THE INTRA-MAMMARY PRESSURE CHANGES
IN THE LACTATING COW

by

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We accept this thesis as conforming
to the required standard

Members of the Division

THE UNIVERSITY OF BRITISH COLUMBIA
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Date **28th April, 1960**.
ABSTRACT

The object of this study was to establish the nature of the intra-mammary pressure changes that occur in the udder of the lactating dairy cow during different phases of lactation. The author was, in addition, interested in the study of the various factors responsible for these changes.

Results have shown marked and rapid fluctuations in the intra-mammary pressure at the time of milking. Stimulation of milk ejection by manipulation of the udder caused a spectacular increase in the initial intra-mammary pressure. On milking a drop in the pressure was recorded. The extent of this fall in pressure was found to be influenced by the amount of milk removed from the udder. On complete milking the intra-mammary pressure dropped to the minimum level.

The pressure in the hind quarters was found to be in most cases higher than in the fore-quarters indicating that the level of the intra-mammary pressure in each quarter was under some influence of the quantity of milk present.

A fall in the intra-mammary pressure of a milked quarter had no effect on that of the other unmilked quarters.

Intra-mammary pressure subsequent to the injection of oxytocin before milking as well as after complete milking was recorded. The administration of oxytocin under both of these conditions resulted in a rise of the pressure, which, however, was not well marked.

Considerable effect of the conditioned reflexes and of the emotional state of the animal on the intra-mammary pressure was observed during this work.
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I. INTRODUCTION

Lactation is the complex physiological mechanism of milk production. Various theories have been extended to explain the onset of lactation after parturition. Lactation itself comprises different phases which may broadly be grouped under two headings: milk secretion and milk removal. The two phases of lactation, though parts of the same process, are quite distinct from each other; a fact that was not recognized until recently. Therefore, the latter phase, the phase of milk removal did not receive much consideration for comprehensive investigation.

Milk after secretion is accumulated in the mammary glands and requires to be removed periodically for efficient lactation. For convenience the phase of milk removal may be sub-divided into: (a) passive withdrawal and (b) milk ejection. It is a well known fact that complete let down of milk or milk ejection is essential to obtain the maximum quantity of milk present in the mammary glands. Without milk ejection, only the milk present in the gland cisterns is obtained on milking which does not include the major portion of the milk stored in the alveoli and smaller ducts of the mammary gland tissue. Milk ejection which has been recognized as a neuro-hormonal mechanism, is stimulated by the act of nursing or hand manipulation of the mammary glands. On milk ejection the pressure within the mammary glands is suddenly increased to its maximum. After complete milking the intra-mammary pressure falls to the minimum level and starts rising again gradually until the next milking. The impact of the level of the intra-mammary pressure on the secretory efficiency of
of the glandular tissue as also on the composition of the milk secreted has been pointed out by Ludwin (39), Gasnier et al. (31), Csiszar (17), and Donker et al. (19). The precise evaluation of the effects of the intra-mammary pressure changes on these aspects of milk secretion is, however, not available from the present literature. Before these effects can be ascertained, a thorough study of the changes in the intra-mammary pressure itself is necessary. This study has been undertaken by a number of investigators working on different animals but the results presented are inadequate and leave a lot of ground for further investigation. The present work is an attempt to understand the fundametals of the problem of the milk ejection and intra-mammary pressure changes in different phases of lactation. These experiments were performed on the dairy cow since this animal is the best specimen for milk production.

Moreover, most of the information collected from the study of the intra-mammary pressure changes is intended to be used to understand and improve the milk production of the cow, an animal which alone contributes the most to meet the human needs of milk.

The work reported here is by no means a complete answer to the important questions related to the problem of milk ejection though the results obtained may prove to be of considerable interest and value. In addition to the observations on the natural milk ejection, the effects of injecting oxytocin, the hormone responsible for milk ejection were also studied.
The findings of this work support the validity of certain theories about the intra-mammary pressure proposed by a number of workers. It is hoped that on further investigation when more knowledge is available about this problem, it will be possible to improve the present practises of the dairy industry to make the industry more productive and profitable. Besides, the knowledge obtained could be useful in understanding the problems of lactation of the other species.
II. LITERATURE REVIEW

A. General

1. Anatomy of the mammary glands

   The anatomy of the bovine udder has been the subject of study by many investigators and has been dealt with comprehensively by Sisson (60) and Turner (65). The udder of the cow comprises of four mammary glands and is located in the inguinal region. Each half of the udder is situated on either side of the median line and each of its quarters has a separate channel and opening to the exterior.

Structure of the Udder

(a) Macroscopic: Within the udder there are two types of tissues:

   i. glandular or parenchymatous; consisting of alveoli and duct system, and:

   ii. the stromal tissue consisting of fibrous and adipose connective tissues.

   The teat of each quarter at the tip has an opening called the papillary duct or streak canal which is 1/4 to 1/2 inch in length. The streak canal is normally kept closed by a circular or sphincter muscle. This arrangement effectively blocks the escape of milk from the udder between milkings. Above the streak canal the cavity of the teat widens to form the teat cistern which at its upper end opens into the gland cistern through a circular opening. The gland cistern, also called the lactiferous sinus, is irregular in shape and has numerous pocket-like processes into which the larger ducts open. The teat cistern and the gland cistern of each individual gland communicate with
each other freely through the circular opening.

(b) **Microscopic:**— The larger ducts rapidly divide into smaller and smaller branches. Ultimately the finest ducts end in elongated or pea shaped dilatations called alveoli. The alveoli are grouped together in clusters within a distinct fibrous capsule. Such a grouping is called a lobule. These lobules themselves group together to form the lobes separated by broader connective tissue septa. It is believed that the sinus-like dilatations along the duct system are storage spaces for milk as it accumulates in the udder during intervals between milkings. The epithelium of the alveoli and the terminal ducts is made up of a single layer of columnar and glandular epithelium. The other ducts of the gland and the teat cisterns are lined with two layers of epithelium.

The nature of the sub-epithelial tissue around the alveoli and inter-calary ducts has been a subject of controversy. Richardson (55) has shown this tissue to be myoepithelium and not the smooth muscle that was thought to be responsible for the contraction of the alveoli during milk ejection.

**Circulatory system:**— The udder is richly supplied with blood. Most of the blood to the udder is supplied by the external pudic arteries. Cranial, epigastric and perineal arteries also contribute. The more important veins of the udder are the external pudic veins. The other route is the sub-cutaneous abdominal or milk vein. The lymph vessels of the udder are
numerous and they pass chiefly to the supra-mammary lymph gland.

Nervous system: The main nerves supplying to the udder are first and second lumbar nerves and inguinal and perineal nerves. These nerves are mainly sensory but they also contain some fibers from the sympathetic nervous system.

2. Physiology of lactation

At parturition, initiation of lactation takes place when the mammary glands of the mother begin to secrete milk. Cowie et al. (9) have presented a classification to describe precisely the different phases of lactation. According to this, the phases of lactation may be set out as follows:

(a) Synthesis of milk by cells of alveolar epithelium.
(b) Passage of milk from cytoplasm of alveolar epithelium into alveolar lumen.

Under this scheme lactation can be divided into two main phases - milk secretion and milk removal. The present study undertaken for this thesis deals with milk removal. However, to make a brief mention about milk secretion would not be out of place.
Several theories have been put forth by different workers, to explain the initiation of lactation at parturition. These theories are reviewed by Smith (62) and are mentioned here briefly.

1. **Mammary growth inhibition theory:** According to this theory lactation is inhibited by the products of pregnancy promoting mammary development. Support for this theory came from Nelson (43) and Nelson et al (44). Lactation during pregnancy is inhibited by cellular growth and lactation is initiated when the stimulus for growth is removed at parturition.

2. **Uterine distension inhibition theory:** Mechanical distension of the uterus by growth of the foetus has been considered as the factor causing inhibition of lactation. Surgical removal of the foetus in late stages of gestation initiated lactation. But if the uterus was distended with paraffin after removal of the foetuses in rats, they failed to lactate. This was shown by Selye (58). Evidence against this theory has come from Bradbury (3).

3. **Placental inhibition:** Nelson (43) showed that presence of placental tissue in the uterus inhibited lactation. On the other hand, Selye (58) observed no inhibition of lactation in lactating mice after they had received placental implants.

4. **Estrogen inhibition:** According to this theory the relatively high level of estrogen in the circulation during pregnancy inhibits lactation by a direct inhibitory effect on the mammary gland and also by preventing the secretion of prolactin (the lactogenic hormone) by the hypophysis. The basis for this theory was the
various reports on the suppression of lactation by the injection of estrogens. These reports were presented by Folley and Kon (23), Robson (57) and Smith and Smith (61).

5. **Progesterone inhibition:** The estrogen theory was criticized by Meites and Turner (41 and 42) who held the view that secretion of prolactin was not inhibited by high level of estrogen in blood which in fact at its high concentration in blood stimulated prolactin secretion. But the inhibitory effect noticed during lactation was only due to the simultaneous presence of progesterone in the blood during pregnancy which nullified the action of estrogen. At parturition, the sudden fall in the progesterone level permitted the secretion of prolactin bringing about the onset of a copious milk secretion. Therefore, corpus luteum was held responsible for inhibiting lactation.

Folley and Malpress (25 and 26) advocated a modification of the estrogen inhibition theory put forth by Nelson (43). They proposed a double threshold concept of estrogen according to which the effect of estrogen on the pituitary gland depends upon the level of estrogen in the blood circulation, a high level being inhibitory to pituitary function while a low level is stimulatory.

Recent observations of Cowie et al. (10) during experiments on ovariectomized goats with estrogens and various dose levels of progesterone led these authors to conclude that while some of their results followed Folley and Malpress double threshold theory, others were more in accord with the progesterone inhibition theory presented by Meites and Turner.
Initiation of milk secretion has been reviewed by Folley (22).

This review points out that:

(a) Measurement of the prolactin content of the pituitary does not give any indication of the rate of prolactin release and is, therefore, of no real guidance.

(b) Low levels of estrogen in the circulation activate pituitary function while higher levels inhibit it.

(c) Lactogenic doses of estrogen may be deprived of lactogenic activity by a suitable dose of progesterone; the combination thus inhibits milk secretion. This inhibiting influence acts during pregnancy. Diminishing levels of progesterone after parturition allows estrogen to assert its lactogenic activity resulting in initiation of lactation.

A theory on the initiation of lactation has been proposed which is based on the neuro-hormonal mechanism concerned in milk ejection. Gaines (29) observed that the milk ejection response of the mammae to post hypophysis hormone was induced only after parturition and not during pregnancy. Colostrum is secreted by the cells and fills the ducts of the mammary glands during pregnancy long before the contractile mechanism is sensitive to pituitrin (oxytocin) or to the stimulus of milking. Gaines pointed out that the contraction of the gland under the influence of pituitrin (oxytocin) runs more closely parallel to the appearance of an actual outflow of milk than does the formation of milk (colostrum) by the secreting cells. Therefore, he considered oxytocin to be an important factor in the onset of lactation.
Petersen (52) proposed a modification of Gaines' theory and suggested that the post parturition milk flow is not due to an initiation of secretion at that time but rather to a beginning of ejection of the alveolar contents.

During the lactation the stimuli of suckling and milking maintain a high level of secretion.

3. Effects of secretions of the different endocrine glands on lactation (milk secretion) in farm animals.

Smith (62) has briefly reviewed the effects of the secretions of the different endocrine glands on lactation in farm animals.

i. Anterior hypophysis and lactation;

Experiments to increase milk production in farm animals by suitable anterior pituitary extracts have been carried out. Azimov and Krouze (2) showed that the milk production of cows could be increased by injecting suitable extracts of anterior pituitary. Folley and Young (28) supported this contention by injecting anterior pituitary extract in cows, otherwise, in declining lactation. But this increase in yield in cows was possible by using anterior pituitary extracts of the bovine origin as injections of sheep or pig pituitary extract rather decreased the yield probably due to antibody formation. No augmentation of yield was observed when cows were treated with this anterior pituitary extract at the peak of their lactation.

The galactopoietic effects of anterior pituitary extract have been shown to bear insignificant relation to their prolactin
content. This was shown by Young (77) who has also given a review. Folley and Young (27) indicated that galactopoietic effects of anterior pituitary extracts appeared to be associated with their content of the diabetogenic factor. When the identity of this factor with the growth factor was revealed, further experiments by Cotes et al (6) showed that the growth hormone appeared to be the all important factor for galactopoietic activity of the crude gland extracts. The observations, however, do not suggest that prolactin and adrenocorticotrophic hormones have no role to play in the galacto-poiesis. Donker and Petersen (18) and Chung et al (5) have confirmed the galactopoietic effects of the growth hormone.

Folley et al (24) showed that increase in milk yield results from the administration of the anterior pituitary extract to cows and goats in which lactation had been artificially induced by estrogen but where estrogen had failed to initiate lactation. The effect of the anterior pituitary extract was both lactogenic and galactopoietic.

ii. Ovarian hormones and milk secretion;

The influence of estrogen and progesterone in initiating and maintaining lactation in collaboration with prolactin and other hormones of the anterior pituitary has already been discussed under theories of milk secretion. Reineke et al (54) suggest that after treatment with estrogen and progesterone in cows and heifers, to induce lactation, there should be a period of treatment with estrogen alone to stimulate the anterior
pituitary to secrete prolactin.

iii. **Thyroid hormone and milk secretion;**

It has been known that the administration of desiccated thyroid or thyroxin to lactating cows results in an increase of milk production up to 20 per cent as shown by Ralston et al (53). Response varies with many factors such as age, stage of lactation, levels of dose etc. Iodinated proteins have been used to increase milk production. It has to be understood that the galactopoietic effect of thyroid preparations is not a specific one on the alveolar cells but is dependent on the role of thyroxine on the general metabolic rate of the animal treated.

Leech and Bailey (37) have shown that the lactation period is shortened if thyroid active substances are used for galactopoietic effects during the first to third lactations.

iv. **Adrenal cortex and milk secretion;**

It has been established that the integrity of the adrenal cortex is essential for the maintenance of lactation. Lactation diminishes in rats when adrenals are removed. Flux (21) has shown that if the ovaries are removed with the adrenals in rats, the milk secretion is diminished still further indicating that the ovaries can to a limited extent replace the adrenal steroids. Cowie (10) has shown that administration of cortisone and desoxycorticosterone restored lactation in adrenalectomized rats.
v. Insulin and milk secretion;
In studies of metabolism of mammary tissues from laboratory animals, insulin has been shown in vitro to potentiate net fatty acid synthesis from small molecules when the tissue has been under the influence of the hormones of the lactogenic or galactopoietic complex of the anterior pituitary. In his review Cowie (7) points out that mammary tissue from the ewe, however, has given no evidence of any insulin response. The role of insulin in milk synthesis in the ruminants may be a minor one as their blood sugar level is relatively low.

vi. Parathyroid and milk secretion;
Not much is known about the role of parathyroid on lactation. Cowie and Folley (8) have indicated that when the parathyroids are removed in rats, a marked impairment in lactation results.
The assumption put forth by Marshall (40) that the udder could not possibly hold all the milk obtained at one milking, and that there is a rapid secretion during milking after stimulation of the udder by nursing, suckling, etc. was proven to be incorrect by the work of Gaines (29). It is well recognized to date that the capacity of the udder is more than enough to contain all the milk that is obtained on milking and milk is actually secreted and held in the udder before milking is commenced. There are two sites for storage of this milk secretion in the udder:

i. the alveoli and small ductules; these two structures have been shown to hold most of the stored milk; and

ii. the gland and teat cisterns and the large ducts; holding part of it.

The ratio between these two amounts vary between species, and individuals of the same species, depending upon the anatomical variations of their udders and other factors.

The milk lying in the gland and teat sinuses can be removed at any time by hand milking or machine milking without stimulating the actual milk ejection procedure. This is not true of the milk stored in the alveoli and ductules since it is held up by capillary forces in the fine ducts and capillaries of the secretory tissue. In the latter case, to obtain milk a complex physiological phenomenon of milk ejection must be triggered.
These two processes of milk removal have been termed:

i. the passive withdrawal; and

ii. milk ejection

Milk ejection and intra-mammary pressure

Milk ejection is the term used to describe the process of milk letting down that follows the reflex stimulation of the mammary glands at the time of milking. The mammary gland tissue has elasticity and thus is capable of exhibiting variations in the intra-mammary pressure at different times of milk secretion and milk ejection.

Theories of milk ejection

Different theories have been presented to explain the process of milk ejection.

i. Erection theory:- Hammond (32) put forth a theory of milk ejection called the erection theory. According to this theory, during milk ejection an erection, comparable to that occurring in the penis, was presumed to be involved. Manipulation of the udder and teats at the time of milking was believed to be the stimulus which contracted smooth muscle fibers in the udder, thereby, occluding the veins which resulted in an engorgement of the udder with blood. The pressure of engorgement was supposed to force the milk from the alveoli and finer ducts into the larger ducts and cisterns. But this theory no longer seems tenable. Peeters et al. (50) experimenting on perfused udder showed that venous engorgement does not produce milk ejection.
ii. Neuro-hormonal theory:-- Ott and Scott (145) had observed that injection of posterior pituitary extract in lactating goats caused an immediate flow of milk. They considered that this was due to an increase in milk secretion as a result of posterior pituitary extract injection.

Later, Gaines (29) put forth quite a new concept of milk secretion and milk ejection. These two processes, he distinctly distinguished from each other. He observed that the milking stimulus in lactating animals brought about a reflex contraction of the mammary gland. This caused milk ejection, a process by which the milk stored in mammary gland tissues became available on milking. These investigations by Gaines established the basis for neuro-hormonal mechanism of milk ejection.

Ely and Petersen (20) carried out a series of experiments on lactating dairy cows to study the phenomenon of milk ejection. They put forth the neuro-hormonal theory of milk ejection which is so well known today. According to this theory, milk ejection is a reflex action brought about by stimulation of secretion of posterior hypophysis by the acts of nursing, suckling, etc. at the time of milking. The secretion of posterior hypophysis is responsible for rise of pressure in the mammary glands which is noticeable on milk ejection.
Tgetgel (64) is one of the pioneer workers who carried out a comprehensive study of the udder pressures in cows. He found that there is a gradual increase in the udder pressure between one milking and the next. When milk ejection is stimulated at milking time, there is a very rapid increase in the udder pressure which in the course of a minute may double. The range of increase is influenced by the udder conformation and milk yield. If the cow is not milked after stimulating milk ejection, the rise in glandular pressure may persist from 10 to 60 minutes, but eventually it falls to the value slightly higher than would have been attained, had the cow not been stimulated. During the milking process the intra-mammary pressure is produced by the height of the accumulated milk in the gland plus the pressure exerted by the milk expulsion mechanism.

Tgetgel has given results of his experiment (Table I.) showing the effect of stimulation on intra-mammary pressure in a number of cows. The variations in their initial pressures and pressures subsequent to stimulation as also between their milk yields are well marked indications of influences of udder size, conformations, and productivity.
### TABLE I.

Showing the effect of udder stimulation on intra-mammary pressure (64)

<table>
<thead>
<tr>
<th>Cow</th>
<th>Pressure in m.m. of Hg.</th>
<th>Increase</th>
<th>Milk Yield lbs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>Maja</td>
<td>25.5</td>
<td>61.5</td>
<td>26.0</td>
</tr>
<tr>
<td>Bronda</td>
<td>27.3</td>
<td>51.5</td>
<td>24.2</td>
</tr>
<tr>
<td>Nora</td>
<td>37.0</td>
<td>59.5</td>
<td>22.5</td>
</tr>
<tr>
<td>Peerla</td>
<td>28.4</td>
<td>44.7</td>
<td>16.3</td>
</tr>
<tr>
<td>Bellina</td>
<td>27.6</td>
<td>43.1</td>
<td>15.5</td>
</tr>
<tr>
<td>Muretta</td>
<td>24.9</td>
<td>37.6</td>
<td>12.7</td>
</tr>
<tr>
<td>Pincha</td>
<td>22.0</td>
<td>37.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Tassa</td>
<td>22.1</td>
<td>35.6</td>
<td>13.5</td>
</tr>
<tr>
<td>Bernina</td>
<td>24.6</td>
<td>27.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Bruna</td>
<td>15.1</td>
<td>21.9</td>
<td>6.8</td>
</tr>
</tbody>
</table>
Prior to Tgetgel's work Gaines (29) studied the intra-mammary pressure changes in the lactating goat. In these investigations he used pituitrin (extract of hypophysis cerebri) to study its effect on milk ejection. He recorded the pressure graphs by inserting a cannula in the teat of a goat and connecting this cannula with a chloroform manometer recording on the revolving drum of a kymograph. He pointed out that the pituitary extract caused contraction of the mammary musculature so forcing out the milk. This reflex contraction is brought about by nursing, milking, etc. with resultant milk ejection, the reflex having a period of delay between 35 to 60 seconds. Gaines showed that all the milk obtained was present as such in the udder at the beginning of milking and is "actively ejected by a reflex contraction of the gland musculature under the stimulus of milking". He also observed that the effect of injecting posterior pituitary extract varies with the stage of activity of the gland and such effect of pituitary extract injection noticed in the lactating goats is apparently absent in the non-lactating goats. Furthermore, during this work, Gaines emphasized that the act of milk ejection is quite different from that of milk secretion. By this work Gaines refuted the theory of Ott and Scott (45) that the posterior pituitary gland extract stimulated milk secretion. This conclusion Ott and Scott derived on noticing an immediate flow of milk in lactating goats following an injection of posterior pituitary extract.

Gaines and Sammann (30) presented further evidence in support of the view that the udder of a cow contains at the beginning of milking not only all the milk that can be obtained during milking but a quantity
in excess of this.

While reviewing the work on the effect of the posterior pituitary extract on milk secretion, Turner and Slaughter (66) suggested that the milk ejection reflex might act through the pituitary gland and that "one of the normal functions of the pituitary gland, so closely connected with the nervous system, is to regulate the discharging phase of milk secretion".

In Germany, Kryzwanek and Bruggemaan (35) and (36) working on the dairy cow recorded measurements of the changes in internal udder pressure following milk ejection and subsequently during milking. The results showed that the magnitude of the variations was affected by the milk yield, udder capacity and udder conformation of the cows though the trends of the changes followed a typical pattern. The interval between stimulation of ejection and the time when maximum pressure was reached, varied from fifteen seconds to over two minutes and if the udder was unmilked the pressure declined slowly to near its original value in 10 to 40 minutes. At the end of milking, if all the strippings were removed, the internal udder pressure fell to zero for a short time because the residual milk in the udder would exert no pressure at the teat.

It was in 1941 that Ely and Petersen (20) put forward their theory of milk ejection which is now well known and accepted. They suggested that milk ejection is a neuro-hormonal mechanism. Many sources of afferent stimuli as nursing, suckling, etc. stimulated the posterior pituitary through the central nervous system to secrete a hormone (oxytocin) which increases the glandular pressure and squeezes out the
milk from the alveoli and smaller ducts whereas different stimuli of a different nature as fright cause the secretion of adrenalin which prevents the ejection of milk. Ely and Petersen utilized the earlier works of Ott and Scott (145) and Gaines (29) which indicated rise in intra-mammary pressure at the time of milk ejection whether the milk was removed from the udder after that or not. Ely and Petersen did a series of experiments on Jersey cows using oxytocin, vassopressin and adrenaline. Denervation of one side (half) of the udder was performed and data obtained which showed that:

i. denervation brought no effect on the rate of milk ejection.

ii. Oxytocin and vassopressin, either of them, caused the gland to drain more completely though oxytocin was comparatively more effective in this regard.

iv. Oxytocin is largely responsible for intra-glandular pressure increase exciting milk ejection. The amount of oxytocin and intra-glandular pressure vary directly.

v. They claimed a new theory of holding up and letting down of milk as a result of these experiments. This left no doubt that milk ejection is a neuro-hormonal mechanism.

In a subsequent paper, Smith and Petersen (63) remarked that there was no measurable increase in mammary pressure during the first hour after milking. Then the pressure gradually increased until the next milking period. The increase in pressure that occurs several hours after milking but prior to expulsion at a succeeding milking, is probably due to the height of the milk that has accumulated in the larger milk ducts and cisterns.
Andersson (1) working in Stockholm in a series of experiments on sheep and goats, recorded milk ejection from the unanaesthetised animals following stimulation of the region of the supra optic nuclei. The response was not affected by denervation of one side of the udder or by complete sacral anaesthesia and that jugular blood withdrawn from stimulated animals produced milk ejection if injected into other animals. More confirmation of the authenticity of the neuro-hormonal mechanism of milk ejection came from Cross and Harris (15). They investigated the effect of electrical stimulation of the supra-optico-hypophysial tract (connected anatomically to neurohypophysis) in rabbit preparations. Such stimulation resulted in milk ejection. The response was reported to be identical to that obtained after an injection of an appropriate dose of anterior pituitary extracts. Cross (11, 12, 13 and 14) at Cambridge in Great Britain has continued to work on this problem and has made valuable contribution to the study of neuro-hormonal mechanism of milk ejection in rabbits.

Peeters et al. (149) studied milk ejection in bovines. They collected blood from four cows, the udders then washed and teats manipulated without milking and further samples of blood withdrawn. Cows were then killed and both halves of each udder perfused with different samples of blood respectively. The milk secretion from different halves were measured. They concluded that a factor aiding milk ejection appears in the blood during preparation of the cow for milking. Their further experiments subsequent to this proved more elaborately that the posterior pituitary provided the hormone for milk ejection at which time the intra-mammary pressure rises.
In 1954, Harris (33) gave a review including an account of the recent work on the mediating role of the hypothalamus in the milk ejection reflex. He suggested referring also to his earlier investigations that milk ejection could be described as a neuro-hormonal reflex on the grounds that the afferent pathway is nervous in character and the efferent, hormonal.

The nature of the contractile tissue of the mamma that plays an important role in milk ejection mechanism has been engaging the attention of a number of workers. Gaines and Sanmann (30) referred to this tissue as "gland musculature". Hammond (32) also referred to the possibility of contractile cells being present around the alveoli but none of these earlier workers could explain the nature of these contractile cells and they took it mostly as the non-striated smooth muscle fibers.

Richardson (55 and 56) did extensive work on the mammary tissues of the goat to study their histology to find out the nature of the contractile tissue responsible for contraction during milk ejection. He found that the presence of the smooth muscle fibers in the glandular tissue is too scarce to bring about this contraction. Instead he discovered the presence of myoepithelium in the mamma (alveoli and ductules) which he believed is responsible for alveolar contraction during milk ejection thus causing a rise in the intra-mammary pressure. In this way the milk contained in alveoli and small ductules is forced into the larger ducts and gland cistern during milk ejection. Richardson demonstrated the presence in large numbers of myoepithelia cells on the stromal surface of the alveoli. The longitudinal or spiral arrangement of the cells on the duct walls probably serves to shorten and widen the duct lumen so that milk is readily transferred from the alveoli to the gland cistern. Linzell (38)
working on other species confirmed these observations of Richardson about the presence of myoepithelium in the mammary tissue.

**Pharmacological Studies On Milk Ejection**

Peeters and his associates (48), (49), and (50) in Belgium have investigated various aspects of this subject in cows. They studied the cistern pressure relationship in the intact and isolated bovine udder. The injection of three units of oxytocin caused an increase in the cistern pressure. In experiments on the effect of various pharmacologically active substances on milk ejection in the isolated udder Peeters et al. found that acetyl choline caused increased blood flow and partial ejection and that this action was inhibited by atropine. The effects of various pharmacologically active substances on milk ejection and blood flow in the perfused mammary gland have been given by Silver (59) in the form of a table. Braude and Mitchell (4) have shown that a complete inhibition of milk ejection in response to oxytocin may be produced by the previous injection of adrenalin. Whether such an effect is the result of vaso constriction in the gland or it represents a direct action on the contractile mechanism rendering it refractory to oxytocin is still a matter of speculation. Earlier work of Ely and Petersen (20) on the cow has indicated another possible way in which the liberation of this hormone in response to sympathetic stimulation may affect the ejection of milk. They showed that the initiation of milk ejection reflex by the milking stimulus could be completely prevented by fright or high doses of adrenalin but that an ejection response could always be elicited in such an animal by the subsequent injection of
of oxytocin. These results indicate that in addition to its peripheral action adrenalin may have a central inhibitory action preventing the release of oxytocin though such a possibility was not considered by Ely and Petersen. Whittlestone (69) tested the activity of a number of posterior pituitary preparations against a standard oxytocin preparation about one hour after normal suckling in sows and came to the conclusion that purified vasopressin has milk ejecting activity equal to one-fifth of that of the equivalent number of oxytocic units but he stresses that further work is required to confirm this. Subsequent to this work, Whittlestone (70) made a study of the effect of adrenalin on the normal milk ejection response of the lactating sow to oxytocin for a limited range of concentrations. His results indicate that even the injected oxytocin fails to produce milk ejection for a short time when given along with adrenalin.

In New Zealand, Whittlestone (69, 70, 71, 72, 73, 74, and 75) has carried out extensive work on the subject of milk ejection in various domestic animals. For most of his work on this subject he used the sow because of the advantages it presents on account of anatomy of its udder and the temperament of the animal. Whittlestone's investigation (69) concludes from his work with sows that the milk ejection hormone not only causes a contraction of the myoepithelium around the alveoli thus increasing the pressure in them but also causes an opening of the small ducts due to the tightening of the longitudinal myoepithelial structures which surrounds these ducts. Whittlestone (71), (72), and (73) made a further study of intra-mammary pressure changes in the
lactating sow. His studies concerned with the effects of oxytocin, vasopressin and acetylcholine on the intra-mammary pressure changes and the effects of level of the dose of oxytocin and the rate of injection. He also studied the effect of adrenaline on the milk ejection response of the sow.

In addition to his work on the sow, Whittlestone (74) studied the intra-mammary pressure changes in the lactating cow during the process of milking. With an electrical pressure recording system, he recorded simultaneously the intra-mammary pressure changes in two quarters, one milked and the other unmilked. The unmilked quarter showed a very slow fall in pressure while as expected the milked quarter showed a steady pressure fall. Restimulation sometime after the beginning of the experiment resulted in a rise and fall in pressure within the milked quarters but no change when the quarter was not milked. In another communication, Whittlestone (75) has presented the results of a study of intra-mammary pressure changes in a lactating ewe. The pattern of the pressure curve recorded in the case of the ewe is shown to be different from both the sow and the cow.

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III. MATERIALS AND METHODS

A. Materials

1. Experimental Animals:

Seventeen lactating cows of the Division of Animal Science dairy unit were used as the experimental animals for the experiments described below. These animals represented different breeds, ages, stage of lactations, milk yields, etc. Complete information about each of these cows is presented in Table II.

2. Hormone Products:

"Oxytoxin", a patent product of the Austin Labs. Ltd., Canada, was used. Each ml. of the preparation contains 20 U.S.P. units of purified oxytocic principle in an aqueous vehicle.

3. The Apparatus:

All measurements of intra-mammary pressure changes were made using the apparatus for recording pressure changes manufactured by the Sanborn Co., U.S.A. It consists of:-

(a) Sanborn Physiologic Pressure Transducer - Model 467-A;
(b) Sanborn Strain Gage Amplifier - Model 140-B;
(c) Sanborn Industrial Recorder - Model 127.

A mercury manometer was permanently fixed with the above apparatus for calibration of pressure recording.

4. Functional Description of the apparatus:

<table>
<thead>
<tr>
<th>Physical Load</th>
<th>Transducer</th>
<th>Signal Voltage</th>
<th>Strain Gage Amplifier</th>
<th>Recorder</th>
<th>Finished Recording</th>
</tr>
</thead>
</table>

Simplified Diagram of Recording System Using a Strain Gage Amplifier
TABLE II.

Detailed information about the experimental animals used.

<table>
<thead>
<tr>
<th>Name</th>
<th>Breed</th>
<th>Date of Birth</th>
<th>Lactation No.</th>
<th>Date of Calving</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Apple</td>
<td>Holstein</td>
<td>July 8, 1956</td>
<td>2</td>
<td>August 11, 1959</td>
</tr>
<tr>
<td>2. Barbara</td>
<td>Holstein</td>
<td>March 15, 1957</td>
<td>1</td>
<td>June 25, 1959</td>
</tr>
<tr>
<td>3. Belle</td>
<td>Ayrshire</td>
<td>February 29, 1952</td>
<td>5</td>
<td>April 7, 1959</td>
</tr>
<tr>
<td>5. Bonnie</td>
<td>Holstein</td>
<td>January 13, 1957</td>
<td>1</td>
<td>February 20, 1959</td>
</tr>
<tr>
<td>6. Cathie</td>
<td>Ayrshire</td>
<td>February 6, 1953</td>
<td>4</td>
<td>November 6, 1958</td>
</tr>
<tr>
<td>7. Dorothy</td>
<td>Ayrshire</td>
<td>February 23, 1954</td>
<td>3</td>
<td>January 16, 1959</td>
</tr>
<tr>
<td>8. Dorrane</td>
<td>Ayrshire</td>
<td>February 24, 1954</td>
<td>3</td>
<td>February 7, 1959</td>
</tr>
<tr>
<td>10. Fleta</td>
<td>Holstein</td>
<td>July 6, 1955</td>
<td>2</td>
<td>January 5, 1959</td>
</tr>
<tr>
<td>11. Grace</td>
<td>Ayrshire</td>
<td>July 17, 1957</td>
<td>1</td>
<td>August 23, 1959</td>
</tr>
<tr>
<td>12. Grade</td>
<td>Holstein</td>
<td>May 12, 1956</td>
<td>2</td>
<td>July 9, 1959</td>
</tr>
<tr>
<td>13. Greta</td>
<td>Ayrshire</td>
<td>August 18, 1957</td>
<td>1</td>
<td>August 13, 1959</td>
</tr>
<tr>
<td>17. View</td>
<td>Holstein</td>
<td>January 24, 1956</td>
<td>1</td>
<td>February 2, 1959</td>
</tr>
</tbody>
</table>
The Strain Gage Amplifier supplies a 2500 cycle excitation voltage to the transducer. The transducer returns a signal voltage to the Sanborn Strain Gage Amplifier.

When the physical variable applied to the transducer is zero, the signal voltage is zero. When a physical load is present, the signal voltage has a magnitude and phasing which represent the magnitude and direction of the physical load.

The Strain Gage Amplifier interprets this signal voltage in terms of the physical load, and moves the galvanometer stylus up or down on the recording paper to show the magnitude and direction of the load on the transducer.

B. Methods:

1. Standardization of Apparatus;

The transducer is connected to the electrical system with a cable eight feet in length and a five pin connector. The transducer is provided with a pair of P1 Luer Locks. By one of these it is connected to the fluid system (a 12 oz. bottle containing physiological saline fixed with the stand at a level higher than that of the transducer) and by the other P1 Luer Lock, the transducer is connected to about 3 ft. long polyethylene tubing (diameter 2/5 cm.) with a cannula at its free end.

Preparing the Transducer:

Before attaching the valves, the transducer is thoroughly but gently flushed with gas free saline and in the same way the catheter is flushed to ensure that the hydraulic system is free
from bubbles and ready for use.

**Theory of Transducer Levelling:**

Physiological pressure measurements are made with respect to an arbitrary zero level. This is done by fastening the transducer at a convenient position and then to balance the transducer electrically with the catheter tip beneath the surface of a jar of saline. The pressure at the transducer due to the weight of the liquid in the tube (between the transducer and the surface of the saline) is balanced out electrically. This makes the transducer output appear as though the Zero Level line on the transducer body were level with the surface of the saline.

**Calibration:**

The Strain Gage Amplifier requires calibration so that the operator can interpret the recording in terms of the variable being measured. This variable here is pressure in mm. Hg. To make this as simple and reliable as possible, "a basic sensitivity" figure is used to express the stylus deflection in terms of the load applied to the transducer.

This "basic sensitivity" is defined as the number of load units which gives one cm. of stylus deflection when the Attenuator is at XL. By using this "basic sensitivity", the variable being measured is read directly from the record, by multiplying the cm. of stylus deflection by the "basic sensitivity" and the Attenuator setting.

The actual calibration of the system is performed by applying a static load to the transducer. For this purpose, the mercury
manometer is used.

The following steps are taken for calibration:

1. A basic sensitivity is selected as mentioned above.
2. A known static load eg. 40 mm. of Hg. is applied.
3. The calibration deflection is calculated.
4. And without changing the static load, the Gain control for the calculated stylus deflection is adjusted.

**Attenuation:**

Attenuation selects the sensitivity of the recording in terms of mm. of Hg. per ten divisions of stylus deflection. For this purpose the attenuator is provided with the front panel data of the Strain Gage Amplifier. The sensitivity for different positions is given below:

<table>
<thead>
<tr>
<th>Position</th>
<th>Sensitivity c 467A Transducer</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1</td>
<td>1 mm. Hg. per 10 divisions</td>
</tr>
<tr>
<td>X2</td>
<td>2 mm. Hg. per 10 divisions</td>
</tr>
<tr>
<td>X5</td>
<td>5 mm. Hg. per 10 divisions</td>
</tr>
<tr>
<td>X10</td>
<td>10 mm. Hg. per 10 divisions</td>
</tr>
<tr>
<td>X20</td>
<td>20 mm. Hg. per 10 divisions</td>
</tr>
<tr>
<td>X50</td>
<td>50 mm. Hg. per 10 divisions</td>
</tr>
<tr>
<td>X100</td>
<td>100 mm. Hg. per 10 divisions</td>
</tr>
</tbody>
</table>
2. Procedure of recording:

The complete recording apparatus is set up before recording in close proximity to the experimental animal. The transducer with the stand is fixed at the level of the mammary quarter concerned. The Strain Gage Amplifier is turned on so that it can warm up and the transducer is connected to the TRANS socket.

After warming up the Strain Gage Amplifier for 30 minutes, the resistance and capacitance unbalance of the transducer circuit and cabling is balanced out; balancing and calibration must be done before filling the pressure head with fluid.

By bringing the stylus to a certain base line on the recording paper by adjustment with the Zero Switch, the Zero Line is established when also the calibration and attenuation is checked and suitably adjusted. Physiological saline is passed through the cannula, by opening the stop cock of the transducer, to ensure that the cannula is filled with saline upto its tip.

The recorder is now switched on. The tip of the cannula is brought close to the tip of the teat and Zero point marked with the MARKER. The cannula is now smoothly passed into the teat canal where the milk of the mammary gland cistern comes in contact with the saline in the cannula and thus the intra-mammary pressure is recorded. The cannula is left in the teat as long as required by the experiment, using the MARKER on the apparatus for designating the points of interest. The point, at which the cannula is taken out, is also marked to facilitate correct and accurate reading and interpretation of the results.
3. **Hormone administration:**

   During experiments with oxytocin hormone, injections of "Oxytoxin" were administered per sub-cutaneous route in the neck region of the animal.

4. **Milk yield:**

   Milk yield was recorded in some experiments that required a study of the milk output of the animal.

5. **Precautions:**

   Certain precautions are required in the operation of recording the intra-mammary pressure for accurate results. These are mentioned below:-

   i. The animal should be standing even on a level floor;

   ii. The animal must be kept in a calm and unexcited state during the experiment;

   iii. The level of the transducer has to be corrected each time that the animal or its stall is changed from one experiment to another;

   iv. To warm up the Strain Gage Amplifier for 30 minutes before operation of the apparatus;

   v. To check and verify the calibration and attenuation shortly before the experiment commences;

   vi. To ensure that the hydraulic system of the transducer is free from air bubbles;

   vii. To flow the Physiological saline through the cannula filling it to the tip each time that the cannula is inserted into a teat.

   viii. The Zero Base line must be marked for every individual recording of the intra-mammary pressure from different quarters of the udder.
ix. As a safeguard against infection of the udder, the cannulae used are to be properly sterilized and other antiseptic conditions maintained.
IV. RESULTS AND DISCUSSION

The importance of the subject of milk secretion cannot be over-emphasized. The study of this complex problem has received attention for many years, but still complete understanding of the phenomenon of milk secretion and milk ejection is lacking. Milk secretion as a separate study from milk ejection was emphasized by Ely and Petersen (20). They postulated that milk ejection is a neuro-hormonal mechanism brought about by the stimulation of secretion of posterior pituitary, resulting into rise of the intra-mammary pressure at the time of milking. Earlier Tgetgel (64) had recorded variations in intra-mammary pressure in lactating cow before and after milking. His results (Table II) show well marked increase in the intra-mammary pressure as a result of udder stimulation at the time of milking. In Germany Kryzwanek and Bruggemaan (35 and 36) recorded measurements of the changes in the intra-mammary pressure in the dairy cow following milk ejection and subsequently during milking. These workers discussed the possible effect of various factors such as milk yield, udder capacity and udder conformation on the intra-mammary pressure of the cow.

Whittlestone (69 to 75) made a valuable contribution towards an understanding of the phenomenon of milk ejection in various animals. He also suggested improvements on the type of equipment and technique to record the intra-mammary pressure changes. Whittlestone's study (74) on the problem of milk ejection in the cow is not comprehensive. It leaves many aspects of the problem for further investigation. A complete understanding of the problem of milk ejection in lactating animals is very essential since with this is associated the hormonal
efficiency, milk yield and health of the animal. The intra-mammary pressure values are reliable indications of the efficiency of the milk ejection reflex in a lactating animal.

The present study deals with recording of the intra-mammary pressure changes during different phases of milk ejection in lactating cows. Various experiments were planned to study different aspects of the problem. As large a number of cows as seventeen was used during these series of experiments. This study is only a preliminary work for the academic and scientific understanding of the mechanism of milk ejection.
Table III

The intra-mammary pressure changes before and after complete milking*

<table>
<thead>
<tr>
<th>Cow</th>
<th>Milking Time</th>
<th>Belle</th>
<th>Dorrane</th>
<th>Cathie</th>
<th>Dorothy</th>
<th>Roberta</th>
<th>Blossom</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RF RH LF LH</td>
<td>RF RH LF LH</td>
<td>RF RH LF LH</td>
<td>RF RH LF LH</td>
<td>RF RH LF LH</td>
<td>RF RH LF LH</td>
<td>RF RH LF LH</td>
</tr>
<tr>
<td>Initial</td>
<td>AM</td>
<td>11 18 21 33</td>
<td>11 13 14 25</td>
<td>9 18 9 18</td>
<td>12 15 20 26</td>
<td>16 21 20 26</td>
<td>13 12 19 25</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>12 11 10 9</td>
<td>9 10 10 13</td>
<td>9 15 8 17</td>
<td>9 9 9 11</td>
<td>12 15 14 17</td>
<td>9 9 13 14</td>
</tr>
<tr>
<td>After</td>
<td>AM</td>
<td>29 x 18 24</td>
<td>23 32 23 32</td>
<td>12 26 11 29</td>
<td>21 26 22 28</td>
<td>22 27 23 27</td>
<td>28 26 35 25</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>22 30 22 32</td>
<td>20 21 19 x</td>
<td>11 25 12 25</td>
<td>19 21 19 21</td>
<td>20 21 21 25</td>
<td>21 18 31 28</td>
</tr>
<tr>
<td>After</td>
<td>AM</td>
<td>7 8 9 8</td>
<td>x 7 8 7</td>
<td>4 x 5 7</td>
<td>7 6 2 6</td>
<td>3 3 4 5</td>
<td>6 6 8 8</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>13 12 9 1</td>
<td>x 8 9 8</td>
<td>3 7 6 8</td>
<td>6 7 6 9</td>
<td>6 5 3 5</td>
<td>6 x 8 8</td>
</tr>
<tr>
<td>Milking</td>
<td>AM</td>
<td>18</td>
<td>17</td>
<td>9.6</td>
<td>22</td>
<td>25.2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>15</td>
<td>13</td>
<td>7</td>
<td>17</td>
<td>19</td>
<td>13</td>
</tr>
</tbody>
</table>

* - Thursday, July 30th, 1959
AM - denotes morning milking at 5.30 A.M.
PM - denotes afternoon milking at 4.30 P.M.
x - unreliable results.
# - Quarters of the udder - right front, right hind, left front and left hind.
Table IV

The intra-mammary pressure changes before and after complete milking.*

<table>
<thead>
<tr>
<th>Cow</th>
<th>Milking Time</th>
<th>Initial</th>
<th>After Stimulation</th>
<th>After Milking</th>
<th>Milk Yield in lbs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>PM</td>
<td>AM</td>
<td>PM</td>
<td>AM</td>
</tr>
<tr>
<td></td>
<td>LH LF RH RF</td>
<td>LH LF RH RF</td>
<td>LH LF RH RF</td>
<td>LH LF RH RF</td>
<td>LH LF RH RF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorrane</td>
<td>28 23 28 22</td>
<td>+ + + +</td>
<td>30 22 29 23</td>
<td>8 9 6 x</td>
<td>16 8 2/3 7/4</td>
</tr>
<tr>
<td>Cathie</td>
<td>10 18 21 20</td>
<td>10 6 9 6</td>
<td>15 12 28 13</td>
<td>6 7 8 9 5 x</td>
<td>31 2/3 7/4</td>
</tr>
<tr>
<td>Dorothy</td>
<td>28 22 29 27</td>
<td>11 11 19 18</td>
<td>33 27 33 27</td>
<td>5 6 x 5 9 6</td>
<td>31 16 14</td>
</tr>
<tr>
<td>Blossom</td>
<td>17 13 16 13</td>
<td>14 12 25 18</td>
<td>33 25 31 26</td>
<td>9 9 8 12 6</td>
<td>16 17 17</td>
</tr>
<tr>
<td>Belle</td>
<td>18 14 16 13</td>
<td>24 16 22 15</td>
<td>38 28 36 29</td>
<td>6 8 5 6 8</td>
<td>22 17 17</td>
</tr>
<tr>
<td>Roberta</td>
<td>25 22 29 25</td>
<td>20 12 18 15</td>
<td>35 32 33 26</td>
<td>5 5 5 6 6</td>
<td>21 19 2</td>
</tr>
</tbody>
</table>

* - Wednesday, August 5th, 1959
AM - Denotes morning milking at 5:30 A.M.
PM - Denotes afternoon milking at 4:30 P.M.
x - Un reliable results.
# - Quarters of the udder - left hind, left front, right hind, right front.
+ - Not recorded.
A. Physiological Studies

1. The intra-mammary pressure (IMP) changes before and after complete milking.

In this experiment, changes in the intra-mammary pressure (IMP) were recorded before milking, after stimulation of the udder by hand and after complete milking (Tables III and IV) on two different days using six cows. The initial IMP readings are indications of the pressure that existed within the udder just before milking and without any stimulation of the udder. After the initial pressure recording, the udder was stimulated by hand manipulation and the IMP recorded. At this stage the animal was completely milked and the IMP recorded subsequently. The milk yield was also recorded. This process of recording the IMP was repeated at the afternoon milking of both days.

The results are tabulated in the order of the animals milked and the teats cannulated to record the IMP i.e., the right front (RF), the right hind (RH), the left front (LF), and the left hind (LH) quarter respectively (Table III) and the LH, the LF, the RH and the RF quarter respectively (Table IV). The order of recording the IMP from different quarters has a special significance especially in the case of the initial IMP values which should be recorded with the least manipulation of the udder. However, the effect of some stimulation of the udder caused by cannulation cannot be prevented completely. This effect is noticeable as one studies the values of the initial IMP recorded going from the first quarter to the last. Due to the possibility of stimulating milk ejection during the process of cannulation, the results recorded as initial IMP are not completely devoid of milk ejection effects. This
important factor cannot be overlooked and, therefore, the initial IMP figures recorded cannot be considered as the true IMP values at the time of milking prior to any stimulation of the udder. The IMP values recorded after stimulation of the udder are without any such side effects and, therefore, are regarded as the accurate or true values. Similarly, the IMP measurements recorded after milking the animal represent the true IMP values except where results were considered unreliable due to the movements of the animal or some other obstruction in the recording procedure.

On studying the results of this experiment (Tables III and IV), a typical pattern of the IMP changes is noticed. It is observed that in general the initial IMP rises instantaneously after stimulation of the udder and after complete milking of the animal, the IMP falls even below the initial IMP levels.

The results also show that the initial IMP values in different quarters of different cows ranged between 9 mm. Hg to 29 mm. Hg pressure except in a few cases (Table III: animal - Cathie, the LF quarter at afternoon milking, 8 mm. Hg. Table IV: animal - Cathie, the LF and the RF quarters at afternoon milking, 6 mm. Hg. Table III: animal - Belle, the LH quarter at morning milking, 33 mm. Hg,) Milk ejection was brought about spontaneously by mechanical stimulation of the udder. The IMP values recorded after milk ejection are much higher than the initial IMP values and range between 18 mm. Hg and 36 mm. Hg with the exception of 5 cases out of 48 where the IMP values recorded ranged between 11 mm. Hg 15 mm. Hg. In many cases it is observed that the IMP value of a quarter after stimulation of the udder is nearly double that of the initial IMP value of the same quarter and sometimes even more than double. This
sudden rise of the IMP after udder stimulation at the time of milking was shown earlier by Tgetgel (61) and Kryzwanek and Bruggemaan (35 and 36). This sudden rise of the IMP is due to the mechanism of milk ejection which is brought about by quick response from the udder stimulation. Ely and Petersen (20) explained the milk ejection process by propounding the neuro-hormonal theory of milk ejection.

The data presented in Tables III and IV also show the IMP values recorded immediately after milking. A very sudden fall is noticed in each quarter and the pressure dropped as low as 3 mm. Hg to 9 mm. Hg. The only exceptions were - (Table III: animal - Belle, the RF, the RH and the LH quarter at afternoon milking, 11 mm. Hg to 13 mm. Hg; Table IV: animal - Blossom, RF quarter at morning milking, 12 mm. Hg.) These results show that the IMP immediately after milking the animal is at its lowest but starts to build up since the previous milking until it reaches a certain high limit at the actual milking process. The initial IMP values when recorded are not influenced by the effect of udder stimulation, represent the udder pressure owing to the amount of milk present in the individual mammary gland cistern as reported by Ely and Petersen (20). At milk ejection as a result of the udder stimulation, the IMP suddenly rises reaching its maximum limits and falls to its minimum limits after milking as observed by Tgetgel (61) and Ely and Petersen (20). The present results tend to prove this current hypothesis by Ely and Petersen.

A noted feature of the results is the comparison of the IMP values of hind quarters to those of the front quarters after stimulation of the
udder. It is noticed that without exception all the IMP readings from both the hind quarters, left and right, are higher than the IMP readings from both the front quarters, left and right, respectively. These differences are noticed from the IMP values recorded at both the morning and the afternoon milking. The range of variations between the IMP values of the front and hind quarters is quite wide but in general the differences are only of a few mm. Hg pressure. It is a well established fact that milk yield from the hind quarters is more than that from the front quarters. If this fact is related to the present findings of higher IMP figures of the hind quarters, it may be considered that the IMP values are influenced by the amount of milk yielded.

Another point of interest that these results present is the comparison of IMP values recorded after stimulation of the udder at morning milking with those recorded at afternoon milking. In general, the morning figures are higher than those recorded in the afternoon. At the same time the present results reveal that without an exception the milk yield of all the individual cows under the experiment at morning milking is higher than that of the afternoon milking. This may be due to the longer interval (13 hours) before morning milking than for afternoon milking (11 hours). These observations tend to support the view that the milk yield has a profound influence on the level of the IMP. To what extent the direct relationship of milk yield and the IMP observed from the present study can be considered valid, is a question that cannot be answered conclusively from this experiment. This requires further investigation using much larger number of cows.
However, the milk yield cannot be considered to be the only factor responsible for the IMP levels in various quarters of the udder. Other factors such as udder conformation, stage of lactation, breed and age of the animal and other individual variations cannot be overlooked.
### Table V.

The effect of increasing time intervals on the intra-mammary pressure.*

<table>
<thead>
<tr>
<th>Cow</th>
<th>Time</th>
<th>Intra-mammary pressure (mm. Hg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Roberta RF LF RH IH</td>
</tr>
<tr>
<td>Initial</td>
<td>At 6:20 A.M. (immediately after milking)</td>
<td>6 6 5 5</td>
</tr>
<tr>
<td>Initial</td>
<td>2 hours after milking</td>
<td>8 10 7 8</td>
</tr>
<tr>
<td>Initial</td>
<td>4 hours after milking</td>
<td>8 11 8 14</td>
</tr>
<tr>
<td>Initial</td>
<td>6 hours after milking</td>
<td>10 13 11 12</td>
</tr>
<tr>
<td>Initial</td>
<td>8 hours after milking</td>
<td>13 16 16 16</td>
</tr>
<tr>
<td>Initial</td>
<td>10 hours after milking</td>
<td>15 17 18 20</td>
</tr>
<tr>
<td>After Milking</td>
<td>At 4:20 P.M. (immediately after milking)</td>
<td>3 4 4 6</td>
</tr>
<tr>
<td>Milk yield in lbs.</td>
<td>Morning milking</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Afternoon milking</td>
<td>19</td>
</tr>
</tbody>
</table>

* - Wednesday, August 5th, 1959.

# - Quarters of the udder - right front, left front, right hind and left hind.
## Table VI

The effect of increasing time intervals on the intra-mammary pressure.*

<table>
<thead>
<tr>
<th>Cow</th>
<th>Time</th>
<th>Fleta LF LH RH RF</th>
<th>Intra-mammary pressure (mm. Hg.)</th>
<th>Grade LF LH RH RF</th>
<th>Milk Yield in lbs</th>
<th>Miranda LF LH RH RF</th>
<th>Milk Yield in lbs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Showed reflex rise of intra-mammary pressure so not recorded.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>At 5.30 AM (morning milking time)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After Stimulation 5.30 AM.</td>
<td>29 37 37 27</td>
<td>-</td>
<td>26 38 35 26</td>
<td>-</td>
<td>35 36 40 35</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After Milking 5.30 AM.</td>
<td>x x 6 6</td>
<td>16</td>
<td>6 7 6 5</td>
<td>15.5</td>
<td>6 6 5 6</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Initial 3 hours after milking</td>
<td>8 8 8 6</td>
<td>-</td>
<td>7 7 6 5</td>
<td>-</td>
<td>6 7 6 6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Initial 6 hours after milking</td>
<td>9 10 7 6</td>
<td>-</td>
<td>8 9 7 6</td>
<td>-</td>
<td>15 11 10 9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Initial 9 hours after milking</td>
<td>21 25 22 19</td>
<td>-</td>
<td>13 11 12 16</td>
<td>-</td>
<td>26 32 39 31</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Initial 12 hours after milking</td>
<td>18 20 23 19</td>
<td>-</td>
<td>22 32 30 21</td>
<td>-</td>
<td>28 34 33 35</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Initial 15 hours after milking (8.30 PM)</td>
<td>18 25 36 25</td>
<td>-</td>
<td>21 45 34 24</td>
<td>-</td>
<td>37 44 45 38</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Initial 18 hours after milking (11.30 PM)</td>
<td>26 39 44 37</td>
<td>-</td>
<td>Left hind quarter was showing signs of inflammation</td>
<td>15</td>
<td>40 46 47 43</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After Stimulation 11.30 PM.</td>
<td>26 45 46 36</td>
<td>-</td>
<td>45 50 50 46</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After Milking 11.30 P.M.</td>
<td>5 6 4 5</td>
<td>13</td>
<td></td>
<td></td>
<td>6 6 7 7</td>
<td>38</td>
</tr>
</tbody>
</table>


x - Unreliable results.

# - Quarters of the udder - left front, left hind, right hind and right front.
2. **The effect of increasing time intervals on the IMP:**

This experiment was performed at two different occasions using different animals each time. It was reported by Tgetgel (64) and Kryzwaneck and Bruggermaan (35 and 36) that there is a gradual increase in the IMP between one milking and the next milking. Korkman (34) demonstrated that the internal udder pressure rises rapidly in the first hour after milking, the rise being due to the hydrostatic pressure of the milk which drains from the secretory tissue to the udder and teat sinuses. After this initial period the rise in pressure is due to the pressure of the milk which is being secreted. At first the increase in pressure is small but the rate accelerates with increasing interval since milking.

The present experiment was undertaken to study the manner in which, after milking, the increasing time interval affects the IMP of the cow. The results shown (Table V) are from two cows, Roberta and Belle. These cows were milked at the morning milking time after which the IMP from each of their quarters was recorded. With increase of the time interval a progressive increase in the IMP is noticed in all cases except the few odd cases showing an insignificant deflection from the general pattern. A sharp drop found in the IMP values after the afternoon milking (Table V) is in accordance with the characteristic pattern discussed under experiment 1, of this work.

Data presented in Table VI were recorded from three cows. A noted feature of this experiment is that the IMP was recorded after the morning milking and subsequently the initial IMP was recorded after every three hours and the cows were not milked till the initial IMP appeared to be reaching a plateau. The data (Table VI) show a very progressive increase
in the initial IMP since the morning milking until the animals were milked after an unusually long interval of 18 hours. The literature presents very little evidence of this type of experiment where the initial IMP was recorded over an interval as long as 18 hours without milking the animals. The object of this experiment was to mark the time after which the initial IMP reached a plateau and also to study its effect on the milk yield. Certain difficulties were quite probable in the way of such an attempt. Repeated cannulation of the teats was obviously disturbing the animals during the experiment. Apart from that the animals showed restlessness on not being milked at the usual afternoon milking time. The large quantity of milk in the unmilked udder was responsible for the higher initial IMP than usual. An increasing stress on the unmilked udders and the animals was noticeable especially in one cow, Grade, which showed signs of inflammation of the udder and was, therefore, milked only 15 hours after the morning milking. In the case of the other two animals also, the experiment was not continued beyond the interval of 18 hours when they were milked in view of the imminent adverse effects.

It is noticed from the results (Table VI) that up until 5:30 P.M., the IMP values recorded, evidently do not indicate any effect of udder stimulation. But for the values recorded at 8:30 P.M. and 11:30 P.M., some effect of udder stimulation can be suspected. However, the IMP values recorded after the udder stimulation are slightly higher than the initial IMP values recorded at this time. If these initial IMP values were without any effect of the udder stimulation, then it may be concluded that in an udder, unmilked over a long interval, the initial IMP keeps on
increasing and may even surpass the usual after stimulation IMP levels which are recorded at the regular milking time. This contention is supported by the present data (Table VI) which show that the initial IMP values recorded at and after 8:30 P.M. are higher than the after stimulation IMP figures of the morning milking. However, this cannot be accepted conclusively because of the possibility of some mechanical or reflex stimulation effects influencing the initial IMP values.

The milk yield after the longer interval (Table VI) was higher than that at the previous milking. The increase, however, is not significant in view of the length of the interval.
Table VII
The effect of partial milking on the intra-mammary pressure.*

<table>
<thead>
<tr>
<th>Cow</th>
<th>Milking time</th>
<th>Intra-mammary pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>PM</td>
</tr>
<tr>
<td></td>
<td>IH LF RH RF</td>
<td>IH LF RH RF</td>
</tr>
<tr>
<td>Initial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roberta</td>
<td>25 22 29 25</td>
<td>18 14 16 13</td>
</tr>
<tr>
<td></td>
<td>20 21 18 25</td>
<td>21 21 22 15</td>
</tr>
<tr>
<td>Belle</td>
<td>35 32 33 26</td>
<td>38 28 36 29</td>
</tr>
<tr>
<td></td>
<td>27 21 29 24</td>
<td>32 21 33 29</td>
</tr>
<tr>
<td>After partial milking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roberta</td>
<td>21 19 16 19</td>
<td>25 20 27 23</td>
</tr>
<tr>
<td></td>
<td>19 20 12 18</td>
<td>20 20 19 18</td>
</tr>
<tr>
<td>Belle</td>
<td>5 5 5 6</td>
<td>6 7 5 9</td>
</tr>
<tr>
<td></td>
<td>6 x 1 3</td>
<td>6 5 6 8</td>
</tr>
<tr>
<td>After complete milking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roberta</td>
<td>12 10</td>
<td>10 9</td>
</tr>
<tr>
<td></td>
<td>2 1 0</td>
<td>1 7</td>
</tr>
<tr>
<td>Belle</td>
<td>24 19</td>
<td>22 17</td>
</tr>
</tbody>
</table>

Milk yield in lbs:

<table>
<thead>
<tr>
<th>After partial milking</th>
<th>AM</th>
<th>PM</th>
<th>AM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>10</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After complete milking</td>
<td>AM</td>
<td>PM</td>
<td>AM</td>
<td>PM</td>
</tr>
<tr>
<td>24</td>
<td>19</td>
<td>22</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

* - Wednesday, August 5th, 1959
# - Quarters of the udder - left hind, left front, right hind, right front.
AM - Denotes morning milking
PM - Denotes afternoon milking
x - Unreliable result.
Table VIII

The effect of partial milking on the intra-mammary pressure.*

<table>
<thead>
<tr>
<th>Cow</th>
<th>Grace</th>
<th>Milk Yield in lbs</th>
<th>Apple</th>
<th>Milk Yield in lbs</th>
<th>Barbaba</th>
<th>Milk Yield in lbs</th>
<th>Fleta</th>
<th>Milk Yield in lbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>After Stimulation</td>
<td>24 31 31 21</td>
<td>-</td>
<td>35 34 39 32</td>
<td>-</td>
<td>33 31 36 28</td>
<td>-</td>
<td>29 35 31 25</td>
<td>-</td>
</tr>
<tr>
<td>After Partial Milking No. 1</td>
<td>19 29 26 18</td>
<td>4.5</td>
<td>27 27 29 25</td>
<td>7</td>
<td>24 24 25 21</td>
<td>4.5</td>
<td>29 31 26 19</td>
<td>4.8</td>
</tr>
<tr>
<td>After Partial Milking No. 11</td>
<td>14 15 15 11</td>
<td>5</td>
<td>19 19 17 10</td>
<td>7</td>
<td>17 15 14 12</td>
<td>5.7</td>
<td>19 15 11 9</td>
<td>4</td>
</tr>
<tr>
<td>After Complete Milking</td>
<td>7 8 4 7</td>
<td>3.8</td>
<td>6 7 6 x</td>
<td>4</td>
<td>7 7 6 4</td>
<td>5.5</td>
<td>6 9 5 5</td>
<td>3.5</td>
</tr>
<tr>
<td>Total Milk Yield</td>
<td>13.3</td>
<td>18</td>
<td>15.7</td>
<td>12.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* - Wednesday, January 6th, 1960 - 3.30 P.M.
# - Quarters of the udder - left front, left hind, right hind, right front.
x - Unreliable result.
3. The Effect of partial milking on the IMP:

It has been demonstrated by various workers that on milk ejection the IMP suddenly rises to its highest levels and it falls back to its lowest values after milking. The pattern of the fall in the IMP during milking was investigated in more detail by Kryzwanek and Bruggemaan (35 and 36). They measured the changes in the internal udder pressure following milk ejection and subsequently during milking at different levels of emptying the udder. They showed that the fall in the IMP after milk ejection depends upon the amount of milk removed and reaches its lowest when the udder is completely emptied.

The present experiment was conducted to study the effects on the IMP when the udder is emptied partially and then completely. The results obtained from this study are presented in Tables VII and VIII.

In one case (Table VII), two cows were used for this experiment and the data recorded at one morning milking and also at the same afternoon milking. At these milking times, the initial IMP and the IMP after stimulation were recorded. Subsequently the animals were milked, removing only approximately half the quantity of the total anticipated milk yield, and the IMP recorded. Finally they were completely milked and the IMP recorded again. The milk yield figures of each milking are also given (Table VII). It is observed from these results that in some cases the initial IMP figures are close to the IMP values recorded after stimulation. This points out to the possibility of some effect of stimulation of the udder on the initial IMP during the recording process. However, the IMP figures after stimulation are correct and without any side effects. After partial milking, a sharp drop in the IMP is observed. Complete milking of
of the animal brings about a further drop in the IMP which reaches its minimum level (Table VII). These results present a typical pattern showing how markedly the amount of milk removed from the udder affects the IMP.

The data presented in Table VIII are from four other cows. Their IMP was recorded after the udder stimulation at the afternoon milking. The animals were milked to remove a part of their anticipated milk yield (quantities of milk removed at each milking are shown in Table VIII) and the IMP recorded; subsequently another portion of milk was removed and the IMP recorded again. Finally the animals were completely milked and the IMP values recorded. These repeated partial milkings were performed one after the other without any interval of time except when the IMP was being recorded. The results presented here (Table VIII) show a very regular fall in the IMP brought about by every removal of milk until the IMP reaches its lowest limits on complete emptying of the udder.

The present study reveals the manner in which the maximum IMP recorded after milk ejection falls on partial and complete milking. According to the present results (Tables VII and VIII) a direct relationship appears to exist between the amount of milk removed and the drop in the IMP. These results are found to be in close conformity with the findings of Kryzwanek and Bruggemaan (35 and 36).
Table IX

The effect of complete milking of the front quarters on the intra-mammary pressure of the unmilked hind quarters.*

<table>
<thead>
<tr>
<th>Cow</th>
<th>Intra-mammary pressure (mm. Hg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fayne #*</td>
</tr>
<tr>
<td></td>
<td>RF LF RH LH</td>
</tr>
<tr>
<td>Initial</td>
<td>16 19 26 24</td>
</tr>
<tr>
<td>After stimulation</td>
<td>Not well Recorded</td>
</tr>
<tr>
<td>After Milking both front Quarters</td>
<td>4 4 33 36</td>
</tr>
<tr>
<td>After complete milking of all quarters</td>
<td>7 6 13 15</td>
</tr>
<tr>
<td>Milk Yield</td>
<td>12.5 lbs</td>
</tr>
</tbody>
</table>

#* - Monday, July 6th, 1959 - 3.30 P.M.
# - Thursday, July 23rd, 1959 - 3.30 P.M.
# - Quarters of the udder - right front, left front, right hind and left hind.
1. The effect of complete milking of the front quarters on the IMP of the unmilked hind quarters.

The anatomy of the bovine udder has been described by Sisson and Grossman (60) and Turner (65). The udder of the cow comprises four quarters, each representing a mammary gland. All the four mammary glands are anatomically separate from one another and no direct communication exists between any two quarters.

Whittlestone (74) recorded simultaneously the IMP changes in two quarters of the cow, one milked and the other unmilked. He demonstrated that there is a very steady pressure fall in the milked quarter which has no influence on the unmilked quarter which shows a very slow fall in the IMP. Little work has been done on this particular subject and thus conformation of Whittlestone's work is not available from the literature. Therefore, the present work was undertaken to study this problem. The present experiment was conducted on one cow at two different occasions (Table IX) to study the effects of completely milking the front quarters on the IMP of the hind quarters.

The data collected (Table IX) show that as usual the initial IMP values appear to have been influenced to some extent by some unavoidable stimulation of the udder. The IMP figures recorded after stimulation are satisfactory and follow the usual pattern. On one occasion (July 6, 1959) the IMP after stimulation could not be recorded satisfactorily owing to the movements of the animal. However, in this case, a study of the IMP figures after complete milking of the front quarters shows that the rise in the IMP following udder stimulation was of the normal level. The IMP readings after milking both the front quarters indicate a sharp drop in the IMP of the milked front quarters. On the other hand, the IMP of
the unmilked hind quarters stands as high as after stimulation and is not affected by the marked drop in the IMP of the milked front quarters. On milking the hind quarters, the characteristic fall in their pressure is noticed (Table IX).

The results of this experiment (Table IX) point out that the IMP of the front and hind quarters is independent of each other as can be expected from a study of anatomy of the udder. These results confirm Whittlestone's observations (74) reported earlier.
### Table X

The effect of milking each individual quarter on the intra-mammary pressure of the other quarters.*

<table>
<thead>
<tr>
<th>Cow</th>
<th>Apple (LF LH RH RF)</th>
<th>Milk Yield in lbs</th>
<th>Fleta (LF LH RH RF)</th>
<th>Milk Yield in lbs</th>
<th>Greta (LF LH RH RF#)</th>
<th>Milk Yield in lbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>After stimulation</td>
<td>- - - -</td>
<td>-</td>
<td>- 31 41 37 25</td>
<td>-</td>
<td>36 45 46 35</td>
<td>-</td>
</tr>
<tr>
<td>After Milking the left front quarter</td>
<td>- - - -</td>
<td>-</td>
<td>19 41 43 30 1.8</td>
<td>11 38 35 25 2.2</td>
<td>x 42 39 34 3.6</td>
<td>-</td>
</tr>
<tr>
<td>After Milking the left hind quarter</td>
<td>- - - -</td>
<td>-</td>
<td>18 14 27 x 4.4</td>
<td>10 8 28 22 1.3</td>
<td>13 12 39 33 4.7</td>
<td>-</td>
</tr>
<tr>
<td>After Milking the right hind quarter</td>
<td>- - - -</td>
<td>-</td>
<td>16 10 16 23 3.8</td>
<td>8 7 7 30 3.3</td>
<td>12 12 14 31 3.7</td>
<td>-</td>
</tr>
<tr>
<td>After Milking the right front quarter</td>
<td>- - - -</td>
<td>-</td>
<td>12 11 9 x 2.0</td>
<td>8 9 7 7 2.5</td>
<td>12 12 13 x 3.5</td>
<td>-</td>
</tr>
<tr>
<td>After Stripping all Quarters</td>
<td>- - - -</td>
<td>-</td>
<td>6 5 x x 1.3</td>
<td>7 8 4 3 2.7</td>
<td>x 5 5 4 1.0</td>
<td>-</td>
</tr>
<tr>
<td>Total Milk Yield</td>
<td>- - - -</td>
<td>-</td>
<td>13.3</td>
<td>12.0</td>
<td>16.5</td>
<td>-</td>
</tr>
</tbody>
</table>

* - Thursday, January 7th, 1960 - 4 P.M.
x - Unreliable results.
# - Quarters of the udder, left front, left hind, right hind, right front.
5. The effect of milking each individual quarter on the IMP of the other quarters:

In the previous experiment (No. 4) of this work it was demonstrated that the milking of both the front quarters together has no effect on the IMP of the unmilked hind quarters. Further investigation of this problem was undertaken under the present experiment in order to study the effect of milking individual quarters on the IMP of the other unmilked quarters. Three animals were used for this experiment. During this experiment, the animals were hand milked; each quarter was milked in quick succession except when recording the IMP.

At the milking time, the udder was stimulated mechanically and the IMP of each quarter recorded. Subsequently one quarter (left front) was milked and the IMP of this and the other three unmilked quarters recorded. Similarly, the other three quarters (left hind, right hind and right front) were milked singly, one after the other. After milking of each individual quarter, the IMP of all the four, milked and unmilked quarters was recorded. Finally all the four quarters were stripped out simultaneously and their IMP recorded. At each milking the milk yield was noted.

The results obtained (Table X) present a characteristic pattern of the IMP as influenced by milking each quarter. After milking the first quarter (left front) a sharp drop in its IMP is noticed. This, however, has no significant effect on the IMP values of the other three unmilked quarters which stand about as high as recorded after stimulation. The same trend in the fall of the IMP is observed on milking the second (left hind) quarter. Milking of this quarter brings about a fall in its IMP without any effect on the other quarters. Some decrease noticed in the IMP of the two unmilked quarters (Table X) at this stage is only a result
of keeping them unmilked. This inference is derived from the observations of Tgetgel (64) and Whittlestone (74). They pointed out that the IMP of the quarters which are not milked subsequent to stimulation gradually subsides to the point that would have been reached had no stimulus been applied. The third (right hind) quarter on milking registers a sharp drop in its IMP. This has no effect on the IMP of the other quarters. A similar drop in the IMP of the fourth (right front) quarter is observed on milking. Finally all the four quarters were stripped simultaneously and their IMP recorded. This resulted into a further drop of their IMP values which reached their minimum level (Table X). This can be explained as the effect of completely emptying the udder by removing the accumulated strippings.

This experiment demonstrates that although each quarter of the udder registers a marked rise in its IMP subsequent to the udder stimulation at the time of milking, the level in the IMP of an individual quarter is not affected by milking any of the other quarters. The simultaneous rise of the IMP of all the four quarters after stimulation is in accordance with the neuro-hormonal theory of milk ejection put forth by Ely and Petersen (20).

B. Pharmacological Studies.

It was reported by Gaines (29) that injection of oxytocin causes milk ejection in a lactating animal. Folley (21a) showed a rise in the IMP in an unanaesthetized goat after the intravenous injection of oxytocin. Up to a certain limit, the rise in the IMP was influenced by the level of the hormone (oxytocin) injected. Silver (59) in his review of the effects of various pharmacologically effective substances on the perfused
udder, pointed out that oxytocin brings about milk ejection and thus raises the IMP. Whittlestone's studies (71 and 73) on the sow demonstrated the effect of oxytocin in causing a rise in the IMP.

In the case of the cow, however, the study of the effects of oxytocin injection is more complex in view of the spontaneous release of oxytocin from the cow's pituitary gland even on slight stimulation of the udder. This makes it difficult to record the unmixed effect of oxytocin injection. Donker (17a) injected various doses of oxytocin into a cow which had become unable to eject milk normally. He used the intravenous route for injection to determine the dose levels effective in causing milk ejection and found 10 I.U. of oxytocin to be more effective. Donker's results are based on the milk yield and he did not study the IMP in his experiment.

The role of oxytocin in the milk ejection mechanism and the IMP was established beyond doubt by Ely and Petersen (20). The present work was undertaken to study the direct effect of oxytocin administration before and after milking, on the IMP of the lactating cow.
Table XI

The effect of the administration of oxytocin prior to milking on the Intra-mammary pressure.

<table>
<thead>
<tr>
<th>Date</th>
<th>Cow</th>
<th>After stimulation</th>
<th>Five Minutes After injection of oxytocin</th>
<th>After Milking</th>
<th>Milk Yield in Lbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>30/11/59</td>
<td>Bonnie</td>
<td>LF IH RH RF</td>
<td>27 33 30 26</td>
<td>2 8 8 3</td>
<td>10½</td>
</tr>
<tr>
<td>18/12/59</td>
<td>Bonnie</td>
<td>LF IH RH RF</td>
<td>22 25 23 20</td>
<td>8 10 x 8</td>
<td>9</td>
</tr>
<tr>
<td>18/12/59</td>
<td>View</td>
<td>LF IH RH RF</td>
<td>29 36 37 28</td>
<td>Not recorded</td>
<td>13</td>
</tr>
<tr>
<td>21/12/59</td>
<td>View</td>
<td>LF IH RH RF</td>
<td>34 43 42 31</td>
<td>6 5 4 3</td>
<td>13½</td>
</tr>
<tr>
<td>21/12/59</td>
<td>Barbara</td>
<td>LF IH RH RF</td>
<td>38 42 45 32</td>
<td>6 11 6 5</td>
<td>17</td>
</tr>
<tr>
<td>22/12/59</td>
<td>View</td>
<td>LF IH RH RF #</td>
<td>30 36 28 32</td>
<td>5 9 8 4</td>
<td>15</td>
</tr>
</tbody>
</table>

XX - 40 U.S.P. units of oxytocin were injected sub-cutaneously at the neck region after stimulation.

x - Unreliable result.

# - Quarters of the udder, left front, left hind, right hind and right front.
1. The effect of the administration of oxytocin prior to milking on the IMP:

Three cows were used for this experiment on different occasions (Table XI). Milk ejection was brought about by stimulating the udder mechanically and the IMP was recorded after stimulation. At this stage a dose of 'oxytocin' * equivalent of 40 U.S.P. units of oxytocin was injected sub-cutaneously at the neck of the animal. After an interval of five minutes from the time of injection, the IMP was recorded again. (This level of the oxytocin dose and of the time interval to record the IMP after the injection was found to be more effective on trying different dose and time interval levels).

A study of the data of this experiment (Table XI) reveals a definite trend towards an increase in the IMP as a result of the oxytocin injection. This rise in the IMP levels, though small, is significant and is evidence of the effect of oxytocin on the IMP. The low values of increase in the IMP may be due to various factors. Firstly; prior to the administration of oxytocin, the IMP recorded was at its maximum level due to the milk ejection brought about by udder stimulation. This limits the scope for further rise in the IMP subsequent to the injection of oxytocin since the capacity of the alveoli of the udder to contract and raise the IMP is limited. Secondly; inspite of all the care taken, the animal resented the prick of injection and was apparently disturbed. This might have resulted in the release of adrenaline from the animal system. It has been shown by Braude and Mitchell (4) and Yokoyama (76) that the presence of

*"Oxytocin" - a purified-oxytocic-principle product of Austin Laboratories Limited, Guelph, Canada.
adrenaline counteracts the effect of oxytocin and inhibits milk ejection. In view of these facts even the small increase in the IMP that was registered each time following the administration of oxytocin after natural milk ejection, leads to significant conclusions. It indicates that level of the IMP brought about by natural milk ejection can be raised by supplementing the supply of oxytocin to the animal system. In view of the important role that the oxytocin hormone plays in milk ejection and milk production, the present findings emphasize the need for further investigation of this problem.
<table>
<thead>
<tr>
<th>Date</th>
<th>Intra-mammary pressure (mm. Hg.)</th>
<th>Cow View</th>
<th>Cow View</th>
<th>Cow View</th>
<th>Cow View</th>
</tr>
</thead>
<tbody>
<tr>
<td>22/10/59</td>
<td>Thursday</td>
<td>LF LH RH RF</td>
<td>LF LH RH RF</td>
<td>LF LH RH RF</td>
<td>LF LH RH RF</td>
</tr>
<tr>
<td>5/11/59</td>
<td>Thursday</td>
<td>24 30 31 24</td>
<td>29 30 39 35</td>
<td>17 21 20 15</td>
<td>22 28 28 23</td>
</tr>
<tr>
<td>9/11/59</td>
<td>Monday</td>
<td>6 6 9 6</td>
<td>11 11 10 x</td>
<td>2 5 9 4</td>
<td>13 13 8 9</td>
</tr>
<tr>
<td>12/11/59</td>
<td>Thursday</td>
<td>9 9 15 10</td>
<td>13 11 12 12</td>
<td>5 6 5 4</td>
<td>14 14 14 11</td>
</tr>
<tr>
<td>Milk Yield in lbs.</td>
<td></td>
<td>15</td>
<td>16 1/2</td>
<td>15 1/2</td>
<td>14 1/2</td>
</tr>
</tbody>
</table>

**XX** - 30 U.S.P. Units of oxytocin were injected sub-cutaneously at the neck region after milking.

**x** - Unreliable result.

**#** - Quarters of the udder - left front, left hind, right hind, right front.
2. The effect of the administration of oxytocin subsequent to milking on the IMP:

In the previous experiment (No. 1.) oxytocin was injected into the cow after stimulating the milk ejection process, but before milking the animal. At that stage the IMP assumes its highest level and, therefore, the increase in the IMP marked as a result of the oxytocin administration was not spectacular. The present experiment was undertaken to study the effect of oxytocin on the empty udder after complete milking, a stage when the IMP was at the minimum level. One cow was employed on four milking occasions for the execution of this experiment. The data presented in Table XII show the IMP values after stimulation, after milking and five minutes after injecting 30 U.S.P. units of oxytocin. The injection was given sub-cutaneously at the neck region.

A study of the data (Table XII) showing the effect of oxytocin on the IMP indicates, in general, a well marked trend. A slight increase in the IMP values is noticed as a result of the oxytocin administration in all quarters except one (right hind quarter on November 9, 1959). In this case the IMP recorded after milking was 9 mm. Hg whilst after injection of oxytocin it dropped to 5 mm. Hg. The higher value of 9 mm. Hg appears to have been affected by a movement of the cow. However, a general slight increase in the IMP as an effect of oxytocin administration is insignificant if compared with the usual rise in the IMP recorded after stimulation as shown in the previous experiments of this work. An explanation for the failure of oxytocin to produce a significant rise in the IMP after milking,
may be found from the observations of Ott and Scott (45). They studied the effects of oxytocin on the udder of the non-lactating goat and found that the oxytocin has no effect on the IMP of the non-lactating animals. This may be due to the absence of the secretory glandular tissue, responsible for milk ejection, from the non-lactating udder. It must be pointed out here that during the present experiment the udder was completely stripped before injecting the oxytocin. Therefore, the behaviour of the completely emptied lactating udder to the administration of oxytocin may be expected to be similar to that of the non-lactating udder.

Further support for the present findings comes from Whittlestone's work. He (74) tried to stimulate milk ejection in the cow after milking the animal. This resulted in the second let-down and rise of the IMP which, however, was far below the IMP increase recorded on stimulation before milking. Although this meager rise of the IMP may be attributed to the smaller quantity of the pituitary extract released on second stimulation, the state of emptiness of the udder is also a factor of no less importance. The results of this experiment (Table XII) indicate that apart from the availability of oxytocin, the degree of fullness of the lactating udder also plays an important role in the IMP levels.
V. GENERAL DISCUSSION

The present work deals with the recording of the intramammary pressure (IMP) changes in different phases of lactation. It is observed that there is a typical pattern of the IMP changes which show well-marked fluctuations on milk ejection and after milking. The initial IMP recorded before the udder stimulation increases suddenly on milk ejection and then falls to the minimum level after milking. These results support the earlier work of Gaines (29), Tgetgel (64), Kryszanek and Bruggemaan (35 and 36) and Whittlestone (74). In the performance of this experiment various important observations were made which require close attention and further investigation. In the cow, milk ejection is caused very suddenly on stimulation of the udder and in some cases it has become a conditioned reflex. The mere sight of the operator and especially cannulation of a teat is liable to cause milk ejection with the result that the recording of the correct values of the IMP becomes very difficult. This is evident from an observation of the initial IMP values recorded during the present work. This situation is peculiar only in the case of the cow since in other species eg. the sow, the goat and the ewe, milk ejection is not stimulated as quickly except when oxytocin is injected. At the same time when the cow is excited and emotionally disturbed or irritated, milk ejection is interfered with and inhibited. This inhibition has been attributed to the release of the hormone adrenaline. This observation has been recorded by Braude and Mitchell (4), Cross (11) and Whittlestone (70). Therefore, the recording of the normal and true values of the IMP in the cow is difficult
if not impossible. Tgetgel (64) appeared to overcome this problem
by leaving the cannula in the teat between two milkings. This itself
causes constant irritation and inconvenience to the animal which may
react unfavourably and thus unnoticeably affect the results. The effect
of this reflex action to stimulate or inhibit milk ejection to some
degree was noticed on the IMP readings in some cases during the present
study.

From the present observations it is found that after milking the
IMP begins to rise and continues to increase with the increasing
interval of time, until the subsequent milking. This supports the
earlier work of Tgetgel (64) and Kryzwanek and Bruggemaan (35 and 36).
However, there is need for further investigation to establish the rate
at which the IMP increases from hour to hour between milkings and also
to study the effect of the increased IMP on the rate of secretion of
milk. The latter aspect is very important since with this is associated
the problem of the frequency of milking.

It has been reported by Gasnier et al. (31) and Hammond (32) that
the milk yield is increased by more frequent milkings. The explanation
offered is that the rate of secretion of milk declines with the increase
in the IMP and that the milk secretion becomes negligible when the IMP
rises beyond a certain limit. On the other hand, Turner (67) has
thrown doubt on whether this is the reason for any increases in yield
that occur with thrice daily milking. However, some effect of the IMP
on the rate of milk secretion cannot be denied. Studies at the University
of Missouri (68) and the work of Petersen (51) demonstrated that the
more frequent and the more complete the milkings, the lower the average
intra-alveolar pressure and the greater the rate of milk secretion; or
what is the same, the longer the interval between milkings, the less the milk production per unit time. The work at the University of Missouri (68) also points out a fall in the fat percentage of milk with the increase in the IMP. As reported above, the present study deals only with the effects of the increasing time intervals on the IMP. The impact of the increasing IMP on the composition and the rate of secretion of milk requires a separate study.

It has been demonstrated by this study that the IMP of each quarter is independent of that of the other. Furthermore, it has been shown that the decrease in the IMP of each quarter after milk ejection depends upon the degree to which that quarter is emptied. These findings support earlier work of Whittlestone (74).

Ely and Petersen (20), Cross and Harris (15) and Whittlestone (69) had reported that oxytocin stimulates milk ejection. Two experiments were performed to study the effects of the oxytocin on the IMP. The results obtained show that there is an increase in the IMP when oxytocin is injected after stimulation, but before milking and also when it is injected after milking. However, the increase in the IMP in both these cases is not spectacular. In the first case this may be attributed to the maximum level of the IMP already attained after stimulation prior to the oxytocic injection. In the second case the absence of a marked rise in the IMP following the oxytocin injection may be due to the complete evacuation of the udder before the injection. Gaines (29) had earlier reported his failure to stimulate milk ejection in the non-lactating goat with the injection of the posterior pituitary extract. From the pharmacological studies of this work it is observed that there is a certain optimum limit of the IMP in an animal beyond which the IMP
cannot rise even with the administration of the milk ejection hormone, the oxytocin. In completely milked mammary glands the oxytocin is almost ineffective to stimulate milk ejection. The presence of both the secretory glandular tissue and of milk in the alveoli of the mammary glands is essential for the stimulation of milk ejection.

As would appear from the present work, the importance of the study of the IMP in general and in the dairy cow in particular, cannot be overemphasized. Dairy industry is an indispensable need of the human population. Efficient lactation is an important factor on which depends the success of this industry so that it can stand competition from other more mechanized fields. The dairy man is to meet the expensive labour problem especially when faced with the task of milking the animals at inconvenient hours. This makes it imperative to find out as to how the animal can be milked at longer intervals than at present without suffering a loss of milk or causing a strain on the health of the animal. Therefore, an understanding of the problem of milk ejection and the IMP with which is connected the efficiency of lactation, becomes of paramount importance. Besides, any improvement on the present method of milking is not possible without a sound knowledge of the changes that take place within the mammary glands at different phases of lactation. Unfortunately the present information about the IMP changes is very limited. Therefore, more fundamental work is required to understand all the aspects of this phenomenon. Only then any practical application of the results can be made.

In view of the influence of various factors such as the breed, age size, temperament and endocrinological state of the animal, conformation and capacity of its udder, the stage of lactation and the milk yield
etc., the study presents difficulties which cannot be avoided. However, a thorough understanding of all the physiological aspects of the IMP changes is absolutely necessary. Only then a statistical study can be undertaken to support any recommendations that may be made to introduce improved methods of milk production. It is hoped that the present study would be a contribution of some value in this direction.
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