caused dilatation of the lateral buds. On the other hand, Trentin and Turner (1947), reported that adrenalectomy in castrate male rats resulted in thin atrophic ducts and complete elimination of lobule-alveolar

development. Although administration of estrogen caused noticeable duct stimulation, it was relatively ineffective in stimulation of alveolar development. Cowie and Folley (1947), reported that regressive changes were frequently, but not invariably, observed following adrenalectomy; increased arborization of the duct system was never observed.

No reports have been found which deal with the combined effects of adrenalectomy and thyroidectomy or thiouracil administration on mammary growth.

PROCEDURE

Seventy-two albino rats were castrated at three weeks of age and divided into eight groups. They were fed a ration for laboratory animals ad libitum, and maintained at constant temperature (76 F.) and humidity. The experimental techniques included adrenalectomy and treatment with thiouracil and estrogen, alone or in various combinations. The litters were divided so that comparison on the basis of estrogen treatment could be made between litter mates. All of the experimental techniques for different groups were performed when the rats were at the same age.

Castration was performed through a ventral median abdominal incision under ether anesthesia. Adrenalectomy was performed two weeks later under nembutal anesthesia, through bilateral paralumbar incisions. The adrenalectomized rats were given one per cent NaCl in the drinking water. Thiouracil was administered at the rate of 0.1 per cent in the feed for 45 days. Diethylstilbestrol was administered subcutaneously in an oil base at the rate of 10 gamma daily during the last ten days of the experimental period. The rats were destroyed on the eleventh day. The right abdominal mammary gland was removed with the skin, mounted on a board, and fixed for 24 hours in

an aqueous solution of 7.5 per cent formalin and 0.5 per cent acetic acid. The mounts were washed in running tap water and the mammary glands were removed from the skin. They were stained *in toto* with Harris's hematoxylin, and destained in acid alcohol. The connective tissue surrounding the glands was teased off under the dissecting microscope, following which they were dehydrated, cleared in xylol, and mounted in Canada balsam between two 2x3 inch slides. The glands were studied under the dissecting microscope and by projection with the lantern. Body weighings were made at the beginning and end of the experimental periods. Examination was made at autopsy for completeness of adrenalectomy and for presence of accessory cortical tissue.

The effects of estrogen on mammary structure of castrate rats

Each of two litters comprising 16 rats was equally divided between groups (1) and (2). These rats were castrated at three weeks of age, then placed in experimental cages and maintained on regular rations for 65 days. During the last ten days group (2) received 10 gamma of diethylstilbestrol subcutaneously in an oil base daily. On the day following the experimental period, both groups were killed and the right abdominal mammary gland was removed. At autopsy the controls showed an average gain of 117 grams and the estrogen injected 109 grams during the last 45 days of the experiment.

All of the mammary glands of the control group showed an uniformly bare duct system with no indication of any lobule-alveolar development. This is the typical picture that is found in the mammary glands of castrated rats. Examination of the mammary glands of the rats injected with estrogen showed extension of the duct system and distinct end bud growth. In some cases definite lobule-alveolar growth was present. These glands showed a typical response to estrogen administered in greater than threshold amounts.

The effects of estrogen on mammary structure of thiouracil treated castrate rats

Each of two litters comprising 18 rats was equally divided between groups (3) and (4). These rats were castrated at three weeks of age. Both groups were placed in experimental cages at seven weeks of age and fed the regular ration to which had been added 0.1 per cent thiouracil. They were maintained on this ration for a 45 day period, the last ten of which group (4) received 10 gamma diethylstilbestrol daily. Both groups were destroyed on the eleventh day and the mammary glands removed. The controls gained an average of 61 grams and the estrogen treated gained an average of 45 grams during the experimental period.

The mammary glands of the control group showed a shortened and thickened duct system. The mammary glands of two rats showed a small amount of lobule-alveolar development. The mammary glands of the estrogen injected rats showed a considerable amount of lobule-alveolar development, especially toward the ends of the ducts. The ducts were shortened and thickened, and the lobule-alveolar growth was much more dense than that of the estrogen treated rats which did not receive thiouracil, (group 2). In comparison with the castrate rats which did not receive thiouracil, (group 1), the mammary glands of group (3) showed shorter and thicker ducts.

The effects of estrogen on mammary structure of adrenalectomized castrate rats

Each of two litters comprising 20 rats was equally divided between groups (5) and (6). These rats were castrated at three weeks of age. All were adrenalectomized at five weeks of age, and placed on one per cent NaCl in the drinking water. A period of ten days was allowed for recovery from the effects of the operation, at which time they were placed in the experimental cages and fed regular rat ration for a period of 45 days. During the last ten days group (6) received 10 gamma of diethylstilbestrol daily. All were destroyed on the eleventh day and the mammary glands removed. The control group gained an average of 86 grams while the estrogen treated group gained an average of 79 grams during the experimental period.

The mammary glands from the control group had long atrophic ducts with no lobule-alveolar development. The ducts were longer and thinner than those of any of the groups which did not receive estrogen. The mammary glands of the rats which received estrogen were also very extensive in area and were greatly thickened grossly as a result of extensive lobule-alveolar development. The overall development of these glands was somewhat greater than that of the estrogen treated castrated rats.

The effects of estrogen on mammary structure of thiouracil treated adrenalectomized castrate rats

Each of two litters comprising 18 rats was equally divided between groups (7) and (8). These rats were castrated at three weeks of age. They were adrenalectomized at five weeks of age and placed on one per cent NaCl in the drinking water. After a ten day recovery period they were placed in experimental cages and fed the regular rat ration to which 0.1 per cent thiouracil had been added. They were maintained on this ration for 45 days the last 10 of which group (8) received 10 gamma of diethylstilbestrol daily. The rats were destroyed on the eleventh day and the mammary glands removed. The average weight gain for the controls was 50 grams and that of the estrogen treated was 37 grams for the experimental period.

The mammary glands of the control group showed very short atrophic ducts. The ducts of these glands were shorter than those of any other group and were thinner than those of either the castrate or the thiouracil treated castrate group. The mammary glands of the estrogen treated group showed a short, thick duct system with considerable lobule-alveolar development. The peripheral alveoli were especially dilated. The overall development of these glands was greater than that of any other group.

Table I on the following page summarizes these results.

TABLE I. The Effects of Estrogen on Mammary Structure of Adrenalectomized and Thiouracil treated Castrate Rats.

Group No.	Treatment other than castration	Wt. gain last 45 da. in gms.	Results
1.	None	117	Bare duct system.
2.	Estrogen *	109	Uniformly developed duct system, slight lobule-alveolar development.
3.	Thiouracil **	61	Short, thick duct system.
4.	Thiouracil Estrogen	45	Short, thick ducts, considerable lobule-alveolar development.
5.	Adrenalectomy ***	86	Long atrophic ducts.
6.	Adrenalectomy Estrogen	79	Extensive duct system, lobule-alveolar development greater than group No. 4.
7.	Adrenalectomy Thiouracil	50	Short atrophic ducts.
8.	Adrenalectomy Thiouracil Estrogen	37	Short, thick ducts, lobule-alveolar greater than group No. 6.

* 10 gamma diethylstilbestrol daily last ten days of experimental period.
** 0.1 per cent thiouracil in feed last 45 days of experimental period.
***Adrenalectomized at five weeks of age.

DISCUSSION

The purpose of the present work was to determine the effects of estrogen on the mammary structure of thiouracil treated, adrenalectomized castrate rats. In order to evaluate the factors responsible for the mammary growth obtained in these rats, it was necessary to set up a series of experiments which would demonstrate the effects of various combinations of treatments. In addition, these control groups served to confirm certain results of earlier workers in similar experiments. The mammary structure of castrate and estrogen treated castrate rats is sufficiently well known to need no further confirmation (Turner and Schultze, 1931; Turner, 1939).

It is also well known that the mammogenic hormones of the pituitary gland are a factor in the response of the mammary gland to estrogen injection. In the present work however, the pituitary gland can be considered merely as one of the many factors operating in the body which affect the mammary gland; i.e., the specific action of the mammogenic hormones on mammary growth need not be considered in these experiments. The response of the mammary gland of this strain of rats to castration and subsequent injection of estrogen was typical, as previously described in this paper. Castration reduced the mammary

gland to a bare duct system. In addition to duct extension, the amount of estrogen injected also caused some lobule-alveolar development.

The results of thiouracil treatment are essentially in agreement with the work of Smithcors (1945) and Smithcors and Leonard (1942). In general the administration of thiouracil inhibited extension of the duct system. Subsequent injection of estrogen resulted in marked lobule-alveolar development without appreciable further duct extension. However, it has been shown that in the mouse, (Mixner and Turner, 1942; Mixner, 1947), thyroidectomy or thiouracil administration decreases the mammary growth response to estrogen and that thyroxine in optimal doses increases the ability to respond with lobule-alveolar development. This difference may be attributed to the fact that the mouse is normally hypothyroid, whereas the rat is normally hyperthyroid. It has been shown that rats respond to minimal doses of thyroxine by retardation of growth, while mice on the same dosage increase their growth rate (Meyer and Wertz, 1938; Koger and Turner, 1943). Minimal doses of thiourea increase the growth rate of the rat (Astwood and Bissell, 1944).

In Smithcors' work it was suggested that failure of thiouracil to induce the changes following thyroidectomy might be due to the relatively short period

of treatment (18-35 days). In the present work the mammary glands of the thiouracil treated rats more closely resembled those following thyroidectomy. This may be due to longer periods of treatment (45 days) in the present work. The shortened and thickened ducts found following thiouracil treatment may result from the modification of the specific action of the pituitary mammogenic or other general growth stimulators in the hypothyroid state.

The results of estrogen administration in the thiouracil treated rats in the present work confirm the results obtained by Smithcors (1945), and are in agreement with the results of Smithcors and Leonard (1942) and Leonard and Reece (1941).

The increased lobule-alveolar development obtained following estrogen administration to thiouracil treated rats might be a result of any one or a combination of the following factors. Since the size of the mammary gland is reduced in rats treated with thiouracil, it might be argued that we are dealing with the effects of a given amount of hormone on a smaller gland. That this is not the case was suggested by Smithcors and Leonard (1942), who reported that the same amount of estrogen administered to a smaller rat with the same size mammary gland as in the case of the hypothyroid rats elicited a normal response, i.e., extension of the duct system.

The other possibilities are that the effectiveness of the injected estrogen is augmented by the hypothyroid state or that the sensitivity of the mammary tissue to injected estrogen is increased by the hypothyroid state. An antagonistic relationship is known to exist between thyroxine and estrogen function, (Tyndale and Levin, 1937), and the hyperthyroid state increases the threshold of response to estrogen (Meyer and Wertz, 1938). Injection of estrogen will lower basal metabolic rate of rats as much as 50 per cent (Sherwood and Bowers, 1936).

It is possible that estrogen in the normal animal may have the potential power to produce both duct and alveolar growth, but the power to produce the latter may be held in check by the functioning of the thyroid. Since the amount of estrogen used in the present work was shown to be capable of stimulating a limited amount of lobule-alveolar growth in the castrate rat, it is possible that the altered metabolic state brought about by thiouracil administration accentuated this response of the mammary gland to injected estrogen. While the present experiments were not designed to discriminate between these two possibilities, it appears likely that both of these factors are operative. If one of the effects of thyroxine in the normal animal is to hold in check the inherent ability of estrogen to produce lobule-alveolar growth, its absence in the

hypothyroid state would favor lobule-alveolar development in a mammary gland known to be capable of making this response to injected estrogen.

The results of adrenalectomy on the mammary growth of castrate rats differs somewhat from those obtained by several other workers. The thin atrophic ducts found in the present work resemble those reported by Trentin and Turner (1947), except that in the present work the ducts are very much longer. Reeder and Leonard (1944), reported an increase in the number of lateral buds and found some increase in end bud growth in their adrenalectomized castrate rats. That end bud growth occured in the present work is obvious from the length of the ducts. The fact that end buds were not abundant at autopsy indicates that the rate of duct extension had decreased some time prior to autopsy.

The difference in appearance of the mammary glands in the present work might be explained by the longer experimental period. Trentin and Turner (1947), used adult male rats which had been castrated for a much longer period of time (41-103 days), and were subjected to adrenalectomy only ten days prior to autopsy. It is apparent that only a small mammary gland was present in these rats at the time of adrenalectomy, and in older rats especially, one would not expect much duct extension in the ten day period unless growth stimulating substances were used. Reeder and Leonard (1944)

used young male rats which were autopsied only 10-12 days following both castration and adrenalectomy.

The mammary gland of the young animal has been shown by Reece and Leonard (1941), and Smithcors and Leonard (1943), to be much more responsive to minimal growth stimuli than that of older animals. In the absence of specific growth stimuli, the lateral buds observed by Reeder and Leonard (1944) in their castrate adrenalectomized rats might have been due to the inherent growth potential of the young animal. Since only slight end bud growth was found in these rats it is apparent that the short experimental period did not permit fullest extension of the duct system.

In the present work with adrenalectomized castrate rats injected with estrogen the mammary glands showed an extensive duct system with very marked lobule-alveolar development. This is not exactly in conformity with previous workers. Reeder and Leonard (1944) found that with injection of estrogen in rats treated as mentioned above, they were able to demonstrate dilatation of stimulated lateral buds and an increase in their number. Trentin and Turner (1947) reported that injection of estrogen caused noticeable duct stimulation, but was relatively ineffective in stimulating mammary alveolar development in adrenalectomized castrate rats. It is the opinion of the writer that the final results may have been different

had the experimental period been longer in duration.

The results of estrogen administration to adrenalectomized castrate rats in the present work confirms substantially those of Reeder and Leonard (1944). In both cases an extensive gland with marked lobule-alveolar development which exceeded that of the estrogen injected castrate rats was found. The relative ineffectiveness of estrogen in stimulating mammary growth in adrenalectomized castrate rats reported by Trentin and Turner (1947) may again be due to differences in experimental procedure.

It was mentioned above that the effect of the thyroid in the normal animal may be to favor duct extension and inhibit lobule-alveolar development. Conversely, the effect of the adrenals in the normal animal may be to restrict duct extension and favor lobule-alveolar development since adrenalectomy causes an exaggeration of the condition found in the young animal with thyroids intact.

The mammary structure of adrenalectomized thiouracil treated castrate rats has not previously been described. Certain of the effects of both procedures were observed in these rats in the present work. The duct system of the mammary gland was short as in the case of the thiouracil treated castrate rat and thin as in the case of the adrenalectomized castrate rat. Apparently thiouracil inhibited the extension of

the duct system observed after adrenalectomy, and conversely, adrenalectomy inhibited the thickening of the duct system caused by thiouracil treatment.

When estrogen was administered to thiouracil treated adrenalectomized castrate rats the degree of duct extension was similar to that of the thiouracil treated castrate rats which received estrogen, but the lobule-alveolar development was greater than in the latter group. The greater degree of lobule-alveolar development of this group is compatible with the findings that estrogen administered to thiouracil treated and to adrenalectomized castrate rats stimulated lobule-alveolar development. While these glands resembled those of the thiouracil treated castrate rats which received estrogen, the additional effect of adrenalectomy could be distinguished by the greater distention of the peripheral alveoli.

Since the effects of thiouracil treatment and of adrenalectomy on mammary growth of castrate rats are diametrically opposed, it is obvious that when these techniques are combined the resulting mammary growth represents a compromise between the several forces acting upon the mammary gland. It is well known that any factor which inhibits growth in general has its greatest effect on cells which are most actively growing at the time this inhibition is applied. The most active cells in the mammary glands of the young

animal are those at the ends of ducts, thus thiouracil which inhibits growth in general would most likely affect end bud growth. The residual growth potential of the mammary gland would then be seen in a thickening of the ducts which were already present. The administration of a potent growth stimulating substance such as estrogen, would also affect structures already present instead of causing new growth. This explanation would seem to account for the thickening of the shortened ducts found upon thiouracil administration, and the lobule-alveolar development of this less extensive gland upon estrogen administration.

The fact that duct extension is the characteristic response of the mammary gland after adrenalectomy, indicates that some inhibiting factor upon growth of the mammary gland has been removed; thus the more rapidly growing part of the gland, i.e. the duct end buds, are stimulated more than the duct system which has already passed its peak of growth. Since in the present experiments the ducts had apparently reached their definitive size at the time estrogen was administered, it is logical that the greatest effect of estrogen would be to stimulate lobule-alveolar development.

In the adrenalectomized castrate rats which received thiouracil, it is apparent in both body growth and in growth of the duct system of the mammary gland

that the thiouracil was highly effective in producing a hypothyroid state. In the absence of duct extension it is obvious that estrogen administration to these animals was most effective in producing lobule-alveolar development. A partial effect of adrenalectomy in counteracting the inhibition of end bud growth was seen in the greater peripheral distention of the alveoli.

SUMMARY

Albino rats were castrated at three weeks of age and divided into eight groups. The experimental techniques included adrenalectomy and treatment with thiouracil and estrogen, alone or in various combinations. The mammary glands were removed at the end of the experimental period and mounted for study.

The mammary glands of castrated rats showed an uniformly bare duct system with no indication of lobule-alveolar development. Administration of a total dose of 10 gamma diethylstilbestrol daily for the last ten days of the experimental period resulted in extension of the duct system of the mammary gland and some lobule-alveolar development.

Thiouracil fed at the rate of 0.1 per cent of ration for 45 days resulted in a shortened and thickened mammary duct system. The administration of estrogen during the last ten days of the thiouracil treatment period resulted in a mammary gland showing shortened and thickened ducts and considerable lobule-alveolar development.

The mammary glands of the rats which had been adrenalectomized for 55 days had long atrophic ducts with no lobule-alveolar development. When estrogen was administered for the last ten days of the experimental period, the mammary glands were very

extensive in area and showed lobule-alveolar development in excess of that of the estrogen treated castrate rats.

The mammary glands of the adrenalectomized, thiouracil treated castrate rats showed a very short atrophic duct system. The administration of estrogen resulted in a mammary gland with a short thick duct system and considerably more lobule-alveolar development than any other group.

BIBLIOGRAPHY

ASTWOOD, E. B. and Abele Bissell
1944 Effects of thiouracil on the iodine content of the thyroid gland.
Endocrin., 34, 282.

ASTWOOD, E. B., C. F. Geschickter and E. O. Rausch
1937 Development of the mammary gland of the rat.
Am. J. Anat., 61, 373.

ASTWOOD, E. B., J. Sullivan, A. Bissell, and R. Tyslowitz
1943 Action of certain sulfonamides and thioureas upon the function of thyroid gland of the rat.
Endocrin., 34, 69.

BANTING, F. G. and S. Gairns
1926 Suprarenal insufficiency.
Am. J. Physiol., 77, 100.

COREY, E. L.
1927 The effect of forcing fluids upon survival after bilateral epinephrectomy.
Am. J. Physiol., 79, 633.

COWIE, A. T. and S. J. Folley
1947 The role of the adrenal cortex in mammary development and its relation to the mammogenic action of the anterior pituitary.
Endocrin., 40, 274.

DEMPSEY, E. W. and E. B. Astwood
1943 Determination of the rate of thyroid hormone secretion to various environmental temperatures.
Endocrin., 32, 509

GARDNER, W. U., G. M. Smith and L. C. Strong
1935 Stimulation of abnormal mammary growth by large amounts of estrogenic hormone.
Proc. Soc. Exper. Biol. and Med., 33, 148.

GAUNT, R.
1933 Adrenalectomy in the rat.
Am. J. Physiol., 103, 494.

HALPERN, S. R. and F. E. D'Amour
1934 Effects of estrin upon gonads, mammary glands and hypophysis of the rat.
Proc. Soc. Exper. Biol. and Med., 32, 108.

HUGHES, A. M.
1944 Cretinism in rats induced by thiouracil.
Endocrin., 34, 210.

KOGER, Marvin and C. W. Turner
1943 The effects of mild hyperthyroidism on growing animals of four species.
Mo. Agri. Exper. Sta. Res. Bul. 373, 24.

LEBLOND, C. P., and H. E. Hoff
1944 Effects of sulfonamides and thiourea derivatives on heart rate and organ morphology.
Endocrin., 35, 229.

LEONARD, S. L. and R. P. Reece
1941 The relation of the thyroid to mammary gland growth of the rat.
Endocrin., 28, 65.

LEWIS, A. A. and C. W. Turner
1940 Effect of stilbestrol on the mammary gland.
Proc. Am. Soc. An. Prod., 710, 63.

LEWIS, A. A. and C. W. Turner
1941 Effect of stilbestrol on lactogenic content of pituitary and mammary glands of female rats.
Proc. Soc. Exper. Biol. and Med., 48, 439.

LEWIS, A. A. and C. W. Turner
1942 Mammogen and unilateral mammary growth in the rabbit.
Endocrin., 30, 985.

MACKENZIE, C. G. and J. B. Mackenzie
1943 Effects of sulfonamides and thioureas on the thyroid gland and basal metabolism.
Endocrin., 32, 185.

MARINE, D. and E. J. Baumann
1927 Duration of life after suprarenalectomy in cats and attempts to prolong it by injections of solution containing sodium salts, glucose and glycerol.
Am. J. Physiol., 81, 86.

MARINE, D. and E. J. Baumann
1921 Effect of suprarenal insufficiency.
Am. J. Physiol., 57, 135.

MARINE, D. and E. J. Baumann
1922 Studies on suprarenal insufficiency.
Am. J. Physiol., 59, 353.

MEYER, A. E. and A. Wertz
1938 Metabolism in normal and thyroidectomized rats as influenced by thyroxine and thyroid globulin feeding.
Proc. Soc. Exper. Biol. and Med., 38, 847.

MEYER, A. E. and A. Wertz
1938 Influence of thyroid hormone on estrin action.
Proc. Soc. Exper. Biol. and Med., 38, 843.

MIXNER, John P.
1947 The influence of thiouracil on mammary lobule-alveolar growth in mice.
J. Dairy Sc., 30, 578.

MIXNER, John P. and C. W. Turner
1942 Influence of thyroxine upon mammary lobule-alveolar growth.
Endocrin., 31, 345.

MIXNER, John P. and C. W. Turner
1942 Role of estrogen in the stimulation of mammary lobule-alveolar growth by progesterone and by the mammogenic lobule-alveolar growth factor of the anterior pituitary.
Endocrin., 30, 591.

NELSON, W. O.
1935 Gonad hormone effects in normal, spayed and hypophysectomized rats.
Anat. Rec., Supp., 64. 32.

NELSON, W. O.
1937 Endocrine control of the mammary gland. Physiol. Rev., 16, 488

NELSON, Warren O., and Jane Hickman
1937 Effect of estrone on hypopsis and reproductive organs of thyroidectomized rats. Proc. Soc. Exper. Biol. and Med., 36, 828.

REEDER, Charles F. and S. L. Leonard
1944 Alterations in mammary structure following adrenalectomy in the immature male rat. Proc. Soc. Exper. Biol. and Med., 55, 61.

SHERWOOD, T. C. and L. M. Bowers
1936 The effect of ovarian hormone on the basal metabolism of experimental hyperthyroid rats. Am. J. Physiol., 115, 645.

SMITHCORS, J. F.
1945 Effects of thiouracil on the mammary gland. Proc. Soc. Exper. Biol. and Med., 59, 197.

SMITHCORS, J. F. and S. L. Leonard
1942 Relation of thyroid to mammary gland structure in the rat with special reference to the male. Endocrin., 31, 454.

STEWART, C. N. and J. M. Rogoff
1925 Studies on adrenal insufficiency. Proc. Soc. Exper. Biol. and Med., 22, 394.

SWINGLE, W. W. and J. J. Pfiffner, H. M. Vars and W. H. Parkins
1934 The effect of sodium chloride administration upon adrenalectomized dogs not receiving extract. Am. J. Physiol., 108, 159.

TRENTIN, J. J. and C. W. Turner
1947 Effect of adrenalectomy on the mammary gland of the castrated and estrogen treated castrated rats. Endocrin., 41, 127.

TRENTIN, J. J. and C. W. Turner
1948 Interrelation of the pituitary and the sex hormones in mammary gland growth.
J. An. Sci., 4, 501.

TURNER, C. W.
1939 The mammary glands.
Sex and Internal Secretions, Chap., 9, 740.
Williams and Wilkins, Baltimore.

TURNER, C. W., A. H. Frank, W. U. Gardner, A. B.
1932 Schultze and E. T. Gomez
The effect of theelin and theelol on the growth of the mammary gland.
Anat. Rec., 53, 227.

TURNER, C. W. and A. B. Schultze
1931 A study of the causes of the normal development of the mammary glands of the albino rat.
Mo. Agri. Exper. Sta. Res. Bul., 157, 5.

TYNDALE, H. H. and Louis Levin
1937 Ovarian weight responses to menopause urine injections in normal, hypophysectomized, hypophysectomized thyroxine treated immature rats.
Am. J. Physiol., 120, 486.

WEICHERT, C. K., R. W. Boyd and R. S. Cohen
1934 A study of certain endocrine effects on the mammary glands of female rats.
Anat. Rec., 61, 21.

WILLIAMS, R. H., A. R. Weinglass, G. W. Bissell and
1944 J. B. Peters
Anatomical effects of thiouracil.
Endocrin., 34, 317.

ROOM USE ONLY