STUDIES OF INFANT FEEDING. XV.

THE CALCIUM OF COW'S MILK IN ITS RELATION TO THE DIGESTION AND ABSORPTION OF CASEIN. PROTEIN CURDS IN STOOLS *

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It is quite generally held that the presence of hard protein curds in the stools from bottle fed infants is indicative of a faulty digestion of the casein fed in the modified cow's milk formulas.¹ Such a view seems to confuse the issue, placing on the baby a fault which should be borne by the person under whose direction the modified milk formulas are prepared, as Brennemann² has clearly demonstrated in four cases in which he was able to produce curds in infants' stools and make them disappear at will by manipulation of the food.

The chemical composition of these hard curds leads me to believe that they are not produced as the result of any fault in the digestive functioning of the infant but that they are produced by a normal digestion, functioning on an unnatural food which is only partly modified to a condition adaptable to the requirements of the infant.

Cow's milk, nature's food for the offspring of the cow, contains inorganic constituents in sufficient amounts to supply the demands of the rapid growth and development of this animal and is especially rich in calcium and phosphoric acid, substances which the young of the cow use to develop rapidly growing bone tissue, but which the infant uses very sparingly. When fed to infants in the proportions found in cow's milk, or modified cow's milk, these substances are greatly in excess of the amounts required to supply the demands of the infant, and hence are eliminated either in the urine or the feces.

In a previous paper³ it has been shown that most modified milk formulas contain an excess of available calcium. In another paper⁴ will be found data concerning the chemical compositions of the caseins from cow's milk and breast milk together with certain facts concerning the action of rennin upon these caseins. In this last paper it was shown that the nature of the curd produced by the action of rennin was dependent on two factors, the amount of soluble calcium and the

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* From the Boston Floating Hospital Laboratories.

1. Courtney, A. M.: *Am. J. Dis. Child.* **3**:1 (Jan.) 1912.

2. Brennemann, J.: *Am. J. Dis. Child.* **1**:341 (April) 1911.

3. Bosworth, A. W.: *Ann. Med.* **1**: No. 4, 1921.

4. Bosworth, A. W.: *Am. J. Dis. Child.* **22**:193 (Aug.) 1921.

degree of acidity in the milk. In another paper ⁵ it was shown how the excess calcium present in cow's milk, when this milk is used as a food for infants, interferes with the assimilation of the fat.

In this paper are presented data concerning the relation between the calcium content of cow's milk and the digestion and absorption of casein by bottle fed infants.

THE CONDITION OF CALCIUM IN COW'S MILK

The calcium content of cow's milk may be separated by mechanical means into three fractions as indicated in Table 1.

TABLE 1.—CONDITION OF CALCIUM IN COW'S MILK

	Gm. per 100 C.e.
Calcium as insoluble phosphate (CaHPO_4).....	0.051
Calcium combined with proteins.....	0.058
Calcium as soluble salts in serum (whey).....	0.045

The insoluble calcium phosphate is inert in so far as its food value to the infant is concerned for it passes through the alimentary canal undigested and unabsorbed and appears in the feces. Its presence in milk, however, is a dangerous factor which must receive proper consideration, for, by the development of acidity within the milk, the calcium combined within the phosphate in an insoluble form is converted into soluble calcium. This soluble calcium, in addition to that naturally present in the milk, together with the developed acidity, have marked influence on the nature of the casein or paracasein curds formed by the action of the gastric enzymes.

Any increase in the amount of soluble calcium in milk also increases the possibility for the production of insoluble calcium soaps, which soaps may cause constipation.

If sanitary conditions are eliminated as a factor, the greatest danger arising from the use of cow's milk, which has been allowed to age, as a food for infants, rests in its content of insoluble dicalcium phosphate.

The other two forms of calcium, that combined with the casein and that present as soluble salts in the whey, are responsible for the loss of fat in the form of calcium soaps in the stools, as has been shown in a previous paper,⁵ and in this paper we will show that they play an important part in causing the protein curds which are found in the stools from some bottle fed infants.

5. Bosworth, A. W., Bowditch, H. I., and Giblin, L. A.: *Am. J. Dis. Child.* 15:397 (April) 1918.

THE CURDLING OF MILK AS INFLUENCED BY ITS CALCIUM PHOSPHATE
CONTENT

Fresh cow's milk contains insoluble dicalcium phosphate which is present in colloidal form. This form of phosphate has practically no influence on the curdling of milk by rennin.

The development of acidity in cow's milk results in the production, from each two molecules of insoluble dicalcium phosphate, of two molecules of soluble calcium salts, i. e., one molecule of calcium lactate and one molecule of soluble monocalcium phosphate.⁶

The presence of these two soluble calcium salts, one of which, the monocalcium phosphate, is an acid salt, has a marked influence on the curdling of milk by rennin.

The effect produced by the development of acidity in milk by the common dairy organism has been shown in Table 5 of a previous paper.⁴

The effect produced by the addition of a mixture of the salts resulting from the development of acidity in milk will be found in Table 2.

TABLE 2.—SHOWING EFFECT ON THE CURDLING TIME OF MILK DUE TO THE ADDITION OF A SOLUTION CONTAINING EQUAL MOLECULAR CONCENTRATIONS OF CALCIUM LACTATE AND ACID CALCIUM PHOSPHATE

	Increase in Acidity Due to Salt Mixture Added	Acidity of 100 C.c. of Milk as N/10	C.c. Rennin Added	Curdling Time	
				Minutes	Seconds
1	0	10.5	1	61	50
2	1.5	12.0	1	10	5
3	3.0	13.5	1	5	45
4	4.5	15.0	1	3	45
5	6.0	16.5	1	3	00
6	7.5	18.0	1	2	15
7	9.0	19.5	1	1	50
8	10.5	21.0	1	1	40
9	12.0	22.5	1	1	25

This table clearly demonstrates the relation in the time required to curdle milk by rennin and if one should repeat the experiment he could not fail to note the increased toughness of curds and greater expression of whey produced by the addition of increased amounts of the salt mixture.

6. Van Slyke, L. L., and Bosworth, A. W.: J. Biol. Chem. **19**:23, 1914; **24**:191, 1916.

THE CONTROL OF THE NATURE OF THE CURD PRODUCED BY THE ACTION OF RENNIN IN MILK

In a previous paper ⁴ it has been shown that the curdling of milk by rennin is dependent upon the nature and amount of soluble bases present in the milk.

The curdling of the milk by rennin can be prevented or the consistency of the curd controlled by the addition to the milk of soluble salts of sodium, potassium or ammonium; the action of these salts being due to an interchange of the bases whereby some of the calcium combined with the casein is replaced by either sodium, potassium or ammonium as the case may be. This is shown in the following reaction:

In the above reaction two potassium atoms have replaced one calcium atom and in this case no curdling could be produced by the action of rennin.

The control of the curdling of milk by the addition of sodium carbonate is shown by the figures given in Table 3.

TABLE 3.—SHOWING THE INFLUENCE OF ADDED SODIUM CARBONATE UPON THE CURDLING OF MILK BY RENNIN

Number	C.c. 1.0% Na ₂ CO ₃ per 100 C.c. Milk	Gm. Na ₂ CO ₃ per 100 C.c. Milk	Amount of Rennin Solution Added, C.c.	Coagulation, Time, Minutes
1.....	0	0	2.5	0.5
2.....	0.5	0.005	2.5	0.75
3.....	1.0	0.01	2.5	2.0
4.....	1.5	0.015	2.5	3.25
5.....	2.0	0.020	2.5	3.50
6.....	2.5	0.025	2.5	6.50
7.....	3.0	0.030	2.5	8.00
8.....	3.5	0.035	2.5	11.50
9.....	4.0	0.040	2.5	13.00
10.....	4.5	0.045	2.5	14.50
11.....	5.0	0.050	2.5	29.00
12.....	5.5	0.055	2.5	38.00
13.....	6.0	0.060	2.5	50.00
14.....	6.5	0.065	2.5	No curd

In connection with the data given in this table it should be stated that a more concentrated extract of rennin was used than was the case in the preceding experiment in order to reduce the curdling time.

The curd formed in Sample 1 was a firm yet soft jelly-like mass. The other curds graded from this consistency toward very fine and soft flakes; in fact, it was rather difficult to measure the curdling time of the last few samples on account of the fine, soft condition of the curd.

A control of the curdling of milk may be obtained by the addition of lime water. The addition of lime water to milk makes it less acid and results in a decrease in the amount of soluble calcium due to the precipitation of insoluble calcium phosphate. If sufficient lime water

is added the soluble calcium can be reduced to such a small amount that the milk will not curdle upon the addition of rennin.

The nature of the curd formed by the action of rennin in milk may also be influenced by boiling the milk and by the action produced by the addition of such substances as malt soup, dextri-maltose No. 3 and Mellen's Food because they contain potassium salts, and barley gruel, etc., because of the physical action.

TABLE 4.—SHOWING THE INFLUENCE OF ADDED LIME WATER ON THE CURDLING OF MILK BY RENNIN

Number	C.c. of Lime Water per 100 C.c. Milk	Gm. Ca(OH) ₂ per 100 C.c. Milk	Amount of Rennin Solution Added	Coagulation Time in Minutes
1.....	0	0	2	0.50
2.....	5	0.00925	2	3.33
3.....	10	0.01850	2	4.16
4.....	15	0.02775	2	5.50
5.....	20	0.03700	2	9.83
6.....	25	0.04625	2	15.00
7.....	30	0.05550	2	24.50
8.....	35	0.06475	2	81.66

RELATION BETWEEN THE CALCIUM IN THE FOOD AND THE NITROGEN IN THE FECES

Cow's milk is the food produced by nature for the nourishment of the calf and as the digestive organs of the calf are quite different from those of the infant it can digest the curds formed from cow's milk completely while the infant can not do so. Breast milk either does not curdle in the stomach of the infant or forms extremely fine soft curds.

TABLE 5.—SHOWING INFLUENCE OF CALCIUM IN THE FOOD ON THE AMOUNTS OF NITROGEN AND FAT ELIMINATED IN THE STOOLS OF INFANTS

	Kind of Food					
	Synthetic 3-6-1.5	Breast Milk 3-6-1.5(?)	Synthetic 3-6-1.5 CaHPO ₄	Synthetic 3-6-1.5 Casein- ate	Modified Cow's Milk 2-6-1.6	Synthetic 3-6-1.5 Caseinate
Formula.....	None	Breast milk salts			Cow's milk salts and acetate	
Casein present as.....	Sodium caseinate	Potassium caseinate (?)	Sodium caseinate	Calcium caseinate	Calcium caseinate	Calcium caseinate
Gm. CaO per 24 hours in food...	0.00	0.40	0.45	0.51	1.13	1.55
Gm. CaO in feces per 24 hours...	0.10	0.20	0.33	0.48	0.94	1.73
Gm. fat in feces per 24 hours....	0.53	0.54	1.36	2.14	5.01	7.90
Gm. N in feces per 24 hours.....	0.16	0.20	0.22	0.41	0.54	0.73

The rate of digestion of the curds formed from milk depends upon the surface exposed. The large tough curds produced from cow's milk will be slowly and incompletely digested in the intestines of the infant, leaving a residue to be excreted in the feces while the fine soft curds produced from breast milk will be rapidly and completely digested.

We know that the removal of the soluble calcium from cow's milk prevents its curdling by the addition of rennin. If calcium is responsible for the curds formed from cow's milk and if the protein curds which appear in the stool are these same curds, undigested, we should expect to find some relation between the calcium and casein intake and the nitrogen eliminated in the stools. That such a relation exists is shown in Table 5.

The table distinctly shows the relation between the calcium in the food and the amount of nitrogen in the stools. As a matter of interest we have also given the figures for the amounts of fat (soaps and fats) eliminated. A relationship will also be noticed between the calcium intake and the fat excretion.

The so-called "synthetic milk" was made from butter fat, lactose, soluble casein, and the salts indicated in the table.

THE NATURE OF THE PROTEIN CURDS PRESENT IN THE STOOLS FROM
SOME BOTTLE FED INFANTS RECEIVING MODIFIED COW'S MILK

A. M. Courtney¹ has given the analyses of many curds separated from infants stools. This work has been repeated by me and extended to include some determinations not made by Miss Courtney. The average results as found by these two sets of analyses are as follows:

TABLE 6.—AVERAGE ANALYSES OF PROTEIN CURDS FROM INFANTS' STOOLS

	Courtney, per Cent.	Bosworth, per Cent.
Protein.....	65	63.0
Fat.....	30	27.0
Ash.....	4	3.8
CaO.....	..	2.2
P ₂ O ₅	..	1.3

These figures show that the curds are composed of protein, fat and ash and that the ash is almost wholly a mixture of calcium oxid and calcium phosphate.

To one familiar with the action of rennin in milk, and especially to one familiar with the chemistry of cheese making, these figures are very significant. When rennin is added to milk the curd formed is a mixture of fat, calcium paracaseinate and dicalcium phosphate. On standing these curds contract into tough masses.

In order to determine the nature of the protein in the curd the following procedure was carried out:

The dry curds were ground to a fine powder and extracted with ether to remove the fat. The protein residue was dissolved in a very weak solution of caustic soda and the protein precipitated by the addition of a little acetic acid. The precipitate was then treated by the

method of VanSlyke and Bosworth⁷ for the preparation of pure casein and paracasein.

The final product was a fine white powder exactly resembling similar preparations of pure casein and paracasein previously made by the author.

It had a nitrogen content of 15.6 per cent. One gram required 2.50 per cent. calcium oxid to make a solution neutral to phenolphthalein. One gram required 2.25 c.c. tenth normal sodium hydroxid to form the soluble compound containing the smallest amount of base. These findings prove the substance to be paracasein.

CONCLUSIONS

1. The calcium and casein in cow's milk are the factors involved in the curdling of this milk by the action of rennin.
2. An increase in either the calcium content or the acidity of milk tends to toughen the curds produced by the action of rennin.
3. The calf has a digestive tract which can digest these curds. The digestive tract of the infant fails to completely digest these curds.
4. The inability of the infant to digest these curds does not indicate an impaired digestive functioning but does indicate a faulty supervision of the feeding of the infant.
5. The nature of the curds formed in cow's milk can be controlled by chemical and physical means.
6. The amount of nitrogen eliminated in the stools of infants may have a relation to the calcium content of the food.
7. The increase in the nitrogen content of stools from infants may be due to the presence of protein curds.
8. These protein curds are composed of protein, fat and dicalcium phosphate.
9. The protein in the curds is calcium paracasein.

7. VanSlyke, L. L., and Bosworth, A. W.: J. Biol. Chem. **14**:203, 1913.