STUDIES ON THE PROTECTIVE COLLOIDS OF MILK.

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All materials of milk such as casein, albumin, enzym, and the fat present as emulsion belong to the colloidal system. Casein is a very important substance from the standpoint of the colloid chemistry of milk, as it is present to such a great extent as 0.67-4.97 % of the total dry matter, the quantity of which is 9.22-19.18 % of milk. The albumin, known as lactoalbumin, is also present to the extent of 0.53-1.55 % and plays also an important rôle in milk. But the application of colloid chemistry to milk by other authors has been concerned chiefly with the protective phenomenon of lactoalbumin to casein. If we compare the average compositions of various milks and the differences of these coagulations, the role of the protective substance in milk will be easily understood.

TABLE I.

Constituents	Cow	Human	Goat	Ewe	Mare	Ass
Specific gravity	1.0316	1.03	1.0305	1.0298	1.0347	1.036
Water	87.27	87.41	85.71	80.82	90.78	89.61
Casein	3.02	1.03	3.20	4.97	1.21	4.67
Albumin	0.53	1.26	1.09	1.55	0.75	1.55
Fat	3.61	3.78	4.78	6.86	1.21	1.64
Milk sugar	4.88	6.21	4.48	4.91	5.57	5.99
Ash	0.71	0.31	0.76	0.83	0.35	0.51

It is well known that an infant can digest human and ass' milk more easily than cows' milk Jacobi and from the above tabel

we see that in cows' milk, the ratio of albumin to casein is only $0.53:3.02=1:5.7$, whereas in human milk it is $1.26:1.03=1:0.8$ and in ass' milk $1.55:0.67=1:0.4$. Koplik (1902) stated as follows:—"Cows' milk is precipitated or coagulated very quickly by the aid of acids or salts. Hence in the infant stomach, cows' milk soon coagulates in large masses even when much acid of the gastric juice is not present. On the other hand, human milk coagulates very slowly and in small masses, after absorbing a large amount of acid of the gastric juice. The difference in the modes of coagulation in the above two cases is of great importance in the study of infant feeding." Brennemann (1910) stated also that "when fresh milk is drunk, the curds formed in the human stomach are very large and hard, but a starchy decoction when added to the milk, causes smaller curds, which are easily digested. So cows' milk can be made to resemble human or ass' milk in their protective qualities by the addition of such colloidal substances as gelatine, gum-arabic, or starchy solution.

Very interesting observations with the ultra-microscope were made by Alexander and Bullock (1910) on this subject. These authors followed the coagulation of milk by heat, rennin and acid, with and without the addition of protective colloids, by the aid of the ultra-microscope. They concluded that "the casein of milk is an irreversible and coagulating colloid, which is protected by lactalbumin, the latter being a reversible and stable colloid; and for infant feeding it is necessary not only to consider the percentage of total proteids, fat etc, present in the milk, but also to see that the casein is adequately protected."

In the coagulation of milk in the stomach, two processes are distinguished, one of which is caused by the action of acid, the other by the action of enzyme. Kreidl and Neumann (1908) stated that "the coagulation process by means of lab-enzyme in milk is similar to that of thrombin in blood and it is an action of both enzyme and Ca-ion . In this reaction, the concentration of enzyme is always decreased in "molke," therefore it is evident that some quantities of enzyme are adsorbed by the coagulated

casein." This fact was also proved by many authors. (Potry, Oppenheimer, Reichel u. Spiro, Spiro, Fuld). If we consider the numerical equation of this reaction, the concentration of enzyme is inversely proportional to the coagulation time i. e. $L \cdot T = \text{constant}$:—where L =lab-concentration, T =coagulation time. Recently, Bauer and Herzfeld (1921) examined this reaction thoroughly, tracing a curve, and proved that it coincides with the above formula. But we have no information concerning the influence of protective colloids on this reaction.

Beside this information we have still many questions which are of importance in infant feeding, especially concerning the protective colloids of milk. If we want to select a good protective colloid, we must pay great attention to the following factors, which must be in agreement with the characters of these colloids.

- (1) The protective colloid must consist of nutritious agent.
- (2) The protective colloid must have a definite protective power and for this purpose it is more convenient when it is a pure chemical compound.
- (3) The colloid must have a protective action against the two processes of coagulation, which are caused by acid and by enzyme.
- (4) The colloid must not spoil easily and at the same time, must have a convenient form for transport.
- (5) The protective colloid must dissolve easily in milk and in water and must have no power to destroy the taste of milk.

Under the considerations of all these factors, the following experiments to select a good protective colloid against milk coagulation were undertaken.

I. PROTECTIVE VALUE AGAINST THE COAGULATION OF GOLD-COLLOID SOLUTION.

A gold colloid solution was prepared by Zsigmondy's method, as follows:— 120 cc. of excellent pure water, which was prepared by redistillation with tin cooler and reserved in a Jena glass flask, was poured into a glass beaker and boiled. It was

mixed with 2.5 cc. of gold hydrogen chloride solution (6 gm. of $\text{AuCl}_3 \cdot \text{H}_2\text{O}$ in 1000 cc. of redistilled water), 3 cc. of 0.18 normal solution of pure K_2CO_3 and 3-5 cc. of dilute formalin solution (0.3 cc. of conc. formalin in 100 cc. of water), stirred vigorously and kept boiling till the mixture showed a bright red colour. In the following experiment, 10 cc. of the gold colloid solution was mixed with the following quantities of 0.5 % colloid solution in a clean test tube and titrated with 2 normal NaCl solution till the mixture showed a bluish purple colour.

Table II.

Colloid-solution.	Quantity of coll. solution.	Quantity of 2N-NaCl solution.	Quantity of coll. solution.	Quantity of 2N-NaCl solution.
Water only	0. cc.	0.45 cc.	—	—
Common rice starch sol.	0.1 cc.	1.40 cc.	0.25 cc.	2.40 cc.
Glutinous rice starch sol.	0.1 cc.	1.50 cc.	0.25 cc.	3.00 cc.
Potato starch sol.	0.1 cc.	0.9 cc.	0.25 cc.	1.60 cc.
Katakuri starch sol.	0.1 cc.	0.75 cc.	0.25 cc.	1.10 cc.
Sweet potato starch sol.	0.1 cc.	0.65 cc.	0.25 cc.	0.95 cc.
Wheat starch sol.	0.1 cc.	0.35 cc.	0.25 cc.	1.10 cc.
Dextrine sol.	0.3 cc.	1.00 cc.	1.00 cc.	1.50 cc.
Alkali-glutinate sol.	0.1 cc.	1.90 cc.	0.25 cc.	3.80 cc.
Egg white sol.	0.1 cc.	1.10 cc.	0.25 cc.	2.70 cc.
Peptone sol.	0.1 cc.	0.60 cc.	0.25 cc.	1.30 cc.
Gelatine sol.	0.1 cc.	8.00 cc.	0.25 cc.	12.00 cc.
Sodium nucleate sol.	0.1 cc.	2.8 cc.	0.25 cc.	4.90 cc.

In the above experiment, all kinds of starch were purified till they showed no protein reaction and their ash contents were decreased to 0.1-0.4 %. Dextrine was made in Kahlbaum which contained no reducing substance. Gelatine, was transparent, showed no colour and gave no reaction of sulphuric acid which was used for purification. Na-nucleate was prepared from yeast nucleic acid and recrystallized from alcohol solution. By the above table, we found the powerful protective action of colloids in the following

order :— Gelatine > Na-nucleate > Alkali-glutinate. The protective power of Na-nucleate was 4-5 times that of starch.

II. PROTECTIVE ACTION OF COLLOIDS AGAINST THE MILK COAGULATION BY ACID.

As already mentioned, Bauer and Herzfeld (l.c.) stated that the coagulation of milk is a function of the concentration of enzyme and the time of coagulation which when traced by the ordinary curve tracing method, shows one curve. On the contrary, in the case of coagulation by acid, it shows a straight line. Hence there is a clear difference between the coagulations by acid and by enzyme. But, for practical purposes the colloid must be able to prevent the coagulation of milk which is caused by either of these agents, as both are met with at the same time in an infant's stomach. Therefore, we examined at first the protective power of different colloids against the coagulation of milk caused by the following method.

10 cc. of fresh cows' milk were mixed with 9 cc. of water or 0.5 % of colloid solution in a large test tube and after addition of acid solution, each test tube was placed in a water bath at 40°C. When the coagulation was observed in the control, all tubes were taken out, from the water-bath and cooled to 17° C and shaken for 10 minutes on a shaking machine. Thus 10 cc. of homogenized coagulated milk were poured into a measuring tube and treated for a definite time by a centrifugal machine and the volume of separated coagulum was measured. If the separation of coagulum was not observable, we continued the centrifugation and determined the time of coagulation and its volume.

The cows' milk used in our experiment, was freshly milked every morning at 6 o'clock and preserved in an ice chamber. In each series (A, B, C, . . .) of the following experiment, the same milk was used which was milked in the same morning. The fresh milk showed almost neutral to litmus paper and during a whole day after milking, there was no change in its hydrogen ion

concentration. The following numbers are the average volume (cc.) of coagulum of 2 samples.

TABLE III.

Solution of protective colloids.	(10 cc. of milk (A)+9 cc. H ₂ O or 0.5 % of colloid solution+the following quantities of N/4 acetic acid solution as coagulating agent.) such as:					(10 cc. of milk (B) +9 cc. H ₂ O or 0.5 % of colloid solution+ the following quan- tities of acetic or lactic acid.)		
						N/50 acetic acid sol.	N/2 lactic acid sol.	
	2.5 cc.	2.5 cc.	2.0 cc.	2.0 cc.	2.0 cc.	2.5 cc.	2.0 cc.	1.5 cc.
Time of coagulation.	30 sec.	3 min.	30 sec.	1 min.	2 min.	1 min.	3 min.	5 min.
H ₂ O (control)	1.25 cc.	—	1.4 cc.	1.2 cc.	—	1.7 cc.	2.3 cc.	1.5 cc.
Common rice starch sol.	1.6 cc.	—	2.5 cc.	1.9 cc.	1.4 cc.	2.9 cc.	2.3 cc.	1.6 cc.
Glutinous rice starch sol.	1.8 cc.	—	2.8 cc.	1.8 cc.	1.6 cc.	2.8 cc.	2.6 cc.	1.6 cc.
Potato starch sol.	1.6 cc.	—	2.7 cc.	—	—	3.1 cc.	3.7 cc.	2.5 cc.
Katakuri-starch sol.	1.4 cc.	—	2.7 cc.	—	—	2.8 cc.	3.1 cc.	1.8 cc.
Sweet potato starch sol.	1.3 cc.	—	2.4 cc.	—	—	3.3 cc.	3.2 cc.	2.0 cc.
Wheat starch sol.	1.3 cc.	—	2.6 cc.	—	—	3.3 cc.	3.0 cc.	2.0 cc.
Corn starch sol.	1.6 cc.	—	2.5 cc.	—	—	3.2 cc.	3.3 cc.	2.0 cc.
Dextrine sol.	—	—	no	4.0 cc.	2.5 cc.	2.6 cc.	—	—
Alkaliglutinate sol.	—	1.8 cc.	—	—	—	—	2.4 cc.	2.7 cc.
Egg white sol.	—	1.3 cc.	—	—	—	—	2.2 cc.	2.3 cc.
Peptone sol.	—	1.3 cc.	—	—	—	—	2.2 cc.	2.5 cc.
Gelatine sol.	—	1.3 cc.	no	no	3.5 cc.	2.4 cc.	2.2 cc.	2.2 cc.
Sodium nucleate sol.	—	no	no	no	no	no	no	no

By this table we observed that the protective action of Na-nucleate against the coagulation of milk by acid is most powerful and the actions of gelatine and dextrine are next in power. This order of the protective powers of colloids against the coagulation

of milk by acid is different from that of coagulation of Au-colloid solution by salts.

III. PROTECTIVE ACTION OF COLLOIDS AGAINST THE MILK COAGULATION BY RENNIN.

The powers of the protective action of colloids against the coagulation of milk by rennin were compared by the same method as above mentioned and the following results were obtained. In this experiment, the viscosity of colloid solution was also compared in order to observe its influence upon the protective action, estimating the flowing time of solution through an Ostwald's viscosimeter.

TABLE IV.
10 cc. of milk (C)+14 cc. H₂O or 0.5 % colloid solution
+1 cc. of 0.1 % rennin solution.
Time of centrifugalization=2 min. Temperature=7.5–8.0°C

Solution of protective colloid.	(Volume of coagulum cc.)	Flowing time.	t/t ₀
Water only (control)	1.9 cc.	9.60 sec.	1.00
Common rice starch sol.	2.6 cc.	12.80 sec.	1.35
Glutinous rice starch sol.	2.5 cc.	13.20 sec.	1.71
Potato starch sol.	2.4 cc.	17.40 sec.	1.81
Katakuri starch sol.	2.6 cc.	13.2 sec.	1.33
Sweet potato starch Sol.	2.4 cc.	11.8 sec.	1.23
Wheat starch sol.	2.5 cc.	11.6 sec.	1.21
Corn starch sol.	2.7 cc.	12.4 sec.	1.29
Dextrine sol.	2.9 cc.	10.6 sec.	1.10
Na-Glutinate sol.	no	—	—
Egg white sol.	2.9 cc.	—	—
Peptone sol.	3.4 cc.	—	—
Gelatine sol.	4.0 cc.	12.20 sec.	1.27
Na-nucleate sol.	no	10.4 sec.	1.08

The above results show that the protective action of na-nucleate and alkali-glutinate are the most powerful, next to which is gelatine with twice the power of starch. At the same time, we know that the protective power has no definite relation to the viscosity. I

we consider these results comparing them with those of the former table, we find that na-nucleate has the most powerful protective action against the coagulation of milk by acid as well as by rennin, while other powerful protective colloids do not show the same power against both kinds of coagulation. Hence, against milk coagulation we recommend na-nucleate as the best protective colloid.

IV. INFLUENCE OF NA-NUCLEATE UPON THE COAGULATION CURVE OF MILK.

In the above description of the reaction of milk coagulation, we stated that the product of the concentration of rennin and the time of coagulation is constant. Hence we determined to ascertain the influence of na-nucleate as the protective colloid upon this reaction. For this purpose, fresh milk was diluted with 0.5 % na-nucleate solution and its coagulation time in the presence of rennin was compared with that of control in a water bath at 38° C. The following numbers are the average of two samples.

TABLE V.

Quantity of milk.	Quantity of H ₂ O and 0.5 % na- nucleate sol.		Quantity of rennin solution.	Dilution of rennin solution	Time of coagulation.		Time of coagulation	
	(H ₂ O)	(Na- nucleate)			(Control.)	(with protect- ive colloid.)	Dilution of rennin.	
							(con- trol)	(pro- tect- ive colloid)
20 cc.	10 cc.	—	1.0 cc. (0.25 %)	1	4.0 min.	—	.0	—
"	—	10 cc.	"	"	—	6.5 min.	—	6.5
20 cc.	10 cc.	—	"	10	12. min.	—	1.2	—
"	—	10 cc.	"	"	—	28. min.	—	2.8
20 cc.	10 cc.	—	"	50	49. min.	—	0.98	—
"	—	10 cc.	"	"	—	170. min.	—	3.40
20 cc.	10 cc.	—	"	100	150. min.	—	1.05	—
"	—	10 cc.	"	"	—	—	—	—
20 cc.	10 cc.	—	"	200	165. min.	—	0.85	—

Quantity of milk.	Quantity of H ₂ O and 0.5 % nucleate sol.		Quantity of rennin solution.	Dilution of rennin solution.	Time of coagulation.		Time of coagulation.	
	(H ₂ O)	(Na- nucleate)			(Control.)	(with protect- tive colloid.	Dilution on rennin.	
							(control.)	(-pro- tect- ive colloid)
20 cc.	10 cc.	—	20 cc.	1	3. min.	—	3.0	—
"	—	10 cc.	"	"	—	3.5 min.	—	3.5
20 cc.	10 cc.	—	"	10	9. min.	—	0.9	—
"	—	10 cc.	"	"	—	21. min.	—	2.1
20 cc.	10 cc.	—	"	50	14. min.	—	0.82	—
"	—	10 cc.	"	"	—	93. min.	—	1.81
20 cc.	10 cc.	—	"	100	101. min.	—	1.01	—
"	—	10 cc.	"	"	—	270. min.	—	2.70
20 cc.	10 cc.	—	2.0 cc.	1	4. min.	—	4.0	—
"	5 cc.	5 cc.	"	"	—	4. min.	—	4.0
20 cc.	10 cc.	—	"	10	8. min.	—	0.80	—
"	5 cc.	5 cc.	"	"	—	9. min.	—	0.90
20 cc.	10 cc.	—	"	50	33. min.	—	0.76	—
"	5 cc.	5 cc.	"	"	—	46. min.	—	0.92
20 cc.	10 cc.	—	"	100	95. min.	—	0.95	—
"	5 cc.	5 cc.	"	"	—	121. min.	—	1.21
20 cc.	10 cc.	—	"	200	203. min.	—	1.01	—
"	5 cc.	5 cc.	"	"	—	287. min.	—	1.43
20 cc.	10 cc.	—	2.0 cc.	1	4. min.	—	4.0	—
"	3 cc.	7 cc.	"	"	—	4. min.	—	4.0
20 cc.	10 cc.	—	"	10	7. min.	—	0.80	—
"	3 cc.	7 cc.	"	"	—	10. min.	—	1.0
20 cc.	10 cc.	—	"	50	35. min.	—	0.70	—
"	3 cc.	7 cc.	"	"	—	46. min.	—	0.90
20 cc.	10 cc.	—	"	100	70. min.	—	0.70	—
"	3 cc.	7 cc.	"	"	—	119. min.	—	1.19
20 cc.	10 cc.	—	"	200	137. min.	—	0.69	—
"	3 cc.	7 cc.	"	"	—	235. min.	—	1.43

From these results we obtained the following conclusions:—

- (1) The time of coagulation of milk is almost proportional to

the concentration of the enzyme.

(2) When the quantity of the protective colloid is constant, the prolongation of the coagulation time is greater than the degree of the quantity of enzyme.

APPENDIX.

Below will be found the results of the examination of the coagulation of human milk by the same method as above.

10 cc. of human milk + 10 cc. H_2O + 0.2 cc. or 2.0 cc. or 4 c.c. or 6.0 cc. of rennin sol.				
(Coagulation time by minutes)	22.0	95.0	42.0	25.0
10 cc. of human milk + 10 cc. H_2O + 1.5 cc. or 2 c.c. or 6.0 cc. of N/4 acetic acid sol.				
(Coagulation)	no	no	no	
10 cc. of human milk + 10 cc. H_2O + 1.5 cc. or 0.2 cc or 2.5 cc. of N/2 lactic acid sol.				
(Coagulation)	no	no	no	

Thus human milk has a great resistance against the coagulation by acid and rennin.

V. SUMMARY.

On the examination of the protective action of six kinds of starches, dextrine, alkaliglutinate, egg white, peptone, gelatine and na-nucleate against milk coagulation, we obtained the following conclusions.

(1) In these colloid solutions, the protective power against the coagulation of gold colloid solution was in the following order:— Gelatine > Na-nucleate > Alkaliglutinate.

(2) The protective power of these colloids against the coagulation of milk by acid was in the following order:— Na-nucleate > Gelatine > Dextrine. In the case of coagulation of milk by rennin the protective action was in the following order:— Na-nucleate > Alkaliglutinate > Dextrine. *Hence in these protective colloids against the coagulation of milk na-nucleate was found to be best.*

(3) The viscosity of the protective colloid solution showed no definite relation to their protective power and the viscosity of na-nucleate solution was found to be very weak though its protective action was very strong.

(4) The coagulation time of the rennin action was delayed in the presence of the protective colloid (Na-nucleate) whereas the

prolongation of time was greater than the decreasing degree of the quantity of enzyme, when the quantity of the protective colloid was constant.

(5) The coagulation of human milk corresponded to that of cows' milk as shown below :—

Milk	Rennin solution.	The protective colloid solution.
10 cc. of human milk+10 cc. of H ₂ O....	2.0 cc. of 0.25 % sol.	without colloid.
10 cc. of cow's milk+10 cc. of H ₂ O....	1.0 cc. of 0.0025 % sol.	without colloid.
10 cc. of cow's milk+10 cc. of na-nucleate sol.	2.0 cc. of 0.005 % sol.	with colloid.

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