THE RELATION BETWEEN THE CALCIUM AND PHOSPHORUS CONTENT AND THE CURD TENSION OF MILK

THESIS FOR THE DEGREE OF M. S.

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Milk

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by

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THESIS

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INTRODUCTION

Within the past several years considerable interest has been aroused among research workers, nutrition students, and the dairy industry by observations made from time to time in research reports on the physical character of milk curds formed subsequently to the action of enzymes. This physical character, or curd tension, as it is commonly called, is subject to wide variation from soft, bodyless, to tough, rubbery consistancies, depending on various physical and chemical phases in the constitution of milk.

Application of the existence of hard and soft curds has been made in the manufacture of cheese, in which a hard curd is advantageous; in infant nutrition, for which a soft curd is required for greater ease of digestion, and to facilitate maximal nutritional advantage; and in the treatment of gastric ulcers, for which the easily digested soft curd is obviously to be preferred.

Many of the curd tension regulating influences have been partially explained or indicated, but the natural complexity of the physics and chemistry of milk invests the studies first, with a difficulty of approach; and secondly, with an ambiguity of results.

It is with the hope of making some contribution toward a clearer understanding of the influences regulating curd tension that this work was undertaken.

HISTORICAL

In 1916 Alleman and Schmidt (1) published a report of their investigations of some of the factors influencing the coagulating properties of cows' milk. The report deals with some of the most fundamental aspects of the phenomenon of coagulation, every one of which must be considered in a discussion of the subject.

For the purpose of measuring the hardness of rennin curds these workers invented a method which was based on the grams of pull measured with a spring scale required to draw through the curd a tool consisting of concentric rings on a horizontal plane attached to the end of a centrally placed, perpendicular rod. The principles involved seem to have been adopted for all succeeding methods of measuring curd tension.

INFLUENCE OF TIME AFTER COAGULATION IS COMPLETE: Alleman and Schmidt (1) found that the curd tension increased in direct proportion to the time allowed for the rennin to act until a maximum of hardness was approached. The period of time elapsing between the moment of coagulation and the measurement of curd tension has been frequently referred to in their report as the "Wartzeit". The longest "Wartzeits"

presented were twelve minutes only. Between the eleventh and twelfth minutes curd tension was increasing at rates of from one to three grams per minute. From their data the authors demonstrated that a constant may be found for any particular sample of milk by dividing the curd tension by the "Wartzeit" in minutes. This constant when calculated for one milk is invalid for other samples of milk with a different degree of reaction to coagulation.

DEPENDENCE OF CURD TENSION ON THE AMOUNT OF RENNIN ADDED: Curd tension and speed of coagulation increased in direct proportion to the amount of rennin added, according to the work of Alleman and Schmidt.

When a "Wartzeit" equal to one tenth (or any given fraction) of the time required for coagulation was established, it was found that for any given sample of milk a constant curd tension developed, regardless of the amount of rennin used.

INFLUENCE OF ACIDITY ON COAGULATION: By coagulating the milk with a constant amount of rennin and adding equal volumes of varying strengths of acetic acid, Alleman and Schmidt demonstrated that increased acidity favored a harder curd.

In this case as before, the curd tension was in direct proportion to the speed of coagulation. The conclusion was drawn that the acid itself has no specific effects, but only creates conditions which favor coagulation. This conclusion was more or less supported by the more recent work of Rona and Gabbe (39) which indicated that the action of rennin in formation of paracasein is most efficient at pHs from 6.0 to 6.4; and the principle, demonstrated by Palmer (34) and Bell (2), that under conditions of increased acidity calcium is made more available for the precipitation of calcium paracaseinate.

INFLUENCE OF CALCIUM SALTS ON COAGULATION: Curd tension increases proportionally to the amount of calcium chloride added. The mathematical relationship found by Alleman and Schmidt (1) between added calcium chloride and curd tension indicates that the salt, like acid, created conditions favorable for coagulation. The authors stated that the calcium had no specific effect, but in view of the function of free calcium recognized by Palmer and Richardson (33) and Hammarsten (17) as the positive radical of an insoluble paracaseinate, the element seems to assume a more significant position.

Addition of calcium hydroxide has a softening influence on the curd according to Bosworth and Bowditch (4).

In connection with the natural calcium content of milk, it has been stated by Weisberg, McCollum and Johnson (50) that soft curd milk contains less of the element than hard curd milk. Espe and Dye (16) found that removal of a large part of the colloidal $CaHPO_4$ by centrifuging has no effects on the curd tension. The results of Hill (20) verified this view, showing that either removal or remixing of cream separator slime, which according to Espe and Dye (16), contains a large part of the colloidal calcium has no effect on curd tension. On the contrary, Weisberg, McCollum, and Johnson (50) have made the statement that the suspensoid phase of milk, including calcium phosphates, through its concentration and mode of distribution controls the curd character.

INFLUENCE OF AMMONIA ON COAGULATION: Alleman and Schmidt (1) have shown that the addition of ammonia slows up coagulation and brings about a decrease in curd tension. This effect is, in some degree, only in opposition to that of addition of acid, but it varies from the latter effect in that the decrease is not in strict proportion to the amount of ammonia added. The curd tension falls at a rate faster than the increase of ammonia additions, which probably indicates a decomposition of the casein, rennin, or the casein-rennin combination which may exist. In this connection it is also noteworthy to recall the demonstration by Van Slyke and Bosworth (43) and Porcher and Brigando (36) that ammonium or sodium radicals may tend to replace the calcium in the caseinate or paracaseinate, and that Rimmington and Kay(37) and Bosworth (6) have shown that weak alkalie tends to remove phosphorus. Hill (20) has recorded that the addition of sodium or potassium in a form not stated decreases curd tension.

INFLUENCE OF TEMPERATURE ON COAGULATION: Alleman and Schmidt (1) confirmed the well known fact that coagulation occurs best at about 40 degrees C. Above or below this point the process becomes slower as the temperature increases or decreases. The change in speed of coagulation is accompanied by a corresponding change of curd tension, but not in the same proportion; for the increase in speed of coagulation decreases per degree as the temperature mounts toward 40 degrees, while the curd tension increases in direct proportion to the temperature.

INFLUENCE OF PHYSICAL TREATMENT OF MILK ON COAGULATION: Muller (32) found that vigorous agitation of milk reduces its capability for coagulating. The accuracy of this earlier observation was later born out by the work of Alleman and Schmidt (1).

Hill (18), (19) and Espe and Dye (16) found that pasteurizing or boiling milk has a softening effect on the subsequently formed curd. Weisberg, McCollum, and Johnson (50) suggested that this effect is due to the precipitation by heat of colloidal $CaHPO_4$, but Espe and Dye (16) have refuted this view on the basis of evidence obtained by the removal of some of the colloidal calcium salts by centrifuging. The curd tension was not altered by this treatment. The same result was obtained by Hill (20) by removal of separator slime which contains a high proportion of the colloidal calcium. Rupp (41) and Bell(2) have shown that heating at pasteurizing temperatures has little or no effect on the amount of soluble calcium in milk filtrates, but Magee and Harvey (27) and Mattick and Hallett (28) have presented evidence to the contrary. Working with colloidal solutions of $CaHPO_4$ stabilized with gelatin, Palmer (34) found it very easily precipitated by heat. To show the effect of calcium in the two mentioned physical states, Palmer proceeded to dialyzed milk until it must have been practically free of all soluble calcium, then tested the effect of rennin on the dialyzed product. Coagulation absolutely failed until a small amount of $CaCl_2$ was added (one drop of 4 molar) upon which the curd formed instantly. Addition of two or three drops of dilute hydrochloric acid also permitted the curd to form. Palmer reached the conclusion that the effect of heat on the curding properties of milk was due to a denaturation or decomposition of the casein, or to a disturbed "conditions which govern what is regarded as normal clotting of calcium paracaseinate".

Although direct evidence in the matter is sparse, the opinion appears to be prevalent that heat causes a definite alteration of the casein molecule. Lacqueur and Sackur (23) showed that casein dried at 94 to 100 degrees C. underwent a cleavage yielding an alkalie soluble fraction that possessed more acid properties than casein, and showed a higher base binding power. Zoller (53) found that pasteurizing caused milk to yield a much softer curd by acid precipitation, and that the curd so formed contained more moisture as the temperature of preheating was increased. The temperatures used were from 50 to 120 degrees C. inclusive. In another study by this worker (52) solutions of casein and sodium hydroxide were heated to 118-135 degrees in sealed tubes. In solutions of pH below 6.5 the caseinates failed to precipitate though held at 135 degrees for forty minutes. In solutions of pH above 6.5 precipitation occurred. During the heating period the pH dropped .18 to .54, the drop being more pronounced in the more alkaline solutions. The coagulum was soluble in acids and alkalies and resembled curd made from heated or sterilized milk. Michaelis and Marui (30) demonstrated that the higher the temperature to which pure casein in alkaline solution was raised the slower was the subsequent process of coagulation by rennin and calcium chloride. The assertion was made in their report that the effect is on the casein itself rather than on the reaction between the calcium and the casein. Kumatsu and Okinaka (22) heated casein with water in stoppered bottles to 110-120 degreesC. and obtained a product, the weight of which, when freed from water, was greater than that of the original sample, evidence which was interpreted as an indication of hydrolysis. It was found in their work that groups, some of which contained diamino nitrogen were removed from the protein molecule by this treatment.

Wright (51), on the other hand, was unable to find any alteration in the optical rotation or racemization of solutions of casein that had been heated as high as 120 degrees C. in an autoclave.

A quantitative conception of the effect of heat on curd tension is readily gained from the work of Hill (19). By heating milk samples from a number of cows to 92 degrees for five minutes and determining curd tensions before and after heating, he arrived at the following averages over a period of several days on which the tests were repeated:

Curd tension of fresh milk	Curd tension after heating to 90 degrees C. for 5 minutes
194	68
57	24
179	56
151	45
167	66
22	6
142	46
78	12
26	4
145	67
51	8
111	34

Furthermore, Hill has followed the changes in curd tension through the commercial processes of evaporation and condensation (2). As a result of this study he has been able to report that heating to 114 degrees F. (48 degrees C.) only slightly affects curd character. In one case the first heating to 180 degrees F. (80 degrees C.) reduced the curd tension almost as low as the final heating to 204 degrees F. (95 degrees C.). The final sterilization of evaporated milk in cans heated to 234 degrees F. (112 degrees C.) has the most pronounced effect of all. A series of curd tensions that might be accepted as typical for mixed herd milk can be compiled as follows:

Treatment	Curd tension
Raw milk	65
Evaporated milk	62
Sterilized and evaporated	25
Boiled milk	19
Evaporated milk, diluted 50%	12
Sterilized and evaporated diluted 50%	5

The precipitation of casein by acid is affected in a way similar to the rennin precipitation, according to Courtney (50), who found that evaporated milks give a bulky and fluid curd, milk dried by the roller process a rather compact and cheesey curd, and fresh milk raw, pasteurized, and boiled, and milk dried by the spray process yielded curds with characteristics between those of the other two groups.

INDIVIDUALITY OF COWS: Alleman and Schmidt (1) and Hill (18) found that curd tensions of milks from different cows show variations as wide as those well known in the chemical composition of milks. Alleman and Schmidt demonstrated that milks from different cows show no constant ratio between "Wartzeit" and curd tension. The individuality of each animal seems to be fairly well established, and to be altered only by the sex cycle, disease, or temporarily by a drastic change of diet. Individual cows show slight variations in the tensions of their milks taken at different periods of the day.

Experiments of Alleman and Schmidt with mixed milks show that when a hard curd milk is mixed with a soft curd milk, the curd formed from the mixture is from 10 to 20 percent harder than would be calculated from the tensions of the separate components. This would indicate that whatever influences tend toward a hard curd extend their action to the softer curd milk upon being mixed with it. This observation has been verified in feeding experiments by Bergeim and co-workers (3).

INFLUENCE OF THE PHYSIOLOGICAL CONDITION OF THE COW: Certain fluctuations in curd tension are found to correspond to the progress of the sex cycle of the animal. The results of Alleman and Schmidt (1) show a decrease in the efficiency of the action of rennin on milk taken during the heat period, but no convincing alterations in curd tension. Hill (18), however, observed that after the cow freshens and as soon as the colostrum is exhausted the milk immediately assumes a somewhat higher curd tension. This condition continues for from four to six weeks. Hill also stated that toward the end of the lactation period the curd usually becomes harder, but sometimes the opposite effect is observed.

Monier and Sommer (31) noted that many low curd tension cows but no high curd tension animals were found to have histories of chronic udder infection. Unpublished results of the work of C. S. Bryan, Michigan State College Department of Bacteriology, have shown that milk from udders in an advanced state of mastitis infection failed entirely to coagulate with rennin.

Washburn and Biglow (48) have found no relation between leucocyte counts of milk and data on coagulation.

EFFECT OF THE COW'S DIET ON CURD TENSION: Alleman and Schmidt (1) found no significant variations in curd tensions resulting from dietary conditions. Hill (18) wrote that he found no indication of any appreciable influence of normal diet on curd tension, but that changes might be brought about by sudden and drastic alterations in diet, the effect of which is, however, only temporary. Very dry diets may cause a harder curd by favoring the production of a more concentrated milk; or diets containing an abundance of water and moisture dispose toward a softer curd by causing dilution.

INFLUENCE OF PROTEIN OF MILK ON CURD TENSION: Weisberg et al (50) make the sweeping statement that the concentration of casein is the major factor in determining curd character; a high concentration favoring a hard curd and vice versa. This idea is considered to be supported by experiments in coagulation of diluted milk. Espe and Dye (16) have observed no difference in alteration of curd tension when milk was diluted with distilled water in one case, and with milk whey in another. Argument is presented to show that in two samples studied the difference in casein content accounted for only 81 percent of the difference in curd tension.

Monier and Sommer (31) have indicated that the ratio of casein to albumin is an influencing factor in coagulation. Albumin apparently favors softness of curd. Van Slyke and Bosworth (45) have found that acid in milk, a factor that tends toward a harder curd, reduces the absorption of albumin by casein. Hill (19) found that curd tension seems to increase along with total protein at the beginning and end of lactation, but the curd tension increases faster than the protein content itself seems to warrant.

Espe and Dye (16) have stated that there is probably no direct proportion between the curd tension and the concentration of any one of the constituents of milk, although there may be marked correlations.

INFLUENCE OF FAT: The actual presence of fat in milk is an influence that softens the curd according to Hill (18). However, as a general rule, it is found that milk of a naturally high fat content yields a hard curd. Weisberg and co-workers (50) included fatty constituents in the suspensoid phase which they claim controls the curd tension.

MILK PHOSPHORUS AND CURD TENSION: The most committal statement in regard to the relation of phosphorus to curd tension has been made by Monier and Sommer (31). They record that although natural phos-

phorus seems to be higher in hard curd milks, added phosphates depress coagulability. Perusual of the reports of Van Slyke and Bosworth (45), Porcher and Birgando (36), Piettre (35) and Rossi (40) convinces one that there is some controversy over the physical and chemical states of both calcium and phosphorus in milk. Weisberg, McCollum, and Johnson (50), however, thought it probable that the mode of distribution of these elements in their inorganic forms has some part to play in the determination of the coagulating properties. Palmer (34) has contributed striking evidence that the calcium phosphates of milk are all present as $CaHPO_4$, some in the colloidal and some in the true solution; that in the true solution monopolizing the functions that this salt performs relative to coagulation.

RELATION OF BREED: Hill (18) has found that the median of curd tensions is lower in the Holstein than in the Jersey breed, and Watson observed (49) that the former breed, in general, yielded milk of a lower buffering capacity than the latter.

RELATIONS OF MINOR CONSTITUENTS: Citric acid has been attributed by Monier and Sommer (31) with an appreciable softening influence on the milk curd. Although it has been suggested by Porcher and

Bergando (36) and Piettre (35) that this effect may result from a propensity of citric acid or citrates to remove calcium by precipitation, Bosworth (5) has shown that the soluble calcium of milk is increased by addition of sodium citrate, and that the less insoluble sodium-calcium caseinate is formed.

Work of Loevenhart (26) indicates that the magnesium precipitates paracasein in a manner that appears to be quite closely comparable to that of calcium. Van Slyke and Bosworth (44) have found magnesium in milk filtrates to the extent of about one sixth of the weight of the calcium. However, no quanitative results have come to light in connection with the effect of natural magnesium on curd tension.

We are equally in ignorance of the influence of chlorine, lactose, and natural sodium and potassium. Loevenhart (26) found that the anions chloride, sulfate, and nitrate were without effect on coagulation of casein.

RELATION OF BUFFERING POWER OF MILK: Notice has been given by Watson (49) and Brennemann (13) to the fact that soft curd milks have a low buffering power. The lack of direct relation between buffering power and curd tension is indicated by comparing the findings of Buchanan and Peterson (8) and Espe and Dye (16). The former workers found that milk may be diluted thirty percent of its volume without any change in buffering power, while the latter have shown that in one case represented the dilution of a sample of milk to the extent of twenty five percent of its volume caused a drop in curd tension of almost forty percent.

There is some disagreement arising in the work of Buckley (9), who found that Jersey and Guernsey milks, which tend toward harder curd than the Holstein and Ayrshire milks, according to Hill (18), are in general, more easily precipitated by weaker dilutions of hydrochloric acid than the milks of the latter breeds, This indicates a lower buffering power among the Jersey animals, and is in direct opposition to the statement of Watson (49).

CURD TENSION AND DIGESTION: Since it is scarcely the function of a thesis bearing on this particular subject to make a thorough review of the very voluminous literature concerned with the digestability of milk, only some representative works will be considered here in order to establish a connection with certain applications of knowledge of curd tension.

There has been considerable dissention over the relative merits of boiled and unboiled milk as a food. The status of the question has been well reviewed by Lane-Claypon (25) for the period previous to

1912, and summed up by conclusions which, as far as our subject is concerned, have had but little contradiction since. Only one phase of the controversy enters in to our discussion; that is, the effect of boiling on digestability.

Brennemann (11) has written that for years European physicians have been feeding milk to babies without the dire results that were considered in America to follow such a diet, and, in fact, with less digestive troubles than were experienced by American infants nurtured on raw milk. The discovery of this situation by American nutrition students led to investigations that have given highly concordant results. Two especially enlightening investigations were carried out by Brennemann (10) and Bergeim et al (3) using the same technique and arriving at almost identical results.

These workers were each fortunate in procuring a human subject who could regurgitate his food at will. By exploitation of this gift the following conclusions were reached. Raw cows' milk coagulated within thirty seconds after it reaches the stomach. At first small curds form which grow, coalesce, and harden, to form chunks as large as a man's thumb, and hard enough to resist breakage when dropped on the floor, These curds grow for one to two hours, then gradually disappear,

disintegrated by peripheral digestion which continues in the stomach for five hours or more.

Boiled milk, on the other hand, forms curds of a soft, flaky nature, which may achieve a size equal to that of a small pea. These curds pass from the stomach in about three hours. Pasteurized milk gave curds with qualities between those of raw and boiled milks, but resembled the raw milk curd most strongly. Milks modified by sodium citrate, sodium carbonates, or lime water; condensed milk and buttermilk yielded soft curds.

In the feeding of raw and boiled milks, very convincing clinical evidence is offered in favor of boiled milk for infants as reported by Brennemann, (43,44,45,46), Variot (47) and Dennett (49). The clinical pictures presented in these reports are so true to form that there is no need of separate discussion of each. Although a great many babies are quite capable of normal and undistressed progress on a raw milk diet, a considerable number of infants do have digestive and nutritional difficulties with raw milk that can be promptly cured by feeding boiled milk. Symptoms of such difficulties often become apparent in the form of regurgitations, dyspepsia, diarrhea, chunks of undigested curd in the stools, liquid stools, foul stools, and even rickets and general malnutrition. A striking number of these afflicted babies have been returned to normal, undistressed growth and development by the simple expedient of boiling their milk. Variot(46) has written of consistent success in treating infant eczema with boiled milk.

It is well known that human milk, being especially designed for human babies, has the property of yielding a curd that is remarkably soft and digestible. Brennemann (13) has made the comment that all milk modifications and substitutes have one factor in common: the curd has been reduced in size and consistancy so as to approach more closely to the qualities of mothers' milk. This is clearly shown by the evidence presented above.

As has been pointed out earlier in the results of Alleman and Schmidt(1) and Hill (18,19,20), the milk of all cows is not the same in curding characteristics. In fact cows milk is to be had that very nearly approaches human milk in this respect without being modified.

The first demonstrations of the use of unmodified cows' milk with much the same effect as boiled milk has been shown to have were made by Hill in collaboration with Blood (18, 19). These workers furnish case reports showing great benefits derived in cases of infant indigestion, malnutrition and rickets, and in unusually satisfying normal development.

Somewhat more quantitative data are presented by Espe and Dye(16) who carried out digestion experiments with dogs, calves, and humans, using for observation Pavlov pouches, roentgenograms, and flouroscopic methods respectively. It was found that an adult dog will digest 300 cc. of milk with a curd tension of 98 grams in from 1.7 to 1.8 hours, while milk with a curd tension of 190 grams remains in the same animal's stomach for from 2.3 to 2.8 hours. Undoubtedly individuality would account for differences in these figures as determined in different animals of the same species.

It was also shown that although the rate of gatric secretion seems not to be influenced by the curd character, the persistance of the secretion does respond to curd toughness.

Returning to heat treated milks in the light of their digestibility, it is interesting that Wallen-Lawrence and Koch (47) report an increase digestibility by Trypsin in vitro of evaporated and boiled milks. The increased ease of digestion seems to be a function of the temperature to which the milk is heated, and the length of time it is held there.

EXPERIMENTAL

In view of the importance that has been attributed to the role of the inorganic constituents of milk in the process of enzymic coagulation, these compounds seem to offer a promising field for investigation. Calcium and phosphorus were selected as the outstanding subjects of this research because precedenting investigations seemed to indicate that a study of these constituents would contribute some light toward an understanding of the controlling factors of curd tension.

The milk used for this work was obtained from the Michigan State College experimental herd of Holsteins. Holstein milk was considered to be most adapted to this work because of the wide variations in curd tension to be found in the breed, and because of the fairly uniform and low fat content. The samples were all taken from the mixture of the total morning milk output of each cow. Precautions were taken to avoid milks from mastitis-infected animals, or from animals in those periods of the sex cycle in which the curd tension might be affected.

Determinations were made of curd tension, total calcium, total phosphorus, acid soluble phosphorus, specific gravity and in some cases total solids and pH.

Curd tension was determined by the use of the apparatus called the "American Curd-O-Meter" manufactured by the Heusser Instrument Company of Salt Lake City, Utah, and claimed by the manufacturers to give results closely comparable to those obtained by the standard Hill apparatus. Coagulation was carried out as prescribed by Hill (18) using as a coagulant a mixture of three parts of .6 percent pepsin solution (1-3000 dry scale) to one part of a calcium chloride solution containing 378 grams of calcium chloride, U.S.P., per liter. A coagulation period of ten minutes was used. A parallel series of determinations of curd tension was made, using three parts of the pepsin solution to one part of distilled water as the coagulant without calcium chloride. Preliminary results obtained by these methods are shown on Table 1.

Calcium and acid soluble phosphorus were determined on a trichloracetic acid filtrate after the procedure of Sanders (42). Sanders found that after precipitating the protein of milk with four volumes of ten percent trichloracetic acid all of the milk calcium (and magnesium) and 18.1 to 31.6 percent of the total phosphorus were converted to a soluble form and could be determined quantitatively in the filtrate with a great saving of time. The results of these determinations were carefully checked by Sanders against those obtained by ashing methods. The filtrate was prepared by placing 20 cc of milk in a 100 cc calibrated volumetric flask and diluting, very slowly at first, and with rotation of the flask, with 10 per cent trichloracetic acid to the mark. This was allowed to stand with occasional shaking for thirty minutes to permit a complete precipitation of the proteins and liberation of the calcium from the colloidal state and the calcium caseinate. The proteins were then filtered off. A 50 cc aliquot of the filtrate was placed in a Kjeldahl flask and ashed wet with 25 cc of a 1-1 mixture of concentrated H_2SO_4 and concentrated HNO_3. In order to assure complete ashing and subsequently a ready precipitation of the phosphorus in the filtrate it was found advisable to boil off an additional 15-20 cc of concentrated nitric acid. The digestive mixture was carefully washed into a 400 cc beaker, using water as required until a piece of blue litmus paper dropped into the flask remains blue. Ninety-five percent alcohol to a volume five times that of the digest and washings was added. The $CaSO_4$ precipitated in this way was allowed to settle over night. The precipitate was filtered off, placed in a crucible, and ashed for the calcium determination. This ash was dissolved in concentrated HCl by digesting 45 minutes at 50-75 degrees, diluted, and examined for calcium content by the Meigs, Blatherwick, Cary modification of Abderhalden's and McCrudden's methods combined (29).

A comparison of this method with a shorter one desribed in the Methods of Analysis of the Association of Official Agricultural Chemists, third edition, page 268, was made. The object was to shorten the procedure by omitting the digestion and precipitating calcium as the oxalate directly from the tric loracetic acid filtrate. The method was slightly mddified in that after the first precipitation of calcium oxalate from the filtrate was complete the precipitate was dissolved in dilute HCl, treated with several drops of concentrated HNO_3 to remove any absorbed organic matter, amd evaporated to dryness. This treatment was repeated once more before proceeding with the final precipitation. Although the results for some milks were very satisfactory others showed wide variations from the values obtained by the longer method. For this reason the procedure recommended by Sanders was followed. The results of the comparison are shown on Table 2.

Acid soluble phosphorus was determined on the filtrate from the calcium separation. This alcoholic filtrate was evaporated on the steam bath; the residual liquor was treated with concentrated HNO_3 drop wise to oxidize organic matter, neutralized with concentrated NH_4OH, and used for the determination of phosphorus by the gravimetric method, Methods of Analysis, A.O.A.C. 3d edition p. 15.

Total phosphorus was determined on a 10 cc sample of the milk evaporated and ashed with the addition of magnesium nitrate. The ashe was wetted with water and dissolved by adding 5-10 cc of concentrated HNO_3, and examining for phosphorus content by the same method used for acid soluble phosphorus. The results of calcium and phosphorus determinations and their relation to curd tension with and without $CaCl_2$ are shown on Tables 3 and 4 and Figure 1.

Total solids determinations were made on weighed 2 cc samples in aluminum dishes. The samples were evaporated to dryness on the steam bath, and then placed in an 80 degree oven (C) to dry for several hours, after which they were cooled in a vacuum dessicator over $CaCl_2$. The oven drying was repeated until the samples lost less than .5 milligrams during the final heating.

The pH was determined by use of the quinhydrone electrode. The results of total solids and pH determinations are recorded on Table 4.

The specific gravity was measured with a lactometer which had been tested for accuracy against pyknometer and Westphall balance determinations.

TABLE 1

Cow	Date	Curd tension Hill	Curd tension Without $CaCl_2$
G7	July 6	57	44
"	" 7	74	38
"	" 8	51	38
"	" 9	80	37
D10	" 6	28	11
"	" 7	33	16
"	" 8	41	24
"	" 9	39	16
D8	" 6	36	21
"	" 7	60	47
"	" 8	36	29
"	" 9	36	29
281	" 12	73	28
"	" 13	90	39
"	" 16	77	50
308	" 13	38	41
"	" 14	31	32
"	" 15	44	44
"	" 16	41	39
167	" 12	37	0
"	" 13	38	0
"	" 14	39	0
"	" 15	38	0
"	" 16	46	0
178	" 12	34	0
"	" 13	33	0
"	" 14	39	0
"	" 15	40	0
"	" 16	46	0
305	" 12	40	40
"	" 13	48	45
"	" 14	40	40
"	" 15	50	47
"	" 16	31	30

TABLE 1

(concluded)

Cow	Date	Curd tension	
		Hill	Without $CaCl_2$
F6	July 6	18	15
"	" 7	33	30
"	" 8	32	27
"	" 9	39	36
"	" 12	30	23
"	" 13	30	23
"	" 14	30	27
"	" 15	34	33
"	" 16	38	38
"	Jan. 21	50	30
D15	July 6	16	12
"	" 7	39	26
"	" 8	28	24
"	" 9	30	24
"	Jan. 7	20	20
"	Feb. 6	55	27
"	Mar. 15	40	21
"	Apr. 11	53	42
"	May 16	55	28

TABLE 2

Cow	Calcium by method of Meigs, Blatherwick, Cary. mg/100 cc	Calcium by modified A.O.A.C. method. mg/100 cc	Error %
F6	110.4	105.8	-4.0
167	102.1	102.8	+ .6
G11	95.3	96.1	+ .8
G4	115.0	114.4	- .5
G11	100.6	96.8	-3.7
G15	128.2	113.2	-11.6
D15	139.4	119.7	-14.1
D9	109.8	128.6	+17.3
D13	126.8	112.5	-11.3

TABLE 3

Cow	Curd tension Hill	Curd tension Without $CaCl_2$	Ca mg 100cc	Total P mg 100cc	Acid sol. P mg 100cc	Acid insol. P mg 100cc
G 11	145	0 #	100.6	77.27	60.60	16.67
G 33	104	43	117.0	103.87	81.95	21.92
"	100	41	139.3	---	---	---
"	79	41	108.2	99.92	78.02	21.90
Herd	102	54	167.7	---	---	---
G 12	100	52	140.9	---	---	---
G 6	91	53	158.4	93.21	78.59	14.62
G 17	90	39	96.3	84.21	64.49	19.72
"	86	48	134.0	---	---	---
"	85	45	118.2	92.02	74.04	17.98
"	52	30	118.7	---	---	---
G 16*	85	45	126.9	---	---	---
"	51	25		78.69	62.00	16.69
G 31	84	29	139.8	---	---	---
"	64	32	131.9	93.97	77.12	16.85
"	57	38	142.8	---	---	---
"	48	20	110.8	93.14	71.58	21.56
D 15	55	28	144.8	86.43	63.13	23.30
"	55	27	139.5	---	---	---
"	53	42	141.7	---	---	---
"	40	21	149.2	---	---	---
C103	41	27	127.6	92.05	76.00	16.05
"	38	16	147.6	95.48	73.54	20.94
167	38	0 @	102.8	84.80	63.32	21.48
D 9	36	24	109.7	---	---	---
F 6	34	33	110.4	82.60	66.70	15.70
G 15	29	9	145.6	---	---	---
D 13****	25	22	126.8	67.33	52.14	15.19

No coagulation in ten minutes.

@ Coagulation but no measureable curd tension in ten minutes.

* Represents one quarter of the udder infected by streptococci.

Note: Each determination recorded in this table represents the average of closely checking duplicates.

Note: Specific gravity was used in each case in calculation of results.

TABLE 4

Cow	Curd tension Hill	Curd tension Without $CaCl_2$	pH Fresh	pH Coag'd Without $CaCl_2$	pH Coag'd Hill method	Total solids percent
G 33	104	43	6.61	---	5.84	12.75
G 33	100	41	---	---	---	12.55
G 17	95	41	---	---	---	10.86
G 6	91	53	---	---	---	12.50
G 17	90	39	---	6.52	5.70	---
G 17	85	45	---	6.52	5.67	11.14
G 31	84	29	---	---	---	11.63
G 33	79	41	---	6,67	5.17	---
G 31	64	32	6.60	6.60	5.87	13.03
D 15	55	28	6.52	6.52	5.92	12.56
D 15	53	42	---	---	---	11.49
G 17	52	30	6.52	6.52	5.68	14.06
G 31	48	20	---	6.35	5.39	---
C103	41	27	6.65	6.65	5.75	10.75
C103	38	16	---	6.70	5.84	---

FIG 1 Relation of phosphorus content to curd tension of milk

DISCUSSION

CALCIUM: The results arrived at in this work fail to corroborate those of preceding reporters, who in general, assert that the calcium content diminishes in relation with the curd tension by the Hill test. The contradiction is supported by comparison of milks taken from the same cow on different days (Table 3). It is notable that in some cases the samples from an individual cow that develop the softest curd prove to have the highest calcium content.

The lack of apparent relation between total calcium and curd tension is not in the least surprising when one considers that it is only the calcium in true solution that takes part in the process of coagulation. Van Slyke and Bosworth (44) working with milk filtrates obtained by passing milk through a porous earthenware filter, have found that in two samples dealt with only 35.16 and 33.33 percent of the total calcium was present in true solution. This evidence suggests that the calcium in true solution would show considerable variation in different samples, depending upon pH and the relative proportions of other constituents of the system rather than on gross calcium content. This argument can apply, however, only in cases in which milk is coagulated without the addition of calcium ions. There is an obvious inconsistency in trying to correlate the effect of naturally occuring calcium with results that are obtained only after the addition of an excess of these ions. A comparison of the curd tensions obtained without and with the added calcium chloride (Tables 1 and 4) convinces one that the treatment is by no means equally effective in all cases. The results shown on Table 3 fail also to show any relation between curd tension determined without calcium chloride addition and natural total calcium.

The relationship in the coagulation process between the activity of rennin and the increased availability of calcium ions under conditions of lower pH would undoubtedly be a moot question at present, but judging from the hardness of the curd formed under different conditions, the total process seems to take place less readily at pH 6.4- 6.0, the zone described as the most advantageous for the conversion of casein to paracasein by rennin, (Rona and Gabbe(39)) than under conditions of greater acidity.

As has been pointed out above, the effect of added calcium chloride on curd tension is not quantitatively constant. In some cases observed the tensions measured without the addition of the salt were scarcely different from those measured with it; while on the opposite extreme were those samples that either failed to coagulate or yielded a curd that was too soft to register on the measuring device, but which yielded comparatively hard curds when coagulated with the added calcium chloride. Most commonly, perhaps, the curd tension was approximately doubled by the use of calcium chloride in the proportions indicated in the Hill test. No relation was found between the natural calcium content of milk and the effect of the calcium chloride addition, although such a relation is suggested in the cases of two milks listed with especially low calcium figures which failed to coagulate to any measurable degree in ten minutes without added calcium ions.

It seems obvious that at least a part of the effect of the calcium chloride addition lies in the depression of the pH (Table 4). It has been pointed out that coagulation of milk by rennin occurs more rapidly and results in a harder curd when the pH is lowered within reasonable limits. When the acidity is induced by the addition of an acid, calcium is made more available for the precipitation of paracasein, but there is no reason to believe that this is the only coagulation-favoring reaction that occurs. When calcium chloride is the cause of the depression of pH, it would seem probable that the increase of available calcium would be accomplished even if the pH were not lowered. The addition of calcium hydroxide, as pointed out above, has a depressing effect on curd tension, which may be the result of a lowering of acidity. Unfortunately, no one has added calcium ions to milk without changing or radically threatening the original pH. Again we are reminded of the assertion of Rona and Gabbe (39) that the change of casein to paracasein is complete only in the range of pH 6.0-6.4. This range of pH is somewhat higher than that which is obtained by the addition of calcium chloride as in the Hill test.

Some lack of uniformity may arise among various workers as a result of using rennin in some cases and pepsin in others for coagulation, for consideration should be given to the fact that pepsin shows a maximum activity at a lower pH than does rennin.

There is no apparent correlation between any of the pH values recorded in Table 4 or the alterations in pH values and the curd tensions.

Although the discussion on calcium ion addition and pH alteration has been limited for the sake of avoiding confusion, such a restricted view is far from adequate. The picture is considerably complicated by variation in casein and fat contents, the effect of which this study is not well adapted to evaluate. In addition there is the colloidal phase of the question, which involves variations in stability of casein and paracasein in solutions of various pH values and conditions of salt equilibrium. Certainly the shift toward the isoelectric point of casein and paracasein which results in varying degrees from calcium chloride addition will exert some regulatory influence on the curd hardness. These physical relations are not at present understood.

PHOSPHORUS: It is observed that both the acid soluble and total phosphorus decrease in a general relation with the curd tension (Table 3 and Figure 1). So far as can be perceived by these results there is no appreciable difference between the activity of total and of acid soluble phosphorus in this relation. This seeming parallelism is affected by the fact that the total phosphorus represents the acid soluble plus the casein phosphorus; and the latter remains fairly constant: 14.6 to 27.0 percent of the total phosphorus. (Sanders: 18.1 to 31.6 percent).

An estimation of the casein content of these milks is possible based on the analysis of casein by Bosworth and Van Slyke (7) showing .71 percent phosphorus; and the conclusion of Lenstrup (25) that the acid insoluble phosphorus of milk is 98.5 percent casein phosphorus. Casein calculated in this way

appears to have no relation to curd tension.

Nothing has been done to specifically demonstrate any activity of the phosphate ions in curd formation. Palmer (34), in fact, produced a ready precipitation of paracaseinate by the addition of calcium chloride to rennin treated milks that had been previously dialyzed until the calcium had been removed, and might therefore be presumed to be fairly free of phosphate ions. Furthermore, Loevenhart(26) found that chloride, sulphate, and nitrate ions had no effect on coagulation; evidence which indirectly favors the assumption that the phosphate radical is impotent, at least in a strictly chemical sense. As has been stated above, it seems probable that the phosphate ion may have some role in stabilization of casein from the physical viewpoint.

Whether or not the principles of calcium buffering which have been worked out by Kugelmass (21) enter into this picture is an interesting question. If such be the case, it must be effective to only a limited extent, for in the Hill test calcium is added in a rather excessive amount: roughly seven to nine times the amount find in true solution, and two and a half to four times the total calcium of milk. However, the conditions outlined by Kugelmass for buffering against calcium ions are not exactly satisfied in milk. These conditions are set up by mixtures of weak acids and their salts, which react to form insloluble normal calcium salts and soluble intermediates.

Since a large part of the soluble phosphorus is considered to be present as a calcium phosphate, it might seem evident that the content of acid soluble phosphorus might be merely an indication of the amount of calcium available for precipitation as the paracaseinate. The case cannot be so simple as that, for the argument in itself is not explanatory of the relation that the phosphates bear to the effect of added calsium chloride. Furthermore, the added calsium would be expected to obliterate the relation between the phosphorous content and curd tension.

Some of the erratic manifestations of the results might be explained on the basis that the percentage, as well as the weight of the phosphorus present in true solution varies. Van Slyke and Bosworth (45) found that of the total milk phosphorus 70.0 and 64.4 percent passed through the earthenware filter in the respective cases of two different samples of milk. A curve in which the curd tension were plotted against the phosphorus in true solution might show a more even relation.

TOTAL SOLIDS: Examination of Table 4 reveals no relation between total solids and the curd tension. Reconsideration in respect to curd tensions determined without the use of calsium chloride is equally fruitless. As in the case of total calsium, this lack of relationship is not surprising in view of the many other modifying factors. Total solids contains colloidal calsium and phosphorus, sodium, potassium, and magnesium salts, citric acid, lactose, and other substances; the effect of which on curd tension is nil, doubtful, or very little understood.

CONCLUSIONS

1. Curd tension as measured by the Hill method is fairly constant for individual cows.

2. The effect of calsium chloride in coagulation differs markedly with milks from different cows. Although calsium chloride has a pronounced effect on the pH of milk, the effect of added calsium is more deeply seated than in a mere alteration of pH.

3. There is no indicated correlation between total solids and curd tension or pH and curd tension.

4. Acid insoluble phosphorus varies slightly in milks of different curd tensions, but there is no indicated correlation in this respect.

5. Acid soluble phosphorus varies with curd tension. In general, milks with a high curd tension have a higher content of acid soluble phosphorus.

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