Increase of Reticulocytes in Mothers with Milk Negative to Arakawa's Reaction.

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By

Shingo Shiraishi.

(白石 信吾)

(From the Department of Pediatrics, Faculty of Medicine, Tohoku Imperial University, Sendai.

Director: Prof. A. Sato.)

Introduction.

Mothers secreting milk negative* to Arakawa's reaction are, as a number of papers¹⁻⁹⁾ from our Laboratory, especially Kimura's paper,⁶⁾ have shown, already in a state of avitaminosis B. On the other hand an increase of reticulocytes or red cells with polychromasia in cases of beriberi is reported by Kan,¹⁰⁾ Kohda,¹¹⁾ Ikuji,¹²⁾ Takasu,¹³⁾ Kurimoto, Aoyama and Sakai.¹⁴⁾

* "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 became blue on the addition of Arakawa's reagent. Here it means that the sample was 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 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.

- 1) T. Arakawa, Tohoku J. Exp. Med., 1930, **16**, 118.
- 2) M. Chiba, Tohoku J. Exp. Med., 1932, **19**, 282 and 486.
- 3) M. Chiba and J. Abe, Tohoku J. Exp. Med., 1932, **19**, 479.
- 4) K. Asakura and H. Ohsako, Tohoku J. Exp. Med., 1933, **20**, 429.
- 5) T. Suzuki, Tohoku J. Exp. Med., 1934, **23**, 23.
- 6) J. Kimura, Tohoku J. Exp. Med., 1934, **23**, 494.
- 7) T. Suzuki and A. Takamatsu, Tohoku J. Exp. Med., 1934, **24**, 202.
- 8) T. Suzuki, Tohoku J. Exp. Med., 1935, **25**, 186 and 201 and 575.
- 9) T. Suzuki and A. Takamatsu, Tohoku J. Exp. Med., 1935, **25**, 588.
- 10) T. Kan, Rinsho Byori Ketsueki-gaku Zasshi (Jap.) 1935, **4**, 677.
- 11) S. Kohda, Jika Zasshi, 1935, No. 420, 607.
- 12) K. Ikuji, Jika Zasshi, 1922, No. 266, 775.
- 13) K. Takasu, Tokyo Igakkai Zasshi, 1903, **17**, 395.
- 14) Y. Kurimoto, T. Aoyama and T. Sakai, Jika Zasshi, 1922, No. 263, 470.

Besides, in an experimental study on animal B-avitaminosis Ogata and his collaborators¹⁵⁾ report an increase of these cells. And such authors as Schilling,¹⁶⁾ Naegeli,¹⁷⁾ Meulengracht,¹⁸⁾ Hertz,¹⁹⁾ Shiroya,²¹⁾ Klima and Rosegger²¹⁾ agree on the point that red cells with reticulofilamentosa are identical with red cells with polychromasia. If apparently healthy lactants with Arakawa-negative milk are really in a state of avitaminosis B, then it will follow that they may show an increase of reticulocytes in their blood, while in mothers with Arakawa-positive milk the increase will not be seen. Or, at least Arakawa-negative mothers may show a larger percentage of reticulocytes than Arakawa-positive lactants. The present paper is the outcome of my own investigations concerning this question.

Method of Experiment.

1. Counting of reticulocytes (Katsunuma's method).²²⁾

One drop of blood was taken with a sterilized glass rod from the ear-lobe vein of each mother and mingled with 1% brilliantcresil blue (in physiological saline) on an object glass. Then a cover glass was set on it and the erythrocytes were microscopically examined with the oil-immersion objective, and thus the rate of reticulocytes to the total erythrocyte count was determined. Erythrocytes were calculated by use of Hayem's solution.

2. Testing of Arakawa's reaction.²³⁾

Milk from each mother was examined with Arakawa's reagent. Cases were divided into three groups according to the intensity of Arakawa's reaction.

a. The Arakawa-negative group: Arakawa's reaction of milk from both breasts is almost or entirely negative after five minutes.

15) T. Ogata etc, Mitteilung aus der Medizinischen Fakultät der Kaiserlichen Universität zu Tokyo, 1925, **32**, 431.

16) V. Schilling, Das Blutbild, Jena 1933, 54.

17) O. Naegeli, Blutkrankheiten und Blutdiagnostik, Berlin 1931, 114.

18) E. Meulengracht, Der chronische hereditäre haemolytische Ikterus, Leipzig 1922, 16.

19) R. Hertz, Dtsch. med. Wschr., 1910, **36**, 2420.

20) B. Shiroya, Jika Zasshi, 1931, No. 368, 1.

21) R. Klima und H. Rosegger, Fol. haemat., 1934, **51**, 414.

22) S. Katsunuma, Nihon Byori Gakkai Kaishi, 1919, **8**, 9.

23) T. Arakawa, Tohoku J. Exp. Med., 1930, **16**, 107.

b. The weakly Arakawa-positive group: Arakawa's reaction of milk from one or both breasts is only weakly positive in one minute.

c. The normally or strongly Arakawa-positive group: Arakawa's reaction of milk from both breasts is normally or strongly positive in one minute.

Results of Experiment.

1. Reticulocyte count in the Arakawa-negative group.

47 Mothers belonged to this group and reticulocytes were from the minimum of 6‰ (absolutely 27,000 cells per cmm.) to the maximum of 23‰ (absolutely 94,300 cells per cmm.). The average was 12‰ (absolutely 56,900 cells per cmm.) (Cf. Tables I and V).

2. Reticulocyte count in the weakly Arakawa-negative (i.e. clinically Arakawa-negative) group.

38 mothers belonged to this group and reticulocytes were from the minimum of 4‰ (absolutely 18,800 cells per cmm.) to the maximum of 14‰ (absolutely 67,200 cells per cmm.). The average was 7‰ (absolutely 36,100 cells per cmm.). One will thus see that the number of reticulocytes was smaller in this group than in the preceding group (Cf. Tables II and V).

3. Reticulocyte count in the normally or strongly Arakawa-positive group.

15 mothers belonged to this group and reticulocytes were from the minimum of 0‰ (absolutely 0 cell per cmm.) to the maximum of 7‰ (absolutely 35,000 cells per cmm.). The average was 3‰ (absolutely 14,300 cells per cmm.). One will thus see that the number of reticulocytes was smaller in this group than in the preceding two groups (Cf. Tables III and V).

Comment.

The normal number of reticulocytes in healthy persons is different according authors, as will be seen from Table IV.

One will, however, see that reticulocytes occur in normal cases below 5‰ or absolute number of 25,000 cells. Now, if my result is judged with the percentage 5‰ as the standard, the normally or strongly Arakawa-positive group with the average of 3‰ reticu-

TABLE I.
Group of the negative or almost negative Arakawa's reaction.

No. of Case	Name	Age y = years	Arakawa's reaction						Reticulocytes		Remarks
			(0')	(1')	(2')	(3')	(4')	(5')	% of erythrocytes (actual counting)	Count in 1 cmm. (theoretical calculation)	
1	S. M.	30 y	(-)	(-)	(-)	(-)	(-)	(-)	18	91,800	Beriberi; has been taking vitamin B
2	Y. C.	25 "	(-)	(-)	(-)	(-)	(-)	(+)	14	70,000	Beriberi
3	F. K.	24 "	(-)	(-)	(-)	(-)	(-)	(+)	14	71,400	
4	H. Y.	28 "	(-)	(-)	(-)	(-)	(+)	(+)	10	56,000	
5	A. I.	28 "	(-)	(-)	(-)	(-)	(-)	(-)	12	55,200	
6	K. O.	22 "	(-)	(-)	(-)	(-)	(-)	(+)	15	93,000	
7	A. O.	23 "	(-)	(-)	(-)	(-)	(+)	(+)	12	52,800	Beriberi; has been taking vitamin B
8	A. T.	37 "	(-)	(-)	(-)	(-)	(-)	(-)	14	74,200	
9	K. S.	22 "	(-)	(-)	(-)	(-)	(-)	(-)	8	40,000	Beriberi 5 years ago
10	Y. K.	26 "	(-)	(-)	(-)	(-)	(-)	(-)	10	56,000	
11	N. I.	35 "	(-)	(-)	(-)	(-)	(-)	(-)	13	71,500	
12	M. K.	30 "	(-)	(-)	(-)	(-)	(+)	(+)	9	44,100	
13	T. K.	31 "	(-)	(+)	(+)	(+)	(+)	(+)	8	40,000	
14	E. K.	30 "	(-)	(-)	(-)	(-)	(-)	(-)	10	50,000	
15	M. W.	24 "	(-)	(-)	(-)	(-)	(-)	(+)	9	43,200	
16	Y. K.	25 "	(-)	(-)	(-)	(-)	(-)	(+)	12	60,000	
17	T. T.	27 "	(-)	(-)	(-)	(-)	(-)	(-)	11	57,200	
18	M. I.	30 "	(-)	(-)	(-)	(-)	(+)	(-)	10	50,000	
19	N. S.	31 "	(-)	(-)	(-)	(-)	(-)	(-)	13	67,600	
20	T. S.	33 "	(-)	(-)	(-)	(-)	(-)	(-)	12	57,000	
21	T. T.	32 "	(-)	(-)	(-)	(-)	(+)	(-)	8	43,200	
22	T. I.	28 "	(-)	(-)	(-)	(+)	(+)	(+)	6	29,400	
23	Y. Y.	32 "	(-)	(-)	(-)	(+)	(+)	(-)	8	38,400	
24	T. B.	27 "	(-)	(-)	(-)	(-)	(-)	(+)	9	44,100	
25	M. M.	26 "	(-)	(-)	(-)	(-)	(-)	(+)	23	94,300	Beriberi

TABLE II.
Group of the weakly positive Arakawa's reaction.

No. of Case	Name	Age y=years	Arakawa's reaction					Reticulocytes		Remarks
			Right		Left			% of erythrocytes (actual counting)	Count in 1 cmm. (theoretical calculation)	
			(0')	(1') (2') (3') (4') (5')	(0') (1') (2') (3') (4') (5')					
1	S. H.	26 y	(+)	(++)	(++)	(++)	(++)	4	18,800	
2	H. K.	28 "	(-)	(+)	(++)	(++)	(++)	6	28,800	
3	T. T.	25 "	(-)	(+)	(++)	(++)	(++)	5	31,000	
4	K. I.	27 "	(-)	(+)	(++)	(++)	(++)	5	24,000	
5	S. K.	36 "	(+)	(++)	(++)	(++)	(++)	5	27,500	
6	T. S.	26 "	(+)	(++)	(++)	(++)	(++)	6	30,000	
7	K. T.	34 "	(-)	(+)	(++)	(++)	(++)	6	30,000	
8	S. S.	26 "	(-)	(+)	(++)	(++)	(++)	7	32,200	
9	M. Y.	28 "	(-)	(+)	(++)	(++)	(++)	4	18,800	
10	Y. G.	32 "	(-)	(+)	(++)	(++)	(+)	10	50,000	
11	M. M.	25 "	(-)	(+)	(++)	(++)	(-)	14	67,200	Has been taking vitamin B
12	K. N.	23 "	(-)	(+)	(++)	(++)	(+)	10	51,000	
13	T. I.	25 "	(-)	(-)	(-)	(-)	(+)	8	41,600	Beriberi; has been taking vitamin B
14	T. T.	25 "	(-)	(+)	(++)	(++)	(+)	12	60,000	Beriberi; has been taking vitamin B
15	F. K.	36 "	(-)	(+)	(++)	(++)	(+)	12	58,800	
16	S. S.	35 "	(+)	(+)	(++)	(++)	(+)	11	44,000	
17	A. T.	38 "	(-)	(-)	(-)	(-)	(++)	9	45,000	
18	M. A.	33 "	(-)	(-)	(-)	(+)	(++)	10	53,000	
19	Y. S.	30 "	(-)	(-)	(-)	(+)	(++)	9	45,000	
20	Y. K.	27 "	(-)	(-)	(+)	(++)	(+)	10	46,000	
21	T. T.	25 "	(-)	(+)	(++)	(++)	(+)	6	36,000	

No. of Case	Name	Age y = years	Arakawa's reaction						Reticulocytes		Remarks
			Right			Left			% of erythrocytes (actual counting)	Count in 1 cmm. (theoretical calculation)	
			(0') (1') (2') (3') (4') (5')		(0') (1') (2') (3') (4') (5')						
22	T. K.	31 y	(±) (++) (++) (++) (++) (++)		(-) (±) (±) (+) (+) (++)		4	24,800	Has been taking vitamin B		
23	H. A.	28 "	(-) (+) (++) (++) (++) (++)		(-) (±) (+) (+) (++) (++)		4	20,000			
24	H. O.	34 "	(-) (-) (±) (±) (±) (±)		(-) (±) (++) (++) (++) (++)		10	46,000			
25	T. S.	30 "	(±) (++) (++) (++) (++) (++)		(-) (±) (+) (++) (++) (++)		8	40,000	Beriberi		
26	M. H.	28 "	(±) (++) (++) (++) (++) (++)		(-) (+) (++) (++) (++) (++)		6	33,000			
27	M. S.	23 "	(±) (++) (++) (++) (++) (++)		(-) (±) (+) (++) (++) (++)		7	35,700			
28	M. T.	24 "	(-) (+) (++) (++) (++) (++)		(-) (-) (±) (±) (+) (+)		5	25,500	Beriberi 1 year ago		
29	Y. S.	25 "	(-) (±) (+) (++) (++) (++)		(-) (-) (±) (±) (+) (+)		8	39,200			
30	T. S.	24 "	(-) (±) (+) (++) (++) (++)		(-) (-) (±) (±) (+) (+)		5	31,000			
31	M. H.	21 "	(-) (±) (+) (++) (++) (++)		(±) (+) (++) (++) (++) (++)		5	30,000			
32	A. O.	26 "	(-) (±) (+) (++) (++) (++)		(-) (±) (+) (++) (++) (++)		11	55,000			
33	Y. M.	27 "	(±) (+) (++) (++) (++) (++)		(-) (-) (-) (-) (-) (-)		7	33,600			
34	H. Y.	27 "	(-) (+) (++) (++) (++) (++)		(-) (±) (+) (+) (+) (++)		8	47,200			
35	Y. O.	25 "	(-) (+) (++) (++) (++) (++)		(-) (-) (-) (-) (+) (+)		11	62,700			
36	M. A.	23 "	(-) (±) (+) (+) (+) (+)		(±) (+) (++) (++) (++) (++)		8	44,000			
37	T. O.	23 "	(-) (+) (++) (++) (++) (++)		(-) (+) (++) (++) (++) (++)		6	29,400			
38	I. M.	20 "	(-) (-) (-) (-) (-) (-)		(-) (±) (+) (++) (++) (++)		8	36,800			
					Average		7	36,100			
					Maximum		14	67,200			
					Minimum		4	18,800			

TABLE IV.
Reticulocyte count in normal human blood.

Author	Reticulocyte count per mille erythrocytes	Literature
R. Hertz	5-20	Dtsch. Med. Wschr., 1910, 36 , 2420.
A. Ferrata	3-4	Fol. Haemat. 1. Teil: Arch., 1910, 9 , 253.
A. M. Luzzatto and F. Ravenna.	2.8	Fol. Haemat. 1. Teil: Arch., 1912, 13 , 102.
R. I. Lee, G. R. Minot, B. Vincent	8	J. Am. Med. Ass., 1916, 67 , 719.
T. D. Cunningham	8	Arch. Int. Med., 1920, 26 , 405.
K. Sato	0.3-1.6	Jikken Ketsuekibyô Gaku, Tokyo 1921, 100.
M. J. Roessigh	4-18	Dtsch. Arch. f. klin. Med., 1922, 138 , 367.
E. Meulengracht	1-2	Der chronische hereditäre hämolytische Ikterus, Leipzig 1922, 16.
F. Amako	♂ 1-8 ♀ 1-14	Iji Shinbun, 1924, No. 1142, 689.
T. Hasuike	3	Okayama Igakkai Zasshi, 1925, No. 431, 1255.
W. Dameshek	5-10	Bost. Med. Surg. J., 1926, 194 , 759.
A. Cohn	0-4	Klin. Wschr., 1926, 5 , 1079.
H. Engel	5-20	Fol. Haemat. 1. Teil: Arch., 1926, 33 , 21.
T. Istomanowa	1-5	Dtsch. Arch. f. klin. Med., 1926, 153 , 106.
C. Seyfarth	1-2	Fol. Haemat. 1. Teil: Arch., 1927, 34 , 7.
S. Davidson	5-20	The Lancet, 1928, 214 , 847.
F. W. Moldawsky	1-2	Med. Welt., 1929, 3 , 1068.
R. I. Feldmann and E. J. Jankelewitsch	1-2	Zbl. f. Gynäk., 1929, 43 , 2729.
G. Takahashi	4	Jika Zasshi, 1930, No. 356, 119.
W. Glitsch	1-2	Zschr. f. g. exp. Med., 1930, 73 , 692.
K. Kitajima	♂ 9.1 ♀ 9.8	Gunidan Zasshi, 1930, No. 205, 1053.
Y. Yamaguchi	1-5.9	Gunidan Zasshi, 1930, No. 205, 1055.
S. Yamaguchi and Y. Suzuki	♂ 4.4 ♀ 3.8	Manshû Igakkai Zasshi, 1931, 15 , 835.
B. Shiroya	4.2-16.1 (11.2)	Zika Zasshi, 1931, No. 368, 1.
L. Heilmeyer	8	Dtsch. Arch. f. klin. Med., 1931, 171 , 123.
O. Naegeli	1	Blutkrankheiten und Blutdiagnostik, 5. Auflage, Berlin 1931, 114.
V. Schilling	1-2	Das Blutbild, Jena 1933, 32.
N. Arakawa	♂ 5.7 ♀ 6.3	Z. f. kl. Pathol. u. Hämatol., (Jap.) 1933, 2 , 19.
S. Takeuchi	♂ 4.1 ♀ 4.8	Hokuetsu Igakkai Zasshi, 1935, 50 , 133.
T. Kan	♂ 6.0 ♀ 6.5	Z. f. kl. u. Pathol. Hämatol., (Jap.) 1935, 4 , 677.
R. Seyderhelm and H. Grebe	1-2	Vitamine und Blut, Leipzig 1935, 12.

locytes may well be considered as normal. Besides, the cases with the reticulocyte count below 5% were 14 in number or 93%, which shows that the lactants of this group were almost all healthy or at least not B-avitaminotic. As to the Arakawa-negative group with the average of 12% cells, no single case showed a reticulocyte count

below 5%. 26 cases out of the lactants or 55% of all the cases of the group were of 6%-10% count, and the remaining 21 cases or 45% of them, of over 10% count. All this shows that, healthy as they might appear, mothers belonging to the Arakawa-negative group were already generally in a state of avitaminosis B. The weakly Arakawa-positive group with the average of 7% red count comes between the two groups above mentioned. And those mothers of the count below 5% were only 10 in number or 26% of all the cases, while the lactants of the count above 5% up to 10% were 22 in number or 58%. In this group there were even 6 lactants of the count over 10% reticulocytes, which thus formed 16% of all the cases (Cf. Table V).

Table VI shows by its summary that the Arakawa-negative mothers show a larger number of reticulocytes than those of the Arakawa-positive group.

It is indubitable that such an increase of reticulocytes in the cases of Arakawa-negative mothers is due to a state of avitaminosis B, because the reticulocytes show a decrease in number on an administration of vitamin B, of which I shall report in the near future in this journal.

Reticulocytes occur in the normal case below 5% and it is not to be disregarded that those authors, who report a high percentage of the normal reticulocyte count in the case of healthy persons, may have been less accurate in the selection of materials.

TABLE V.

Distribution of cases of the three different groups according to the reticulocyte count.

Reticulocytes %	Arakawa's reaction		Negative
	Normally or strongly positive	Weakly positive	
0	2	0	0
1	1	0	0
2	4	0	0
3	4	0	0
4	1	4	0
5	2	6	0
	(93%)		
6	0	6	3
7	1	3	0
8	1	6	7
9		2	5
10		5	11
	(7%)		(55%)
11		3	1
12		2	7
13		0	3
14		1	4
15			2
16 and more			4
	(16%)		(45%)
Total number of cases	15	38	47

TABLE VI.

Reticulocyte count according to the three different groups of Arakawa's reaction.

Reticulocyte count in 1 cmm.	Arakawa's reaction			Total number of cases
	Negative group	Weakly positive group	Normally or strongly positive group	
Below 25,000	0	5 cases (28%)	13 cases (72%)	18
25,100 } 50,000	19 cases (40%)	26 cases (55%)	2 cases (5%)	47
above 50,100	28 cases (80%)	7 cases (20%)	0	35

Conclusions.

1. The normal reticulocyte count in the blood of healthy mothers is below 5% (absolutely 25,000 cells per cmm.).

2. Mothers secreting milk negative to the Arakawa's reaction showed an increase of reticulocyte count, which is probably an early sign of B-avitaminosis.