WHY SODIUM CITRATE PREVENTS CURDLING OF MILK BY RENNIN*

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The practice of adding sodium citrate to milk used as infant food has been common for many years. It has found application especially in the treatment of certain types of "feeding-cases" in which untreated milk, after entering the stomach, forms abnormally large chunks of tough curd, shown by Talbot¹ to consist of casein. These lumps of curd may pass practically unchanged through the entire intestinal canal, causing mechanical irritation, which often results in serious interference with the process of normal digestion. Empirical practice has shown that this abnormal curdling of milk may, to some extent, be modified or controlled by the addition of sodium citrate at the rate of 1 or 2 grains per ounce of milk. While various suggestions have been offered to explain the results observed, these have been based so little on demonstrated chemical facts as to partake largely of the nature of guesswork.

In our work² on the compounds of casein and paracasein we obtained certain results which appeared to suggest a simple and satisfactory explanation of the marked effect produced by the addition of sodium citrate to milk. Work has been done to test the application of the suggested explanation and the results are presented in this paper.

In order that the details of our investigation may be more readily understood we will call attention to certain fundamental facts which have been brought out in our former work before we give the details of our present investigation. In the work to which reference is made in the paragraph preceding, the following points may be regarded as being established as far as the data now at hand enable us to reach any conclusions:

1. Casein is a protein showing the characteristic property of an acid in that it combines with metals or bases to form compounds known as caseinates.

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1. Talbot, F. B.: *Boston Med. and Surg. Jour.*, June 11, 1905, p. 205, and Jan. 7, 1909, p. 13.

2. Van Slyke and Bosworth: *Jour. Biol. Chem.*, 1913, No. 14, p. 206, and Technical Bull. No. 26, N. Y. Agricultural Experiment Station; also Bosworth: *Jour. Biol. Chem.*, 1913, No. 15, p. 231, and Technical Bull. No. 31, N. Y. Agricultural Experiment Station.

2. The molecular weight of casein is 8888, and it can combine with eight equivalents of a monovalent metal or base. For example, the compound of casein containing the largest amount of a monovalent metal like sodium could be represented by the formula Na_8 casein (sodium caseinate); the corresponding calcium compound is Ca_4 casein (calcium caseinate).

3. Casein is present in milk in combination with calcium as calcium caseinate. It has not been definitely settled yet which particular compound is in milk, but it is probably either tetra-calcium or tri-calcium caseinate.

4. When the calcium caseinate of milk is acted on by rennin, it is changed into another compound called calcium *paracaseinate*. By this action one molecule of calcium caseinate is split into two molecules of calcium paracaseinate. Thus, assuming for the sake of our illustration, that the caseinate present in milk is the tetra-calcium compound, we can represent the change from calcium caseinate to calcium paracaseinate in the following manner:

5. Paracasein, like casein, possesses acid properties, but has a molecular weight of 4444, only one-half that of casein. Paracasein, as an acid, has only one-half the combining power of casein; that is, its highest combining power is equal to four equivalents of a monovalent metal; for example, Na_4 paracasein (sodium paracaseinate), Ca_2 paracasein (calcium paracaseinate).

6. Calcium paracaseinate is *less soluble* than the corresponding calcium caseinate present in milk from which it is formed, and, therefore, it is precipitated as a solid, or, in ordinary language, the milk curdles.

7. If rennin is added to a solution of sodium caseinate, the caseinate is split into two molecules of sodium paracaseinate (for example, Na_8 caseinate = Na_4 paracaseinate + Na_4 paracaseinate), but no precipitation or curdling takes place. This is explained by the fact that sodium paracaseinate is very soluble. If, however, to this same solution of sodium paracaseinate we add a small amount of some soluble calcium salt (calcium chlorid, for example), curdling occurs at once, the curd being calcium paracaseinate. This precipitation or curdling is the result of a chemical reaction or double decomposition, which can be illustrated in the following manner:

This reaction or equilibrium can be made to proceed in either direction at the will of the experimenter; for example, addition of excess of sodium chlorid changes insoluble calcium paracaseinate back into soluble

sodium paracaseinate. These facts appeared to us to furnish an explanation of the action of sodium citrate when added to milk, in that there is formed calcium citrate and sodium caseinate, which latter compound is converted by rennin into sodium paracaseinate, a compound so soluble as not to curdle or form a precipitate. This hypothesis furnished the basis of our present investigation.

The facts stated above raise the query whether or not sodium citrate reacts with the calcium caseinate in milk to form sodium caseinate and calcium citrate? If such a reaction takes place, we should be able to determine the amount of calcium thus transferred from the caseinate to the citrate. How this determination can be made we will indicate briefly.

When milk is filtered under pressure through unglazed porous porcelain (in the form of a Chamberland filter), the serum containing the filterable soluble portions passes through the filter, while the insoluble portion, consisting largely of caseinate and insoluble calcium phosphate, remains on the filter. If, therefore, milk treated with sodium citrate is filtered through a Chamberland filter, the amount of calcium in the filtered serum should increase with the amount of citrate added up to a certain point, provided that calcium citrate (or, perhaps, a double salt of calcium sodium citrate) is formed. It may be added here that calcium citrate is soluble to the extent of about 0.090 gm. per 100 c.c. of water at ordinary room temperatures, while the amount of calcium citrate formed by such a reaction in milk containing 3.2 gm. of casein per 100 c.c. has been found by us to be not over 0.066 gm. per 100 c.c.; it all, therefore, remains in solution, passing through the filter into the serum.

If, then, we determine in milk the amount of soluble and insoluble calcium and then add to the milk sodium citrate, filtering and determining the calcium in the filtrate, there should, on the basis of our hypothesis, be an increased amount of calcium in the filtrate, showing how much calcium is transferred from the form of calcium caseinate to the form of calcium citrate.

In carrying out our experimental work, we proceeded in the following manner: Fresh separator skim-milk was used, in which the amounts of casein, soluble and insoluble calcium, magnesium and phosphorus were determined. To prevent bacterial action, 3 c.c. of 40 per cent. formaldehyd solution was added to each liter of milk. The milk was then divided into nine equal parts and to each part was added, in varying amounts, crystallized sodium citrate (containing 27.7 per cent. of water of crystallization), as indicated in the table given below. The milk was then allowed to stand long enough for the reaction to reach equilibrium. Each portion was then filtered through a Chamberland filter and the amounts of calcium, magnesium and phosphorus were determined in the filtered serum. The results are given in Table 1. Experiments were

TABLE 1.—EFFECT OF SODIUM CITRATE IN INCREASING SOLUBLE CALCIUM, ETC., IN MILK

No. of Experiment	Grams of Sodium Citrate Per 100 c.c. of Milk	Equal to Grains of Sodium Citrate Per Ounce of Milk	Effect of Adding 2 c.c. of Rennet Solution to 100 c.c. of Milk at 37 C. (98.8 F.)	Grams Per 100 c.c.					
				Calcium		Phosphorus		Magnesium	
				Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
1	0.0	0.0	Curdled	.045	.101	0.56	0.35	.010	.004
2	0.130	0.55	Curdled	.052	.094	0.55	0.36	.010	.003
3	0.260	1.10	Curdled	.054	.093	0.55	0.36	.010	.003
4	0.390	1.65	Curdled	.056	.090	0.55	0.36	.010	.003
5	0.520	2.20	Not curdled	.059	.087	0.56	0.37	.011	.002
6	0.650	2.75	Not curdled	.064	.082	0.61	0.30	.011	.002
7	0.780	3.30	Not curdled	.069	.078	0.61	0.30	.011	.002
8	0.910	3.85	Not curdled	.072	.074	0.62	0.29	.012	.001
9	1.040	4.40	Not curdled	.075	.071	0.64	0.27	.012	.001

also made to test the effect of sodium citrate on the curdling action of rennin, the results of which are given in Table 2.

Attention is called to the following points in connection with a study of the results contained in Table 1.

1. *Changes in solubility of calcium.* In the columns headed "calcium" we give the amounts of soluble and insoluble calcium in the original untreated milk used in the experiments, and then, following, the amounts of soluble and insoluble calcium in the milk after treatment with amounts of sodium citrate varying from 0.130 to 1.040 gm. per 100 c.c. of milk (equivalent to 0.55 to 4.40 grains per ounce of milk). As previously stated, the soluble calcium is the portion appearing in the serum after filtering the milk under pressure through a Chamberland filter, while the insoluble calcium is that which fails to pass through the

TABLE 2.—EFFECT OF SODIUM CITRATE ON THE CURDLING OF MILK BY RENNIN

Experi- ment No. of	Grams of Sodium Citrate Added to 100 c.c. of Milk	Equal to Grains of Sodium Citrate in 1 Ounce of Milk	Amount of Rennet Solution Used Per 100 c.c. of Milk	Minutes Required for Milk to Curdle
1	0.0	0.0	2	6
2	0.050	0.20	2	7½
3	0.100	0.40	2	8½
4	0.150	0.65	2	11
5	0.200	0.85	2	31
6	0.250	1.00	2	37
7	0.300	1.25	2	47
8	0.350	1.50	2	62
9	0.400	1.70	2	Not curdled
10	0.450	1.90	2	Not curdled
11	0.500	2.10	2	Not curdled

filter. An examination of the figures in the table shows that the amount of soluble calcium in 100 c.c. of the original milk is 0.045 gm. and this increases quite uniformly after each addition of increasing amounts of sodium citrate, the insoluble decreasing in essentially the same amounts. The only interpretation of these results that we can give is that some of the calcium of the caseinate or phosphate in the milk has been replaced by the sodium of the added citrate in the manner already discussed.

2. *Changes in solubility of phosphorus.* The question suggests itself as to whether or not the increase of soluble calcium may come from action of sodium citrate on the insoluble calcium phosphate in the milk, forming sodium phosphate and calcium citrate. An examination of the figures in the columns under "Phosphorus" shows that there is no increase of soluble phosphorus until we have added more than 0.520 gm. of sodium citrate per liter of milk (equivalent to 2.20 grains per ounce),

an amount sufficient to prevent curdling, and even with larger additions the increase of soluble phosphorus is relatively small. The increase of soluble calcium comes, therefore, largely from the calcium that is combined with casein in the milk.

3. *Changes in solubility of magnesium.* Owing to the small amount of magnesium in milk, the observed increase of solubility is slight but is in the direction shown by calcium, which would be expected.

In Table 2 we give the results obtained by treating 100 c.c. of milk with 2 c.c. of rennet solution (Shinn's liquid rennet) at 37 C. (98.8 F.). The rennet test was applied to untreated milk and also to samples of milk containing the varying amounts of crystallized sodium citrate given in the table.

Inspection of the results in this table makes it obvious that the presence of sodium citrate in milk, even in small amounts, delays very markedly the time of rennet curdling, while increase of citrate increases the time required for curdling, until we reach a point (0.400 gm. per 100 c.c. of milk or 1.7 grains per ounce), where no curdling takes place under the conditions of our experiments. It should be stated, in addition, that the character of the curdled milk varied in a characteristic way with the amount of sodium citrate added. Sample 1, untreated milk, gives a firm curd; the treated samples give curd of increasing softness with increase of sodium citrate.

The experimental results embodied in Tables 1 and 2 show that when sodium citrate is added to normal milk, (1) the amount of soluble calcium increases; (2) this increase is largely due to reaction of sodium citrate with the calcium caseinate in the milk, forming sodium caseinate or a double salt (calcium-sodium caseinate) and calcium citrate; and (3) use of increased amounts of sodium citrate lengthens the time required for the milk to curdle with rennet action or entirely prevents the curdling.

Without going into full details, it will answer our purpose here to offer, on the basis of the facts developed, the following explanation as to why the presence of sodium citrate in milk delays or prevents curdling by rennin: Knowing the amount of soluble calcium formed by addition of sodium citrate to milk and knowing also the amount of casein present, we are able to ascertain that, at the point at which the rennet solution fails to curdle the milk, we have in place of the calcium caseinate of the normal milk a double salt of calcium sodium caseinate. This double salt, on addition of rennin, forms a calcium sodium paracaseinate containing one equivalent of sodium; and this compound, owing to the presence of the sodium, is not curdled by rennin. The increasing softness of the curd, accompanying the addition of increasing amounts of sodium citrate, is due to the presence of increasing amounts of calcium

sodium paracaseinate. A point is finally reached when all the calcium caseinate becomes calcium sodium paracaseinate and the milk fails to curdle.

SUMMARY

1. The addition of sodium citrate to milk in infant feeding is a frequent practice in cases in which the use of normal milk results in the formation of large lumps of tough indigestible curd in the stomach. The favorable results attending such use of sodium citrate have never been explained on the basis of actual investigation.

2. Work previously done by the authors suggested a chemical explanation of the observed facts and led them to test the matter by an experimental study of the action of sodium citrate on milk.

3. The addition of sodium citrate to normal milk increases the amount of soluble calcium in the milk, this increase resulting from a reaction between the calcium caseinate of the milk and sodium citrate, by which is formed sodium caseinate (or calcium-sodium caseinate) and calcium citrate. The reaction is reversible.

4. The curdling of milk by rennin is delayed by the presence of sodium citrate; when there is added 0.400 gm. of sodium citrate per 100 c.c. of milk (equal to 1.7 grains per ounce), no curdling takes place.

5. The curd produced by rennin in the presence of small amounts of sodium citrate (0.050 to 0.350 gm. per 100 c.c. or 0.20 to 1.5 grains per ounce) increases in softness of consistency as the amount of sodium citrate in the milk increases.

6. The results of our work indicate that at the point at which rennin fails to curdle milk we have in place of the calcium caseinate of normal milk a double salt of calcium-sodium caseinate; this double salt, when rennin is added, is changed to a calcium sodium paracaseinate which, owing to the presence of the sodium, is not curdled.

7. The practice of adding sodium citrate to milk at the rate of 1 to 2 grains of citrate per ounce of milk appears to have a satisfactory chemical basis in the reaction between the sodium citrate and the calcium caseinate of the milk. The amount added is governed by the object in view, viz., whether it is desired to prevent curdling or only modify the character of the curd in respect to softness.