Report on Chemical Study of Human Milk (3)

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I. Introduction

We have shown some results about mother's milk pH in report 1 and 2. On continuing in this report, we can show the results which are obtained by analyseing the composition of milk. The cases of this report are as follows: 4 cases are the same as in report 2 and another new case is also investigated.

II. Methods

It was impossible to get a great deal of samples, therefore we planned so that all the operations could be made with milk of 10 ml. at most.

(*a*) *Fat.*

Procedures:

i) Fat was removed from a milk sample by centrifugeing for 30 minutes at the rate of about 3000 r. p. m. At this juncture, 10 ml. of milk diluted twice was used as the sample.

ii) We measured the stratum of fat which was separated by the height of the fat layer in the centrifuge tube.

iii) Next, we took off the fat from the tube, and it was dried in the desiccater.

iv) The weight of fat was weighed by Chemical Balance and this weighing was continued until constant weight was obtained. This weight is taken as quantity of fat.

(*b*) *Protein.*

The nitrogen of milk sample was analysed by Micro-Kjeldahl method.

i) For the analysis, separated mother's milk which was removed from fat was used as the sample in order to prevent the foaming.

ii) The catalyser which we used, was as follows.

i. 0.5 ml. of solution obtained by adding 5 gm. of red mercuric oxide to 6 ml. of sulfuric acid plus 44 ml. of distilled water.
ii. 0.5 gm. of potassium sulfate.

iii) Decomposition was continued until the solution becomes clear.

iv) Total protein nitrogen was converted to protein with the facter 6.37.

(*c*) *Casein protein and whey protein.*

The distribution of casein protein and whey-protein nitrogen in the nitrogen of milk sample was determined by the modified Rowland's methods.*

Namely, add a few drops of mixed solution of N Hydrochloric acid and N Acetic acid (1 : 2) to the separated fat-free milk, until pH of the solution becomes 4.7 which is isoelectrice point of casein protein. And by steeping it in hot water for about five minutes, casein protein clots soon. The casein was fitered off, and whey protein, the filtrate which contained lactalbumin and lactglobulin, was analysed by Micro-Kjeldahl method.

Then, we determined the quantity of casein protein by subtracting whey protein from total protein which was previously analysed.

Regarding to each quantity of lactalbumin and lactglobulin, we are now studying by the Electrophoresis method, but can not get the results of it at present.

And we are studying about what buffer solution is most suitable.

(*d*) *Lactose.*

We are owing to Plumel's method of lactose.**

1) The following reagents are used.

i) Ferriccyanide solution.

Dissove 0.66 gm. of potassium ferricyanide, 0.02 gm. of potassium ferrocyanide, 1.06 gm. of sodium carbonate in distilled water and diluted to 100 ml.

ii) Fluoride solution.

Dissolve 1.26 gm. of sodium fluoid, 1.36 gm. sodium actate in distilled water and diluted to 100 ml..

iii) Fe-alum solution.

Dissolve 1.2 gm. of ferric alum, 13.76 ml. of sulphuric acid in distilled water and diluted to 100 m..

2) Standard lactose solution.

The working standard is made to contain 0.7 mg. lactose per cc.

3) Determination.

To the sample (5 ml. of solution) add 1 ml. of ferriccyanide solution, heat in a boiling-water bath for exactly 14 minute, and cool. Add 1 ml. of fluoride solution as stabilizer for prussian blue, 2 ml. of Fe-alum solution, and dilute the solution to 20 ml. and then measure the color with a photometer or colorimeter.

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* Rowland: J. Dairy Research **2**. 42. (1938)

** M. Plumel: Ann. biol. chem. (Paris) **9**. 307–17 (1951) *C. A.* 1947. 11082 c./

III. Results and Discussion

Table 1 is the list of individual results.

(*a*) *Quantitave of fat*

i) Fig. 1 presents the average and individual measurement of fat. It shows pretty individual difference among many mother's milk as there are cases with much or less of fat.

Besides, the quantity of fat for an individual changes remarkably.

ii) Why does the quantity of fat for an individual change remarkably?

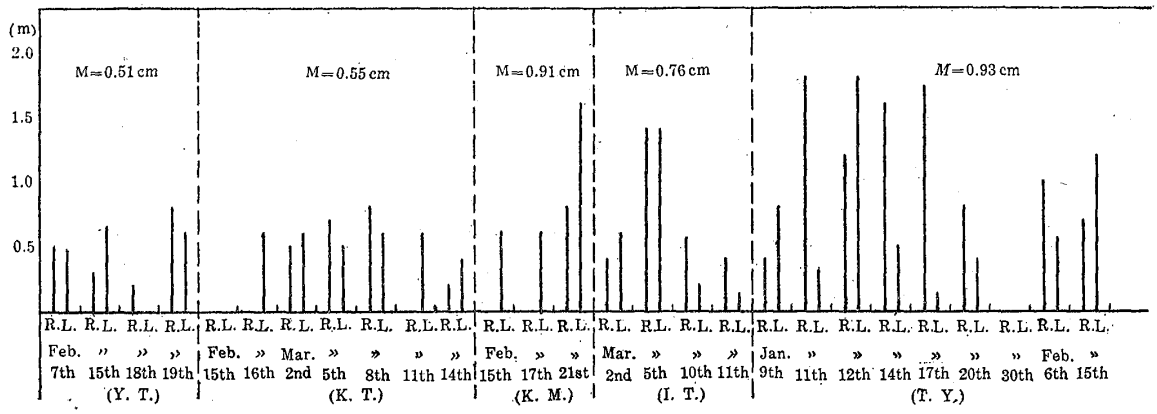

Fig. 1.

As the means to know the reason, we studied the relation between quantities of fat and the time which is needed to full mother's milk in the breast. Relation between quantity of fat and the elapsed time since the last suckling is as Fig. 2.

Fig. 2 shows a tendency that fat is rich when the elapsed time is short, but the milk is thin when the interval becomes longer.

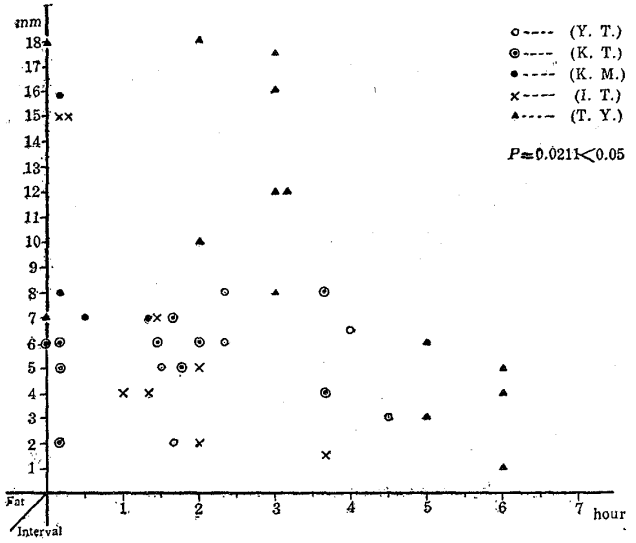

Fig. 2.

Therefore, there are the fact that fat of the milk after suckling is more than that of before. Its tendency is remarkable about case five. (Fig. 2 points)

This tendency can be also recognized in the other cases, though they do not show remarkable changes like case five.

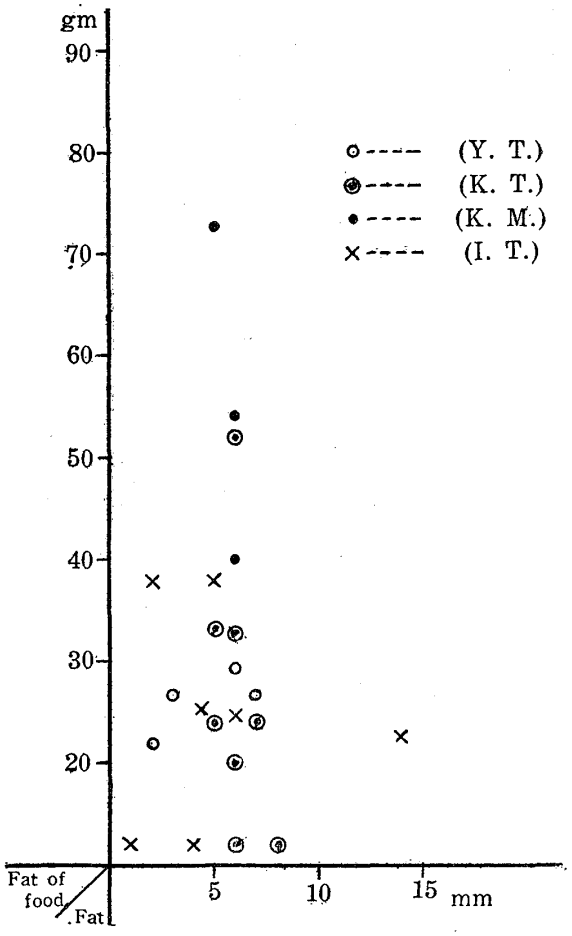

Fig. 3.

But there is clearly some difference between the case five and others. This difference may perhaps due to the fact that in case five it is milked regularly and in the other cases it is not so.

iii) Relation between the milk fat and the fat of food which mother took the day before.

As shown in Fig. 3, it is not recognized any relation between them.

We suppose that the amount of fat is influenced by the interval than the fat of food which mother took.

Yet this relation between the milk fat and the fat of food which mother took, must be studied precisely with other means.

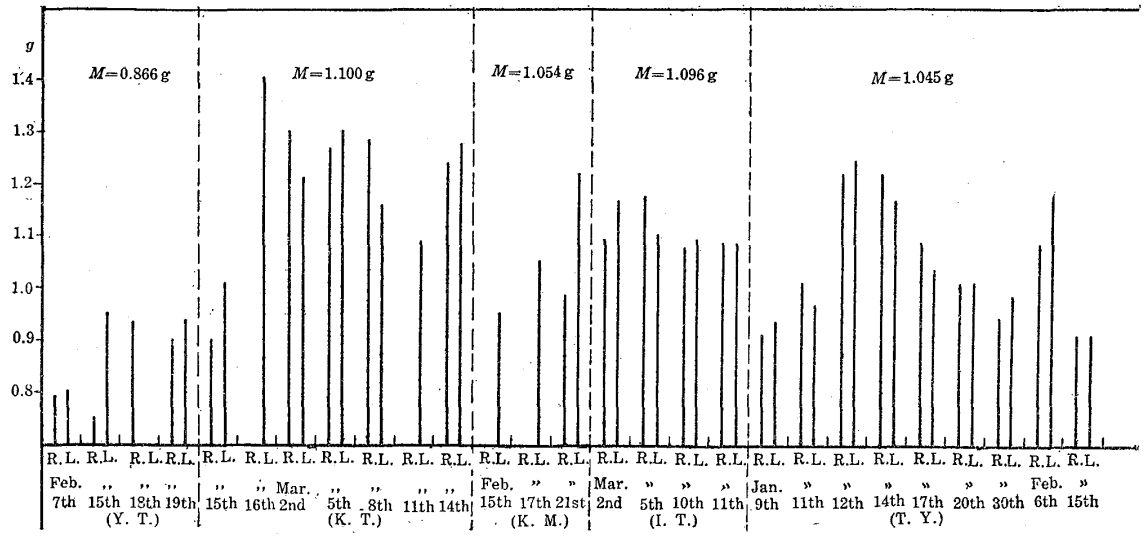

Fig. 4.

Table I.

Name	Mother Age	Delivery number	Infant Age (month)	Suckling method	Family	Measurement date (1955)	Suckling frecuency (a day)	Sleeping hours	Milking time	Before* suckling hour	The interval
Y. T.	26	1	5	Irregularly	Father Mother Children (1)	Feb. 7th	5	9	10:05	R. 8:30 L. 5:00	1:35 5:05
						〃 15〃	5	8	15:00	R. 10:31 L. 12:50	4:29 4:10
						〃 18〃	5	7	11:00	R. 9:20 L. 9:20	1:40 1:40
						〃 19〃	5	9	14:30	R. 12:00 L. 12:00	2:30 2:30
K. T.	28	2	4	〃	Father Mother Children (2)	〃 15〃	9	8	15:00	R. 14:00 L. 14:00	1:00 1:00
						〃 16〃	8	7	8:00	R. L. 6:30	 1:30
						Mar.2nd	9	7.5	12:00	R. 11:50 L. 10:00	:10 2:00
						〃 5th	7	7	10:45	R. 9:00 L. 9:00	1:45 1:45
						〃 8〃	9	5	11:30	R. 7:00 L. 11:00	3:40 :00
						〃 11〃	7	7	8:20	R. 4:00 L. 8:20	4:20 :00
						〃 14〃	8	6.5	10:30	R. 10:20 L. 6:50	:10 3:40
K. M.	34	3	3	〃	Grand f. Grand m. Father Mother Children (3) Aunt	Feb.15〃	9	8	19:50	R. 18:30 L.	1:20
						〃 17〃	9	8	19:10	R. 18:40 L.	:30
						〃 21st			11:20	R. 11:10 L. 11:10	:10 :10
I. T.	25	2	8	〃	Father Mother Children (2)	Mar.2nd	6	7	10:30	R. 9:00 L.	1:30
						〃 5th	7	7	10:45	R. 10:30 L. 10:30	:15 :15
						〃 10〃	7	6.5	9:30	R. 7:30 L. 7:30	2:00 2:00
						〃 11〃	7	8	8:00	R. 7:00 L. 4:20	1:00 3:40
T. Y.	23	1	40 days	Regularly	Father Mother Children (1)	Jan. 9th	8	6	21:00	R. L.	
						〃 11〃	8	7	13:00	R. 11:30 L. 8:30	2:00 5:00
						〃 12〃	9	6	16:00	R. 13:00 L. 16:00	3:00 :00
						〃 14〃	8	6	10:50	R. 7:50 L. 4:50	3:00 6:00
						〃 17〃	8	7	17:00	R. 14:60 L. 11:00	3:00 6:00
						〃 20〃	9	7	7:00	R. 4:00 L. 1:00	3:00 6:00
						〃 30〃	9	7	20:00	R. 17:00 L. 13:30	3:00 6:00
						Feb. 6〃	9	6	21:00	R. 19:00 L. 16:00	2:00 5:00
						〃 15〃	8	7	7:00	R. 7:00 L. 4:00	:00 3:00

* {R. .... Right breast
L. .... Left breast

** The distributions method of casein protein and whey protein nitrogen in the nitrogen of milk

pH	Food			Analysis				Fat/ Protein	Casein/ Abb.+ Glob.	Fat/ Casein	Casein/ Protein × 100
	Protein	Fat	Calolie	Protein gm./ 100 ml.	Casein gm./ 100 ml.	Abb.+ Glob. gm./ 100 ml.	Fat *** mm./ 10 ml.				
7.56	131.6	73.01	3124.7	0.786			5	6.36			
7.47				0.800			5	6.25			
7.57	82.83	27.37	2831.3	0.745			3.3	4.42			
7.57				9.945			6.6	6.99			
7.53	121.95	22.08	3086.6	0.939	0.389	0.550	2	2.11	0.708	5.150	30.7%
7.52	119.63	29.81	3230.7	0.907	0.370	0.537	8	8.83	0.690	21.61	40.7 〃
7.44				0.942	0.315	0.627	6	6.37	0.503	19.05	33.4 〃
7.77	97.46	58.21	2552.2	0.900							
7.59				1.016							
7.53	81.04	50.99	2417.6	1.400			6	4.28			
7.28	121.26	33.49	2052.6	1.299			5	3.86			
7.24				1.181	0.411	0.770	6	5.07	0.534	14.61	34.8 〃
7.41	69.87	24.37	1643.8	1.268	0.483	0.785	7	5.53	0.615	14.49	38.9 〃
7.28				1.293	0.446	0.847	5	3.86	0.527	11.20	34.5 〃
7.68	76.90	12.23	1668.9	1.282	0.557	0.726	8	6.24	0.766	14.34	43.5 〃
7.26				1.157	0.630	0.527	6	5.18	1.194	9.53	54.4 〃
7.32	87.60	20.62	1552.4								
7.78				1.093			6	5.49			
7.55	64.81	84.21	1781.7	1.243			2	1.61			
7.44				1.272			4	3.14			
7.57	119.7	54.73	3611.3	0.958			6.3	6.58			
7.38	77.75	40.89	3169.0	1.052	0.320	0.732	6	5.71	0.437	18.75	30.4 〃
7.32				0.988	0.458	0.530	8	8.08	0.865	17.46	46.3 〃
7.49				1.220			16	13.10			
7.66	78.2	25.80	2884.5	1.098			4	3.64			
7.46				1.156			6	5.19			
7.68	87.5	23.50	2358.0	1.178	0.373	0.805	14	11.89	0.464	37.50	31.6 〃
7.50				1.001			14	13.90			
7.51	83.43	38.65	3096.4	1.180			5.6	5.18			
7.56				1.093	0.544	0.549	2.0	1.83	0.991	36.82	49.8 〃
7.59	55.51	12.27	2107.6	1.082	0.240	0.842	4	3.70	0.284	16.63	22.0 〃
7.40				1.082	0.293	0.789	1.4	1.29	0.359	4.78	27.0 〃
				0.915	0.063**		4	4.37			
				0.926	0.072**		8	8.64			
				1.008			18	17.85			
				0.962	0.092**		3	3.12			
				1.220			12	9.84			
				1.240	0.910**		18	14.50			
				1.222	0.220**		16	13.12			
				1.170	0.200**		5	4.27			
				1.088			17.5.	16.08			
				1.030			1.3	1.09			
				1.007			8	7.98			
				1.008			4	3.97			
				0.942							
				0.987							
				1.080			10	9.27			
				1.180	0.440**		5.8	4.87			
				0.912	0.170**		7	7.68			
				0.910	0.129**		12	13.19			

sample has not been determined.

*** The value measured by the stratum of fat which was separated by the height of the fat layer in the centrifuge tube.

(*b*) *Total protein*

i) Fig. 4 shows individual protein quantity and its average. It shows very close results between four cases: it is nearly in accordance to the results which appear in many literatures. Protein of one case (Y. T.) is lower than average. But we can not give the reason for it.

ii) Protein quantitave change for an individual is not so remarkable as fat.

iii) Relation between protein and protein of food which mother took the day before is as Fig. 5. It is even recognized the tendency that milk protein of the mother who has taken in great quantities of protein the day before, shows lower value. Why does it so?

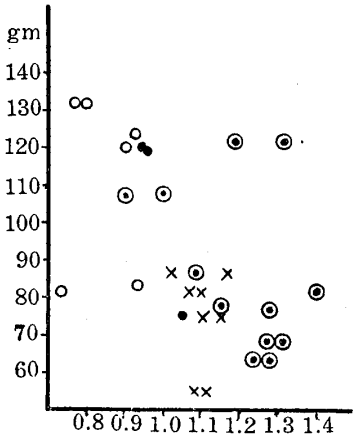

Fig. 5.

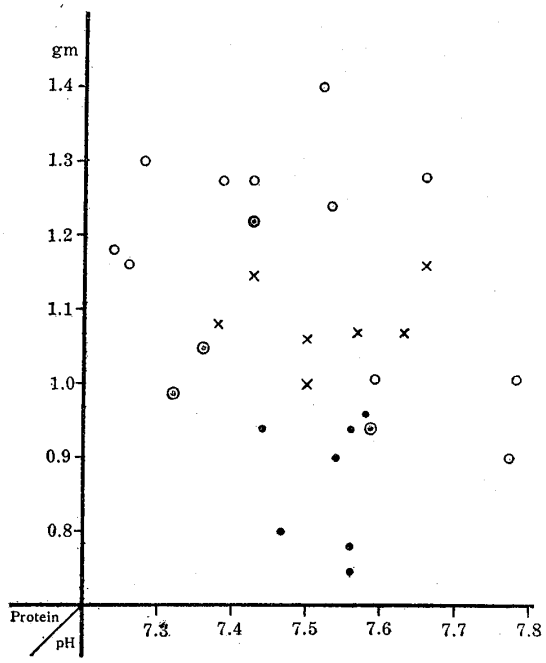

Fig. 6.

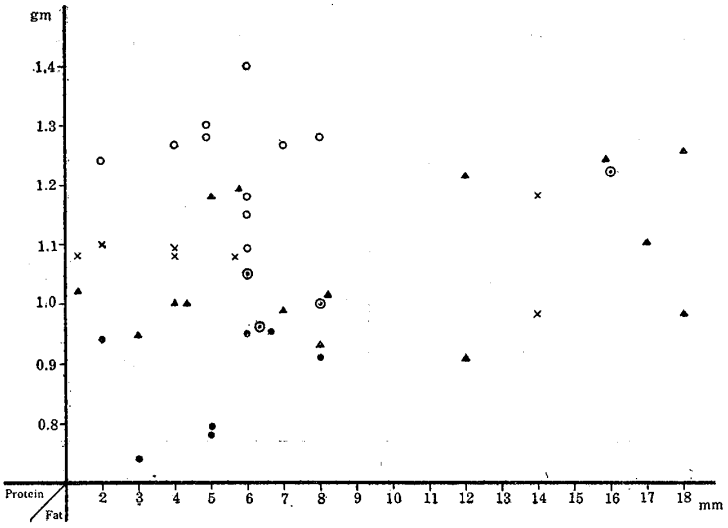

Fig. 7.

iv) Relation between milk pH and protein.

No significant relation between them was recognized. (Fig. 6)

v) In regard to casein, lactalbumin and lactglobulin, we shall publish by increasing the cases some other day.

vi) Relation between milk fat and protein.

No relation between them is recognized as Fig. 7. We are apt to think, if mother's milk contains much fat, it should also contain much protein. But this supposition was denied.

Summary and Conclusions.

The protein of milk shows constant quantity in an individual, although it shows pretty individual difference among many mother's milk. But the quantity of fat changes remarkably in an individual. The quantity of fat is probably dominated by the elapsed time since the last suckling.

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