UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

F. B. Mumford, Director

A Study of the Involution of the Mammary Gland of the Goat

C. W. Turner and E. P. Reineke

(Publication Authorized February 28, 1936)



COLUMBIA, MISSOURI

UNIVERSITY OF MISSOURI

Agricultural Experiment Station

EXECUTIVE BOARD OF CURATORS .- H. J. BLANTON, Paris; GEORGE WILLSON, St. Louis: J. H. WOLPERS, Poplar Bluff.

STATION STAFF, MARCH, 1936

FREDERICK A. MIDDLEBUSH, Ph.D., President

F. B. MUMFORD, M. S., D. Agr., Director S. B. SHIRKY, A. M., Asst. to Director MISS ELLA PAHMEIER, Secretary

AGRICULTURAL CHEMISTRY

A. G. HOGAN, Ph.D.
L. D. HAIGH, Ph.D.
E. W. COWAN, A.M.
LUTHER R. RICHARDSON, Ph.D.
S. R. JOHNSON, A.M. VIRGIL HERRING, B.S.

AGRICULTURAL ECONOMICS ·

O. R. Johnson, A.M. Ben H. Frame, A.M. F. L. Thomsen, Ph.D. †C. H. Hammar, Ph.D.

AGRICULTURAL ENGINEERING

J. C. WOOLEY, M.S. MACE M. JONES, M.S. G. W. GILES, M.S. in A. E.

ANIMAL HUSBANDRY

ANIMAL HUSBANDRY
E. A. TROWBEIDGE, B.S. in Agr.
L. A. WEAVER, B.S. in Agr.
A. G. HOGAN, Ph.D.
F. B. MUMFORD, M.S., D. Agr.
F. F. MCKENZIE, Ph.D.*
J. E. COMFORT, A.M.*
H. C. MOFFETT, A.M.
VICTOR BERLINER, M.S.
C. S. WILLIAMS, B.S.
C. E. MURPHEY, B.S.

BOTANY AND PATHOLOGY

W. J. ROBBINS, Ph.D. C. M. TUCKER, Ph.D. C. G. SCHMITT, A.B.

DAIRY HUSBANDRY

DAIRY HUSBANDRY
A. C. RAGSDALE, M.S.
WM. H. E. REID, A.M.
SAMUEL BRODY, Ph.D.
C. W. TURNER, Ph.D.
H. A. HERMAN, A.M.
E. R. GARRISON, A.M.
WARREN C. HALL, A.M.
E. P. REINERE, B.S.
E. T. GOMEZ, A.M.
C. W. McIntyre, M.S.
LLOYD E. WASHBURN, M.S.
RALPH P. REECE, M.S.
R. C. CUNNINGHAM, B.S. in
W. R. GRAHAM, Ph.D. R. C. CUNNINGHAM, B.S. in E.E. W. R. GRAHAM, Ph.D.

ENTOMOLOGY LEONARD HASEMAN, Ph.D. T. E. BIRKETT, A.M. H. L. KOCH, B.S.

LEE JENKINS, B.S. C. H. BALDWIN, B.S. FIELD CROPS

W. C. ETHERIDGE, Ph.D.

*In cooperative service with the U. S. Department of Agriculture.

C. A. Helm, A.M. L. J. Stadler, Ph.D.* B. M. King, A.M.* E. Marion Brown, A.M.* MISS CLARA FUHR, M.S.*

HOME ECONOMICS MABEL CAMPBELL, A.M. MABEL CAMPBELL, A.M.
JESSIE ALICE CLINE, A.M.
ADELLA EPPLE GINTER, M.S
BERTHA BISBEY, Ph. D
JESSIE V. COLES, PhD..
BERTHA K. WHIPPLE, M.S.
MINERVA GRACE, A.M.
MARY I. SHELL, A.M.
ADELIA WEIS, A.M.
F. VALDENG DVED A.M.

ELIZABETH DYER, A.M. HORTICULTURE

HORTICULIURE
T. J. TALBERT, A.M.
A. E. MURNEER, Ph.D.
H. G. SWARTWOUT, A.M.
GEO. CARL VINSON, Ph.D.
H. F. MAJOR, B.S.
R. H. WESTVELD, B.S., M.F.
R. A. SCHROEDER, B.S. in Agr.
GEORGE E. SMITH, B.S. in Agr.
AUBREY D. HIBBARD, M.A.

POULTRY HUSBANDRY

H. L. Kempster, M.S. E. M. Fune, A.M. RURAL SOCIOLOGY

E. L. Morgan, Ph.D. Melvin W. Sneed, B.S. in B.A.

SOILS

M. F. MILLER, M.S.A.
H. H. KRUSEKOPF, A.M.
W. A. ALBRECHT, Ph.D.
HANS JENNY, Ph.D.
†L. D. BAVER, Ph.D.
H. F. WINTERGORN, Ph.D.

VETERINARY SCIENCE

A. J. DURANT, A.M., D.V.M.
J. W. CONNAWAY, D.V.M., M.D.
CECIL ELDER, A.M., D.V.M.
O. S. CRISLER, D.V.M.
ANDREW ÜREN, A.M., D.V.M.
HAROLD C. McDOUGLE, A.M.
P. L. PIERCY, D.V.M.

OTHER OFFICERS R. B. PRICE, B.L., Treasurer LESLIE COWAN, B.S., Sec'y of University A. A. JEFFREY, A. B., Agricultural Editor L. R. GRINSTEAD, B.J., Ass't. Agr. Editor J. F. BARHAM, Photographer LEON WAUGHTAL, Assistant Photographer JANE FRODSHAM, Librarian

†On leave of absence.

TABLE OF CONTENTS

	Page
INTRODUCTION	5
Operative Technique	6
Histological Technique	7
INVOLUTION OF THE MAMMARY GLAND WHEN MILKING IS SUSPENDED:	
Review of Literature	7
Plan of Investigation	8
Experimental Findings	8
Gross Examination of Glands	8
Microscopic Examination	9
Summary	10
THE EFFECT ON INVOLUTION OF THE STIMULATION OF MILKING:	
Review of Literature	12
Effect of Stimulation of Milking during Early Lactation	13
Effect of Stimulation during Advanced Lactation	14
Summary	14
THE RATE OF INVOLUTION DURING THE PERIOD OF DECLINING LACTATION:	
Experimental Animals	16
Histological Study	
Summary	
DISCUSSION	20
SUMMARY AND CONCLUSIONS	22
T TYPR A YTIRE CIYED	23

ABSTRACT

A study is reported of the rate of involution of the mammary gland of the goat when milking is entirely suspended; when milking is continued in one-half; and during the declining phase of lactation. When milking is stopped 30 days after parturition, the lobule-alveolar system gradually degenerates. Within 75 days after the last milking, the gland epithelium consists essentially of a duct system similar to that found in a virgin gland. When milking is stopped in one side of the gland, the stimulation provided by milking the opposite side retards the rate of involution in the side that is not milked. The epithelial cells become tall and vacuolated, but a large number of them are still present after 65 days. The effect of stimulation is apparently lessened during advanced lactation. Evidence is presented to show that the mammary gland undergoes a gradual involution with advancing lactation. In some areas the alveoli continue to secrete milk, while in others considerable numbers of inactive, but apparently intact alveoli may be found. In far advanced lactation, however, involution of the mammary gland is practically complete. The few alveoli that remain are very small in diameter, and the gland consists of a duct system, surrounded by extensive amounts of connective tissue.

ACKNOWLEDGMENT

The authors wish to express their appreciation to A. C. Ragsdale, Professor of Dairy Husbandry, University of Missouri, for his constructive suggestions and criticisms in the preparation of the manuscript for this bulletin.

A Study of the Involution of the Mammary Gland of the Goat*

C. W. Turner and E. P. Reineke

During the last ten years, the part played by the hormones of the ovary and the anterior pituitary in the regulation of the growth and functional activity of the mammary gland has been well established. It is generally agreed that the growth of the duct system of the mammary gland is stimulated by the estrogenic hormone. The growth of the lobule-alveolar system is activated by a hormone (corporin, progesterone) secreted by the corpus luteum, acting simultaneously with the estrogenic hormone. The stimulus to milk secretion has been shown to be provided by the hormone, galactin (prolactin) secreted by the anterior lobe of the pituitary gland.

In all laboratory animals studied it has been found that when milk is no longer removed from the mammary gland the secretory tissue soon regresses to an inactive state similar to that found in virgin animals. This regression or involution is accomplished by degeneration of the lobule-alveolar structure of the gland, leaving only the duct system and the surrounding connective tissue. Before such animals can be stimulated to full lactation with the lactogenic hormone, their mammary glands must be developed by treatment with the ovarian hormones. It appears, therefore, that the response to the lactogenic hormone is dependent, at least in part, upon the amount of secretory tissue present.

Cows that go dry and subsequently fail to breed cause considerable losses to the dairyman every year. The increased knowledge of endocrine functions gives us hope that eventually such cows may be brought into milk production by stimulating their udders to activity with the hormones.

Another question of considerable interest is that concerning the causes of the decline in milk production with advancing lactation. Is this decline due to a gradual loss of secretory cells, or is the hormonal stimulation insufficient to maintain high production as the period of lactation advances? If the period of maximum

^{*}Submitted by the junior author to the Department of Dairy Husbandry in partial fulfillment of the requirements for the degree of Master of Arts, 1936.

production could be prolonged by hormonal stimulation, it would mean a tremendous economic gain to the dairyman.

Before attempting to decide on the form of hormonal treatment needed to stimulate the secretion of milk during these periods, it is desirable to know to what extent the mammary gland is developed during the period of declining lactation and during the dry period.

If the udder of the cow involutes in a manner similar to that observed in laboratory animals, it would be futile to attempt to bring dry cows into lactation by administering the lactogenic hormone, unless the lobule-alveolar system had been developed previously.

Little is known about the causes for the normal decline in lactation. It is believed that a study of the anatomy of the mammary gland at various stages of lactation may provide a clue to the fundamental causes, and also indicate to what extent the decline may be prevented by means of hormonal stimulation.

The dairy goat is rapidly coming into use as a laboratory animal for experiments in milk secretion. Therefore, it seemed desirable to determine the extent of development of the goat udder at various stages of lactation in order to provide the basis for studies on the hormonal stimulation of lactation in this animal.

The following questions have been studied and are reported in this paper:

- 1. Involution of the mammary gland when milking is suspended.
 - 2. The effect on involution of the stimulation of milking.
- 3. The rate of involution during the period of declining lactation.

Operative Technique

The hair was shaved from the gland, and it was washed with soapy water, followed by 70 per cent alcohol. The area surrounding the point to be incised was painted with a dilute alcoholic iodine solution. The gland was anaesthetized by subcutaneously injecting 2.5 per cent apothesine solution around the point of operation. A deep injection in the region of the inguinal canal was often successful in desensitizing the inguinal nerve.

The tissue was taken in most cases from the posterior-dorsal portion of the udder, about 2 inches removed from the supramammary lymph gland. A dorsal-ventral skin incision about 2 inches long was made, and the underlying connective tissue parted by

blunt dissection. Since the mammary gland is highly vascular, care had to be taken to avoid the larger blood vessels. Small vessels were tied off with catgut at two points, and severed between the sutures. When the gland proper had been exposed, a portion about three-fourths of an inch in diameter and one-half to three-fourths of an inch thick, was isolated by running a circle of catgut sutures around it in order to tie off the milk ducts, and then the tissue was cut out with a sharp scalpel. Although all the large ducts were tied off by this method, milk often continued to ooze out of the finer ducts into the wound, and caused considerable infection in some cases. Four or five stitches were taken through the skin and underlying connective tissue to close up the incision, and then it was swabbed with a tar-phenol mixture in order to minimize the danger of infection.

Histological Technique

The tissue was fixed in Bouin's fluid, in which it was allowed to remain for two or three days. It was dehydrated in a graded series of alcohols (from 50 per cent to absolute), cleared in alcohol-xylol and xylol, and imbedded in paraffin. Picric acid was removed by saturating the 70 per cent alcohol used in dehydration, with lithium carbonate.

Sections were cut at a thickness of 6 to 10 microns; fixed to glass slides with Mayer's egg albumen fixative; cleared in xylol and xylol-alcohol; run down through a graded series of alcohols; and stained. Part of the sections from each tissue sample was stained in Delafield's hematoxylin, and counter-stained with eosin. The remainder were stained in Mallory's aniline blue connective tissue stain, and counter-stained with acid fuchsin.

INVOLUTION OF THE MAMMARY GLAND WHEN MILKING IS SUSPENDED

Review of Literature

The gross histological changes taking place in the mammary gland when the young are weaned normally, or when they are taken away at parturition, have been described for a number of species, including the rat (Myers and Myers, 1921; Maeder, 1922), the mouse (Turner and Gomez, 1933; Cole, 1933), the guinea pig (Kuramitzu and Loeb, 1921; Turner and Gomez, 1933), the cat (Turner and DeMoss, 1934), the dog (Turner and Gomez, 1934),

the rabbit (Hammond, 1925; Turner, 1932), and the squirrel (Deanesly and Parkes, 1933).

There is a striking similarity in the mode of regression of the mammary gland of all the animals studied. When the young are taken away from a lactating animal, the milk glands first are distended by the pressure of the accumulated secretion. This turgidity persists for a period varying from 24 to 48 hours and then becomes lessened through resorption of the milk. The epithelial cells lining the alveoli gradually degenerate, many of them being cast out into the lumina, and finally the point is reached where only the ducts and the gland stroma remain. At this point the mammary gland very closely resembles that of a virgin animal.

The main difference in species is in the length of time required for involution of the lobule-alveolar system to reach completion. This time ranges from 10 or 12 days in the mouse to 40 days or more in the dog.

These observations are supported in general by the cytological findings of Da Fano (1922), Weatherford (1929), and Jeffers (1935a).

The first section of the present paper will include the results of a study of the histological changes taking place in the mammary gland of the goat when milking is stopped at the peak of the lactation curve.

Plan of the Investigation

Milking was suspended in four goats approximately 30 days after parturition. It was estimated that lactation would be at the maximum at this time. A fifth goat which had been dried up about five months previously was also used.

Pieces of mammary gland tissue were removed at biopsy in order to get a series at approximately 15 day intervals. The tissue specimens were removed alternately from the right and left. halves of the udder, and the operations were spaced so as to allow at least 30 days to elapse between two operations on any particular goat.

The external appearance of the glands was also observed from time to time, and any evidences of secretion were noted at the time of operation.

Experimental Findings

Gross Examination of Glands.—When milking was stopped the udders filled up with milk and in some cases were so distended that they seemed painful, and caused the goats difficulty in walk-

ing. In two goats (Nos. 11 and 83) the pressure had gone down noticeably by 15 days after the last milking. Their udders were small in capacity in comparison to the other two. Goats No. 17 and No. 57-A had excessively large and capacious udders and apparently had a high capacity for milk production. Their gland cisterns remained completely distended with milk for about 25 days after the last milking, and then the pressure gradually subsided.

A long period of time was required for complete resorption of the milk from the storage system of the udder. Goat No. 57-A died (cause unknown) 28 days after milking was suspended. Although the pressure had gone down noticeably by this time, 1700 cc. of secretion were milked from one-half of the udder after it had been removed from the carcass. In goat No. 17 the gland cisterns were still filled with a watery secretion 75 days after the last milking. All the milk had been resorbed from the udders of goats No. 11 and No. 83 after 45 days. This difference may be explained by the fact that Goats No. 17 and No. 57-A had much larger milk storage systems than the other two.

Microscopic Examination.—Fifteen days after milking was suspended the alveoli were small but most of them were intact. A small amount of secretion was present. A few alveoli had collapsed, and were marked by the infiltration of numerous leucocytes. The connective tissue of the gland had thickened considerably, chiefly because of the contraction resulting when the alveoli were no longer distended with milk.

In 28 days involution had progressed considerably (Fig. 2). In some areas the alveoli were entirely collapsed. Alveolar remains were represented by groups of epithelial cells having no definite arrangement. Some alveoli decreased in size by losing individual cells, as evidenced by tiny vacuoles in the gland stroma, with one to four epithelial cells clinging to their peripheries. A few alveoli were still normal in size but the cells were exceedingly tall, extending far out into the lumina. Many of the cells had a vacuolated appearance. In one goat (No. 57-A) involution had not progressed to such a marked extent. Numerous alveoli were present in some areas while in others the alveoli were collapsed and many disorganized groups of epithelial cells were observed in the gland stroma.

Forty-eight days after the last milking a further loss of alveoli had occurred (Fig. 3). The connective tissue had thickened

in proportion to the loss of epithelium. Only a few small alveoli still persisted, and most of these were in various stages of degeneration. By the 75th day involution was near completion but was still progressing slowly (Fig. 4).

Sections taken from a goat udder five and one-half months after the last milking (Figs. 5 and 6) resembled very closely the condition found in virgin glands. All that remained was a duct system surrounded by connective and adipose tissue.

Summary

When milking is stopped 30 days after parturition, the goat mammary gland undergoes a progressive involution. The alveoli are lost by collapse of the entire alveolus, and by shedding individual cells into the lumen. Involution is nearly completed after 48 days but continues at a decreased rate. Seventy-five days after the last milking only a few of the more persistent alveoli remain. These have all disappeared at the end of five and one-half months, leaving only a duct system comparable to that in a virgin gland.

PLATE I (See opposite page)

Rate of Involution When Milking is Suspended.

- Fig. 1.—Cross-section of a mammary gland in full lactation. The alveoli are large and distended with secretion. (x282)
- Fig. 2.—Section of a mammary gland taken after 28 days involution. The lobule-alveolar system had degenerated considerably. The alveoli are situated irregularly, and many of them have collapsed. The connective tissue has thickened because of the contraction of the gland parenchyma. (x66)
- Fig. 3.—Section of mammary gland tissue taken after 48 days involution. Degeneration of the lobule-alveolar system is almost complete. Numerous alveolar remnants, surrounded by connective tissue, may be seen. (x66)
- Fig. 4.—Section of a mammary gland in advanced involution. The longitudinal section of a duct shows only a few side branches. Taken from Goat No. 17 after 75 days involution. (x57)
- Fig. 5.—A completely involuted mammary gland. In this section, taken from Goat No. 15 after 5½ months involution, the udder has involuted completely, leaving only the ducts, and the surrounding connective tissue. (x66)
- Fig. 6.—A duct ending, showing the branching end buds in a completely involuted mammary gland. (same section as Fig. 5, highly magnified). (x282)

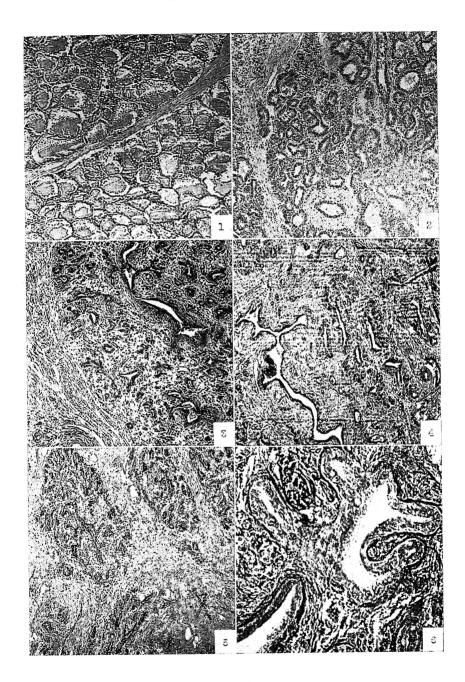


PLATE I

THE EFFECT ON INVOLUTION OF THE STIMULATION OF MILKING

Review of Literature

Until quite recently it was believed that the involution of the mammary gland was due entirely to the pressure of the accumulated secretion, and that the act of suckling or milking was concerned in the maintenance of the secretory cells only by bringing about the removal of milk.

In support of this view, Kuramitzu and Loeb (1921) found that when one nipple is tied off in lactating guinea pigs and suckling continues, the occluded side undergoes involution similar to that observed after weaning. Hammond (1925) ligated the nipples on one side of two lactating rabbits, painted the duct openings with collodion and cotton wool, and allowed the young to continue nursing. On the sixteenth and seventeenth day after the ligation, the rabbits were killed and the weights of the mammary glands compared, with the following results:

Rabbit No.	Occluded Glands	Normal Glands
93	8 grams per gland	32 grams per gland
91	11 grams per gland	34 grams per gland

From these results Hammond concluded that the stimulation of suckling had no effect on retarding involution of the mammary gland. Since the histology of the glands was not studied the results are inconclusive. The loss in weight might have been due to the resorption of milk rather than the loss of gland epithelium.

Selye (1934) obtained a decidedly different response in the rat. The main milk ducts at each of the nipples of seven lactating rats were severed three days after parturition and the young were allowed to suckle. Since no milk was obtained, they were alternated with normal litters in order to keep the young alive. Rats killed as much as 14 days after the operation were reported to have mammary glands with alveoli well preserved and turgid with milk, while the controls showed considerable evidence of involution six to eight days after the young were removed. Likewise, when the milk ducts were excised on one side and the nipples cut off on the other, and also when the nipples on one side were cut off and the other side was untouched, milk was still present in glands on both sides of the body after fourteen days. A similar response was reported in the mouse (Selye and McKeown, 1934-c).

It was also shown that bilateral ovariectomy does not prevent the stimulation response in the rat.

In the light of these findings it seemed desirable to determine whether or not the stimulation of milking plays a part in the maintenance of the mammary gland of the goat.

Effect of Stimulation of Milking During Early Lactation

Milking was suspended on one side of the udders of two goats (Nos. 135 and 254) about 30 days after parturition, while the other side was milked regularly. Biopsy specimens were taken at various intervals and the tissue was sectioned and stained for microscopic examination.

There was an increase in milk pressure on the side not milked, followed by a gradual decrease in pressure and resorption of milk. Fifteen days after the last milking little secretion was present in the alveoli on the side not milked, although the gland cistern was still distended (Fig. 7). Most of the alveoli were still intact, but in a few limited areas the connective tissue was thickened considerably, and alveoli which had decreased in size to the extent that some of them contained only three or four cells in cross section were observed. The histological picture obtained when goats were stimulated by milking on one side was distinctly different from that observed during normal involution. The alveolar cells were greatly lengthened and protruded into the lumen in a characteristic fashion. Many cells appeared to be distended with secretion which they were unable to discharge.

The 32-day stage (Figs. 8 and 9) showed little change from the condition observed at 15 days. The majority of the alveoli were essentially intact, although they had decreased slightly in diameter.

Sixty-five days from the beginning of the experiment the alveolar structure was still well defined (Figs. 10 and 11). There had been a further loss of alveoli as evidenced by a thickening of the connective tissue in some areas, but a good many alveoli were still present.

A comparison of the results obtained on the two goats that were subjected to the stimulus of milking, with the first series, shows that involution of the udder is retarded considerably when the stimulus is supplied. Involution was nearly complete in the first series after 48 days, while the goats receiving the milking stimulus showed large numbers of well defined alveoli after 65 days.

Effect of Stimulation During Advanced Lactation

Milking was suspended on one side of the udder of a goat which had been milking for approximately six months. At this time she was producing three pounds of milk per day.

Tissue obtained 34 days later showed that the side of the gland that was not milked was fairly well involuted (Fig. 12). Only the duct system with a few alveolar remnants, and a large proportion of connective tissue remained.

Summary

In two goats in which milking was suspended on one side of the udder 30 days after parturition, involution was greatly retarded by milking the opposite side. Although the alveoli contained little secretion by the fifteenth day, a large proportion of the alveolar tissue was still present 65 days after the beginning of the experiment. The cells lining the alveoli were greatly distended and enlarged, bulging out into the lumen in a characteristic manner quite distinct from that observed after "drying up" a goat.

Stimulation apparently had very little effect in maintaining the mammary gland in a goat that had been lactating for approximately six months.

PLATE II (See opposite page)

Effect of the Stimulation of Milking on Involution.

Fig. 7.—Effect of the stimulation of milking during early lactation. This section was taken from the side of a goat udder in which milking had been suspended 15 days previously, while milking was continued on the opposite side. Most of the alveoli are intact and some of them are distended with secretion.

Fig. 8.—Effect of the stimulation of milking during early lactation. Section taken from the side of a goat udder in which milking had been suspended 32 days previously, while milking was continued on the opposite side. It will be noted that the aveoli have decreased in diameter, but a large proportion of

the epithelial cells remain. (x66)

Fig. 9.—Highly magnified view showing the effect of the stimulation of milking in early lactation. Milking had been suspended in this side 32 days previously. The epithelial cells are beginning to extend into the lumen. (x282)
Fig. 10.—Effect on involution of the stimulation of milking during advancing

Fig. 10.—Effect on involution of the stimulation of milking during advancing lactation. A section taken from the side of a goat udder in which milking had been stopped 65 days previously, while milking was continued on the opposite side. There has been very little loss of epithelium. The alveolar cells appear vacuolated and many of them extend far out into the lumina. (x66)

Fig. 11.—Effect of the stimulation of milking on involution with advancing lactation. High magnification of the section shown in Fig. 10. Alveoli are well defined; the epithelial cells are extremely tall and vacuolated. (x282)

Fig. 12.—Effect of the stimulation of milking during advanced lactation. Sections taken from a goat udder 34 days after milking was suspended on one side, following six months lactation. The lobule-alveolar system degenerated rapidly. In addition to the ducts and connective tissue, a few alveolar remains are present. (x231)

are present. (x231)

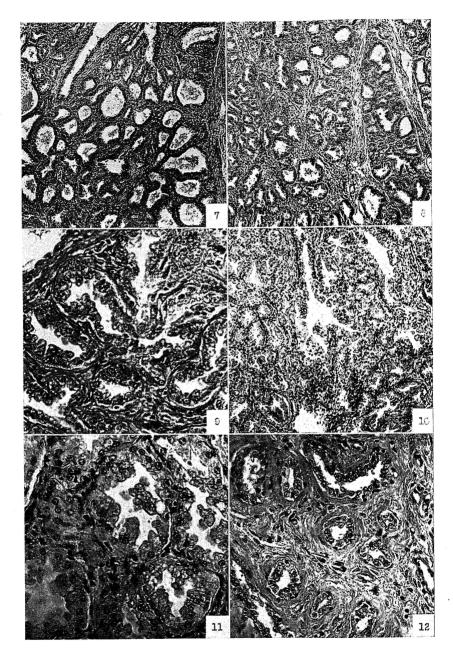


PLATE II

THE RATE OF INVOLUTION DURING THE PERIOD OF DECLINING LACTATION

The question whether or not the mammary gland involutes during normal lactation is of considerable interest in considering the possibility of increasing milk production of animals in various stages of lactation by stimulation with the lactogenic hormone. If the secretory cells degenerate in proportion to the decline in lactation, it would obviously be impossible to increase milk production appreciably without first stimulating growth of the lobule-alveolar system. If a large proportion of the milksecreting cells is maintained during advancing lactation, it should be possible to bring production up, or to hold it to a high level without such preliminary treatment. It has been observed that when lactating mice are suckled over a long period of time, the mammary gland finally involutes in spite of a strong suckling stimulus (Selve and McKeown, 1934-c). It has also been observed that in cows which have been milking for a long time (16 to 18 months), the parts of the udder most removed from the gland cistern are in various stages of involution (Lenfers, 1907).

Experimental Animals

Mammary gland tissue was collected from three non-pregnant lactating goats (No. 135, 20-A and 27-A) which were in advanced stages of lactation. Goat No. 135 had freshened February 14, 1935, and milking had been suspended in the left half of her udder although the right half was milked regularly. Tissue was taken from the lactating side 146 days after parturition (approximately 5 months). Goats No. 20-A and No. 27-A had been purchased recently, and their exact freshening dates were not known. Goat No. 20-A had been milking approximately six months, and was producing two pounds of milk daily when she died. A sample of udder tissue was taken at post mortem. A biopsy specimen was taken from Goat No. 27-A on October 19, 1935, when she was producing 0.5 pounds of milk daily. A post mortem sample was obtained on the same goat 38 days later. At this time production had dropped to about 0.2 pounds of milk per day.

Histological Study

After five months lactation, the alveoli were small, but most of them were filled with secretion (Fig. 13). A few of the lobules showed definite signs of involution as indicated by collapsed al-

veoli, and still other alveoli which had lost all but a few of their epithelial cells (Fig. 14).

In the tissue taken in the sixth month of lactation, large numbers of the alveoli contained no secretion although most of them still retained their structure (Fig. 15). Many of the epithelial cells were extremely tall, extending well out into the lumina (Fig. 16). Considerable numbers of epithelial cells had been sloughed into the lumina of the alveoli, and some alveoli were completely filled with extruded cells and cellular debris. Limited areas showed alveoli in the final stages of degeneration.

Goat No. 27-A (nearly dry) showed further evidence of slow but gradual, involution (Fig. 17). The amount of connective tissue present in a given area had increased greatly because the gland stroma had drawn together, following the decrease in the amount of milk present and the loss of epithelial cells. A small amount of secretion was present in some alveoli, while others appeared to be inactive.

In tissue taken from the same goat 38 days later, involution was practically completed (Fig. 18). A few very small alveoli still remained, and a small amount of secretion was present in some cases, but the mammary gland consisted essentially of a duct system surrounded by fat and loose connective tissue.

Summary

Study of the mammary glands of a limited number of goats during advanced stages of lactation indicated that a gradual involution takes place. Considerable amounts of alveolar tissue appear to be maintained for some time after they have stopped secreting, while other areas remain active. Nearly all of the alveoli were lost, however, in a goat that was practically dry.

PLATE III (See opposite page)

The Rate of Involution during Advanced Lactation

- Fig. 13.—Extent of involution after five months lactation. Most of the alveoli are small, but filled with secretion, although a few are inactive. (x57)
- Fig. 14.—Extent of involution after five months lactation. High magnification of the section shown in Fig. 13. Most of the alveoli are filled with secretion, but a few inactive and apparently involuting alveoli may be found. (x231)
- Fig. 15.—Extent of involution after approximately six months lactation. Although the alveoli are small, and little secretion is present, a large proportion of the gland epithelium is intact. (x57)
- Fig. 16.—High magnification of mammary tissue taken from a goat after about six months lactation. Some alveoli are degenerating, but most of them are fairly well preserved. The epithelial cells are becoming tall and vacuolated. (x231)
- Fig. 17.—Section of a mammary gland from a goat in far advanced lactation. A few alveoli are filled with secretion, while others are in various stages of involution. (x231)
- Fig. 18.—Section of mammary tissue at the end of the lactation period. From the same goat as the section shown in Fig. 17, but taken 38 days later. Involution is practically complete. Only a few alveolar remnants surrounded by connective tissue remain. A few active alveoli were present, however, in other sections of the same tissue. (x231)

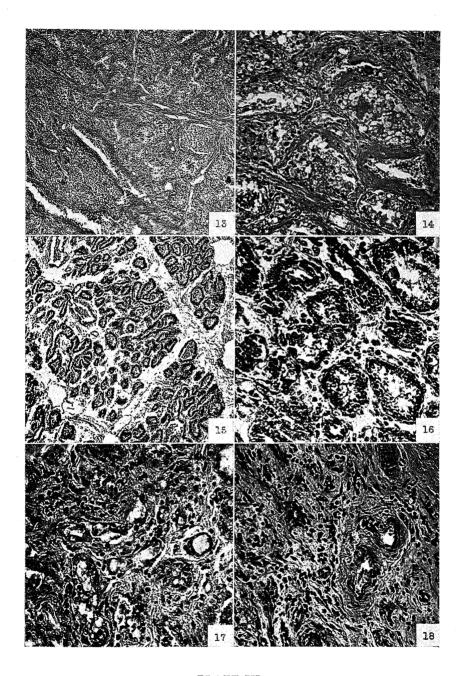


PLATE III

DISCUSSION

The possibility of applying the facts known about the lactogenic hormone to the practical problems of dairy husbandry is dependent on the amount of secretory tissue present. Secondly, it is necessary that the gland epithelium be in a condition to respond to the lactogenic hormone.

The results of the investigations reported make it clear that in virgin or dry animals, where only a duct system is present, it would be impossible to induce more than a slight amount of secretion even if it were possible to stimulate all of the secretory cells present to lactation.

The evidence presented shows that the mammary gland of the goat gradually loses its alveoli, and regresses to a duct system when milking is suspended. From this we can infer that stimulation of the mammary gland to lactation during the dry period by the injection of the lactogenic hormone, would meet with little success unless the development of the gland had been induced previously.

The causes for the decline in the rate of milk production with advancing lactation have been the subject of considerable speculation. Does milk production decline because of a gradual loss of secretory tissue? Do all of the cells continue to secrete, although at a slower rate? Do certain areas become inactive, although still intact, while the remainder of the alveolar cells continue to secrete at an undiminished rate? Or is the decline in lactation due to several of these factors?

It was found that the stimulation of milking retards the rate of involution when milking is completely suspended in one side of the udder. During the period of declining lactation considerable areas of alveolar tissue are maintained although apparently not secreting. From this it is evident that involution of the mammary gland proceeds at a slower rate than the rate of decline in milk production would lead us to expect, were the decline due primarily to the loss of secretory cells.

Therefore, it should be possible, by supplying the proper stimulus, to bring these inactive cells back to activity, and so increase milk production, or at least to prevent for a time the normal decline in lactation. This view is supported by the fact that several investigators have reported significant increases in lactation during this period, following the administration of alkaline extracts of

the anterior pituitary (Stricker and Grueter, 1921; Asdell, 1932; Kabok and Margulis, 1935; Azimov et al., 1935). Recent reports show also that increases in milk and milk fat production are stimulated by thyroid feeding (Graham, 1934a), or by intravenous injections of thyroxin (Graham, 1934b; Jack and Bechdel, 1934).

While these results leave little doubt that the mammary gland has the capacity to respond to added stimulation during advanced lactation, they do not show which agent is responsible. Most of the crude extracts of lactogenic hormone contain the thyreotropic hormone as well. Could it be possible that the effect obtained with pituitary extracts is due to stimulation of the animals' own thyroid by the thyreotropic hormone? Graham concluded that the effect of thyroid feeding on milk and milk fat production is secondary to the factors controlling the lactation cycle, causing its effect by raising the metabolism. Jack and Bechdel, on the other hand, proposed that thyroxine stimulates milk secretion indirectly by causing an increased secretion of lactogenic hormones by the hypophysis.

The tendency toward maintenance of the lobule-alveolar system is markedly decreased during far advanced lactation. Involution of the mammary gland was almost complete in a goat which was nearly dry. The stimulation of milking was also ineffective in maintaining the secretory tissue during advanced lactation. Although this evidence is limited, it indicates that attempts to increase the milk flow by hormonally stimulating animals that are nearly dry, without first inducing growth of the secretory tissue would meet with failure. This view is supported also by the fact that Jack and Bechdel failed to obtain significant increase in milk production when thyroxine was injected at the extreme end of the lactation period.

SUMMARY AND CONCLUSIONS

- 1. A method is described for securing biopsy specimens from the udder of the goat.
- 2. When milking is suspended 30 days after parturition, the lobule-alveolar system of the mammary gland degenerates and is gradually lost. Approximately 75 days after the last milking, only a duct system remains.
- 3. When milking is stopped on one side of the udder, and the other side is milked regularly, involution is retarded in the side that is not milked.
- 4. The stimulation of one side of the mammary gland apparently had no effect in retarding involution of the opposite side in a goat that had been milking for about six months.
- 5. In goats that were well advanced in lactation a large proportion of the alveoli were still intact, although many of them were not secreting.
- 6. Involution of the mammary gland was practically complete in a goat that was at the extreme end of her lactation period.
- 7. The application of these findings to the hormonal stimulation of lactation is pointed out.

LITERATURE CITED

Asdell, S. A. 1932 The effect of the injection of hypophyseal extract in advanced lactation. Am. J. Physiol., vol. 100, p. 137.

Azimov, G., Pchelina, V., Parjskaia and Chwatov, B. P. 1935. The influence of anterior pituitary hormones upon lactation in cows. The Problems of Zootechnical and Experimental Endocrinology, vol. 2, p. 261 (trans.) Cole, H. A. 1933 The mammary gland of the mouse during the oestrous cycle,

pregnancy and lactation. Proc. Roy. Soc. B., vol. 114, p. 136.

Da Fano, C. 1922 On Golgi's internal apparatus in different physiological conditions in the mammary gland. J. Physiol., vol. 16, p. 459.

Graham, W. R., Jr. 1934a The effect of thyroidectomy and thyroid feeding on

the milk secretion and milk fat production of cows. J. Nutr., vol. 7, p. 407.

Graham, W. R., Jr. 1934b The action of thyroxine on the milk and milk fat

production of cows. Biochem. J., vol. 28, p. 1368.

Grueter, F., and Stricker, P. 1929 Action of a hormone of the anterior lobe of the hypophysis on secretion of milk. Klin. Wchnschr., vol. 8, p. 2322. (Title trans.)

Hammond, John. 1925 Reproduction in the Rabbit. Oliver and Boyd, Edinburgh. Tweedale Court, London; 33 Paternoster Row, E. C.

Jack, E. L., and Bechdel, S. I. 1934 A study of the influence of thyroxin on milk secretion. J. Dairy Sci., vol. 18, p. 195.

Jeffers, Katherine R. 1935a Cytology of the mannary gland of the albino rat.

I. Pregnancy, lactation and involution. Am. J. Anat., vol. 56, p. 257.

Jeffers, Katherine R. 1935b Cytology of the mannary gland of the albino rat.

II. Experimentally induced conditions. Am. J. Anat., vol. 56, p. 279.

Kabok, J. M., and Margulis, F. S. 1935 The influence of endocrine preparations on lactation in sheep. Transactions on the Dynamics of Development, vol. 9, p. 161 (trans.) Kuramitzu, C., and Loeb, L. 1921 The effect of suckling and castration on

the mammary gland in rat and guinea pig. Am. J. Physiol., vol. 56, p. 40.

Lenfers, P. 1907 Zur Histologie der Milchdruse des Rindes. Inaug. Diss. Giessen and Zeit. f. Fleisch. u Milchhygiene, vol. 17, pp. 340, 383, and 424.

Selye, Hans. 1934 On the nervous control of lactation. Am. J. Physiol., vol. 107, p. 535.

Selye, H. and McKeown, T. 1934a Production of pseudo-pregnancy by mechanical stimulation of the nipples. Proc. Soc. Exp. Biol. and Med., vol. 31, p. 683.

Selye, H. and McKeown, T., 1934b The effect of mechanical stimulation of the nipples on the ovary and sexual cycle. Surg. Gynec. and Obst., vol. 59, p. 886.

Selye, H. and McKeown, T. 1934c Further studies on the influence of suckling.

Anat. Rec., vol. 60, p. 323. Selye, H., Collip, J. B, and Thomsen, D. L. 1934 Nervous and hormonal factors in lactation. Endocrinology, vol. 18, p. 237.

Turner, C. W. 1932 The Manmary Glands. Sex and Internal Secretions, p. 545. Williams and Wilkins Co., Baltimore.

Turner, C. W., and DeMoss, W. R. 1934 The normal and experimental development of the mammary gland. I. The male and female domestic cat. Mo. Agr. Exp. Sta. Res. Bul. 207.

Turner, C. W., and Gomez, E. T. 1932 The normal development of the mammary C. W., and Gomez, E. T. 1932 The normal development of the mammary can be seen to the control of the mammary can be seen to the control of the mammary can be seen to the control of the mammary can be seen to the control of the mammary can be seen to the control of the control of the mammary can be seen to the control of the con

mary gland of the male and female mouse. Mo. Agr. Exp. Sta. Res. Bul. 182.

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

Turner, C. W., and Gomez, E. T. 1934 The normal and experimental development of the mammary gland. II. The male and female dog. Mo. Agr.

Exp. Sta. Res. Bul. 207.

Weatherford, H. L. 1929 A cytological study of the mammary gland: Golgi apparatus, trophospongium, and other cytoplasmic canaliculi; mitochondria. Am. J. Anat., vol. 44, p. 99.