MILK CURD—ITS MECHANISM AND MODIFICATION*

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The mechanism of milk clotting has been discussed in a previous report¹. Each of the clotting constituents of milk affect not only the course of coagulation but the character of the clot. At first a colloidal, non-settling precipitate forms which agglomerates to a dense mass, the curd. The set gel contracts and squeezes out the whey, a phenomenon called syneresis. The character of the clot has been studied to determine the modes of modifying milk in order to obtain a clot favourable for infants' digestion.

Curd-toughness of milk was determined by Hill's method² as shown in figure 1. It affords a quantitative measure of the tensile strength of milk curd upon peptic digestion. A series of 100 c.c. samples of milk were brought to 37° C. in special glass jars, the curd knife placed in position, 10 c.c. of coagulant added and after ten minutes a spring balance hooked through a loop in the knife. It was then drawn through the curd by even tension, the amount required being read directly on the balance. The lower the resistance the greater the accuracy of reproduction. The appearance of the cut curds of various milks are shown in figure 2.

Cow's milk varies greatly in curd tension. It is an individual characteristic of the cow rather than the breed, and cows from the same breed give milks with curds ranging from very soft to very hard. What determines the character of the clot? Several workers³ have studied a variety of factors which take part in the production of curd tension of milk, but consideration of the previous paper¹ indicates that the only factors determining the toughness of the curd are the clotting constituents of milk—casein, calcium ions, rennet. All others modify the clot superficially.

(A) Effect of milk clotting constituents on curd tension

The concentration of the clotting constituents were correlated with the curd tension of milk. Casein was determined by the method of Waterman, calcium by the method of Clark, and rennet added in increasing amounts.

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FIG. 1.

FIG. 2.—Character of curd.

TABLE 1.

EFFECT OF MILK CLOTTING CONSTITUENTS ON CURD TENSION AND CLOTTING TIME.

CASEIN PER CENT.	CURD TENSION GM.	CLOTTING TIME MINUTES	CALCIUM PER CENT.	CURD TENSION GM.	CLOTTING TIME MINUTES	RENNET C.C.	CURD TENSION GM.	CLOTTING TIME MINUTES
1.0	15	40	0.10	5	40	2	6	50
1.5	20	32	0.11	10	25	3	7	40
2.0	27	15		15	15	4	9	25
2.5	45	10	0.13	30	11	5	15	18
3.0	95	7	0.14	55	9	6	19	15
3.5	130	4	0.15	70	7	7	22	13
4.0	160	2	0.16	85	6	8	26	12
			0.17	100	5	9	35	9
						10	39	8
						14	40	7
						12	50	6

There is practically a direct relationship between each of these clotting constituents and curd tension; the higher their concentration the greater the toughness of the curd. But the casein effect is most determining upon the tensile strength of the milk curd. The auto-catalytic curve is almost upright (fig. 3). At low casein concentrations there appear a linear relationship between the casein content and the curd tension of milk. The minimum transition point at which milk develops hard curd is about the 2.5 per cent. level of casein. Slight rises in the casein content markedly increase the curd tension. Indeed, between the 2.5 per cent. casein and the 3.5 per cent. casein represents a difference in curd tension from about 30 gm. to about 150 gm. in this series. Above 3.5 per cent. casein content the rate of increase in curd tension abates asymptotically. The physico-chemical significance of this S-shaped curve will be considered below in discussing the volume changes during clot formation with varying concentration of casein in milk. Apparently soft curd milk is delimited by an upper casein concentration of 2.5 per cent. The calcium and rennet relationships are linear, the former affecting the curd tension more than the latter. A calcium content of about 0.125 per cent. delimits soft curd milk, for above this value the greater the increase in the calcium content the greater the curd tension. And conversely, the higher the clotting time the lower the curd tension. Rennet has the least effect upon the tensile strength of the curd for relatively large amounts of rennet raise the curd tension but slightly in comparison with the other clotting constituents. But there is a gradual and steady rise in the curd tension with increasing concentrations of rennet. Curd tension thus varies inversely with the clotting time.

(B) Effect of milk dilution with water on curd tension

Hard curd milks obtained from Sheffield Farms were diluted with increasing amounts of water and the curd tension of each clotted mixture

EFFECT OF MILK CLOTTING
CONSTITUENTS ON CURD
TENSION

FIG. 3.

determined. The relationship between dilution and curd tension is parabolic (fig. 4). The curd tension diminishes rapidly with dilution. The slope of the curve indicating a transition point which marks the beginning of soft curd milk at 50 per cent. dilution of raw milk. In other words if dilution

EFFECT OF MILK DILUTION WITH WATER ON CURD TENSION

FIG. 4.

alone were the only modification of raw milk in infant feeding the one to one ratio of milk to water would suffice to soften the curd without any other procedure. The fact that half-diluted milk initiates soft curd formation also follows from a consideration of the previous experiment. It has been seen that hard curd milk is relatively high in casein and in calcium and the half

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dilution brings it approximately into the soft curd range. But dilution of the clotting constituents need not necessarily be with water for the addition of any other non-clotting factor diminishes the percentage composition of clotting constituents. Therefore the addition of milk modifiers must be included as diluents of the clotting constituents and thus contribute somewhat to the formation of soft curds. Slight dilution, less than 10 per cent., produces too small an effect on the curd tension to be significant. In infant feeding practice it is a useless procedure.

TABLE 2.
WATER DILUTION AND CURD TENSION.

MILK PER CENT.	CURD TENSION GM.
100	120
90	80
80	65
70	50
60	40
50	25
40	20
30	10
20	0
10	5

(C) The effect of heating on the curd tension of milk

Heating alters the clotting constituents of milk and consequently the character of the curd. The temperature effect was determined, maintaining the time of heating constant according to previous procedures. Figure 5 presents the average temperature curve from 20° to 100° C. There is a sharp maximum at a little over 40° in the curd tension determination with definite falls in the tension at both lower and higher temperatures than 40° C. In other words a slight rise of temperature of milk to body heat actually increases the curd tension while subsequent heating diminishes it. This is significant in infant digestion. On ingestion of raw milk at room temperature raised to body temperature within the infant's stomach the curd tension of milk is increased beyond its initial value. This temperature curve for curd tension corresponds to that for clotting time determinations of milk. In both there is a maximum at about 40° C. with a gradual diminution on either side of this transition temperature. Apparently clotting time varies inversely with curd tension for optimum clotting at body temperature parallels the maximum curd tension of milk.

But the time of heating is as significant as is the temperature. Experiments were therefore set up to determine the effect of heating milk at 60° C. for varying lengths of time. Figure 5 shows a definite drop in the curd tension on maintaining milk for the initial length of time at 60° C. Increase in the duration of heating at this temperature produced gradually diminish-

EFFECT OF MILK MODIFICATION BY HEATING ON CURD TENSION

FIG. 5.

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ing effects on the curd tension. The higher the temperature of heating the more marked is the initial drop in the curd tension after the first few minutes. On boiling milk there is little advantage in prolonging the procedure beyond five minutes. Generally, however, the duration of heating at a constant temperature has much less effect upon the character of the curd than the degree of heating for a definite time.

Heat changes the physico-chemical behaviour of milk. The calcium caseinate is denatured and hydrolyzed until finally there is an association of the molecules to form larger aggregates. The higher the temperature the greater the amount of water bound by the caseinate until elevated temperatures are reached when the colloid states are reversed. Initially the caseinate is dispersed in water as a dispersion medium. Upon hydration and aggregation of these large molecules the viscous caseinate separates slowly in the presence of substances which tend to keep it dispersed and so that it becomes the dispersion medium for water as the dispersed phase. The curd shows the characteristics of a gel which is identical with a soft curd. Without considering the simultaneous chemical changes involved in heating milk this hydration phenomenon results in a thickening prior to clotting. It is due to a release of the caseinate or paracaseinate from its combined state with calcium. Both casein and paracasein molecules then swell to capacity, a condition conducive to soft curd formation.

TABLE 3.
EFFECT OF HEATING OF HARD CURD MILK ON THE CURD TENSION.

TEMPERATURE OF HEATING °C.	CURD TENSION GM.	CLOTTING TIME MINUTES	TIME OF HEATING MINUTES	CURD TENSION GM.
20	40	20	Control	75
30	50	12	10	60
40	60	8	20	55
50	55	13	30	50
60	35	18	40	48
70	24	25	50	45
80	18	30	60	40
90	15	35	70	35
100	13	42	80	32
			90	30
			100	30

(D) The effect of hydrogen ion concentration on the curd tension of milk

Acid and alkali modify the course of milk coagulation and the character of the curd¹. The curd tension at each pH level from 3 to 9 was determined for pasteurized milk. To 100 c.c. a mixture of 0.14 N-hydrochloric acid and 0.1094 N-sodium hydroxide was added in a fine stream by means of a burette, the mixture rotated constantly to prevent any local excess of reagent and the curd tension then determined as in the preceding tests. A

condensed curve of averages is plotted in figure 6 representing the average curd tension of milk at various pH levels combined from nine complete titrations.

Pasteurized milk shows a maximum curd tension at about pH 6.0. On the acid side of this maximum the rapid decrease in curd tension with increased acidity is caused by the removal of casein from colloid solutions

EFFECT OF MILK MODIFICATION WITH ACID AND ALKALI ON CURD TENSION

FIG. 6.

by the precipitation of acid which leaves less and less of the protein to react with the pepsin. The curd tension on the alkali side of the maximum is due chiefly to the inactivation of the pepsin by hydroxions. Upon addition to milk modifiers, crystalloids (sugars) or colloids (powdered banana, gelatin), the maximum inflection point is displayed by a flattened zone from about 5.5 to about 6.5 and zero curd tension is reached at less acid reaction than does milk alone. A zero curd tension with modified milk is also reached on the alkaline side of the isoelectric point of casein. No

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attempt was made to extend these curves beyond 8.5 since the quinhydrone electrode ceases to give reliable results beyond this range.

The hydrochloric acid used in these titration experiments with pasteurized milk produced a very soft curd near the isoelectric zone of casein. Its use in the modification of milk is less frequent in this country than on the continent. The character of the curd was compared with other acids. The curd from phosphoric is comparable to that of hydrochloric, acetic is slightly firmer, citric is very firm and so is the lactic acid curd produced naturally or artificially. Not only is there a difference in the casein curd formed with different acids but also with different milks. Evaporated milk gives soft spongy curds, powdered milk (spray process) compact curds and pasteurized milk intermediate between them. When powdered milk is roller processed the curd obtained is soft.

TABLE 4.

pH AND CURD TENSION.

ACID pH	CURD TENSION (AVERAGE) GM.	ALKALI pH	CURD TENSION (AVERAGE) GM.
3.0	0	7.0	46
3.5	2	7.5	42
4.0	5	8.0	32
4.5	9	8.5	17
5.0	18	9.0	10
5.5	30		
6.0	35		
6.5	35		
7.0	26		

TABLE 5.

PROPERTIES OF MILK CURD.

RENNET COAGULATION	ACID COAGULATION
Calcium paracaseinate formed	Casein formed
Elastic, retractile	Inelastic, non-retractile
Spongy, plastic	Floccular, sticky
Curd tension high	Curd tension low
pH zone 6-7	pH zone 4-5
Mineral content high	Mineral content low
Whey exudation great	Whey exudation slight
Serum clear	Serum cloudy

(E) Effect of milk modification with colloids on curd tension

The character of the curd upon addition to milk of banana, gelatin and gruels respectively has been determined. The colloid was weighed into a curd tension jar, the milk added and allowed to stand at room temperature for ten minutes. The jar was then placed in a water bath at 60° C. with occasional stirring until completely dispersed. The container was then transferred to a water bath at 37° C. and the curd tension measured at equilibrium. The results of this study are given in table 6, the curd tension values being the average of duplicate determinations.

Whatever the milks used, be it hard or soft curd, the curd tension is lowered by the addition of any of the colloidal modifiers used. Each represented a characteristic colloidal state, barley a hydrophobe, banana intermediate between hydrophobe and hydrophile and gelatin a hydrophile colloid. The diminution in curd tension parallels this order of degree of hydration of the colloid in milk. Assuming that a curd tension of 30 gm. or less represents a soft curd milk, 90 per cent. (51) yielded soft curd milk upon addition of 3 per cent. gelatin, 5 per cent. banana, 8 per cent. barley. The curd tension of the original milks having been from 40 to 60 gm. The most pronounced lowering occurred in the milk which had the highest curd tension. The curves (fig. 7) represent the average curd tension of the milks with a progressive softening of the casein upon increasing the amounts of colloid. An addition effect of adding a colloid to milk is to make it more acid by 0.1 to 0.3 pH units. From the curve in figure 6 such a change in pH produces no lowering of curd tension. The decrease in curd tension from 20 to 60 per cent. of the original values is therefore due to protective action of the colloidal modifier of milk.

TABLE 6.

COLLOIDS AND CURD TENSION.

BARLEY PER CENT.	CURD TENSION GM.	BANANA PER CENT.	CURD TENSION GM.	GELATIN PER CENT.	CURD TENSION GM.
0	45	0	42	0	40
1	42	1	39	1	32
2	39	2	36	2	27
3	37	3	33	3	23
4	35	4	30	4	21
5	33	5	28	5	18
6	32	6	27	6	17
7	31	7	26	7	15
8	31	8	24	8	14
9	31	9	22	9	13
10	30	10	21	10	12

EFFECT OF MILK MODIFICATION WITH PROTECTIVE COLLOIDS ON CURD TENSION

FIG. 7.

TABLE 7.

EFFECT OF COLLOID ON THE CURD TENSION OF MILK.

AVERAGE CURD TENSION IN GRAMMES.							
PERCENTAGE OF COLLOID.							
Barley	0	5	6	7	8	9	10
Banana	0	3	4	5	6	7	8
Gelatin	0	1	2	3	4	5	6

CERTIFIED MILK.							
36							
27	28	25					
21	19	24	17				
41	38	35	33	35			
37	31	31	27	23	25		
53	45	39	25	23	27	21	
36	24	15	18	19	17	14	

SOFT CURD MILK.							
23						14	
26						11	
18	15	15	17	11			
30	28	28	25	20			
35	23	23	21	21	16		
32	31	26	21	19	14	16	
23	17	17	14	14	15	13	

PASTEURIZED MILK.							
30		29					
21			11				
28			21				
32			31				
34				29			
50					20		
30							19
34	37		45				
35		19		16			
38					22		
29							19
23	22	14	14				
30				17			
39	33	27		15			12
27	19	21	19	16			19
32	30	27	26	25			20

FIG. 8.—Effect of cow's milk modifications on the character of the curd.

FIG. 9.

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TABLE 8.

EFFECT OF GELATIN ON THE pH AND CURD TENSION OF MILK.

MILK ALONE		MILK—5 PER CENT. OF GELATIN		LOWERING OF CURD TENSION
pH	CURD TENSION GM.	pH	CURD TENSION GM.	PER CENT.
6.59	43	6.45	20	54
6.58	20	6.32	12	40
6.58	19	6.36	15	21
6.54	32	6.30	12	63
6.54	20	6.33	16	20
5.77	36	5.46	16	56

(F) Effects of various milk modifications on the swelling of the clot

Every change in milk affects its physical behaviour. This changes the character of the curd. On modifying milk according to previous procedures the clot was obtained, centrifugalized and dried and the volume determined by displacement. Figure 9 shows the relative increase in volume with modification by heat, dilution, acidification, alkalization and colloid additions, in comparison with the clot obtained from unmodified milk. Upon addition of the clotting constituents, rennet, calcium and casein to milk a diminution in volume occurred with the formation of hard compact curds respectively. Apparently clots obtained from clotting constituents in milk below certain percentage composition will swell while those of higher content will lose water and shrink. Between the swelling and shrinking of the clot there is a transitional milk composition which will neither swell nor shrink on coagulation⁵.

By an increase in volume is meant, generally speaking, the difference in total volume of all the participants before and after the process. The volume change that occurs when NH_4Cl is dissolved in water is not the difference between the volume of the solution and that of the water but the difference between the volume of the solution on one hand and the combined volumes of water and salt on the other; hence the observation of the increase in volume of a gel immersed in liquid cannot be interpreted by saying that the process of swelling takes place with an increase in volume. The two things to be compared are the swollen gel and the dry gel plus fluid. This comparison leads to the inference that swelling is characterized by a decrease in volume. Fluid is forced into capillary spaces and increase in surface brings about compression of the dispersed phase.

Paracaseinate gels are two phase, three component systems. The solid phase consists of particles or micellae of calcium paracaseinate, some of which is dissolved and some dispersed in the liquid phase. Each micella is a distinct system with an insoluble elastic wall or network containing an internal liquid phase. The paracaseinate anions are not in solution in the strictest sense since they form part of the gel network, the calcium ions

existing in the interstitial solution. The calcium ions are balanced only by the negative electrical charges on the network of paracaseinate. In consequence there is an unequal distribution of all ions between the interstitial solution and the external solution, the total concentration of ions in the gel being always greater than in the main solution. The cations of the calcium paracaseinate tend to diffuse into the external solution and so exert an attraction on the anions which form part of the gel structures. This causes the volume of the gel to increase in direct proportion to the excess of concentration of diffusible ions of the gel over that of the main solution. The greater the proportion of paracaseinate existing in the ionic state the greater the swelling produced.

Shrinking, the converse of swelling, is represented by a phenomenon called syneresis. It appears as a separation of whey squeezed out from the contracting clot. Syneresis is produced by the elastic stress in the micellae of the paracaseinate solution. Elastic stress in the micellae is due to the unusually high concentration of the paracaseinate or other clotting constituents in the internal liquid phase. When they become supersaturated paracaseinate separates out of solution. The force within the micellae which keeps the micellae stretched is therefore diminished. The elastic walls of the micellae contract with the result that the liquid expelled from both

EFFECT OF VARIOUS MILK MODIFIERS ON THE SWELLING OF THE CLOT

FIG. 10.

micellae and interspaces is syneresis fluid. The size of the particles increases continuously. Several lyophobic portions appear on one and the same particle. These gradually tend to cohere thus necessitating contraction of the gel and expulsion of the fluid in the pores.

(G) The relation between viscosity and curd tension of milk

The course of milk coagulation was followed for viscosity measurement in a previous communication. It was observed that the viscosity curve was autocatalytic. After a latent period of clotting wherein viscosity changes were slight, a sudden and marked rise in viscosity characterized the clotting stage of milk. Each modification of milk altered this viscosity maxima. This was particularly observed in measuring the viscosity maxima of milks of various curd tensions. Figure 10 shows that hard curd milk is 50 per cent. higher in viscosity at its maximum than is soft curd milk. There is also a striking difference in the duration and degree of contraction of the clot, hard curd squeezing out the whey more rapidly and more effectively than does the soft curd. The maximum so attained is thus a function of the concentration of the clotting constituents, casein, calcium and rennet. The factors that tend to swell the clot produce low viscosity maxima as is illustrated in figure 10 with acid milk. Very slight changes in the curd tension of milk suffice to produce marked effects in the viscosity maxima attained during the clotting of milk.

Summary

1. The curd tension of milk is determined by the concentration of the clotting constituents, casein, calcium and rennet. A casein content of 2.5 per cent. delimits soft curd milk, a slight rise in the concentration increasing markedly the curd tension. A calcium content of 0.125 per cent. delimits soft curd milk, increasing concentrations raising the curd tension but moderately. The rennet concentrations have the least effect upon the tensile strength of the curd. Curd tension generally varies inversely with the clotting time of milk.

2. The curd tension of milk diminishes rapidly with dilution. Fifty per cent. dilution delimits soft curd milk. Milk modifiers diminish the curd tension as diluents of the clotting constituents.

3. Heating alters the curd tension. A rise in the temperature of milk to about 40° C. actually increases curd tension while subsequent heating diminishes it with a maximum at body heat. The time of heating at various temperatures likewise modifies the character of the curd, the diminution in curd tension being more marked the higher the temperature of heating.

4. The curd tension in a function of the hydrogen ion concentration of milk with a maximum at about pH 6.0. On both the acid and alkaline sides of this maximum there is a marked diminution in curd tension. Additions of milk modifiers displace this maximum to a flattened zone extending from about pH 5.5 to 6.5. Each acid produces a characteristic

curd at the same pH in the following order, hydrochloric, phosphoric, acetic, lactic, citric, hydrochloric producing the softest curd and citric the firmest.

5. Rennet clotting is characterized by the formation of calcium paracaseinate, retractility, plasticity, high curd tension, pH zone around 6.5, high mineral content, exudation of clear whey. Acid coagulation is characterized by the formation of casein, non-retractility, floccular, low curd tension, pH zone around 4.5, low mineral content, slight but cloudy whey formation.

6. Colloidal milk modifiers diminish the curd tension progressively in the following order, sugars, barley, banana, gelatin.

7. Milk modifiers alter the volume of the clot. Heat, dilution, acidification, alkalization and colloids produce swelling of the clot while additions of the clotting constituents, casein, calcium and rennet produce a shrinking of the clot. Swelling of the clot is conducive to soft curd formation and shrinking to hard curd.

8. Hard curd milk shows a viscosity 50 per cent. higher at this maximum than soft curd milk while acid milk shows the viscosity maximum 50 per cent. lower than that of soft curd milk.

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