A MICROIMPELLETATION TECHNIQUE FOR STUDYING THE LOCALIZED ACTION OF HORMONES AND SOME RESULTS OF ITS USE IN THE MAMMARY GLAND

By

KWOH HSIONG LI

Bachelor of Science

University of Nanking

Nanking, China

Submitted to the faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1956

A MICROIMPELLETATION TECHNIQUE FOR STUDYING THE LOCALIZED ACTION OF HORMONES AND SOME RESULTS OF ITS USE IN THE MAMMARY GLAND

Thesis Approved:

William R. Kirkham
Thesis Adviser

Lauris MacVicar

Lauris MacVicar
Dean of the Graduate School

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. William R. Kirkham, under whose direction this study was conducted, for his guidance and supervision throughout his graduate work. He also wishes to express appreciation to Dr. Allen D. Tillman for supplying the facilities and to Mr. A. A. Deen for the care of the animals in the Department of Animal Husbandry. The author is grateful to Dr. L. Michaud of Merck and Company for supplying cortisone and hydrocortisone; to Dr. I. Bunding of Armour Research Laboratories for supplying ACTH, growth hormone and prolactin; and to Dr. C. D. Kochakian for the use of his pelleting machine.

The author is pleased to acknowledge the friendliness and spirit of cooperation displayed by staff and students of the Department of Agricultural Chemistry. Grateful acknowledgement is extended to the Oklahoma Experiment Station for financial support of this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The development of the mammary gland from a primary duct system to a fully differentiated lobule-alveolar system producing milk in the lactating female involves the sequential action of a number of endocrine factors. Many investigators have attempted to elucidate the nature and the role of specific hormones in mammary gland development. Ovarian, adrenal and hypophyseal factors have been implicated in a direct action. Other endocrine factors; thyroid, parathyroid, testicular and placental have been shown, in some instances, to have subsidiary roles in mammary development and function. In comprehensive reveiws of the literature pertaining to this problem (Turner, 1949a; Folley and Malpress, 1948; Folley, 1952) attempts have been made to integrate the voluminous work in this field into a concise concept which adequately explains all the phenomena observed. Although the roles of some hormones for some species appear to be adequately explained, there are many questions left unanswered.

Turner (1939b) has given extensive consideration to the anatomy and normal development of the mammary gland. Development varies from species to species, so that any concise treatment of this subject meets with exceptions. In the following discussion, unless otherwise indicated, the remarks will be confined to mammary development in the rat.

The development of mammary tissue is conveniently divided into four phases. The peripheral extension of the duct system may be considered the first phase. The endocrine factors promoting this activity have been

difficult to study. Although the pituitary gland has been implicated (Mixner and Turner 1943), specific hormones registering their effect have been ill-defined.

With the onset of pregnancy in the adult animal the mammary gland begins a period of proliferation of the duct system and as gestation proceeds an extensive lobule-alveolar system develops. The changes in endocrine balance accompanying pregnancy have been related to the stimulation of this widespread proliferative activity of the mammary parenchyma. Estrogens and progestins have been shown to have paramount roles in this stage of mammary development.

The third phase of mammary development begins shortly before or at the time of parturition and continues thereafter. At this time proliferative growth of the mammary parenchyma is greatly diminished and the tissue becomes engorged with milk. As the milk is removed by the suckling young the tissue comes into full lactation and milk production is maintained until the young are weaned. Cessation of suckling and the retention of milk in the mammary tissue leads to the involution of this tissue. The lobule-alveolar system and the extensive duct system regress in the period of a few days to a duct system only slightly more elaborate than existed in the animal before the onset of pregnancy.

The foregoing description of events taking place in the animal during the times of pregnancy and lactation points out the extreme variation in histological appearance and functional capacity of the mammary tissue. There can be no doubt that these changes are directly relatable to various hormone levels and balances within the animal. The elucidation of the various endocrine factors which promote such profound changes in this tissue has been the quest of numerous investigators. Their research has taken many forms and employed numerous techniques.

The classical procedures of endocrine research have been employed in most studies in this field. These consisted of extirpation of glands and substitution with various hormones. Many of these studies were carried out with hormones of questionable purity. Administration of hormones in almost all studies has been by such a route as to effect a systemic stimulation of the animal, i.e.; the hormones were injected, implanted or otherwise administered in such a manner that all tissues would come under the influence of the hormones.

This approach has been fruitful and almost all the evidence we have today on endocrine factors affecting mammary tissue is attributable to this experimental method. However, the data obtained from such studies has often been difficult to interpret and occasionally it has been in direct conflict with results from other laboratories, or results reported by the same laboratory at a different time. Furthermore, it has not been possible from the results of such studies, to enumerate clearly the specific route of activity of a given hormone. For example, systemic administration of estrogens induces proliferation of the parenchyma. It is uncertain whether the action of estrogens is directly on the parenchyma, or whether the action is mediated via the pituitary. It has not been possible to ascertain that the hormone administered is actually the hormone affecting the mammary tissue. It is entirely possible and probable in some cases, that the hormones administered is not, as such, an active stimulator of the mammary tissue. The hormone may first undergo some alteration, perhaps in a remote area of the animals system, and the metabolite of the administered hormone is effective. A perusal of the review literature clearly shows that interpretation of the experimental data and observations is difficult in many cases. When one visualizes the variability of this tissue in terms of histological structure and biochemical

function, it is apparent that a comprehensive understanding of the nature and role of the endocrine factors involved is most difficult. It is not surprising that, though much work has been done in this field, there are many phenomena which are left unexplained and for which an explanation would entail a comprehensive detailed understanding of the direct effect of the endocrine factors on the mammary parenchyma.

No one study can hope to furnish information to bring a greater degree of order to this subject, but there is a definite need for study of the effect of hormones on mammary tissue in isolated conditions. It is toward this goal that this study was directed. Here, attempts have been made to develop a technique of administering hormones directly to the mammary parenchyma in such a way as to maintain a high hormone level in the vicinity of certain areas of the mammary system, but to avoid absorption of the hormone into the animal's system in quantities that would affect a reaction with all of the tissue, as is done in the usual methods of hormone administration. It was further hoped that this method of adminstration would demonstrate the direct effects of various steroid and protein hormones on the mammary parenchyma and suggest avenues of research which would lead to a clearer understanding of the complicated endocrine control of development of this biochemically unique tissue.

REVIEW OF LITERATURE

Extensive reviews of the literature concerning the endocrine control of mammary growth and lactation have been given by Turner (1939a), Folley and Malpress (1948), and Folley (1952). Less comprehensive reviews of this literature and more recent information pertinent to this field of study have been given by Elliott and Turner (1953), Peterson (1948), Lyons (1950), Selye (1954), Meites (1954). In view of this excellent material no attempt at a complete review of the literature will be made, but particular attention will be given to certain works and ideas of various authors which have significance to the observations in the studies presented in this thesis.

I. SYSTEMIC TREATMENTS AND RESULTS

In order to maintain some degree of order in the literature cited, specific hormones or groups of hormones will be listed and work pertaining to the effects of these hormones on the mammary gland will be cited.

A. Ovarian Hormones

1. Estrogens. Estrogens have long been implicated in the development of mammary glands. Lacquer (1928) and Turner and Schultz (1931) were first to report that estrogens promoted development of the duct system in rats. Confirmation of this came from Astwood, et.al. (1934) and Weichert, et.al. (1934). Since these early studies, many workers have investigated this role of estrogens. In large doses, estrogens have been reported to induce development of the lobule-alveolar system in the rat (Nelson 1935;

Lewis and Turner 1941; Smith 1955). However, it is generally agreed that the most outstanding effect of systemically administered estrogens has been in duct growth stimulation. The question of the direct or indirect action of these hormones in duct development is one which remains unanswered (Folley and Malpress 1948).

The relation of estrogens to area of duct extension has been studied by Silver (1953), Elliott and Turner (1951) and Smith (1955). This latter author reports no significant increase in the area of duct extension over that appearing in untreated animals. High estrogen dosage stunts the growth of the mammary gland (Curtiss 1949).

The action of estrogens in the development of the mammary gland system varies widely with the species of the animal and the prior or simultaneous treatment which the animal undergoes. In mice the result of estrogen treatment above is primarily duct growth, whereas, in the guinea pig complete mammary development ensues (Folley 1952).

2. Progesterone. Various workers (Corner 1930, Selye et.al. 1936a, Turner and Schultze 1931, Curtiss 1949) have reported that progesterone alone is ineffective in promoting lobule-alveolar development in the rat. Selye (1940a) however, reported that the administration of 15 mg. of progesterone daily for ten days to ovariectomized rats gave marked gland growth with lobule formation. Mixner and Turner (1943) reported the amount of progesterone needed for alveolar development in ovariectomized mice was six times as great as when estrogen was given simultaneously. Reece and Bivins (1942) noted the effectiveness of large doses of progesterone alone. Smith and Braverman (1953) observed some duct growth and extension of end buds from 4 mg of progesterone daily in immature ovariectomized rats.

Progesterone has been indicated as an inhibitor of lactation. Selye (1940b) reported that estrogens always promoted some secretion, but that

it was completely inhibited by progesterone. Desclin (1952) showed that progesterone prevented secretion in the rat and that this inhibition was overcome only by large simutaneous doses of prolactin. These observations are similar to those of Meites and Sgouris (1953, 1954) for the rabbit, in which they showed that progesterone administration inhibited the onset of lactation. This inhibition was overcome only by high levels of prolactin or by withdrawl of the progesterone treatment.

3. Progesterone and Estrogens. The most fruitful hormone treatments in promoting mammary development in the rat have been the combined use of estrogens and progesterone. The interrelation or synergism between these two hormones has been discussed in detail by Courrier (1950) in which this synergism is described as having effect on the uterus, vagina, mammary glands and other tissues.

In the rat, many investigators have attempted to find the optimum progesterone-estrogen synergism for mammary growth. Astwood and Gesichter (1938) reported a dosage of 1 mg progesterone and 5 ug of estrone for 8 days induced no demonstrable lobule-alveolar growth. Meites and Turner (1948) reported limited lobule-alveolar development in the castrate rat receiving 15 mg progesterone and 5 ug of estrone daily for 10 days. Lyons and coworkers (1952, 1953) used 4 mg of progesterone and 1 ug estrone and obtained good mammary development. Curtiss (1949) showed that 4 mg progesterone daily and 1 ug estradiol twice weekly gave optimal mammary development. Kirkham and Turner (1954) and Elliott and Turner (1953) showed that 5 mg progesterone and 1 ug estradiol-benzoate gave mammary development comparable to that obtained in normal pregnancy. Treatment for 26 days with this ratio of the hormones gave good mammary gland development. Rats so treated could be brought into copious lactation by cessation of all treatment and were found to lactate sufficiently to maintain foster

litters. Lactation was initiated only on cessation of treatment. The work of Smith (1955) further demonstrates that this synergism is highly effective in promoting mammary development in the rat.

Interesting related observations have been made by Nelson and coworkers (1954) who showed that 4 mg progesterone and 1 ug estradiol administered daily was sufficient to prevent resorption of litters in pregnant rats on severe nutritional deficiencies such as protein free, pyridoxine deficient or potassium deficient diets.

B. Pituitary Hormones.

Stricker and Grueter (1928) showed that the pituitary to have a factor which has an effect on initiating lactation in the developed mammary gland. Many investigations have centered around the pituitary in attempts to elucidate the puzzling role of this gland in the development and function of the mammary gland.

Numerous workers have shown that estrogen and progesterone, hormones which will stimulate gland growth in the ovariectomized rat, fail to do so in the absence of the pituitary gland (Selye and Collip 1936; Gomez and Turner 1937; Leonard and Reece 1942; Leonard 1943). Mammary development in hypophysectomized rats was stimulated with estrogen and implanted pituitaries, or estrogens and pituitary extracts (Gormez and Turner 1937; Nathanson et.al. 1939).

C. W. Turner proposed the concept of mammogenic hormones of the pituitary which are caused to be released by ovarian hormones and thus stimulate mammary development. Estrogen was visualized as stimulating the pituitary to the secretion of a duct growth factor "Mammogen I". A second pituitary factor, "Mammogen II" was believed to be produced by pituitary and released on the stimulus of progesterone or estrogen and progesterone in combination (Mixner et.al. 1940, Mixner and Turner 1943).

These factors have never been fully characterized so that it is difficult to relate them to any well known pituitary hormone. Indeed, one of these factors was postulated to be soluble in organic solvents, a postulate which was readily denied, so that the very existence of two distinct factors is questioned. None the less, the bulk of evidence points to the presence of a pituitary factor which is directly involved in mammary growth.

Nathanson et.al. (1937) and Reece and Leonard (1941) found that the mammary gland could be developed by treatment of hypohpysectomized rats with estrogen and pituitary growth hormone. Mammary gland growth has been obtained in hypophysectomized male mice by the administration of estrogen and prolactin (Gardner and White 1941) or with a "lactogenic" preparation (Trentin and Turner 1948). Cowie and Folley (1947a) showed that cattle pituitary extracts exhibited memmogenic activity, both in the presence and absence of the adrenals. Stimulation of duct growth was most prominent in the young rats, while in the old animals alveolar development also occured.

Lyons et.al. (1952) reported that injection of 1 ug esterone and 4 mg progesterone together with 60 I.U. of prolactin and 1 ug of growth hormone daily to castrate hypophysectomized rats stimulated lobule-aveolar growth. If growth hormone was absent, the lobule-alveolar growth obtained was not equal to that obtained in ovariectomized rats with estrogen and progesterone. Growth hormone at levels of 0.1 to 6.0 mg daily for 10 days with esterone and progesterone failed to stimulate lobule-alveolar growth, indicating a role of prolactin in stimulating lobule-alveolar growth.

Lyons (1942) obtained evidence for the localized action of prolactin in promoting enlargement of a locally treated sector of the gland of ovariectomized rabbit. The animals were pretreated with estrone and

progesteone and then given prolactin into a single duct. The sector of the gland draining by this duct showed full lactation while neighboring sectors were not lactating. The gland had to be weel developed before this action of initation of lactation could be seen.

When estrone, progesterone and pituitary mammotropin were administered to castrate C_3H male mice mammary development was greater than in similar animals receiving only estrogen and progesterone (Thomas and Lyons 1954).

C. Adrenal Cortical Hormones.

In a number of experimental studies, the adrenal cortex has been found to have some relation to mammary growth and lactation. Brownell et.al. in 1933 suggested division of cortical principles into two groups, one necessary for life and the other substances, "corticolactin", necessary for lactation.

The mammary gland rapidly regresses following adrenalectomy (Cowie and Folley 1947b; Trentin and Turner 1947, 1948). Mixner and Turner (1942b) reported that desoxycorticosterone acetate evoked alveolar growth in the mouse. These authors and Cowie and Folley (1947b) have shown that descxycorticosterone acetate showed one-third the activity of progesterone in promoting lobule-alveolar growth in ovariectomized female mice.

Smith and Braverman (1953) reported that 2 and 5 mg. of desoxycorticosterone acetate administered daily for 12 days induced mammary duct growth in immature ovariectomized rats but induced no alveolar formation. Increased "spreading factor" activity of mammary glands was noted by Elliott and Turner (1953) in the ovarectomized rat receiving 1 ug estradiol benzoate and 1.5 mg cortisone or 3 mg of desoxycosticosterone acetate daily for 10 days. The estrogen alone did not give this effect.

The mechanism of adrenal cortical action in mammary growth and lactation is little understood. Smith and Braverman (1953) suggested that

the action of desoxycorticosterone acetate was by way of its conversion to progesterone in vivo. Cowie and Folley (1947b) suggested that this effect of the adrenal gland may reside in its ability to produce progesterone. Trentin and Turner (1948) explained the action of estrogen as being stimulatory to the adrenal glands for the production of progesterone or another like compound.

Some investigators got stimulation of lobule-alveolar development of the mammary system with desoxycosticosterone acetate in hypophysectomized rats (Gardner 1940, Nelson 1941), while Leonard and Reese (1942) observed that partially hypophysectomized rats showed growth of the mammary gland in response to desoxycorticosterone acetate, but no response in those animals in which hypophysectomy was completed.

In adrenalectomized lactating rats milk production can be maintained by administration of desoxycorticosterone acetate, partially restored by cortisone and fully restored by a combination of these hormones (Cowie 1952). Other adrenal and adrenal-like compounds have been shown to have an effect in maintaining lactation. Such compounds are 17-hydroxy-11-desoxycorticosterone (Flux 1955), 9a-chlorohydrocortisone acetate, 9-fluorohydrocortisone acetate and hydrocortisone (Cowie and Tindal 1955).

Selye (1954) has shown as outstanding effect of adrenal hormones on the mammary gland, when administered with estrogens. Hydrocostisone (0.5 ug daily) plus estradiol (5 ug daily) administered to adrenalectomized-ovariectomized rats resulted in mammary glands whose ducts were greatly enlarged and engorged, suggesting lactation. Administered individually, neither hormone was particularly effective. Cortisone was shown to have an effect comparable to hydrocortisone (Selye 1954a).

Other experiments have shown a less well defined action of adrenal hormones. Lyons et.al. (1955) have consistently used adrenal hormones

(cortisone) along with lactogenic hormone, growth hormone, estrogen and progesterone in hypophysectomized-adrenalectomized-ovariectomized rats to promote mammary development. These hypophysectomized -adrenalectomized-gonadectomized rats (both males and females) showed lobule-alveolar growth following continued injections of estrone, progestrone, lactogenic hormone and growth hormone for 1 week. When further injections of lactogenic hormone and growth hormone plus hydrocortisone were continued for an additional week, the glands secreted an abundance of milk (Chen et.al. 1955).

D. Other Hormones Effecting Mammary Development.

1. The Thyroid. Mixner and Turner (1942c) found that the lobule-alveolar response of ovariectomized rats given simultaneous progesterone and estrogen injection was decreased by thyroidectomy, and was increased by thyroid feeding. Since thyroxin effects the metabolic rate, it may be expected to influence both lobule-alveolar development and lactation. Cowie and Folley (1945) showed parathyroidectomy adversely affected lactation.

2. Androgenic Hormones. Selye et.al. (1936) reported that testosterone and other androgenic hormones induced mammary growth in rats. Nelson and Gallagh (1936) showed similar effects on ovariectomized virgin rats. Various workers have further demonstrated this mammary stimulating effect of androgens in rats. (Folley and Malpress 1948)

3. Placental Factors. Some placental factors have a specific effect aiding estrogen and progesterone in their stimulation of lobule-alveolar mammary growth. They also promote lactation similar to interior pituitary factors (Lyons 1944, 1950; Leonard 1945; Lyons et.al. 1955).

It is exceedingly difficult to assess the factors or conditions essential for mammary gland growth in realization of the variously

different observations made in the literature. One wonders if the mammary gland responds specifically to the various hormones, or if part of the conflicting nature of the experimental data might be due to a non-specific response of the mammary gland comparable to that seen in the pigeon crop sac. The literature is further complicated by the inability of workers to find suitable test conditions to fully differentiate the individual endocrine factors and their effect on the mammary gland. The question of the active form of the hormone at the site of the mammary tissue is one which, at the present time, is impossible to answer.

The complex nature of the hormonal control of mammary development is reflected by the following listing of observed effects in rats:

1. Duct growth and extension is promoted by estrogens.

2. Lobule-aveolar growth is stimulated by progesterone.

3. Lobule-alveolar growth is excellent under the effects of a synergistic ratio of estrogens and progesterone.

4. Progesterone potentiates the action of estrogen on the lobule-alveolar system.

5. Estrogen or progesterone stimulates the pituitary to secrete mammogenic hormone to induce mammary growth.

6. Desoxycorticosterone promotes mammary growth perhaps due to its conversion to progesterone.

7. Cortisone and hydrocortisone combined with estrogen give good mammary development.

8. Progesterone inhibits the action of lactogenic hormone in the near-lactation gland.

9. Estrogens give a local mammary growth response by inunction.

10. Androgens promote gland growth under certain conditions.

II. LOCALIZED TREATMENTS AND RESULTS.

In numerous cases, hormones have been shown to have effects when applied directly to tissues. Outstanding examples of this action are the response of the chick's comb to externally applied testosterone or androgenic hormones; the response of the endometrium to the intrauterine application of progesterone; the response of the vaginal mucosa to the direct application estrogens; the response of the sexual skins of monkeys to estrogens; and other less well demonstrated actions.

The direct action of hormones on the mammary gland may also be included in the catagory of having been well established. Zondek (1935, 1938) reported that estrone in oil, ointment, benzol, ether, or alcohol can be absorbed through the skin. By rubbing an estrone containing ointment on the skin in the region of the mammary gland of a male guinea pig, first the treated, and then the untreated mamma enlarged, and finally the gland secreted milk. At the same time, it was shown that subcutaneous administration of estrogen led to lactation. A local effect of inunction was indicated by the preferential development of gland. Jadassohn et.al. (1937) verified this result by showing that the inuncted mamma showed a more rapid enlargement than the untreated gland. Estrogens alone are sufficient to promote lactation in this species (Folley and Malpress 1948).

Lyons and Sake (1940) conducted a similar experiment on two months old male rabbits. The estrone in sesame oil (3 I.U. per dose) was rubbed on the skin over the gland area. The glands in these animals were rudimentary structures. Oil was rubbed on the right side as this gland served as a control. Treatment was continued five weeks. The hormone treated gland showed a more extensive duct growth and some alveolar

development. The area of the gland was about six to ten times greater than the area of the control. Nipple growth was also accelerated by this hormone. In similar experiments, Ruinen (1932) got an equal mammary gland response on either side due to the application of excessive amounts of estrogen. This evidence supports the view that estrogen is a direct mammary stimulant in these species.

A cutaneous method of application of estrone in 95% alcohol (Estrogen 0.05 mg./ml. of 75 days) was used on immature male (Speert 1940) and immature female (Chamberlin et.al. 1941) rhesus monkeys. On the side receiving the estrogen, there was greater growth of the nipple and development of the glandular tissue, consisting of large branching ducts, terminating in thickened ends and some lobule-alveoli.

Tests on male mice also have been reported (Gardner and Chamberlin 1941). Leonell et.al. (1937) transplanted fragments of ovarian carcinoma to the mammary gland of male mice. The mammary gland grew into an extension duct system similiar to that seen in male mice which had received optimal amount of estrogen. This phenomenon was interpreted as resulting from the secretion of estrogen by the carcinoma tissue.

Stilbene tablets implanted under the skin of the cow's udder caused the enlargement of the gland and lactation (Peeters et.al. 1949). The same results were obtained by implantation of pellets in front of the shoulder (Meites 1950).

Lyons (1942) developed a delicated intraductal injection method for studying the localized action of prolactin on the mammary gland. Ovariectomized virgin rabbits were pretreated with estrogen and progesterone which initiated development of the mammae. A prolactin solution was administered into the duct draining one of the sectors of a single mamma. Other sectors were not treated and served as control tissues. Administration

of prolactin to such pretreated rabbits led to a distinct localized enlargement of the alveoli in the treated sector. The treated sector was conspicious by its engorgement with milk, which could be expressed from the gland 48 hours after the injection. This experiment demonstrated that the hypophyseal lactogenic hormone acts directly on the secretory tissue, and, at least in such pretreated rabbits, this hormone is effective in inducing milk formation in the lobule-alveolar system.

Another area in which a local response to prolactin is used in the assay of this hormone (Emmens 1950). When administered intradermally into the skin of a pigeon over the crop sac, this organ is induced to proliferation. Continued stimulation will lead to the secretion of "crop milk" by this species.

MATERIALS AND METHODS

The primary purpose of this study was to develop a method whereby hormones could be applied directly to the mammary parenchyma without evoking a systemic response. There were a number of factors to be considered and overcome if such a technique were to be developed.

One fundamental consideration was the size of the dose to be administered. Any dosage should be sufficiently large for a local response and small enough that a systemic stimulation was avoided. This meant that careful consideration should be given to the rate of absorption of the administered dosage. Ideally, the level in the vicinity of the mammary parenchyma would be high enough to promote a local reaction, but it would be present in such a small quantity that it would be below the threshold for a systemic response.

The duct system of the mammary gland, as found in the mature virgin rat of about 170 grams body weight, is a fan shaped reticular type structure, supported by spongy connective tissue. Each gland has an area of about 10-13 square centimeters (inguinal gland). The nature of this tissue suggested that hormones might be implanted into one area of the mammary gland as small pellets or emulsified droplets and if the diffusion of the hormone was restricted other areas of the gland might remain unaffected. Thus, the tissue of the same gland could be visualized to serve as its own control. Even more encouraging was the possibility that a gland on one side might be treated without the gland on the opposite side responding. This paried gland might also be considered as

the control simplifying detection of localized effects.

In this study a series of experiments were conducted to develop techniques to be used in the implantation of small pellets of hormones into an area of one mammary gland of rats of various ages and treatments. The results were compared with studies of the effect of systemic treatments under closely controlled conditions. Due to the length of time required in treating the animal and making histological preparations of the tissues, the number of rats used in any one group was small. In instances of uncertainity of response, the treatment was repeated.

Various methods of administration were tried in an attempt to achieve a local response to the hormones. Crystalline or powdered hormones were made into aqueous emulsions by the use of an emulsifying agent, "Aquaphor". Compounded in this way, the emulsified hormone preparation could be injected as a small globule, with a No. 25 or 27 hypodermic needle directly into the mammary gland site. As an added convenience, small quantities of particulate gold or carbon black could be added to the emulsion to aid in locating the site of administration following histological preparation of the tissues. Other liquid carriers were also used but the results were in general negative. In some cases there was indication of altered cellular growth in tissue adjacent to the injected emulsion, but responses were weak and the technique was considered unsuccessful.

On the other hand, when small pellets of pure hormones were placed directly in the gland area, it was readily recognized that this was far superior to the attempted injection method by the nature of the responses obtained.

PREPARATION OF FUSED HORMONE PELLETS.

Various steroid hormones available as crystalline powders, were

transformed to fused pellets (Hohn 1953) by heating the powder to its melting point, allowing it to fuse and cool. On cooling, the fused hormones solidified and cracked into small pieces. Selected pieces served as pellets in these studies. They were irregular in shape and in a size range of 0.2-0.3 cubic millimeters.

PREPARATION OF PRESSED HORMONE PELLETS.

Pressed hormone pellets were made in a hand operated pelleting machine.[1] This machine consisted of a mold of about 1 mm. diameter and a plunger. The hormone to be pelleted was added to the mold and the plunger moved within the mold at high pressures. Pellets formed by this machine could be cut into proper size for implantation.

PREPARATION OF HORMONES FOR SUBCUTANEOUS ADMINISTRATION.

Steroid hormones were prepared for subcutaneous administration by dissolving into sesame oil in most cases.

HORMONES USED IN THE FOLLOWING EXPERIMENTS.

The Hormones used in the following experiment were:

A. Fused or pressed pellets:

1. Cortisone Acetate (C.A.)
2. Hydrocortisone Acetate (H.C.A.)
3. Estradiol Benzoate (E.B.)
4. Progesterone (Prog.)
5. Estradiol Benzoate - progesterone (E.B. - Prog.)

B. Pressed pellets:

1. Growth Hormone (G.H.)
2. Prolactin (Prol.)

[1] The hormone pelleting machine of Dr. C. D. Kochakian of the Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma was used to make the pressed pellets.

ANIMALS USED.

Albino rats of a uniform inbred strain were used in all experiments. These rats were obtained either from the Holtzman Rat Company, Madison, Wisconsin, or were raised in the stock colony at the Department of Animal Husbandry, at the Oklahoma A. and M. College.

METHOD OF IMPLANTATION OF PELLETS.

Rats were first anesthetized with ether, stretched on a board with their ventral side up and immobilized by tying their four feet. Anesthesia was continued throughout the implantation. A midline incision of about 1 inch in length was made. On retraction of the skin the mammary gland was found to be adhering to the skin. The mammary gland was carefully raised, the mammary tissue was exposed in clear view through the incision. The teat served as a guide to the location of the gland. The gland exists as a delicate structure in fatty connective tissue and is difficult to discern, unless stained or otherwise treated. In most experiments implantations were confined to the inquinal glands as explained here. A small incision was made by piercing the mammary tissue with the points of a watchmaker's forceps. The same forceps were used to pick up the pellet and place it in the incised areas of the tissue, making sure that the pellet remained in place. Several factors were noted from experience which facilitated the conduct of this total technique. If the incision into the mammary gland is extensive there may be adhesion to the body wall, making subsequent removal of the mammary gland more difficult. The mammary pad is a thin sheet tissue, almost membranous in character, so that the fine incision must be made in a manner that does not permit complete penetration through this membranous tissue. Implantation of a single pellet into an incision is recommended since it is difficult to

manipulate the small fused pellets without their shattering. Two or three pellets were implanted in different areas of the mammary gland, trying in all instances to locate the pellet in a region peripheral to the lateral extension of the mammary ducts. It was further found convenient to use the gland on the right side as the treated gland and the left as the control.

FIXATION AND PREPARATION OF TISSUES.

After rats had been subjected to the local action of the hormones for the desired period of time, they were sacrificed and the mammary glands removed for fixation and staining. Skin was removed from the animals starting with a dorsal incision and loosening the skin from the body wall. Dissection was carefully continued in the inguinal region. A large section of the skin from the mid region of the rat was tacked to a board. When the skin was removed in this way the mammary glands were removed with it and they were conveniently handled. The board with the skin attached was immersed in a vat of Bouin's solution[1] for fixation. The skins were removed after 24-48 hours and immersed in running water until free of the fixative.

The mammary tissues were stained with gallocyanin-chrom-alum stain[2] for 24 to 48 hours, the length of time required for staining depending upon the nature of the tissue and the age of the stain. This stain stains the mammary parenchyma a dark blue color and the background spongy

1. Bouin's solution.
   75 parts saturated picric acid.
   20 parts formaldehyde
   5 parts glacial acetic acid
2. Gallocyanin-chrom-alum stain:
   Gallocyanin 0.3 gm.
   Chrom alum $Cr_2K_2(SO_4)_2\ 24H_2O$ 10.0 gm.
   Distilled water. 200 ml.
   Boil 5 minutes, cool and use.

connective material remains unstained. Occasionally, it was noted that the stained tissue faded to a brown color after standing for a period of time. The stain was washed off with running tap water, the glands were dried with absorbant paper and they were then dehydrated in 70, 80 and 95 percent ethyl alcohol. They were cleared in xylene and allowed to remain in contact with xylene until time of mounting.

Under the dissecting microscope the mammary glands were carefully freed of excessive connective tissue by the use of fine dissecting forceps. After careful dissection the glands were prepared for observation without excessive connective tissues to obscure one's vision. So prepared, the mammary glands were mounted on glass slides in balsam and covered with a cover glass. Pressure was applied for 3 to 4 days by placing a weight on the cover glass so that a flat preparation could be obtained. One particular difficulty was encountered here in that the mammary gland has lymph nodes which when fixed become hard and make it difficult to obtain flat preparations.

EXPERIMENTAL RESULTS

In presenting the results of experiments the treatment and results will be described briefly, and the experimental treatment will be presented in tabular form for easy reference.

A series of sixteen experiments, employing some one-hundred sixty rats of various ages and systemic treatments, was carried out in an effort to demonstrate a localized action of various steroid and protein hormones administered as an aqueous emulsion with "Aquaphor". In some of these experiments there were indications of a slight effect in the immediate vicinity of the site of injection into the mammary tissue, but these effects were unimpressive. When hormones were injected into the gland area as an oil solution a greater effect was noted. When small hard pellets of the hormones were implanted into the mammary sites, the activities of the hormones on this tissue was demonstrable. Studies were carried out on the effects of hormones administered in this way.

A quantitative estimation of mammary gland growth has been a real problem in studies of the development of this tissue. Almost all investigators have used as arbitary scale to rate the degree of development of mammary tissues studied. A rating scale similar to that used by Smith (1955) has been employed in these studies. The scale of 1 to 5 indicates total mammary development as follows:

1: Lobules distributed very sparsely on the ductal system, "incipient" alveoli on a sparse duct system.

2: Lobular proliferation light, but more uniformly distributed.

3. Lobular development moderate with some elements on each duct, highly branched duct system, bunching of lobules.

4. Lobular growth moderate to heavy, not over the entire gland, many clusters and packed lobules.

5. Lobules crowded together over almost all of the gland area obscuring ductal structures giving a "packed" appearance, excellent growth of alvoeli.

Glands rated as intermediate between any two of these ratings were given the rating of + or -, e.g., a gland showing lobules forming on a fairly widely dispersed duct system would receive a rating of 1 or 2. It might be pointed out that it is rather difficult to apply this rating system to a large number of memmae, but familiarity with the tissues aids in placing them in the proper category.

Another scale was devised and applied to give some relative values to the response to local implantation of hormone pellets. This arbitary scale results from the comparison of the treated gland on the right side of the animal to the inguinal gland of the left side, the left being the untreated control gland. The scale is as follows:

1. Only slight increase in lobule size indicating a minimum of stimulation.

2. Lobules 1-1/2 to 2 times larger than lobules in control gland, not well developed and increasing general density of pelleted area.

3. Lobules 2 to 3 times larger then control, moderately dense nature of tissue.

4. Marked lobulation, lobules 3 to 4 times as large as on the control side, lobules showing some tendency to spread.

5. Lobules with marked alvoeli, effect registered in large area in vicinity of pellet, lobules 5 or more times larger than those of control

side.

The following list of abbreviations apply to the tables:

C.A.	Cortisone acetate	Cast.	Castrate
H.C.A.	Hydrocortisone acetate	Fus.	Fused hormone pellet
E.B.	Estradiol-benzoate	Pre.	Pressed hormone pellet
Prog.	Progesterone	Liq.	Liquid hormone
G.H.	Growth hormone	Cont.	Control
Immat.	Immature	Gms.	Grams
Mat.	Mature	Ml.	Milliliter
Ovar.	Ovariectomized	Ug.	Microgram
Prol.	Prolactin	Mg.	Milligram
Adren.	Adrenalectomized		

A column is given in the table entitled "Figures". Photographs representing these figures will be found in the appendix and serve as a guide to the type of mammary growth obtained by a given treatment.

EXPERIMENT I.

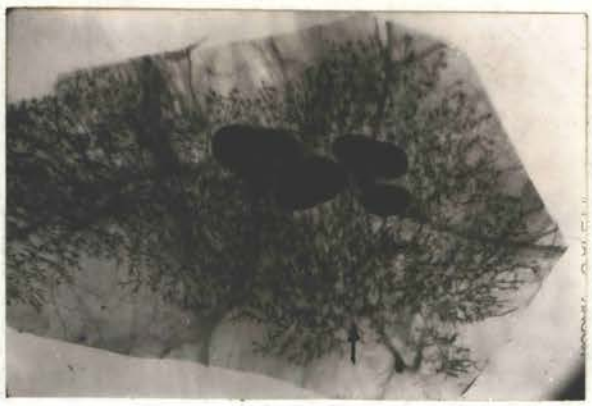
In this experiment, 70 to 110 gm. immature female rats were ovariectomized and implanted with hormone pellets into mammary gland areas in one operation. After nine days the glands were removed for examination. Five groups of 3 rats received respectively cortisone acetate, hydrocortisone acetate, progesterone, estradiol benzoate, and mixed estradiol benzoate-progesterone pellets into the mammary gland. The degree of development of the mammary system is indicated in Table I.

Examination of glands of the cortisone acetate and the hydrocortisone acetate treated group revealed that the pellets had been placed in a position peripheral to and not in contact with, the mammary parenchyma. Even under these conditions, the influence of the hormone could be seen by the slightly increased lobulation near the area of the pellet. In

Table I. Effect of microimpelletation on the Mammary Glands of Immature Ovariectomized Rats.

Group	Number of Rats	Body Weight gms.	Treatment		Degree of Total Mammary Growth	Degree of Local Lobular Growth	Figure Number
			Systemic	Impelletation*			
1	3	70-100	Ovar.	C.A. fus.	3- to 3+	1	--
2	3	70-100	Ovar.	H.C.A. fus.	2+ to 3-	2	1a
3	3	70-100	Ovar.	Prog. fus.	2 to 2+	3.5	1b
4	3	70-100	Ovar.	E.B. fus.	3+ to 4	1	1c
5	3	70-100	Ovar.	E.B. Prog. fus.	3- to 3+	4.5	1d

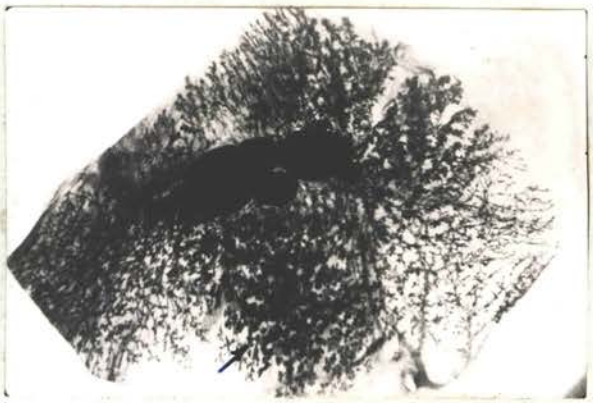
* Pelleted 9 days before sacrifice.



1a. Group 2, hydrocortisone acetate implanted. Treated gland.

1b. Group 3, progesterone implanted. Treated gland.

1c. Group 4, estradiol benzoate implanted. Control gland left, treated gland right.



1d. Group 5, estradiol benzoate and progesterone fused pellet. Treated gland.

Figure 1. Localized stimulation of mammary glands of immature ovariectomized rats by various hormones. Large dark areas are lymph nodes. The site of implantation is marked by arrows. Magnification X3. (see Table I).

the rats receiving the progesterone pellets, a local stimulation of lobule development was seen. The stimulatory effect of the hormone diffusing from the pellet was distributed over a fairly large area of the treated gland. The affected tissue stained more intensely. In the estradiol benzoate pelleted rats a definite systemic stimulation of mammary growth was obtained. There was some suggestion of a slightly increased lobulation and an increase in size of the ducts in the treated glands but this was observed in only one animal. Both a systemic and a localized lobulation resulted from treatment with the mixed estradiol benzoate-progesterone pellet. The progesterone brought the lobules of a fairly large area of the treated gland into a state of near complete development. The estrogen was judged not to have been restricted in its effect since the control gland showed considerable development.

EXPERIMENT II.

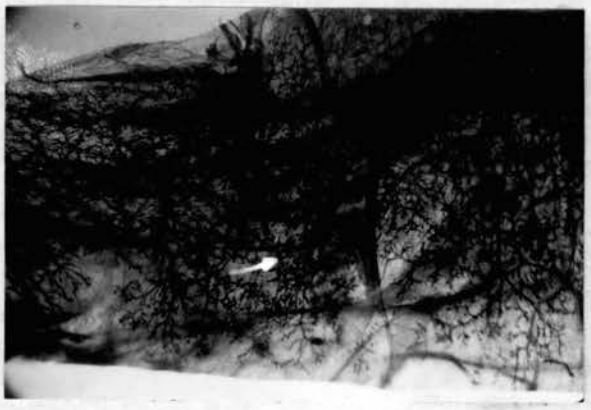
This experiment was a repeat of the previous experiment, except that female rats employed were of a more advanced age and a weight range of 140-180 gm. Ovariectomy and impelletation were performed in one single operation. The rats were sacrificed after two weeks.

In the glands examined the progesterone and fused progesterone-estradiol benzoate pellets promoted greater lobulation of the tissues in an area around the implanted site. Some systemic effect was apparent in rats receiving the impelletation of estradiol benzoate. Cortisone acetate and hydrocortisone acetate promoted growth of the lobules. The lobules were less developed than those obtained with progesterone, but the alveoli were swollen and distinct, with some evidence of the presence of a distending fluid, indicating lactation. They differed from the lobules formed under the stimulation of progesterone. The latter were more dense and gave a more intensely staining preparation.

Table II. Effect of Microimpelletation on the Mammary Glands of Mature Ovariectomized Rats.

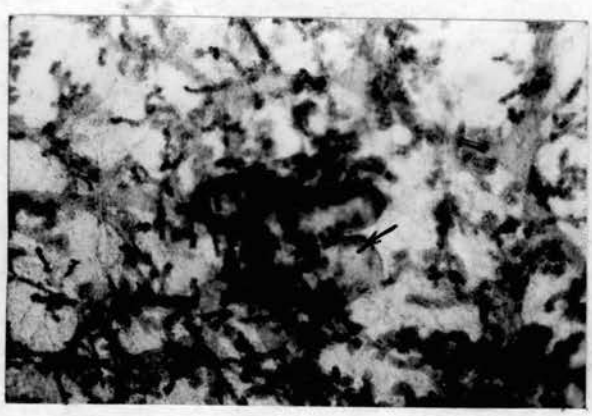
Group	Number of Rats	Body Weight gms.	Treatment		Degree of Total Mammary Growth	Degree of Local Lobular Growth	Figure Number
			Systemic	Impelletation*			
1	4	140-180	Ovar.	C.A. fus.	2 to 3	2, 1	2a
2	4	140-180	Ovar.	H.C.A. fus.	2- to 2	2	2b, 2c
3	4	140-180	Ovar.	Prog. fus.	3- to 3	4, 4, 2	2d, 2e 2f
4	4	140-180	Ovar.	E.B. fus.	3+ to 4	1	2g
5	3	140-180	Ovar.	E.B. Prog. fus.	3- to 3+	2, 2	2h
6	2	140-180	Ovar.	Cont.	3- to 3+	--	--

* Pelleted 2 weeks before sacrifice.

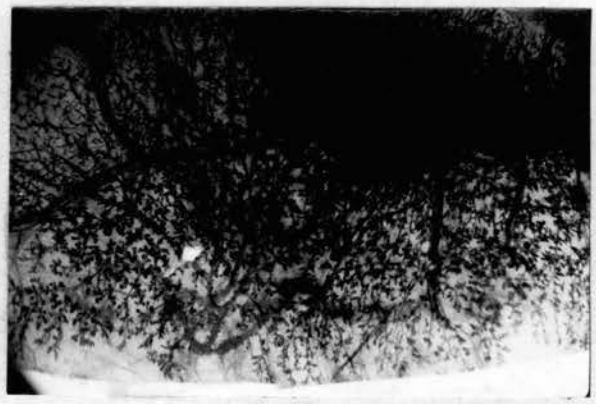


2a. Group 1, cortisone acetate implanted. Treated gland X3.

2b. Group 2, hydrocortisone acetate implanted. Treated gland X3.

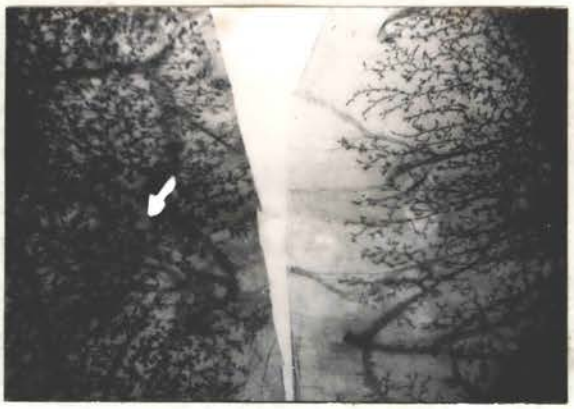


2c. Same as 2b showing details of pellet site, distended lobule-alveolar system and diffuse staining X15.

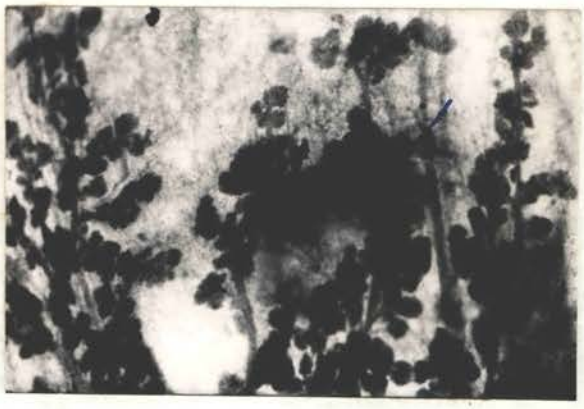


2d. Group 3, progesterone implanted. Treated gland X3.

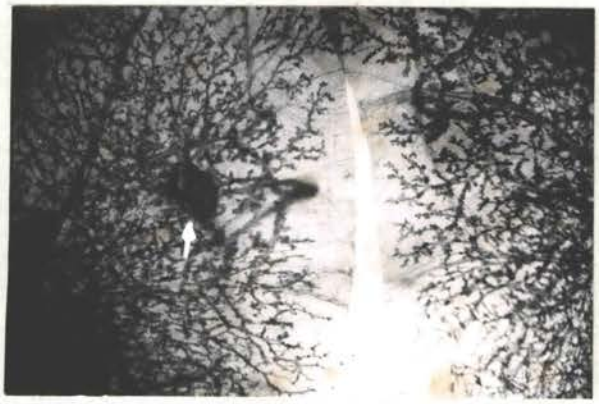
Figure 2. Localized stimulation of mammary glands of mature ovariectomized rats by various hormones. The site of pellet implantation is marked by arrows. (see Table II).



2e. Group 3, progesterone implanted. Control side right, treated gland left, X3.



2f. Group 3, progesterone implanted Details of pellet site, compact lobule-alveolar system, dense staining, X15.



2g. Group 4, estradiol benzoate implanted. Treated gland, X3.

2h. Group 5, estradiol benzoate-progesterone fused pellet implanted. Treated gland, X3.

Figure 2 (continued)

When the control glands of rats in this series (the glands on the left side of the rats) were compared there was no sign of gross differentiation of these glands beyond that seen in the control animals, except in those cases in which estradiol benzoate was implanted. In the latter case, the estradiol benzoate gave a systemic effect promoting extension of the duct system and budding, but not clear lobule-alveolar formation. These observations indicate that the hormones cortisone acetate, hydrocortisone acetate and progesterone give a local action on the gland, but are not present in sufficient quantity in the general circulation to extend this effect to all mammary tissue.

The control animals in this group showed rather well developed mammary systems for ovariectomized animals. The two week estrogen free period usually promotes regression of the gland to a simple duct system with few if any buds.

EXPERIMENT III.

This short experiment was used to test the action of cortisone acetate in male rats (Table III). Castrate rats were implanted with cortisone acetate pellets on the fourth day following castration. The implanted pellets provoked lobulation in the treated gland. Alveoli were observed in a state of distension with fluid. There was no doubt of a localized stimulation of both lobule formation and milk secretion in the treated gland, whereas the control gland was primarily a duct system with few buds. Non-castrate 100 to 120 days old male rats treated with cortisone acetate pellets responded in the same manner as the castrate male.

EXPERIMENT IV.

Immature female rats of weights ranging from 65 to 80 grams were simultaneously ovariectomized and implanted with cortisone acetate, hydro-

Table III. Effect of Microimpelletation on the Mammary Gland of Castrate and Non-Castrate Male Rats.

Group	Number of Rats	Body Weight gms.	Treatment		Degree of Total Mammary Growth	Degree of Local Lobular Growth	Figure Number
			Systemic	Impelletation			
1	2	100-120	Non-Cast.	C.A. fus.*	2	3	--
2	2	160-170	Cast.	C.A. fus.+	2 to 3-	5	3

* Pelleted 11 days before sacrifice.
\+ Pelleted 10 days before sacrifice.

cortisone acetate or estradiol benzoate pellets. Subcutaneous injections of either sesame oil or estradiol benzoate, cortisone acetate or hydrocortisone acetate in sesame oil, were made daily for ten days starting the day following ovariectomy and implantation. On the eleventh day the glands were removed and examined.

This was the first experiment to demonstrate a copious lactation response to the adrenal cortical hormones when administered either locally or systemically. In the vicinity of either the locally implanted cortisone acetate or hydrocortisone acetate pellet the lobules were well developed and the alveoli were swollen and full of liquid, indicating lactation. The size and degree of alveolar distention decreased with the distance of the tissue from the pellet indicating a concentration gradient around the pellet.

When systemic treatment with estradiol-benzoate (1 ug./day) accompanied impelletation with cortisone acetate and hydrocortisone acetate, both lobule growth and lactation in the vicinity of the pellet was enhanced. Systemic administration of cortisone acetate (0.5 mg./day) and hydrocortisone acetate (0.5 mg./day), led to well developed lobule-alveolar systems in the glands, but the lateral extension of the duct system and hence the total gland area was conspicuously reduced. The glands showed characteristics of early lactation with distension of the alveoli.

The effect of this systemic treatement was outstanding. These immature glands were apparently greately stimulated to a functional capacity with the result that the gland appeared to come into a precocious lactation before all the anatomical elements of the gland were developed. Consequently, in many cases, the swelling of the duct system could be seen as the gland came into a lactating capacity.

Table IV. Effect of Cortisone Acetate, Hydrocortisone Acetate and Estradiol Benzoate on the Mammary Glands of Ovariectomized Rats.

Group	Number of Rats	Body Weight gms.	Treatment			Degree of Total Mammary Growth	Degree of Local Lobular Growth	Figure Number
			Systemic	Impelletation*	Subcutaneous Injection			
1	3	65-80	Ovar.	C.A. fus.	Sesame Oil 0.1 ml./day	3-	4, 4, 3	4a
2	3	65-80	Ovar.	C.A. fus.	E.B. Liq. 1 ug./day	4	4, 4, 4	4b
3	3	65-80	Ovar.	H.C.A. fus.	Sesame Oil 0.1 ml./day	2+ to 3+	3, 4	--
4	3	65-80	Ovar.	H.C.A. fus.	E.B. Liq. 1 ug./day	4+ to 5	4, 4, 3	4c
5	3	65-80	Ovar.	E.B. fus.	C.A. Liq. 0.5 mg./day	5+		4d
6	3	65-80	Ovar.	E.B. fus.	H.C.A. Liq. 0.5 mg./day	5		4e

* Pelleted 11 days before sacrifice.

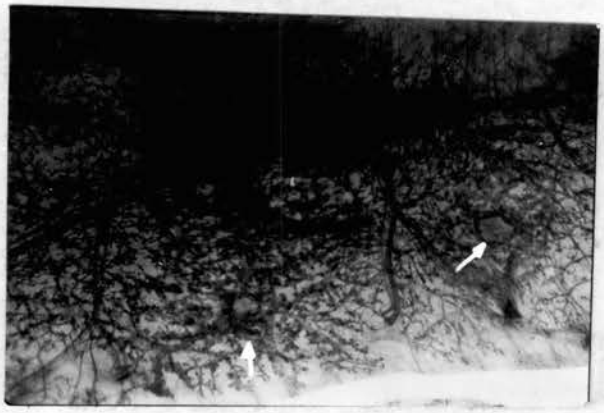
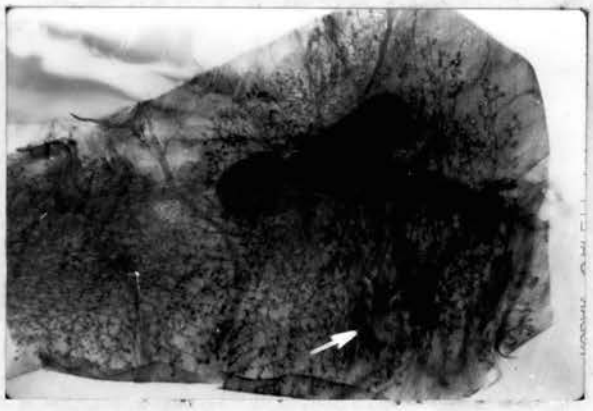
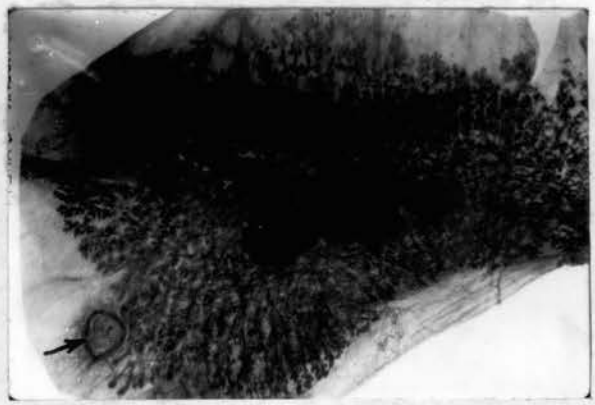


Figure 3, Group 2, response of the mammary gland of a mature castrate rat to cortisone acetate implanted pellets. Pellets indicated by arrow, X3. (see Table III).

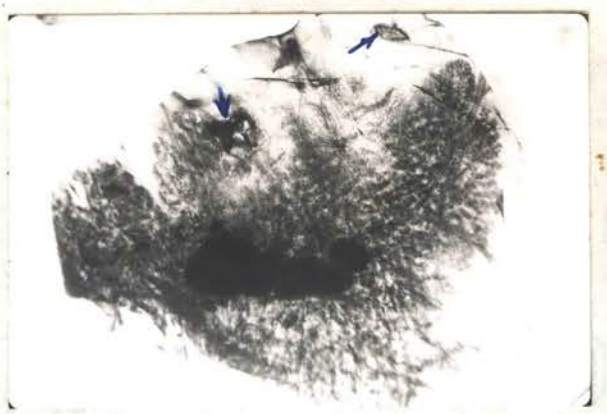


4a. Group 1, cortisone acetate implanted, sesame oil parenterally. Treated gland.



4b. Group 2, cortisone acetate implant, estradiol benzoate parenterally. Treated gland.

Figure 4. Localized effects of cortisone acetate, hydrocortisone acetate and estradiol benzoate administered as pellets. Treatment was combined with these same hormones administered parenterally. Responses in immature ovariectomized rats. Magnification X3. (see Table IV).



4c. Group 4, hydrocortisone acetate implanted, estradiol benzoate parenterally. Treated gland.

4d. Group 5, estradiol benzoate implanted, cortisone acetate parenterally. Treated gland.

4e. Group 6, estradiol benzoate implanted, hydrocortisone acetate parenterally. Treated gland left, control gland right.

Figure 4 (continued).

Cortisone acetate and hydrocortisone acetate appear to play a role in the initiation of lactation, a role which is enhanced by the systemic effect of estradiol benzoate.

EXPERIMENT V.

Mature rats were ovariectomized and adrenalectomized by a dorsal approach in one single operation. All rats received the same diet and a one per cent sodium chloride solution. On the ninth day following operation, cortisone acetate and hydrocortisone acetate pellets were implanted. Subcutaneous administration of 1 ug estradiol-benzoate or sesame oil was started on the day following implantation. Ten days later, the glands were removed for examination.

The results in the absence of the adrenals was similar to those of Experiment IV and are shown in Table V. Lobule-alveolar development with the signs of initiation of lactation was apparent in the area of the pellets in all cases. Hydrocortisone acetate appeared to give a slightly greater localized stimulation to lobulation than did cortisone acetate. The localized stimulatory effect of both of these hormones was enhanced by the subcutaneous administration of estradiol benzoate.

Ovariectomy and adrenalectomy leads to regression of the gland to a duct animals in the time of 9 days following operation. Even in this highly involuted stage cortisone and hydrocortisone alone promote growth and development; in the presence of systemic estrogen the response greatly enhanced.

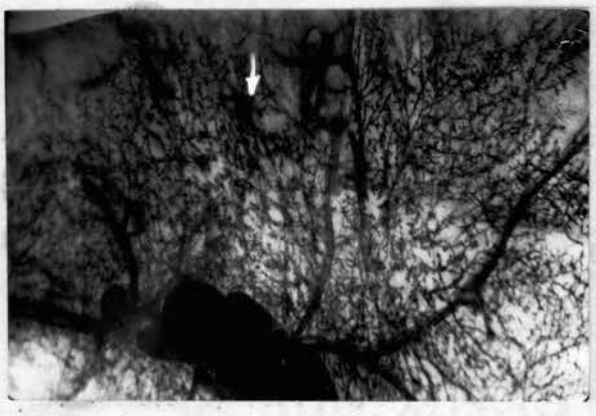
EXPERIMENT VI.

In this experiment the systemic effect of cortisone and progesterone were compared since both of these compounds had given localized response by the pellet treatment. Immature female rats (80-100 gm.) were administered sesame oil, cortisone acetate 0.5 mg, progesterone 5 mg, estradiol

Table V. Effect of Microimpelletation on the Mammary Glands of Ovariectomized Adrenalactomized Rats.

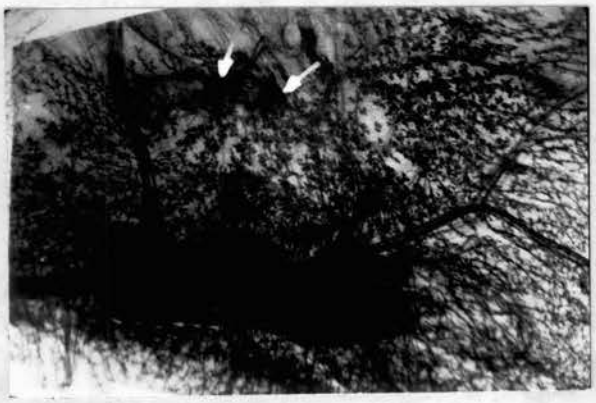
Group	Number of Rats	Body Weight gms.	Treatment Systemic	Impelletation	Subcutaneous Injection	Degree of Total Mammary Growth	Degree of Local Lobular Growth	Figure Number
1	2	140-160	Ovar. Adren.	C.A. fus.	Sesame Oil 0.1 ml./day	3	1	5a
2	2	140-160	Ovar. Adren.	C.A. fus.	E.B. Liq. 1 ug./day	3+ to 4-	3, 2	5b
3	2	140-160	Ovar. Adren.	H.C.A. fus.	Sesame Oil 0.1 ml./day	2 to 3	3, 4	5c
4	2	140-160	Ovar. Adren.	H.C.A. fus.	E.B. Liq. 1 ug./day	4- to 4	2, 4	5d

* Pelleted 9 days after ovariectomy and adrenalectomy, sacrificied 10 days later.



5a. Group 1, cortisone acetate implanted, sesame oil parenterally. Treated gland.

5b. Group 2, cortisone acetate implanted, estradiol benzoate parenterally. Treated gland.



5c. Group 3, hydrocortisone acetate implanted, sesame oil parenterally.

5d. Group 4, hydrocortisone acetate implanted, estradiol benzoate parenterally. Treated gland.

Figure 5. Effect of systemic administration of estradiol benzoate on the localized stimulation of lobule-alveolar development by implanted pellets of cortisone acetate and hydrocortisone acetate in ovariectomized-adrenalectomized mature rats. Magnification X3. (see Table V).

benzoate 1 ug, estradiol benzoate 1 ug plus cortisone acetate 0.5 mg, or estradiol benzoate 1 ug plus progesterone 5 mg daily for 13 days. They were sacrificed two days later. The results are summarized in Table VI.

Treatment with cortisone acetate, progesterone or estradiol benzoate as the only hormone resulted in about the same level of mammary development as was obtained by the use of sesame oil alone. Little if any stimulatory activity was attributed to these hormones under these conditions. When estradiol benzoate was administered simultaneously with cortisone acetate or progesterone the lobule-alveolar system was well developed. The previously noted distinction between the tissues stimulated by these hormones, when they were applied as pellets, was more pronounced in this method of treatment. All the glands in the estradiol-benzoate and progesterone treated rats showed good lobule-alveolar development. The lobules were more dense and the alveoli were not as apparent as in the cortisone acetate group. In this latter group all the glands showed good lobule-alveolar growth with the alvoeli being very prominent as distended with fluid. They stained more diffusely. This response of the glands to cortisone acetate indicates that this hormone may play a role in the initiation of lactation. Progesterone on the other hand appears to promote growth of the parenchyma but does not seem to have an active role in promoting lactation. As will be discussed later, evidence exists that progesterone may actively inhibit the onset of lactation.

EXPERIMENT VII.

This was a repeat of the previous experiment with mature rats using the same hormone administration as in that experiment. Thirteen daily injections were given and the glands were removed three days later. The results are summarized in Table VII.

The whole gland growth was slightly accelerated by treatements with

Table VI. Effect of Various Hormones in the Mammary Gland Growth of Immature Female Rats by Subcutaneous Injection.

Group	Number of Rats	Body Weight gms	Treatment Systemic	Treatment Subcutaneous Injection*	Degree of Total Mammary Growth	Average Degree of Total Mammary Growth	Figure Number
1	2	80-100	Intact	Sesame Oil 0.1 ml./day	3-	3-	≠
2	3	80-100	Intact	C.A. Liq. 0.5 mg./day	3- to 3	3	≠
3	3	80-100	Intact	Prog. Liq. 5.0 mg./day	3 to 4-	3+	≠
4	3	80-100	Intact	E.B. Liq. 1 ug./day	3- to 3+	3	≠
5	3	80-100	Intact	1 ug. E.B. - 0.5 mg. C.A. daily.	3+ to 4	4-	≠
6	3	80-100	Intact	1 ug. E.B. - 5.0 mg. Prog. daily.	4 to 4+	4	≠

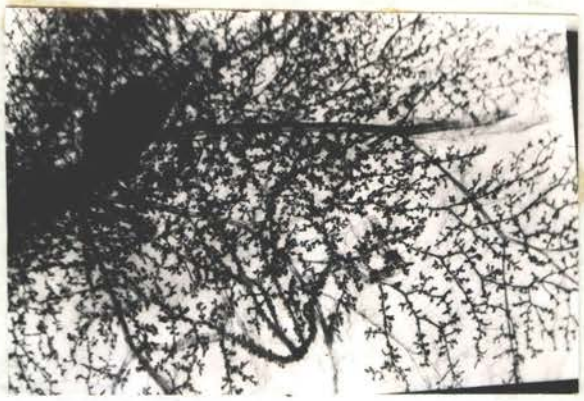
* Subcutaneous injection for 13 days.

≠ Results similar to following experiment, see Table VII for comparable figures.

Table VII. Effect of Various Hormones on the Mammary Gland Growth of Mature Female Rat by Subcutaneous Injection.

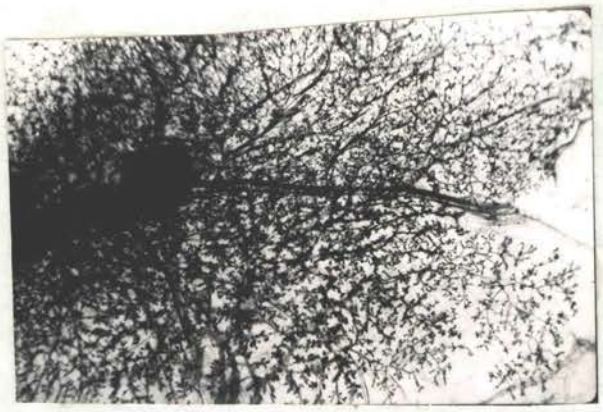
Group	Number of Rats	Body Weight gms.	Treatment		Degree of Total Mammary Growth	Average Degree of Total Mammary Growth	Figure Number
			Systemic	Subcutaneous Injection*			
1	5	160-180	Intact	Sesame Oil 0.1 ml./day	2+ to 4-	3 to 3+	--
2	5	160-180	Intact	C.A. Liq. 0.5 mg./day	3- to 4	3 to 3+	6a
3	5	160-180	Intact	Prog. Liq. 5.0 mg./day	3+ to 4	4-	6b
4	5	160-180	Intact	E.B. Liq. 1 ug./day	2+ to 5	3 to 3+	6c
5	5	160-180	Intact	1 ug. E.B. - 0.5 mg. C.A. daily.	3+ to 5+	5- to 5	6d
6	5	160-180	Intact	1 ug. E.B. - 5.0 mg. Prog. daily	5	5	6e

* Subcutaneous injection for 13 days.



6a. Group 2, cortisone acetate 0.5 mg. daily.

6b. Group 3, progesterone 5 mg. daily.



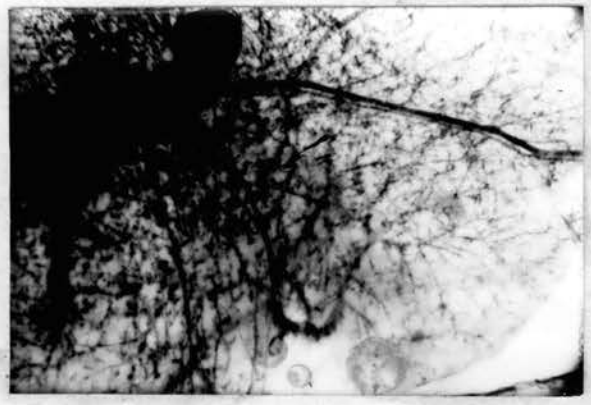
6c. Group 4, estradiol benzoate 1 ug. daily.

6d. Group 5, cortisone acetate 5 mg. and estradiol benzoate 1 ug. daily.

Figure 6. Effect of systemic treatment with cortisone acetate, hydrocortisone acetate and progesterone alone and combined with estradiol benzoate on growth of the mammary system in mature rats. Magnification X3. (see Table VI).

6e. Group 6, progesterone 5 mg. and estradiol benzoate 1 ug. daily.

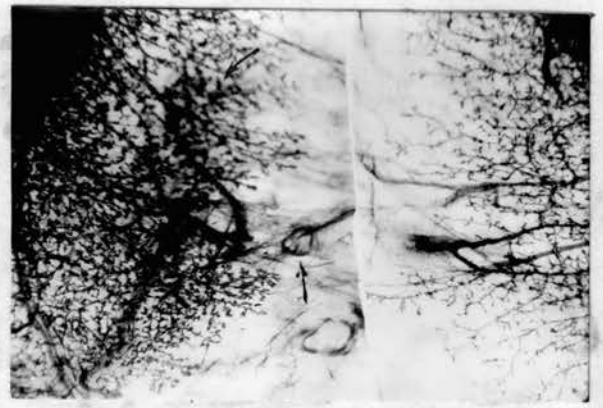
Figure 6 (continued).



7a. Group 1, cortisone acetate implanted. Treated gland.

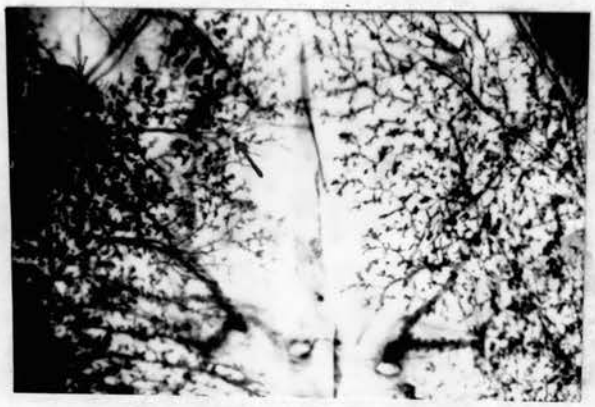
7b. Group 2, hydrocortisone acetate implanted. Treated gland.

Figure 7. The local response of mammary tissue to cortisone acetate, hydrocortisone acetate, progesterone, progesterone-estradiol benzoate and estradiol benzoate pellets in ovariectomized mature rats after about 20 days regression of the gland. Magnification X3. (see Table VIII).



7c. Group 3, progesterone implanted Control gland right, treated gland left.

7d. Group 4, estradiol benzoate implanted. Treated gland.



7e. Group 5, progesterone and estradiol benzoate fused pellet implanted. Control gland right, treated gland left.

7f. Group 6, control, no treatment.

Figure 7 (continued).

estradiol benzoate, cortisone acetate and progesterone. When estradiol benzoate administration was combined with cortisone acetate or progesterone treatment the lobule-alveolar system in all rats was well formed. The same difference in character of the lobule-alveolar system was noted as was noted in the previous experiment.

EXPERIMENT VIII.

Mature virgin rats were ovariectomized, and deferred from any treatment for 19 or 20 days in order to allow the mammary gland system to regress. These rats were implanted with hormone pellets into the gland area as shown in Table VIII and the glands were removed for examination on the ninth day. No systemic treatment was given; the control glands showed a duct system with very few buds.

Treatment with estradiol benzoate locally gave the best mammary growth of any of the treatments. Some systemic effect was noted by development in all the glands. In two animals evidence of a localized action of this hormone was noted. Some alveoli were distended and near the lactation phase.

The estradiol benzoate-progesterone fused pellets demonstrated a local action and a systemic effect. Systemically, the number of buds was increased over those seen in control rats. Locally, the lobule-alveoli were stimulated to further development possessing a more dense nature as described previously to have resulted from progesterone treatment.

Cortisone, hydrocortisone and progesterone all three gave local stimulation to lobule-alveolar development, but to a lesser extent than previously noted. Perhaps this diminished action was due to the low level of endogenous estrogen. The gland may have regressed to such an extent that it was refactory to these hormones.

Table VIII. Effect of Microimpelletation on the Mammary Glands of Mature Ovariectomized Rats, after Regression.

Group	Number of Rats	Body Weight gms.	Treatment		Depression Days	Degree of Total Mammary Growth	Degree of Local Lobular Growth	Figure Number
			Systemic	Impelletation*				
1	3	150-200	Ovar.	C.A. fus.	19	2+ to 3	1, 1, 2	7a
2	3	150-200	Ovar.	H.C.A. fus.	19	3- to 3+	2, 3	7b
3	3	150-200	Ovar.	Prog. fus.	19	3- to 3+	2, 2, 4	7c
4	3	150-200	Ovar.	E.B. fus.	20	4-		7d
5	2	150-200	Ovar.	E.B. Prog. fus.	20	3+ to 4+	3, 3	7e
6	1	150-200	Ovar.	Cont.	20	2		7f

*Pelleted 14-20 days following ovariectomy. Sacrificed 9 days after impelletation.

EXPERIMENT IX.

The same hormones used in the previous experiment were implanted locally into the gland area of castrate male rats (100-130 gm) at the end of a two week recovery period. The animals were sacrified 7 days after implanting and the glands examined.

As in the previous experiment, the control rats showed only a duct system with a few suggestions of buds. No systemic effect resulted from the local implantation of cortisone, hydrocortisone and progesterone. The treatments employing estradiol benzoate on the other hand, resulted in some lobule-alveolar development in the gland system.

Progesterone in the absence of any estrogen treatment gave a local stimulation of lobule development. In some cases, the localized tissue approached an advance stage of lobule-alveolar growth.

Both cortisone and hydrocortisone evoked local lobule-alveolar growth and distension. In general, this effect was not as outstanding as was seen in rats of less advanced age and with gonads intact or only recently removed.

In these castrate male rats, estradiol benzoate in addition to provoking a systemic reaction gave a localized stimulation. Although this effect was slight, its action was definite resulting in an increase in the number of developing lobules in the area of the pellet.

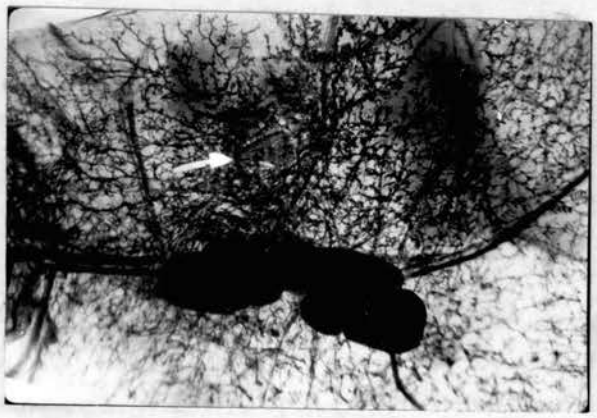
EXPERIMENT X.

Many attempts were made to introduce prolactin and growth hormone into the mammary gland area in order to study their effects. Very slight effects were noted. In this experiment pressed prolactin and growth hormone pellets were available for implantation. Pellets of these hormones were implanted into the glands of mature (180-200 gm.) and immature (80-100 gm.) female rats. This was the only treatment. After 9 days the glands were

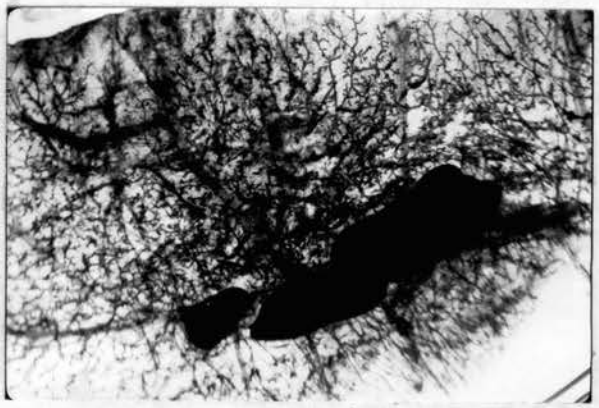
Table IX. Effect of Microimpelletation on the Mammary Glands of Mature Castrate Male Rats.

Group	Number of Rats	Body Weight gms.	Treatment		Degree of Total Mammary Growth	Degree of Local Lobular Growth	Figure Number
			Systemic	Impelletation*			
1	4	100-120	Cast.	C.A. fus.	2- to 2	2, 4	8a
2	4	100-120	Cast.	H.C.A. fus.	2 to 2+	3, 3	8b
3	4	100-120	Cast.	Prog. fus.	2- to 3	3, 5	8c
4	4	100-120	Cast.	E.B. fus.	3 to 4		8d, 8e
5	4	100-120	Cast.	E.B. Prog. fus.	3 to 4-	3, 3	8f
6	4	100-120	Cast.	Cont.	2 to 2+		8g

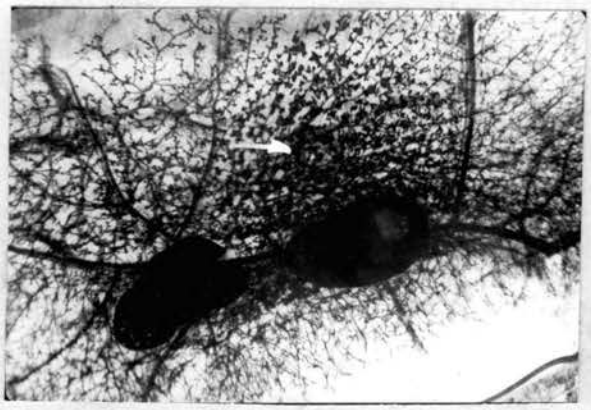
*Pelleted on 14th day following castration. Sacrificed 7 days later.



8a. Group 1, cortisone acetate implanted. Treated gland.



8b. Group 2, hydrocortisone acetate implanted. Treated gland.

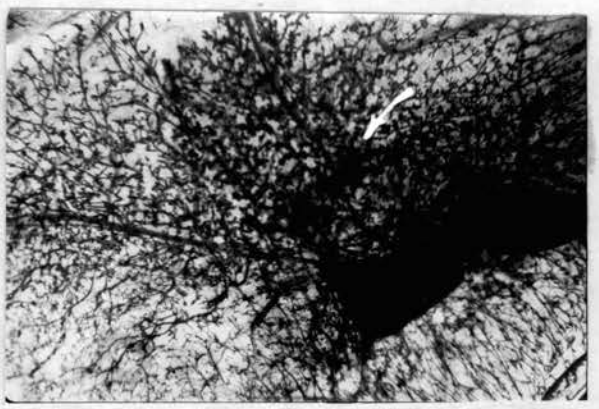


8c. Group 3, progesterone implanted. Treated gland.

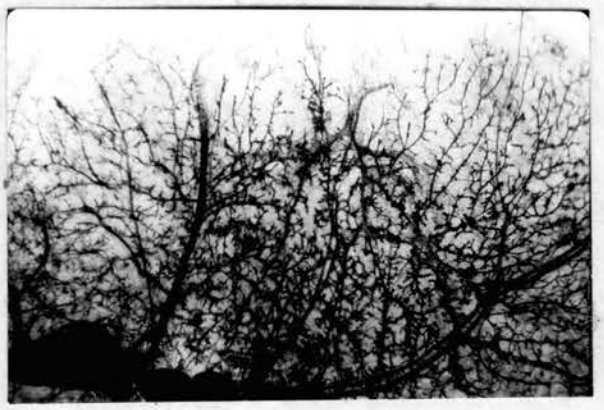
8d. Group 4, estradiol benzoate implanted. Treated gland.

Figure 8. The local response of mammary tissue to cortisone acetate, hydrocortisone acetate, progesterone, progesterone-estradiol benzoate and estradiol benzoate pellets in castrate mature male rats. Treatment started 14 days after castration. The arrows indicate pellet sites. Magnification X3. (see Table IX).

8e. Group 4, estradiol benzoate implanted, control gland right, treated gland left.



8f. Group 5, combined progesterone-estradiol benzoate pellet implanted. Treated gland.



8g. Group 6, control group.

Figure 8 (continued).

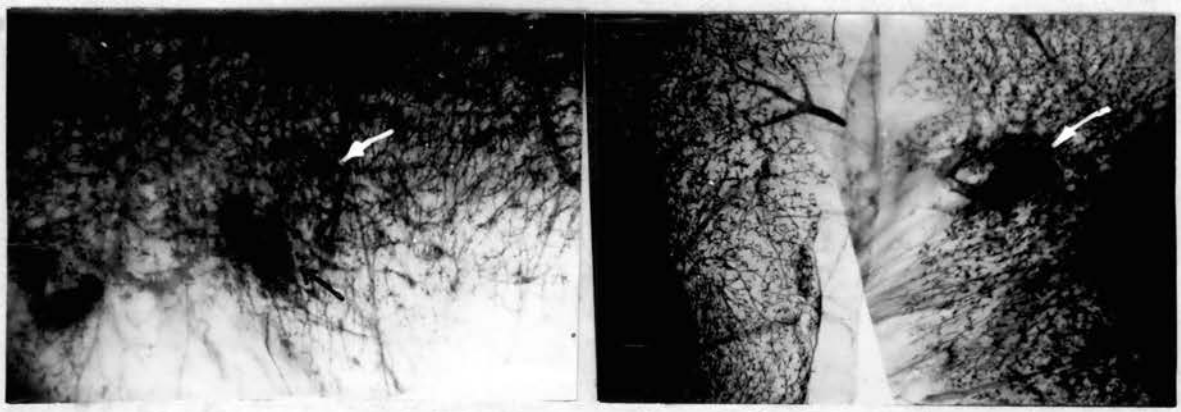
removed for examination. The results are tabulated in Table X.

In rats treated with prolactin a localized lobule-alveolar growth was noted. The somewhat distended nature of the alvoeli indicated early formation of milk which was most apparent in the mature animals. The growth hormone increased the number of buds in the vicinity of the pellets. All glands showed a weak systemic stimulation resulting from the growth hormone.

Table X. Effect of Microimpelletation of Prolactin and Growth Hormone on the Mammary Glands of Mature and Immature Female Rats.

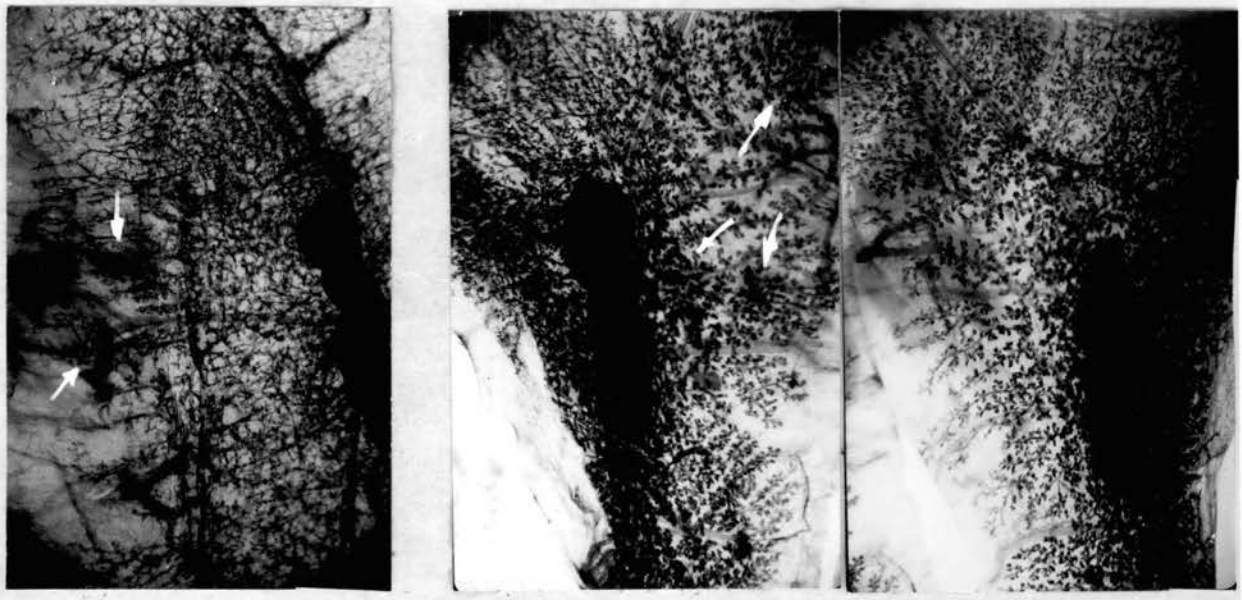
Group	Number of Rats	Body Weight gms.	Age	Treatment		Degree of Total Mammary Growth	Degree of Local Lobular Growth	Figure Number
				Systemic	Impelletation*			
1	2	180-200	Mat.		Prol. Pres.	2+	2, 3	9a
2	2	80-100	Immat.		Prol. pres.	2 to 2+	1, 3	9b
3	1	180-200	Mat.		G.H. pres.	2	2	9c
4	2	80-100	Immat.		G. H. pres.	3 to 3+	1, 2	9d

*Pelleted 9 days before sacrifice.



9a. Group 1, prolactin implanted. Treated gland from mature rat.

9b. Group 2, prolactin implanted. Treated gland from immature rat.



9c. Group 3, growth hormone implanted. Treated gland from mature rat.

9d. Group 4, growth hormone implanted in immature rat. Control gland right, treated gland left.

Figure 9. The local response of the mammary gland to prolactin and growth hormone in immature and mature female rats. Arrows indicate pellet sites. Magnification X3. (see Table X).

DISCUSSION

The micro-impelletation technique as described here has several qualities which make evaluation of results difficult. One serious disadvantage as it was used here was the inability to control the dosage level. It was originally believed that compounding the hormones with a non-aqueous material would restrict diffusion of the hormone and permit some degree of control over absorption. This method was unsuccessful and had to be replaced by the use of fused or pressed pellets with the consequent loss of control over absorption, and thus dosage level. Some hormones are more soluble in tissue fluid than others and there was always some question in the experiments reported here concerning the length of time the hormone remained in contact with the surrounding tissues. Estradiol in microgram quantities has a stimulatory effect on mammary growth. Even with the implantation of small pellets some degree of systemic effect was obtained.

The response of the mammary gland to various hormones depends to some extent upon the previous treatment and the concurrent treatment. To promote and study localized reactions of the mammary gland to hormones it would seem advisable to carefully control the pretreatment stage of the animal and the simultaneous systemic treatment. Considerable care was given to these factors.

The microimpelletation technique of studying the action of hormones on the mammary gland has yielded information on the local action of several hormones in spite of the complicated nature of the endocrines

involved. In some cases a zone of differentiation could be detected in the tissue indicating a zone of diffusion of the hormone from the site of implantation. This is ample proof that the hormones have a direct action on the mammary tissue.

The observations made in the experiments conducted in this investigation are not radically different from those made by systemic administration of the hormones, the main difference being that the results in some cases were more striking.

Estrogens stimulate extension of the duct system when administered systemically. In some cases limited lobule-alveolar growth has been obtained (Nelson 1935, Lewis and Turner 1941), although Smith (1955) reported that estrogens inhibited the peripheral extension of the gland. He reported lobule development on dosages of 0.5 ug or more daily.

Following microimpelletation of estrodiol benzoate pellets, no localized effect could be seen on bud growth in most animals studied. An exception to this was the slight bud development in the vicinity of the hormone pellets in experiments VIII and IX. These rats had been castrated for a period of time before use. Furthermore, it was consistently noted that the implantation of estrogen pellets always promoted a slight growth of the gland. This was noticable in all the mammary glands of the animals, truly a systemic effect. In general, only slight differentiation of tissue was apparent in the vicinity of the estrogen pellets.

Another inescapable observation made throughout this study was that estrogen potentiated or primed the effect of other hormones. Cortisone, hydrocortisone, and progesterone all demonstrated a more striking localized action in systemically estrogen treated rats. (Tables IV, V, VI, and VII). The consistent recurrence of this observation suggests that the estrogens themselves render a profound effect on this tissue, or that they

act indirectly by stimulating another gland, the pituitary, to the production of mammogenic hormones. This latter idea has been advanced by Turner and coworkers (Mixner and Turner 1943).

Progesterone promoted a local stimulation of lobule-alveolar development in the mammary parenchyma (Table I, II, VIII and IX). A distinct enlargement of the lobules was seen in a number of cases in the vicinity of the progesterone pellet. By subcutaneous administration of 5 mg daily no significant lobule-alveolar growth was obtained (Table VI and VII). Numerous workers have reported a similar lack of mammary stimulation by use of progesterone alone. On the other hand, higher doses (15 mg daily) evoke lobule-alveolar response (Selye 1940a). The stimulation obtained by impelletation would agree with both these observations if one considers the high level of progesterone in the vicinity of the pellet. This level may be considered to be sufficiently high to stimulate lobule-alveolar development by local action, whereas parenteral administration of 5 mg per day would not suffice for this action.

Both a local and systemic effect was observed from the implantation of pellets of estradiol benzoate and progesterone fused into the same pellet. The stimulation of all glands to growth was undoubtedly due to the estrogen present. The localized lobule-alveolar development was due to the presence of progesterone (Table I, II, VIII, and IX). Smith (1955) found that systemically administered progesterone by itself was without effect. However, he described its action as potentiating the action of estrogen on the lobule-alveolar system. The local action of progesterone in promoting lobule-alveolar development, even in the absence of estrogens, suggests that the reverse is true, that is, that estrogen potentiates the action of progesterone. The progesterone as well as other hormone responses were always more pronounced in the presence of estrogen.

The lobule-alveolar system developed by progesterone was strikingly different than that developed by cortisone and hydrocortisone. The lobules appeared to be of a more solid nature, with no particular signs of fluid distension, and stained a deeper color. Even though the lobules were large, they did not appear to have fluid present as seen after treatment with cortisone and hydrocortisone. This apparent lack of fluid distension suggests that progesterone stimulates development of the lobule alveolar system and also inhibits the initiation of lactation. This latter idea is suggested by numerous works previously cited.

In the experiments reported here cortisone acetate (11-dehydro-17-hydroxycorticosterone-21-acetate) and hydrocortisone acetate (17-hydroxy-corticosterone-21-acetate) were shown to stimulate both the development of the lobule-alveolar system and lactation (Tables I, II, III, IV, VIII, and IX). Selye (1954) found that these two hormones promoted lobule-alveolar development and lactation in ovariectomized-adrenalectomized rats if simultaneous estrogen treatment was carried out. This general systemic effect is in good agreement with the results of a number of the experiments reported here.

Particularly outstanding was the effect of systemically administered estradiol benzoate and cortisone or hydrocortisone on immature rats. Here the mammary system is infantile but the stimulation brought about by these hormones caused great distension of the immature buds and ducts. Yellow concretions within the lobules suggested that secretory function was initiated (Table IV, X). In many instances a highly abnormal "overstimulated" gland resulted.

Implanted pellets of cortisone or hydrocortisone gave a local stimulation of the mammary parenchyma as just described but confined to only a portion of a single gland. It could be seen that within the

immediate vicinity of the pellet the tissue had shown a large degree of lobule-alveolar development as compared to the remainder of the gland, or the opposite control gland. In addition, the tissues in this region were distended with fluid. The alveoli were prominent in these preparations as though poised for lactation (Tables I, II, III, IV, V).

In adrenalectomized-gonadectomized rats following regression of the gland parenchyma (Table V, VIII, IX), the response to local implantation to cortisone acetate or hydrocrotisone acetate was slight. In the vicinity of the pellets lobules were stimulated, but they were small as compared to lobules of a more mature gland. Even so, alveoli could be seen to be swollen, as if distended with fluid, suggesting the localized initiation of lactation under these severe conditions.

The similarity of progesterone and adrenal cortical hormones has received much discussion. Progesterone has been isolated from the adrenals (Callow and Parker 1936, Becal 1938). Some adrenal cortical extracts have shown progestational activity (Gaunt and Nelson 1938), or adrenal compounds may be converted to progesterone and vice versa. (Lazo-wasem and Zanow 1955, Manerva, et al 1952, Farrow, et.al. 1950). The similarity of these hormones is seen in the lobule-alveolar responses they provoke. Courrier (1954) however, wrote "cortisone is not progestationally active, it can even interfere with the normal development of pregnancy". He indicated that cortisone is an antagonist to progesterone. The action of these two hormones on the mammary gland is alike in that they both stimulate lobule-alveolar growth by local action. On the other hand, they differ in that cortisone promotes the initiation of lactation within the parenchyma as evidenced by the distended alveoli, and the diffuse staining of the gland system. This is in opposition to the observed effect of progesterone, which gave good lobule-alveolar development, but

the lobules were more dense, deeper staining, and showed no evidence of lactation. In the initiation of lactation these hormones appear to be antagonistic to each other.

Are progesterone or cortisone interconverted at the site of the implant? This is a just question which cannot be answered with our fragmentary knowledge. Is the response seen in the treatment of the gland a non-specific response resulting from tissue injury? The different responses to the various hormones implanted may be interpreted or demonstrating that it is the hormone ifself which is the stimulating agent. The similarity of local activity and that seen by parenteral administration suggests that these are indeed specific responses.

The lack of response of the mammary gland to prolactin throughout the conduct of these experiments was unexpected, particularly in view of the well demonstrated fact that prolactin initiates lactation in the well developed gland of various animals. One possible explanation lies in the fact that the prolaction may be absorbed too readily into the system to evoke a localized response. In those cases in which pressed pellets were administered (Table X) some local response was noted. Most noticeable was an increase in the number of buds, but not in their size. Growth hormone gave a similar response locally, with some indication of a systemic stimulation. More detailed studies are needed before the action of these two hormones can be correlated with a particular role in mammary development, although both hormones have been implicated in a number of ways.

Endocrine control of duct growth has been extremely difficult to study. In these experiments there were faint suggestions of localized duct growth stimulation, but nothing calling for specific studies of this

subject. This problem could probably be better studied in the absence of pituitary factors.

In conclusion it may be said that hormone pellets may be successfully implanted into the mammary gland area (and perhaps other tissues as well) and be studied for their stimulatory effects on the mammary parenchyma. The studies presented here suggest that by varying the systemic treatment of the animal and the microimpelletation, the specific endocrine control of mammary development and function may be studied. Observations of a preliminary nature suggest that estrogens, progesterone and adrenal cortical hormones (cortisone and hydrocortisone) all can have a role in normal development of this gland. More extensive studies using this technique are needed to determine the action of these hormones in the absence of the pituitary gland.

SUMMARY

1. A technique has been described in which small hormone pellets were implanted into the mammary gland tissues of rats to study the direct effect of these hormones on development of the mammary parenchyma. The technique has been called "microimpellètation". Results of the use of this technique suggest that it might be used to an advantage in elucidating the direct action of various hormones on the mammary gland.

2. The microimpelletation technique was combined with various systemic treatments in order to describe the conditions under which the implanted hormones were most active.

3. Estradiol benzoate stimulated whole gland growth when administered systemically. This hormone was without noticeable localized effect in most cases, but even when implanted as small pellets it gave a systemic stimulation to all of the glands. It augmented the localized actions of progesterone, cortisone acetate and hydrocrotisone acetate.

Progesterone promoted lobule-alveolar growth in a small portion of the gland when implanted. This lobule-alveolar system showed no particular signs of milk formation.

Cortisone acetate and hydrocortisone acetate stimulated lobular growth in an area around the implanted pellet. In addition the alveoli showed early signs of lactation.

Both prolactin and growth hormone gave a week stimulus to lobular growth in the experiments conducted.

4. The results were interpreted as a truly direct effect of the hormones on the mammary gland, particularly in view of the divergence of

responses of the gland to the different types of hormones. The responses to microimpelletation were in agreement with other observations made in this study by the parenteral administration of hormones or with observations reported in the literature.

5. The microimpelletation technique has been tested for its fitness in studying problems of this nature. It is believed to have the advantage of eliminating possible complex reactions which favor misinterpretation of results. It has definite possibilities of application to the study of other endocrine problems where the knowledge of the direct action of the hormone is desired.

A SELECTED BIBLIOGRAPHY

Astwood E. B. and C. F. Geschickter, 1938. Changes in mammary gland of the rat produced by various glandular preparations. Arch. Surg. 36: 672.

Astwood, E. B., C. F. Geschickter and E. O. Bausch, 1937. Development of the mammary gland of the rat. A study of normal, experimental and pathological changes and their endocrine relationships. Am. J. Anat. 61: 373.

Becal, D., 1938. Isolation of progesterone and 3:20-allopregnanolone from ox adrenals. J. Biochem. 32: 1957.

Brownell, K.A., J. E. Lockwood and F. A. Hartman, 1933. A lactation hormones of the adrenal cortex. Proc. Soc. Exper. Biol. Med. 30: 783.

Callow, R. K., and A. S. Parkes, 1936. The occurrence of oestrin and progestin in adrenal, testis and hypohysis. J. Physiol. 87: 28

Chamberlin, T. L., W. U. Gardner and E. Allen, 1941. Local responses of the "sexual skin" and mammary glands of monkeys to cutaneous applications of estrogen. Endocrin. 28: 753.

Chen, T. T., R. E. Johnson, W. R. Lyone, C. H. Li, and R. D. Cole, 1955. Hormonally induced mammary growth and lactation in the absence of the thyroid. Endocrin. 57: 153.

Corner, G. W., 1930. The hormonal control of lactation. Am. J. Physiol. 95: 43.

Courrier, R., 1950. Interactions between estrogens and progesterone. In R. S. Harris and K. V. Thimann: Vitamins and Hormones. Vol. VIII, p. 179.

Courrier, R., 1954. Ann. d'endocrin. 15:1. Cited by Selye, H. 1954. The effect of cortisol upon the mammary glands. Acta. Endocrin. 17: 394.

Cowie, A. T., 1952. Influence on the replacement value of some adrenal cortex steroid of dietary sodium and synergism of steroid in lactating adrenalectomized rats. Endocrin. 51: 217.

Cowie, A. T. and S. J. Folley, 1945. Parathyroidectomy and lactation in the rat. Nature, 156: 719.

Cowie, A. T. and S. J. Folley, 1947a. The measurement of lactational performance in the rat in studies of the endocrine control of lactations. J. Endocrin. 9: 13.

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

Cowie, A. T. and J. S. Tindal, 1955. Maintainance of lactation in adrenalectomized rat with aldosterone and 9 -halo-derivatives of hydrocortisone. Endocrin. 56: 612.

Curtiss, C., 1949. Factors influencing lobule-alveolar development and mammary secretion in rat. Endocrin. 45: 284.

Desclin, L., 1952. Recherches sur le Determinisme des phenomenes de secretion dans la Glande Mammaire du Rat. Ann. d'endocrin. 13: 120.

Elliott, J. R. and C. W. Turner, 1951. Some hormones involved in elaboration or activation of the mammary spreading factor. Proc. Soc. Exper. Biol. Med. 77: 320.

Elliott, J. R. and C. W. Turner, 1953. The mammary gland spreading factor. Agr. Exper. Sta. Res. Bull. 537.

Emmens, C. W., 1950. Hormone Assay. New York Academic Press. New York.

Flux, D. S., 1955. The value of some steroids in replacement therapy in adrenalectomized and adrenalectomized-ovariectomized lactating rats. J. Endocrin. 12: 57.

Folley, S. J., 1952. Lactation. In Marshall's Physiology of Reproduction. Vol. II. Ongmans, Green and Co. London.

Folley, S. J. and F. H. Malpress, 1948. Hormonal control of mammary growth. Hormonal control of lactation. In G. Pincus and K. V. Thimann: The Hormones. Academic Press Inc. Publishers. New York.

Gardner, W. U., 1940. Growth of mammary glands in hypophysectomized mice. Proc. Soc. Exper. Biol. Med. 45: 835.

Gardner, W. U. and T. L. Chamberlin, 1941. Local action of estrone on mammary glands of mice. Yale J. Biol. Med. 13: 461.

Gardner, W. U. and A. White, 1941. Mammary gland growth in hypophysectomized male mice receiving estrogen and prolactin. Proc. Soc. Exper. Biol. Med. 48: 590.

Gaunt, R. and W. O. Nelson, 1938. Cortical hormone-like action of progesterone in rats. Anat. Rec. 72: 58.

Gaunt, R., W. J. Eversole and E. C. Kendall, 1942. Influence of some steroid hormones on lactation in adrenalectomized rats. Endocrin. 31: 84.

Gomez, E. T. and C. W. Turner, 1937. Hypophysectomy and replacement therapy in relation to the growth and secretory activity of the mammary gland. Mo. Agr. Exper. Sta. Res. Bull. 259.

Hohn, E. O., 1954. Direct progestational action of progesterone and certain related steroids on the endometrium of the rhesus monkey. J. Endocrin. 10: 358.

Jadassohn, H., E. Uchlinger, and W. Zuescher, 1937. The enlargement of the nipple of the breast of guinea pigs by means of hormone. The local folliculin effect. Klin. Wochschr. 16:314.

Kirkham, W. R. and C. W. Turner, 1954. Introduction of mammary growth in rats by estrogen and progesterone. Proc. Soc. Exper. Biol. Med. 87: 139.

Laquer, E. 1928. Uber weihliches sexual-hormone, Menforumon V. Uber den femins urenden einfluss des menformons auf de unentwickelte brustdruse. Deutsch. Med. Wochenschr. 53: 867.

Lazo-wasem, E. A. and M. X. Zarrow, 1955. The conversion of desoxycorticosterone acetate to a progesterone-like substance. Endocrin. 56: 511.

Leonard, S. L. 1943. Stimulation of mammary glands in hypophysectomized rats by estrogen and testosterone. Endocrin. 32: 229.

Leonard, A. L., 1945. The relation of the placenta to the growth of the mammary gland of the rat during the last half of pregnancy. Anat. Rec. 91: 65.

Leonard, S. L. and R. P. Reese, 1942. Failure of steroid hormones to induce mammary growth in hypophysectomized rats. Endocrin. 30: 32.

Leonell, C. S., W. U. Gardner, and R. T. Hill, 1937. Production of estrogenic hormone by a transplantable ovarian carcinoma. Endocrin. 21: 268.

Lewis, A. A. and C. W. Turner, 1939. The mammogenic hormones of the anterior pituitary. I. The duct growth factor. Mo. Agr. Exper. Sta. Res. Bull. 310.

Lewis, A. A. and C. W. Turner, 1941. Effect of stilbestrol on the mammary gland of the mouse, rat, rabbit and goat. J. Dairy Sci. 24: 845.

Lewis, A.A., C. W. Turner and E. T. Gomez, 1939. The biological assay of the mammogenic duct growth factor of the anterior pituitary. Endocrin. 24: 157.

Lyons, W. R. 1942. The direct mammotrophic action of lactogenic hormone. Proc. Soc. Exper. Biol. Med. 51: 308.

Lyons, W. R. 1944. Evidence of placental mammotrophin. Anat. Rec. 88: 446.

Lyons, W. R., 1950. Lobule-alveolar mammary growth in the rat. Colloque International du CNRS, Strasbourg.

Lyons, W. R. and Y. Sakot, 1940. Direct action of estrone on the mammary gland. Proc. Soc. Exp. Biol. Med. 44: 398.

Lyons, W. R. C. H. Li, R. E. Johnson, 1952. The enhancing effect of somatotropin on the mammary growth induced in rats with estrin, progestin and mammotropin. J. Clin. Endocrin. 12: 937.

Lyons, W. R.,C. H. Li, R. E. Johnson and R. D. Cole, 1955. Internations symposium on hypophyseal growth hormone, nature and actions. Blakiston Co. Inc. New York.

Mancera, O., A. Zaffaroni, B.A., Rubin, F., Sondheimer, G. Rosenkranz and C. Djerassi, 1952. A ten step conversion of progesterone to cortisone. J. Am. Chem. Soc. 74: 3711.

Meites, J. 1950. Artificial induction of mammary growth and lactation in sterile heifer with stilbestrol-progesterone implantations. Mich. Agr. Exper. Sta. Quart. Bull. 32:445.

Meites, J., 1954. Recent studies of the mechanisms controlling the iniation of lactation. Revue Canad. de Biol. 13: 359.

Meites, J. and J. T. Sqouris, 1953. Can the ovarian hormones inhibit the mammary response to prolactin. Endocrin. 53: 17.

Meites, J. and J. T. Sqouris, 1954. Effect of altering the balance between ovarian hormones and prolactin on initiation of lactation in rabbits. Fed. Proc. 13: 134.

Meites, J. and C. W. Turner, 1948. Studies concerning the induction and maintenance of lactation. Mo. Agr. Exper. Sta. Res. Bull. 415.

Mixner, J. P. and C. W. Turner, 1942a. 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.

Mixner, J. P. and C. W. Turner, 1942b. Progesterone-like activity of some steroid compounds and of diethylstilbestrol in stimulating mammary lobule-alveolar growth. Endocrin. 30: 706.

Mixner, J. P. and C. W. Turner, 1942c. Influence of the thyroxine upon mammary lobule-alveolar growth. Endocrin. 31: 345.

Mixner, J. P. and C. W. Turner, 1943. The mammogenic hormones of the anterior pituitary. II. The lobule-alveolar growth factor. Mo. Agr. Exper. Sta. Res. Bull. 378.

Mixner, J. P., A. A. Lewis, and C. W. Turner, 1940. Evidence for the presence of a second mammogenic (lobule-alveolar) factor in the anterior pituitary. Endocrin. 27: 888.

Nathanson, I. T., D. T. Shaw and C. C. Franseen, 1939. Effect of growth complex and estradiol on mammary growth of hypophysectomized rat. Proc. Soc. Exper. Biol. Med. 42: 652.

Nelson, M. M. and H. M. Evans, 1954. Maintenance of pregancy in the absence of dietary protein with estrone and progesterone. Endocrin. 55: 543.

Nelson, W. O., 1935. Gonad hormone effects in normal, sprayed and hypophysectomized rats. Anat. Rec. 64: (Suppl. 1) 52.

Nelson, W. O., 1941. The relation of the adrenal cortex to the production of sex hormones. Twenty-fifth Annual Meeting and Scientific Session of Association for the Study of Internal Secretions. p. 24.

Nelson, W. O. and T. F. Gallagher, 1936. Some effects of androgenic substances in the rat. Science, 84: 230.

Peterson, W. E., 1948. Hormonal control of lactation. Recent Progress in Hormone Research, 2: 133.

Peeters, G. L., M. R. Coussens and M. Vandeplaasche, 1949. Implantation of synthetic estrogens in the udder of heifers. Arch. Intern. Pharmacodynamic 78: 548.

Ray, E. W., S. C. Averill, W. R. Lyons and R. E. Johnson, 1955. Rat placental hormonal activities corresponding to those of pituitary mammotropin. Endocrin. 56: 359.

Reece, R. P. and J. A. Bivins, 1942. Progesterone effect on pituitary lactogen content and on mammary gland of ovariectomized rats. Proc. Soc. Exper. Biol. Med. 49: 582.

Reece, R. P. and S. L. Leonard, 1941. Effect of estrogen, gonadotropin and growth hormone in mammary glands of hypophysectomized rats. Endocrin. 29: 297.

Ruinen, F. H., 1932. Uber den Angriffspunkt der Mammawirkung von Menformon. Acta. Brev. Neerl. 2: 161.

Selye, H. 1940a. Activity of progesterone in sprayed females not pretreated with estrin. Proc. Soc. Exper. Biol. Med. 43: 343.

Selye, H., 1940b. Effect of chronic progesterone overdosage on the female accessory organs of normal, ovariectomized and hypophysectomized rats. Anat. Rec. 78: 253.

Selye, H., 1954. The effect of cortisol upon the mammary glands. Acta. Endocrin. 17: 394.

Selye, H., 1954a. Stress and lactation. Revue Canad. de Biol. 13: 377.

Selye, H. and J. B. Collip, 1936. Fundamental factors in the interpretation of stimuli influencing endocrine glands. Endocrin. 20: 667.

Selye, H., H, S. L. Browne and J. B. Collip, 1936a. Effect of large doses of progesterone in the female rat. Proc. Soc. Exper. Biol. Med. 34: 472.

Selye, H., C. S. McEuen and J. B. Collip, 1936b. Effect of testosterone on mammary gland. Proc. Soc. Exper. Biol. Med. 34: 201.

Silver, M., 1953. A quantitative analysis of the role of estrogen in mammary development in the rat. J. Endocrin. 10:17.

Smith, T. C., 1955. The effect of estrogen and progesterone on mammary gland development in the rat. Endocrin. 57: 33.

Smith, T. C. and L. E. Braverman, 1953. The action of desoxycorticosterone acetate on the mammary glands of the immature ovariectomized rat. Endocrin. 52: 311.

Speert, H., 1940. Mode of action of estrogen on the mammary gland. Science 92: 461.

Stricker, P. and F. Grueter, 1928. Action du Lobe Anterieur de l'Hypophyse sur la Montee Laiteuse. Compt. Rend. Soc. de Biol. 99: 1978.

Thomas, A. D. and W. R. Lyons, 1954. Effect of estrone, progesterone and pituitary mammotropin on the mammary glands of castrate C3H male mice. Endocrin. 55: 191.

Trentin, J. J. and C. W. Turner, 1947. Effect of adrenalectomy on the mammary glands of the castrated and estrogen-treated castrated male rat. Endocrin. 41: 127.

Trentin, J. J. and C. W. Turner, 1948. The experimental development of the mammary gland with special reference to the interaction of the pituitary and ovarian hormones. Mo. Agr. Exper. Sta. Res. Bull 418.

Turner, C. W., 1939a. The mammary gland. In Allen, Danforth and Doisey: Sex and Interual Secretion. The William and Wilkins Co. Baltimore. 2nd. Edit.

Turner, C. W., 1939b. The comparative anatomy of the mammary glands. University Cooperative Store, Columbia, Mo.

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. Exper. Sta. Res. Bull. 157.

Weichert, C. K., R. W. Boyd and P. S. Cohen, 1934. A study of certain endocrine effect on the mammary gland of female rat. Anat. Rec. 61:21.

Zarrow, M. X., F. L. Hisaw and F. Bryans, 1950. Conversion of desoxycorticosterone acetate to progesterone in vivo. Endocrin. 46: 403.

Zondek, B. 1935. Hormone d. ovarium du.d. Hypophysenvorderlappens. Vienna, p. 104.

Zondek, B. 1938. Cutaneous application of folliculin Lancet. 234: 1107.

VITA

Kwoh Hsiong Li

Candidate for the Degree of

Master of Science

Thesis: A MICROIMPELLETATION TECHNIQUE FOR STUDYING THE LOCALIZED ACTION OF HORMONES AND SOME RESULTS OF ITS USE IN THE MAMMARY GLAND

Major: Biochemistry

Biographical and Other Items:

Born: July 1, 1916 at Canton, Kwangtung, China.

Undergraduate Study: University of Nanking, 1934-1937; 1938-1939.

Graduate Study: Oklahoma Agricultural and Mechanical College, 1954-1956.

Experience: The First Fuel Plant of the Ministry of War of China, 1940-1945. Pei Pue Coal Tar Plant of the National Resource Committee of China, 1945-1946. Cheluchien Sugar Factory of Taiwan (Formosa) Sugar Corporation, 1946-1954. Graduate Assistant in the Agricultural Chemistry Department of Oklahoma Agricultural and Mechanical College, 1955-1956.

Member of: Phi Lambda Upsilon and American Chemical Society.

Date of Final Examination: May, 1956.