Chlorine Content of Mother's Urine and Their Arakawa's Reaction.

136th Report of the Peroxidase Reaction.
(83rd Human Milk Study.)

By

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Introduction.

In 1934 it was published by Nozaki¹⁾ (Cf. 46th Report of the Peroxidase Reaction) from this Laboratory as a surprising and unexpected fact that human milk positive to Arakawa's reaction* is a chlorine-poor milk, and that Arakawa-negative milk is a chlorine-rich milk. This assertion has lately been confirmed by Ishii (Cf. 87th Report of the Peroxidase Reaction),²⁾ who made a further investigation into the relation in a much larger number of materials. Now I thought, if the amount of milk chlorine is considerably different according as lactating mothers are Arakawa-positive or -negative, then what would become of that surplus of the salt corresponding to the difference? Of course they must be probably taking an approximately equal amount of chlorine, for it is utterly impossible that mothers with milk positive to Arakawa's reaction are daily taking much more table salt than

1) J. Nozaki, Tohoku J. Exp. Med., 1934, 23, 60.

* Arakawa-positive may be used in two different senses. One of these is: Arakawa-positive in a biochemical sense. A sample of human milk is said to have become Arakawa-positive, when it becomes blue on the addition of Arakawa's reagent. There it means that the sample is not negative to Arakawa's reaction. Arakawa-positive in a clinical sense: A sample of human milk is clinically Arakawa-positive only when it shows such a reaction as ++ or ## in one minute of the addition of Arakawa's reagent. Another sample of human milk may be Arakawa-positive in the first described sense, but yet clinically negative.

2) M. Ishii, Tohoku J. Exp. Med., 1937, 31, 580.

Arakawa-negative mothers. So I wanted to investigate the relation between chlorine content of mothers' urine and their Arakawa's reaction.

Experimental Method.

The method which has generally been used for the volumetric micro determination of chlorine in biological fluids in our Laboratory is that of Rusznyak³⁾ for blood chlorine. I applied, for reasons of economy, the micro method to human urine using 0.1 c.c., and the following application of the method of Rusznyak was found to be satisfactory for my purpose.

The principle is the oxydation of the organic substances in urine with potassium permanganate, and then the precipitation of the chlorides by a standard solution of silver nitrate and the subsequent titration of the excess silver nitrate by ammonium thiocyanate, using iron alum as an indicator. The end point is the first permanent red tint due to the formation of ferricyanate.

Procedure.

In a 20 c.c. volumetric flask place 0.1 c.c. of urine accurately measured, 4 c.c. of 0.01 N silver nitrate solution, 1 c.c. of concentrated nitric acid. Boil gently with a drop of potassium permanganate solution 1 N until it becomes tinged with rust colour, and add a small amount of pure grape sugar powder until the colour disappears. Cool the flask in running water or at room temperature and titrate the excess silver nitrate with 0.01 N ammonium thiocyanate solution, adding a few drops of saturated iron alum as an indicator.

Determination.

Subtract the number of c.c. of ammonium thiocyanate used in the titration from the number of c.c. of 0.01 N silver nitrate solution; the difference is the volume of the silver solution used to precipitate the chlorine in 0.1 c.c. of the urine examined. Since 1 c.c. of the silver nitrate solution is equivalent to 0.00035 grms. of chlorine, the calculation is as follows:—

$$\frac{(a-b) \times 0.000355}{0.1} \times 100 = \text{chlorine (grms.) in 100 c.c. of urine}$$

a = the number of c.c. of silver nitrate solution.

b = the number of c.c. of ammonium thiocyanate.

3) St. Rusznyak, Biochem. Z., 1921, 114, 23.

Accuracy.

To test the accuracy of the method, determinations were made of the chlorine content of the urine in a number of cases. Then a theoretical solution of sodium chloride was added and determinations were performed again. The figures are given below (Cf. Tables 1 and 2):—

TABLE 1.
*Recovery of NaCl added to
human urine.*

No. of sample	Determined (grms.)	Added (grms.)	Recovered (grms.)	Theoretical value (grms.)
1	1.26	0.100	1.36	1.36
2	0.77	0.100	0.88	0.87
3	1.53	0.100	1.64	1.63
4	1.06	0.100	1.16	1.16
5	1.42	0.100	1.52	1.52
6	0.88	0.100	0.98	0.99

TABLE 2.
*Comparison of results obtained
with Volhard-Salkowski's
and author's method.*

No. of sample	Volhard- Salkowski's method (grms.)	Author's method (grms.)
1	1.04	1.02
2	0.61	0.59
3	1.45	1.44
4	1.45	1.40

As shown in Tables 1 and 2, the present method is not so exact as the macro method, but it is sufficiently accurate for the present purpose (see later).

Materials.

All the 202 urine samples were mostly from mothers of out-patients of our Department (Cf. Table 3). Cases of mastitis, mammary tumours and cases with menstruation just at the time of examination were excluded. The urine was obtained in the morning (9–10 a.m.). At the same time mother's milk was examined with Arakawa's reagent in each case. Cases within one month after the delivery were discarded from this paper.

Result of Experiment.

As is seen from Table 3 (Cf. Table 3), chlorine content in urine samples from mothers is subject to a very remarkable variation, but it is interesting to find in Tables 5 and 6 (Cf. Tables 5 and 6) that the samples of Arakawa-negative mothers are the poorest in chlorine content (0.70 grms. per 100 c.c. on an average). Samples of intermediate reactions show intermediate values (0.882, 0.893, 0.990, 0.820, 0.885,

TABLE 3.
Chlorine content of urine of lactating mothers.

No. of experiment	Month of experiment	Age of mother or infant y=years m=months	Side of breast R=right L=left	Arakawa's reaction	Chlorine (grms. per 100 c.c.)
1	X (1936)	28 y 17 m	R L	1(+) [±] 1(+)	0.91
2	"	30 y 8 m	R L	6(±) 2(+)	0.86
3	"	19 y 6 m	R L	1(+) 4(-)	0.93
4	"	27 y 2 m	R L	7(±) 7(±)	0.62
5	"	25 y 12 m	R L	16(-) 1(±)	0.71
6	"	25 y 18 m	R L	7(±) 16(-)	0.89
7	"	19 y 11 m	R L	4(+) 5(+)	0.78
8	"	25 y 2 m	R L	10(-) 6(±)	1.04
9	"	36 y 8 m	R L	10(-) 10(-)	1.00
10	"	32 y 2 m	R L	7(±) 7(±)	0.43
11	"	27 y 11 m	R L	16(-) 16(-)	0.50
12	"	29 y 14 m	R L	4(-) 4(-)	0.77
13	"	25 y 7 m	R L	12(-) 16(-)	1.06
14	"	38 y 18 m	R L	11(-) 3(-)	0.99
15	"	25 y 2 m	R L	16(-) 16(-)	0.71
16	"	20 y 9 m	R L	2(+) 3(+)	1.32
17	"	25 y 9 m	R L	14(-) 16(-)	0.67
18	"	26 y 2 m	R L	7(+) 3(±)	0.99
19	"	23 y 1 m	R L	2(-) 2(-)	0.78
20	"	26 y 14 m	R L	2(-) 16(-)	0.68
21	"	38 y 13 m	R L	3(+) 3(+)	0.89
22	"	39 y 5 m	R L	9(+) 9(±)	0.96
23	"	27 y 6 m	R L	10(-) 14(-)	0.79

No. of experiment	Month of experiment	Age of mother or infant y=years m=months	Side of breast R=right L=left	Arakawa's reaction	Chlorine (grms. per 100 c.c.)
24	"	21 y 23 m	R L	7(±) 12(-)	0.80
25	"	30 y 4 m	R L	1(+) 3(+)	0.99
26	"	25 y 5 m	R L	1(±) 1(±)	0.89
27	"	29 m 14 m	R L	11(-) 3(-)	0.82
28	"	27 y 3 m	R L	11(±) 9(+)	0.92
29	"	31 y 14 m	R L	1(+) 7(-)	0.79
30	"	31 y 6 m	R L	1(+) 2(+)	1.08
31	"	31 y 1 m	R L	16(-) 15(-)	0.48
32	"	23 y 13 m	R L	15(-) 15(-)	1.08
33	"	28 y 20 m	R L	11(±) 11(±)	0.89
34	"	25 y 4 m	R L	16(-) 16(-)	0.74
35	"	24 y 17 m	R L	4(+) 4(+)	1.21
36	"	21 y 12 m	R L	4(+) 4(+)	1.17
37	"	20 y 4 m	R L	9(+) 5(±)	0.39
38	"	28 y 1 m	R L	* 5(±)	0.84
39	"	33 y 2 m	R L	*** 16(-)	0.53
40	"	22 y 4 m	R L	5(+) 5(+)	0.97
41	"	28 y 2 m	R L	13(-) 13(-)	0.92
42	"	25 y 2 m	R L	2(-) 16(-)	0.57
43	"	27 y 12 m	R L	7(+) 5(±)	0.92
44	"	41 y 6 m	R L	1(±) 1(±)	0.73
45	"	39 y 13 m	R L	2(+) 5(+)	0.98
46	"	40 y 10 m	R L	15(-) 16(-)	1.34

No. of experiment	Month of experiment	Age of mother or infant y=years m=months	Side of breast R=right L=left	Arakawa's reaction	Chlorine (grms. per 100 c.c.)
47	"	29 y 4 m	R L	11(±) 11(±)	0.80
48	"	28 y 11 m	R L	2(+) 2(+)	0.51
49	"	21 y 5 m	R L	3(±) 8(±)	0.85
50	"	29 y 18 m	R L	3(±) 5(++)	0.87
51	"	40 y 10 m	R L	10(±) 16(++)	1.26
52	"	24 y 2 m	R L	3(±) 5(++)	0.92
53	"	28 y 16 m	R L	11(-) 11(-)	0.60
54	"	25 y 5 m	R L	11(±) 11(±)	0.64
55	"	26 y 2 m	R L	16(-) 16(-)	0.92
56	"	25 y 2 m	R L	8(-) 8(-)	0.81
57	"	26 y 8 m	R L	14(-) 13(±)	0.81
58	"	26 y 8 m	R L	16(-) 16(-)	0.75
59	"	29 y 13 m	R L	10(-) 6(-)	0.89
60	"	32 y 4 m	R L	16(-) 5(±)	0.89
61	"	25 y 10 m	R L	16(-) 5(±)	0.75
62	"	21 y 11 m	R L	16(-) 14(-)	1.21
63	"	25 y 1 m	R L	16(-) 4(+)	0.53
64	"	20 y 2 m	R L	14(-) 15(-)	0.91
65	"	28 y 14 m	R L	15(-) 4(-)	0.91
66	"	25 y 7 m	R L	16(-) 14(-)	0.53
67	"	26 y 9 m	R L	5(±) 14(-)	0.85
68	"	25 y 3 m	R L	16(-) 16(-)	0.85
69	"	23 y 3 m	R L	4(±) 3(-)	1.14
70	"	25 y 18 m	R L	16(-) 16(-)	0.50
71	"	23 y 9 m	R L	1(-) 7(+)	1.04

No. of experiment	Month of experiment	Age of mother or infant y=years m=months	Side of breast R=right L=left	Arakawa's reaction	Chlorine (grms. per 100 c.c.)
72	"	28 y 10 m	R L	16(-) 16(-)	0.89
73	"	25 y 2 m	R L	12(-) 12(-)	0.64
74	"	28 y 1 m	R L	16(-) 15(-)	0.43
75	"	30 y 6	R L	14(-) 14(-)	0.82
76	"	25 y 1 m	R L	16(-) ***	0.78
77	"	32 y 6 m	R L	10(+) 6(-)	0.82
78	"	24 y 5 m	R L	13(±) 4(±)	0.99
79	XII	23 y 5 m	R L	2(-) ****	0.93
80	"	40 y 17 m	R L	7(±) 6(-)	0.59
81	"	45 y 20 m	R L	16(-) 14(-)	0.71
82	"	29 y 5 m	R L	7(+) 7(+)	0.76
83	"	34 y 2 m	R L	14(-) 15(-)	0.39
84	"	25 y 4 m	R L	16(-) 16(-)	0.44
85	"	27 y 4 m	R L	15(-) 15(-)	0.50
86	"	21 y 2 m	R L	15(-) 16(-)	0.57
87	"	27 y 11 m	R L	16(-) 16(-)	0.41
88	"	24 y 11 m	R L	14(-) 10(+)	0.82
89	"	27 y 10 m	R L	16(-) 16(-)	0.89
90	"	41 y 15 m	R L	15(-) 16(-)	0.94
91	"	29 y 16 m	R L	1(+) 1(+)	0.99
92	"	41 m 19 m	R L	1(+) 1(±)	0.76
93	"	34 y 12 m	R L	7(±) 8(-)	0.91
94	"	20 y 8 m	R L	11(±) 11(±)	0.64
95	"	26 y 18 m	R L	6(-) 6(±)	1.02
96	"	26 y 1 m	R L	12(-) 16(-)	0.89

No. of experiment	Month of experiment	Age of mother or infant y=years m=months	Side of breast R=right L=left	Arakawa's reaction	Chlorine (grms. per 100 c.c.)
97	"	25 y 2 m	R L	10 (-) s	0.82
98	"	26 y 2 m	R L	15 (-) 4 (-)	1.01
99	"	28 y 4 m	R L	16 (-) 16 (-)	0.80
100	"	24 y 19 m	R L	16 (-) 16 (-)	0.99
101	"	27 y 21 m	R L	5 (+) 4 (+)	0.82
102	"	24 y 10 m	R L	16 (-) 16 (-)	0.57
103	"	23 y 14 m	R L	16 (-) 14 (-)	1.08
104	"	26 y 1 m	R L	14 (-) 16 (-)	0.75
105	"	31 y 10 m	R L	4 (-) 4 (-)	0.50
106	"	23 y 2 m	R L	11 (+) 7 (-)	0.80
107	"	28 y 15 m	R L	16 (-) 16 (-)	1.16
108	"	26 y 2 m	R L	7 (-) 7 (-)	1.14
109	"	30 y 2 m	R L	16 (-) 16 (-)	0.72
110	"	31 y 7 m	R L	8 (-) 7 (±)	0.71
111	I (1937)	34 y 1 m	R L	12 (-) 12 (-)	1.17
112	"	31 y 7 m	R L	14 (-) 16 (-)	0.86
113	"	27 y 14 m	R L	6 (±) 6 (±)	0.85
114	"	23 y 1 m	R L	15 (-) 14 (-)	0.64
115	"	32 y 3 m	R L	16 (-) 16 (-)	0.97
116	"	26 y 2 m	R L	ss sss	0.53
117	"	25 y 5 m	R L	15 (-) 13 (-)	0.89
118	"	31 y 3 m	R L	12 (-) 12 (-)	0.89
119	"	29 y 4 m	R L	6 (±) 6 (±)	1.08
120	"	25 y 7 m	R L	16 (-) 16 (-)	0.78
121	"	23 y 9 m	R L	2 (+) 5 (+)	0.83

No. of experiment	Month of experiment	Age of mother or infant y=years m=months	Side of breast R=right L=left	Arakawa's reaction	Chlorine (grms. per 100 c.c.)
122	"	27 y 2 m	R L	6 (-) 6 (-)	1.10
123	"	29 y 16 m	R L	16 (-) 16 (-)	0.78
124	"	25 y 15 m	R L	16 (-) 16 (-)	0.24
125	"	30 y 15 m	R L	16 (-) 16 (-)	0.93
126	"	25 y 10 m	R L	6 (-) 12 (-)	1.07
127	"	25 y 5 m	R L	16 (-) 16 (-)	0.71
128	"	31 y 3 m	R L	11 (±) 11 (±)	0.89
129	"	30 y 15 m	R L	16 (-) 11 (±)	0.75
130	"	28 y 3 m	R L	16 (-) 6 (+)	1.03
131	"	27 y 2 m	R L	16 (-) 16 (-)	0.58
132	"	32 y 15 m	R L	12 (-) 16 (-)	0.82
133	"	26 y 12 m	R L	16 (-) 10 (±)	0.71
134	"	25 y 13 m	R L	16 (-) 8 (-)	0.68
135	"	25 y 3 m	R L	11 (±) 11 (±)	0.99
136	"	25 y 10 m	R L	3 (±) 16 (-)	0.92
137	"	30 y 7 m	R L	9 (+) 14 (±)	0.82
138	"	33 y 7 m	R L	16 (-) 16 (-)	0.21
139	"	34 y 12 m	R L	16 (-) 12 (-)	0.96
140	"	27 y 10 m	R L	16 (-) 15 (-)	0.11
141	"	26 y 9 m	R L	16 (-) 16 (-)	0.76
142	"	28 y 4 m	R L	16 (-) 16 (-)	0.10
143	"	27 y 9 m	R L	16 (-) 16 (-)	0.11
144	"	29 y 6 m	R L	15 (-) 16 (-)	0.92
145	"	28 y 12 m	R L	4 (-) 8 (-)	0.68
146	"	27 y 11 m	R L	10 (±) 6 (+)	0.60

No. of experiment	Month of experiment	Age of mother or infant y = years m = months	Side of breast R = right L = left	Arakawa's reaction	Chlorine (grms. per 100 c.c.)
147	"	22 y 4 m	R L	12 (-) 3 (-)	0.94
148	"	33 y 6 m	R L	16 (-) 1 (+)	0.90
149	"	25 y 2 m	R L	8 (-) 16 (-)	0.98
150	"	25 y 1 m	R L	16 (-) 16 (-)	0.84
151	"	28 y 11 m	R L	7 (±) 7 (±)	0.84
152	"	24 y 10 m	R L	7 (±) 11 (-)	1.19
153	"	25 y 2 m	R L	6 (-) 10 (-)	0.85
154	"	22 y 7 m	R L	9 (+) 4 (-)	1.03
155	II	28 y 3 m	R L	SSSS 13 (±)	1.04
156	"	25 y 4 m	R L	16 (-) 16 (-)	0.57
157	"	25 y 9 m	R L	12 (-) 16 (-)	0.50
158	"	28 y 6 m	R L	16 (-) 4 (-)	0.92
159	"	34 y 6 m	R L	2 (+) 4 (+)	0.75
160	"	34 y 17 m	R L	16 (-) 8 (-)	0.69
161	"	28 y 8 m	R L	16 (-) 6 (-)	0.82
162	"	36 y 9 m	R L	6 (-) 7 (±)	1.21
163	"	30 y 4 m	R L	16 (-) 16 (-)	0.64
164	"	25 y 26 m	R L	16 (-) 16 (-)	0.41
165	"	34 y 4 m	R L	15 (±) 7 (±)	0.83
166	"	23 y 9 m	R L	10 (+) 5 (+)	0.89
167	"	25 y 9 m	R L	12 (-) 16 (-)	0.89
168	"	30 y 1 m	R L	15 (-) SSSS	0.71
169	"	29 y 6 m	R L	11 (±) 9 (+)	0.89
170	"	25 y 5 m	R L	7 (±) 5 (+)	0.99
171	"	26 y 9 m	R L	5 (+) 10 (+)	0.92

No. of experiment	Month of experiment	Age of mother or infant y = years m = months	Side of breast R = right L = left	Arakawa's reaction	Chlorine (grms. per 100 c.c.)
172	"	30 y 9 m	R L	4 (+) 5 (±)	0.96
173	"	29 y 11 m	R L	9 (+) 4 (+)	0.85
174	"	23 y 1 m	R L	3 (-) 15 (±)	0.62
175	"	25 y 9 m	R L	3 (-) 14 (-)	1.08
176	"	23 y 6 m	R L	1 (+) 6 (±)	0.82
177	"	23 y 10 m	R L	15 (±) 11 (+)	0.92
178	"	33 y 12 m	R L	11 (+) 15 (±)	0.78
179	"	38 y 20 m	R L	7 (+) 7 (-)	0.99
180	"	35 y 20 m	R L	4 (+) 4 (+)	0.85
181	"	26 y 2 m	R L	5 (±) 14 (±)	0.92
182	"	32 y 15 m	R L	4 (+) 5 (+)	1.17
183	"	27 y 2 m	R L	4 (+) 4 (+)	0.96
184	"	31 y 5 m	R L	7 (±) 7 (±)	1.17
185	"	32 y 4 m	R L	8 (-) 16 (-)	0.60
186	"	26 y 22 m	R L	14 (-) 16 (-)	0.75
187	"	26 y 7 m	R L	11 (+) 11 (+)	0.99
188	III	30 y 6 m	R L	10 (-) 16 (-)	0.70
189	"	24 y 4 m	R L	7 (+) 7 (+)	1.31
190	"	29 y 19 m	R L	12 (-) 12 (-)	0.87
191	"	27 y 2 m	R L	16 (-) 16 (-)	0.36
192	"	25 y 3 m	R L	4 (+) 4 (+)	0.89
193	"	29 y 5 m	R L	11 (+) 4 (+)	1.03
194	"	23 y 1 m	R L	7 (±) 16 (-)	0.84
195	"	27 y 2 m	R L	10 (+) 10 (+)	1.26
196	"	23 y 3 m	R L	1 (±) 1 (±)	1.06

No. of experiment	Month of experiment	Age of mother or infant y=years m=months	Side of breast R=right L=left	Arakawa's reaction	Chlorine (grms. per 100 c.c.)
197	"	27 y 9 m	R L	1(##) 2(+)	1.17
198	"	25 y 7 m	R L	1(+) 2(+)	1.27
199	"	22 y 8 m	R L	1(##) 1(##)	1.17
200	"	24 y 8 m	R L	1(##) 1(##)	1.34
201	"	26 y 12 m	R L	1(##) 1(##)	1.16
202	"	25 y 8 m	R L	1(+) 2(+)	1.36

* Cf. Table 4.

	1'	2'	3'	4'	5'
*	-	+	+	+	+
**	-	+	+	+	+
***	-	+	+	+	+
****	+	##	##	##	##
§	-	-	+	+	+
§§	-	+	+	+	+
§§§	-	+	+	+	+
§§§§	-	+	+	+	+
§§§§§	-	-	-	-	+

TABLE 4.

Table of signs for showing different Arakawa's reaction.

		1'	2'	3'	4'	5'			1'	2'	3'	4'	5'	
1.	1(##)	stands for	##	##	##	##	25.	8(±)	stands for	±	±	+	+	##
2.	1(+)	"	+	+	##	##	26.	9(±)	"	±	±	+	+	##
3.	2(+)	"	+	+	##	##	27.	10(±)	"	±	±	+	+	##
4.	3(+)	"	+	+	+	##	28.	11(±)	"	±	±	+	+	+
5.	4(+)	"	+	+	+	##	29.	12(±)	"	±	±	±	+	+
6.	5(+)	"	+	+	+	+	30.	13(±)	"	±	±	±	+	+
7.	1(+)	"	+	+	##	##	31.	14(±)	"	±	±	±	±	+
8.	2(+)	"	+	+	##	##	32.	15(±)	"	±	±	±	±	±
9.	3(+)	"	+	+	+	##	33.	1(-)	"	-	±	+	+	##
10.	4(+)	"	+	+	+	##	34.	2(-)	"	-	±	+	+	##
11.	5(+)	"	+	+	##	##	35.	3(-)	"	-	±	+	+	##
12.	6(+)	"	+	+	+	##	36.	4(-)	"	-	±	+	+	+
13.	7(+)	"	+	+	+	##	37.	5(-)	"	-	±	±	+	+
14.	8(+)	"	+	+	+	##	38.	6(-)	"	-	±	±	+	+
15.	9(+)	"	+	+	+	##	39.	7(-)	"	-	±	±	±	+
16.	10(+)	"	+	+	+	##	40.	8(-)	"	-	±	±	±	±
17.	11(+)	"	+	+	+	+	41.	9(-)	"	-	-	±	+	##
18.	1(±)	"	±	+	+	##	42.	10(-)	"	-	-	±	+	+
19.	2(±)	"	±	+	+	##	43.	11(-)	"	-	-	±	±	+
20.	3(±)	"	±	+	+	##	44.	12(-)	"	-	-	±	±	±
21.	4(±)	"	±	+	+	##	45.	13(-)	"	-	-	-	±	+
22.	5(±)	"	±	+	+	##	46.	14(-)	"	-	-	-	±	±
23.	6(±)	"	±	+	+	##	47.	15(-)	"	-	-	-	-	±
24.	7(±)	"	±	+	+	+	48.	16(-)	"	-	-	-	-	-

Explanation to the table:—

Take, for instance, the sign: 2(+). This stands for Arakawa's reaction with the course (+)1' (+)2' (##)3' (##)4' (##)5'. The sign does not express any prompt result of the reaction, so the prompt reaction of the sign: 2(+) may be (-)0', (±)0', (+)0' or even (+)0', but this will not matter much, as the result of the reaction in one minute is the most important.

0.840 and 0.808 grms. per 100 c.c. respectively on an average. Cf. Tables 5 and 6).

TABLE 5.

Chlorine content (grms. per 100 c.c.) of mother's urine according Arakawa's reaction.

Group	Arakawa's reaction R=right L=left	Case	Average	Maximum	Minimum
1	R (##)1' or R or L (##)1' L (##)1' or L or R (##)1' (Arakawa-positive case)	14	1.062	1.355	0.725
2	R or L (++)1' L or R (+)1'	5	0.882	1.172	0.753
3	R or L (++)1' L or R (±)1'	5	0.893	0.994	0.817
4	R (+)1' L (+)1'	18	0.990	1.318	0.511
5	R or L (+)1' L or R (±)1'	13	0.820	0.994	0.391
6	R or L (+)1' L or R (-)1'	12	0.885	1.030	0.532
7	R (±)1' L (±)1'	16	0.840	1.171	0.432
8	R or L (±)1' L or R (-)1'	19	0.864	1.193	0.621
9	R (-)1' L (-)1'	67	0.807	1.335	0.107
10	R (-)5' L (-)5' (Completely Arakawa-negative cases)	33	0.700	1.065	0.212
	Total	202	0.874	1.355	0.107

TABLE 6.

Chlorine content (grms. per 100 c.c.) of mothers' urine according to clinically positive and negative Arakawa's reaction.

	Arakawa's reaction	Case	Average	Maximum	Minimum
Arakawa-positive case	(##)1' (++)1'	14	1.062	1.355	0.725
Weakly Arakawa-negative case	(+)1' (±)1' (-)1'	155	0.873	1.335	0.391
Completely Arakawa-negative case	(-)5'	33	0.700	1.065	0.212

The chlorine content of completely Arakawa-negative mothers shows an average figure -34% deviation as compared with that of Arakawa-positive mothers (Cf. Table 7), and as to the maximum of chlorine content (Cf. Table 7), the deviation of the urine of completely Arakawa-negative mothers from that of Arakawa-positive mothers is

TABLE 7.
Chlorine content in urine of Arakawa-positive and completely negative mothers.

	Chlorine content in urine of Arakawa- positive mothers (14 cases in number)	Chlorine content in urine of completely Arakawa-negative mothers (33 cases in number)	Deviation of Arakawa-negative cases from positive cases
Average chlorine content (grms. per 100 c.c.)	1.062	0.700	-34%
Maximum (grms. per 100 c.c.)	1.355	1.065	-21%
Minimum (grms. per 100 c.c.)	0.725	0.212	-70%

TABLE 8.
Distribution of all the samples according to Arakawa's reaction and to the amount of chlorine.

Group* Grms. per 100 c.c.	1 (Cases)	2 (Cases)	3 (Cases)	4 (Cases)	5 (Cases)	6 (Cases)	7 (Cases)	8 (Cases)	9 (Cases)	10 (Cases)
1.000 or over	7	1		7		3		5	13	3
0.900 " "	4		2	4	6	3	3	2	9	4
0.800 " "	2	2	3	5	2	3	6	6	15	5
0.700 " "	1	2		1	3	2	1	4	8	8
0.600 " "				1	1		3	1	10	1
0.500 " "								1	5	4
0.400 " "							1		5	5
0.300 " "					1				1	1
0.200 " "										2
0.100 " "									1	

* The number of group is the same as in Table 5.

TABLE 9.
Distribution of all the samples according to Arakawa's reaction and to the amount of chlorine.

Groups Grms. per 100 c.c.	Arakawa- positive (cases)	Weakly Arakawa- negative (cases)	Completely Arakawa- negative (cases)
1.000 or over	7	30	3
0.900 " "	4	29	4
0.800 " "	2	42	5
0.700 " "	1	21	8
0.600 " "		16	1
0.500 " "		7	4
0.400 " "		6	5
0.300 " "		2	1
0.200 " "			2
0.100 " "		1	

less remarkable (-21%), while the minimum value of chlorine content deviation shows an average figure of -70% (Cf. Table 7).

It will be thus seen that, in general, chlorine content of urine is larger in Arakawa-positive mothers than Arakawa-negative ones. One will notice the fact above mentioned from Table 8, too (Cf. Tables 8 and 9).

Literature and Comment.

According to Isono,⁴⁾ the chlorine content in urine of healthy (Japanese) male adults is 0.871 grms. per 100 c.c. on an average. I cite here part of a table compiled by Isono (Cf. Table 10).

TABLE 10.
Chlorine content of urine of healthy Japanese male adults.

	5) Osawa	6) Kajita	7) Huna- oka	8) Mori	9) Inaba	10) Yu- kawa	11) Satoda	12) Isono
Chlorine (grms. per 100 c.c.)	1.222	0.825	0.866	0.930	1.023	0.775	1.290	0.871

The average chlorine content in all my cases, 202 in number, is 0.874 grms. per 100 c.c. on an average.

Now the Arakawa-negative milk is milk from a *B-avitaminotic body, as has been shown by a number of works¹³⁻²¹⁾ published from our Department.

Omori, Ohashi, Nakanishi, Hara and Ota²²⁾ fed their patients with slight affection of beriberi on B-avitaminotic diet, and found decrease of urine chlorine in the stage of exacerbation of the disease and an increase of it in the stage of convalescence. Then they investigated the feeding of healthy persons on B-avitaminotic diet

4) S. Isono, Kaigun Gunikai Kaiho, 1913, 5, 76.

5) O. Osawa, Tokyo Igakkai Zasshi, 1889, 3, 667.

6) K. Kajita, Tokyo Igakkai Zasshi, 1890, 4, 275 and 1190.

7) E. Hunaoka, Tokyo Igakkai Zasshi, 1901, 15, 8.

8) R. Mori, Arbeiten aus der Kaiserl. Japanischen militärärztlichen Lehranstalt, 1892, 1, 1.

9) R. Inaba, Tokyo Igakkai Zasshi, 1907, 21, 1025.

10) G. Yukawa, Tokyo Igakkai Zasshi, 1909, 23, 259.

11) G. Satoda, Tokyo Igakkai Zasshi, 1909, 23, 1143; 1910, 24, 411 and 991.

12) S. Isono, Kaigun Gunikai Kaiho, 1913, 5, 76.

* In the present paper vitamin B means vitamin B₁.

13) T. Arakawa, Tohoku J. Exp. Med., 1930, 16, 83 and 118.

14) A. Sato, Jikken Iho, 1933, 19, 1554.

15) A. Sato, Jikken Iho, 1935, 21, 523.

16) K. Asakura and H. Osako, Tohoku J. Exp. Med., 1933, 20, 429.

17) T. Suzuki, Tohoku J. Exp. Med., 1934, 23, 23.

18) A. Takamatsu, Tohoku J. Exp. Med., 1934, 23, 46.

19) A. Takamatsu, and A. Sato, Tohoku J. Exp. Med., 1934, 23, 506.

20) J. Kimura, Tohoku J. Exp. Med., 1934, 23, 494.

21) A. Takamatsu, Tohoku J. Exp. Med., 1935, 27, 439.

22) K. Omori, Y. Ohashi, T. Nakanishi, M. Hara and T. Ota, Keio Igaku, 1923, 3, 197.

and found a decrease of urine chlorine.

Uehara, So and Fujinami²³⁾ made an investigation into chlorine metabolism in beriberi and B-avitaminosis. An administration of vitamin B in avitaminotic cases with edema would, they asserted, result in the excretion of 3~4 times as much as chlorine in the urine as taken in. Miura²⁴⁾ and Hasui²⁵⁾ also found a decrease of urine chlorine in the stage of exacerbation of beriberi.

These results above cited are generally in conformity with the result of my own experiment (Tables 6 and 7). The abundance of chlorine (1.062 grms. per 100 c.c. in Table 7) in the urine of Arakawa-positive mothers and the paucity of that of Arakawa-negative mothers (0.700 grms. per 100 c.c. in Table 7) are very interesting facts. Even the minimum chlorine content in the former is larger (0.725 grms. in Table 7) than the average figure of 0.70 grms. in the latter. We know that Arakawa-negative milk is generally a milk from a B-avitaminotic body and a "bad"* milk. One can easily conceive the existence of a close relation between Arakawa's reaction and the chlorine content of urine. Now with the result shown above we shall safely be able to conclude that the Arakawa-negative milk may pass for a B-avitaminotic milk also from the chlorine point of view.

Now in 1934 Nozaki²⁶⁾ concluded that human milk negative to Arakawa's reaction was essentially higher in chlorine content than the Arakawa-positive milk. In 1938, Ishii²⁶⁾ (Cf. 95th Report of the Peroxidase Reaction) reported that chlorine content in the blood of mothers with negative Arakawa's reaction is slightly richer than in that of mothers with positive Arakawa's reaction.

Considering solely the chlorine content of milk, and of urine, the Arakawa-negative mother is rich in milk chlorine and poor in urine chlorine. So there is highly probably a tendency to chlorine retention in an Arakawa-negative mother.

Summary.

Human milk negative to Arakawa's reaction is generally milk from a B-avitaminotic body as shown by a number of papers from our

* "bad" milk:—generally such a human milk with weak or negative Arakawa's reaction.

23) I. Uehara, T. So and H. Fujinami, Tokyo Igakkai Zasshi, 1922, **36**, 1421.

24) K. Miura, *Ergeb. d. inn. Med. u. Kinderhk.*, 1909, **4**, 280.

25) N. Hasui, Nippon Naikagakkai Zasshi, 1922, **10**, 169.

26) J. Nozaki, Tohoku J. Exp. Med., 1934, **23**, 60.

Laboratory. Viewed from the chlorine content of human urine, the Arakawa-negative mother shows an essentially lower content. And it is evident from the above cited literature that a B-avitaminotic body shows lower content of chlorine in urine. It is very probable that, viewed from the chlorine content of mother's urine, the Arakawa-negative human milk will pass for a B-avitaminotic milk even to the exclusion of other possibilities.

Conclusion.

1. Urine of Arakawa-negative mothers is essentially lower in chlorine content than that of Arakawa-positive mothers.
2. Chlorine content in the urine of Arakawa-negative mothers shows an average figure of -34% deviation as compared with that of Arakawa-positive mothers.
3. Viewed from the chlorine content of milk (Nozaki and Ishii), blood (Ishii) and urine (author), it is not difficult to presume that there is a tendency to abnormal chlorine retention in Arakawa-negative mothers.