FEBRUARY, 1936 RESEARCH BULLETIN 234

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
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The Effect of Udder Irrigation and Milking Interval on Milk Secretion

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(Publication Authorized February 6, 1936)

COLUMBIA, MISSOURI

ABSTRACT

The effect on milk secretion of the interval between milkings and the injection into the udder of water, solutions of salt and lactose, milk, oil and oxygen has been studied.

The changes in milk secretion observed following irrigation of the udder with any of the above substances were: (1) a decrease in milk yield; (2) a decrease in lactose content; and (3) an increase in chlorine, fat, protein, catalase, leucocytes, and pH of the milk for several subsequent milkings. The degree of change produced varied with the individual cows and with the amount and kind of substance injected.

The detrimental effects of solutions increased with the concentration of solute injected. Distilled water had less effect on milk secretion when introduced into the udder than any solution investigated, including milk.

When the milking interval exceeded 18 or 20 hours, the composition of the milk secreted was slightly altered in composition.

A theory explaining the changes in the composition of milk secreted following irrigation or other conditions impairing the activity of the udder is presented.

ACKNOWLEDGMENT

The writers wish to acknowledge their indebtedness to Dr. W. R. Graham, Jr. for his constructive criticism of the manuscript.

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E. R. Garrison and C. W. Turner

The large milk cistern and the extensively branching duct system of the cow's udder serve as a reservoir to hold the milk that is secreted during the interval between milkings. As the milk accumulates, the pressure within the udder increases, thus gradually decreasing the rate of secretion and probably altering the composition of the milk then secreted (Turner, 1935).

The influence of pressure within the udder on milk secretion may be studied experimentally in several ways: (1) by the injection of water; (2) by the injection of solutions of various osmotically active chemical compounds such as salt and lactose; (3) by the injection of non-osmotically active substances not easily absorbed from the mammary gland, such as oil; (4) by the re-injection of milk; (5) by the injection of gases such as air or oxygen into the udder; (6) and by determining the yield and composition of milk after increasing intervals between milkings.

The above studies, in addition to their more or less theoretical interest in relation to milk secretion, will yield information that may be applied to certain practical problems. For example, one proposed method for the treatment of mastitis involves the injection of an antiseptic dye into the affected quarters of the udder. The question arises as to what carrier or diluent of the dye will have the least detrimental effect on the subsequent milk secretion. Would distilled water, an isotonic solution, or a mixture of the osmotically active constituents in approximately the same proportions as found in milk, be the most satisfactory?

The object of this bulletin is to present the results of a study involving the points enumerated above and to indicate their theoretical and practical application to milk secretion.

GENERAL PROCEDURE

Six purebred Holstein and two purebred Jersey cows, free from mastitis and varying from one to nine months in lactation were milked at 12-hour intervals and the yield of each quarter recorded. During the course of this investigation the individual quarters of these cows were irrigated with one or more of the following substances: distilled water, thin paraffin oil, milk, oxygen, solutions of calcium chloride, sodium chloride and lactose in various concentrations, and a solution containing 0.12 per cent sodium chloride and 3.5 per cent lactose.

Uniform amounts (750 ml.) of each solution were introduced into each quarter by gravity through a teat canula and allowed to remain for 12 hours. All liquids, except milk, were sterilized in one-liter Erlenmeyer flasks, cooled to body temperature, and injected immediately after the evening milking. When milk was returned to the udder it was drawn into a sterile flask and the entire volume promptly re-injected into the quarter from which it was obtained. The amounts varied from 600 to 900 ml.

The necessary aseptic precautions such as wiping the teat with alcohol were taken during the injection in order to reduce to a minimum the possibility of udder infection. The bacterial content of the milk from each quarter was periodically determined by plating on beef infusion agar. During the course of this study three quarters were rejected because of the development of a high bacterial count.

An interval of about one week or such longer period of time as was required for the milk to return to normal composition intervened between each injection.

In addition to the above experiments, a study was made of the effect of eliminating a regular milking and of increasing the interval between milkings on the yield and composition of the milk. Individual quarters of several cows were not milked for 24 hours for the suspended milking studies, while two cows were milked at periods of increasing lengths of one, two and three hours, up to 24 hours for the interval-between-milkings study.

Daily composite samples for two days preceding the injection were analyzed, as a check, while after injection each milking was analyzed separately for two days and daily composite samples for an additional five days or more.

The determinations made and the methods of analysis were as follows:

1. Butterfat by the Babcock method.

2. Total protein by the formaldehyde titration method using potassium oxalate as outlined by Pyne (1932).

3. Lactose by means of the polariscope using $Hg(NO_3)_2$ as a clarifying agent. However, during the latter part of this work a method was developed whereby the proteins in the abnormal milks were more completely precipitated by the use of a solution of zinc sulphate and sodium hydroxide (Garrison, 1935).

4. Chlorine by the digestion method developed by Davies (1932).

5. The catalase content was estimated by the standard method of adding 5 ml. of 1 per cent hydrogen peroxide to 15 ml. of milk. The amount of gas liberated was read after standing three hours at 37 degrees Centigrade.

6. The pH determinations were made with a potentiometer using the quinhydrone electrode against a saturated calomel electrode.

During that part of the work where a study was made of the effect of the interval between milkings, a more complete analysis of the milk was made involving, in addition to the above, determination of specific gravity by means of the Westphal balance, ash, total protein, casein and heat coagulable proteins (albumin) by official A.O.A.C. methods*.

GRAPHICAL PRESENTATION OF RESULTS

As the original data were considered too voluminous to include, it was decided to present the average results in graphic form. The first point at the left of each graph (over zero days) is the average value of the composite samples for the two days preceding the injection for all quarters similarly treated. The next point represents the average for the milking 12 hours after injections. The next three milkings were individually analyzed, after which daily composite analyses are shown.

PRESENTATION OF EXPERIMENTAL RESULTS

In order to determine the influence of the volume of solution on the disturbance to milk secretion, increasing amounts of distilled water were injected at weekly intervals into the same quarter of a cow's udder (Fig. 1). The per cent lactose decreased with the volume of liquid injected while the per cent chlorine, in general, gradually increased. It will be seen that the 250-ml. injection produced relatively little effect but this volume of liquid is probably insufficient to irrigate the individual glands of the average cow. While individual quarters vary greatly in capacity (Turner, 1934), it was considered that in general a volume of 750 ml. would be sufficient to penetrate to all parts of the average quarter of the cows used and yet not cause an excessive disturbance to milk secretion. A volume of 750 ml. was therefore used in this study.

*The writers are indebted to Mr. A. J. Bergman and Mr. W. H. McShan for the analysis of the milk proteins.

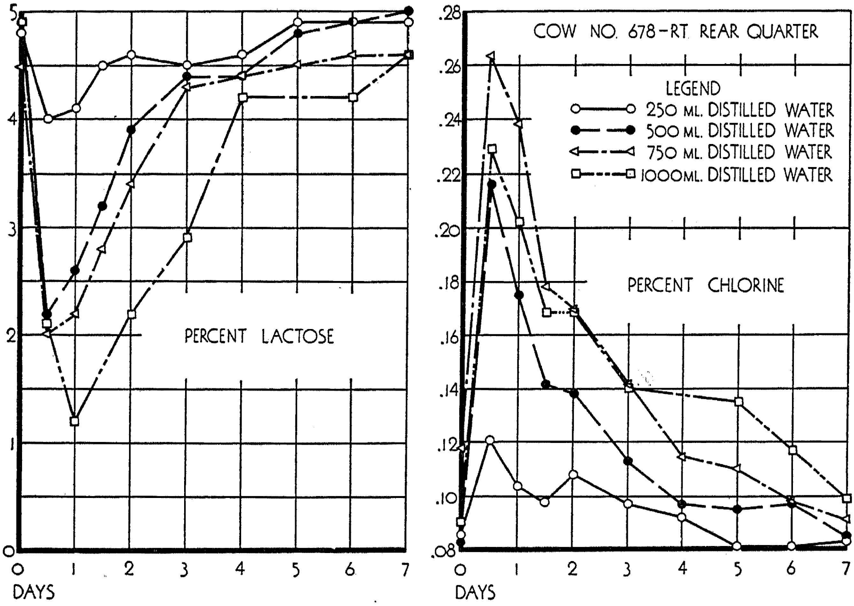

Fig. 1.—The effect of injecting increasing volumes of distilled water into the same quarter on the lactose and chloride content of milk. As the volume of water was increased from 250 to 1000 ml. there was a gradual depression of the lactose and a corresponding increase in the chlorine.

Comparison of Distilled Water with a Dilute Solution of Salt and Lactose

One proposed method for the treatment of mastitis involves the irrigation of the udder with an antiseptic dye solution. The question immediately arises as to the most suitable carrier to use. Clearly, the solution which, when injected alone, has a minimum effect on the gland epithelium as indicated by subsequent yield and composition of milk, would appear to be the most desirable carrier of the dye.

Work on this problem is rather limited. Davidson (1926) injected 200 and 300 ml. of distilled water into two quarters of a cow's udder and found that the disturbances to milk secretion were less pronounced than those caused by the injection of 300 ml. of isotonic salt and lactose solutions.

Petersen and Rigor (1932b) also compared the effect of distilled water and salt and lactose solutions injected into the udder in amounts equal to the volume of milk removed. They likewise found that distilled water had less effect on milk secretion than any of the concentrations of salt or lactose used (3/4 isotonic and higher).

Hucker and Lee (1932), however, concluded as a result of their investigations, that a solution containing 0.12 per cent sodium chloride and 3.5 per cent lactose was less injurious than distilled water when the same quantities (250 ml.) were injected into the mammary gland.

As these results were conflicting and leave some doubt as to the most suitable diluent to use for the carrier of dyes in the treatment of mastitis, it seemed desirable to extend these observations and obtain further information on the effects of various solutions on the mammary gland.

Experiment 1.—The first study to be undertaken was concerned with a comparison of distilled water and a solution containing 0.12 per cent sodium chloride and 3.5 per cent lactose. During this investigation 25 quarters of seven cows were irrigated with each of these liquids. In most instances the two quarters on one side of the udder were injected with 750 ml. of distilled water, while the other two quarters received the same amount of the salt-lactose solution. One week later the injections were reversed, the quarters which received the distilled water now received the salt-lactose solution and vice versa. By this method any differences in the response made by individual quarters as well as any possibility of the second injection producing more severe results than the first injection because of increased sensitiveness of the gland tissue were eliminated since these differences, if they existed, would be equally favorable or unfavorable for each liquid.

The changes that occurred in the composition of the milk after injection were essentially the same as have been observed by other investigators following irrigation of the udder with various solutions (Fig. 2). The changes noted were an increase in fat, protein, chlorine, catalase, and pH and a decrease in lactose for several milkings after injection. There followed a slow return to normal which was not reached, for certain constituents, until about the fifth day. The fat and protein content, however, decreased at the first milking, rose to a maximum at the second, then slowly declined. The amount of secretion decreased to a minimum at the second milking, then steadily increased until normal production was attained on about the fourth or fifth day. The physical condition and color of the milk was also usually altered after the injections. The first milking was generally thin and watery but sometimes flaky, while

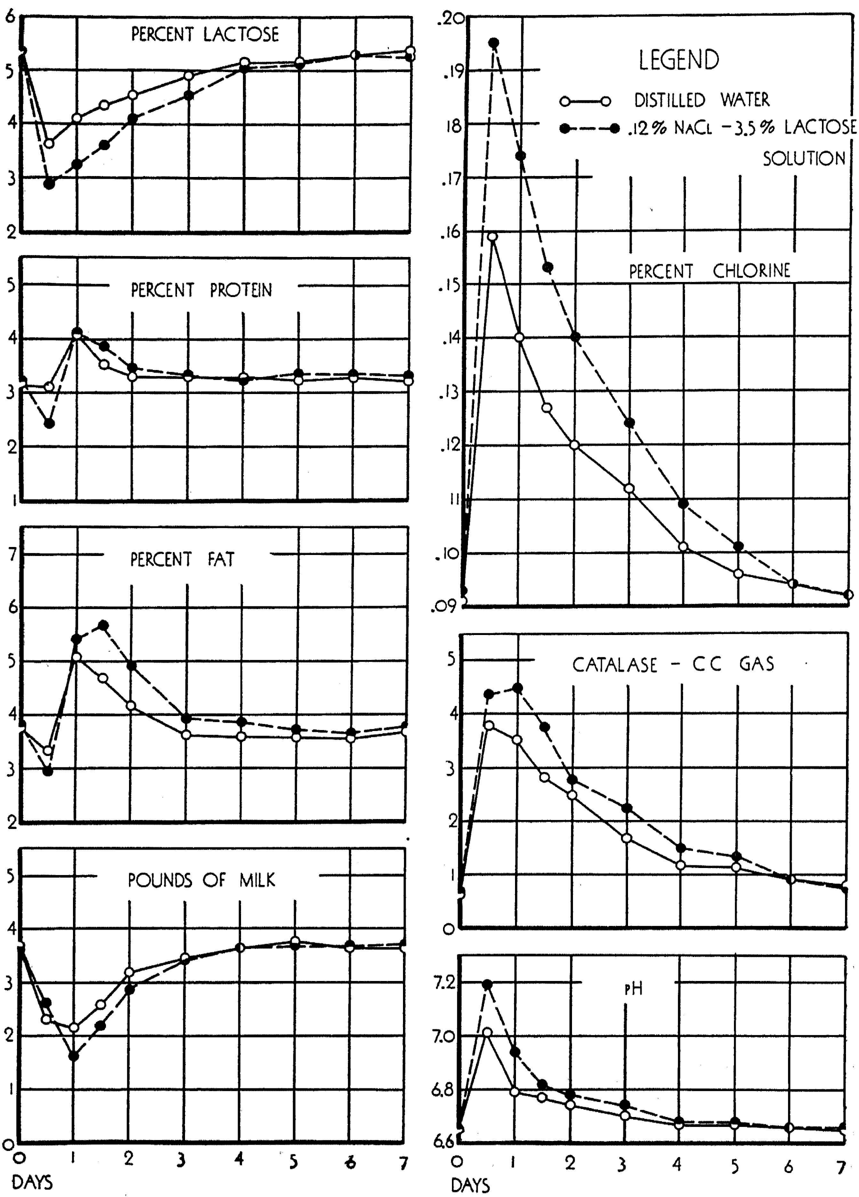

Fig. 2.—Changes in yield and the composition of milk following the injection of distilled water and a solution containing 0.12 per cent sodium chloride and 3.5 per cent lactose into the same quarters. The superiority of distilled water over the salt-lactose solution as an irrigant of the mammary gland is clearly shown.

the second and later milkings were usually thick and contained particles of slimy material which settled out upon standing. The increased consistency tended to disappear as the protein decreased and was probably due to an increased amount of albumin and globulin in the milk. The change in physical condition was usually proportional to the extent of the other changes which occurred in the composition of the milk.

The depression in yield and the degree of alteration in the composition of the milk after the injection of either water or salt-lactose solution varied with different cows and with individual quarters of the same cow, only slight changes occurring after injection in some cases, while in other quarters the effects were quite severe. In general the highest producing quarters were affected the least by the introduction of either liquid. However, the reduction in yield and the alteration in the composition of the milk were not as great following the injection of distilled water as occurred after treatment with the salt-lactose solution. It was also noted that the physical condition and color of the milk obtained for several milkings after udder irrigation was affected the least when water was introduced.

These results clearly indicate that distilled water is superior in all respects to a solution containing 0.12 per cent sodium chloride and 3.5 per cent lactose as an irrigant of the mammary gland.

The Injection of Sodium and Calcium Chloride Solutions

The effect on the mammary gland of the injection of various salt solutions has also been studied by dairy physiologists. Davidson (1926) injected 300 ml. of an isotonic salt solution (sodium and potassium chlorides and citrates) into one quarter of the udders of two cows after they were milked dry. The injection caused a marked increase in per cent fat, a noticeable increase in per cent protein plus ash and a decrease in the per cent lactose in the milk secreted for several milkings. The yield of milk was also noticeably reduced for three days after the injection, the lowest production occurring at the second milking following the irrigation.

Petersen and Rigor (1932b) concluded as the result of an experiment in which isotonic, hypotonic (3/4 isotonic) and hypertonic (1¼ isotonic) solutions of saline and Ringer's were injected into the udder in amounts equal to the volume of milk removed, that the detrimental effect on the subsequent amount and composition of the milk increased with the concentration of the solutes injected.

There was no significant difference between the effects of saline and Ringer's solution when injected at the same concentration.

Hucker and Lee (1932) injected several quarters with 250 ml. of sodium chloride solutions ranging in concentration from 0.05 to 1.5 per cent. They concluded that the minimum effect on the appearance and pH of the subsequent milk obtained was produced by a solution containing 0.12 to 0.14 per cent salt. They found the injection of Ringer's solution to have a very severe effect on the mammary gland, the milk flow being markedly decreased or entirely stopped.

Experiment 2.—Petersen and Rigor found progressively increasing injurious effects from 3/4 isotonic solutions and above. The work of Hucker and Lee, on the other hand, indicated that solutions containing 0.12 to 0.14 per cent salt produced the minimum effect. It seemed desirable to compare distilled water with a series of solutions of increasing salt content. Solutions containing 0.12, 0.24, 0.48 and 0.96 per cent sodium chloride were therefore injected into various quarters of six cows and the effect on the subsequent milk secretion determined. Six quarters of five cows were treated with distilled water and each salt concentration. Each quarter, however, did not receive all of these salt solutions.

The results obtained fail to show any definite relation between the salt concentration of the solutions injected and the effect on milk secretion (Fig. 3). In most cases the 0.96 per cent solution caused the least disturbance, which is contrary to what would be expected from the work of other investigators. The more favorable showing of this solution is believed to be due principally to the larger capacity of the quarters so treated, as indicated by the higher production level. These results indicate that the response made to injections of different salt concentrations is affected by the size of the cistern system or storage capacity and the production level of the particular quarter injected. The above results, therefore, probably do not show the true relationship between salt concentration and the disturbance to milk secretion because the variation in the capacity of the individual quarters was not taken into consideration.

In addition to the variation in the capacity of the udder, it was observed also that individual cows appear to vary in their response to the injection of solutions irrespective of the size of the udder. An illustration of this difference in sensitivity is shown by the difference in the response made by one Holstein and two Jersey

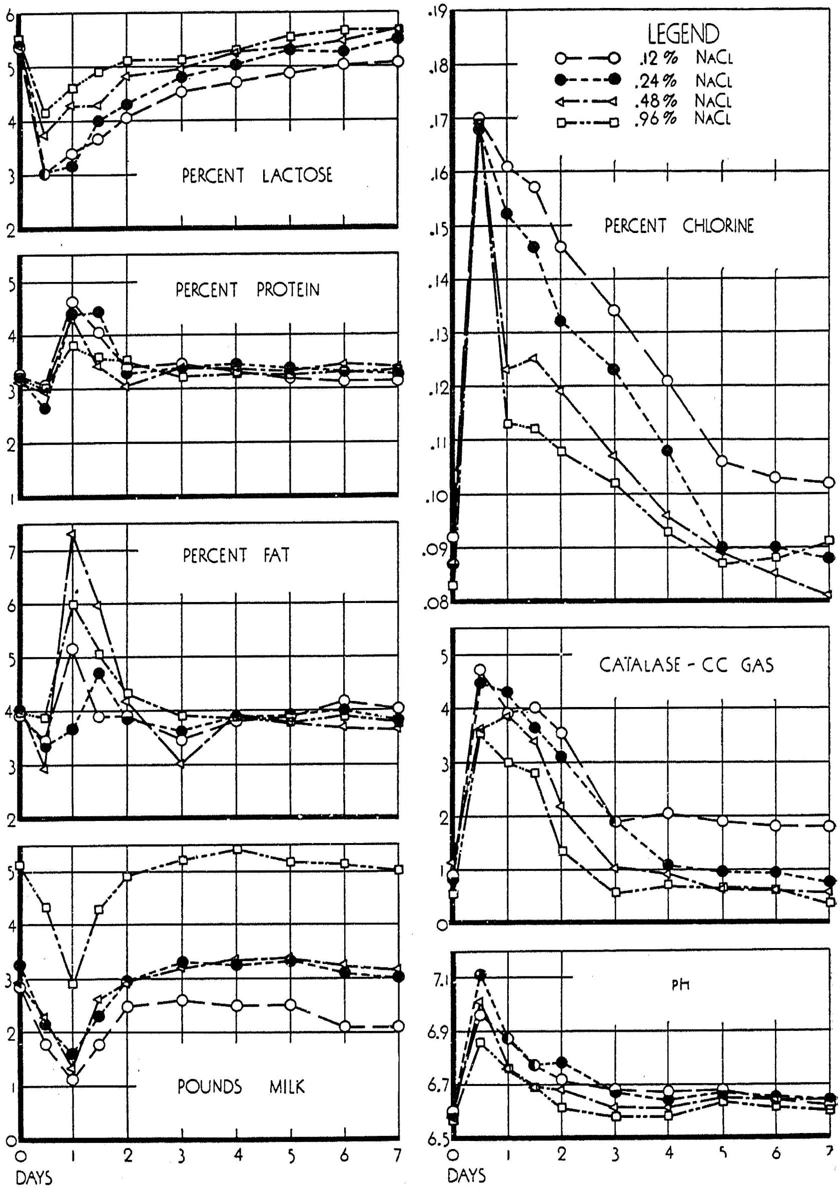

Fig. 3.—The effect of injecting solutions containing increasing amounts of salt on the subsequent yield and composition of the milk. The true relationship between salt concentration and the disturbance to milk secretion is probably not shown due to variation in the capacity and sensitivity of the individual quarters injected.

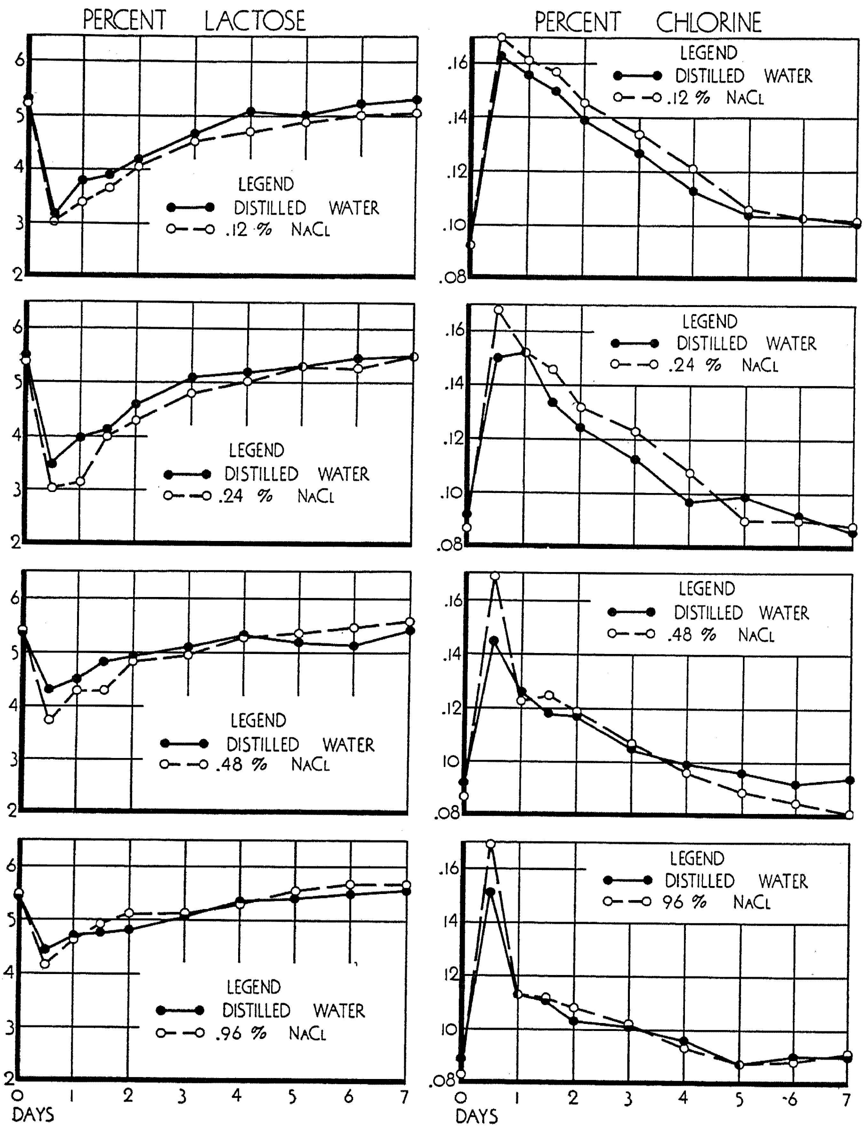

Fig. 4.—Comparison of salt with distilled water. The data from Fig. 3 for chlorine and lactose was replotted to show the comparable effect produced by each salt concentration and distilled water in the same quarters. It will be noted that in all cases distilled water was less detrimental.

cows which were injected with 750 ml. of 0.12, 0.24, 0.48 and 0.96 per cent saline solutions in the left front, left rear, right front, and right rear quarters, respectively.

The Holstein cow (No. 578) was more sensitive to all the solutions than the two Jersey cows (No. 822 and No. 828), even though the production of this cow was considerably higher than that of the other two. Apparently, individual cows vary in their sensitivity to injections and are affected by other factors than the capacity of the udder.

While these data fail to show a definite relation between the salt concentration of the solutions injected and the effect on milk secretion, it is interesting to note the effect of distilled water on these same quarters (Fig. 4). In all instances each salt concentration caused a slightly greater depression in lactose and a larger increase in the chlorine content of the milk than occurred after the injection of distilled water into the corresponding quarters.

Experiment 3.—As the above experiments were not believed to show the true influence of increasing salt concentrations on milk secretion it was decided to repeat certain parts of the work and use the same quarters for all solutions. Two quarters of each of two cows were each injected at weekly intervals with solutions containing 0.48, 0.24 and 0.96 per cent sodium chloride in the order named. By following this method of treatment any variations made by the individual quarters because of any differences in size or response would be eliminated since each quarter was injected with each salt concentration. The changes which occurred in the yield and certain components of the milk, particularly lactose and chlorine, secreted after injections, increased in severity with the concentration of the solute in the liquid injected (Fig. 5).

The amount of chlorine recovered in the milk at the first milking after injection of the 0.24, 0.48 and 096 per cent solutions was 104.8, 82.3 and 35.6 per cent, respectively, of the chlorine injected in the form of sodium chloride. This indicates that chlorine is absorbed from the mammary gland when strong solutions of sodium chloride are injected. It should be appreciated, however, that a certain amount of milk in the udder cannot be removed at milking time (Turner and Slaughter, 1930).

From Experiments 2 and 3 it seems evident that distilled water is less detrimental to milk secretion than injections of sodium chloride in any of the concentrations studied (0.12 to 0.96 per cent), and that the detrimental effects of such solutions increase with the concentration of the solute (0.24 to 0.96 per cent). This agrees with the results obtained by Petersen and Rigor but does not entirely agree with the results obtained by Hucker and Lee. The

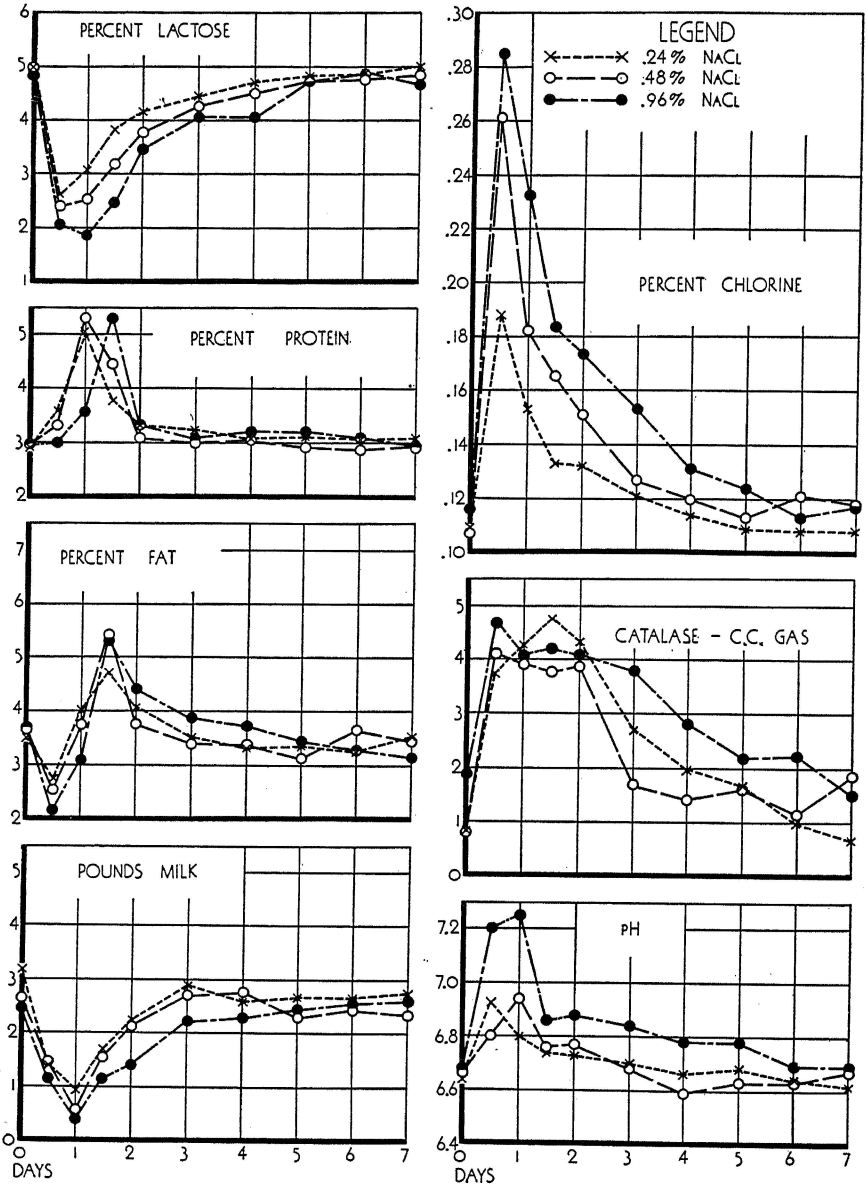

Fig. 5.—The effect of injecting solutions of increasing salt concentration into the same quarters on yield and composition of the milk. The disturbance to milk secretion increased with the concentration of the solute injected.

results obtained by the latter may, however, be due to the small amount of fluid injected (Fig. 1).

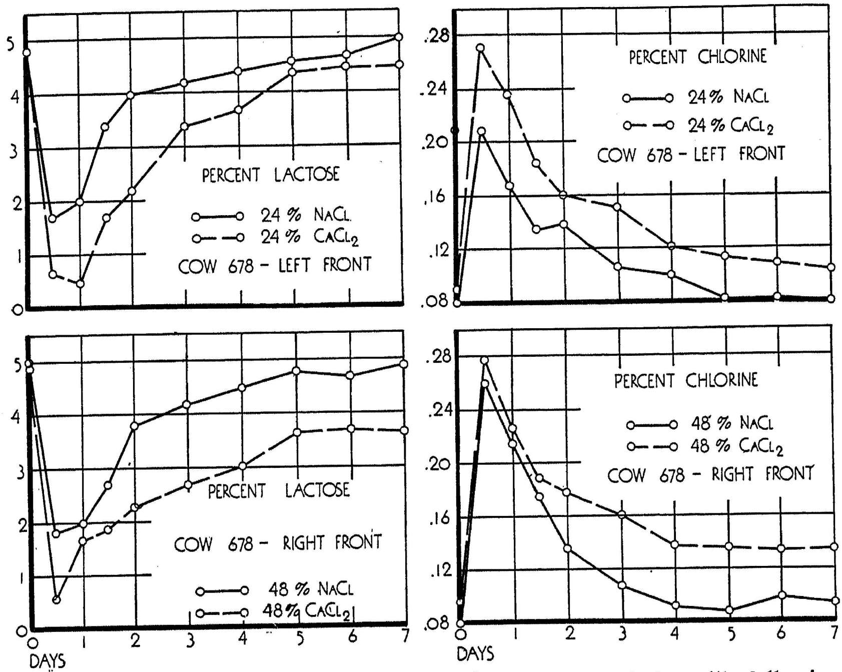

Fig. 6.—Changes in the lactose and chlorine content of the milk following injection of sodium and calcium chloride solutions. Calcium chloride caused more pronounced changes than sodium chloride solutions of the same percentage concentration.

Experiment 4.—Reports in the literature on the results obtained from injecting calcium chloride solutions into the cow's udder are unknown to the authors.

In order to learn the comparative effects of sodium and calcium chloride on milk secretion, 0.24 and 0.48 per cent solutions of these salts were injected into each of two quarters of one cow. Although a complete analysis of the milk was made, the effect of the injections on only the lactose and chlorine content of the milk later secreted is considered here (Fig. 6).

The reaction to each of the calcium chloride solutions was quite pronounced and more severe than that produced by the injection of sodium chloride solutions of similar percentage concentration.

The Injection of Lactose Solutions

A limited amount of work on the effect of injecting lactose solutions into the cow's udder has been done by previous investigators. Davidson (1926) injected 300 ml. of an isotonic lactose (8.8

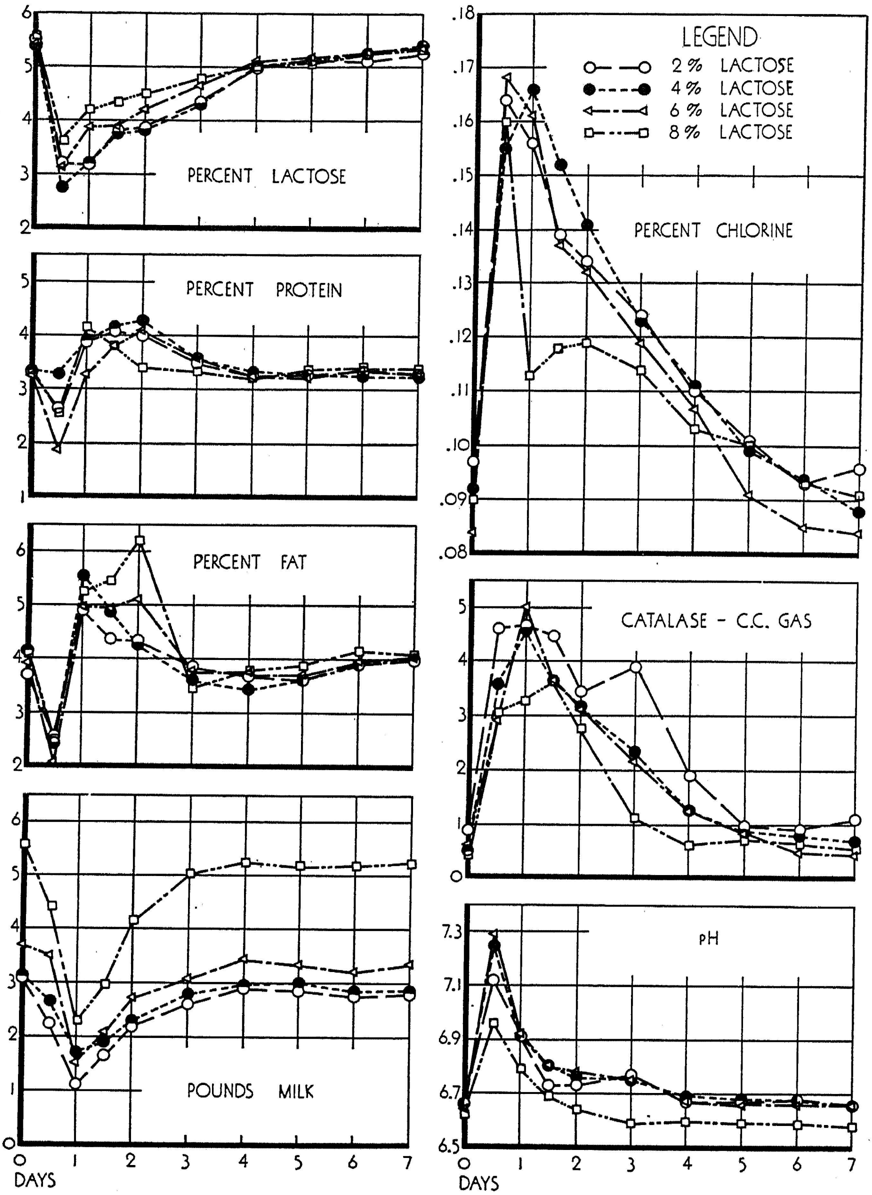

Fig. 7.—The effect of injecting solutions of increasing lactose concentration on the yield and composition of milk. The effect of the increasing lactose concentration is believed to be obscured by the differences in size and sensitivity of the individual quarters.

per cent) solution into one quarter of a cow's udder and observed essentially the same disturbances in the composition of the milk as was produced by the injection of the same volume of isotonic salt solution into another quarter of the same udder. The first milking after the lactose injection was not as low in percentage lactose as the second milking, presumably because of the large amount of this substance injected.

Petersen and Rigor (1932b) injected both isotonic and hypotonic (3/4 isotonic) solutions of lactose into the udder in amounts equal to the volume of milk removed and observed that the composition of the milk subsequently secreted was altered most by the isotonic solution.

Experiment 5.—Solutions of 2, 4, 6, and 8 per cent lactose were injected into various quarters of five cows, four quarters being treated with each concentration. The general procedure followed here was the same as that used in the first part of the study with solutions of sodium chloride and consequently the same factors must be considered in the interpretation of the results. The 8 per cent solution showed the least effect on milk secretion but no consistent relationship existed in the effects produced by the solutions of other concentrations (Fig. 7). The more favorable showing of the 8 per cent solution is probably due to the injection of two quarters of a high producing cow with this solution and not treating any quarters of this cow with any of the weaker solutions. This cow showed very little response to the injections and indicates the influence of the producing capacity of the quarter on the effects produced by the injection of a uniform volume of liquid.

All the quarters irrigated with lactose solutions were also injected with distilled water either prior or subsequent to the lactose treatment. The lactose and chlorine values for the milk secreted after injection by the quarters that received the same lactose concentration were averaged and compared to the lactose and chlorine content of the milk obtained from these quarters after being injected with distilled water (Fig. 8). In all cases each lactose concentration caused a greater decrease in lactose and a larger increase in the chlorine content of the milk for several days following the treatment than was produced by the introduction of distilled water into the corresponding quarters.

Experiment 6.—In order to obtain more complete information on the question of the relationship between lactose concentration and the disturbances to milk secretion, another series of injections

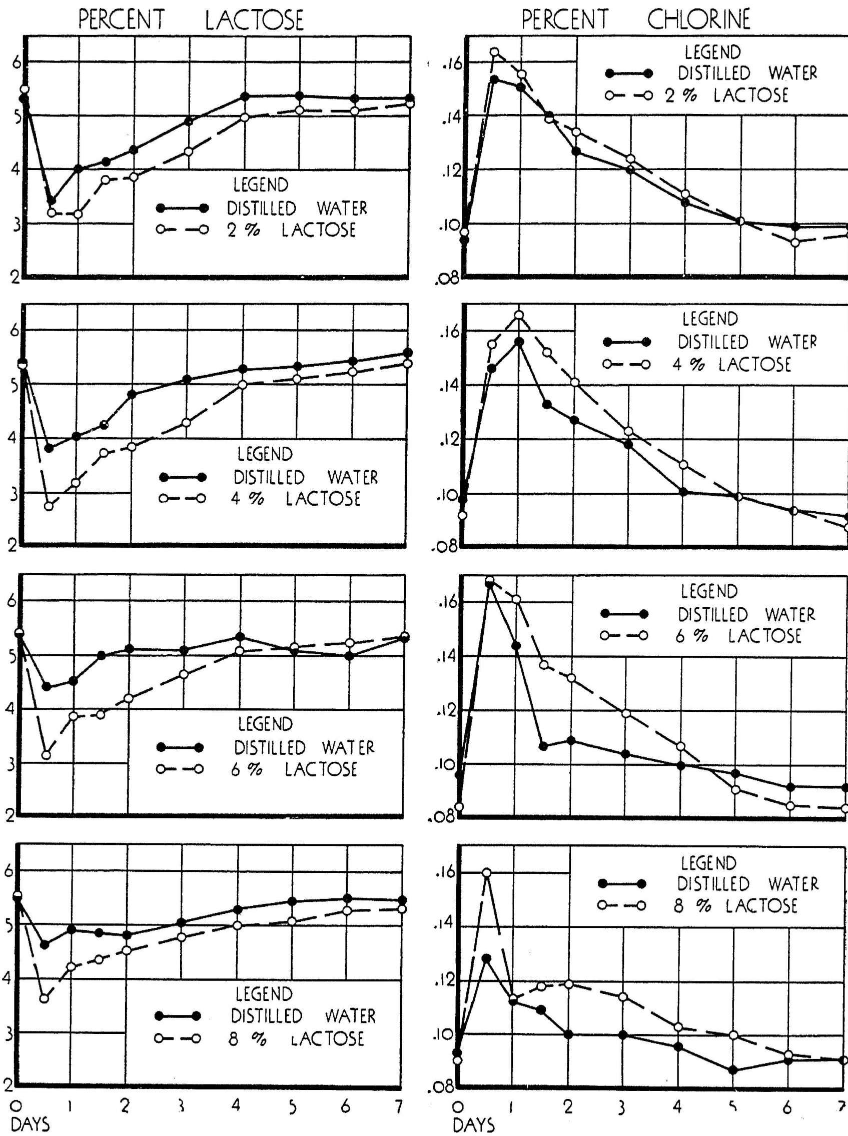

Fig. 8.—Changes in the lactose and chlorine content of the milk following the injection of lactose solutions and distilled water into the same quarters. Regardless of the lactose concentration distilled water always produced less alteration in the composition of the milk.

was made. Solutions containing 3, 6, and 9 per cent lactose were injected into two quarters of each of two cows, according to the plan outlined in Experiment 3. This method was believed to be

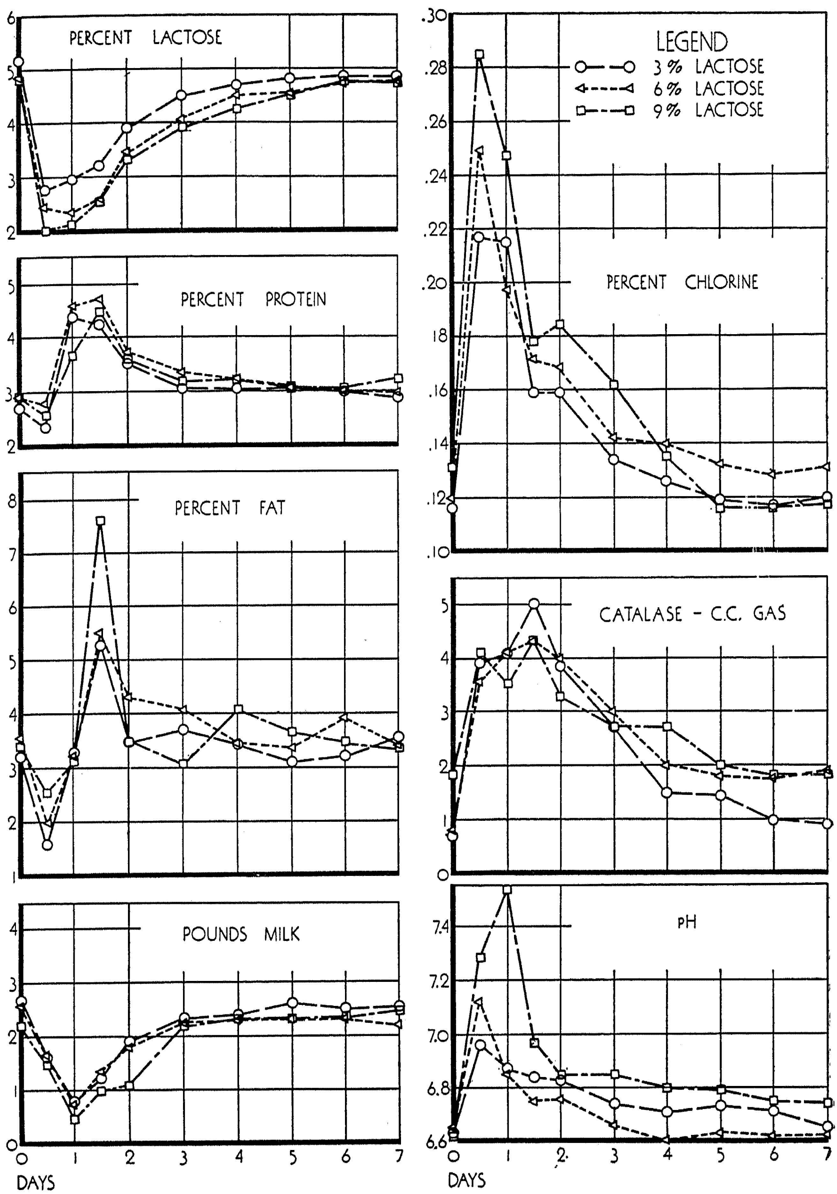

Fig. 9.—The effect of injecting solutions of increasing lactose concentration into the same quarters on yield and composition of the milk. The disturbance to milk secretion increased with the lactose concentration injected.

the most reliable way of ascertaining the relative injurious effects of these solutions.

In general the effect on yield and composition of the milk increased with the concentration of lactose injected, the lactose and chlorine changes being particularly significant (Fig. 9). The changes in the composition of the milk after lactose injections are essentially the same as those which occurred following the injection of the solution previously described.

The percentage of lactose was usually lowest at the first milking regardless of the lactose concentration injected, instead of at the second milking as found by Davidson (1926). However, a larger volume of solution was used in this study (750 ml.) than was injected by Davidson (300 ml.) which may account for this difference. The average amount of lactose recovered at the first milking after injection of the 3, 6, and 9 per cent solutions was 88.9, 40.3, and 20.0 per cent, respectively, of the lactose injected. This may indicate that a rather marked absorption of lactose from the mammary gland occurred following the injection of the more concentrated lactose solutions.

It may be concluded, as a result of these studies, that the injurious effect on milk secretion increases with the concentration of lactose in the solution injected.

The Injection of Thin Paraffin Oil

Experiment 7.—Since mineral oil is probably not appreciably absorbed from the udder, it was considered desirable to learn what effect the injection of such a substance would have on milk secretion and to compare this effect with that produced by the injection of distilled water into the same quarter. Two quarters of a cow were, therefore, injected with 750 ml. of a thin paraffin oil and a similar volume of distilled water. Although this oil was quite thin, it was introduced into the udder with more difficulty than was encountered with water, indicating that it did not readily penetrate into the small ducts of the gland. In one trial the cow became restless during the injection and a part of the oil was lost, only about 600 ml. going into the udder. This quarter showed less effects from the treatment than the quarter which received the full amount, but the results were very pronounced in both cases.

The comparative effect on the lactose and chlorine content of the milk secreted after the injection of paraffin oil and distilled water into the left rear quarter of this cow (No. 678) is shown in Fig. 10. Recovery following the oil injection was very slow and the milk did not return to normal composition until more than 15

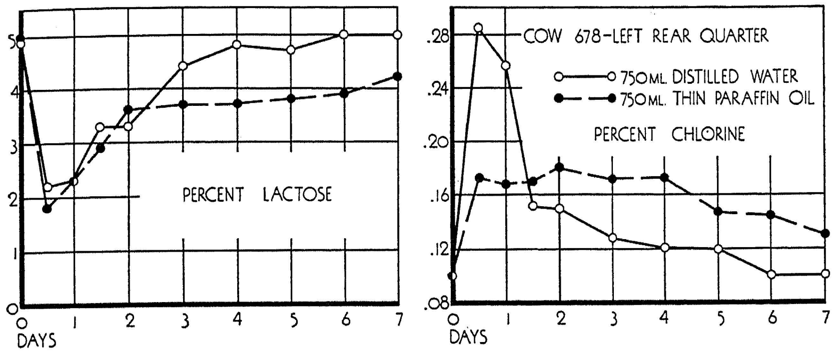

Fig. 10.—Changes in the lactose and chlorine content of milk following the injection of paraffin oil and distilled water into the same quarters. Recovery from the oil injection was very slow.

days had elapsed. The oil was present in large quantities in the milk at the first two milkings after injection and could be detected in small quantities for six or seven days. The large amount of oil present diluted the milk and thus prevented an accurate analysis of the first two milkings. The lactose analyses showed about the same effect from the injection of paraffin oil and distilled water for the first two days, but after that the lactose content of the milk increased much more rapidly when water was injected. The chlorine analyses do not show as large an increase in chlorine the first two milkings following the oil injection as occurred when distilled water was introduced, but after the first day the chlorine content of the milk was highest following the oil injection. The low chlorine figures for the first two milkings after the introduction of oil were probably due to the dilution of the milk with oil. The effect on yield and the other constituents of the milk resulting from these injections followed the characteristic trend.

The results of this study indicate that irrigation of the udder with paraffin oil causes as much disturbance to milk secretion as the introduction of distilled water and that recovery occurs much more slowly following the oil injection.

The Effect of Oxygen

Petersen and Rigor (1932) introduced air into the udder at different levels of pressure for a period of 6 hours and determined the effect on the amount of milk secreted. They demonstrated that the rate of milk secretion decreased as the pressure in the udder increased up to 25 mm. Hg., at which time milk production practically ceased. The yield and composition of the subsequent milk produced was not reported.

Experiment 8.—Since it was shown that air pressure above 25 mm. practically inhibited milk secretion, it was considered desirable to determine if such treatment would affect the subsequent yield and composition of the milk. Six quarters of two cows were subjected to a constant oxygen pressure equal to 20 mm. and 40 mm. Hg. for 12 hours. The oxygen was introduced through sterile cotton and the desired pressure maintained by means of a regulatory escape valve.

Twenty mm. Hg. pressure produced slight but noticeable changes in the composition of the milk secreted for three days after the treatment, the increase in catalase and chlorine content being the most pronounced (Fig. 11). The higher pressure (40 mm. Hg.) had a very marked effect on both yield and composition of the milk for three or four days after the treatment, the results being far more severe than those produced by the injection of 750 ml. of distilled water. In contrast to Petersen's and Rigor's observations, secretion was not entirely inhibited during the time that the pressure (40 mm. Hg.) was maintained. In a preliminary trial, the pressure was raised to 40 mm. Hg. for 15 or 20 minutes, then discontinued with the result that the composition of the milk secreted was affected for several milkings afterward.

These results appear to indicate that gas pressure, as well as hydrostatic and osmotic pressure, affects the secretion of the mammary gland.

Suspended Milking vs. the Reinjection of Milk

Jackson and Rothera (1914) returned a portion of the milk removed to the udder and observed a decrease in lactose and an increase in the soluble ash content of the milk for several subsequent milkings.

In a similar experiment Davidson (1926) returned 200 and 300 ml. of milk to two quarters of a cow's udder and secured a reduction in yield, a depression in lactose, and a marked increase in protein plus ash in the milk for about five days afterward; the fat content increased markedly during the second or third milking, then became quite variable.

A number of investigators, including Regan and Mead (1921), Fitch et al. (1922), Wylie (1923), and Davidson (1924), have observed that the subsequent yield of milk and the fat percentage increased when part of the milk was not removed from the udder.

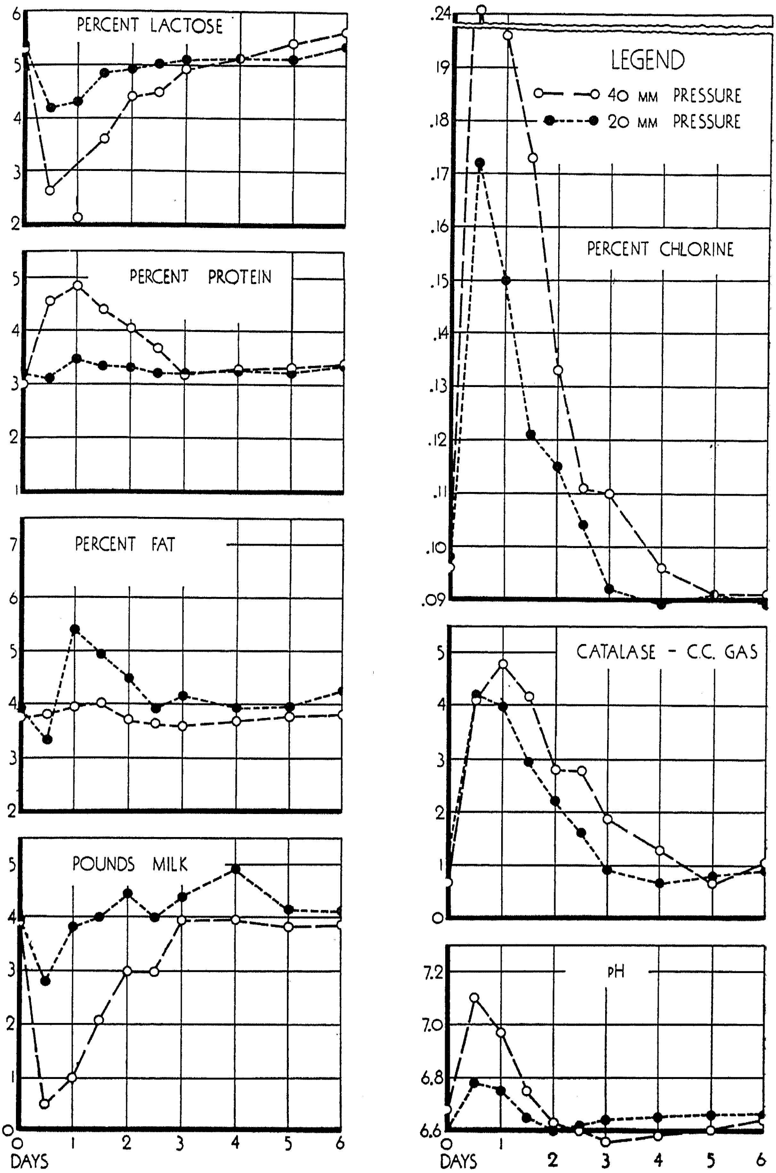

Fig. 11.—The effect of two levels of oxygen pressure on the yield and composition of milk. While the lower pressure (20 mm. Hg.) produced noticeable effects the higher pressure (40 mm. Hg.) caused very severe alterations in the yield and composition of the milk.

The degree of change in the composition of the milk was influenced by the amount of milk left in the udder and the length of time it was allowed to remain there.

Petersen and Rigor (1932a) allowed milk to remain in the udder for periods of 24, 36, and 120 hours and compared the composition of the milk then obtained with that drawn at the regular 12 hour interval. Yield increased during the 24 and 36 hour periods, then decreased after 120 hours. The protein and total solids content, and the pH of the milk increased, while the lactose content decreased, with the length of the period the milk was retained. The ash content increased to 36 hours, then fell slightly at 120 hours, while fat was not affected until the 120 hour period, when it decreased.

Roadhouse and Henderson (1932) milked two cows at different intervals and found no effect on the flavor or the chlorine and lactose content of the milk when the intervals between milkings were varied from 12 to 10 and 14 hours.

Experiment 9.—Since milk is normally in contact with the mammary gland it would not be expected to be injurious. It was considered interesting, therefore, to learn the comparable effects on milk secretion produced by the injection of milk and distilled water and of suspending one regular milking. A study was carried out in which all the milk produced (600 to 900 ml.) by each of seven quarters of five cows was returned immediately after milking and the results secured compared to the effects produced by the injection of 750 ml. of distilled water into the same quarters (Fig. 12).

Returning milk to the udder caused the usual changes in milk secretion which occur following the injection of other solutions. The average fat and protein content did not reach a maximum, however, until the third milking after the milk was returned instead of at the second milking. The general effect of returning milk to the udder was very pronounced and much more severe than was produced by the injection of approximately the same volume of water into the same quarters. These results, while rather surprising, are in keeping with what might be expected when considering the changes produced by the injection of lactose and salt solutions.

The effect of omitting a regular 12-hour milking was studied, using eight quarters of four cows. Some of the quarters were stimulated to let down their milk at the time of the regular milk-

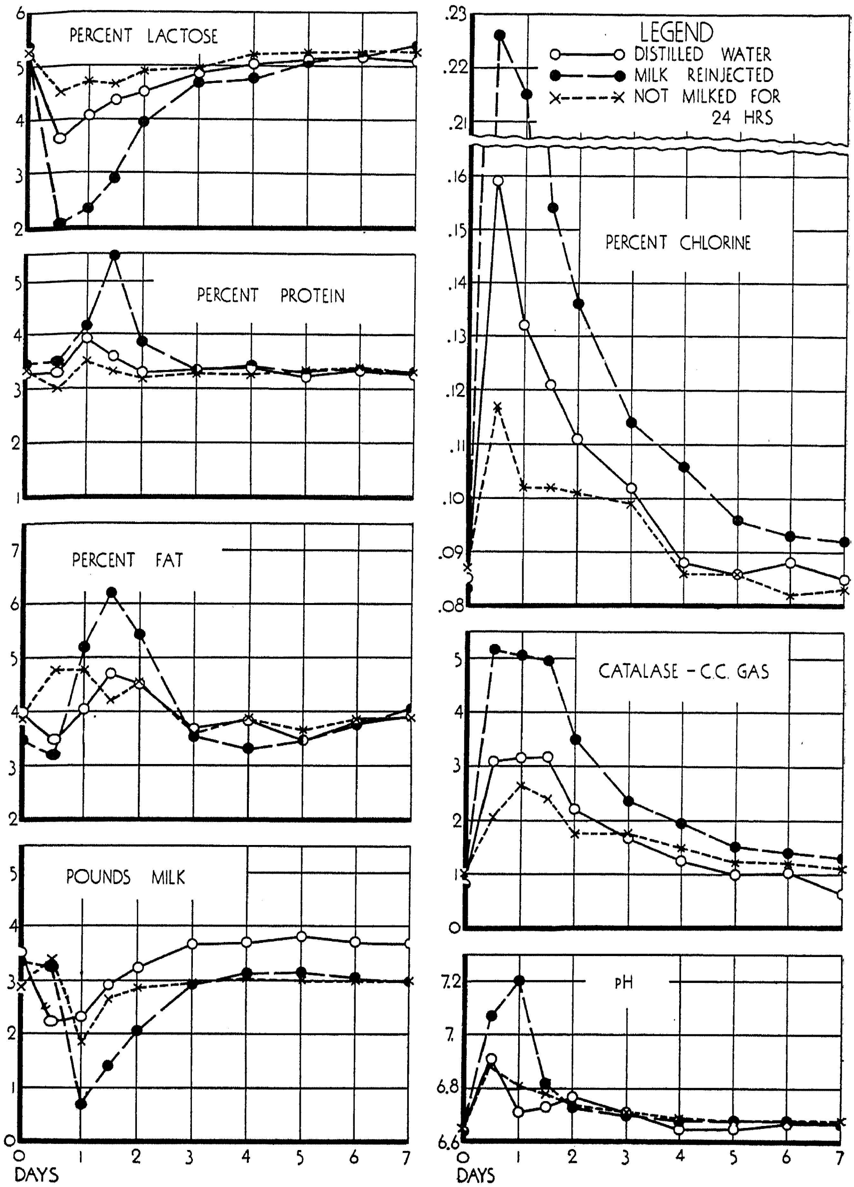

Fig. 12.—Changes in yield and composition of milk following suspended milking and the injection of milk and water into the udder. Suspended milking had only a slight effect while the return of milk to the udder caused very pronounced changes.

ing, while on one occasion the cow was not fed or approached in any way by the milker in order not to encourage the letting down of the milk in the udder. The average results secured are also presented in Fig. 12.

The individual cows varied in the response made to a suspended milking, the milk from some cows being affected only slightly by the practice, while in others the results were more pronounced. The production level of the various cows may have been a factor in this respect as the higher producing cows seemed to be affected more by this practice than others more advanced in lactation and low in production. Stimulation of the udder at the regular milking time when milking was omitted had no apparent effect on the results secured.

Suspended milking for 24 hours had a noticeable effect on yield and composition of the milk for three or four days afterward, but did not produce nearly as much disturbance as the injection of distilled water or the return of milk to the udder. The higher chlorine and slightly lower lactose content of the milk for three days after a suspended milking is interesting from the standpoint of the effect that this might have on the flavor of milk. In keeping with the results secured by others, the fat content of the milk was noticeably raised for four milkings after a suspended milking, but the yield, except for the first milking, was reduced during this period instead of increased as they observed.

Increased Interval Between Milkings

In a previous experiment at this Station, reported by Ragsdale, Turner and Brody (1924) four cows were milked at regular intervals night and morning, followed by a single experimental milking at one hour, two hours, three hours, etc. . . . . . up to 36 hours after the last regular milking. After each such experimental period, the cows were again milked at regular intervals night and morning until they returned to normal. It was observed that as the interval between milkings increased, there was a gradual decline in the rate of milk secretion. The fat percentage showed a marked decline with the increasing interval up to about the 16th hour. As the experiment required considerable time, the decline in milk yield with the advance of the lactation period was considerable.

Experiment 10.—In extending the study concerned with the influence of the interval between milkings on the yield and composition of milk, it seemed desirable to reduce the length of the experiment so that the decline in yield with the advance of the stage of lactation would be reduced to a minimum. Further, more complete analysis of the milk was made. Two Holstein cows, after a preliminary control period, were milked one hour after the normal period, then two hours, three hours, ... up to 24 hours after the previous milking without a normal milking period (12 hours) intervening, as indicated in the following table.

Day	Time of Milking	Hours Since Last Milking
	6 a. m.	Normal period
	7 a. m.	1
	9 a. m.	2
1	12 noon	3
	4 p. m.	4
	9 p. m.	5
	3 a. m.	6
2	10 a. m.	7
	6 p. m.	8
	3 a. m.	9
3	1 p. m.	10
	12 night	11
4	12 noon	12
.	.	.
§	§	§
§	§	§
13	6 p. m.	24

The rise in the yield of milk with increasing time intervals was observed to follow almost a straight line for a period of 12 hours and then the rate of increase fell off rather sharply (Fig. 13). A reexamination of the data presented by Ragsdale, Turner and Brody showed a similar trend. These results appear to indicate that during the normal interval between milkings (i. e., an interval that the cow has become accustomed to and whose production has reached a degree of equilibrium) the rate of milk secretion is rather uniform. Only as the storage system reaches near capacity is the rate of secretion reduced by the increasing pressure.

By this method of milking, the downward trend in fat percentage for the first 16 hours previously observed was not obtained (Fig. 14). This difference may be explained as follows: The residual milk left in the udder when normal milking is completed is high in fat. This milk is diluted as additional milk is secreted. After short milking intervals, the milk obtained would be rich in fat but as the interval increased the residual milk would be diluted more and more. This would account for the gradual decline in

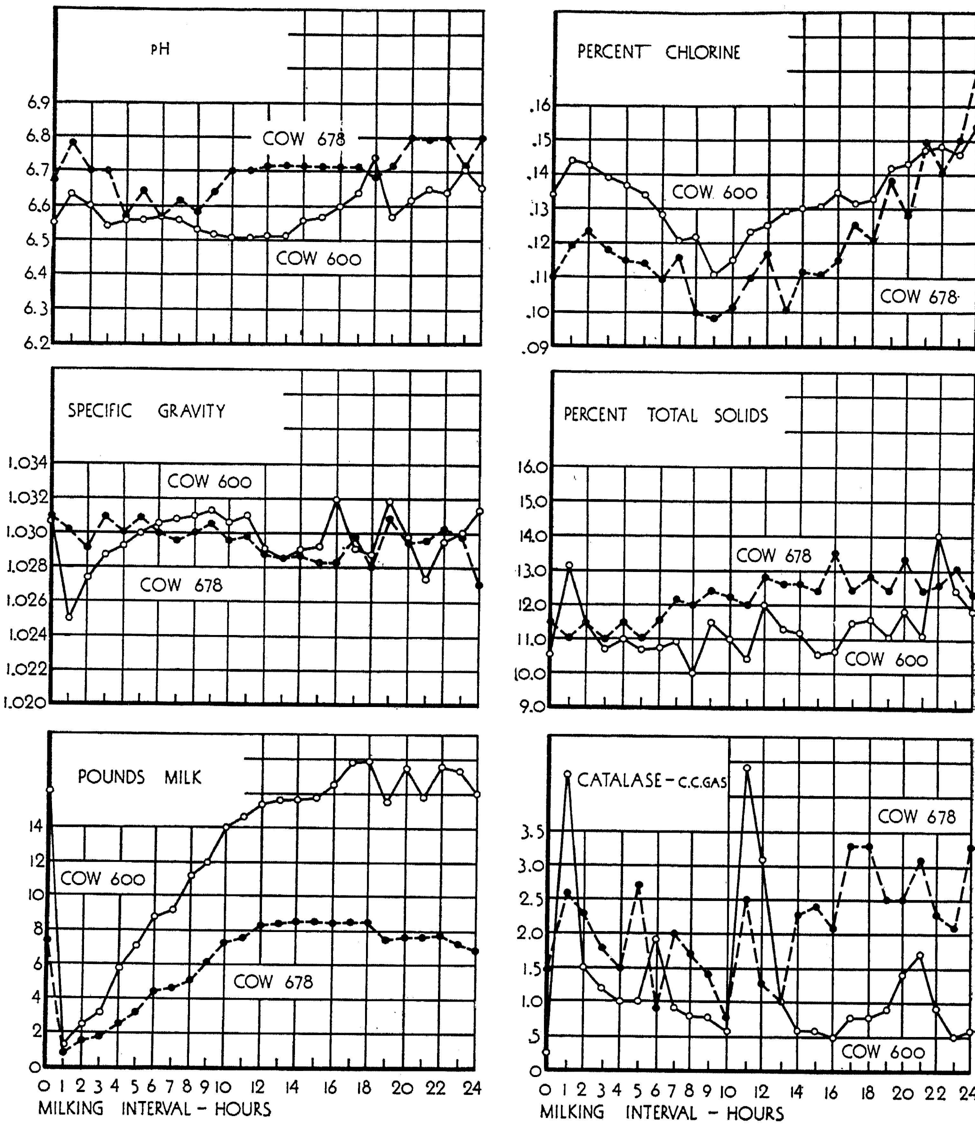

Fig. 13.—The effect of increasing intervals between milkings on yield and composition of of the milk. While the yield was seriously depressed after twelve hour intervals, alterations in the composition of the milk only occurred after intervals of eighteen hours or more.

the fat percentage with increasing interval between milkings noted in the previous experiment as each experimental period followed a normal 12 hour period. In the present experiment, the milking periods followed without normal 12 hour periods intervening. As

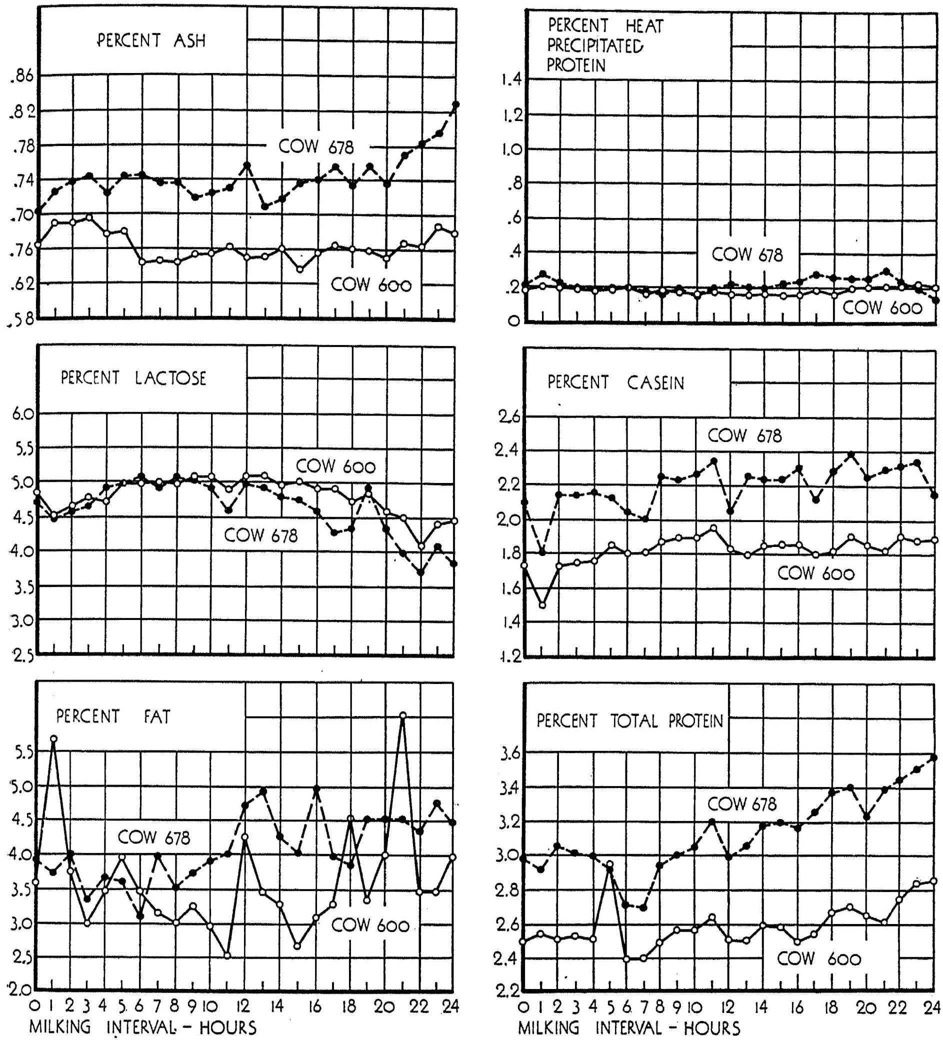

Fig. 14.—The effect of increasing intervals between milkings on the composition of the milk. Continuation of Fig. 13.

a result the milk obtained resembled more nearly in composition the milk actually secreted during the period

Although milk secretion was practically inhibited after an interval of about 16 hours, there were no significant changes in the composition of the milk secreted until after an interval of 18 or 20 hours. After 18 hours there was a tendency for the lactose to decrease slightly and the chlorine to increase correspondingly. In Cow No. 678 the percentage ash and the total protein also showed a slight gradual rise with the greater intervals.

These data indicate that the rate of milk secretion of the cows used was depressed only after a period of 12 hours. The composition of the milk was not seriously affected until after an interval of about 18 hours, at which time chlorine, lactose, ash and total protein tended to show some slight alterations.

The Effect of Udder Irrigation on the Leucocyte Content of Milk

Sjollema and Van der Zande (1922) observed, in addition to other changes, an increase in the leucocyte content of the milk secreted by one quarter of a healthy udder following the injection of a 0.2 per cent solution of silver nitrate. They concluded that aseptic and bacteriological inflammations have the same general influence on the chemical composition of the milk secreted.

In order to gain further information on the effect of udder irrigation on the number of leucocytes in milk the leucocyte content of the milk of several cows before and after injection was determined by the direct microscopic method. A large increase in the number of body cells occurred, following injection, in the milk of all cows examined and remained above normal for about five or six days. The results secured for different methods of treatment for one cow (No. 578) are shown in the following table.

COW NO. 578—LEUCOCYTES PER ML. OF MILK

Days after treatment	Quarter				
	Left Front	Left Rear	Right Front	Right Front	Right Rear
	750 ml. Salt-lactose solution	Not milked for 24 hours	750 ml. distilled water	Oxygen (40 mm. Hg. pressure)	Milk returned
Before treatment	163,000	76,000	258,000	415,000	92,000
1st day a. m.	73,000,000	127,000	25,560,000	1,400,000	12,900,000
1st day p. m.	60,800,000	150,000	14,190,000	30,650,000	3,510,000
2nd day a. m.	36,800,000	152,000	7,120,000	36,400,000	2,900,000
2nd day p. m.	----------	90,000	----------	----------	2,850,000
3rd day	8,330,000	-------	2,153,000	24,700,000	1,920,000
4th day	2,700,000	57,000	847,000	5,760,000	678,000
5th day	918,000	49,000	233,000	2,280,000	215,000
6th day	530,000	53,000	247,000	650,000	134,000

Correlation of Milk Constituents Under Experimental Conditions

Many attempts are being made to secure knowledge of the complicated physico-chemical processes involved in milk secretion. The relation of the various constituents to one another may indicate something of the process under normal physiological conditions. Correlation studies have shown that there is a very definite relation between the secretion of fat and protein. Similarly, variations in lactose influence the secretion of the ionizable mineral constituents (Tocher, 1925; Gaines, 1925; Overman, Sanmann and Wright, 1929; and Black and Voris, 1934).

Under the experimental conditions described in the present work, the rate of secretion of the various constituents of milk was altered considerably. It is of great interest to determine whether there are alterations in the proportions of the several constituents of milk when the normal process is affected. Correlation studies have, therefore, been made between the more important constituents of the milk analyzed. As the first milking contained the injected fluid, it was rejected from the study. The results are shown in the following table.

CORRELATION OF MILK CONSTITUENTS

Constituents	Experimental Conditions		Black and Voris (1934) Normal Holstein milk (134 samples)
	Number of Samples	Coefficient of Correlation (r)	Coefficient of Correlation (r)
Lactose-chlorine	1543	−.854±.0046	−.7030±.0295
Lactose-protein	1577	−.536±.0121	−.1134±.0575
Lactose-fat	1554	−.180±.0165	+.1264±.0573
Lactose-pH	1485	−.625±.0107	--------------
Lactose-catalase	1528	−.747±.0076	--------------
Chlorine-protein	1615	+.626±.0102	+.2197±.0555
Chlorine-fat	1597	+.055±.0168	−.1194±.0574
Chlorine-pH	1613	+.691±.0088	--------------
Chlorine-catalase	1681	+.712±.0081	--------------
Protein-fat	1573	+.314±.0153	+.7000±.0297
Catalase-pH	1596	+.602±.0107	--------------

As would be expected, a high negative correlation between lactose and chlorine is observed in both normal and abnormal milks. The increased negative correlation between lactose and protein in milk produced under experimental conditions might be explained on the basis of the increased permeability of the gland cells to plasma protein. The low negative correlation of lactose and fat in this study is interpreted as indicating the independence of the secretion of these two constituents. The high positive correlation between fat and protein (probably casein) in normal milk and the lowered correlation in the experimentally produced milk is believed to indicate that the mechanism regulating the secretion of these two constituents does not hold for the passage of the plasma proteins into the milk. The high negative correlation between lactose and catalase and pH and the high positive correlation between chlorine and catalase and pH will be noted.

If it is assumed that a lowered lactose content is an indication of abnormal milk, then the degree of correlation with chlorine, catalase, pH and protein should indicate the relative value of tests for these constituents in the detection of abnormal milk.

DISCUSSION

In considering the meaning of the series of experiments just described, it is important to have in mind the anatomical basis of milk secretion—the alveolus made up of an aggregate of epithelial cells. Under normal conditions, the cells take the precursors of milk from the blood plasma and synthesize the various milk constituents which are then discharged into the lumen of the alveolus.

If various fluids are injected into the udder and pass up the duct system into the lumini of the alveoli, how are the changes in the subsequent yield and composition of milk to be explained?

At the outset, it appears clear that the injection of gas or liquid into the mammary gland does affect the epithelial cells in some manner so that several days elapse before the secretion of normal milk is resumed. That this damage does not lead to an extensive destruction of the secreting cells may be indicated by the fact that the rate and composition of milk secreted soon returns to normal.

It would appear more logical to assume that the presence of a gas or a solution in the lumen of the alveolus interferes with the normal secretion and discharge of the formed milk and that the interference with and depression of milk secretion causes the alteration in the composition.

How, it may be asked, does the presence of a gas or a liquid interfere with milk secretion and why do the various solutions affect the gland as they do? As a tentative theory, it is suggested that the reduction in the rate of milk secretion and the subsequent alteration in the composition of the milk is due to the impaired activity of the mammary gland resulting from the pressure of the gas or liquid on the secreting cells. Further, that as the pressure is increased (as a result of injecting larger amounts of liquid or gas), the effect on milk secretion will be more extreme. Similarly, the duration of the pressure will affect the extent of the alterations produced.

It is believed that the injection of distilled water causes a minimum disturbance to the mammary gland due to the rapid resorption of water by the gland epithelium and its discharge into the blood stream. With the resorption of water, the pressure is rapidly reduced and milk secretion soon returns to normal. When a salt or sugar solution is injected into the gland, the passage of water into the blood stream is interfered with due to osmotic equilibrium. As a consequence, the pressure is maintained for a longer period and the effect upon lactation is accentuated. Hypertonic solutions tend to draw water from the blood, increasing the pressure and prolonging the inhibiting action on milk secretion until equilibrium is attained by the passage of salt into the blood. In other words, it is believed that the magnitude of the effect on the secretory tissue depends first on the original pressure and second on the time required to relieve this pressure by osmotic or other activity.

The injection of paraffin oil, which is probably not appreciably resorbed from the gland prolongs the effect of pressure and causes a long continued effect upon milk secretion. In addition it is quite likely that the paraffin interferes with the discharge of milk from the cells due to the consistency of the oil.

The effect of the introduction of oxygen at a constant pressure also falls in line with the theory advanced. The higher pressure inhibited the secretion of milk quite effectively and altered the subsequent composition.

The results obtained by the reinjection of milk might be thought difficult to explain. When it is considered that milk contains not only considerable lactose and salt (which are isotonic with the blood) but protein as well, which is not really resorbed, the pressure produced by the injected milk would be long continued. There is considerable difference between reinjected milk and milk allowed to accumulate in the gland. In the latter case a portion of the milk present is located within the epithelial cells and is released from the cells during the milking process as the pressure within the gland cistern is reduced. The same milk when reinjected is forced into the ducts and lumini of the alveoli and against the epithelial cells in a collapsed condition and the further secretion of milk is retarded in proportion to the pressure exerted. When milk is allowed to accumulate in the gland the alterations produced are very slight until a number of hours have elapsed and considerable pressure developed.

The characteristic alteration in the composition of milk following the injection of various fluids and gas throws light on the mode of secretion of the various constituents of milk and the interrelations between them. Under the experimental conditions described there was a reduction in milk yield with an increase in catalase and pH and the percentage of fat, protein and chlorine. Lactose was alone seriously depressed. Why do these changes occur?

Available evidence indicates that the changes in the composition of milk noted above usually follow a depression in the yield of milk. The causes of the depression of milk yield may be placed in several categories. First there is the normal decline in lactation due to a decrease in the lactogenic hormone; second, pressure in the udder; third, infection or inflammation of the udder; fourth, depression of the precursors of the milk constituents in the blood; and fifth, nervous disturbance such as pain and fright. While this investigation was concerned with the second factor (pressure) the explanation of the results requires an understanding of certain physico-chemical aspects of milk secretion.

The studies of Dreser (1892), Winter 1895-96), Mundula (1909-10), Stoecklin (1911), van der Laan (1915-16) and others, have shown that blood and milk under all conditions maintain osmotic equilibrium (usually shown by the depression of the freezing point). Even in diseases affecting the freezing point of the blood, or in diseases affecting the udder, no alteration in the osmotic equilibrium of the blood and milk was observed.

Jackson and Rothera (1914) pointed out that the substances which are chiefly responsible for the osmotic pressure of milk are lactose and soluble salts. To maintain osmotic equilibrium, alterations in lactose would require changes in the salts or alterations in the salts would require adjustment of the lactose content of milk. On the basis of all available evidence, it is suggested that at least two conditions are involved in the relation between lactose and salts.

When the milk yield is depressed because of the partial or complete withdrawal of food (Overman and Wright, 1927, and Gowen and Tobey, 1931) or upon the reduction of the precursors of lactose, the glucose of the blood by experimental means such as the use of insulin or phlorizin (Gowen and Tobey, 1931, and Petersen and Brown, 1934) the marked reduction of lactose in the milk secreted may be logically assumed to be due to the lack of the precursor. The normal osmotic equilibrium of the blood and milk in such cases is maintained by the passage of larger amounts of salts from the blood into the milk. In other words, *the low lactose is the cause* and the high chloride is simply the expression of the physicochemical mechanism of the secreting cell for regulating osmotic equilibrium.

The low lactose content of colostrum, and milk near the end of the lactation period appears to be due to a different mechanism. As milk secretion gradually declines, certain cells cease secreting entirely and in others the rate of milk formation is reduced. Under these conditions the milk produced tends to approach more nearly the composition of blood. It would appear that instead of the cells synthesizing the precursors of milk, as in the active cell, the normal selective absorption of the semi-permeable cell membrane is reduced, permitting the passage of certain constituents of blood to pass into the milk unchanged.

Thus, such milk is characterized by increased amounts of the enzyme, catalase, which is normally high in blood but very low in milk. The pH of milk increases approaching that of blood. Serum globulin and possibly albumin (Peskett and Folly, 1933) pass unchanged into milk in increasing amount under the same conditions. Finally, the chloride content of the milk shows a marked rise (Sharp and Struble, 1935).

These observations suggest that *the increased permeability of the cells to chlorides is the cause* and the low lactose secretion the result of the cells effort to maintain osmotic equilibrium. Of course, it might be argued that the cells in the process of "drying up" no longer secrete lactose normally, and the increase in chloride simply takes care of the deficiency of lactose.

The alterations in the composition of milk from quarters of the udder affected with mastitis are in general similar to those described above. The affected cells either partly or entirely cease to secrete normal milk and apparently permit the passage of certain components of the blood to pass through the cells into the milk. The increased flow of chlorides thus depresses the secretion of lactose.

Coming finally to the experimental observations reported in this bulletin, the writers are lead to the opinion that the alterations in the composition of the milk following the injection of various solutions and gas into the quarters are due primarily to the impaired activity resulting from the pressure exerted upon the secreting cells of the alveoli depressing milk yield and increasing the permeability of the cells so affected to certain constituents of the blood, including the chlorides. As a consequence, there is an increase in pH, and in the content of catalase, chlorine and protein, while lactose is depressed. Fat secretion is more or less independent of the other constituents of milk, being inversely related to milk yield.

The practical application of these data in connection with the treatment of mastitis is rather obvious. As a carrier of antiseptic dyes, it would appear that distilled water is superior to all other solutions which have been investigated. It causes the minimum change in both yield and composition of milk when injected in amounts sufficient to properly irrigate the cisterns, ducts, and lumina of the entire gland.

SUMMARY AND CONCLUSIONS

The effect on milk secretion of the interval between milkings and the injection into the udder of water, solutions of salt and lactose, milk, oil and oxygen has been studied.

The changes in milk secretion observed following irrigation of the udder were a decrease in milk yield, a decrease in lactose content, and an increase in chlorine, fat, protein, catalase, leucocytes, and pH of the milk for several subsequent milkings. The maximum effect occurred at the first or second milking following injection, after which there was a slow return to normal which was not reached for certain constituents until about the fifth or sixth day. The degree of change produced varied with the individual cows and with the amount and kind of substance injected.

The injection of 750 ml. of distilled water into 25 quarters of seven lactating cows had less effect on milk secretion than the injection of a similar volume of a solution containing 0.12 per cent sodium chloride and 3.5 per cent lactose into the same quarters.

The injection of uniform volumes (750 ml.) of distilled water and sodium chloride solutions of increasing concentration from 0.12 to 0.96 per cent showed that distilled water was less detrimental to milk secretion than any of the chloride solutions studied and that the injurious effects of such solutions increase with the concentration of the solute.

Calcium chloride solutions of 0.24 and 0.48 per cent concentration were more detrimental to milk secretion than injections of sodium chloride solutions of similar percentage concentration into the same quarters.

Comparisons of various concentrations of lactose solution (2 to 9 per cent) with distilled water again showed that distilled water was less injurious than lactose and that the injurious effects on milk secretion increased with the concentration of lactose in the solution injected.

The irrigation of the udder with paraffin oil caused as much disturbance to milk secretion as the introduction of distilled water, but recovery occurred much more slowly following the oil injection.

The introduction of oxygen into the udder at pressures equal to 20 mm. and 40 mm. Hg. for 12 hours affected the yield and composition of milk similar to the injection of liquids, thus indicating that gas pressure as well as hydrostatic and osmotic pressure affects the secretion of the mammary gland.

Suspended milking for 24 hours had a noticeable effect on the yield and composition of milk for three or four days afterward but did not produce nearly as much disturbance as the injection of distilled water or the return of milk to the udder.

Milk secretion was practically inhibited when the milking interval exceeded 16 hours and the composition of the milk became slightly altered when the period between milkings exceeded 18 or 20 hours.

The changes in the composition of milk secreted following irrigation or accumulation of milk in the udder is believed to be due to the impaired activity resulting from the pressure exerted on the gland epithelium which increases the permeability of the secreting cells to certain constituents of the blood, including the chlorides; osmotic pressure between blood and milk is then maintained by a depression in lactose synthesis.

It is concluded that distilled water is preferable to any of the solutions studied as an irrigant for the mammary gland.

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