EFFECTS OF ADRENAL CORTICAL AND PITUITARY HORMONES ON

INITIATION AND MAINTENANCE OF LACTATION IN RATS

by

Robert Merrill Johnson

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology and Pharmacology

1957

Approved by *Joseph Meites*

ProQuest Number: 10008533

ProQuest.

ProQuest 10008533

Published by ProQuest LLC (2016). Copyright of the Dissertation is held by the Author.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

Robert Merrill Johnson

1. In Experiment I, physiological saline (0.85 percent) or 2 mg. doses of ACTH, cortisone or hydrocortisone were injected daily for 10 days into 42 intact, mature female rats. All three hormones produced growth of ducts, lobule-alveolar development, and secretory activity in the mammary glands. This was most marked for cortisone and least for ACTH at the dose levels employed.

2. Cortisone increased pituitary prolactin content by about 23 percent, hydrocortisone by about 41 percent, and ACTH by about 71 percent.

3. In Experiment II, 100 mature female rats were ovariectomized and divided into ten groups of 10 rats each, according to weight. Eight groups were divided into two major groups of 40 rats each (four groups of 10 each) and the remaining two groups (20 rats each) were used for controls. The treatment of the groups was as follows:

(1) controls, saline only,

(2) controls, 1 mg. cortisone only.

The two major groups received either 5 ug. or 10 ug. of estrone. Within these two major groups cortisone was administered to each sub-group as follows: 1) none, 2) 1 mg. daily, 3) 2 mg. daily, 4) 4 mg. daily. All injections were made for 10 days.

4. Cortisone alone stimulated lobule-alveolar development and secretory activity in the mammary glands of the ovariectomized rats. However, mammary growth was not as pronounced as observed in the intact rats of Experiment I. On the other

hand, when cortisone and estrone were given together, marked lobule-alveolar development was elicited and secretory activity was greatly increased.

5. Cortisone augmented the pituitary prolactin content of ovariectomized-estrone treated rats at the 1 mg. level. However, when 4 mg. of cortisone was given daily with 10 ug. of estrone, there was an inhibition of estrone action on the pituitary.

6. In Experiment III, the effects of cortisone on galactopoiesis was studied in 30 mature female rats. These rats were bred and at parturition their litters were reduced to 7 young each. The dams were injected daily during an 18-day post-partum period with 0.25, 0.5 or 1.0 mg. of cortisone. The lactational response was measured by the use of litter growth rate. The rats receiving 0.5 mg. cortisone showed a significant increase in milk yield during the peak (6th-10th day post-partum) and during the declining phase of lactation (11th-18th day post-partum).

Cortisone at the 1.0 mg. level only slightly increased the average litter growth during the 18-day experimental period. However, during the declining phase of lactation, significant increases in litter weight gain were noted over that of the controls.

6. The results of Experiment III were confirmed in Experiment IV. In addition, injections of cortisone were continued for 10 days after the young were removed (18th-28th

day), in order to study its effects on mammary involution. It was found that cortisone at the 1.0 mg. level markedly retarded mammary involution. These mammary glands were comparable to those of an untreated rat 5 days after removal of the young. Cortisone at the 0.5 mg. level produced slightly less retardation of mammary disintegration, comparable to that of an untreated rat six days after removal of its litter.

7. In Experiment IV the effect of growth hormone, prolactin, oxytocin and ACTH on galactopoiesis and mammary involution were studied. Prolactin given at a dosage of 1 mg. daily increased the average litter weight throughout the 18-day post-partum period. These increases were about equal to those of the rats treated with 0.5 mg. of cortisone daily. When cortisone, prolactin and growth hormone were administered together, the response was of about the same magnitude as cortisone or prolactin alone. Thus no synergistic action on galactopoiesis was exerted by these hormones. Growth hormone, when given alone, did not increase lactation.

8. Prolactin (1 mg.) or prolactin, cortisone and growth hormone given together, retarded mammary involution comparable to that of a mammary gland of an untreated rat 5 days after removal of the young. Growth hormone alone at the level employed did not retard mammary involution.

9. Oxytocin and ACTH exhibited galactopoietic effects in parturient rats comparable to those of prolactin or cortisone-treated rats. However, the former two hormones

showed no ability to retard mammary involution following re-
moval of the young for 10 days.

10. In Experiment V, suckling decreased the pituitary
prolactin content in lactating rats. Electrical stimulation
of the cervix of lactating rats appeared to increase pituitary
prolactin content over that of non-suckled or suckled rats.
Injections of oxytocin appeared to produce a large increase
in pituitary prolactin content over that of suckled, non-suckled
or electrically stimulated rats. It appears, therefore, that
neither oxytocin nor electrical stimulation of the cervix in-
duces a release of prolactin from the pituitary.

EFFECTS OF ADRENAL CORTICAL AND PITUITARY HORMONES ON

INITIATION AND MAINTENANCE OF LACTATION IN RATS

by

Robert Merrill Johnson

A THESIS

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology and Pharmacology

1957

Dedicated

to

my wife

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENTS

INTRODUCTION

It is widely accepted that mammary growth requires estrogen for duct growth, and progesterone and estrogen in combination for lobule-alveolar development. In some species, i.e., the guinea pig, cow, goat, etc., estrogens alone in sufficient amounts are capable of producing lobule-alveolar growth. A possible explanation is that the guinea pig and other species may be able to secrete progesterone or other adrenal cortical hormones from the adrenal cortex under estrogen stimulation, which may stimulate lobule-alveolar mammary growth (Höhn, 1957).

Desoxycorticosterone (DCA) has about one-third the mammary growth activity of progesterone in mice (Mixner and Turner, 1943) and it is able also to stimulate mammary growth in guinea pigs (Van Heuverswyn, et al, 1939; Nelson, 1937).

The importance of the adrenal cortical hormones for the maintenance of lactation is well established. Adrenalectomy during lactation results in cessation of lactation, but if the adrenal cortical hormones are replaced, lactation can be maintained. It has not been definitely established whether these hormones are only necessary for the general maintenance of carbohydrate, protein and electrolyte metabolism in the body as a whole, or if they have a more direct role in the initiation and maintenance of lactation.

It has been established that lactation in most mammals rapidly reaches a peak and then slowly declines over a long period of time. Attempts have been made to increase milk yield during lactation by the use of hormones. Crude anterior pituitary extracts given during the declining phase of lactation in cattle produces a temporary increase in milk secretion (Asimov and Krouze, 1937; Folley and Young, 1938). A more pronounced increase can be obtained by the injection of growth hormone while the galactopoietic effect of purified prolactin is relatively small (Folley, 1955). This suggests that prolactin is not the only hormone responsible for the galactopoietic potency of the pituitary, and that other hormones of the pituitary may act synergestically with prolactin. Despite its low galactopoietic potency, prolactin is essential for the initiation and maintenance of lactation.

Other factors leading to the decline in lactation are the involutionary changes in the mammary gland (Selye and McKeown, 1934; Turner and Reineke, 1936). It is well established that suckling or milking causes the release of pitocin and prolactin which may be at least partially responsible for the maintenance of the mammary gland. Williams (1945) has shown that prolactin is capable of retarding mammary involution in parturient, non-suckled rats. However, little is known of the action of other hormones on the maintenance of the mammary gland during lactation. The adrenal cortex may play a role

since Gregoire (1947) and others have shown that ACTH is re-
leased during suckling.

This thesis is an attempt to provide additional in-
formation on the relation of the adrenal cortex and pituitary
hormones on the initiation and maintenance of lactation. The
specific problems studied were: (1) the effects of adrenal
glucocorticoids and ACTH on mammary growth and secretion;
(2) the effects of ACTH and glucocorticoids on pituitary pro-
lactin content; (3) the possible galactopoietic effects of
cortisone, prolactin, oxytocin, ACTH and growth hormone in
lactating rats; (4) the effects of all these hormones on the
retardation of mammary involution after parturition, and
(5) the effects of oxytocin on prolactin release in the lac-
tating rat.

REVIEW OF LITERATURE

Hormonal Control of Mammary Growth

Estrogens

It was concluded in earlier work that duct growth could be induced with estrogen alone, but that complete development of the lobule-alveolar systems required progesterone as well. While estrogen stimulates duct growth in all species, the lobule-alveolar response to estrogens varies among different species. For example, in the mouse, estrogen-induced growth has been reported to be limited to the duct system with no lobule-alveolar development (Bradbury, 1932; Turner and Gomez, 1934). In the rat estrogen induces a limited lobule-alveolar development (Turner and Schultz, 1931), while in the guinea pig estrogen will cause complete lobule-alveolar development (Nelson, 1937).

With the development of synthetic estrogens a number of workers have been able to produce fairly large milk yields in dairy cows and goats from estrogenic treatment alone, which suggests that this hormone may induce some lobule-alveolar development. (Mixner, Meites and Turner, 1944, in goats; Folley, Steward and Young, 1944, in cows; Walker and Stanley, 1940, in spayed heifers.)

Mixner and Turner (1943) reported that lobule-alveolar development in the goat udder stimulated by diethylstilbestrol was not normal in appearance. The alveoli were oversized and papillomatous outgrowths of the epithelium projected into the lumen. However, when progesterone was administrated along with diethylstilbestrol, normal alveolar development occurred. It should be pointed out that in these experiments and in those reported by Nelson (1937) in the guinea pig, there is the possibility of intervention by extra-ovarian progesterone or other steroids from the adrenal cortex. Thus the ability of estrogens to induce some lobule-alveolar growth does not rule out the need for progesterone or progesterone-like compounds.

Progesterone

That the corpus luteum of pregnancy has a definite role in developing the mammary gland was shown early by Nelson and Pfiffner (1930, 1931). They were able to obtain lobule- alveolar development in male and spayed female rabbits, rats and guinea pigs with injections of crude corpus luteum extracts. However, Turner and Schultze (1931) observed that crude progesterone itself had very little effect on mammary growth unless estrogen was given first as a primer. Gardner and Hill (1936), on the other hand, showed that large doses of progesterone alone were capable of producing good lobule- alveolar development in mice. Mixner and Turner (1943) suggested that the ability of small doses of progesterone to

elicit lobule-alveolar development in the presence of estrogen was due to the action of estrogen in producing hyperaemia of the mammary stroma, leading to an increased vascular permeability. This provides an easy access to the gland of progesterone and other mammary stimulating hormones needed for the growing tissues.

Anterior Pituitary Hormones

The work of Corner (1930) and Lyons and Catchpole (1933) demonstrated a mammary growth response to anterior pituitary extracts, suggesting that this gland plays a role in normal mammary development. However, other workers failed to stimulate mammary growth with estrogen alone or with estrogen-progesterone combinations in hypophysectomized animals. (Reece, Turner and Hill, 1937; Gomez and Turner, 1937a, in the rat; Gomez and Turner, 1937a, in the guinea pig.)

As a result of the above findings Turner postulated the "mammogen theory." He stated that the ovarian hormones, estrogen and progesterone, do not exert their growth stimulus directly on the mammary gland but evoke their effects through the anterior pituitary, which secretes a "duct-stimulating hormone" and a "lobule-alveolar hormone." These were named "Mammogen I," the duct growth factor stimulated by the action of estrogen, and "Mammogen II" the lobule-alveolar factor elicited by progesterone stimulation. To support this theory, Turner and his students in a series of papers, (Gomez and

Turner, 1937a; Gomez, Turner and Reece, 1937 ; Gomez and Turner, 1938) showed that pituitaries from estrogen-primed rats would induce mammary growth while pituitaries from untreated rats had no effect in the hypophysectomized male guinea pig. When pituitary extracts from pregnant cows were injected into rats, a greater degree of mammary growth was produces than with pituitary extracts from non-pregnant cows. Nelson (1941) and Folley et al (1940) showed that topical application of estrogens to rudimentary mammary glands of guinea pigs and goats respectively, produced mammary development while adjacent non-treated glands showed little or no growth. This suggested that estrogens acted directly on the mammary gland. Mixner and Turner (1943) interpreted these results to mean that topical applications elicited a localized hyperaemia in the gland which enabled sub-threshold levels of mammogens to become effective.

Lyons (1942) reported convincing evidence that pituitary prolactin may be a growth-stimulating factor by obtaining localized alveolar growth following intraductal injection of prolactin in the teats of rabbits. In hypophysectomized rats, Lyons (1943) was able to obtain well developed mammary glands by injecting estrogen and prolactin combinations. Lyons et al (1955) showed that in hypophysectomized, gonadectomized immature male rats, it was possible to produce different degrees of development of the female mammary glands by the following sequence of injections: (1) estrone and growth hormone

produced mammary duct growth, (2) estrone, progesterone and
prolactin produced lobule-alveolar growth comparable to early
pregnancy, (3) estrone, progesterone, lactogenic and growth
hormone produced lobule-alveolar development comparable to
late pregnancy, (4) prolactin, growth hormone and hydrocortisone
acetate initiated milk secretion in the fully developed glands.
It seems logical to conclude therefore, that the ovarian hor-
mones stimulate mammary growth best in the presence of anterior
pituitary hormones. The "mammogenic hormones" of the anterior
pituitary appear to be prolactin, ACTH and growth hormone.

Adrenal Cortex

The role of the adrenal cortex in mammary development
is not yet fully understood. Trentin and Turner (1947) reported
that adrenalectomy reduced mammary growth in rats, while Smith-
cors and Johnston (1948) found an increase in mammary growth
in adrenalectomized rats. Van Heuverswyn et al (1939) and
Nelson (1937) noted that desoxycorticosterone (DCA) stimulated
duct growth in mice and guinea pigs respectively. Mixner and
Turner (1943) reported that DCA had about 1/3 the mammary growth
activity of progesterone in mice. Turner and Meites (1947)
observed that DCA did not augment the pituitary prolactin con-
tent in female rats.

Recently the mammary growth effects of ACTH and the
glucocorticoids have become the subject of investigation.
Nelson (1941) noted that crude ACTH preparations stimulated

mammary growth in hypophysectomized rats. However, Flux (1954) observed that cortisone, hydrocortisone and ACTH produced inhibition of mammary growth when injected into estrone-treated, ovariectomized mice of the CHI strain. When given alone, ACTH and the glucocorticoids were without effect on the mammary glands of ovariectomized mice.

In contrast Selye (1954a) found substantial mammary development and secretory activity when hydrocortisone and ACTH were injected into ovariectomized rats, previously primed with estradiol. Selye (1954b) further reported that adrenalectomized and ovariectomized rats treated with hydrocortisone and estradiol showed marked mammary development and secretion. When hydrocortisone was given alone, mammary growth was reduced and confined to the ducts.

Höhn (1957) has recently reported that in the adrenalectomized guinea pig, estrone alone was incapable of producing lobule-alveolar development and elicited only duct growth, suggesting an adrenal involvement in mammary growth by estrogen. The hormones responsible for the lobule-alveolar growth of the mammary gland may be progesterone or other adrenal-cortical hormones with progesterone-like activity. This probably explains why Nelson (1937) was able to obtain full mammary growth in the intact guinea pig with estrogen injections alone.

Involution of the Mammary Gland during Lactation

Failure to remove milk from the mammary glands leads
to rapid disintegration of the lactating glad to a resting
condition, which closely resembles that seen in virgin ani-
mals. Williams (1942) noted that in the mouse there is a
rapid engorgement of the gland with secretion upon the removal
of the young. This is followed by absorption of the milk. By
the third day after removal of the young the mammary glands of
the dams are still morphologically intact. However, from the
fourth day onward there is a rapid regression of the paren-
chyma. The alveoli collapse and the small interlobular ducts
disintegrate, leaving the collapsed alveoli as isolated cell
masses. Finally the site of the alveoli and small ducts are
infiltrated with corpuscles. The larger ducts show very little
degeneration but are reduced in size.

Cessation of milking or the removal of young are not
the only factors responsible for mammary involution. After
the peak of lactation has been reached, a period of decline
follows in which secretory activity gradually decreases
despite the fact that the animal still produces milk. Whether
this is due to decrease in hormones stimulating lactation,
decreased secretory rate of the individual alveoli, or to
degeneration of the alveoli is not definitely known.

Selye and McKeown (1934) observed that despite a
strong and continuous suckling stimulus, the mammary glands of

mice will involute over a period of time. Turner and Reineke (1936) reported that in advanced stages of lactation in the goat, involution was almost complete with only a small number of active alveoli still present. When milking was suspended on one side of the udder of an actively lactating goat, while continuing milking on the other side, they observed that involution was retarded in the side not milked. This seems to demonstrate that the milking stimulus is necessary to maintain the integrity of the gland.

Selye (1934) also demonstrated that suckling is of prime importance in preventing the rapid involution of the mammary gland. If he ligated the galactophores of a rat, thus preventing milk from being withdrawn, and permitted active suckling by the young, rapid involution did not take place. When some of the nipples were excised to prevent suckling, mammary involution was retarded provided the other nipples were suckled. Hooker and Williams (1940, 1941) observed the same phenomenon in mice although they did note some involution that was not characteristic of post-weaning involution.

Hooker and Williams (1941) applied turpentine to the nipples of lactating mice isolated from their young in an attempt to mimic the suckling stimulus by irritation. They reported that mammary involution was retarded in the treated glands and even in the untreated glands to a lesser degree.

Reece and Turner (1937a) reported that pituitary prolactin content decreased in cows upon resumption of milking

after a period of milking. This was also demonstrated to be true when the galactophores in rats were ligated, thus showing that decreased milk secretion was related to the milking stimulus rather than to the removal of milk from the gland. It was further demonstrated that suckling in rats and rabbits maintained a higher prolactin secretion than in unsuckled animals (Meites and Turner, 1948b).

Williams and Hooker (1941) and Williams (1945) showed that prolactin injections of 20-60 I. U. daily retarded mammary involution in mice after the young were removed. Prolactin maintained lobule-alveolar and duct system similarly to mammary gland of mice at parturition when injected over a seven-day period.

That the suckling stimulus influences the release of other anterior pituitary hormones than prolactin has been demonstrated by Desclin (1947). He showed that when rats were spayed at parturition and the galactophores were ligated, the appearance of castration cells in the pituitary could be prevented if suckling was continued. Gregoire (1947) reported that thymic involution resulting from gestation could be maintained in rats spayed at parturition if the suckling stimulus was continued.

Factors Controlling the Initiation and
Maintenance of Lactation

Role of the Anterior Pituitary

Stricker and Grueter (1928) were the first to demonstrate
that lactation could be initiated in ovariectomized, pseudo-
pregnant rabbits by injections of anterior pituitary extracts.
This was the first step in demonstrating that lactation was
not only inhibited by the products of pregnancy, but a definite
hormonal stimulus was required for the initiation of lactation.
The factor necessary for this stimulus was demonstrated to be
prolactin (Riddle and Bates, 1939; Lyons, 1942; Meites and Turner,
1948). The latter two groups of workers showed that small quan-
tities of prolactin injected into the galactophores of non-
lactating rabbits with fully developed mammary glands evoked a
localized lactation, indicating that prolactin acts directly
on the epithelial cells.

The reports of several workers (Gomez and Turner, 1936,
1937b; Nelson and Gaunt, 1936, 1937b) suggested that other
anterior pituitary hormones than prolactin were necessary for
the initiation of lactation in hypophysectomized animals since
crude extracts of the anterior pituitary initiated lactation
in such animals while purified prolactin extracts did not.
ACTH appears to be one of these hormones, since lactation can
be initiated in hypophysectomized guinea pigs with purified
prolactin extracts and ACTH or adrenal cortical extracts (Gomez
and Turner, 1936, 1937b; Nelson and Gaunt, 1937b). Recently

Lyons et al (1953) have shown in hypophysectomized rats that lactation can be initiated with combinations of prolactin, growth hormone and cortisone or ACTH, provided the mammary glands are fully developed. This is further proof of the necessity for other pituitary hormones than prolactin to initiate lactation in hypophysectomized animals. Prolactin alone, however, can initiate lactation in intact animals with developed mammary glands, with the possible exception of the rat (Meites and Turner, 1948).

Adrenal Cortex

It has been clearly established that adrenalectomy interrupts established lactation. Adrenalectomy before parturition does not prevent the initiation of lactation but lactation will not be maintained (Gaunt, 1933; Carr, 1931). However, there is some disagreement as to which cortical steroids are responsible for the maintenance of lactation. Gaunt et al (1942) reported that cortisone was able to completely maintain lactation in rats adrenalectomized the day after parturition. However, DCA only partially maintained lactation.

Folley and Cowie (1944) and Cowie and Folley (1947) have shown that DCA is more effective than cortisone in maintaining lactation in rats adrenalectomized on the fourth day after parturition. However, there was not a complete restoration of lactation with these compounds when given individually.

Even when dietary protein was increased by 50 percent, so as to favor the action of the glucocorticoids, the lactational response was subnormal. Cowie (1952) was able to obtain complete maintenance of lactation in adrenalectomized rats by the implantation of cortisone and DCA pellets. Reece (1939) initiated a higher degree of milk secretion (+++) in pseudo-pregnant rats when prolactin was given together with adrenal cortical hormones and 20 percent glucose solution, than with prolactin alone (++).

Brownell, Lockwood and Hartman (1933) postulated the existence of a specific adrenal cortical hormone, "cortilactin." However, Hurst, Meites and Turner (1942) were unable to detect any pigeon crop-stimulating activity in adrenal extracts from several species.

The circulating levels of the adrenal corticoids (17-hydroxicorticosteroids) begin to rise early in pregnancy and remain high until shortly after parturition (Gemzell, 1953). Venning (1946) has also shown a progressive rise in excretion of the glucocorticoids in women beginning early in pregnancy.

There is a significant reduction of circulating leukocytes during suckling in the lactating rat and mouse. However, this may be due to removal of leukocytes via the milk. Tabachnick and Trentin (1951) have suggested a possible involvement of the adrenal in this lymphopenia of lactation, since they found a significant decrease in the mean ascorbic acid of the adrenals in suckled as compared to non-suckled mice.

Folley and his group reported the effects of hormones on the metabolism of mammary gland slices in vitro. After parturition the R. Q. of the mammary gland in the presence of glucose or glucose plus acetate was above unity (Folley and French, 1949). A composite respiratory curve showed that mammary gland slices from 20-day pregnant rats, incubated in bicarbonate saline buffer with glucose plus acetate added, produced an over-all fall in pressure while mammary tissue from lactating rats exhibited a rise in this curve (Balmain and Folley, 1952). When prolactin was added to mammary gland slices from rats pregnant 20 days there was no effect on respiration. However, cortisone produced an increase in respiration. Cortisone and prolactin did not give any greater increase in respiration than cortisone alone.

These English investigators further found that prolactin added to mammary slices of rats 1-5 days after parturition elicited an increase in respiratory activity. They suggested that cortisone conditions the mammary gland before parturition and that after parturition the gland is able to respond to prolactin. This remains to be proved.

Suckling Reflex

Suckling is known to evoke the release of oxytocin through a neurohormonal reflex (Ely and Peterson, 1941; Cross, 1955a, b). Reece and Turner (1937) have also demonstrated that suckling results in release of prolactin from the pituitary,

and Meites and Turner (1948) showed that suckled rats and rabbits contained more prolactin in their pituitaries than non-suckled animals. Suckling is not necessary for the initiation of lactation. At parturition, although neither maximum milk production nor maximum pituitary prolactin content is attained without it.

Benson and Folley (1956) reported that release of prolactin could be stimulated by treatment with oxytocin, as judged by the appearance of secretion in the mammary gland. However, they did not actually measure prolactin secretion. These investigators inhibited the decline in milk secretion in rats which had their young removed, by injecting 1 I. U. of oxytocin three times daily from the fourth to the thirteenth day after parturition. They suggested that impulses arising from sensory fibers of the mammary gland stimulates the posterior pituitary to release oxytocin, which in turn produces a release of prolactin.

Attempts to Increase Lactation (Galactopoiesis) or Maintain Established Lactation with Hormones

It has been established that lactation in most mammals rapidly reaches a peak and then slowly declines over a long period of time. Attempts have been made to increase milk yield during lactation by the use of hormones. Folley and Young (1939) have classified hormones with this ability as "galacto-poietic." It is important to emphasize that these "galactopoietic"

agents may not necessarily play a role in the initiation of lactation (lactogenesis).

Azimov and Krouze (1937) were the first to demonstrate galactopoietic activity in crude anterior pituitary extracts during the declining phase of lactation in cattle. They showed that injections of ox anterior pituitary into cows produced a marked but temporary increase in milk (lasting 5 to 6 days). Folley and Young (1938) confirmed this work in cows, using purified prolactin and growth hormone preparations. These workers found that a single injection produced a mean increase in milk yield of about 10 percent.

Folley and Young (1939, 1940) were able to substantially increase yields in cows by 15-20 percent by injecting crude ox pituitary extracts every other day over a three-week period. The lactation curves obtained by pituitary treatment during the declining phase of lactation were identical in slope with untreated cows. They also showed that crude pituitary extracts did not affect the peak of lactation nor delay the onset of the decline of lactation.

Cotes et al (1949) showed that growth hormone given as a single injection of 30 mg in cows, substantially increased milk yields. This was confirmed by Donker and Petersen (1951). Shaw (1955) treated a few cows at parturition with growth hormone and was able to increase lactation during the entire lactational curve over that of untreated control cows.

ACTH injections exerted an inhibitory effect on lactation in cows (Cotes et al, 1949). This action does not seem to

be in agreement with the view that ACTH and the adrenal-cortical hormones are necessary for the maintenance of lactation in the adrenalectomized animals described earlier. However, the dosages used may have influenced these results. Roy (1947) claimed that large doses of purified ACTH injected every other day, increased lactation during the declining phase as much as 20 percent. When prolactin was added to the ACTH preparation there was no significant increase above that of ACTH alone. Later work by Folley (1955) indicated that ACTH is only capable of inhibiting rather than augmenting lactation in cows.

EXPERIMENT I. EFFECTS OF ACTH AND ADRENAL CORTICAL
HORMONES ON MAMMARY GROWTH AND PITUITARY
PROLACTIN CONTENT IN INTACT RATS

Procedure

This experiment was performed to determine the effects
of cortisone, hydrocortisone and ACTH on mammary development
and pituitary prolactin content in intact female rats.

Forty-two mature female albino rats, of the Carworth
strain, were divided into four groups and injected daily for
10 days as follows: 1, controls, 0.85 percent saline; 2, 2 mg
cortisone; 3, 2 mg hydrocortisone; and 4, 2 mg (2 I. U.) ACTH
(Armour's). All injections were made subcutaneously in 0.1 cc
volumes. On the 11th day the rats were killed and the pitui-
taries were removed, weighed and prepared for prolactin assay.

The prolactin content of the pituitaries from each
group of rats was assayed in 5 or 6 white Carneau pigeons by
the sensitive intradermal method of Reece and Turner (1937).
In one group of 6 pigeons, the control pituitaries were injected
over one crop sac and directly compared with the pituitaries
from the hydrocortisone-treated rats, injected over the other
crop sac. In another group of 5 pigeons, the pituitary sus-
pension from the cortisone-treated rats was directly compared
with the pituitaries from the ACTH-treated rats. Thus a total
of 2 rat pituitaries were injected over each crop sac during a

four-day period. On the 5th day the pigeons were killed and the crop glands were removed and rated visually for degree of proliferation.

The right inguinal mammary gland was dissected from each rat and prepared for gross mounting and for fixing in Bouin's Fluid. Standard histological staining procedures were employed, using hematoloxylin and eosin. Each of the excised mammary glands was rated (0-4) for degree of development and secretion.

Results

These findings are summarized in Table 1 and Figure 1. Cortisone and particularly hydrocortisone inhibited body growth while the dose of ACTH employed had no effect on body growth. The mammary glands of the controls showed mostly bare ducts with little branching, and few to moderate number of ductal buds. Cortisone and hydrocortisone induced marked branching of the ducts and considerable lobule-alveolar development. ACTH was least effective in eliciting mammary growth, but produced moderate branching of the ducts and limited lobule-alveolar growth.

Cortisone was the most effective in inducing secretory activity in the mammary glands while hydrocortisone was only moderately effective and ACTH was least effective. There was no evidence of secretion in the control glands. Representative mammary glands are shown in Figure 1.

The pituitary weights were not altered by any of the hormone treatments when compared on a body weight basis. However, all three hormones increased the prolactin content of the pituitary. Cortisone and hydrocortisone increased it about 23 and 41 percent respectively, and ACTH about 71 percent (on a body weight basis).

TABLE 1

EFFECTS OF CORTISONE, HYDROCORTISONE AND ACTH ON MAMMARY GROWTH
AND PITUITARY PROLACTIN CONTENT OF RATS

Treatment	Av. Body Wt.		Av. Pituitary Wt.		Mammary Gland		Av. No. Pigeon Units		
	Initial g	Final g	Actual Wt. mg	Per 100g Body Weight	Av. Growth (0-4)	Av. Secretion (0-4)	Per Pit.	Per mg. Pit.	Per 100g B.W.
Controls (12)*	163.3	178.5 ±8.6**	10.0 ±0.6	0.158 ±0.012	0	0	.375 ±.06	.0375	.229
E^1, 2 mg daily (10)*	164.8	168.0 ±3.0	10.3 ±0.9	0.165 ±0.01	1.6(1-3)	3	.465 ±0.11	.0451	.282
F^2, 2 mg daily (10)*	162.6	149.1 ±4.4	10.2 ±0.5	0.137 ±0.012	0.85(0-2.5) ±0.25	2	.525 ±0.21	.0316	.325
ACTH, 2 I.U. daily (10)*	162.3	174.6 ±3.9	10.3 ±.06	0.170 ±0.003	0.7(0-2.0) ±.29	1	.640 ±0.12	.0382	.394

*Number of rats per group
**Standard error of mean
E^1 = Cortisone acetate
F^2 = Hydrocortisone acetate

Figure 1.   Histological sections of representative
            mammary glands (100x) from each group:

            (1) 0.85 percent saline
            (2) 2 mg. cortisone
            (3) 2 mg. hydrocortisone
            (4) 2 I. U. ACTH
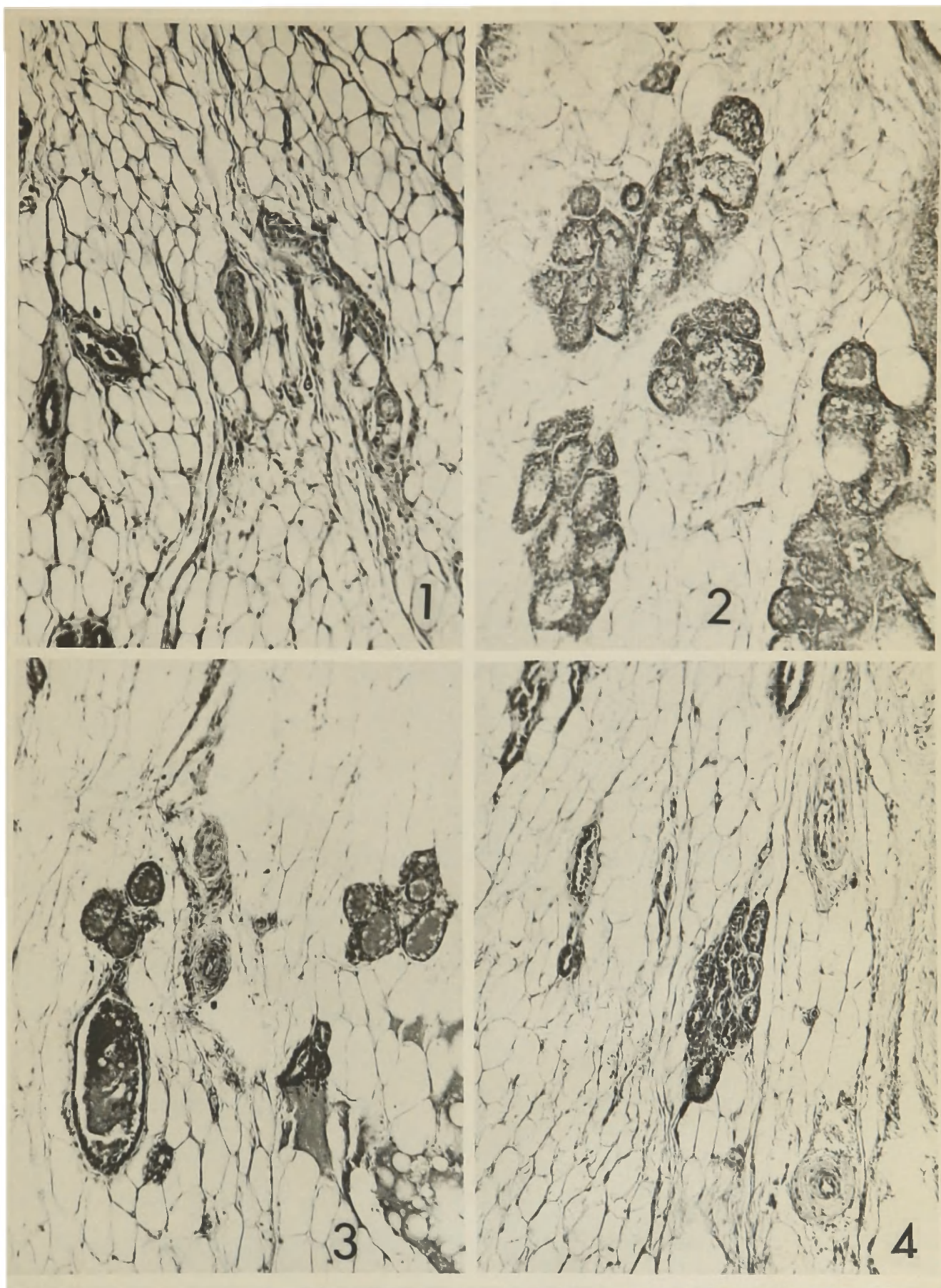
Figure 1.

EXPERIMENT II.   EFFECTS OF ESTRONE AND CORTISONE ON
PITUITARY PROLACTIN CONTENT AND MAMMARY
GROWTH OF OVARIECTOMIZED RATS

Procedure

This experiment was performed in an attempt to compare
the results of the previous experiment in intact rats with
ovariectomized rats, and also to see whether there was any
interaction between estrogen and cortisone on pituitary pro-
lactin content and mammary growth.   One hundred mature female
albino rats of the Carworth strain were ovariectomized.   After
a period of 14 days the rats were divided by weight into eight
uniform groups of 10 rats each.   These were further divided
into two major groups of 40 rats each (four groups of 10), and
the remaining two groups (20 rats) were used for controls.   The
treatments of the groups were as follows:

(1) controls, saline injections only; (2) controls,
1 mg. cortisone acetate only.   The two major groups received
(1) 5 ug. of estrone, and (2) 10 ug. of estrone.   Within these
two major groups, cortisone was administered to the four sub-
groups as follows: (1) no cortisone, saline only; (2) 1 mg.
cortisone daily; (3) 2 mg. cortisone daily; and (4) 4 mg.
cortisone daily.   All injections were given subcutaneously in
0.1 cc. volumes of physiological saline, daily, for a period
of 10 days.   On the 11th day the rats were killed and the
pituitaries were removed and prepared for prolactin assay.

The prolactin content of the pituitaries was assayed in 25 white Carneau pigeons by the sensitive intradermal method of Reece and Turner (1937). These were divided into 5 groups of 5 pigeons each and were injected as follows: (1) control pituitaries were directly compared with pituitaries from estrone-treated (5 ug.) rats; (2) pituitaries from cortisone-treated rats were directly compared with the pituitaries from estrone (5 ug.) and cortisone-treated (1 mg) rats; (3) pituitaries from estrone (5 ug.) and cortisone-treated (2 mg.) rats were directly compared with pituitaries from the estrone (5 ug.) and cortisone-treated (4 mg.) rats; (4) pituitaries from estrone-treated (10 ug.) rats were directly compared with pituitaries from the estrone (10 ug.) and cortisone-treated (1 mg.) rats; and (5) pituitaries from estrone (10 ug.) and cortisone-treated (2 mg.) rats were directly compared with pituitaries from the estrone (10 ug.) and cortisone-treated (4 mg.) rats.

A total of 2 rat pituitaries were injected over each crop sac for a period of four days. On the 5th day the pigeons were killed and the crop glands were rated for degree of proliferation. The right inguinal mammary gland from each rat was removed, stretched gently on a small piece of cork, fixed in Bouin's fluid over night and prepared for histological examination.

Results

The mammary glands of the controls (group 1) showed
duct growth and budding, while the cortisone-treated (1 mg.)
controls (group 2) showed a larger number of ductal buds and
some degree of lobule-alveolar development. The rats receiving
estrone alone (groups 3 and 7) exhibited duct growth only. How-
ever when cortisone was administered to the estrogen-treated
rats (groups 4, 5, 6, 8, 9, and 10), lobule-alveolar development
was substantially stimulated by all levels of cortisone. A con-
siderable amount of secretion was noted in the mammary glands
of the rats treated with cortisone and estrone. Representative
mammary glands are shown in Figure 3.

It can be seen in Table 2 that there was a good weight
gain in the control rats (group 1) and in those animals which
received the lower levels of cortisone (groups 4 and 8). How-
ever, at the higher levels of cortisone (groups 5 and 9), there
was a definite inhibition of body growth, and at the highest
level of cortisone (groups 6 and 10) an actual loss of body
weight.

When pituitary prolactin contents were compared, no
significant change was found between the controls given saline
only (group 1) or those injected with 1 mg. of cortisone (group
2). Estrone alone (groups 3 and 7) produced a definite increase
in pituitary prolactin content at both levels (5 ug. and 10 ug.)
over that of the controls (groups 1 and 2). When 1 mg. of cor-
tisone was added to the estrone (groups 4 and 8) the pituitary

prolactin content was significantly augmented above that produced by estrone alone (groups 3 and 7). Only the largest dose of cortisone employed (group 10) inhibited the action of estrone in increasing pituitary prolactin content.

TABLE 2

EFFECT OF ESTRONE AND CORTISONE ON PITUITARY PROLACTIN CONTENT

Group No. and Treatment	Av. Body Wt.		Av. Pit. Wt.		Av. No. Pigeon Units		
	Initial g	Final g	Actual mg	Per 100g B.W. mg	Per Pit.	Per mg. Pit.	Per 100g B.W.
1) Controls	201.5	278.8±7.93*	13.25±.84	4.76	.47±.10	.03	.16
2) Cortisone 1 mg daily	183.5	230.5±8.42	14.40±.51	7.91	.52±.10	.03	.22
3) Estrone 5 ug daily	191.5	243.1±6.22	16.65±.73	9.51	.91±.06	.05	.52
4) Estrone 5 ug daily Cortisone 1 mg daily	185.5	231.5±5.23	16.50±.47	7.14	1.20±.14	.07	.51
5) Estrone 5 ug daily Cortisone 2 mg daily	189.5	229.0±5.76	16.30±.95	7.11	1.11±.14	.06	.48
6) Estrone 5 ug daily Cortisone 4 mg daily	197.0	162.27±3.54	13.07±.56	6.63	1.07±.08	.08	.66
7) Estrone 10 ug daily	190.0	203.9±4.76	16.32±.80	8.00	1.15±.11	.07	.56
8) Estrone 10 ug daily Cortisone 1 mg daily	189.4	187.2±4.49	15.90±.52	8.50	1.35±.07	.08	.72
9) Estrone 10 ug daily Cortisone 2 mg daily	192.0	180.2±4.45	14.86±.63	8.25	1.10±.08	.07	.61
10) Estrone 10 ug daily Cortisone 4 mg daily	200.0	165.6±2.88	15.10±.47	9.15	.70±.06	.04	.42

*Standard error of the mean

Figure 2. Effect of estrone and cortisone on
          pituitary prolactin content.
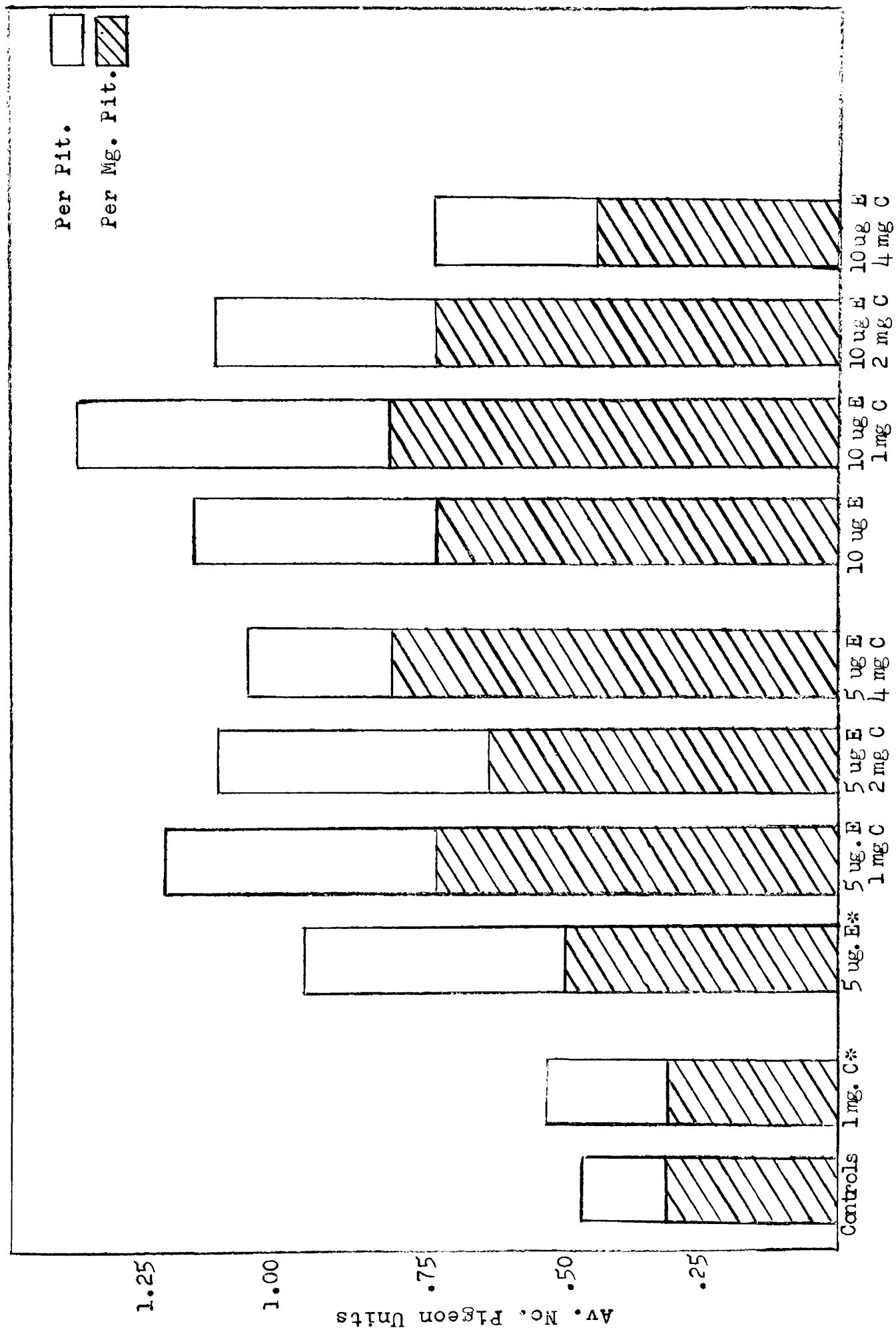
Figure 2

*C=Cortisone
E=Estrone

Figure 3.   Histological sections of representative
mammary glands (80x).

(1) 0.85 percent saline
(2) 10 ug. estrone
(3) 1 mg. cortisone
(4) 10 ug. estrone and 1 mg. cortisone
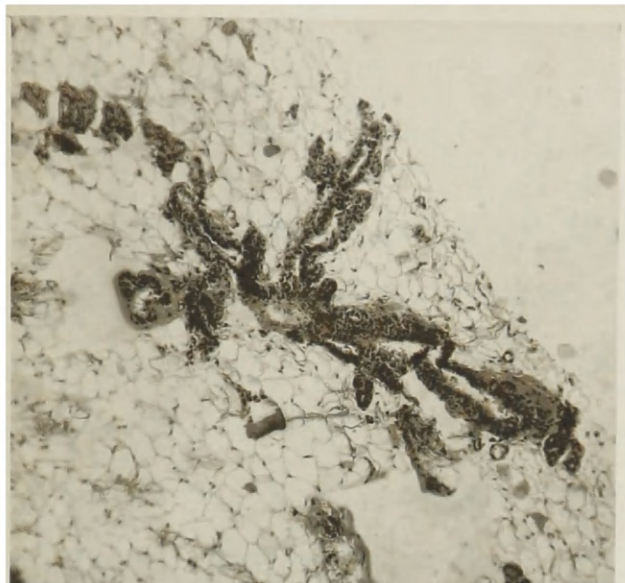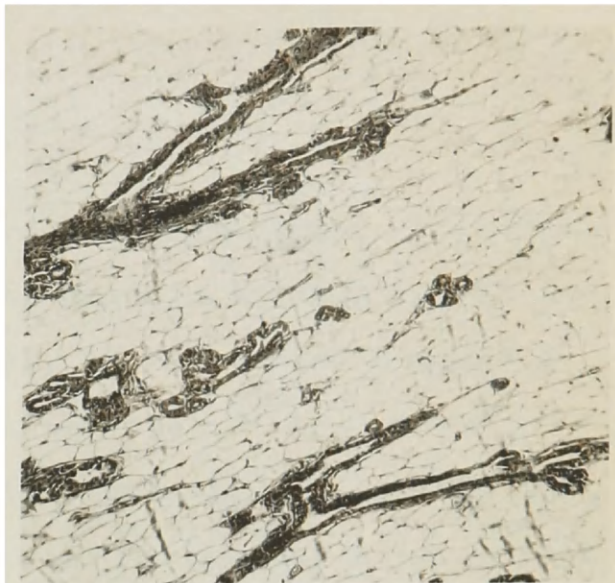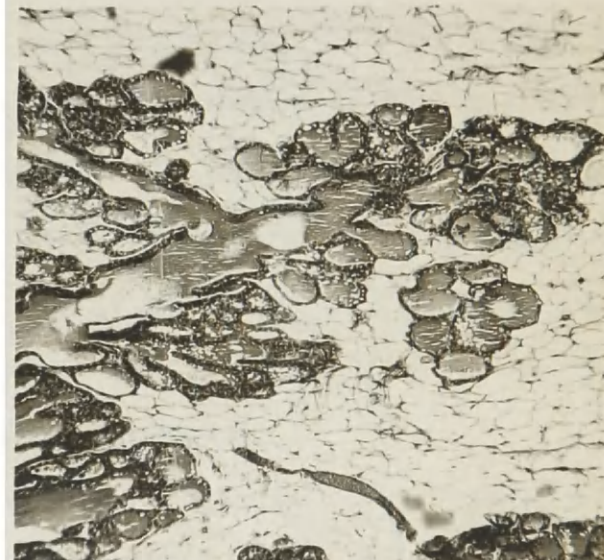
Figure 3.

EXPERIMENT III. EFFECTS OF CORTISONE ON GALACTOPOIESIS IN THE RAT

Procedure

From the results of the previous experiments it seemed reasonable to assume that if cortisone had the ability to increase pituitary prolactin content, it might also produce a galactopoietic effect in the suckling rat. It is extremely difficult to accurately measure the lactational response in small laboratory animals when compared to larger animals such as the cow and goat. Since it is difficult to actually measure milk output, it is necessary to employ survival of litters and litter growth curves as measures. In this experiment the latter were employed. However, it should be pointed out that this is not an absolute but only a relative index of lactational response. In litter growth curves the daily milk yield is not directly calculable, since the daily weight loss due to excreta and insensible perspiration are not measured.

Cowie (1946) used a logistic equation and plotted a mean growth curve for litters of lactating dams from birth to 16 days of age and obtained a sigmoid curve. However from the 6th to the 11th days of lactation, the points on this curve were in a straight line, and he therefore claimed that during this 5-day period the mean total increase in litter weight was approximately constant as well as at a maximum for each rat.

This gain was termed "litter growth index" and he used it as a quantitative measure for lactational responses in rats. For reasons pointed out above, this is not believed to be a true reflection of milk yield but is estimated by Cowie to be roughly 50-60 percent of the true output.

Thus, if this period (6th to 10th day) of lactation represents the peak of lactation in rats, the two extremes of this sigmoid curve could then be called the initial phase of the declining curve of lactation. The average litter weight gain in the present experiment was divided into three periods for comparison as follows: (1) initial phase (0-5th day); (2) peak phase, Cowie's "litter growth index" (6th-10th day); and (3) declining phase (11th-18th day).

Brody (1945) took into consideration the growth rate of an animal at a given age and related it to body size at a given age, and developed a logarithmic equation for showing growth rate of an animal during a given unit of time.

$$k = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

or simplified,

$$k = \ln \frac{W_2}{W_1} \div t$$

where   k = daily instantaneous rate of growth

$\dfrac{W_2}{W_1}$ = ratio of the surviving proportion of litter weights

t = time interval in days between observations.

The "instantaneous growth rate" index has been used in the present experiment merely as a refinement of previous measurements and to see to what extent it agrees with them.

Thirty mature female albino rats of the Carworth strain were bred. Once pregnancy was established, the dams were placed in individual cages. These cages were designed to make the dam's ration inaccessible to the young throughout the experimental post-partum period of 18 days. This insured that the young received food only from their mothers. On the day of parturition the litters were reduced to 7 young each and these were randomly grouped as follows: (1) controls (9 rats), saline only; (2) 0.25 mg. cortisone (8 rats); (3) 0.5 mg. cortisone (7 rats); and (4) 1.0 mg. cortisone (6 rats). All injections were administered subcutaneously, daily, for the eighteen-day period after parturition. The litters were weighed daily and the dams weekly until termination of the experiment. On the 19th day the dams were killed and the adrenals were removed and weighed on a Roller-Smith balance.

Results

It can be seen in Table 3 that there was no significant weight loss in the control dams (group 1) during the experimental period. However, in each of the three treated groups (groups 2, 3 and 4) there was a significant weight loss of approximately 18, 19 and 46 grams each, respectively.

In the rats receiving 0.5 mg. cortisone (group 3)
the average litter weight gain during the experimental period
was significantly increased over that of the controls (group 1).
Groups 2 and 4, which receiver 0.25 mg. cortisone and 1.0 mg.
cortisone each daily, exhibited no significant litter weight
gain over the controls. However, it is interesting to note
that even though the dams receiving 1.0 mg. cortisone showed
a larger body-weight loss, they still maintained their young
at the same or at a slightly higher growth rate than in the
control rats.

Table 4 shows a somewhat more complete picture of the
lactational response in these rats. The rats receiving 0.5
mg. cortisone (group 3) showed no effect during the initial
phase of lactation (0-5th day). However, significant increases
over that of the controls can be seen in this group during the
peak of lactation (6th-10th days and during the declining phase
(11th-16th days). Although the rats which received 1.0 mg. cor-
tisone (group 4) did not show a significant litter weight gain
over the controls during the entire 18-day experimental period,
they did exhibit a significant increase during the declining
phase of lactation (11th-18th day). When "instantaneous growth
rates" are employed this same trend is noted (Table 5). Figure
4 is a graphical representation of these experimental data.
These results suggest that cortisone may increase the lactational
response in the intact female lactating rat.

TABLE 3

EFFECT OF CORTISONE ON BODY WEIGHTS OF DAMS AND LITTERS, AND ON ADRENAL WEIGHT

Group No. and Treatment	Av. Litter Size	Av. Dam Wt. at Parturition g	Av. Dam Wt. at 18 Days Post-part. g	Adrenal Weight mg	Av. Litter Wt. at 18 Days Post-part. g
1) Controls Saline daily (9)*	10.5	244.50 ±7.04**	239.50 ±3.40	63.10 ±1.33	171.22 ±4.89
2) 0.25 mg Cortisone daily (8)	8.5	262.62 ±6.52	240.88 ±5.68	47.84 ±2.18	168.37 ±4.39
3) 0.5 mg. Cortisone daily (7)	9.8	269.60 ±4.23	250.00 ±9.99	52.57 ±2.54	216.00 ±4.94
4) 1 mg. Cortisone daily (6)	8.6	260.17 +7.73	214.67 ±8.14	45.02 ±2.46	183.83 ±3.92

*Number of rats per group
**Standard error of the mean

TABLE 4

EFFECTS OF CORTISONE ON LITTER WEIGHT GAIN IN RATS

Average Litter Growth Rate

Group No. and Treatment	0-5 days Total g	0-5 days Daily g	6-10 days Total g	6-10 days Daily* g	11-18 days Total g	11-18 days Daily g	18 days Total g	18 days Daily g
1) Controls (9)**	42.89 ±1.69***	8.59 ±1.07	49.11 ±2.28	9.81 ±1.45	79.11 ±3.21	9.42 ±1.31	171.22 ±4.89	9.51 ±.89
2) Cortisone (8) 0.25 mg daily	39.37 ±2.27	7.78 ±.44	53.87 ±1.74	10.77 ±.11	75.12 ±5.25	9.42 ±.64	169.37 ±4.39	9.34 ±.80
3) Cortisone (7) 0.5 mg daily	44.43 ±2.52	8.88 ±.50	63.28 ±1.97	12.65 ±.39	108.43 ±1.39	13.55 ±.02	216.00 ±4.94	11.98 ±.88
4) Cortisone (6) 1 mg daily	39.33 ±3.02	7.86 ±1.91	53.50 ±2.37	10.70 ±1.50	91.00 ±2.94	11.35 ±1.21	183.83 ±3.92	10.23 ±.70

*Litter Growth Index
**Number of rats per group
***Standard error of the mean

TABLE 5

EFFECTS OF CORTISONE ON INSTANTANEOUS GROWTH RATE OF LITTERS

Treatment	Instantaneous Growth Rate			
	0-5 days	6-10 days	11-18 days	18 days
Controls (9)*	.134 $\pm$.0028**	.091 $\pm$.0032	.058 $\pm$.0020	.083 $\pm$.0037
Cortisone (6) 1 mg daily	.127 $\pm$.007	.102 $\pm$.0097	.064 $\pm$.003	.092 $\pm$.0014
Cortisone (7) 0.5 mg daily	.139 $\pm$.006	.108 $\pm$.0026	.067 $\pm$.0014	.098 $\pm$.0014
Cortisone (8) 0.25 mg daily	.130 $\pm$.0046	.101 $\pm$.0043	.054 $\pm$.0042	.088 $\pm$.002

*Number of litters per group
**Standard error of the mean

Figure 4.   Effects of cortisone on litter weight
gains in the rat.
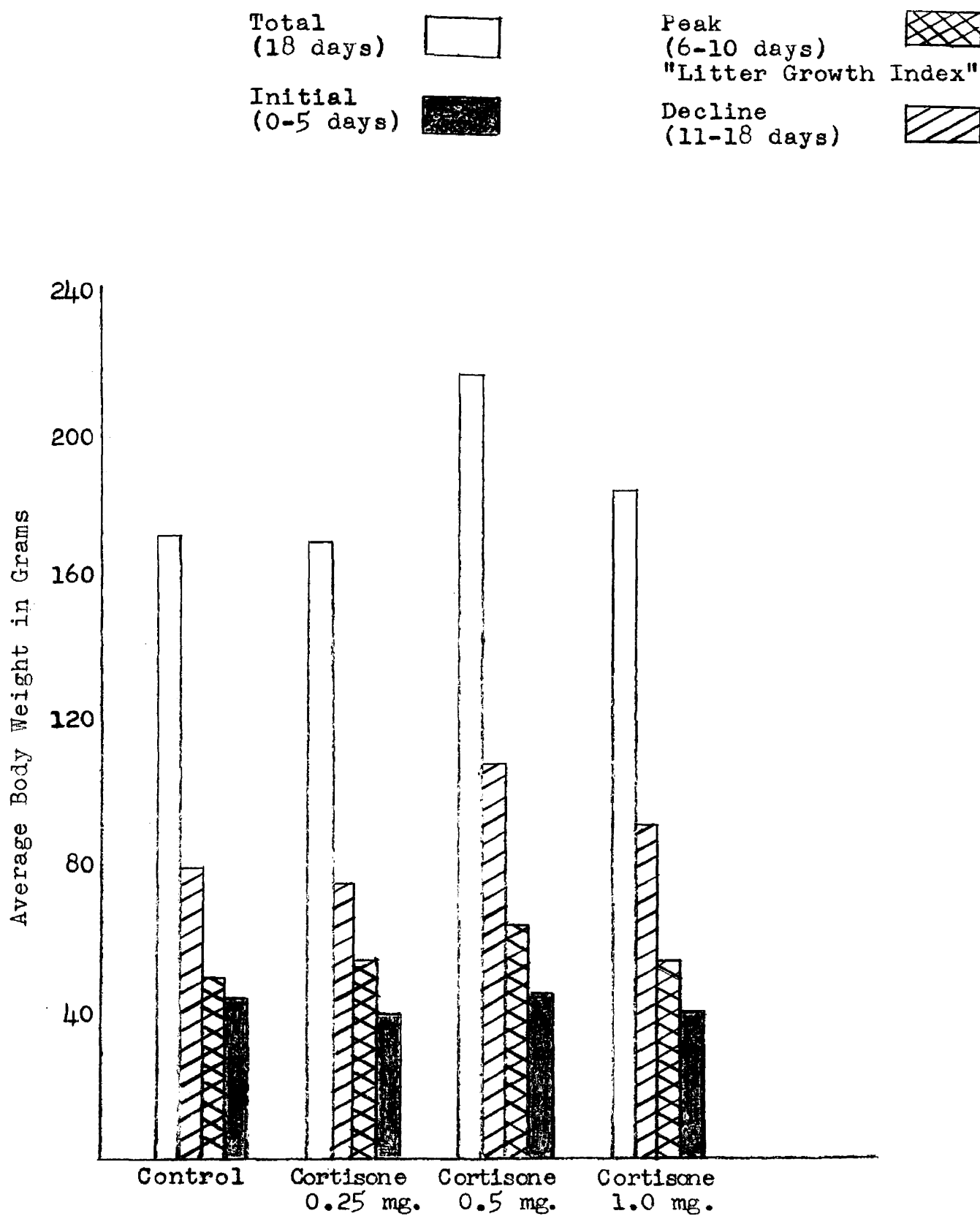
Figure 4

EXPERIMENT IV. EFFECTS OF CORTISONE, GROWTH HORMONE, PROLACTIN, OXYTOCIN AND ACTH ON LACTATION AND INVOLUTION OF MAMMARY GLANDS IN RATS

Procedure

This experiment was performed to determine whether cortisone as well as growth hormone, prolactin, oxytocin and ACTH could exert galactopoietic effects and retard mammary involution in lactating rats.

In this experiment 94 mature female rats of the Carworth strain were used. The experimental design was the same as in Experiment III except for the following modifications: (1) at parturition the litters were reduced to 6 young each instead of 7, in order to enable a larger percentage of litter survival and to insure that at least this many were alive on the day of parturition. (2) All injections were made subcutaneously once daily, with the exception of oxytocin which was injected twice daily. The injections were carried out for an additional 10 days (18th-28th day) after removal of the young. (3) On the 28th day, the dams were killed and the right inguinal mammary glands were removed and prepared for histological examination. (4) The control group was divided into two sub-groups, one (group 1) of which was used to determine mammary involution at the end of the 28-day experimental period while the other (group 2) was used to follow normal daily involutionary

changes of the mammary glands from the 18th to the 27th day (see Appendix, Figures 14-23).

The total number of litters reported in the data are 68 and these were treated as follows: (1) controls, saline only (17 rats); (2) cortisone, 1 mg. (8 rats); (3) cortisone, 0.5 mg. (8 rats); (4) growth hormone, 1 mg. (8 rats); (5) prolactin, 1 mg. (8 rats); (6) growth hormone, 1 mg. cortisone, 0.5 mg. and prolactin, 1 mg. (8 rats); (7) oxytocin, 1 I. U. (6 rats) (this was injected twice daily, 0.5 I. U. per injection); (8) ACTH, 1 mg. (5 rats).

An additional group of 8 rats were added to the latter control series in order to further determine the degree of daily involution after removal of the young on the 18th day. Only litters which were healthy at 18 days of age were used as a measure of milk secretion. Dams which developed infection were discarded.

The control groups (1 and 2) and the two groups given cortisone (3 and 4) did not develop any infection in the dams and the young were all normal at 18 days. The dams in some of the other groups developed abnormal litters as follows: growth hormone (group 5), six litters were stunted; prolactin, (group 6), two litters were stunted, and one dam was killed because of infection; growth hormone, cortisone and prolactin (group 7), three stunted litters; oxytocin (group 8), three litters were stunted and ACTH (group 9), two litters were stunted and one dam died of infection.

Results

As can be seen in Table 6, there was no significant
weight loss in the control dams (groups 1 and 2) over the
entire experimental period (28 days). Both levels of corti-
sone (groups 3 and 4) produced a weight loss. However, this
was not as great as that exhibited in Experiment III. Both
growth hormone (group 5) and ACTH (group 9) increased the body
weight of the dams. The other groups showed no significant
changes in body weight.

In the rats receiving cortisone, prolactin, oxytocin or
ACTH (groups 3, 4, 6, 8 and 9), there was a significant litter
weight gain during the experimental period (18 days), while
0.5 mg. cortisone (group 4) produced the greatest increase in
litter weight gain over that of the controls. In the rats re-
ceiving growth hormone (group 5) there was no significant gain
in litter weight. The rats receiving the combination of hor-
mones (group 7) also showed a significant increase in the average
litter weight gain, but it was no greater than that in the rats
given 0.5 mg. cortisone (group 4). Therefore, prolactin and
cortisone do not seem to have a synergistic action on milk
secretion as judged by gain in weight of the young.

The results in Table 7 show that cortisone at the 0.5
mg. level (group 4) only slightly increased lactation during
the initial phase (0-5th day). However, milk secretion was
significantly increased between the 6th and 10th day and during
the declining phase (11th-18th day) over that of the controls

(groups 1 and 2). The rats treated with all three hormones (group 7) and the prolactin-treated rats (group 6) showed the same trends as the rats treated with 0.5 mg. cortisone (group 4). Growth hormone (group 5), on the other hand, resulted in no greater gains in litter weight during these periods than in the controls (groups 1 and 2).

It is interesting to note the results obtained with oxytocin (group 8) and ACTH (group 9). Although their stimulating effect on lactation was similar to that of cortisone, prolactin or the hormone-combination group during the peak phase and declining phase of lactation, there was no effect at all during the initial phase of lactation.

When computed in terms of "instantaneous growth rates," the same trends were noted in litter weight gain (Table 8). Figure 5 shows a graphical representation of these experimental data.

Histological examination of the mammary glands reveal striking differences between the different groups (Figures 6-13). The control mammary glands (Figure 6) show marked involution. All the alveoli are collapsed or in an advanced state of disintegration. Also, marked fibrosis of the lobules can be seen, despite an apparently normal ductal epithelium. Cortisone (Figures 7 and 8), prolactin (Figure 10), and the hormone-combination group (Figure 11) showed definite maintenance of alveolar structure as compared to the controls.

In the above hormone-treated groups the alveolar epithelium was intact in most of the sections cut, although some degeneration was noted in a few lobules. The greatest amount of degeneration can be seen in the cortisone-treated (0.5 mg.) rats (Figure 8). Inspissated secretions were seen throughout these sections. These mammary glands (groups 3, 4, 6 and 7) were comparable to control mammary glands from rats five days after the young had been removed. Cortisone at the 0.5 mg. level (group 4) produced mammary glands which resembled a control mammary gland six days after the young we're removed (see Appendix, Figures 19 and 20).

Growth hormone, oxytocin and ACTH appeared to have little or no effect in retarding involution of the mammary gland. Sections from the mammary glands of these rats showed no marked differences from those in the controls (Figures 9, 12 and 13). The oxytocin and ACTH-treated groups did show some very sparse but intact alveoli. However, this was also seen in some control glands.

Macroscopic examination of the mammary glands from all groups revealed very few differences at the end of the 10-day post-weaning period. In the controls and all rats treated with hormones, the larger lobular ducts were intact. However, the smaller interlobular ducts had disintegrated and the lobules were arranged in clusters throughout the parenchyma. In the cortisone-treated rats (groups 4 and 5), in the prolactin rats (group 6), and in the rats given the combination of hormones

(group 7), the lobules appeared more dense and the individual alveoli were larger than those in the control group.

These results show a similar galactopoietic activity for cortisone as in Experiment III, but in addition indicate that prolactin, ACTH and oxytocin also possess an ability to increase lactation. They also demonstrate that cortisone and prolactin were able to markedly retard mammary involution.

TABLE 6

EFFECTS OF CORTISONE, GROWTH HORMONE, PROLACTIN, OXYTOCIN AND ACTH ON BODY WEIGHT OF MOTHERS AND LITTERS

Group No. and Treatment	Av. Litter Size	Av. Dam Wt. at Parturition g	Av. Dam Wt. at 18 Days Post-part. g	Av. Dam Wt. at 28 Days Post-part. g	Av. Litter wt. at 18 Days Post-part. g
1) Controls (8)* Saline daily	9.1	237.87 ±7.74**	233.75 ±7.55	239.75 ±7.12	142.50 ±2.89
2) Controls (9)	7.1	262.25 ±5.08	253.62 ±5.41	---	137.00 ±2.61
3) Cortisone (8) 1 mg daily	8.7	247.00 ±7.45	226.87 ±7.52	222.12 ±6.43	163.75 ±4.18
4) Cortisone (8) 0.5 mg daily	8.2	252.00 ±7.96	242.00 ±5.64	238.12 ±6.08	172.37 ±4.61
5) Growth hormone (8) 1 mg daily	7.4	253.12 ±6.54	264.12 ±5.45	276.37 ±3.86	137.13 ±3.46
6) Prolactin (8) 1 mg daily	7.8	262.44 ±11.55	250.33 ±6.30	265.77 ±9.35	165.87 ±6.31
7) Growth hormone 1 mg Cortisone 0.5 mg Prolactin 1 mg (8)	9.6	263.87 ±8.11	253.37 ±7.72	263.62 ±6.44	169.12 ±5.85
8) Oxytocin (6) 1 I.U. daily	9.5	278.50 ±8.38	281.16 ±9.77	287.00 ±7.19	155.00*** ±7.05
9) ACTH (5) 1 mg daily	6.4	278.80 ±9.46	296.60 ±7.46	283.80 ±10.00	160.40*** ±6.58

*Number of rats per group
**Standard error of the mean
***Based on seventeen days

TABLE 7

EFFECTS OF CORTISONE, GROWTH HORMONE, OXYTOCIN, PROLACTIN AND ACTH ON BODY WEIGHTS OF LITTERS

Group No. and Treatment	Average Litter Growth Rate							
	0-5 days		6-10 days		11-18 days		18 days	
	Total g	Daily g	Total g	Daily0 g	Total g	Daily g	Total g	Daily g
1) Controls (8)*	36.25 ±1.56**	7.25 ±.102	41.37 ±3.08	8.27 ±.62	66.12 ±3.30	8.17 ±.45	142.50 ±2.89	7.90 ±.16
2) Controls (9)	31.92 ±1.29	6.32 ±.26	41.75 ±.93	8.35 ±.17	63.62 ±3.08	7.95 ±.38	137.00 ±2.61	7.58 ±.38
3) Cortisone 1 mg daily (8)	40.25 ±2.65	8.05 ±.53	48.87 ±2.76	9.60 ±.53	71.37 ±3.92	8.96 ±.50	163.75 ±4.18	8.94 ±.69
4) Cortisone (8) 0.5 mg	42.62 ±1.88	8.50 ±.37	50.37 ±1.90	10.07 ±.37	78.25 ±2.89	9.80 ±.36	172.37 ±4.61	9.57 ±.25
5) Growth hormone (8) 1 mg daily	36.12 ±2.17	7.22 ±.45	40.75 ±2.26	8.15 ±.43	60.25 ±1.76	7.53 ±.21	137.13 ±3.46	7.62 ±.20
6) Prolactin (8) 1 mg	40.87 ±2.26	8.17 ±.37	49.37 ±2.97	9.87 ±.59	75.63 ±4.07	9.47 ±.53	165.87 ±6.31	9.20 ±.33
7) Growth Hormone 1 mg Cortisone 0.5 mg Prolactin 1 mg (8)	42.12 ±2.30	8.42 ±.36	47.12 ±2.58	9.42 ±.51	79.62 ±3.81	9.96 ±.47	169.12 ±5.58	9.39 ±.33
8) Oxytocin (6) 1 I.U. daily	32.66 ±2.24	6.53 ±.43	47.50 ±3.03	9.50 ±.60	74.83*** ±4.04	10.68*** ±.56	155.00# ±7.05	9.10# ±.41
9) ACTH (5) 1 mg.	33.40 ±2.34	6.68 ±.45	51.60 ±3.77	10.32 ±.75	75.40*** ±5.66	10.78*** ±.72	160.40# ±6.58	9.44# ±.38

O Litter growth index
*Number of litters per group
**Standard error of the mean

***Based on seven days
#Based on seventeen days

TABLE 8

EFFECTS OF CORTISONE, GROWTH HORMONE, PROLACTIN, OXYTOCIN, AND ACTH ON "INSTANTANEOUS GROWTH RATE" OF LITTERS

Group No. and Treatment	Instantaneous Growth Rate			
	0-5 days	6-10 days	11-18 days	18 days
1) Controls (8)*	.133 ±.0041**	.087 ±.006	.056 ±.0033	.086 ±.003
2) Controls (9)	.130 ±.005	.096 ±.0038	.059 ±.0034	.088 ±.002
3) Cortisone (8) 1 mg daily	.140 ±.0067	.093 ±.0037	.054 ±.0033	.089 ±.0017
4) Cortisone (8) 0.5 mg daily	.152 ±.0036	.098 ±.0026	.058 ±.0031	.095 ±.002
5) Growth hormone (8) 1 mg daily	.123 ±.0065	.089 ±.0046	.039 ±.0033	.082 ±.0014
6) Prolactin (8) 1 mg daily	.145 ±.0038	.095 ±.0059	.058 ±.0028	.091 ±.0022
7) Growth hormone 1 mg (8) Cortisone 0.5 mg Prolactin 1 mg	.154 ±.005	.093 ±.004	.060 ±.003	.095 ±.0014
8) Oxytocin (6) 1 I.U. daily	.125 ±.0094	.102 ±.0062	.105# ±.0048	.097*** ±.0028
9) ACTH (5) 1 mg daily	.120 ±.0059	.103 ±.0053	.098# ±.0053	.094*** ±.0028

*Number of litters per group
**Standard error of the mean
***Based on seventeen days
#Based on seven days

Figure 5.   Effects of cortisone, growth hormone,
             prolactin, oxytocin and ACTH on body
             weights of litters.
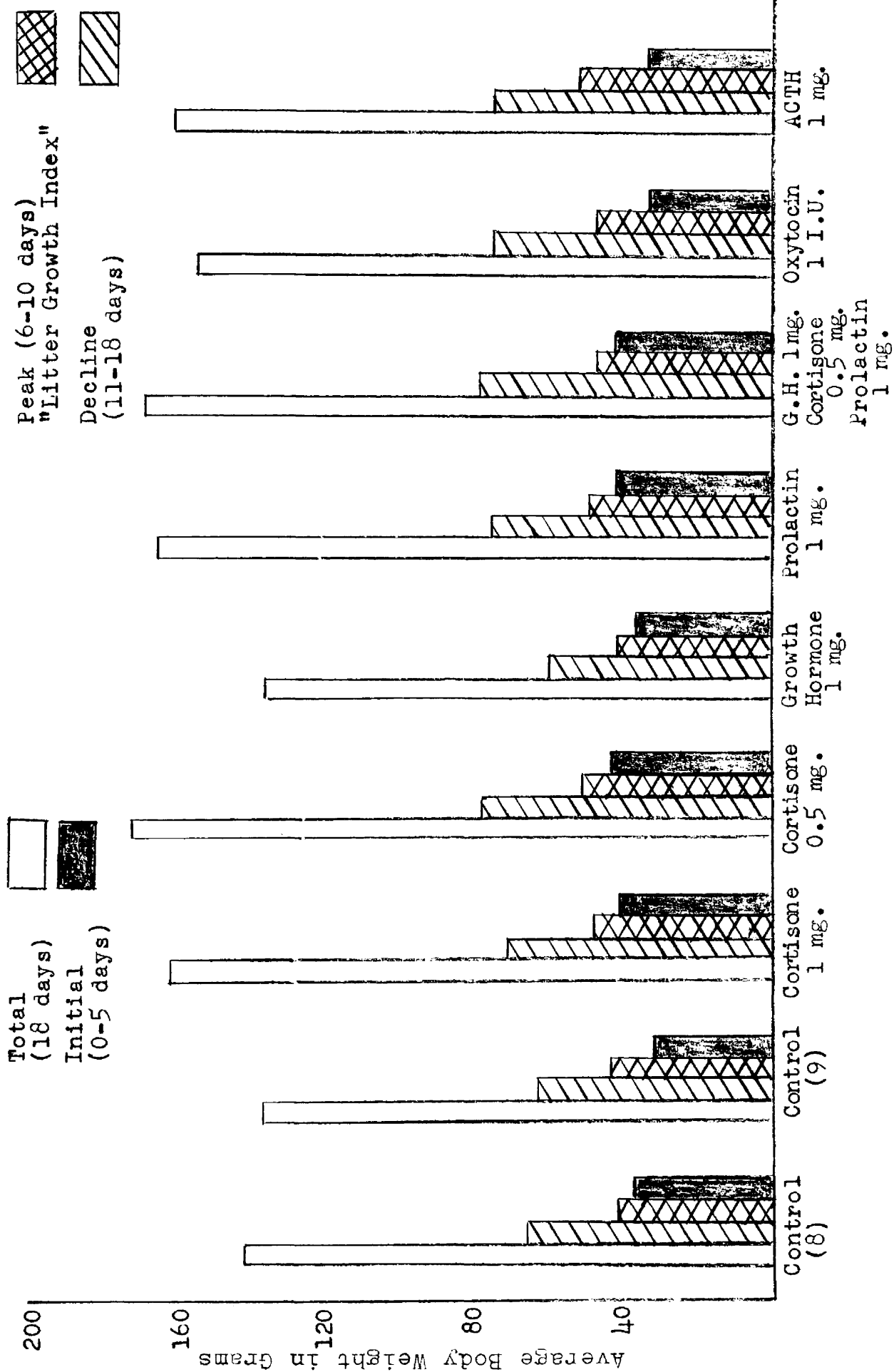
Figure 5

Figure 6.   Control mammary gland from a rat 10 days
            after young have been removed.   Top 130x,
            bottom 250x.   Stained with hematoxylin
            and eosin.

Figure 6.

Figure 7. Mammary gland from a rat receiving
1.0 mg cortisone daily for 10 days
after young were removed. Top 130x,
bottom 250x. Stained with hematoxylin
and eosin.

Figure 7.

Figure 8.   Mammary gland from a rat receiving
            0.5 mg. cortisone for 10 days after
            young were removed. Top 130x,
            bottom 250x.   Stained with hematoxylin
            and eosin.

Figure 8.

Figure 9. Mammary gland of a rat receiving
1 mg. growth hormone for 10 days
after young were removed. Top 130x,
bottom 250x. Stained with hematoxylin
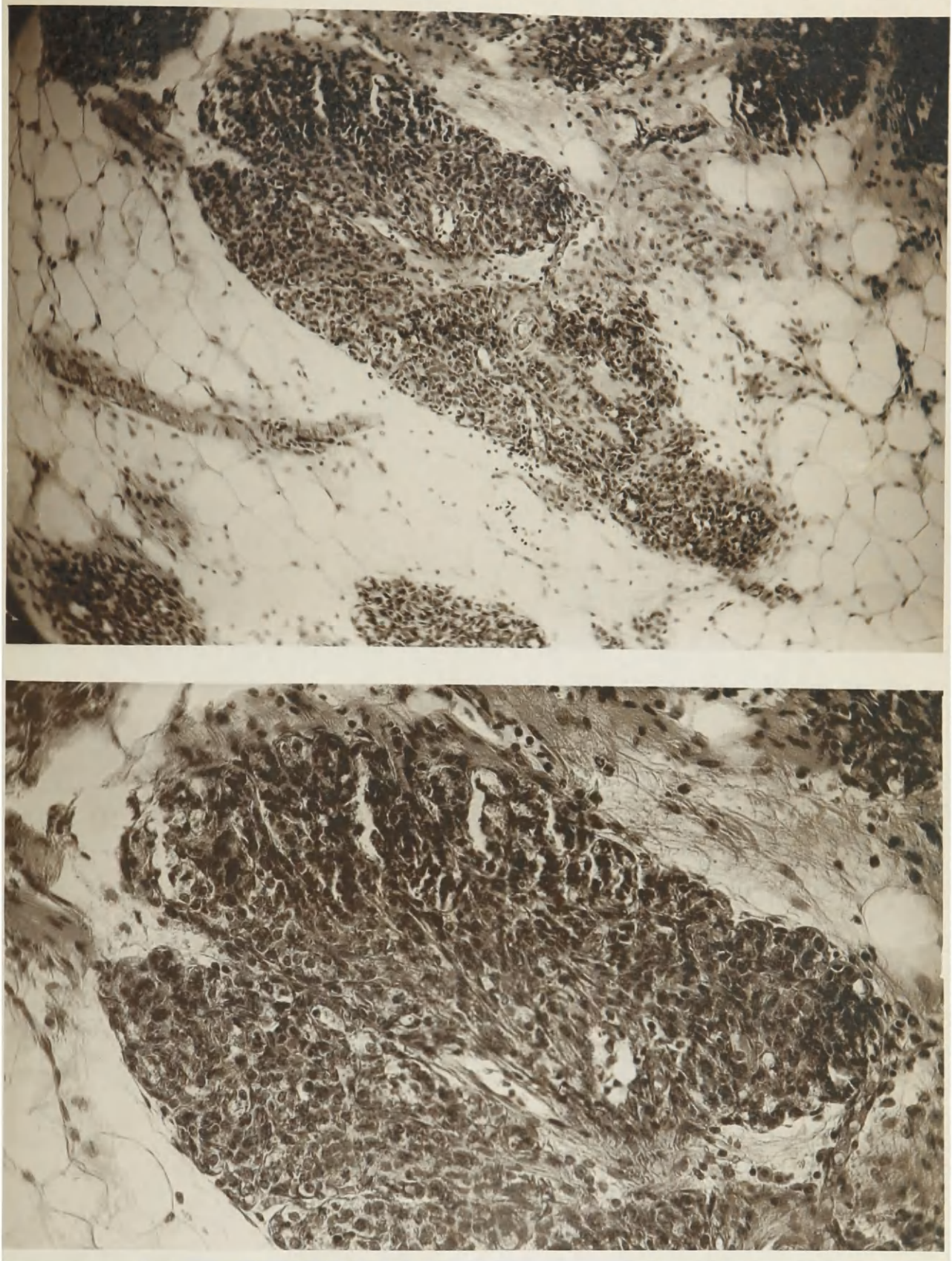and eosin.

Figure 9.

Figure 10.   Mammary gland of a rat receiving
             1 mg. prolactin daily for 10 days
             after young were removed. Top 130x,
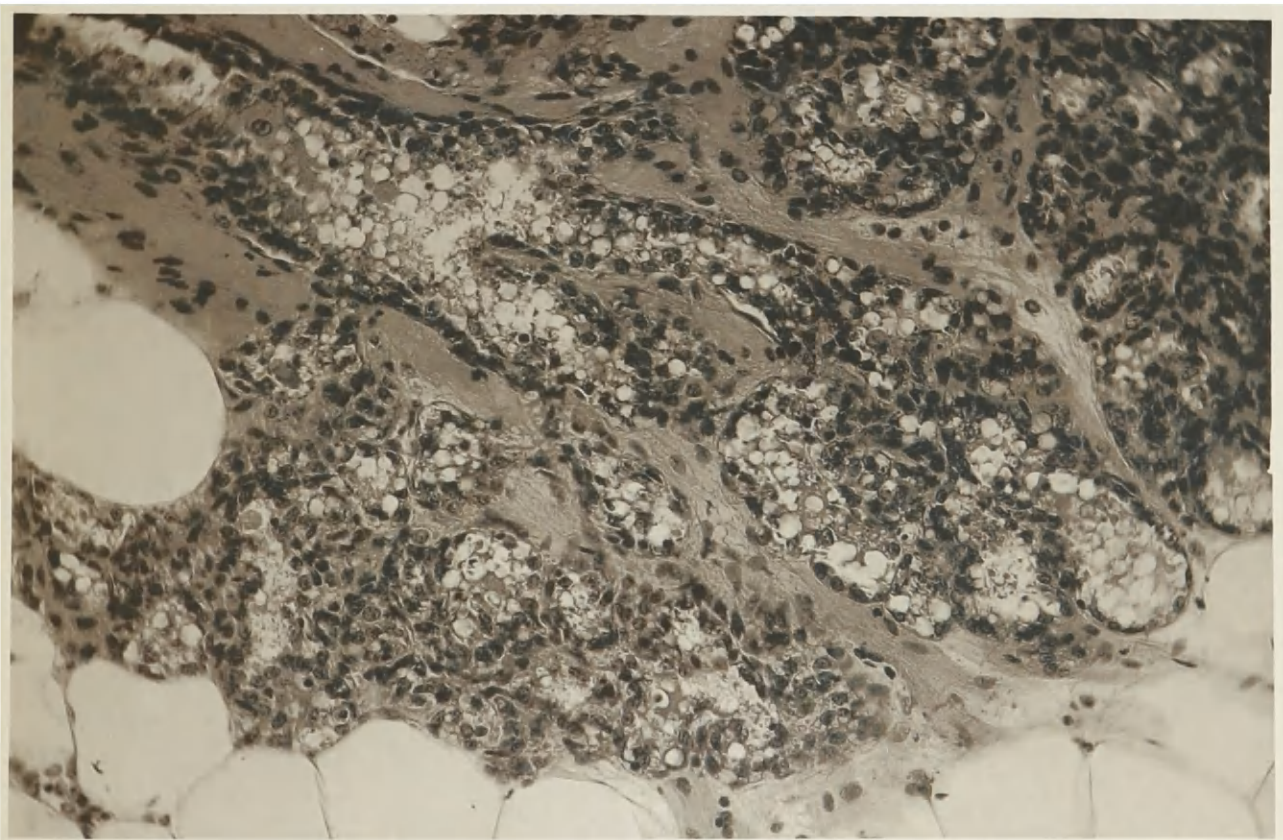   bottom 250x. Stained with hematoxylin
             and eosin.

Figure 10.

Figure 11.   Mammary gland of a rat receiving cortisone 0.5 mg., prolactin 1.0 mg. and growth hormone 1.0 mg. for 10 days after young were removed. Top 130x, bottom 250x. Stained with hematoxylin and eosin.
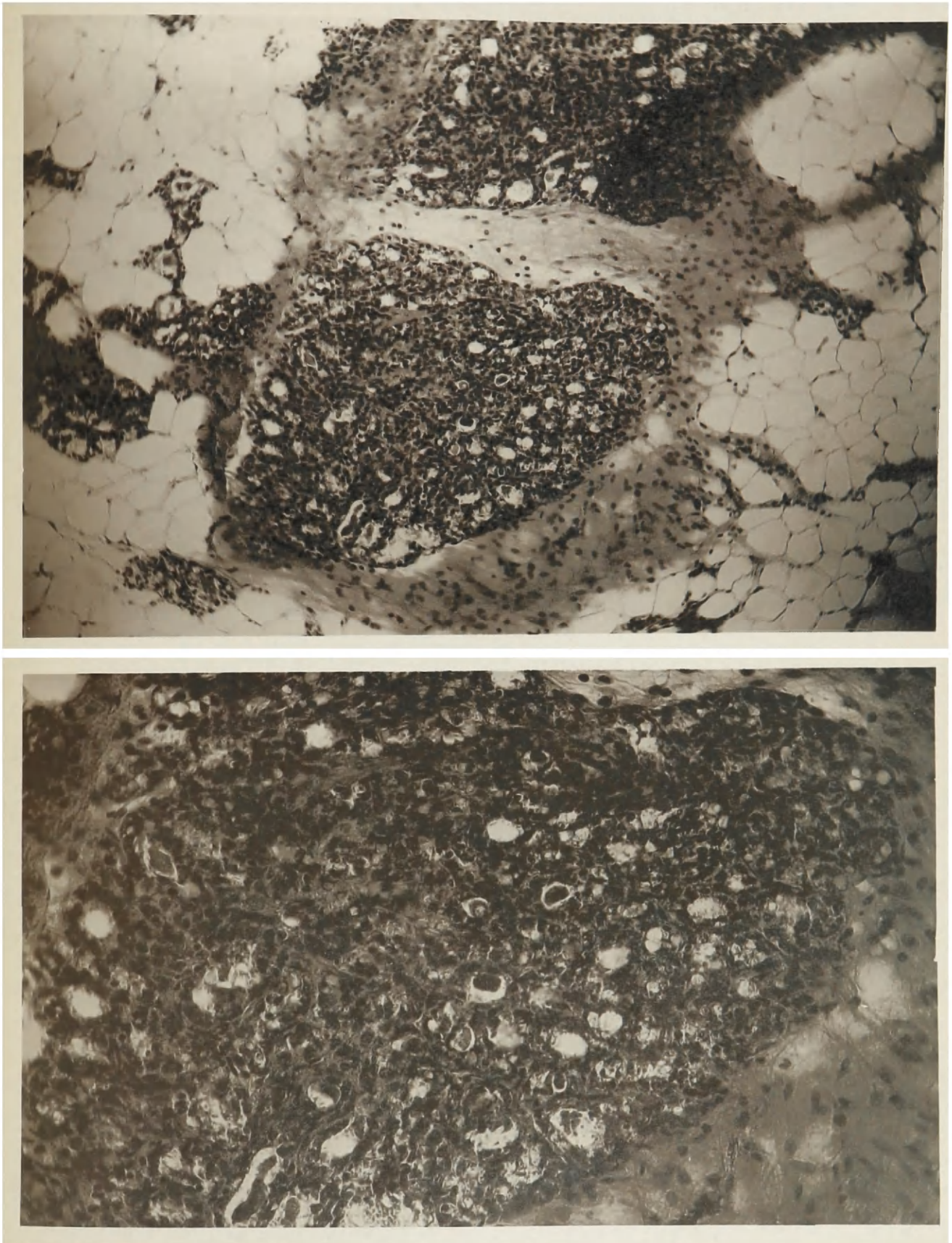
Figure 11.

Figure 12.   Mammary gland of a rat receiving
             oxytocin 0.5 I. U., twice daily,
             for ten days after young were re-
             moved. Top 130x, bottom 250x.
             Stained with hematoxylin and eosin.
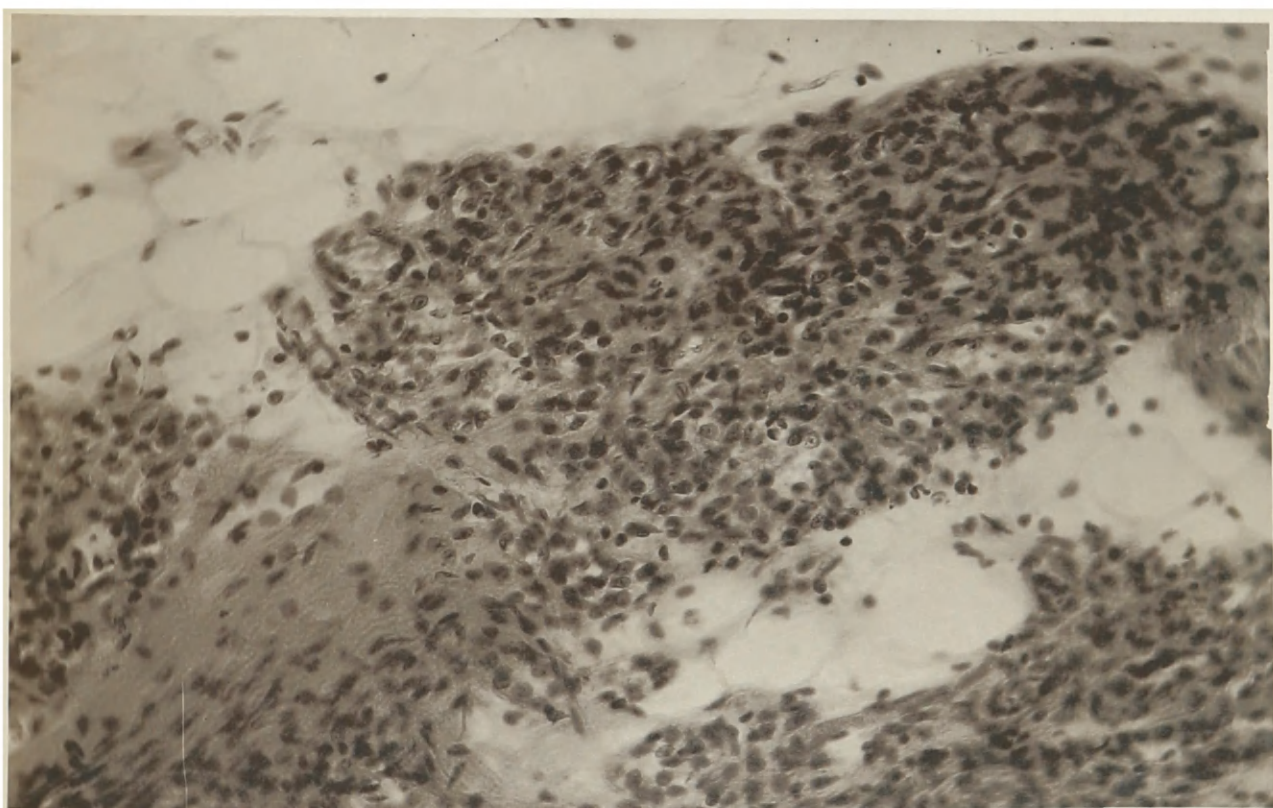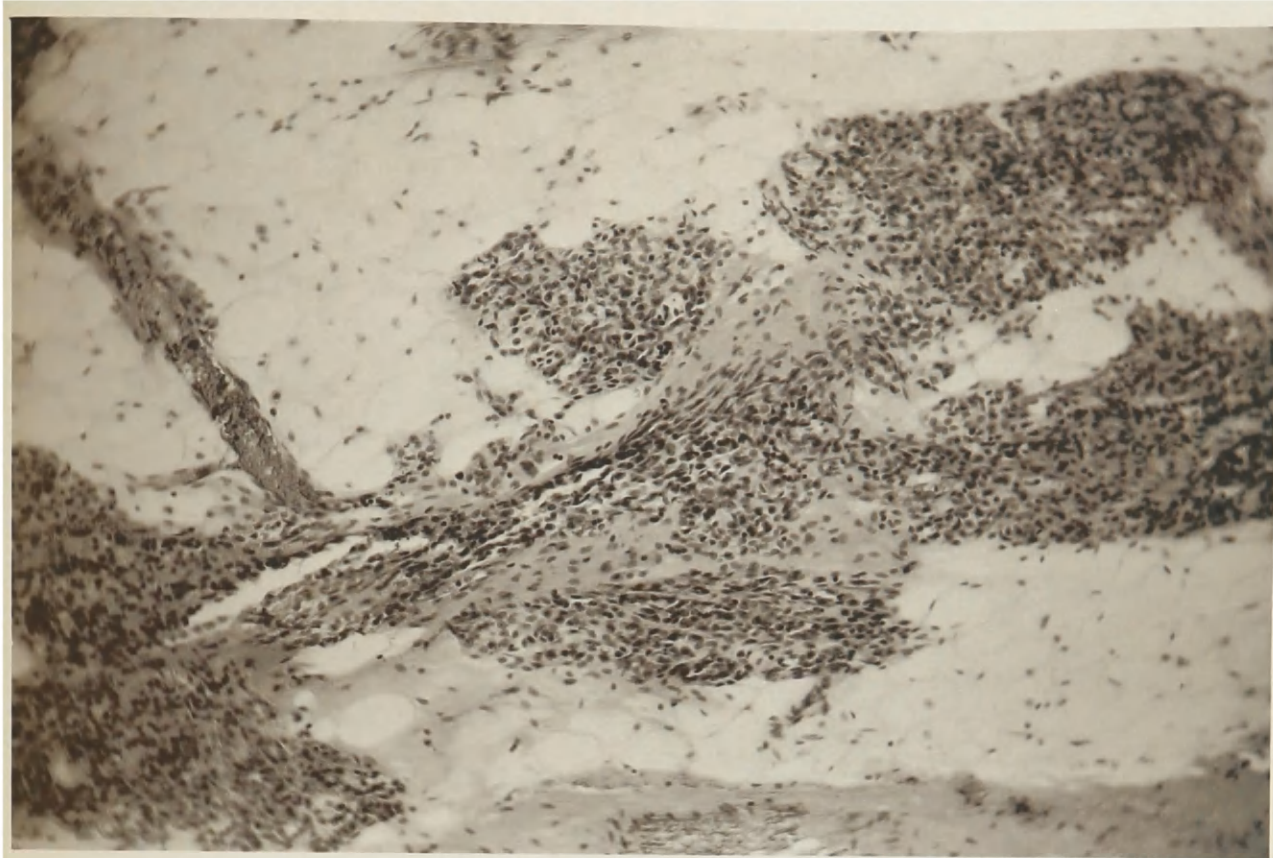
Figure 12.

Figure 13.   Mammary gland of a rat receiving ACTH
             1 mg. for 10 days after young were re-
             moved.   Top 130x, bottom 250x.   Stained
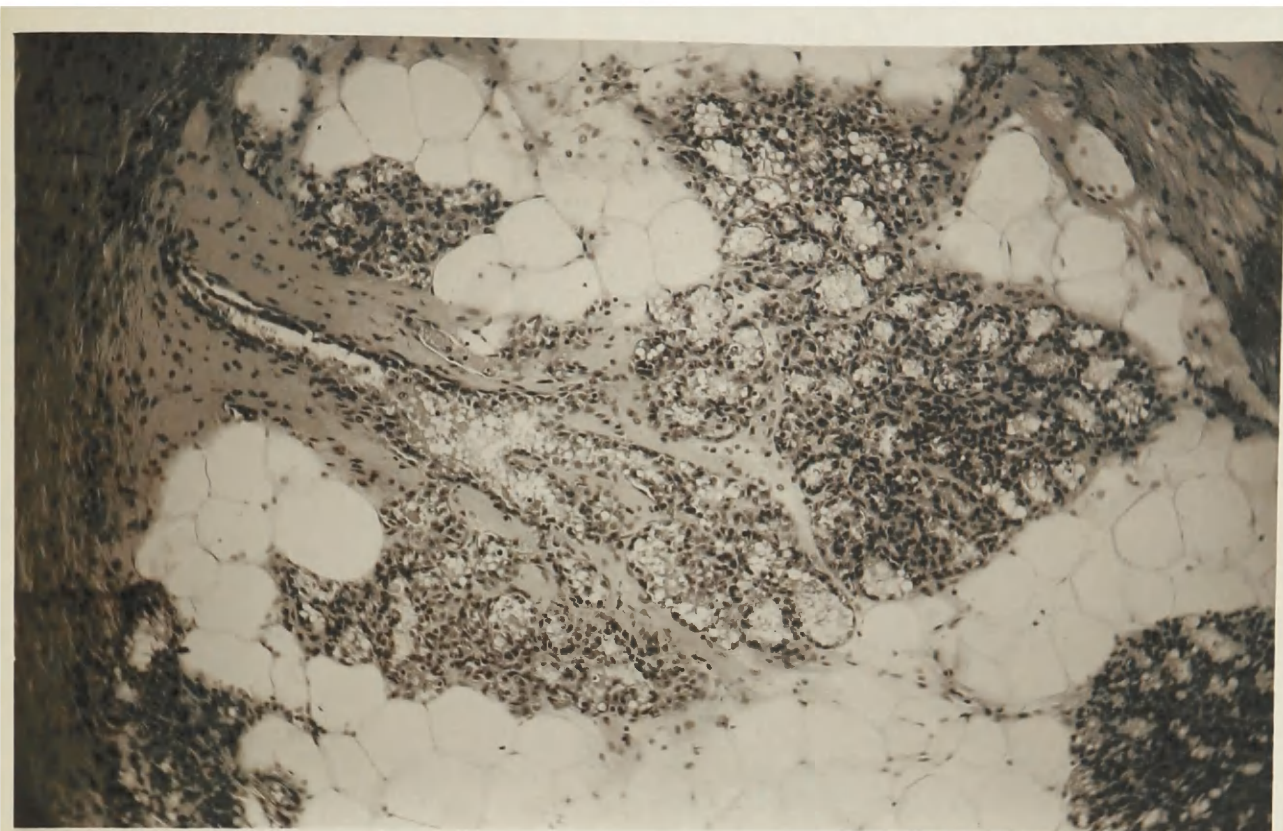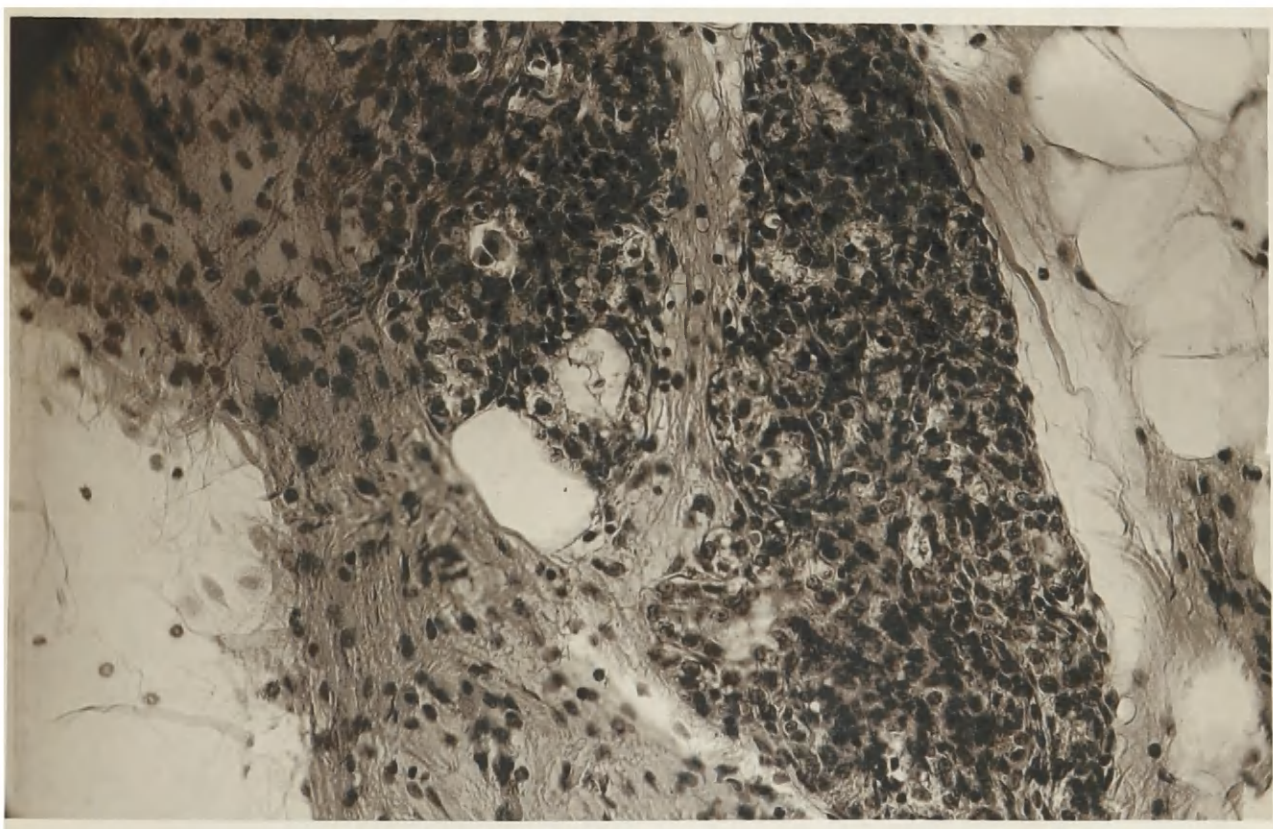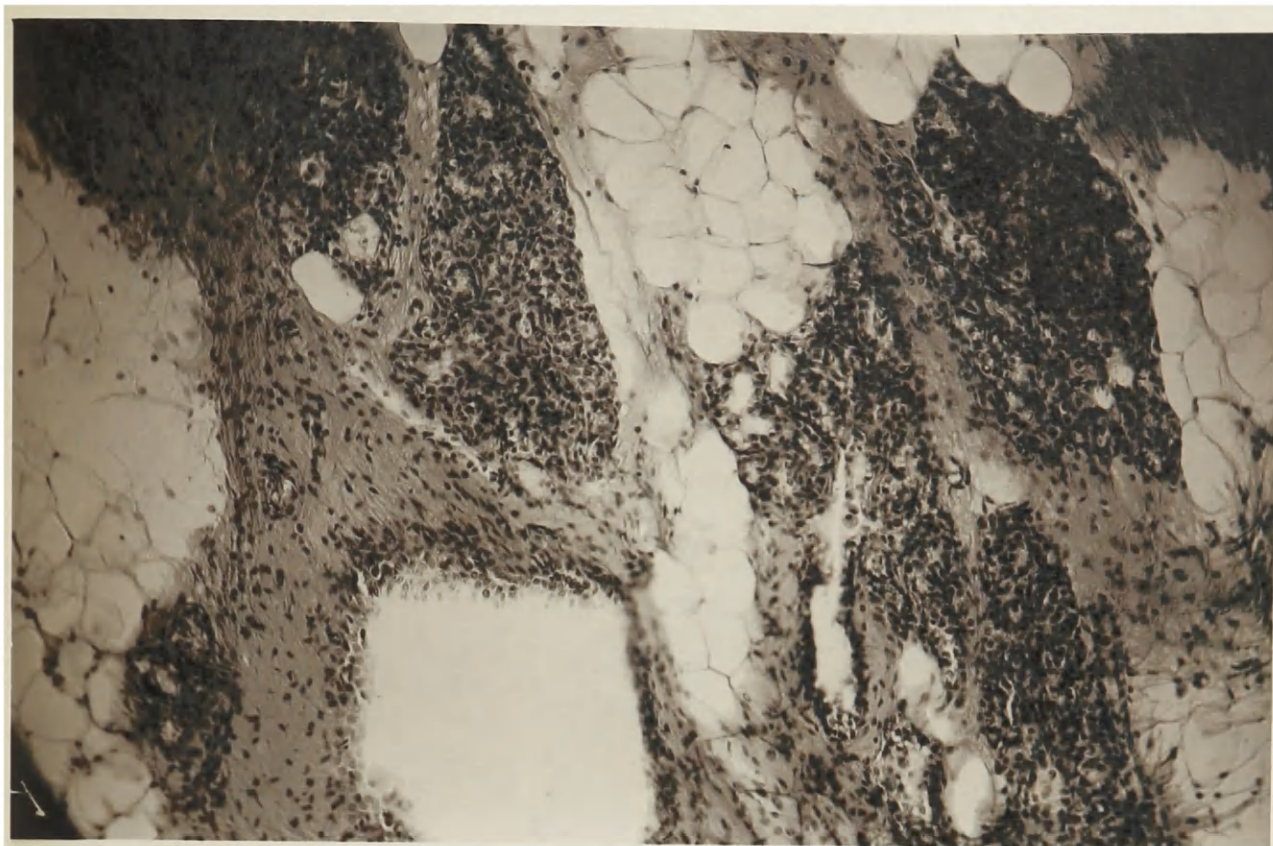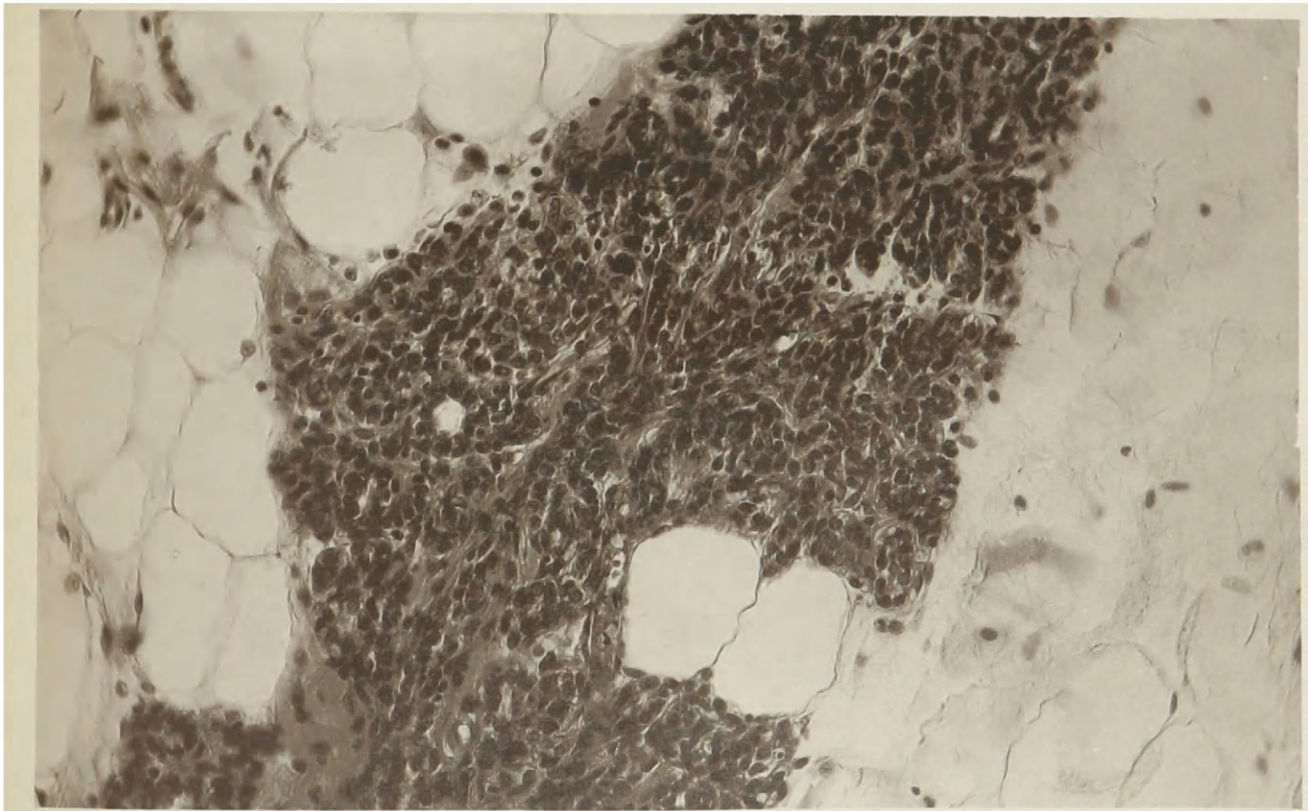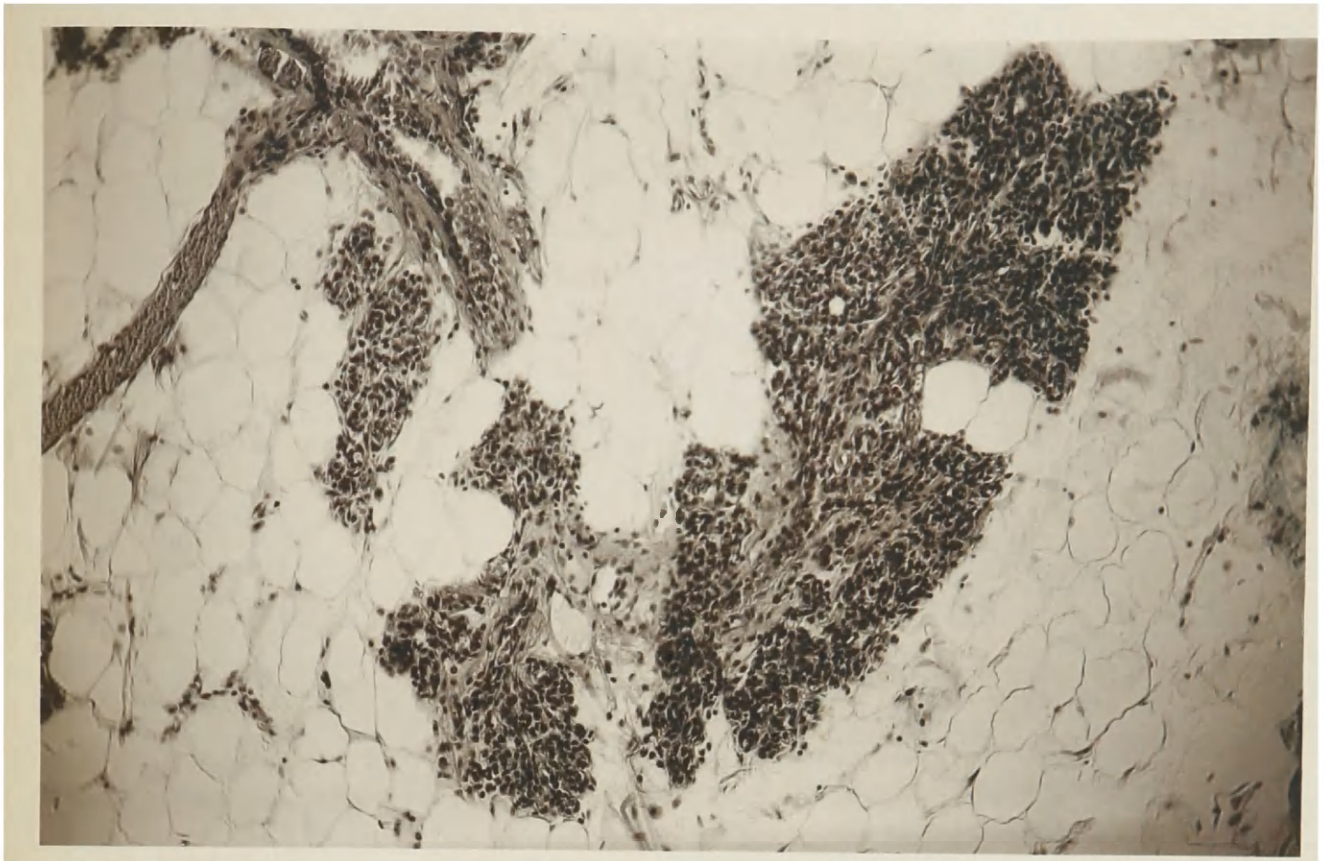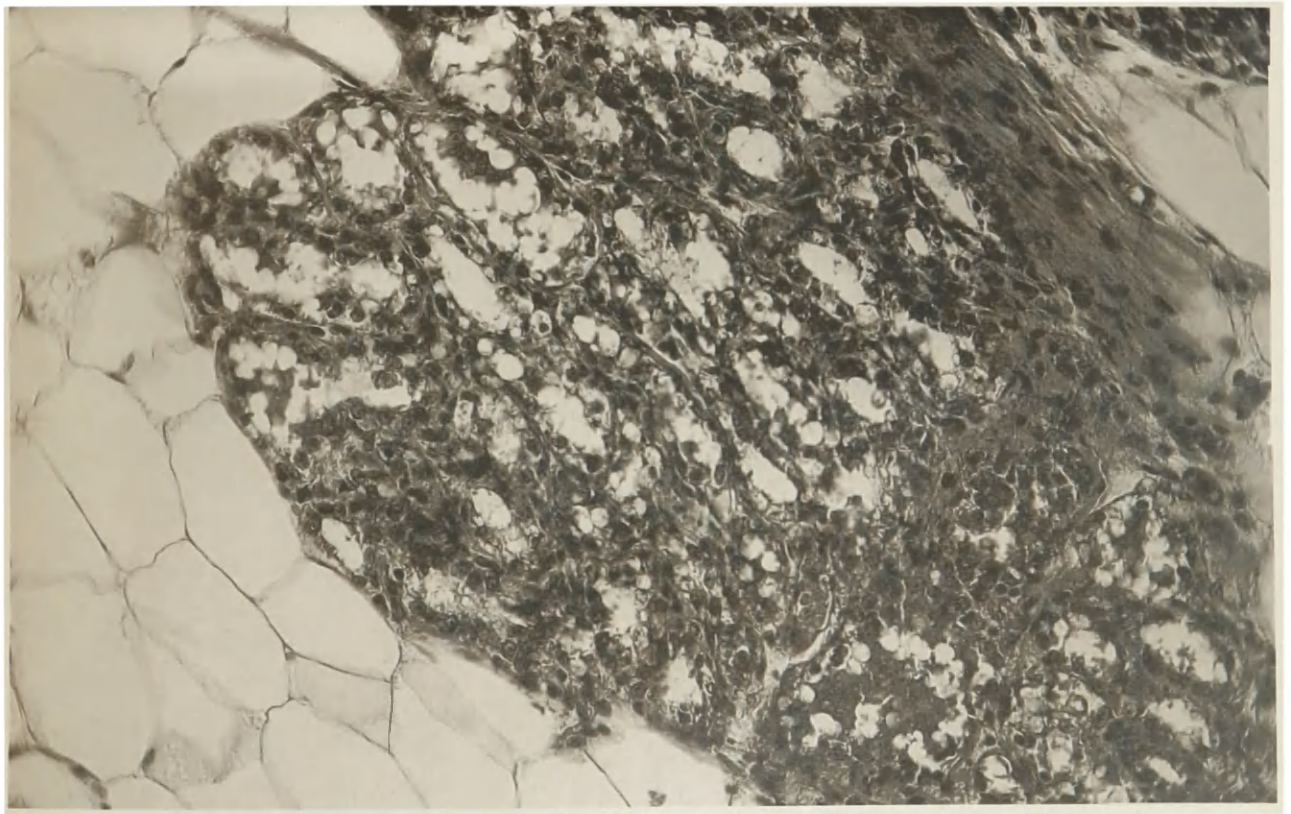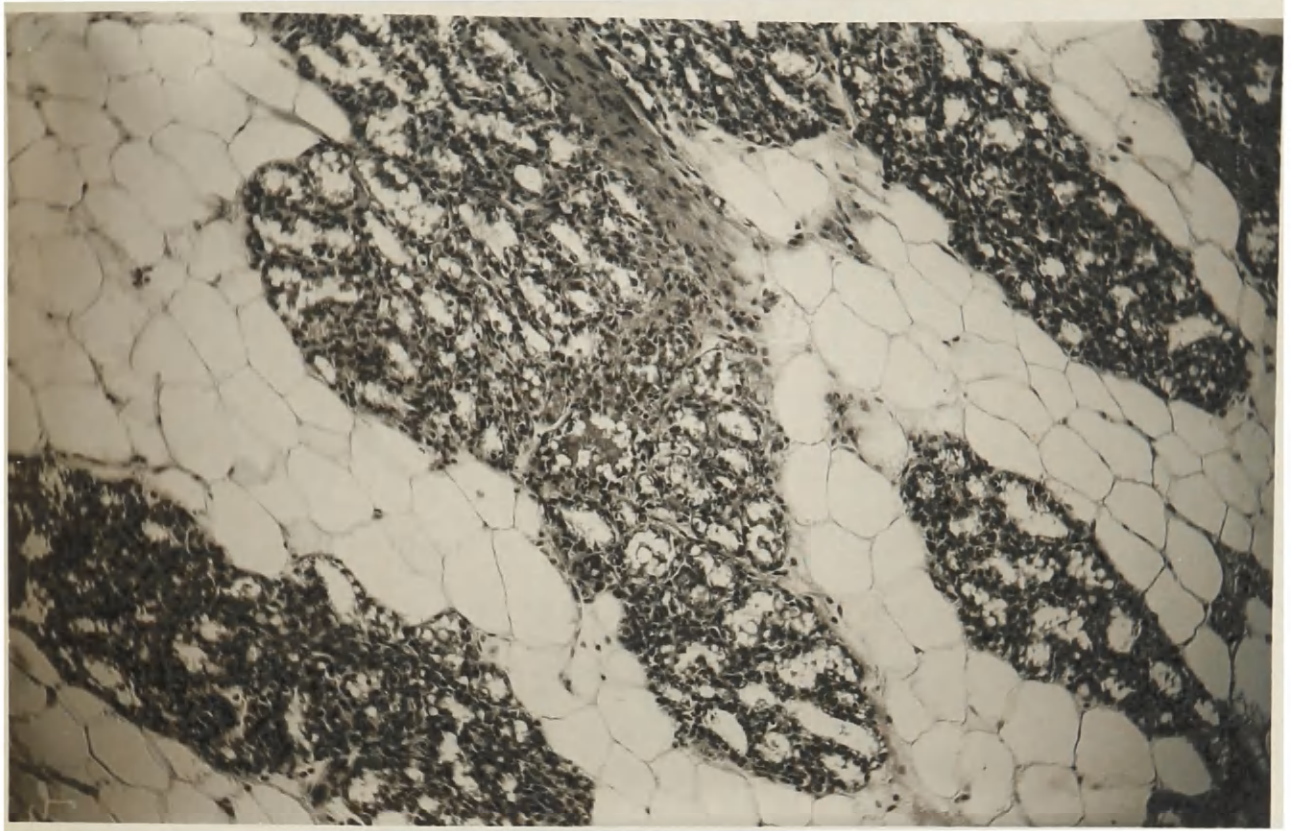   with hematoxylin and eosin.
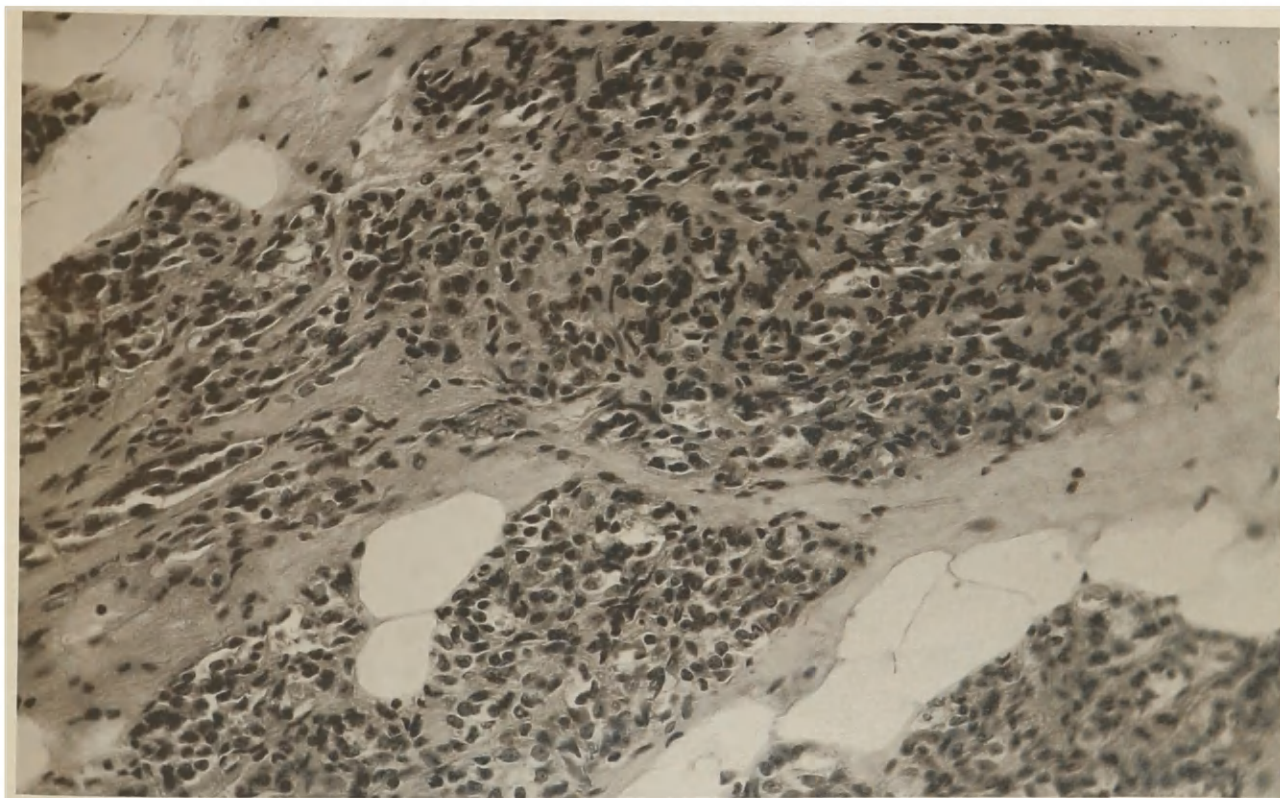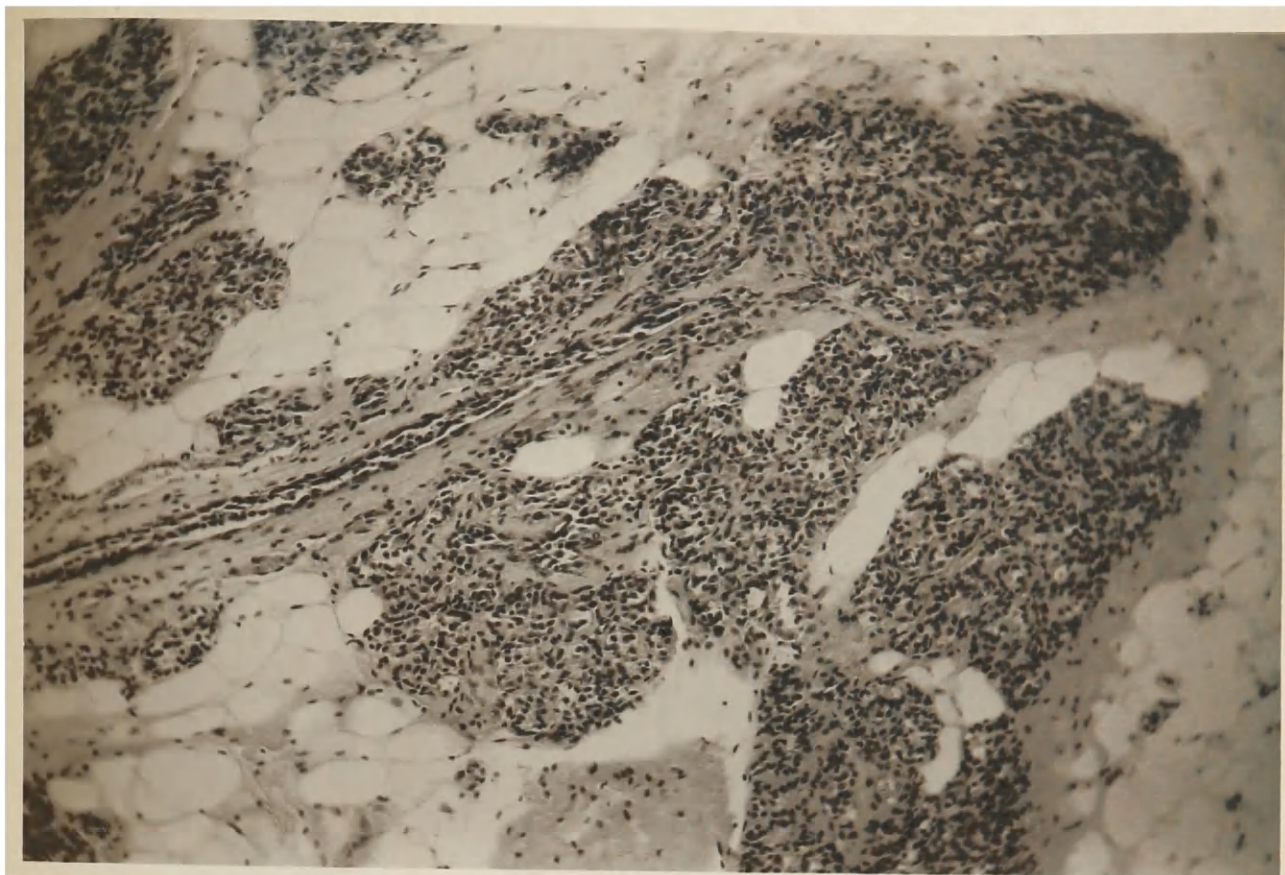
Figure 13.

EXPERIMENT V.   FACTORS INFLUENCING THE RELEASE OF
PROLACTIN IN SUCKLING MOTHER RATS

Procedure

The object of this experiment was to determine whether
injections of pitocin or a nervous stimulation which produced
pitocin release from the pituitary (electrical stimulation of
the cervix) results in release of pituitary prolactin of
lactating rats. For this investigation, 25 mature female
albino rats of the Carworth strain were bred. On the third
day after parturition, the dams were removed from their young
and were divided into 4 groups of 5 each. These were treated
as follows: (1) Negative controls. The dams were separated
from their young for 15 hours and were then killed. (2) Posi-
tive controls. After 12 hours of separation from their young,
the mothers were returned to their litters for a period of
three hours of suckling, at the end of which time the dams
were killed. (3) After 12 hours of separation from their young,
the cervices of the mother rats were electrically stimulated
for a period of one minute with 25 volts, on the 12th, 13th,
14th and 15th hours. The dams were killed immediately after
the last stimulation. (4) After 12 hours of separation from
their young, these rats were injected subcutaneously with 1.0
I. U. pitocin on the 12th, 13th, 14th and 15th hours. The
dams were killed immediately after the last injection.

In all four groups, the pituitaries were removed from the mother rats immediately after sacrifice on the 15th hour, and were weighed and prepared for prolactin assay. The prolactin content of the pituitaries from each group of rats was assayed in 20 white Carneau pigeons by the intradermal method of Reece and Turner (1937).

In one group of 10 pigeons, the positive control (group 2) pituitaries were injected over one crop sac and directly compared with the pituitaries from the electrically stimulated rats (group 3), injected over the other crop sac. In another group of 10 pigeons, the pituitary suspension from the negative control rats (group 1) were directly compared with the pituitaries from the pitocin-treated rats (group 4). One-quarter of a pituitary was injected over each crop sac during a 4-day period. On the 5th day the pigeons were killed and the crop glands were removed and rated visually for degree of proliferation.

Results

The pituitary weights, when compared on a body weight basis, did not show any significant alteration as a result of the different treatments. The negative controls (group 1) showed a significant increase in prolactin content of the pituitary by all three measures of comparison over that of the positive control (group 2). The electrically-stimulated rats (group 3) also showed a marked increase in pituitary prolactin

content over that of both control groups, while the greatest increase occurred in the pitocin-treated rats (Table 9).

These results show that suckling decreased pituitary prolactin content, while electrical stimulation of the cervix and pitocin injections apparently increased it.

TABLE 9

RELEASE OF PROLACTIN IN SUCKLING MOTHER RATS

Group No. and Treatment	Av. Body Wt. g.	Av. Pituitary Wt.		Av. No. Pigeon Units		
		Actual	Per 100g B.W.	Per Pit.	Per mg. Pit.	Per 100g B.W.
1) Controls (5)* (non-suckled)	242.50 ±6.80	10.78 ±1.07	4.44 ±.42	1.22 ±.07	.11	.504
2) Controls (5)* (suckled)	220.60 ±4.45**	12.12 ±.98	5.48 ±.41	0.70 ±.062	0.06	.318
3) Electrically stimulated (5)	241.20 ±10.89	10.92 ±.92	4.49 ±.28	1.40 ±.10	.137	.580
4) Pitocin (5)	240.00 ±7.24	10.46 ±.51	4.35 ±.17	2.20 ±.41	.206	.850

*Number of rats per group
**Standard error of the mean

DISCUSSION

The results of Experiment I show that cortisone, hydrocortisone and ACTH can induce mammary growth and secretion in intact female rats. This is in accord with similar findings of Selye (1954a, b) in ovariectomized, estrogen-primed rats. Although the reports of Selye (1954a, b) had not yet appeared when this study was completed, these results confirm and extend his observations. In addition they show that ACTH and glucocorticoids can increase the prolactin content of the pituitary and suggest that this mechanism is responsible for the initiation of secretion. In this study the greatest degree of mammary growth and secretion were produced by cortisone rather than by ACTH, as reported by Selye (1954a). The latter employed almost 25 times as much ACTH as was used in the present experiment.

The lesser degree of mammary growth noted with hydrocortisone as compared to cortisone in the present experiment may be due to over-dosage with the former, as indicated by the considerable loss of body weight in these rats.

In Experiment II, cortisone injected alone into ovariectomized rats induced some degree of mammary growth, but this growth was more limited than in intact rats. The alveoli were very small and duct growth was only slightly increased over that of the controls or estrone-primed rats.

Secretion in the cortisone-treated rats was not as marked as in the intact rats treated with cortisone in Experiment I. When cortisone was injected into estrone-primed rats, alveolar growth and secretion were greatly increased over that of the rats treated with cortisone alone. It appears therefore, that there is a synergistic action between cortisone and estrone in ovariectomized rats. It has been noted in the Review of Literature that in normal mammary growth, estrogen may produce a hyperaemia of the mammary stroma which could lead to increased vascular permeability. This in turn is believed to lead to easy access to the gland of progesterone and other mammary stimulating hormones (Mixner and Turner, 1943). This may explain the increased alveolar growth seen in the ovariectomized rats treated with both cortisone and estrone as compared to those treated with cortisone alone.

The explanation for the action of cortisone in inducing mammary growth and secretion is not fully understood, but it is believed that cortisone may exert a direct action on the mammary gland as well as on the anterior pituitary. Since secretory activity was not prominent when cortisone or estrone were given alone, it is apparent that the two hormones were most effective when given together. This is not surprising since both have been shown to increase pituitary prolactin content.

The glucocorticoids and ACTH appear to possess considerably less ability to increase pituitary prolactin content in rats than estrogen, although they are as much or more potent

in this respect than testosterone or progesterone (Meites and Turner, 1948).

The adrenals are believed to be essential for maintaining lactation in rats (Nelson and Gaunt, 1937; Cowie and Folley, 1947). However, the adrenal hormones have also been reported to inhibit established lactation. Flux et al (1954) and Shaw (1954, 1955) reported a decrease in cows. Meites and Reineke (unpublished, 1955) found that 100 mg. of cortisone injected daily into goats during the declining phase of lactation had no effect. In Experiment III and IV, cortisone increased the lactational response during the peak of lactation (Cowie's litter-growth index) and during the declining phase of lactation (11th-18th day post-partum). Although this increase was only moderate, it was of the same magnitude as attained with prolactin. When these two hormones and growth hormone were given in combination, the response was the same as with prolactin or cortisone alone. Apparently there was no synergistic action between the levels of cortisone, prolactin and growth hormone used in this experiment. It thus appears that cortisone may stimulate or inhibit lactation, depending on dosage and species used.

In Experiment IV the results indicate that both cortisone and prolactin retarded mammary involution when administered to rats for 10 days after suckling had been terminated. Hooker and Williams (1941) had previously shown that prolactin could retard mammary involution in mice. If cortisone acts on the

anterior pituitary to cause a release of prolactin, then its effects in retarding mammary involution may be mediated, in part at least, through this mechanism. Since cortisone also can induce mammary growth in the rat, it is probable that both these actions account for its ability to retard mammary involution.

The inability of growth hormone to increase the lactational response or to retard mammary involution is difficult to explain. It is not in agreement with the positive results of Cotes et al. (1949), Donker and Petersen (1951) and Shaw (1955) in the cow, and of Meites and Reineke (1955) in the goat. In about 40 percent of the rats treated with growth hormone, there were one or two abnormal young in each litter, while the litters in the controls remained normal. This suggests that the growth hormone used may have been toxic, or that it was detrimental when given to lactating as opposed to non-lactating rats.

In Experiment IV there was a strong indication that oxytocin and ACTH produced a galactopoietic effect. While these two hormones proved capable of increasing milk secretion during the peak and declining phase of lactation, they did not have as great an effect on the initial phase as cortisone or prolactin. Also, despite their ability to increase lactation, ACTH and oxytocin did not show any notable ability to retard mammary involution after the young were removed from the dams. This is difficult to explain in the case of ACTH. Presumably ACTH

stimulates adrenal cortical secretion, which in turn should increase pituitary prolactin content and maintain mammary growth. It is possible however, that the dose of ACTH employed was suboptimal for both of these actions. Closer examination of the histological sections indicate that there was a more definite alveolar pattern in the ACTH-treated glands than in the control glands.

The action of oxytocin in increasing litter weight gains may have been directly on the myoepithelium of the alveoli, making available a greater supply of milk to the young through its easier removal. Benson and Folley (1956) were able to markedly retard loss of secretory activity with intraperitoneal injections of 1 I. U. of oxytocin three times daily given from the fourth to thirteenth day after parturition in rats which had their young removed on the fourth day after parturition. The present results are not entirely in agreement with these workers. However, it should be pointed out that their studies were made on rats whose mammary glands were at the height of activity (4th-10th day), when involutionary changes would be expected to be minimal, while the present study was made on rats towards the end of lactation (18 days), when a larger degree of involution might be expected.

Benson and Folley (1956) suggested that the effects of oxytocin in retarding loss of secretory activity were not mediated through a direct action on the mammary gland but rather to a release of prolactin from the anterior pituitary.

Prolactin has previously been shown to inhibit mammary involution in the mouse (Hooker and Williams, 1941), and in this study, in the rat.

The results in Experiment V show that in lactating rats, suckling causes a release of prolactin, which confirms the original report of Reece and Turner (1937). Oxytocin does not have this effect. On the contrary these results suggest that oxytocin in the lactating rat may increase the prolactin content of the pituitary. Meites and Turner (1948) have previously reported that injections of posterior pituitary extracts do not elicit a release of prolactin from the pituitary of rats, guinea pigs and rabbits. These results are therefore not in agreement with the explanation of Benson and Folley (1956), and suggest that another mechanism is responsible for the favorable action of oxytocin in inhibiting decline in secretory activity. It is possible that oxytocin may cause the release of other anterior pituitary hormones favorable to lactation, such as ACTH. Posterior pituitary extracts have been shown to induce a release of ACTH (Saffran et al, 1955), and it has also been demonstrated that suckling induces a release of ACTH (Gregoire, 1946). It has not been demonstrated that oxytocin either stimulates or maintains mammary development.

SUMMARY

1. In Experiment I, physiological saline (0.85 percent) or 2 mg. doses of ACTH, cortisone or hydrocortisone were injected daily for 10 days into 42 intact, mature female rats. All three hormones produced growth of ducts, lobule-alveolar development and secretory activity in the mammary glands. This was most marked for cortisone and least for ACTH at the dose levels employed.

2. Cortisone increased pituitary prolactin content by about 23 percent, hydrocortisone by about 41 percent, and ACTH by about 71 percent.

3. In Experiment II, 100 mature female rats were ovariectomized and divided into ten groups of 10 rats each, according to weight. Eight groups were divided into two major groups of 40 rats each (four groups of 10 each) and the remaining two groups (20 rats each) were used for controls. The treatment of the groups was as follows:

(1) controls, saline only,

(2) controls, 1 mg. cortisone only.

The two major groups received either 5 ug. or 10 ug. of estrone. Within these two major groups cortisone was administered to each sub-group as follows: 1) none, 2) 1 mg. daily, 3) 2 mg. daily, 4) 4 mg. daily. All injections were made for 10 days.

4. Cortisone alone stimulated lobule-alveolar development and secretory activity in the mammary glands of the ovariectomized rats. However, mammary growth was not as pronounced as observed in the intact rats of Experiment I. On the other hand, when cortisone and estrone were given together, marked lobule-alveolar development was elicited and secretory activity was greatly increased.

5. Cortisone augmented the pituitary prolactin content of ovariectomized-estrone treated rats at the 1 mg. level. However, when 4 mg. of cortisone was given daily with 10 ug. of estrone, there was an inhibition of estrone action on the pituitary.

6. In Experiment III, the effects of cortisone on galactopoiesis was studied in 30 mature female rats. These rats were bred and at parturition their litters were reduced to 7 young each. The dams were injected daily during an 18-day post-partum period with 0.25, 0.5 or 1.0 mg. of cortisone. The lactational response was measured by the use of litter growth rate. The rats receiving 0.5 mg. cortisone showed a significant increase in milk yield during the peak (6th-10th day post-partum) and during the declining phase of lactation (11th-18th day post-partum).

Cortisone at the 1.0 mg. level only slightly increased the average litter growth during the 18-day experimental period. However, during the declining phase of lactation,

significant increases in litter weight gain were noted over that of the controls.

6. The results of Experiment III were confirmed in Experiment IV. In addition, injections of cortisone were continued for 10 days after the young were removed (10th-20th day), in order to study its effects on mammary involution. It was found that cortisone at the 1.0 mg. level markedly retarded mammary involution. These mammary glands were comparable to those of an untreated rat 5 days after removal of the young. Cortisone at the 0.5 mg. level produced slightly less retardation of mammary disintegration, comparable to that of an untreated rat six days after removal of its litter.

7. In Experiment IV the effect of growth hormone, prolactin, oxytocin and ACTH on galactopoiesis and mammary involution were studied. Prolactin given at a dosage of 1 mg. daily increased the average litter weight throughout the 18-day post-partum period. These increases were about equal to those of the rats treated with 0.5 mg. of cortisone daily. When cortisone, prolactin and growth hormone were administered together, the response was of about the same magnitude as cortisone or prolactin alone. Thus no synergistic action on galactopoiesis was exerted by these hormones. Growth hormone, when given alone, did not increase lactation.

8. Prolactin (1 mg.) or prolactin, cortisone and growth hormone given together, retarded mammary involution comparable to that of a mammary gland of an untreated rat

5 days after removal of the young. Growth hormone alone at the level employed did not retard mammary involution.

9. Oxytocin and ACTH exhibited galactopoietic effects in parturient rats comparable to those of prolactin or cortisone-treated rats. However, the former two hormones showed no ability to retard mammary involution following removal of the young for 10 days.

10. In Experiment V, suckling increased the pituitary prolactin content in lactating rats. Electrical stimulation of the cervix of lactating rats appeared to increase pituitary prolactin content over that of non-suckled or suckled rats. Injections of oxytocin appeared to produce a large increase in pituitary prolactin content over that of suckled, non-suckled or electrically stimulated rats. It appears, therefore, that neither oxytocin nor electrical stimulation of the cervix induces a release of prolactin from the pituitary.

BIBLIOGRAPHY

Azimov, G. J. and Krouze, N. K. The lactogenic preparations from the anterior pituitary and the increase of milk yield in cows. J. Dairy Sci. 20:289-306, 1937.

Balmain, J. H., and Folley, S. J. In vitro effects of prolactin and cortisone on the metabolism of rat mammary tissue. Arch. Biochem. Biophys. 39:188-194, 1952.

Balmain, J. H., Folley, S. J. and Glascock, R. F. Inhibition of fatty acid synthesis in rat mammary gland slices by cortisone in vitro and its antagonism by insulin. Nature 169:447-449, 1952.

Benson, G. K. and Folley, S. J. Oxytocin as stimulator for the release of prolactin from anterior pituitary. Nature 177:700, 1956.

Bradbury, J. T. Study of endocrine factors influencing mammary development and secretion in the mouse. Proc. Soc. Exper. Biol. and Med. 30:212-213, 1932.

Brody, S. Bioenergetics and Growth. Reinhold Publishing Corp., N. Y., pp. 507-517, 1945.

Brownell, K. A., Lockwood, J. E., and Harman, F. A. A lactation hormone of the adrenal cortex. Proc. Soc. Exper. Biol. and Med. 30:783-784, 1933.

Carr, J. L. Effect of Swingle's extract upon lactation in the adrenalectomized white rat. Proc. Soc. Exper. Biol. and Med. 29:131, 1931.

Clemenko, D. R., and McChesney, E. W. Influence of epinephrine and adrenal cortical extract on the lactogenic property of prolactin. Endocrinology 28:710-714, 1941.

Corner, G. W. The hormonal control of lactation. I. Non-effect of the corpus luteum. II. Positive action of extracts of the hypophysis. Am. J. Physiol. 95:43-54, 1930.

Cotes, P. M., Crichton, J. A., Folley, S. J. and Young, F. G. Galactopoietic activity of purified anterior pituitary growth hormone. Nature 164:992-993, 1949.

Cowie, A. T. The relative growth of the mammary gland in normal gonadectomized and adrenalectomized rats. J. of Endocrinology 6:145-157, 1949.

Cowie, A. T. Influence on the replacement value of some adrenal-cortex steroids of dietary sodium and synergism of steroids in lactating adrenalectomized rats. Endocrinology 51:217-225, 1952.

Cowie, A. T., and Folley, S. J. Effects of adrenalectomy and anterior pituitary injection on mammary development. Nature 154:302-303, 1944.

Cowie, A. T. and Folley, S. J. The measurement of lactational performance in the rat in studies of the endocrine control of lactation. J. Endocrinology 5:9-13, 1947a.

Cowie, A. T. and Folley, S. J. Adrenalectomy and replacement therapy in lactating rats. 2. Effects of deoxycorticosterone acetate on lactation in adrenalectomized rats. J. Endocrinology 5:14-23, 1947b.

Cowie, A. T. and Folley, S. J. The role of the adrenal cortex in mammary development and its relation to the mammogenic action of the anterior pituitary. Endocrinology 40:274-285, 1947.

Cross, B. A. The hypothalmus and the mechanism of sympatheticoadrenal inhibition of milk ejection. J. Endocrinology 12:15-28, 1955a.

Cross, B. A. Neurohormonal mechanisms of emotional inhibition of milk ejection. J. Endocrinology 12:29-37, 1955b.

Cross, B. A. and Harris, G. W. The role of the neurohypophysis in the milk ejection reflex. J. Endocrinology 8:148-161, 1952.

Desclin, L. Concerning the mechanism of diestrum during lactation in the albino rat. Endocrinology 40:14-29, 1947.

Donker, J. D. and Petersen, W. E. The milk secretion response of the bovine to injected growth hormone. J. Anim. Sci. 10:1074, 1951.

Espe, D. and Smith, V. R. Secretion of Milk. The Iowa State College Press, Fourth edition, 1952.

Ely, F. and Petersen, W. E. Factors involved in the ejection of milk. J. Dairy Sci. 24:211-223, 1941.

Flux, D. S. Growth of the mammary duct system in intact and ovariectomized mice of the CHI strain. J. Endocrinology, 11:223-237, 1954a.

Flux, D. S. The effect of adrenal steroids on the growth of the mammary glands, uteri, thymus and adrenal glands of intact, ovariectomized and oestrone-treated ovariectomized mice. J. Endocrinology 11:238-254, 1954b.

Flux, D. S., Folley, S. J. and Rowland, S. J. The effect of adrenocorticotrophic hormone on the yield and composition of the milk of the cow. J. Endocrinology 10: 333-339, 1954.

Folley, S. J. The adrenal cortex and the mammary gland. The Supra-adrenal Cortex, Academic Press Inc., N. Y., 85-94, 1952.

Folley, S. J. Effects of somatotropin and other pituitary hormones on the lactating mammary gland. Hypophyseal. Growth Hormone, Nature and Action, McGraw-Hill Book Co., Inc., N. Y., pp. 473-486, 1955.

Folley, S. J. and Cowie, A. T. Adrenalectomy and replacement therapy in lactating rats. Yale J. Biol. Med. 17: 67-74, 1944.

Folley, S. J. and French, T. H. The intermediary metabolism of the mammary gland. 2. Respiration and acid production of mammary tissue during pregnancy, lactation and involution in the rat. Biochem. J. 45:270-275, 1949.

Folley, S. J., Scott Watson, H. M., and Bottomly, A. C. Induction of lactation in goats with diethylstilbestrol dipropionate. J. Physiol. 98:15-16, 1940.

Folley, S. J., Steward, P. L. and Young, F. G. Experiments on the use of tables containing 50 percent hexoestrol for artificial induction of lactation in bovine. J. Endocrinology 4:43-52, 1944 (Cited by Espe, D. and Smith, V. R., Secretion of Milk, 1952.)

Folley, S. J. and Young, F. G. The effect of anterior pituitary extracts on established lactation in the cow. Proc. Roy Soc. B. 126:45-76, 1938.

Folley, S. J. and Young, F. G. The effect of continued treatment with anterior pituitary extracts on milk volume and milk fat production in the lactating cow. Biochem. J. 33:192-197, 1939.

Folley, S. J. and Young, F. G. Further experiments on the continued treatment of lactating cows with anterior pituitary extracts. J. Endocrinology 2:226-236, 1940. (Cited by Espe, D. and Smith, V. R. Secretion of Milk, 1952.)

Gardner, W. U., and Hill, R. T. Effect of progestin upon the mammary gland of the mouse. Proc. Soc. Exper. Biol. and Med. 34:718-720, 1936.

Gaunt, R. Adrenalectomy in the rat. Am. J. Physiol. 103:494-510, 1933.

Gaunt, R. Lactation in adrenalectomized rats. Am. J. Physiol. 115:588-598, 1936.

Gaunt, R., Eversole, W. J., and Kendall, E. C. Influence of some steroid hormones on lactation in adrenalectomized rats. Endocrinology 31:84-88, 1942.

Gemzell, C. A. Blood levels of 17-hydroxycorticosteroids in normal pregnancy. J. Clin. Endo. and Metab. 13:898-902, 1953.

Gomez, E. T. and Turner, C. W. Effects of hypophysectomy and replacement therapy on lactation in guinea pigs. Proc. Soc. Exper. Biol. and Med. 34:404-406, 1936.

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

Gomez, E. T. and Turner, C. W. The adrenotropic principle of the pituitary in relation to lactation. Proc. Soc. Exper. Biol and Med. 36:78-80, 1937b.

Gomez, E. T. and Turner, C. W. Further evidence for a mammogenic hormone in the anterior pituitary. Proc. Soc. Exper. Biol. and Med. 37:607-609, 1938.

Gomez, E. T., Turner, C. W., and Reece, R. P. Growth of mammary gland of hypophysectomized guinea pigs. Proc. Soc. Exper. Biol. and Med. 36:286-287, 1937.

Gregoire, C. Factors involved in maintaining involution of the thymus during suckling. J. Endo. 5:66-87, 1946.

Hohn, E. D. Estrogen response of mammary gland in adrenalectomized guinea pigs. Fed. Proc. 16:60, 1957.

Hooker, C. W. and Williams, W. L. Retardation of mammary involution in the mouse by irritation of the nipples. Yale J. Biol. Med. 12:559-564, 1940.

Hooker, C. W. and Williams, W. L. Retardation of mammary involution in mice by injection of lactogenic hormone. Endocrinology 28:42-47, 1941.

Hurst, J., Meites, J. and Turner, C. W. Assay of adrenals for lactogenic hormone. Proc. Soc. Exper. Biol. and Med. 49:592-594, 1942.

Johnston, R. F. and Smithcors, J. F. The effects of estrogen on mammary structure of adrenalectomized and thiouracil-treated castrated rats. Endocrinology 43:193-201, 1948.

Lyons, W. R. The direct mammotrophic action of lactogenic hormone. Proc. Soc. Exper. Biol. and Med. 51:308-311, 1942.

Lyons, W. R. Lobule-alveolar mammary growth induced in hypophysectomized rats by injections of ovarian hypophyseal hormones. Essay in Biology. University of California Press, Los Angeles, 315-330, 1943.

Lyons, W. R., Choh, H. F., Cole, R. D. and Johnson, R. E. Some of the hormones required by the mammary gland in its development and function. J. Clin. Endo. and Metab. 13:836-837, 1953.

Lyons, W. R., Johnson, R. E., Cole, R. D., and Li, C. H. Mammary growth and lactation in male rats. Hypophyseal Growth Hormone, Nature and Action. McGraw-Hill Book Co., Inc., 461-472, 1955.

Lyons, W. R. and Catchpole, H. R. Availability of the rabbit for assay of the hypophyseal lactogenic hormone. Proc. Soc. Exper. Biol. and Med. 31:305-309, 1933.

Meites, J. General discussion. Hypophyseal Growth Hormone, Nature and Action. McGraw-Hill Book Co., Inc., N. Y., 493-495, 1955.

Meites, J., Trentin, J. J. and Turner, C. W. Effect of adrenalectomy on the lactogenic hormone and initiation of lactation. Endocrinology 31:607-612, 1942.

Meites, J. and Turner, C. W. Studies concerning the induction and maintenance of lactation. I. The mechanism controlling the initiation of lactation at parturition. Res. Bull. Mo. Agri. Exper. Sta. 415, 1948.

Meites, J. and Turner, C. W. Studies concerning the induction and maintenance of lactation. II. The normal maintenance and experimental inhibition and augmentation of lactation. Res. Bull. Mo. Agri. Exper. Sta. 416, 1948b.

Mixner, J. P., Meites, J. and Turner, C. W. The stimulation and inhibition of milk secretion in goats with diethylstilbestrol. J. Dairy Sci. 27:957-964, 1944.

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

Nelson, W. O. Endocrine control of the mammary gland. Physiol. Rev. 16:488-526, 1936.

Nelson, W. O. Studies on the physiology of lactation. VI. The endocrine influences concerned in the development and function of the mammary gland of the guinea pig. Am. J. Anat. 60:341-366, 1937.

Nelson, W. O. Growth of the mammary gland following local application of estrogenic hormone. Am. J. Physiol. 133:397, 1941.

Nelson, W. O. and Gaunt, R. Initiation of lactation in the hypophysectomized guinea pig. Proc. Soc. Exper. Biol. and Med. 34:671-673, 1936.

Nelson, W. O. and Gaunt, R. The adrenal-pituitary relationship. Symposium on Quant. Biol., Cold Spring Harbor, 5:398-402, 1937a.

Nelson, W. O., and Gaunt, R. The adrenal and pituitary in initiation of lactation. Proc. Soc. Exper. Biol. and Med. 36:136-138, 1937b.

Nelson, W. O. and Pfiffner, J. J. An experimental study of the factors concerned in mammary growth and in milk secretion. Proc. Soc. Exper. Biol. and Med. 28:1-2, 1930.

Nelson, W. O. and Pfiffner, J. J. Studies on the physiology of lactation. I. The relation of lactation to ovarian and hypophyseal hormones. Anat. Rec. 51:51-83, 1931.

Reece, R. P. Initiation of lactation in the albino rat with lactogen and adrenal cortical hormones. Proc. Soc. Exper. Biol. and Med. 40:25-27, 1939.

Reece, R. P. and Turner, C. W. The lactogenic and thyrotropic hormone content of the anterior lobe of the pituitary gland. Res. Bull. Mo. Ag. Exper. Sta. 266, 1937a.

Reece, R. P. and Turner, C. W. Effects of stimulus of suckling upon galactin content of the rat pituitary. Proc. Soc. Biol. and Med. 35:621-622, 1937b.

Reece, R. P., Turner, C. W. and Hill, R. T. Mammary gland development in the hypophysectomized albino rat. Proc. Soc. Exper. Biol. and Med. 34:204-207, 1937.

Richardson, K. C. Measurement of the total area of secretory epithelium in the lactating mammary gland of the goat. J. Endocrinology 9:170-184, 1953.

✓ Riddle, O. and Bates, R. W. Chapter XX in Sex and Internal Secretions. The Williams and Wilkins Co., 2nd edition, Baltimore, 1939.

Roy, A. The galactopoietic activity of hormones in cows in declining lactation. J. Endocrinology 5:35, 1948.

Saffran, M., Schally, A. V. and Benney, B. G. Stimulation of the release of corticotropin from the adenohypophysis by a neurohypophyseal factor. Endocrinology 57:439-444, 1955.

Selye, H. On the nervous control of lactation. Am. J. Physio. 107:535-538, 1934.

Selye, H. Stress and lactation. Revue Canadienne de Biologie 13:377-384, 1954a.

Selye, H. The effect of cortisol upon the mammary glands. Acta Endocrinologia 17:394-401, 1954b.

Selye, H. and McKeown, T. Further studies on the influence of suckling. Anat. Rec., 60:323-332, 1934.

Shaw, J. C. Influence of growth hormone on the mammary gland on human metabolism. The Hypophyseal Growth Hormone, Nature and Actions, McGraw-Hill Book Co., Inc., N. Y., 486-492, 1955.

Shaw, J. C., Chung, A. C., and Bunding, I. The effect of pituitary, growth hormone and adrenocorticotrophic hormone on established lactation. Endocrinology 56: 327-334, 1955.

Silver, M. A quantitative analysis of the role of oestrogen in mammary development in the rat. J. Endocrinology 10:17-34, 1953.

Stricker, P., and Grueter, F. Recherches experimentales sur les functions du lobe anterieur de l'hypophyse. Influence des extraits du lobe anterieur sur l'appareil genital de la lapine et sur la montee laiteuse. Presse. Med. 37:1268, 1929. (Cited by Meites, J. and Turner, C. W., Res. Bull. Mo. Agri. Exper. Sta. 413, 1938.

Tabachnick, I. I. A. and Trentin, J. J. Effect of suckling on adrenal ascorbic acid level of mice. Fed. Proc. 10:339-340, 1951.

Trentin, J. J. and Turner, C. W. Effect of adrenalectomy on the mammary gland of the castrated and estrogen-treated castrated male rat. Endocrinology 41:127-134, 1947.

Turner, C. W. and Gomez, E. T. The normal development of the mammary gland of the male and female guinea pig. Res. Bull. Mo. Agri. Exper. Sta. 194, 1933.

Turner, C. W. and Gomez, E. T. The experimental development of the mammary gland. I. The male and female albino mouse. Res. Bull. Mo. Agri. Exper. Sta. 206, 1934.

Turner, C. W. and Meites, J. Effects of desoxycorticosterone on pituitary and lactogen content. Proc. Soc. Exper. Biol. and Med. 47:232-234, 1947.

Turner, C. W. and Reineke, E. P. A study of the involution of the mammary gland of the goat. Res. Bull. Mo. Agri. Exper. Sta. 235, 1936.

Turner, C. W. and Schultze, A. B. A study of the causes of the normal development of the mammary gland of the albino rat. Res. Bull. Mo. Agri. Exper. Sta. 157:1931.

Van Heuverswyn, J., Folley, S. J., and Gardner, W. U. Mammary growth in male mice receiving androgens, estrogens and desoxycorticosterone acetate. Proc. Soc. Exper. Biol. and Med. 41:389-392, 1939.

Venning, E. H. Adrenal function in pregnancy. Endocrinology 39:203-223, 1946.

Walker, S. M. and Stanley, A. J. Induced lactation in virgin heifers. <u>Anat. Rec.</u> 78:142, 1940.

Walker, S. M. and Stanley, A. J. Effect of diethylstilbestrol dipropionate on mammary development and lactation. <u>Proc. Soc. Exper. Biol. and Med.</u> 48:50-53, 1941.

Williams, W. L. Normal and experimental mammary involution in the mouse as related to the inception and cessation of lactation. <u>Am. J. Anat.</u> 71:1-41, 1942.

Williams, W. L. The effect of lactogenic hormone on post-parturient unsuckled mammary glands of the mouse. <u>Anat. Rec.</u> 93:171-181, 1945.

APPENDIX

Figures 14 to 23 are histological sections of mammary glands from normal parturient rats showing daily involutionary changes in mothers from the first to ninth day after removal of their young. In this series the young were removed 18 days post-partum, and the mothers were sacrificed each day thereafter until the 27th day post-partum. All figures are at a magnification of 130x and stained with hematoxylin and eosin.

Figure 14.   Eighteenth day post-partum or immediately
            after removal of young. Sections are
            comprised almost completely of large
            lobules with dilated alveoli containing
            copious secretion. The nuclei lie at the
            cell bases. The thin connective tissue
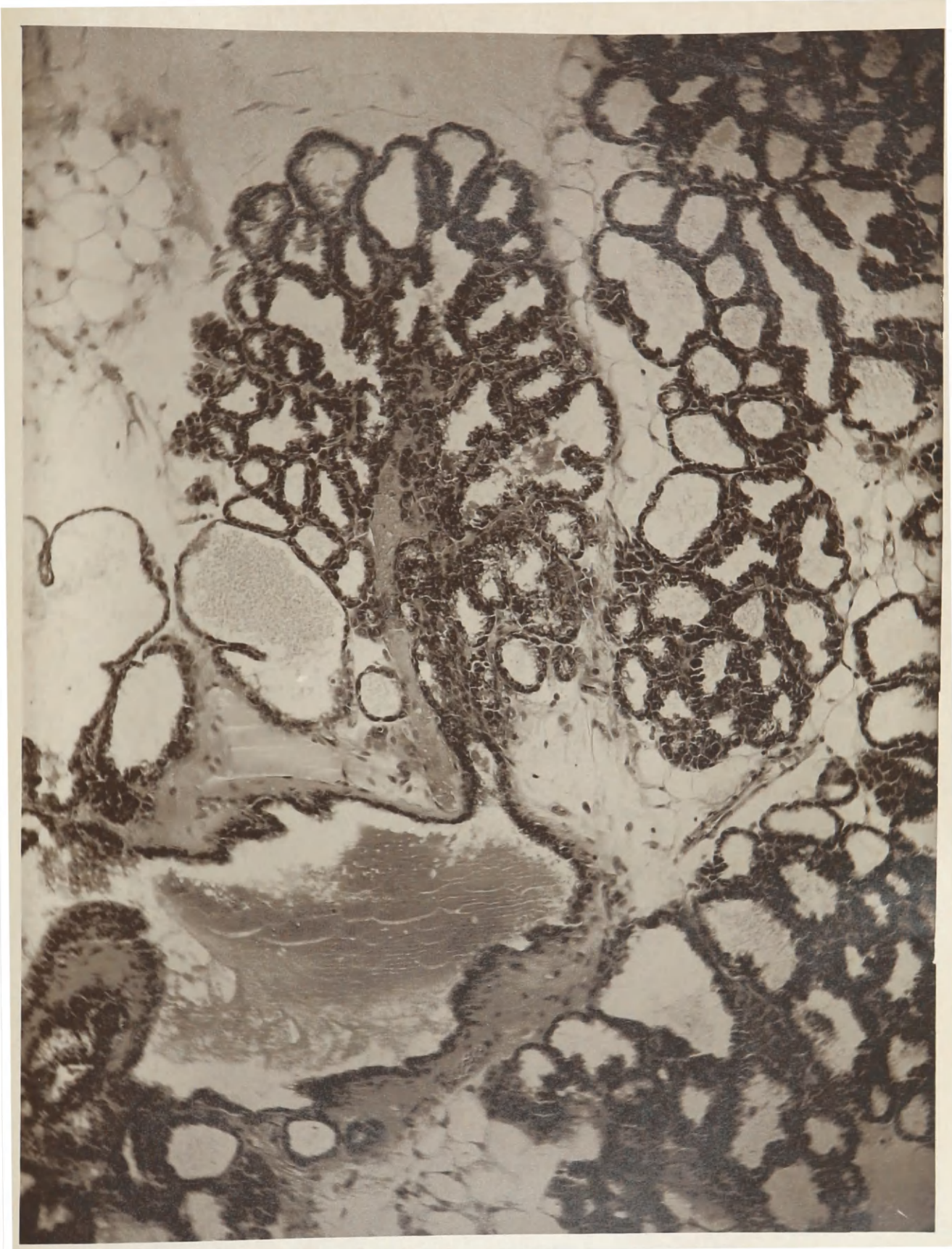   septa contain greatly dilated, engorged
            blood vessels.

Figure 14.

Figure 15.   Nineteenth day post-partum or first
              day after removal of young. Sections
              show alveoli with increased dilation,
              and filled with secretion. The epi-
              thelium is flattened. Other factors
              are the same as on the previous day.

Figure 15.

Figure 16.   Twentieth day post-partum or second day
             after removal of young. The alveoli are
             engorged with secretion and the epithelium
             is slightly ragged. In other areas the
             alveoli are filled with secretions. Other
             factors are the same as on the previous
             two days.

Figure 16.

Figure 17. Twenty-first day post-partum or third
day after removal of young. These
sections show alveoli and ducts markedly
engorged with secretions. There is an
even greater flattening of the epithelium
with less secretion in the cells.

Figure 17.

Figure 18.   Twenty-second day post-partum or fourth
             day after removal of young. These sec-
             tions show a little more flattening of
             the epithelium and less cellular secre-
             tion. Some epithelial cells have lost
             their nuclei, indicating early degenera-
             tive changes.
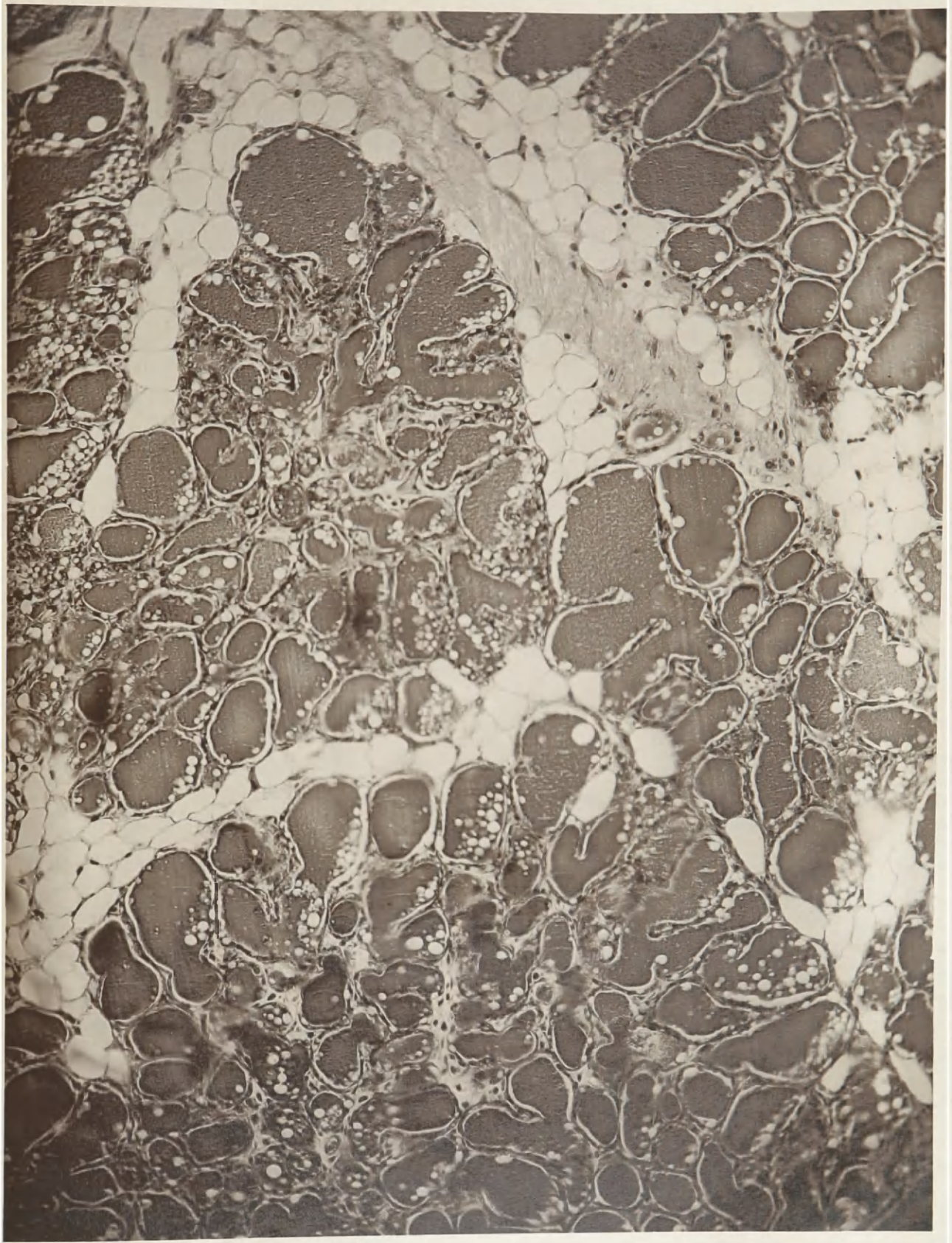
Figure 18.

Figure 19.   Twenty-third day post-partum or five
days after removal of young. These
sections show that the lobules are small
and isolated, with increased fibrosis.
The alveoli are rapidly degenerating and
are small. Infiltration of fat has
increased. Inspissated secretions are
found in the degenerating alveoli. The
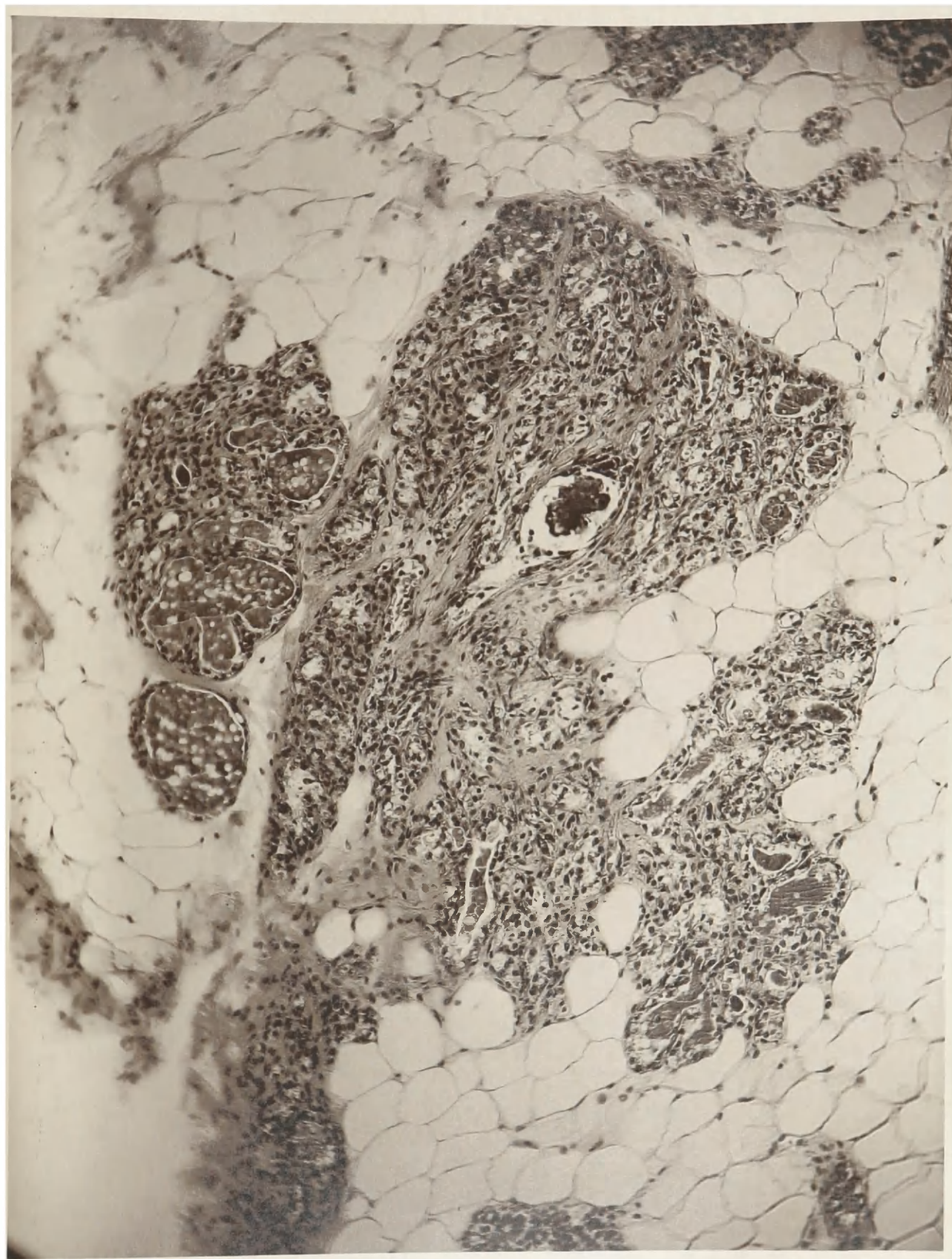ducts are lined by cuboidal cells and
nuclei are ovoid.

Figure 19.

Figure 20.   Twenty-fourth day post-partum or six
days after removal of young. These
sections show a more pronounced fibrosis
in the lobules with further degeneration
of the alveoli. There are still a few
actively secreting alveoli. The ducts
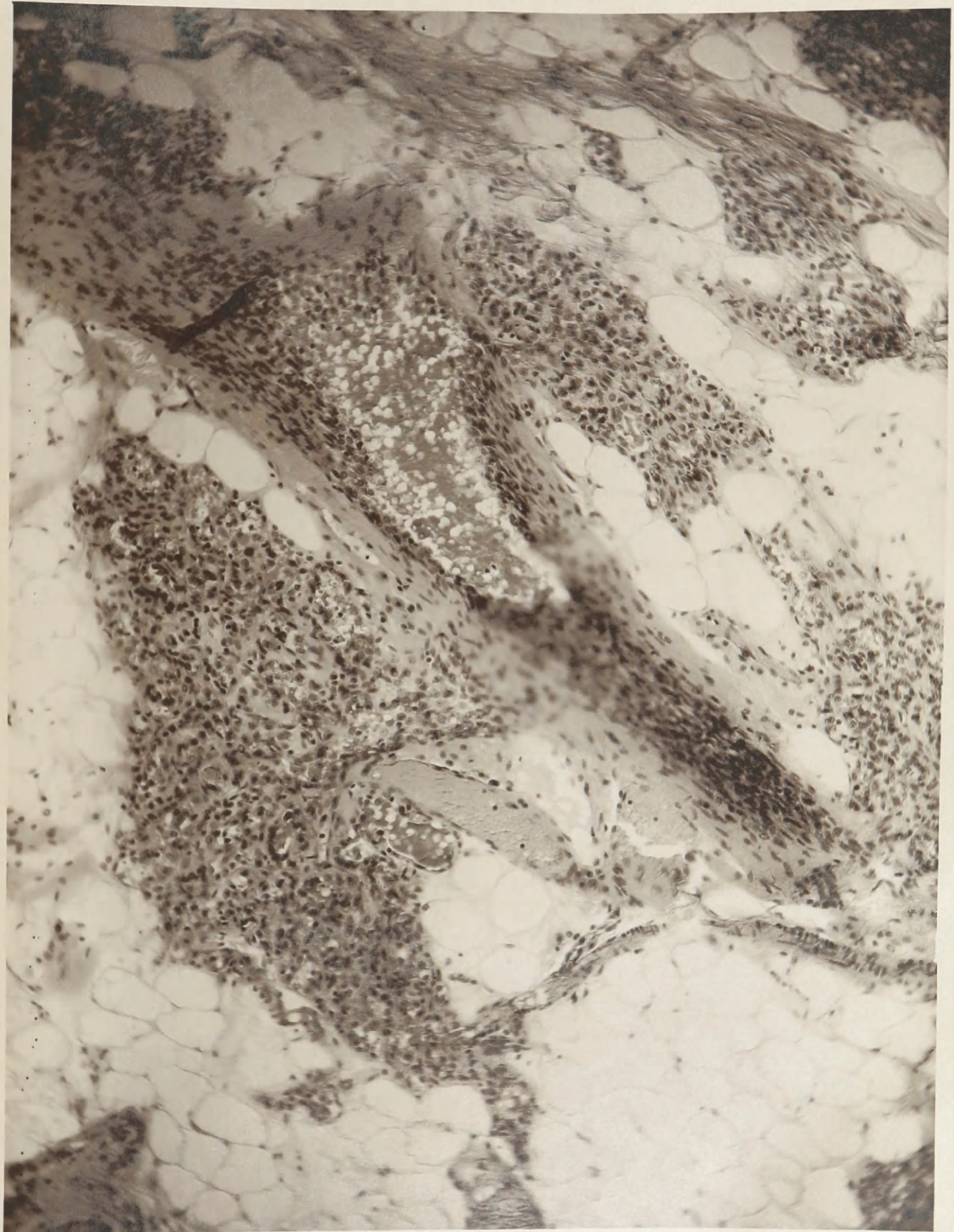are filled with secretion.

Figure 20.

Figure 21. Twenty-fifth day post-partum or seventh
day after removal of young. These sec-
tions show a large increase in fat.
The lobules are very small and an oc-
casional alveolus contains inspissated
secretion. Little secretory material
is found in the ducts and the ductal
epithelium has about reached its maxi-
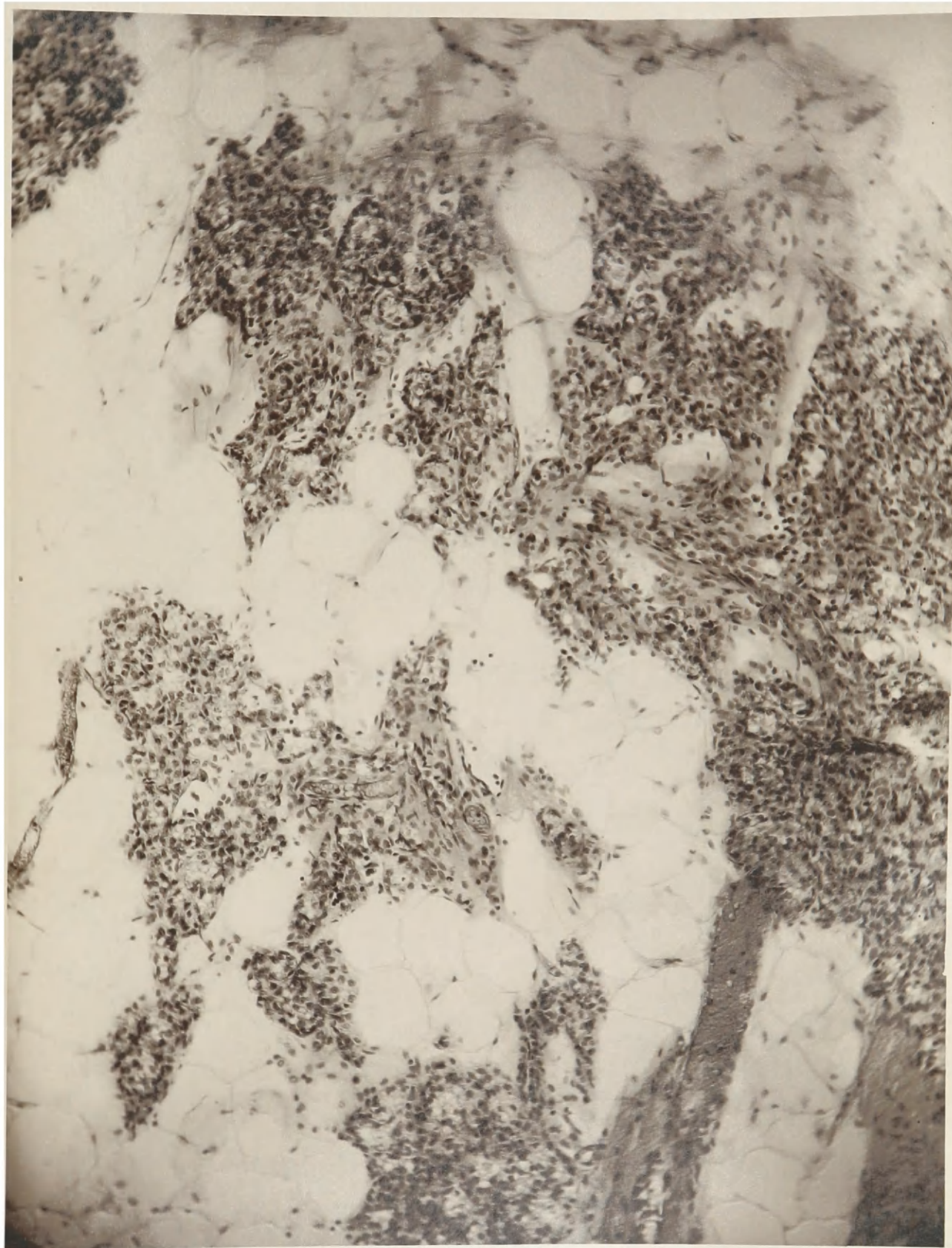mum height.

Figure 21.

Figure 22.   Twenty-sixth day post-partum or eighth
              day after removal of young. These
              sections show no pronounced change
              from the previous day. Early lympho-
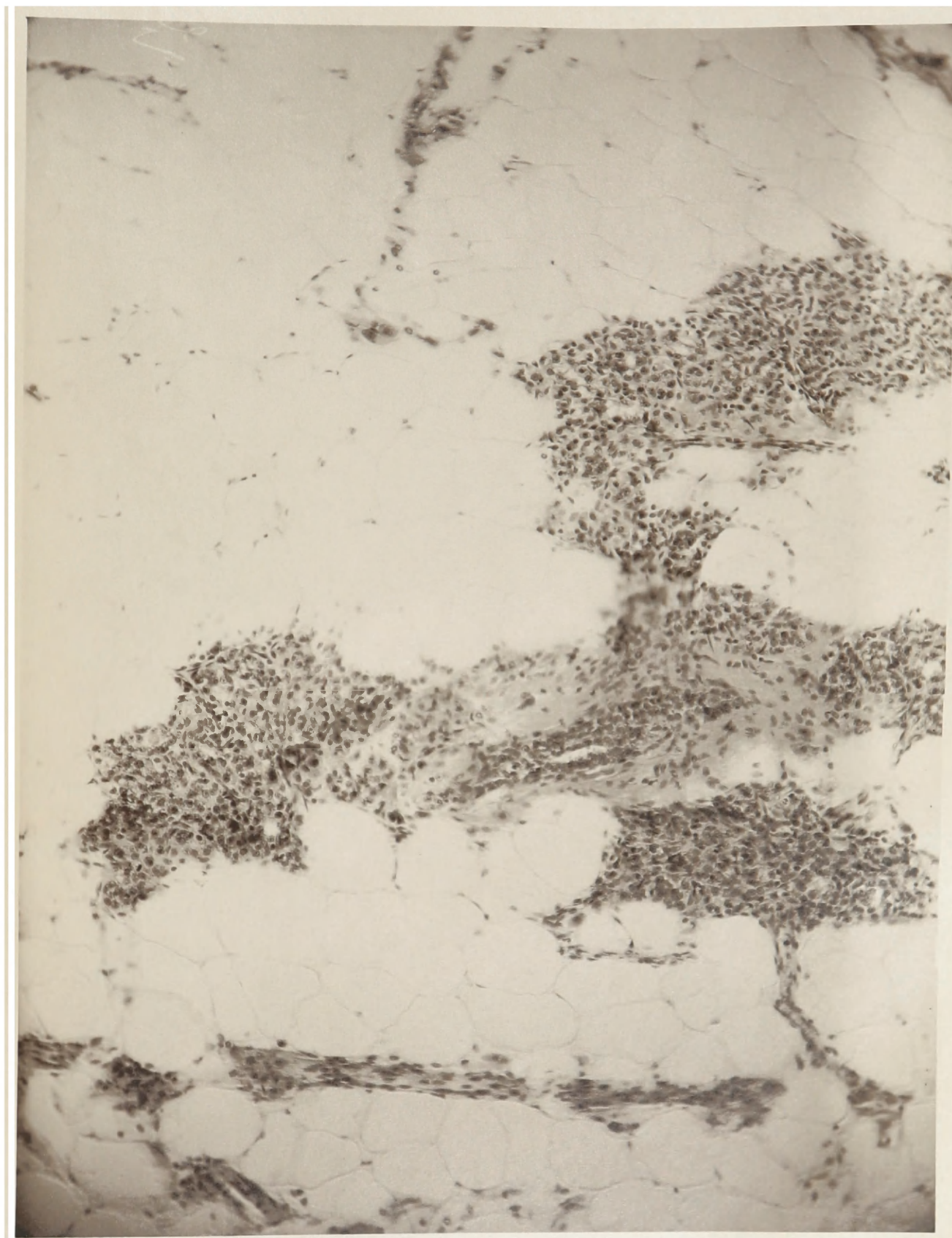   cytic infiltration can be seen.

Figure 22.

Figure 23.   Twenty-seventh day post-partum or ninth
              day after removal of young. These sec-
              tions show a large amount of glandular
              degeneration. The lobules are very small
              and fibrotic. Lymphocytic infiltration
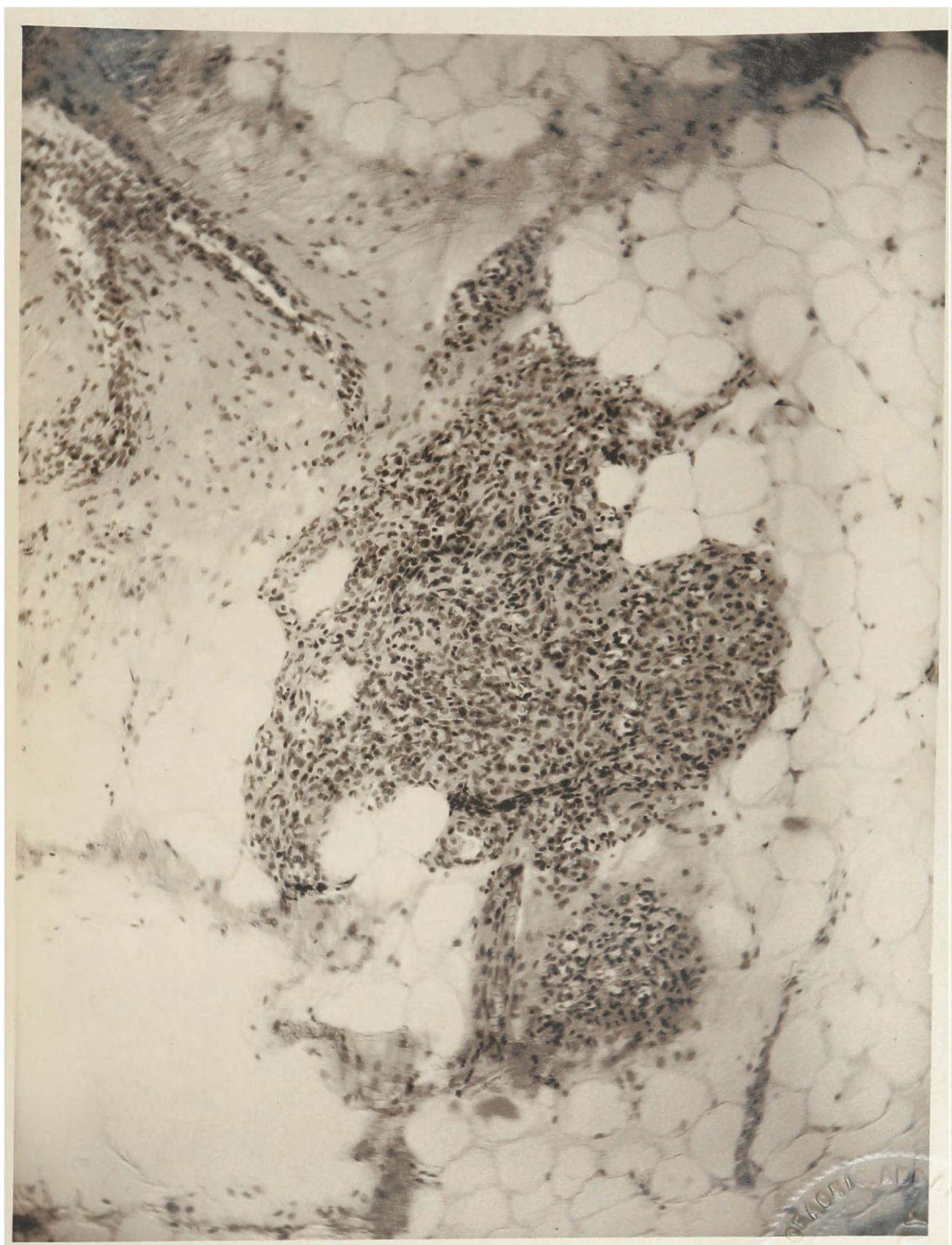   is scattered throughout the lobules.

Figure 23.