Study on the Iodine Reaction of Blood Neutrophiles in Cases of Lactating Mothers.

145th Report of the Peroxidase Reaction.
(92nd Human Milk Study.)

By

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Introduction.

According to Ogata,¹⁾ Hosokawa,²⁾ Hirai³⁾ and Asai⁴⁾ blood sugar content increases in B-avitaminosis. A decrease of the glycolytic enzyme in the blood of rice-diseased animals was proved by Gentile⁵⁾ and Takayama,⁶⁾ and further Takemoto,⁷⁾ Sasaki,⁸⁾ Ikebe,⁹⁾ Tiwaki,¹⁰⁾ Usuki¹¹⁾ and Era¹²⁾ confirmed that glycolysis in blood was disturbed in cases of B-avitaminosis or rice-disease. Thus, a disturbance of glycolysis will happen and a hyperglycemia will result in the B-avitaminotic body. Then, it is natural to deduce that an increase of glycogen content of leucocytes will occur in B-avitaminosis. This was confirmed by Miyakawa,¹³⁾ who saw an in-

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- 1) T. Ogata, Nissin Igaku, 1923 1924, **13**, 332.
 - 2) Sy. Hosokawa, Nihon Naikagakkai Zassi, 1924 1925, **12**, 116.
 - 3) R. Hirai, Nihon Byorigakkai Zassi, 1921, **11**, 80.
 - 4) H. Asai, Nihon Naikagakkai Zassi, 1924 1925, **12**, 736.
 - 5) F. Gentile, Arch. di Fisiol., 1927, **25**, 21.
 - 6) S. Takayama, Tyugai Izi Simpo, 1925, No. 1078, 357.
 - 7) H. Takemoto, Zikken Igaku Zassi, 1927, **11**, 165.
 - 8) K. Sasaki, Nagasaki Igakkai Zassi, 1929, **7**, 54.
 - 9) K. Ikebe, Nagasaki Igakkai Zassi, 1930, **8**, 457.
 - 10) Z. Tiwaki, Nihon Seikwagakkai Kaiho, 1932, **7**, 63.
 - 11) Z. Usuki, Nagoya Igakkai Zassi, 1936, **43**, 1119.
 - 12) K. Era, Nagoya Igakkai Zassi, 1936, **44**, 1091.
 - 13) M. Miyakawa, Nagoya Igakkai Zassi, 1938, **47**, 1077.

crease of glycogen content of leucocytes in rice-diseased pigeons. If mothers secreting milk negative to Arakawa's reaction are, as has been shown by a number of reports¹⁴⁾ from our Laboratory, more or less in a state of B-avitaminosis, then there will presumably be an increase of glycogen content of leucocytes in cases of Arakawa-negative mothers.

Ehrlich's,¹⁵⁾ iodine reaction is a method which is used to examine an increase of glycogen content of leucocytes, because glycogen is an iodophilic substance. Kiyokawa¹⁶⁾ published that Ehrlich's iodine reaction was remarkably positive in 6 cases out of 8 sucklings suffering from beriberi, the disease which is closely related to B-avitaminosis.

In the present treatise I described iodine reaction by the use of Ehrlich's method in the cases of Arakawa-positive and Arakawa-negative mothers compared with the result of the reaction between these two groups of lactating mothers.

Method of Investigation.

1. Iodine reaction of blood neutrophils :—

Kiyokawa's modification of Ehrlich's method was used. The technique is as follows :

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- 14) T. Arakawa, Tohoku J. Exp. Med., 1930, **16**, 118.
 K. Suzuki and T. Arakawa, Ibid., 1930, **16**, 228.
 M. Chiba and J. Abe, Ibid., 1932, **19**, 479.
 M. Chiba, Ibid., 1932, **19**, 284 and 486.
 T. Suzuki, Ibid., 1934, **23**, 23.
 T. Suzuki, Ibid., 1935, **25**, 186.
 T. Suzuki, Ibid., 1935, **25**, 575.
 T. Suzuki, Ibid., 1937, **31**, 280.
 A. Takamatsu, Ibid., 1934, **23**, 46.
 A. Takamatsu, Ibid., 1934, **23**, 372.
 J. Nozaki, Ibid., 1934, **23**, 60.
 T. Suzuki and A. Takamatsu, Ibid., 1934, **24**, 202.
 T. Suzuki and A. Takamatsu, Ibid., 1935, **25**, 588.
 J. Kimura, Ibid., 1934, **23**, 494.
 J. Kimura, Ibid., 1935, **27**, 360.
 J. Kimura, Ibid., 1935, **27**, 374.
 S. Siraishi, Ibid., 1936, **28**, 44.
 S. Siraishi, Ibid., 1937, **31**, 268.
 S. Siraishi, Ibid., 1937, **31**, 510.
 S. Siraishi, Ibid., 1938, **32**, 73.
 S. Siraishi, Ibid., 1938, **33**, 60.
 H. Ohta, and T. Suzuki, Ibid., 1937, **31**, 247.
 M. Ishii, Ibid., 1937, **31**, 580.
 - 15) Z. Ehrlich, Zschr. f. klin. Medizin, 1883, **6**, 33.
 - 16) Y. Kiyokawa, Rinsyo Shonika Zassi, 1932, No. 9, 25.

Blood was taken from an ear lobe of the mother. The blood film, dried in the air, is fixed for one minute on the copper plate heated over a flame. Then the fixed film is kept in a vapour of iodine in a vessel with many crystals of iodine on the bottom of it. The film is taken out from the vapour of iodine after 3 to 5 minutes and is mounted in cedar oil.

Neutrophiles are made objects of microscopic examination. The protoplasma of neutrophiles positive to the iodine reaction looks yellow or yellowish brown. I used signs to denote the intensity of the reaction in this paper. Sign (++) stood for neutrophiles entirely filled with brownish yellow granules, and sign (+) for neutrophiles filled with yellow granules.

2. Arakawa's reaction of mother's milk :—

Milk was obtained from mothers of breast-fed infants and was examined with Arakawa's reagent. The mothers were divided into three groups according to the intensity of Arakawa's reaction.

a. The normally or strongly Arakawa-positive group :

Arakawa's reaction of milk from both breasts was normally or strongly positive in one minute.

b. The weakly Arakawa-positive group: Arakawa's reaction of milk was of intermediate strength.

c. Arakawa-negative group: Arakawa's reaction of milk from both breasts was completely or almost completely negative after five minutes.

The different course of Arakawa's reaction during five minutes was shown by special signs (Cf. Table 1) for simplification.

Mothers who had been taking preparations of vitamin B, and those with normally positive Arakawa's reaction on one breast and completely negative Arakawa's reaction on the other breast were excluded in this treatise, because I intend relating about such cases in subsequent reports.

Results of Investigation.

1. Iodine reaction of neutrophiles in the group of normally or strongly Arakawa-positive mothers (Cf. Table 2).

Fifty mothers belonged to this group and the iodine reaction was utterly negative in 37 cases (74 %). Even in 13 cases (26 %) showing a positive iodine reaction neutrophiles with (++) were not seen, while neutrophiles with (+) were 8.8% in each case on an average.

TABLE 1.

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 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.

2. Iodine reaction of the neutrophile in the group of weakly Arakawa-positive mothers* (Cf. Table 3).

There were 50 mothers belonging to this group and the iodine reaction was utterly negative in only 10 cases (20%). Most of the cases i.e. 40 cases (80%) showed a positive iodine reaction, and even the reaction of (++) was seen in 22 cases (44%). Neutrophiles with

* "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. Here it means that the sample is not negative to Arakawa's reaction. The other of these two senses is: Arakawa-positive in a clinical sense. A sample of human milk is clinically Arakawa-positive only when it shows such a reactions 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.

TABLE 2.

Iodine reaction of normally or strongly Arakawa-positive group.

Case No.	Name	Age (years)	Arakawa's reaction		Iodine reaction of blood neutrophils		
			Right	Left	(-)	(+)	(++)
1	S. O.	26	1(##)	1(##)	100	0	0
2	N. S.	23	1(+)	1(##)	100	0	0
3	U. S.	29	1(##)	1(+)	100	0	0
4	H. K.	28	1(+)	1(##)	100	0	0
5	T. H.	31	1(+)	1(##)	100	0	0
6	T. K.	27	1(##)	1(##)	100	0	0
7	H. A.	22	1(##)	1(##)	100	0	0
8	N. I.	27	1(##)	1(##)	100	0	0
9	M. A.	25	1(##)	1(##)	100	0	0
10	K. K.	23	1(+)	1(##)	100	0	0
11	Y. T.	24	1(##)	1(+)	88	12	0
12	T. Y.	31	1(##)	3(++)	100	0	0
13	M. S.	28	1(##)	1(+)	100	0	0
14	Y. H.	27	1(##)	2(++)	0	100	0
15	R. S.	23	1(+)	1(+)	100	0	0
16	U. M.	31	1(+)	1(+)	100	0	0
17	T. Y.	36	1(+)	1(+)	100	0	0
18	M. M.	26	1(+)	1(+)	100	0	0
19	H. Y.	40	3(++)	1(##)	100	0	0
20	H. S.	31	3(++)	1(##)	100	0	0
21	S. S.	25	1(+)	2(++)	72	28	0
22	S. Y.	25	4(++)	1(##)	10	90	0
23	A. S.	25	2(++)	2(++)	100	0	0
24	Y. O.	26	2(++)	2(++)	100	0	0
25	H. K.	36	4(++)	1(+)	84	16	0
26	K. M.	30	1(##)	4(++)	80	20	0
27	M. A.	42	3(++)	1(+)	100	0	0
28	T. S.	25	1(##)	3(++)	100	0	0
29	S. S.	34	2(++)	2(++)	100	0	0
30	K. T.	23	2(++)	2(++)	88	12	0
31	H. S.	29	2(++)	2(++)	100	0	0
32	H. U.	25	2(++)	2(++)	100	0	0
33	Y. Y.	28	1(+)	3(++)	100	0	0
34	H. I.	24	3(++)	2(++)	80	20	0
35	U. M.	25	5(++)	2(++)	100	0	0
36	T. S.	26	3(++)	5(++)	100	0	0
37	T. H.	39	3(++)	3(++)	100	0	0
38	T. S.	28	4(++)	3(++)	100	0	0
39	M. T.	27	3(++)	3(++)	100	0	0
40	T. T.	26	4(++)	2(++)	100	0	0
41	Y. S.	27	5(++)	2(++)	80	20	0
42	K. S.	25	2(++)	4(++)	90	10	0
43	S. S.	23	2(++)	3(++)	100	0	0
44	M. Y.	36	5(++)	2(++)	100	0	0
45	H. Y.	25	3(++)	4(++)	95	5	0
46	K. O.	35	4(++)	4(++)	8	92	0
47	K. K.	25	3(++)	2(++)	100	0	0
48	K. K.	35	4(++)	4(++)	85	15	0
49	S. E.	31	4(++)	4(++)	100	0	0
50	S. E.	30	6(++)	5(++)	100	0	0

only (+) were in most cases from 20% to 75% (on an average 55.7%), and neutrophils with (++) were in most cases from 20% to 80% (on

TABLE 3.
Iodine reaction of weakly Arakawa-positive group.

Case No.	Name	Age (years)	Arakawa's reaction		Iodine reaction of blood neutrophiles		
			Right	Left	(-)	(+)	(++)
1	M. K.	24	8(+)	1(++)	100	0	0
2	S. A.	23	7(+)	3(++)	92	8	0
3	H. N.	39	3(±)	1(++)	68	32	0
4	H. S.	26	1(++)	1(-)	84	26	0
5	S. T.	31	4(+)	3(+)	100	0	0
6	M. K.	33	5(+)	5(+)	92	8	0
7	T. N.	25	5(+)	4(+)	0	100	0
8	Y. I.	26	13(±)	1(++)	100	0	0
9	S. S.	26	3(±)	9(+)	40	60	0
10	K. M.	30	4(++)	12(±)	0	0	100
11	T. K.	31	5(+)	6(±)	44	56	0
12	K. S.	26	6(±)	8(+)	100	0	0
13	N. I.	27	2(+)	5(-)	16	84	0
14	M. T.	30	7(±)	5(+)	100	0	0
15	K. S.	29	5(±)	5(±)	100	0	0
16	T. H.	21	10(±)	2(±)	0	80	20
17	T. S.	26	7(±)	4(±)	0	64	36
18	R. M.	32	2(+)	10(-)	0	0	100
19	Y. M.	29	14(±)	7(+)	64	36	0
20	T. K.	34	11(±)	8(±)	12	76	2
21	M. H.	31	6(-)	4(±)	15	70	15
22	T. Y.	27	8(±)	13(±)	52	48	0
23	T. N.	26	8(-)	5(+)	100	0	0
24	A. M.	21	3(-)	11(±)	25	75	0
25	M. O.	30	15(±)	9(±)	100	0	0
26	M. T.	33	15(±)	6(±)	0	28	72
27	R. E.	28	7(-)	6(±)	0	90	10
28	H. S.	43	12(±)	14(±)	0	100	0
29	K. K.	21	14(±)	13(±)	0	100	0
30	H. H.	31	7(±)	11(-)	0	0	100
31	T. T.	29	4(+)	16(-)	50	50	0
32	M. K.	29	12(-)	4(±)	100	0	0
33	S. M.	30	6(-)	6(-)	100	0	0
34	T. S.	23	10(±)	13(-)	60	40	0
35	S. A.	26	12(-)	2(-)	0	40	60
36	N. O.	28	6(-)	9(-)	40	60	0
37	T. E.	41	9(±)	14(-)	0	48	52
38	H. S.	44	11(±)	8(-)	0	12	88
39	M. N.	23	12(±)	11(-)	80	20	0
40	S. W.	29	9(±)	15(-)	0	76	24
41	Y. H.	20	14(±)	8(-)	0	100	0
42	H. S.	23	13(±)	13(-)	0	40	60
43	I. A.	35	7(±)	14(-)	0	40	60
44	Y. M.	32	7(-)	7(-)	0	60	40
45	Y. S.	22	1(-)	16(-)	0	0	100
46	M. K.	26	15(-)	3(-)	0	90	10
47	T. T.	23	15(-)	12(±)	0	20	80
48	Y. W.	25	11(±)	14(-)	24	70	6
49	S. K.	25	6(-)	14(-)	0	80	20
50	Y. T.	29	16(-)	4(-)	0	88	12

an average 45.8%).

3. Iodine reaction of the neutrophile in the group of Arakawa-

TABLE 4.
Iodine reaction of Arakawa-negative group.

Case No.	Name	Age (years)	Arakawa's reaction		Iodine reaction of blood neutrophils		
			Right	Left	(-)	(+)	(++)
1	H. E.	38	16(-)	16(-)	0	0	100
2	T. T.	28	16(-)	16(-)	0	100	0
3	N. S.	35	16(-)	16(-)	0	0	100
4	H. K.	24	16(-)	16(-)	0	94	6
5	A. N.	31	16(-)	16(-)	0	85	15
6	M. T.	32	16(-)	16(-)	14	82	4
7	T. O.	31	16(-)	16(-)	20	80	0
8	E. A.	32	16(-)	16(-)	20	80	0
9	S. I.	25	16(-)	16(-)	0	0	100
10	K. S.	25	16(-)	16(-)	0	100	0
11	M. U.	25	16(-)	16(-)	0	28	72
12	M. T.	33	16(-)	16(-)	60	40	0
13	Y. S.	25	16(-)	16(-)	0	0	100
14	T. A.	20	16(-)	16(-)	0	64	36
15	K. M.	27	16(-)	16(-)	0	0	100
16	N. S.	30	16(-)	16(-)	0	0	100
17	S. S.	35	16(-)	16(-)	0	15	85
18	T. S.	21	16(-)	16(-)	10	90	0
19	M. K.	27	16(-)	16(-)	0	0	100
20	Y. H.	24	16(-)	16(-)	0	0	100
21	K. M.	29	16(-)	16(-)	5	30	65
22	K. I.	23	16(-)	15(-)	0	0	100
23	H. Y.	27	16(-)	16(-)	0	0	100
24	A. T.	23	15(-)	15(-)	0	10	90
25	T. Y.	30	16(-)	14(-)	0	60	40
26	T. E.	24	16(-)	14(-)	32	68	0
27	M. H.	24	14(-)	16(-)	0	40	60
28	Y. A.	28	14(-)	16(-)	35	65	0
29	H. S.	30	14(-)	16(-)	0	0	100
30	T. E.	25	15(-)	15(-)	0	0	100
31	S. M.	28	16(-)	14(-)	0	0	100
32	M. S.	25	15(-)	15(-)	0	0	100
33	T. S.	29	12(-)	16(-)	0	20	80
34	E. H.	31	16(-)	12(-)	0	60	40
35	N. O.	23	16(-)	14(-)	0	35	65
36	T. M.	20	14(-)	16(-)	17	33	50
37	Y. K.	30	8(-)	16(-)	0	74	26
38	T. H.	27	15(-)	14(-)	0	60	40
39	M. O.	24	12(-)	16(-)	0	80	20
40	S. A.	25	12(-)	16(-)	0	100	0
41	T. M.	27	14(-)	16(-)	0	100	0
42	T. S.	39	16(-)	8(-)	0	68	32
43	K. O.	22	14(-)	15(-)	0	100	0
44	H. M.	26	12(-)	16(-)	28	72	0
45	M. M.	21	14(-)	14(-)	0	100	0
46	H. H.	24	8(-)	15(-)	0	100	0
47	H. A.	23	12(-)	12(-)	0	0	100
48	H. S.	35	12(-)	8(-)	37	63	0
49	H. O.	25	8(-)	8(-)	15	85	0
50	H. S.	27	16(-)	16(-)	0	100	0

negative mothers (Cf. Table 4).

A total of 50 mothers belonged to this group, and there was no

case showing negative iodine reaction. In most cases, i.e. in 33 cases (66% of all the cases) the iodine reaction of (++) was seen. Neutrophils with (++) in most cases from 50% to 100% (on an average 70.5%), and neutrophils with (+) were in most cases from 40% to 90% (on an average 74.8%).

Comment.

Of the normally or strongly Arakawa-positive mothers 74%, and of the weakly Arakawa-positive mothers 20 % showed negative iodine reaction of neutrophils, but there was not a single case with the neutrophils with negative iodine reaction in the group of Arakawa-negative mothers (Cf. Table 5). As to a positive iodine reaction of the neutrophile, on the contrary, none of normally Arakawa-positive mothers showed the reaction of (++) , but 44% of the weakly Arakawa-positive mothers and 66% of the Arakawa-negative mothers showed the reaction of (++) (Cf. Table 5). In other words, lactating women show an inverse proportion between their Arakawa's reac-

TABLE 5.

Distribution of the three different groups of lactating mothers according to the iodine reaction of neutrophils.

Iodine reaction of neutrophils	Lactating mothers		
	Normally or strongly Arakawa-positive group	Weakly Arakawa-positive group	Arakawa-negative group
—	37 cases (74 %)	10 cases (20 %)	0 case (0 %)
+	13 " (26 ")	18 " (36 ")	17 cases (34 ")
++	0 case (0 ")	22 " (44 ")	33 " (66 ")

Foot note to the table :—

* Cases showing only the reaction of (+).

** Cases showing the reaction of (++) .

tion and their iodine reaction of neutrophils ; the weaker the Arakawa reaction, the more intense the iodine reaction of neutrophils is. This relation may also be shown by the following table (Cf. Table 6). Table 6 shows that the number of neutrophils intensely positive to the iodine reaction increased as Arakawa's reaction became weaker.

TABLE 6.

*Average percentage of neutrophiles positive to the iodine reaction
seen respectively in three groups of mothers.*

Group of mothers	Neutrophiles positive to the iodine reaction		
	+*	++**	Total (+ and ++)
Normally or strongly Arakawa-positive group	8.8 %	0 %	8.8 %
Weakly Arakawa-positive group	55.7 "	45.8 "	78.5 "
Arakawa-negative group	74.8 "	70.5 "	94.1 "

Foot note to the table:—

* Cases showing only the reaction of (+).

** Cases showing the reaction of (++)

Now, it became clear, also from my own experiment, that mothers secreting milk negative to Arakawa's reaction are abnormal or pathological on principle. Reports concerning Arakawa's reaction from our Laboratory have shown, as stated in the introduction, that Arakawa-negative mothers are more or less in a state of B-deficit. If Kiyokawa's paper is taken into consideration, it is to be ascribed to B-avitaminosis that mothers with milk with negative[§] Arakawa's reaction show a positive iodine reaction of neutrophiles.

I shall publish in the near future a report upon the experimental demonstration of the relation between the iodine reaction of neutrophiles and B-deficit in lactating women.

Conclusion.

Mothers secreting milk negative to Arakawa's reaction show a positive iodine reaction of blood neutrophiles, which is probably a sign of B-avitaminosis.

[§] Weakly positive and completely or almost completely negative Arakawa's reaction.