The Change of Reticulocyte Count and of Arakawa's Reaction on Administration of Vitamin B* in Lactating Mothers.

94th Report of the Peroxidase Reaction.

By

Sh. Shiraishi.

(白石 信吾)

*(From the Department of Pediatrics, Faculty of Medicine,
Tōhoku Imperial University, Sendai.
Director : Prof. A. Sato.)*

Introduction.

In my^{1,2)} preceding paper I reported that mothers secreting milk negative to Arakawa's reaction showed an increase of reticulocyte count and a reticulocyte shift to the left, which was, as I reported, probably an early sign of B-avitaminosis. In the present paper I desire to report the change of the reticulocyte count on an administration of vitamin B.

Method of Experiment.

1. Counting of reticulocytes (Katsunuma's method³⁾).

One drop of blood was taken with a sterilized glass rod from the ear-lobe vein of each mother and mingled with 1% brilliant cresil blue (in physiological saline) on an object glass. Then a cover glass was set on it and the reticulocytes 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

* Vitamin B₁ is meant in the present paper.

1) Sh. Shiraishi, Tohoku J. Exp. Med., 1936, 28, 44.

2) Sh. Shiraishi, Tohoku J. Exp. Med., 1937, 31, 510.

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

of Hayem's solution. The classification of reticulocytes was determined by Heilmeyer's method.⁴⁾

2. Testing of Arakawa's reaction.*:5)

Milk from each mother was examined with Arakawa's reagent.

Result of Experiment.

Arakawa's reaction will, as Arakawa's experiment⁵⁾ shows, change from negative to positive on an administration of vitamin B, though the amount of this differs according to cases. This experiment was further confirmed by Takamatsu.⁶⁾ Now, lactating mothers with Arakawa-positive milk seen in daily practice are not acute cases of B-avitaminosis, of course, and a daily administration of vitamin B in a not very large amount cannot easily make an Arakawa-negative case Arakawa-positive, but when mothers with Arakawa-negative milk are taking vitamin B, the milk is losing in its toxicity or its content in a glyoxal-like substance or substances, even if it still remains negative to Arakawa's reaction all the same. This is what was shown by Takamatsu.⁶⁾

In the present work, it was difficult to examine mothers for a long time, because as soon as their infants had recovered from illness they stopped visiting our Dispensary.

Most of the 34 cases given in Table 1 and Table 2 showed an improvement of Arakawa's reaction on a daily administration of vitamin B, and all the cases showed a decrease in the reticulocyte count. In Cases No. 4, No. 5, No. 6 and No. 8 in Table 1 and in Cases No. 3, No. 4, No. 5, No. 8, No. 11, No. 12, No. 17 and No. 24 in Table 2, Arakawa's reaction did not improve at all, as far as the reaction during the first five minutes was concerned. Yet vitamin B must have decreased⁶⁾ the toxicity of each milk, with the reticulocyte count showing a decrease

4) L. Heilmeyer, Dtsch. Arch. f. klin. Med., 1931, **171**, 123.

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

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

** "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 the reagent. Another sample of human milk may be Arakawa-positive in the first described sense, but yet clinically negative.

5	Y.K.	25 "	-	-	-	-	-	-	-	±	-	±	-	5.0 5.0	12 8	60,000 40,000	9 days after daily administration of liq. oryzanin (30 c.c.).
6	T.T.	27 "	-	-	-	-	-	-	-	-	-	-	-	5.0 5.2	11 9	55,000 46,800	5 days after daily administration of liq. oryzanin (20 c.c.).
			-	-	-	-	-	-	-	-	-	-	-	5.3	7	37,100	14 days after daily administration of liq. oryzanin (20 c.c.).
7	M.L.	30 "	-	-	±	-	±	+	±	±	±	±	±	5.0 5.7	10 7	50,000 39,900	5 days after daily administration of liq. oryzanin (20 c.c.).
			-	+	±	±	±	±	±	±	±	±	±	5.5	5	27,500	12 days after daily administration of liq. oryzanin (20 c.c.).
8	N.S.	31 "	-	-	-	-	-	-	-	-	-	-	-	5.2 5.1	13 9	67,600 45,900	7 days after daily administration of liq. beriberol (20 c.c.).
9	S.S.	26 "	-	-	-	-	-	-	-	-	±	±	+	5.4 4.9	10 7	54,000 34,400	5 days after daily administration of liq. oryzanin (20 c.c.).
10	M.T.	25 "	-	-	+	-	±	±	±	±	±	±	±	4.7 4.9	10 7	47,000 34,200	5 days after daily administration of liq. oryzanin (20 c.c.).
			-	±	±	±	±	±	±	±	±	±	±	6.2	5	31,000	27 days after daily administration of liq. oryzanin (20 c.c.).

* Liq. oryzanin (Sankyō), 1.0 c.c. = vitamin B, then claimed to contain 6 International Units in it.

Case of No.	Name	Y= years	A r a k a w a's reaction												Ery- thro- cytes (milli- ons)
			Right						Left						
			0'	1'	2'	3'	4'	5'	0'	1'	2'	3'	4'	5'	
1	A.T.	25 Y	—	±	+	+	+	+	—	±	+	+	+	+	5.1
			—	—	±	±	++	++	—	—	±	±	++	++	5.1
			—	±	+	++	++	++	—	±	+	++	++	++	5.1
2	Y.T.	31 "	—	—	±	+	+	+	—	—	±	+	+	+	5.2
			—	—	—	—	—	±	—	±	±	±	±	+	5.4
3	T.S.	35 "	—	—	—	—	±	+	—	±	+	+	++	++	4.9
			—	—	—	±	±	±	—	±	±	+	+	+	5.0
4	I.S.	32 "	—	—	—	—	—	±	—	—	—	—	—	—	5.3
			—	—	—	—	—	—	—	—	—	—	—	—	5.3
5	I.O.	27 "	—	—	—	—	—	—	—	—	—	—	—	—	4.2
			—	—	—	—	—	—	—	—	—	—	—	—	4.7
6	I.K.	34 "	±	++	++	++	++	++	—	—	±	±	±	±	4.5
			±	++	++	++	++	++	—	±	+	++	++	++	4.6
7	K.I.	25 "	—	—	—	±	±	±	—	—	—	±	±	±	4.6
			—	—	—	—	—	—	—	±	±	+	+	+	4.6
8	Y.H.	29 "	—	—	—	—	—	—	—	—	—	—	—	—	4.9
			—	—	—	—	—	—	—	—	—	—	—	—	5.5
9	K.K.	29 "	—	±	±	±	±	±	—	—	—	—	—	—	4.3
			—	±	±	+	+	++	—	±	±	±	±	±	4.3
			±	++	++	++	++	++	—	+	++	++	++	++	4.6
10	T.A.	21 "	—	—	—	—	—	—	—	—	—	—	—	—	4.0
			—	—	—	—	—	—	—	—	—	—	±	±	4.5

2.

reticulocyte shift on administration of vitamin B.

Reticulocytes per mille						Reticulo- cytes (absolute count)	Remarks
0	I	II	III	IV	Total		
0	1	7	6	2	16	81.600	4 days after daily administration of liq. oryzanin* (20 c.c.) and liq. paranutrin (20 c.c.); and injection of oryzanin fortior** (1 c.c.).
0	0	6	10	0	16	81.600	
0	0	2	6	0	8	40.800	9 days after daily administration of liq. oryzanin (20 c.c.) and liq. paranutrin (20 c.c.); and injection of oryzanin fortior (1 c.c.) or beriberol (3 c.c.) and since 5 days injection of yakriton (0.5 c.c.) every day.
0	0	6	4.5	1	11.5	59.800	6 days after daily administration of liq. oryzanin (30 c.c.) and injection of yakriton (0.5 c.c.).
0	0	2	3	1	6	32.400	
0	0	3.5	2.5	1.5	7.5	36.800	Has been taking vitamin B.
0	0	0	1	4	5	25.000	Has been taking vitamin B for a long time.
0	0.5	6.5	5.5	0.5	13	68.900	9 days after daily administration of liq. oryzanin (20 c.c.).
0	0	3	6	0	9	47.700	
0	0	9	3	2	14	58.800	7 days after daily administration of vitamin B.
0	0	2	4	1	7	32.900	
0	0	1	3	1	5	22.500	10 days after daily administration of vitamin B.
0	0	0.5	2.5	0.5	3.5	15.800	
0	0	5	2.5	0.5	8	26.800	Has been taking vitamin B and injection of vitamin B preparation for a long time.
0	0	2	2	1	5	23.000	22 days after daily administration of vitamin B and injection of vitamin B preparation and injection of yakriton (0.5 c.c.).
0	0	7	5	1	13	63.700	7 days after daily administration of liq. oryzanin (20 c.c.).
0	0	2.5	3.5	1.5	7.5	41.300	
0	0	6	6	0	12	51.600	11 days after daily administration of liq. oryzanin (20 c.c.).
0	0	4	5	1	10	43.000	
0	0	3	3.5	0.5	7	32.200	22 days after daily administration of liq. oryzanin (20 c.c.).
0	0	9	9	1	19	76.000	9 days after daily administration of vitamin B.
0	0	5.5	7.5	1	14	63.000	

[illegible]

Reticulocytes per mille						Reticulo- cytes (absolute count)	Remarks
0	I	II	III	IV	Total		
0	0	6	5	2	13	57.200	5 days after daily administration of liq. oryzanin (20 c.c.) and liq. beriberol (20 c.c.); and injection of yakriton (0.5 c.c.).
0	0	5.5	5.5	1	12	58.800	
0	0.5	8.5	5	0.5	14.5	76.900	Has been taking vitamin B sometimes.
0	0	4	4.5	0.5	9	45.900	Has been taking vitamin B for a long time and sometimes injection of oryzanin fortior (1 c.c.).
0	0	4.5	3	0.5	8	36.800	4 days after daily administration of liq. oryzanin (20 c.c.) and injection of oryzanin (1 c.c.)* and injection of yakriton (0.5 c.c.).
0	0	4	4	0	8	40.600	
0	0	3.5	4.5	0	8	40.000	11 days after daily administration of liq. oryzanin (20 c.c.) and injection of oryzanin (1 c.c.) and injection of yakriton (0.5 c.c.).
0	0	3	3.5	0.5	7	32.900	20 days after daily administration of liq. oryzanin (20 c.c.) and injection of oryzanin (1 c.c.) and injection of yakriton (0.5 c.c.).
0	0.5	9	4.5	0	14	70.000	5 days after daily administration of liq. oryzanin (20 c.c.) and injection of yakriton (0.5 c.c.).
0	0	5.5	4.5	0.5	10.5	53.600	
0	0	2.5	2.5	1	6	30.600	10 days after daily administration of liq. oryzanin (20 c.c.) and injection of yakriton (0.5 c.c.) and since 5 days injection of paranutrin (3 c.c.).
0	0.5	5.5	4	0	10	54.000	Has been taking vitamin B.
0	0	4.5	5.5	1	11	52.800	10 days after daily administration of liq. oryzanin (20 c.c.) and injection of yakriton (0.5 c.c.).
0	0	3	4.5	0.5	8	38.400	16 days after daily administration of liq. oryzanin (20 c.c.) and injection of yakriton (0.5 c.c.).
0	1	9.5	4	0	14.5	78.300	8 days after daily administration of liq. oryzanin (20 c.c.).
0	0	6	4	0	10	50.000	
0	0	3.5	4	1	8.5	42.500	14 days after daily administration of liq. oryzanin (20 c.c.) and injection of yakriton (0.5 c.c.) and since 6 days injection oryzanin fortior (1 c.c.).
0	0	7.5	5	0	12.5	58.800	7 days after daily administration of liq. paranutrin (10 c.c.) and liq. beriberol (10 c.c.).
0	0	4.5	5.5	0.5	10.5	57.800	

Case of No.	Name	Y= years	Arakawa's reaction												Ery- thro- cytes (milli- ons)
			Right						Left						
			0'	1'	2'	3'	4'	5'	0'	1'	2'	3'	4'	5'	
18	K.Y.	34 Y	-	-	±	±	±	+	-	±	+	+	+	++	5.1
			-	-	-	-	-	-	-	±	±	+	+	++	4.9
			-	-	-	-	±	±	±	+	+	++	++	++	4.9
19	T.A.	27 „	-	-	-	±	±	±	-	-	-	-	-	-	4.6
			-	-	±	+	+	+	-	-	-	±	±	+	4.8
20	I.K.	25 „	-	-	-	-	-	-	-	-	-	-	-	-	4.9
			-	-	±	±	±	±	-	-	±	±	±	±	4.9
			-	±	±	+	+	++	-	-	±	±	±	+	4.9
21	T.N.	30 „	-	-	-	-	-	±	-	-	-	-	-	-	4.8
			±	+	++	++	++	++	-	±	±	±	±	±	5.2
22	M.A.	30 „	-	-	±	+	+	+	-	-	-	-	-	±	5.8
			-	±	+	+	+	++	-	±	+	+	++	++	5.6
23	T.W.	34 „	-	±	+	+	+	+	-	±	+	+	+	+	5.0
			-	-	±	+	++	++	-	-	±	±	+	++	5.0
			-	±	+	++	++	++	-	±	+	++	++	++	4.6
24	T.K.	25 „	-	-	-	-	-	-	-	-	+	+	++	++	4.3
			-	-	-	-	-	-	-	-	-	-	-	-	4.1
			-	-	-	-	-	-	-	-	-	-	-	-	4.2

* Liq. oryzanin. (Sankyo) 1 c.c. = vitamin B, claimed to contain 6 International
 ** Oryzanin fortior (Sankyo) 1 c.c. (injection) = vitamin B, claimed to contain
 *** Oryzanin (Sankyo) 1 c.c. (injection) = vitamin B, 33 International Units in
 **** Oryzanin (Sankyo) 1 grm. = vitamin B, claimed to contain 40 International
 ***** Ebios (Tanabe) 1 grm. = vitamin B, claimed to contain 15-27 International

Reticulocytes per mille						Reticulo- cytes (absolute count)	Remarks
0	I	II	III	IV	Total		
0	0	5.5	2	0.5	8	40.800	7 days after daily administration of liq. paranutrin (20 c.c.).
0	0	2	3.5	0.5	6	29.400	
0	0	1	2	0	3	14.700	14 days after daily administration of liq. paranutrin (20 c.c.) and since 7 days injection of yakriton (0.5 c.c.) every other day.
0	0	6	4	0	10	46.000	7 days after daily administration of vitamin B.
0	0	1.5	3	0.5	5	24.000	
0	1	5	4	0.5	10.5	51.500	5 days after daily administration of liq. paranutrin (20 c.c.).
0	0	2.5	3.5	0.5	6.5	31.900	
0	0	1	3	2	6	29.400	14 days after daily administration of liq. paranutrin (20 c.c.) and 8 days after daily injection of beriberol (5.0 c.c.) and injection of yakriton (0.5 c.c.).
0	0	4.5	4	1	9.5	45.600	6 days after daily administration of liq. oryzanin (20 c.c.) and liq. beriberol (20 c.c.); and injection of yakriton (0.5 c.c.).
0	0	2	4	1	7	36.400	
0	0.5	6	4	1	11.5	66.700	Since yesterday administration of liq. oryzanin (20 c.c.) and injection of spelzon (5 c.c.).
0	0	2	3	1.5	6.5	36.400	8 days after daily administration of liq. oryzanin (20 c.c.) and injection of spelzon (5.0 c.c.).
0	1	8	8	1	18	90.000	10 days after daily administration of oryzanin***** (6 grms.).
0	0.5	5	5.5	1.5	12.5	62.500	16 days after daily administration of oryzanin (12 grms.).
0	0	5	5	2	12	55.200	1 month after daily administration of oryzanin (12 grams.).
0	1	6	5	2	14	60.200	1 month after daily administration of oryzanin (5 grms.) and ebios***** (3.0 grms.) and injection of yakriton (0.5 c.c.) every other day.
0	0	2.5	4.5	1	8	32.800	
0	0	2	3	1.5	6.5	27.300	7 days after daily administration of oryzanin (5 grms.) and ebios (3.0 grms.) and injection of yakriton (0.5 c.c.) and oryzanin fortior (1 c.c.) every other day.

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too. Table 2 shows that the reticulocyte count decreased on an administration of vitamin B and the reticulocyte shift to the left was made nearer and nearer the normal.

Summary.

In my preceding paper¹⁾²⁾ I asserted that mothers with Arakawa-negative milk are already in a state of avitaminosis B. In the present paper I was able to make the reticulocyte count smaller in mothers of negative or weakly Arakawa's reaction by an administration of vitamin B to them, which fact shows again that Arakawa-negative cases are already in a state of B-avitaminosis.

Conclusion.

Mothers with weakly or negative Arakawa's reaction show a decrease of reticulocyte count and a restoration of reticulocyte shift to the normal on an administration of vitamin B. The abnormality of the reticulocyte picture in such lactating mothers is due to a state of B-avitaminosis.