STUDIES ON THE INORGANIC CONSTITUENTS OF MILK *

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I. CHLORIDS IN HUMAN MILK

In discussions of the composition of human milk and of modifications of cow's milk, much stress is laid on the absolute and relative proportions of fats, carbohydrates and protein, but, as a rule, no mention is made of the concentration of the mineral constituents. As an example, we may cite the fact that in many clinics the most premature infant is given mixtures of whey, which may provide certain advantages in regard to the quality of the protein, but which contain 300 per cent. more chlorids than does human milk. A similar relation exists in connection with several of the other inorganic constituents.

This practice is, of course, based on the assumption that an excess of inorganic material is present even in human milk, and that this excess is not injurious, an assumption not based on experimental evidence. Theoretically, it is, of course, perfectly conceivable that such an excess of salts may not only increase the work of the kidneys to a dangerous degree, but may even induce intestinal disturbances.

Stimulated by recent work which has demonstrated the importance of the mineral elements of the diet, we began about one year ago a study of the mineral constituents of human milk and of the various modifications of cow's milk ordinarily used in infant feeding.

In this paper, which is the first of a series dealing with this subject, we shall consider only findings on the chlorids of human milk.

METHOD OF ANALYSIS

Before attempting the collection of data, it was first necessary to find an analytical method by means of which it would be possible to obtain accurate results with a moderate outlay of time, and with relatively small amounts of material. Most of the milk chlorid determinations recorded in the literature were made either by gravimetric methods, usually from the weight of silver chlorid, or by the Volhard titration. In either case, a considerable volume of milk had to be evaporated to dryness, ashed in a platinum dish at low heat with the

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* From the Children's Medical Department and the Clinical Laboratory of the Massachusetts General Hospital.

help of an alkaline fusion mixture, and the ash then extracted with water. Such a process was tedious and time consuming, and one requiring an elaborate equipment and considerable chemical experience. The micro methods, which during the past ten years have been applied so extensively to the chemical study of the blood, can be used, in many cases, with slight modifications for the examination of milk.

Two methods for the determination of chlorids in blood are in current use, namely, Rappelye's¹ modification of the Volhard titration, and the idonitric titration of McLean and Van Slyke.²

An attempt to utilize Rappelye's modified Volhard titration was unsuccessful because we were unable by means of it to obtain complete precipitations of the milk protein. We, therefore, have made use of the McLean-Van Slyke technic, with a few modifications made necessary by the nature of our problem.

Attempts to employ the Van Slyke-Donleavy³ modifications, which involve the use of picric acid as a protein precipitant in place of the magnesium sulphate-acetic acid mixture of the original method, were unsuccessful, as it was found impossible also to obtain complete precipitation of the milk proteins in human milk by this technic. It was found, however, that if the proteins were first precipitated by means of a saturated solution of picric acid containing a little acetic acid and the acid silver nitrate solution then added without preliminary filtration, a clear filtrate could almost invariably be obtained.

In view of the fact that human milk contains only one-tenth as much chlorin as blood, it was necessary to make a few changes in the quantity and concentrations of the reagents. Although no claim for originality is made in this connection, it has seemed wise to furnish a brief description of our technic.

The titration as used by us, is as follows: To 3 c.c. of human milk contained in a 100 c.c. Erlenmeyer flask is added 9 c.c. of a 1.2 per cent. solution of picric acid containing 2 c.c. of glacial acetic acid per liter. After the mixture has stood for five minutes, 3 c.c. of the standard nitrate-nitric acid solution is added. After ten minutes the liquid is poured on a chlorid free filter. To 10 c.c. of the clear filtrate is added 2 c.c. of the starch indicator, and the excess of silver is then titrated by means of standard potassium iodid solution.

As it seemed best in this work to express our results in terms of milligrams of chlorin per 100 c.c. of milk, we have, in order to avoid the use of a factor, made a slight change in the concentrations of the

1. Rappelye, W. C.: *J. Biol. Chem.* **25**:509, 1918.

2. McLean, F. C., and Van Slyke, D. D.: *J. Am. Chem. Soc.* **37**:1128, 1915.

3. Van Slyke, D. D., and Donleavy, J. J.: *J. Biol. Chem.* **37**:551, 1919.

standard silver nitrate and standard potassium iodid solutions prescribed by McLean and Van Slyke.

SOLUTION 1

Silver nitrate 4.780 gm.
Nitric acid, sp. gr. 1.42..... 250. c.c.
Distilled water, add to.....1,000 c.c.

One c.c. of this solution is equivalent to 1 mg. chlorin.

SOLUTION 2

Potassium iodid 1.5 gm.
Water, add to.....1,000. c.c.

This solution is standardized against the silver nitrate and so diluted that 20 c.c. are equivalent to 5 c.c. of the silver solution.

SOLUTION 3

This consists of a solution of starch, sodium citrate and sodium nitrate made up according to the directions of Van Slyke and Donleavy.

It has recently been shown by Austin and Van Slyke⁴ that the Van Slyke-Donleavy procedure, when used on whole blood, gives results greatly in excess of the truth. To remedy this they suggest the preliminary precipitation of proteins by means of picric acid, and the removal of the precipitate so obtained before the addition of the standard silver nitrate solution. Although our work on the subject had been concluded before the publication of this paper, we have made a few determinations by this method, and have, by means of it, obtained results essentially similar to those obtained by the technic described above.

TABLE 1.—COMPARISON OF RESULTS OBTAINED BY COAGULATION AND BY FUSION METHODS

Sample	Source	Mg. Chlorin by Coagulation	Per 100 C.c. Milk by Fusion
1	Human.....	75	77
2	Human.....	81	82
3	Human.....	32	35
4	Human.....	47	47
5	Goat.....	165	165

The corrections of our results have also been checked by means of Volhard titration used after the removal of the organic material by means of fusion with sodium carbonate.⁵ In Table 1 a comparison of the results of determinations of the chlorin content of six samples of milk made by coagulation and fusion methods is shown.

4. Austin, J. H., and Van Slyke, D. D.: J. Biol. Chem. **41**:345, 1920.

5. Bull. 107 (revised), U. S. Dept. Agriculture.

METHOD OF COLLECTION OF SPECIMENS

The specimens were collected by expressing the milk manually from the nipple into specially washed bottles. A breast pump was used in some instances, and it was found that the mechanical stimulation produced by this process caused no change in the chlorin content of the secretion. The nipples were carefully washed with water before collections were made and the use of boric acid and similar solutions was avoided.

The specimens were obtained in most instances about three minutes after the beginning of nursing, when the flow of milk had been established.

TABLE 2.—SHOWING CHLORIN CONTENT OF "FORE" AND "AFTER" MILK FROM NORMAL WOMEN

Number	Mg. Chlorin in "Fore" Milk	Mg. Chlorin in "After" Milk	Period of Lactation, Weeks
21-22.....	53.0	64.0	1
23-24.....	52.0	61.0	1
25-26.....	25.9	34.0	2
27-28.....	48.3	32.7	1
29-30.....	71.0	74.5	1
31-32.....	14.1	42.0	2
33-34.....	43.0	34.0	2
35-36.....	107.0	96.6	1
45-46.....	59.0	50.0	7
52-53.....	93.0	10.2	7
62-63.....	72.0	10.2	2
86-87.....	40.0	54.0	30
102-103.....	76.5	40.6	1
127-128.....	42.0	42.0	3
316-317.....	40.8	36.0	9
318-319.....	36.0	35.0	9
320-321.....	37.8	37.8	9
322-323.....	41.4	38.4	9
312-313.....	29.4	30.0	52
314-315.....	36.0	28.8	52
177-178.....	22.9	29.8	17
168-169.....	75.0	87.0	3
149-150.....	48.0	54.0	11
143-144.....	51.0	51.0	32
127-128.....	42.0	42.0	3
125-126.....	42.0	47.0	3
14-15.....	28.2	24.6	10

This method was adopted as larger specimens were obtained with less difficulty, and was used only after we had satisfied ourselves that no constant differences were noted in the "fore" and "after" milk, as can be seen from Table 2. The specimens used in this table were obtained from women who had large amounts of milk.

SOURCE OF MATERIAL

The specimens of milk were obtained from various sources, namely, from the Outpatient Department of the Massachusetts General Hospital, the Boston Lying-In Hospital, the Clinics of the Boston Baby Hygiene Association, the Boston Wet Nurse Directory, and from private patients.

In Table 3 we have arranged, according to the period of lactation, the results obtained on all the 327 samples of milk examined. In many instances, the results represent averages of as many as ten specimens collected from one woman. Figure 1 shows a curve of the average concentration of chlorin and the maximum variation for each week as based on the results in Table 3.

TABLE 3.—AVERAGE OF RESULTS ON DETERMINATION OF CHLORIN IN 327 SPECIMENS OF HUMAN MILK TAKEN FROM THE FIRST TO THE SEVENTY-FOURTH WEEK OF LACTATION

Mg. Chlorin per 100 C.c. of Milk																	
1st	2d	3d	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	15th	16th	17th	18th	
Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.
120.0	30.0	49.0	73.5	21.9	64.5	54.3	36.2	44.7	50.0	31.0	48.0	96.0	112.0	71.0	60.0	75.0	
81.5	28.0	39.7	86.0	49.0		71.9		49.2	26.9	43.3		73.8	45.7	105.0	111.0	40.8	
36.0	41.0	36.0	69.0	39.0		36.0		60.8	52.0	51.0		60.0	43.8		47.0		
81.0	114.0	37.2	30.0	43.5		39.0		38.0		61.5		42.0			28.3		
61.0	117.0	97.0	72.8	31.0		45.0									33.0		
111.0	111.0	94.0	77.7												34.0		
51.0	111.0	44.5	82.0														
46.5	99.0	42.0	36.0														
58.5	60.0	42.0															
61.0	87.0	81.0															
40.5	38.9	52.0															
72.7	75.0	55.1															
101.8	49.8	40.6															
75.0	58.6																
111.4	102.3																
42.0	65.0																
158.3	56.0																
78.0	50.6																
	60.0																
20th	21st	22d	23d	24th	26th	28th	29th	34th	39th	43d	47th	48th	51st	52d	74th		
Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	Wk.	
38.2	30.0	43.5	72.5	43.8	19.5	38.4	53.6	82.8	36.6	23.4	67.0	49.2	31.0	60.0	34.8		
47.3	66.0	106.0	24.0		52.2		56.7	73.8						18.0			
45.4	40.2	24.9												35.6			
54.0	42.0	41.1															
		101.3															

The results presented in Table 3 show that our average after the first two weeks of lactation is 52.6 mg. chlorin per 100 c.c. of milk. The average, if all periods of lactation are considered, is 58.2. This approaches the average results to be found in the literature as completed by Holt, Courtney and Fales.⁶ As has been noted formerly, there is a tendency toward slightly high concentration of chlorin to occur during the first week, as shown in the accompanying illustration, after which the average remains about constant up to the seventeenth month.

Extreme variations were noted in the chlorin content of specimens from women in the same period of lactation and in specimens collected from individuals during a single day. Table 3 and the second curve in the illustration are based on averages, and show, in a measure, only the

6. Holt, L. E.; Courtney, A. M., and Fales, H. L.: *Am. J. Dis. Child.* **10**: 229 (Oct.) 1915.

frequency and the degree of these variations. In some instances, there was more than 300 per cent. difference in the chlorin content in individual specimens taken from one woman during twenty-four hours, more than 1,000 per cent. in specimens from different women at the same period of lactation. Figures as low as 14.1 mg. and as high as 156.8 have been obtained. This extreme variation may be accounted for by the fact that the specimen of milk was obtained from a woman who had had extreme symptoms of one of the toxemias of pregnancy and also because of her small daily output of milk. Variations nearly as great, however, may occur between specimens from normal women in the same period of lactation. In searching for a possible cause for these extreme variations of chlorin content in human milk, the data have been studied from different points of view. The influence of the

Curves of maximum (upper curve), average (middle curve) and minimum (lower curve) amounts of chlorin in human milk.

period of lactation, of the volume of the secretion, of the time of collection during the day, of the ingestion of food and of psychic stimuli have been considered. As a basis for comparing the possible effects of these factors, it was thought advisable to establish, first, a normal figure. For this purpose, we have collected the results obtained on the milk of women whose environment, diet and temperament were most accurately known. These data were obtained largely from private patients living under most favorable circumstances and who were considered to be normal, physically and psychically, and who were giving an adequate amount of milk of such a quality that their babies developed normally for a period of at least six months.

The results obtained on the milk of these selected subjects are presented in Table 4.

TABLE 4.—CHLORIN CONTENT OF SPECIMENS FROM NORMAL SUBJECTS (LACTATING TYPES)

Name	Number	Mg. Chlorin per 100 C.c. of Milk			Period of Lactation, Weeks	Approximate Amount of Milk in 24 Hours, C.c.
		Before Nursing	After Nursing	Middle Nursing		
Mrs. W.	2	47.4			11	750
Mrs. W.	3		39.3		..	750
Mrs. T.	25	25.9			..	750
Mrs. T.	26		34.2		2	750
Mrs. H.	13			36.0	3	600
Mrs. H.	88			32.0	10	750
Mrs. S.	16	37.2			3	680
Mrs. St.	45	59.0			7	720
Mrs. St.	46		50.0		..	750
Mrs. St.	149	48.0			11	450
Mrs. St.	150		54.0		11	750
Mrs. R.	92			43.0	5	750
Mrs. Ra.	98			44.0	5	750
Mrs. F.	108		49.4		20	900
Mrs. F.	109		45.3		20	900
Mrs. F.	132			47.0	20	900
Mrs. F.	133	47.0			20	900
Mrs. F.	134	42.0			20	900
Mrs. F.	135	47.0			20	900
Mrs. F.	136	47.0			20	900
Mrs. F.	138	42.0			20	900
Mrs. F.	139	36.0			20	900
Mrs. F.	140	37.0			20	900
Mrs. F.	141	35.0			20	900
Mrs. F.	143	51.0			30	750
Mrs. F.	144		51.0		30	750
Mrs. I.	312	29.4			52	1,200
Mrs. I.	313		30.0		52	1,200
Mrs. Str.	298			30.0	5	750
Mrs. Str.	299			31.0	5	750
Mrs. Str.	300			32.0	5	750
Mrs. Str.	301			32.0	5	750
Mrs. Str.	302			32.0	5	750
Mrs. Str.	303			30.0	5	750
Mrs. Str.	304			30.0	5	750
Mrs. Wi.	294			30.0	24	750
Mrs. K.	251	40.2			22	750
Mrs. B.	201	38.4			28	750
Mrs. Kv.	166	40.8			17	840
Mrs. Q.	125	42.0			3	750
Mrs. Q.	126	47.0			3	750
Mrs. D.	101	30.0			4	750
Mrs. Sp.	14	24.6			10	600
Mrs. Sp.	15		28.2		10	600
Mrs. G.	316	40.8			9	630
Mrs. G.	317		36.0		9	630
Mrs. G.	318	37.0			9	630
Mrs. G.	319		35.0		9	630
Mrs. G.	320	37.8			9	630
Mrs. G.	321		37.8		9	630
Mrs. G.	322	41.4			9	630
Mrs. G.	323		38.4		9	630
Mrs. Sti.	324			30.0	39	1,100
Mrs. Sti.	325			32.5	..	750
Mrs. Sti.	326			34.0	..	750
Mrs. Sti.	327			40.0	..	750

From the results presented in Table 4, it will be seen that the chlorin content of milk from so-called strictly normal subjects has a relatively narrow range of variation, after the first week of lactation, namely, from 30 to 50 mg. per 100 c.c. of milk (average 38.1 mg.). Attention should also be called to the fact that in these subjects little variation is noted in specimens taken before and after nursing and at

various periods during the day. The effect of the period of lactation on the amount of chlorin in human milk can be seen from the determinations recorded in Tables 3 and 4. Table 3, in which specimens from all types of patients are considered, shows that during the first two weeks of lactation there is a definite tendency for the chlorin content to be high, and that after this period no constant change takes place. Specimens with high and with low chlorin content will be found to occur at all stages of lactation. Table 4, on the other hand, shows that in "normal" subjects such variations do not occur during the first six

TABLE 5.—CHLORIN CONCENTRATION OF SPECIMENS OF HUMAN MILK FROM NORMAL WOMEN COLLECTED AT INTERVALS DURING THE DAY

Number	Name	Time	Amount Collected, Oz.	Daily Amount, Oz.	Mg. Chlorin	Period of Lactation, Weeks
210	Mrs. L.	6 a.m.	...	25%	43.7	21
211	Mrs. L.	9 a.m.	...		37.5	21
212	Mrs. L.	12 m.	...		36.0	21
213	Mrs. L.	3 p.m.	...		39.0	21
214	Mrs. L.	6 p.m.	...		45.0	21
215	Mrs. L.	9 p.m.	...		41.4	21
192	Mrs. F.	8 a.m.	6%		15.0	26
198	Mrs. F.	8 p.m.	6½	34½	19.5	26
186	Mrs. A.	6 a.m.	3½		32.0	17
187	Mrs. A.	9 a.m.	4%		40.8	17
188	Mrs. A.	12 m.	2		32.0	17
189	Mrs. A.	3 p.m.	2%		31.8	17
190	Mrs. A.	6 p.m.	3		31.8	17
191	Mrs. A.	3 p.m.	2½		32.0	17
170	Mrs. W.	6 a.m.	5%	35	28.8	22
171	Mrs. W.	9 a.m.	8		27.0	22
172	Mrs. W.	12 n.	4%		24.0	22
173	Mrs. W.	3 p.m.	...		26.8	22
174	Mrs. W.	6 p.m.	5		22.8	22
175	Mrs. W.	9 p.m.	...		26.8	22
176	Mrs. T.	3 p.m.	...		32.3	22
177	Mrs. T.	12 n.	...		26.3	22
134	Mrs. F.	12 n.	...		44.5	22
136	Mrs. F.	3 p.m.	...		47.0	22
64	Mrs. Q.	12 m.	...	25	108.0	1
65	Mrs. Q.	3 p.m.	...		108.0	1
66	Mrs. Q.	6 p.m.	...		108.0	1
67	Mrs. Q.	10 p.m.	...		108.0	1
68	Mrs. Q.	2 a.m.	...		112.0	1
69	Mrs. Q.	5 a.m.	...		117.0	1
70	Mrs. Q.	9 a.m.	...		112.0	1
316	Mrs. G.	3 p.m.	...		38.4	12
318	Mrs. G.	6 p.m.	...		36.0	12
320	Mrs. G.	10 p.m.	...		37.8	12
322	Mrs. Q.	6 a.m.	...		39.9	12

months of lactation. From these observations we feel justified in concluding that after the first two weeks of milk secretion the concentration of chlorin in human milk is not related to the period of lactation. The effect of the volume of the milk and of the time of collection during the day on the concentration of the chlorids is shown in Table 5. The subjects represented in this table were wet nurses at the Boston Wet Nurse Directory, who were normal mothers giving relatively large amounts of milk. It will be noted that the average figure, as in the table of "normal" subjects, is about 40 mg., and that the chlorin concentration of the milk varies little in the specimens collected at three-

hour intervals during the day and bears no constant relation to the volume of milk obtained at three hour periods. There is, however, a tendency for somewhat higher figures to occur in the early morning and late evening specimens.

In Table 6 we have collected all the specimens of milk with the chlorin concentration above 50 mg. per 100 c.c. Although the subjects from whom these specimens were secured, were not known to us personally in all instances, they were mothers who were giving an amount of milk inadequate for the growth of the infant. From Tables 5 and 6 it will thus be seen, that after the supply of milk has been established, normal mothers, giving relatively large amounts of milk, always have between 30 and 50 mg. chlorin per 100 c.c. of milk, that this concentration does not vary constantly with the amount of milk obtained at three hour periods and that high chlorin concentrations tend to occur among mothers who for some reason cannot be regarded as "lactating types."

TABLE 6.—CHLORIN CONTENT OF SPECIMENS OF NORMAL SUBJECTS OF NONLACTATING TYPE

Number	Name	Mg. Chlorin per 100 C.c.	Period of Lactation, Weeks	Daily Approximate Amount of Milk, C.c.
295	Mrs. S.	82.0	4	210
308	Mrs. S.	69.0	6	180
309	Mrs. S.	60.0	6	180
289	Mrs. P.	114.0	22	300
290	Mrs. P.	102.0	22	300
291	Mrs. P.	98.0	22	300
257	Mrs. S.	90.6	5	200
258	Mrs. S.	114.0	5	200
227	Mrs. W.	67.0	48	15 oz. 450
168	Mrs. H.	75.0	3	15 oz. 450
169	Mrs. H.	87.0	3	15 oz. 400
197	Mrs. H.	77.7	5	12 oz. 360
117	Mrs. S.	96.0	13	10 oz. 350
59	Mrs. P.	112.0	15	250
248	Mrs. P.	51.6	24	120
249	Mrs. P.	52.8	24	120
54	Mrs. P.	86.0	4	15 oz. 400
89	Mrs. W.	59.0	4	10 oz. 300

The results presented in Table 5 indicate also that the chlorin content of milk is not influenced by the food as the values of milk chlorids in the first morning nursing, when no food had been taken since the evening of the previous day, were essentially the same as those obtained in the late afternoon and early evening, a time when one would expect the effects of ingested food to be apparent. It is, of course, conceivable that the chlorid content of milk might be altered by feeding diets poor or rich in sodium chlorid for relatively long periods, or by the administration of relatively large amounts of the same for short periods. For lack of suitable experimental material, it was impossible, unfortunately, to extend this line of inquiry in connection with our studies of human

milk. We have, however, carried out a considerable number of experiments dealing with this subject on goats and cows, the results of which work will be published at an early date.

TABLE 7.—CHLORIN CONTENT OF MILK FROM "UNSTABLE" SUBJECTS

Number	Name	Mg. Chlorin per 100 C.e. of Milk			Approximate Daily Amount of Milk, C.e.	Period of Lactation, Weeks
		Middle Nursing	Before Nursing	After Nursing		
8	Mrs. R.		25.0		600	11
9	Mrs. R.			37.1	600	11
10	Mrs. R.		32.0		600	11
11	Mrs. R.			64.0	600	11
102	Mrs. H.			40.6	350	1
103	Mrs. H.		76.5		350	1
180	Mrs. B.	30.0				
184	Mrs. B.		68.0		600	3
185	Mrs. B.			60.0		
198	Mrs. S.		45.0		600	13
199	Mrs. S.			102.7		13
240	Mrs. S.	72.0				17
241	Mrs. S.	66.0				17
216	Mrs. N.		40.0		600	3
219	Mrs. N.	78.0			600	3
47	Mrs. D.		90.0		250	4
48	Mrs. D.		57.0		250	4
309	Mrs. S.		41.4		700	5
310	Mrs. S.			31.2	700	5
296	Mrs. M.		48.0			2
297	Mrs. M.			72.0		2
268	Mrs. K.	50.0				2
269	Mrs. C.	50.0				2
		55.0			700	2
272	Mrs. C.	84.0			700	2
273	Mrs. C.	47.0			700	5
275	Mrs. C.	49.0			700	2
282	Mrs. G.	49.0			700	5
283	Mrs. G.	49.0				5
284	Mrs. G.	54.0				5
285	Mrs. G.	59.0				5
286	Mrs. G.	42.0				5
264	Mrs. I.	36.0				5
265	Mrs. I.	43.8				3
266	Mrs. I.	49.8				3
267	Mrs. I.	33.0				3
221	Mrs. N.	72.0			600	3
205	Mrs. N.	49.8			600	2
216	Mrs. N.	40.0			600	3
217	Mrs. N.	43.8			600	3
218	Mrs. N.	42.0			600	3
219	Mrs. N.	78.0			600	3

In view of the well recognized effect of psychic stimuli on the composition of the urine and of the saliva, it seemed possible that this factor might be a cause for the variation in the concentration of chlorin in human milk. It was noted that in most instances the variations were found in specimens from women whose babies were growing unsatisfactorily, and who for this or other reasons may be classified as irritable or emotional mental types. In some instances, the daily volume of milk was sufficient for the baby but was subject to considerable variation. In Table 7 we have arranged the results of the analyses of specimens of milk from these so-called "unstable" types. It will be seen that in the milk of such mothers variations of as much as 100 per cent. occur in the chlorin content of specimens of milk obtained at different periods.

SUMMARY

A study of the chlorin content of 327 specimens of human milk has been made by the use of the McLean-Van Slyke titration method. It has been shown from these determinations that the average chlorin content of human milk from all types of mothers and at various stages of lactation is 38.1 mg. per 100 c.c. of milk. It has been noted that during the first two weeks of lactation, the chlorin content of human milk is somewhat higher. Specimens containing high and low chlorin concentration may be found in all stages of lactation. It has been found that the milk of strictly normal women giving a normal amount of milk has a relatively narrow range of variation in the chlorin concentration of their milk.

Observations on the relation of ingestion of food, of the time of collection of the specimens, of the volume of secretion and the effect of psychic stimuli, to the variation in the chlorin concentration of human milk have been made. It was found that in normal subjects the food intake and the time of day that the collection is made cause no outspoken change in the chlorin content of the milk. There is a slight tendency toward somewhat higher concentrations in specimens collected in the early morning and late evening. In women whose daily output is very large, there is an unmistakable tendency to the production of milk of a low chlorin content, and conversely those with a small amount of milk more frequently secrete milk containing a relatively high concentration of chlorids. This fact probably accounts for the statements in the literature to the effect that the concentration of the mineral constituents of the milk increase with each month of lactation, a finding which would, of course, go hand in hand with the decreased daily volumes usually noted in the later stages of milk production.

Our results would seem to indicate that the chlorin content of the milk can be affected markedly by psychic stimuli, as in phlegmatic mothers living under good conditions the chlorin content of the secretion remains constant from day to day and hour to hour. In the case of mentally unstable types, women also living under favorable conditions, large variations, frequently amounting to as much as 100 per cent., may occur. It has been noted that the babies with mothers of this type very frequently show evidences of intestinal indigestion and that as a rule the mothers are able to nurse their babies for relatively short periods.

CONCLUSIONS

1. The "salt" content of human milk is subject to great variations during all periods of lactation.
2. Estimation of the chlorin content of human milk can be obtained only by examining specimens from all nursings during the day.

3. The average chlorin concentration of milk from all types of women at all periods of lactation is 58.2 mg. per 100 c.c. of milk; the average after the second week of lactation is 52.6 mg.

4. "Normal" mothers, giving large amounts of milk, show a minimum variation in the chlorin concentration of their milk, with an average of 38.1 mg.

5. The greatest variation in the chlorin content of milk is found in specimens from "nervous" women.

6. Diet is not a cause for the variation in the chlorin concentration of human milk.

7. The chlorin concentration of milk from women, after the second week of lactation, who give large amounts of milk tends to be low and conversely the milk from women giving small amounts of milk is high in chlorin.