Further Study on the Short-timed Peroxidase Reaction of Blood Leucocytes; Prolongation of the Reaction in Avitaminosis B.

44th Report of the Peroxidase Reaction.

By

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Introduction.

Different modifications of the original method of the copper peroxidase reaction devised by Prof. Sato and Sekiya¹⁾ have been reported by a number of authors in- and outside our country. A very short-timed peroxidase method was reported in 1915 from our laboratory by K. Suzuki,²⁾ who found in collaboration with Arakawa³⁾ that in B-avitaminotic pigeons blood leucocytes would become weakly peroxidase-stained with a short-timed peroxidase method. Chiba⁴⁾ of our laboratory made a special study on the relation of a very short-timed peroxidase reaction and the state of B-avitaminosis in human cases as well as in pigeons. His conclusions are to the effect that: 1) Blood leucocytes of pigeons, fed on B-avitaminotic food, will become

1) A. Sato and S. Sekiya, Tohoku Igaku Zasshi, 1924, 6, 534 and Tohoku J. Exp. Med., 1926, 7, 111. (Cf. also A. Sato and Sh. Yoshimatsu, Amer. J. Diseases. Children, 1925, 29, 301.) Sato and Sekiya's Method is as follows: To a blood smear apply Solution I (0.5% Copper sulphate solution). Pour off most of it and apply Solution II (Saturated benzidin solution, to which add 2 drops of 3% hydrogen peroxid per 100 c.c.) for two minutes. Then wash with water. The peroxidase granules of the leucocytes are stained a deep bluish green.

2) K. Suzuki, Jikwa Zasshi, 1925, 1416 and Tohoku J. Exp. Med., 1932, 19, 262. In fact before him H. Ohara tried in 1922 a short-timed modification of Sato-Sekiya's original method, but the time of application of Solution II was 20 seconds, thus very short. (Nippon-no Ikai, 1922, 23, 690).

3) K. Suzuki and T. Arakawa, Tohoku J. Exp. Med., 1930, 16, 228.

4) M. Chiba, Tohoku J. Exp. Med., 1932, 19, 282 and 486.

weakly stained with the short-timed peroxidase method, but become normally stained again on the administration of vitamin B, and 2) blood leucocytes of human cases that have something to do with B-avitaminosis will show a prolongation of the short-timed peroxidase reaction, but on the administration of vitamin B, the prolongation will be made good. He used in human cases always "three seconds method" as short-timed peroxidase reaction, because he believed that human neutrophile leucocytes became just perceptibly stained with the "three seconds method." Three seconds was the time during which Solution II of the Sato-Sekiya stain was to be applied on a blood film, while Solution I might be applied for any number of seconds.

Method of Our own Short-timed Peroxidase Reaction.

It may seem unnecessary to write about the short-timed peroxidase reaction, because my method is almost the same as Chiba's, yet I describe it here for the reason which I shall give later.

The method used was as follows:

1. The direction of use of Solution I was entirely the same as in the original method. Stain time of Solution II was shortened to three or two seconds (timed by use of a chronometer), while with the original method it is as long as two minutes. Three or two seconds is the time when the neutrophile leucocytes on a fresh blood smear from a healthy individual begin to take the stain.

2. Solution II was prepared fresh at each examination. This was again different from the original method, in which there is no absolute necessity for a fresh preparation.

3. Blood smears were tested within a definite time; always within two hours of blood taking.

4. The reaction was tested always at the room temperature.

A large number of blood smears were prepared from each patient, so that a good smear, homogenous and not too thick, might be chosen from among them. Neutrophiles were made the objects of microscopic examination.

The original copper method of Sato and Sekiya, i.e. with 2 minutes stain time of Solution II, stains myeloid leucocytes distinctly greenish-blue so that in general no one will have them stained any longer. But this original method stains blood leucocytes in both normal and B-avitaminotic subjects were equally distinctly greenish-blue. The difference between normal and B-avitaminotic leucocytes will, as

stated above, be shown only with a very short-timed peroxidase reaction. And Chiba used the "three seconds method" exclusively, because he considered 3 seconds as the shortest possible time for copper peroxidase reaction (with the Solution II).

I learned* the "three seconds method" first, but after some months of practice, I have come to consider 2 seconds for Solution II as the normal shortest stain time.

In the former series (Part I) of my own cases the normal shortest stain time for peroxidase reaction was three seconds, and in the latter series (Part II) two seconds. In my future reports concerning the shortest peroxidase-stain time, this of course will always remain two seconds instead of three.

PART I.

Three Seconds as the Shortest Stain Time for Copper Peroxidase Reaction.

In Part I the shortest time, viz. the normal time for the peroxidase reaction was considered to be three seconds.

I. Cases of Sucklings with Apparent B-deficit and with Nutritional Disturbance.

These cases were fed with an apparently false formula with an evident B-deficit, and there were six cases (40%) with a prolongation of the shortest peroxidase stain time. And two cases (Cases Nos. 6 & 8 in Table I) with such a prolongation showed, when tested later after some time of a daily administration of vitamin B, no longer any prolongation. I was unable to test the other cases. Cf. Table I.

II. Cases of Allaitement Mixte (with Such Human Milk either Negative or Weakly Positive to Arakawa's Reaction§).

In these 16 cases with allaitement mixte there were 9 cases (56.2%) with a prolongation, of which 3 cases were fed on human milk entirely or almost negative to Arakawa's reaction. And I was able to test

* The original method of Sato and Sekiya is very easy and can be performed with success by a beginner, while such a very short-timed method as the three seconds one can be attained only after long practice.

§ T. Arakawa, Tohoku J. Exp. Med., 1930, 16, 83 and 107.

TABLE I.

Cases of sucklings with apparent B-deficit and with nutritional disturbance. 3 seconds as the shortest peroxidase stain-time.

No.	Name	Age d.=days m.=months y.=years	Diagnosis	Kind of nutrition	Stain- time	Remark
1	K. Kash.	10 m.	Luxatio coxae	Condensed milk, rice-gruel	3"	
2	H. Suz.	5 m.	Hypotrophy+obstipation	Kinomeal*	4"	
3	K. Wat.	1 y. 2 m.	Atrophy	Milk, omoyu**	3"	
4	H. Kam.	10 m.	Atrophy+dyspepsia	Omoyu	3"	
5	I. Ind.	82 d.	Laes congenita+dys- pepsia	Omoyu	3"	
6	H. Hak.	5 m.	Convulsions (consequ- ence of B-avitaminosis)	Omoyu, milk	4"	
7	Y. Kaw.	105 d.	Atrophy+dyspepsia	Omoyu, milk	3"	12 days after daily administra- tion of vitamin B. No paroxysm.
8	Y. Nak.	7 m.	Atrophy+dyspepsia	Flour of rice	4" 5" 3"	22 days after daily administra- tion of vitamin B.
9	M. Hom.	1 y. 1 m.	Dyspepsia	Milk, condensed milk, omoyu	5"	
10	H. Sho.	6 m.	Grave dyspepsia	3 Milk	6"	Dead 6 days later.
11	T. Nar.	7 m.	Atrophy+dyspepsia	Condensed milk	3"	
12	T. Yam.	1 y. 2 m.	Atrophy+ecthyma	Condensed milk, omoyu, juice of fruit	3"	
13	S. It.	6 m.	Atrophy	Kinomeal, omoyu, juice of fruit	3"	
14	K. Mai.	6 m.	Laes congenita+dys- pepsia	Flour of rice	3"	
15	S. Mor.	10 m.	Grave nutritional distur- bance	Omoyu, milk	3"	

* Kinomeal is a dried milk powder preparation with an addition of carbohydrate and mineral matter.

** Omoyu is a mucous or colloidal solution prepared from rice-gruel; very frequently polished rice is used.

TABLE II.
Cases of *allaitement mixte*. 3 seconds as the shortest peroxidase stain-time.

No.	Name	Age d.=days m.=months y.=years	Diagnosis	Kind of nutrition	Arakawa's reaction		Stain-time		Remark
					Right (0') (1') (3') (5')	Left (0') (1') (3') (5')	Infant	Mother	
1	S. On	5 m.	Rhinopharyngitis	Milk, omoyu, mother's milk (only at night)	(-)(-)(-)(-)	(-)(-)(-)(-)	5"	3"	7 days after daily administration of vitamin B.
2	K. Oi	1 y.	Obstipation + anaemia levis	Omajiri,* mother's milk	(±)(+)(+)(+)	(±)(+)(+)(+)	4"	3"	
3	A. Mom.	8 m.	Bronchitis acuta	Chiefly milk	(-)(+)(+)(+) not examined	(±)(+)(+)(+)	5" 3"	3"	
4	K. Im.	5 m.	Atrophy	Lactogen, omoyu, milk of wet-nurse	not examined		4"		
5	N. Chi.	52 d.	Atrophy + Thrush	Kinomeal, mother's milk	(-)(±)(+)(+)	(-)(±)(+)(+)	3"	3"	
6	T. Ter.	8 m.	Fever after vaccination	Mother's milk, omoyu, condensed milk	(-)(-)(-)(-)	(-)(-)(-)(-)	4"	3"	
7	I. Wak.	1 y. 2 m.	Hypotrophy	Omoyu, mother's milk	(-)(-)(±)(±)	(-)(-)(+)(+)	4"	3"	
8	Y. Mat.	8 m.	Anaemia	Milk, mother's milk	(-)(-)(-)(-)	(-)(-)(-)(-)	3" 4"	4"	
9	H. Ab.	1 y. 3 m.	Bad state of nutrition after pertussis	Kinomeal, mother's milk	not examined				
10	C. Miy.	1 y.	Dyspepsia	Milk, mother's milk	(±)(+)(+)(+)	(-)(+)(+)(+)	4" 3"	3"	
11	K. Ab.	9 m.	Dyspepsia + decomposition	Milk of wet-nurse, milk	not examined				
12	Y. Ko.	1 y. 5 m.	Hypoplasia + hypotrophy	Milk of wet-nurse, milk	not examined		4"		
13	N. Ko.	7 m.	Dyspepsia	Milk, mother's milk	(-)(+)(+)(+)	(-)(+)(+)(+)	3" 3"	3"	
14	E. Kik.	1 y.	Dyspepsia + pertussis	Milk (chiefly), mother's milk	(-)(+)(+)	(-)(±)(+)			
15	Y. Kum.	5 m.	Pharyngitis acuta	Milk (chiefly), Lactogen, mother's milk	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	3"	
16	H. Nit.	5 m.	Dyspepsia	Lactogen (chiefly), mother's milk	not examined		3"		

* Omajiri is a diluted rice-gruel.

only one case (Case No. 3 in Table II) again after a week of daily administration of vitamin B, when it no longer showed any prolongation of the shortest peroxidase stain time. And it is to be noted here that most of the infants in Table II suffered from nutritional disturbance. Cf. Table II.

III. Cases of Breast-fed Infants with Human Milk (either Negative or Weakly Positive to Arakawa's Reaction).

In these 24 cases of breast feeding, there were 6 infants (25%) with a prolongation of the shortest peroxidase stain time. And 18 out of these 24 suffered from nutritional disturbance of some kind or other. It was possible to test Cases No. 1 and No. 3 in Table III again after resp. 4 and 33 days of daily administration of vitamin B, when their shortest peroxidase stain time was already restored to 3 seconds (thus to normal). Cf. Table III.

PART II.

Two Seconds as the Shortest Stain Time for Copper Peroxidase Reaction.

While I was working on the shortest stain time for Sato and Sekiya's copper peroxidase reaction, I have, as stated before, come to consider not three seconds, but two seconds as the shortest possible stain-time for it, because neutrophile leucocytes are just perceptibly blue-stained during two seconds' time. And I have put the new method to test in similar cases as those shown in Part I.

I. Cases of Sucklings with Apparent B-deficit and with Nutritional Disturbance.

There were four cases (30.8%) with a prolongation of the shortest peroxidase stain-time. And one case (Case No. 10 in Table IV), which was the only case I was able to examine more than once, showed, as will be seen from Table IV, a gradual restoration of the prolonged peroxidase stain-time to the normal. This case will again show that an administration of vitamin B in a large amount will bring back a prolonged peroxidase stain-time to the normal. Cf. Table IV.

TABLE III.
Cases of infants with human milk. 3 seconds as the shortest peroxidase stain-time.

No.	Name	Age d.=days m.=months y.=years	Diagnosis	Arakawa's reaction			Stain-time		Remark
				Right (0') (1') (3') (5')	Left (0') (1') (3') (5')		In- fant	Mo- ther	
1	T. Ooi	1 y. 5 m.	Dyspepsia	(-)(-)(+)(±)(±) not examined	(-)(±)(+)(+)(++)		4" 3"	3"	4 days after daily administration of vitamin B to infant and mother. 35 days after daily administration of vitamin B to infant and mother.
2	T. Fuj.	68 d.	Hypogalactia+eczema	(±)(+)(+)(+)(++)	(±)(+)(+)(+)(++)		4" 5"	3" 4"	
3	I. Kaw.	8 m.	Anaemia + bronchitis	not examined	not examined		3" 3"	3"	
4	Y. Uji.	1 y. 4 m.	Dyspepsia	(±)(+)(+)(+)(++)	(±)(+)(+)(+)(++)		3"	3"	
5	J. Uch.	3 m.	Dyspepsia	(-)(+)(+)(+)(++)	(-)(±)(+)(+)(++)		3"	3"	
6	T. Id.	67 d.	Obstipation	(-)(±)(±)(±)(±)	(-)(±)(+)(+)(++)		3"	3"	
7	S. Tak.	51 d.	Dyspepsia + bronchitis	(±)(+)(+)(+)(++)	(±)(+)(+)(+)(++)		3"	3"	
8	Y. Yom.	6 m.	Infantile preberiberi	(-)(±)(+)(+)(+)	(-)(-)(-)(-)(±)		3"	3"	
9	S. Kat.	6 m.	Dyspepsia	(-)(+)(+)(+)(++)	(±)(+)(+)(+)(++)		3"	3"	
10	T. Ko.	1 y.	Dyspepsia + pertussis	(-)(-)(-)(±)(±)	(-)(-)(-)(-)(+)		3"	3"	
11	K. Nit.	1 y. 1 m.	Dyspepsia	(+)(+)(+)(+)(++)	(-)(-)(±)(+)(+)		3"	3"	
12	S. Im.	6 m.	Poor state of nutrition + hypogalactia	(+)(+)(+)(+)(++)	(+)(+)(+)(+)(++)		3"	3"	
13	R. Cbi.	62 d.	Infantile preberiberi	(±)(+)(+)(+)(+)	(±)(+)(+)(+)(+)		3"	3"	
14	K. Tak.	8 m.	Obstipation	(-)(+)(+)(+)(+)	(-)(-)(-)(-)(-)		3"	3"	
15	S. Kan.	56 d.	Infantile preberiberi	(-)(-)(-)(-)(±)	(-)(-)(+)(+)(+)		3"	3"	
16	K. Wat.	30 d.	Doubt of icterus catarrhalis + meningitis	(+)(+)(+)(+)(+)	(+)(+)(+)(+)(+)		4"		
17	T. Sho.	5 d.	Icterus neonatorum	(±)(+)(+)(+)(+)	(±)(+)(+)(+)(+)		4"		
18	Sat.	1 y.	Infantile diarrhea	(-)(-)(-)(-)(-)	(-)(-)(-)(-)(-)		3"	3"	
19	R. Kat.	33 d.	Dyspepsia	(-)(-)(-)(-)(-)	(-)(-)(-)(-)(-)		3"	3"	
20	K. Sat.	4 d.	Icterus neonatorum	(-)(-)(-)(-)(-)	(-)(-)(-)(-)(-)		4"		
21	K. Tak.	43 d.	Infantile preberiberi	(-)(-)(-)(-)(-)	(-)(-)(-)(-)(-)		3"	3"	
22	T. Mas.	3 m.	Atrophy	(-)(-)(-)(-)(-)	(-)(-)(-)(-)(-)		3"	3"	
23	A. Nat.	69 d.	Dyspepsia	(±)(+)(+)(+)(+)	(±)(+)(+)(+)(+)		3"	3"	
24	T. Uch.	65 d.	Atrophy + dyspepsia	(-)(+)(+)(+)(+)	(-)(+)(+)(+)(+)		3"	3"	

TABLE IV.

*Cases of sucklings with apparent B-deficit and with nutritional disturbance.
? seconds as the shortest peroxidase stain-time.*

No.	Name	Age d.=days m.=months y.=years	Diagnosis	Kind of nutrition	Stain- time	Remark
1	M. Ai.	5 m.	Lues congenita	Milk, omoyu	2"	
2	Y. Sat.	51 d.	Atrophy	Omoyu	2"	
3	S. Ko.	7 m.	Dystrophy	Milk, omoyu	2"	
4	K. Ar.	85 d.	Atrophy	Condensed milk	2"	
5	T. Suz.	1 y.	Severe atrophy	Milk 100 c.c. $\times$ 1, omoyu 200 c.c. $\times$ 5	3"	
6	K. Oo.	8 m.	Möller-Barlow's disease	Milk	2"	
7	T. Wat.	11 m.	Möller-Barlow's disease	Milk	2"	
8	K. Tak.	7 m.	Dystrophy	Milk 200 c.c. $\times$ 2, omoyu	2"	
9	R. Ow.	11 m.	Healthy	Milk 200 c.c. $\times$ 2, condensed- ed milk	2"	
10	N. As.	45 d.	Atrophy + obstipation		4" 3"	1 week after daily administration of vitamin B. Obstipation mitigated. 2 weeks after daily administration of vitamin B. Became worse in spite of one week's treatment.
11	T. Nak.	53 d.	Vomiting (B-avitaminosis)	$\frac{1}{3}$ Milk	2"	
12	K. Hor.	11 m.	Deficit in different vitamins (abnormal posture of hands)	Condensed milk	5"	
13	K. Kan.	1 y.	"Mehlnährschaden" probable + angina	Condensed milk, rice flour	6" 2"	

II. Cases of Allaitement Mixte (with such a Human Milk as was either Negative or Weakly Positive to Arakawa's Reaction).

Most of the cases tabulated in Table V suffered from nutritional disturbance, and there was 9 cases (39.1%) with a prolongation of the shortest peroxidase stain-time, of which 5 cases were fed on human milk with completely or almost negative to Arakawa's reaction. And I was able to test 2 cases (Cases No. 2 and No. 10 in Table V) again after some days of daily administration of vitamin B to mothers and infants, when a prolongation was no longer seen in either of them. Cf. Table V.

III. Cases of Breast-fed Infants with Human Milk (either Negative or Weak to Arakawa's Reaction).

Of these 81 cases with exclusive breast feeding there were 72 cases with negative or weakly positive to Arakawa's reaction, and 37 cases (51.4%)* had a prolongation of the shortest peroxidase stain-time and most of these suffered from nutritional disturbance of some kind or other, especially from infantile beriberi or infantile preberiberi. Of these 37 cases I was able to examine the shortest stain-time in 8 cases after a lapse of time. All the cases except two were restored to the normal as to the peroxidase reaction after from 4 to 11 days of daily administration of vitamin B, and in one case (Case No. 16 in Table VI) of the two exceptions the shortest stain-time (4'') was shortened to three seconds, so that it may be assumed that it must have been restored to normal (two seconds' time) later after a continuous use of the vitamin B. And in the other case (Case No. 47 in Table VI) the shortest peroxidase stain-time (3'') was gradually prolonged up to five seconds and the infant began to vomit in spite of daily administration of vitamin B to mother and infant, probably because the mother suffered at that time more severely from the disease, which presumption was not without reason from the fact that the prolongation of the shortest peroxidase stain-time had now begun to manifest itself in the mother's blood too. More interesting is the case No. 35 in Table VI, in which the stain-time of the infant's blood was two seconds at the time when infant and mother were taking vitamin B, while, after giving up vitamin B for a week, the stain-time

* In contrast to 37.0% of Chiba; Cf. M. Chiba, Tohoku J. Exp. Med., 1932, 19, 487.

TABLE V.

Cases of allaitement mixte. 2 seconds as the shortest peroxidase stain-time.

No	Name	Age d.=days m.=months y.=years	Diagnosis	Kind of nutrition	Arakawa's reaction		Stain-time		Remark
					Right (0') (1') (3') (5')	Left (0') (1') (3') (5')	Infant	Mother	
1	M. Sug.	7 m.	Dyspepsia	Mother's milk, milk	(-)(+)(+)(+)	(-)(-)(-)(-)	3"	2"	
2	I. Kim.	31 d.	Hypogalactia	Mother's milk, milk	(-)(-)(-)(-)	(-)(-)(+)(+)	4"	2"	
					Not examined		2"		12 days after administration of vitamin B to infant and mother.
3	C. Oo.	7 m.	Hypotrophy	Milk (chiefly), mother's milk	(-)(-)(-)(-)	(-)(-)(-)(-)	2"	2"	
4	F. Hay.	6 m.	Eczema	Meal (chiefly), mother's milk	(±)(+)(+)(+)	(±)(+)(+)(+)	2"	2"	
5	R. Im.	5 m.	Dyspepsia + rhinopharyngitis acuta	Milk (chiefly), mother's milk	(-)(-)(-)(-)	(-)(-)(-)(-)	2"	2"	
6	H. Kum.	24 d.	Erythema neonatorum toxicum	$\frac{1}{3}$ Milk 100 c.c. $\times$ 3, milk of wet-nurse	Not examined		4"		
7	K. Ko.	8 m.	Obstipation	Condensed milk (6 times a day), mother's milk	(-)(+)(+)(+)	(-)(+)(+)(+)	4"	3"	Right ovary of mother is extirpated through ovarian cyst.
8	K. Mat.	1 y.	Atrophy + anaemia levis (doubt of lues congenita)	Rice, mother's milk	Not examined		2"		
9	I. It.	1 y. 3 m.	Dyspepsia	Rice, mother's milk	(-)(±)(+)(+)	(-)(±)(+)(+)	2"	2"	
10	T. Mar.	2 m.	Dyspepsia	Milk 200 c.c. $\times$ 1, mother's milk	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	2"	

				(-)(-)(-)(-)	(-)(-)(-)(-)	2"	6 days after administration of vitamin B to infant and mother.
11	H. Tam.	1 y. 3 m.	Bronchitis acuta + infantile pre-beriberi	Rice, mother's milk	(-)(±)(±)(±)	2"	2"
12	S. Sat.	1 y. 2 m.	Lymphadenitis + peritonis tuberculosa + conjunctivitis	Rice, mother's milk	(-)(-)(-)(-)	2"	2"
13	M. Sai.	1 y. 2 m.	Influenza	Rice, mother's milk	(±)(+)(+)(+)	2"	2"
14	H. Tak.	1 y. 1 m.	Influenza	Milk 200 c.c. × 1, rice, mother's milk	(-)(-)(-)(±)	2"	2"
15	K. Hos.	32 d.	Dystrophy	Milk 200 c.c. × 1, rice, mother's milk	(-)(-)(-)(±)	4"	3"
16	K. Mur.	3 m.	Atrophy (previously beriberi probable)	Condensed milk, milk of wet-nurse (twice a day)	Not examined	4"	Vomits present.
17	Y. Hor.	52 d.	Dyspepsia	Milk 200 c.c. × 2, mother's milk	(±)(+)(+)(+)	3"	Icterus neonatorum continued long.
18	R. Tsu.	2 y.	Dyspepsia	Rice, mother's milk	(-)(-)(+)(+)	2"	
19	H. Fuj.	6 m.	Serious dyspepsia + symptom of rachitis	Milk of wet-nurse, 3 milk		2"	
20	H. Sat.	1 y. 7 m.	Dyspepsia	Rice, mother's milk		2"	2"
21	A. Mat.	1 y. 10 m.	Dyspepsia	Rice, mother's milk		2"	
22	T. Su.	2 y. 7 m.	Bronchitis	Rice, mother's milk	(-)(±)(+)(+)	2"	2"
23	K. Tak.	1 y. 7 m.	Dyspepsia	Rice, mother's milk (chiefly)	(-)(-)(+)(+)	3"	2"

TABLE VI.

Cases of infants with human milk. 2 seconds as the shortest peroxidase stain-time.

No.	Name	Age d.=days m.=months y.=years	Diagnosis	A r a k a w a's reaction				Stain-time		Remark
				Right (0') (1') (3') (5')	Left (0') (1') (3') (5')	In- fant	Mo- ther			
1	K. Wash.	47 d.	Infantile preberiberi	(-)(-)(-)(-)	(-)(-)(-)(-)	2"	2"	7 days after daily administration of vitamin B to infant and mother.	2"	
2	T. Got.	49 d.	Infantile preberiberi	(-)(+)(+)(+)	(-)(+)(+)(+)	2"	2"		2"	
3	I. It.	63 d.	Eczema	(-)(+)(+)(+)	(-)(+)(+)(+)	2"	2"		2"	
4	S. Jui.	7 m.	Rhinopharyngitis	(-)(-)(-)(-)	(-)(-)(-)(-)	2"	2"		2"	
5	K. Shi.	34 d.	Infantile preberiberi	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	3"		3"	
6	T. Suz.	1 y.	Atrophy + dyspepsia	(-)(+)(+)(+)	(-)(+)(+)(+)	2"	2"		2"	
7	S. Sai.	52 d.	Infantile preberiberi	(-)(-)(-)(-)	(-)(-)(-)(-)	2"	2"		2"	
8	T. Tak.	41 d.	Infantile preberiberi	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	3"		2"	
9	R. Yam.	6 m.	Dyspepsia	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	2"		2"	
10	R. Suz.	49 d.	Hypotrophy	(-)(+)(+)(+)	(-)(+)(+)(+)	2"	2"		2"	
11	R. Sat.	40 d.	Infantile preberiberi	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	2"		2"	
12	H. Yus.	9 m.	Lues congenita	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	2"		2"	
13	A. Ab.	3 m.	Rhinopharyngitis acuta	(-)(-)(-)(-)	(-)(-)(-)(-)	2"	2"		2"	
14	S. Hay.	62 d.	Lues congenita	(-)(-)(-)(-)	(-)(-)(-)(-)	2"	2"	11 days after daily administration of vitamin B to infant and mother. Mother is ill of beriberi, but already has been taking vitamin B.	2"	
15	T. It.	1 y.	Hoarseness	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	2"		2"	
16	Y. Fun.	46 d.	Lues congenita	(-)(-)(-)(-)	(-)(-)(-)(-)	4"	3"		2"	
17	J. Tak.	11 m.	Dyspepsia	(-)(+)(+)(+)	(-)(-)(-)(-)	2"	2"	2"	6 days after daily administration of vitamin B to infant and mother. Mother is ill of beriberi, but already has been taking vitamin B.	
18	M. Tak.	4 m.	Infantile preberiberi	(-)(-)(-)(-)	(-)(-)(-)(-)	4"	2"	2"		
19	T. Yor.	57 d.	Infantile preberiberi	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	3"	2"		
20	Y. His.	52 d.	Infantile preberiberi	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	3"	2"		
21	M. Chi.	53 d.	Dyspepsia + doubt of Rachitis	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	3"	2"		

22	S. Tak.	1 y. 2 m.	Disturbance of nutrition after pertussis	(-)(-)(-)(-)(-)	Not examined	(-)(-)(-)(-)(-)	5"	2"	11 days after daily administration of vitamin B to infant and mother. Weight of body increased.
23	Y. Hay.	31 d.	Infantile beriberi	(-)(-)(-)(-)(-)		(-)(-)(-)(-)(-)	8"	2"	Died next day. The most remarkable prolongation of the shortest peroxidase stain-time ever experienced; note the normal stain-time of mother.
24	S. Kim.	4 m.	Rhinopharyngitis acuta	(-)(-)(+)(+)(+)		(-)(-)(±)(±)(±)	2"	2"	Mother is ill of beriberi, but has never taken vitamin B.
25	I. Suz.	44 d.	Diarrhea (consequence of B-avitaminosis)	(±)(+)(+)(+)(+)		(±)(+)(+)(+)(+)	3"	2"	
26	T. Tak.	8 m.	Rubeola + bronchitis acuta	(+)(+)(+)(+)(+)		(+)(+)(+)(+)(+)	2"	2"	
27	M. Sat.	4 m.	Rhinopharyngitis acuta	(-)(±)(+)(+)(+)		(-)(-)(+)(+)(+)	3"	2"	9 days after daily administration of vitamin B to infant and mother.
28	T. Hash.	63 d.	Obstipation (consequence of infantile preberiberi)	(-)(-)(-)(+)(+)		(-)(+)(+)(+)(+)	3"	3"	
29	E. Mit.	6 m.	Rhinopharyngitis acuta	(-)(±)(+)(+)(+)	Not examined	(±)(+)(+)(+)(+)	2"	2"	10 days after daily administration of vitamin B to infant and mother.
30	E. Ko.	55 d.	Infantile preberiberi	(±)(+)(+)(+)(+)		(±)(+)(+)(+)(+)	2"	2"	
31	R. Fuk.	28 d.	Dyspepsia	(-)(-)(±)(±)(±)		(-)(-)(-)(-)(-)	2"	2"	
32	K. Hat.	34 d.	Infantile beriberi probable	(-)(-)(-)(-)(-)		(-)(-)(-)(-)(-)	3"	2"	
33	S. Suz.	7 m.	Anaemia	(-)(±)(±)(±)(±)		(-)(±)(±)(±)(±)	2"	2"	Infant and mother under administration of vitamin B.
34	K. Suz.	2 m.	Avitaminotic dyspepsia	(±)(+)(+)(+)(+)		(+)(+)(+)(+)(+)	2"	2"	
35	K. Kat.	2 m.	Pharyngotracheitis + doubt of infantile Preberiberi	(-)(-)(-)(-)(-)		(-)(-)(-)(-)(-)	2"	2"	
				(-)(-)(-)(±)(±)		(-)(-)(-)(-)(-)	3"	2"	1 week after interruption of administration of vitamin B. Vomiting increased.
				(-)(-)(-)(±)(+)		(-)(-)(-)(-)(±)	2"	2"	4 days after resuming of administration of vitamin B.

No.	Name	Age d.=days m.=months y.=years	Diagnosis	Ara kawa's reaction				Stain-time		Remark			
				Right (0') (1') (3') (5')	Left (0') (1') (3') (5')	In- fant	Mo- ther						
36	T. Sat.	2 m.	Bronchitis acuta	(-)	(-)	(-)	(-)	(±)	(+)	(+)	4"	2"	Mother has taken vitamin B. 7 days after daily administration of vitamin B to infant and mother. 17 days after daily administration of vitamin B to infant and mother. Infant vomits.
37	T. Got.	1 y. 2 m.	Anaemia+hernia	(+)	(+)	(+)	(+)	(+)	(+)	(+)	2"	2"	
38	M. Tan.	25 d.	Infantile beriberi probable	(-)	(-)	(-)	(+)	(-)	(±)	(+)	3"	3"	
39	S. Tan.	2 m.	Infantile beriberi probable	(-)	(±)	(+)	(+)	(-)	(±)	(+)	3"	2"	
40	H. Ed.	5 m.	Dyspepsia	(-)	(-)	(±)	(±)	(-)	(±)	(±)	2"	2"	
41	K. Hir.	25 d.	Dyspepsia	(-)	(-)	(±)	(±)	(-)	(±)	(±)	2"	2"	
42	S. Kur.	3 m.	Vomiting and obstipation	(-)	(±)	(+)	(+)	(±)	(+)	(+)	2"	2"	
43	K. Mat.	3 m.	Bronchitis	(-)	(±)	(+)	(+)	(±)	(+)	(+)	2"	2"	
44	T. Yor.	4 m.	Eczema	(-)	(-)	(-)	(±)	(-)	(±)	(±)	2"	2"	
45	T. Koz.	22 d.	Bronchopneumonia	(-)	(-)	(-)	(-)	(-)	(-)	(-)	2"	2"	
46	Y. Iwa.	6 m.	Eczema	(-)	(-)	(-)	(-)	(±)	(+)	(+)	2"	2"	
47	M. Uj.	2 m.	Severe Atrophy	(-)	(-)	(-)	(-)	(-)	(-)	(-)	3"	4"	
48	A. Yosh.	3 m.	Rhinopharyngitis + doubt of infantile preberiberi	(-)	(-)	(-)	(-)	(-)	(-)	(-)	5"	3"	
49	K. Bab.	7 m.	Dyspepsia	(-)	(+)	(+)	(+)	(±)	(+)	(+)	2"	2"	
50	K. Ish.	2 m.	Rhinopharyngitis	(-)	(+)	(+)	(+)	(-)	(+)	(+)	2"	2"	
51	F. Kik.	12 d.	Infantile beriberi	(-)	(-)	(-)	(-)	(-)	(-)	(-)	4"	2"	
52	T. Sak.	4 m.	Eczema + rhinopharyngitis acuta	(-)	(±)	(+)	(+)	(±)	(+)	(+)	2"	2"	
53	T. Tach.	3 m.	Rhinopharyngitis acuta	(-)	(±)	(+)	(+)	(±)	(+)	(+)	2"	2"	
54	T. Mun.	34 d.	Infantile beriberi	(-)	(-)	(-)	(-)	(-)	(-)	(-)	5"	2"	
55	S. Koz.	59 d.	Influenza	(-)	(±)	(+)	(+)	(-)	(±)	(+)	2"	2"	
56	S. Wat.	9 m.	Rhinopharyngitis acuta + lues congenita	(-)	(-)	(-)	(-)	(-)	(-)	(-)	3"	3"	Infant was once injected with vitamin B.

57	Y. Mor.	3 m.	Lues congenita	(-)(-)(-)(-)	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	2"
58	Y. Tak.	6 m.	Dyspepsia	(+)(+)(+)(+)	(+)(+)(+)(+)	(+)(+)(+)(+)	2"	2"
59	T. Tak.	76 d.	Infantile preberiberi	(+)(+)(+)(+)	(+)(+)(+)(+)	(+)(+)(+)(+)	3"	2"
60	M. Kik.	46 d.	Infantile preberiberi	(-)(+)(+)(+)	(-)(+)(+)(+)	(-)(+)(+)(+)	2"	2"
61	T. Wat.	4 m.	Dyspepsia	(+)(+)(+)(+)	(+)(+)(+)(+)	(+)(+)(+)(+)	2"	2"
62	M. Kon.	29 d.	Doubt of infantile beriberi	(-)(-)(-)(-)	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	2"
63	K. Tak.	70 d.	Atrophy+doubt of lues congenita	(+)(+)(+)(+)	(+)(+)(+)(+)	(+)(+)(+)(+)	3"	2"
64	M. Hig.	46 d.	Subicterus	(-)(+)(+)(+)	(-)(+)(+)(+)	(-)(+)(+)(+)	2"	2"
65	K. Sai.	38 d.	Doubt of avitaminosis B	(+)(+)(+)(+)	(+)(+)(+)(+)	(+)(+)(+)(+)	2"	2"
66	S. Koz.	3 m.	Pharyngitis	(+)(+)(+)(+)	(+)(+)(+)(+)	(+)(+)(+)(+)	2"	2"
67	K. Kam.	87 d.	Hypotrophy+doubt of infantile beriberi	(-)(-)(+)(+)	(-)(-)(+)(+)	(-)(-)(+)(+)	3"	3"
68	T. Hat.	5 m.	Rhinopharyngitis acuta	(-)(+)(+)(+)	(-)(+)(+)(+)	(-)(+)(+)(+)	2"	2"
69	R. Eg.	33 d.	+hypotrophy Doubt of infantile beriberi	(-)(-)(-)(-)	(-)(-)(-)(-)	(-)(-)(-)(-)	4"	2"
70	S. Nog.	103 d.	Meningitis tuberculosa	(-)(+)(+)(+)	(-)(+)(+)(+)	(-)(+)(+)(+)	2"	Infant and mother have taken vitamin B.
71	O. Kum.	1 y. 2 m.	Dystrophy+stomatitis	(+)(+)(+)(+)	(+)(+)(+)(+)	(+)(+)(+)(+)	2"	
72	T. Yam.	8 m.	Bronchopneumonia	(+)(+)(+)(+)	(+)(+)(+)(+)	(+)(+)(+)(+)	2"	Infant and mother have taken vitamin B.
73	H. Oo.	23 d.	Infantile beriberi probable	(-)(-)(+)(+)	(-)(-)(+)(+)	(-)(-)(+)(+)	3"	
74	X. Tak.	39 d.	Hypotrophy+doubt of infantile preberiberi	(-)(+)(+)(+)	(-)(+)(+)(+)	(-)(+)(+)(+)	2"	Infant and mother have taken vitamin B.
75	K. Tak.	3 m.	Pharyngobronchitis	(-)(+)(+)(+)	(-)(+)(+)(+)	(-)(+)(+)(+)	2"	
76	S. Tan.	1 y. 2 m.	Dyspepsia+bronchitis+thrush	(+)(+)(-)(-)	(+)(+)(-)(-)	(+)(+)(-)(-)	2"	Infant and mother have taken vitamin B.
77	T. Ko.	1 y. 3 m.	Dyspepsia	(+)(+)(+)(+)	(+)(+)(+)(+)	(+)(+)(+)(+)	2"	
78	T. Kur.	3 m.	Dyspepsia	(+)(+)(+)(+)	(+)(+)(+)(+)	(+)(+)(+)(+)	2"	Infant and mother have taken vitamin B.
79	T. Yas.	21 d.	Doubt of infantile preberiberi+icterus neonatum	(-)(-)(-)(-)	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	
80	R. Yos.	37 d.	Obstipation (consequence of hypogalacty)	(+)(+)(+)(+)	(+)(+)(+)(+)	(+)(+)(+)(+)	2"	
81	H. Sho.	36 d.	Hypotrophy+rhinopharyngitis	(-)(-)(-)(-)	(-)(-)(-)(-)	(-)(-)(-)(-)	3"	

(2'') was prolonged to three seconds, and then, after taking vitamin B again for 4 days the prolonged stain-time (3'') was shortened again and restored to the normal.

It is to be noted here that mothers were, from the peroxidase point of view, suffering from avitaminosis B in a less degree than their babies were, because there were only 6 cases (9.1%)* with a prolongation of the shortest peroxidase stain-time out of 65 mothers, who were secreting milk negative or weakly positive to Arakawa's reaction. And we can say with Chiba and Abe⁵⁾ that "assuming that the prolongation of the stain-time is due to the grade of avitaminosis B, it is a possibility that a mother secreting B-avitaminotic milk can make her suckling more avitaminotic than herself." I was able to examine 17 babies of infantile preberiberi and among them I found 11 cases with a prolongation of the shortest peroxidase stain-time. (Chiba⁴⁾ examined 8 cases of infantile preberiberi, all with a prolongation of the shortest peroxidase stain-time).

As to the case of the diagnosis of infantile beriberi and that of infantile B-avitaminosis, there was no case out of all the 13 patients without a prolongation of the shortest peroxidase stain-time; the stain-time was 3 seconds in nine cases, 4 seconds in two, 5 seconds in one, and in the other remaining case, as long as 8 seconds. But then, there were only two out of the whole 13 mothers with a prolongation of the shortest peroxidase stain-time. Chiba⁴⁾ examined 17 cases of infantile beriberi and there were 9 babies and only 2 mothers with a prolongation of the shortest peroxidase stain-time. Here again I repeat the above cited quotation from the paper of Chiba and Abe.⁵⁾ As to the shortest possible peroxidase stain-time as my experience described in Part II shows, it is two seconds instead of three, and two seconds will be considered as normal for the short-timed peroxidase stain.

Final Comparison between Two and Three Seconds Method in Different Diseases.

Here a comparison between the "two" and the "three seconds method" in different diseases will be given. This comparison is not, however, that between the two methods in one and the same cases, but that between the "three seconds method" in one series of cases

* In contrast to 17.0% of Chiba; Cf. M. Chiba, *Tohoku J. Exp. Med.*, 1932, **19**, 492.
5) M. Chiba and J. Abe, *Tohoku J. Exp. Med.*, 1932, **19**, 479.

TABLE VII.

Cases of different diseases including healthy. 3 seconds as the shortest peroxidase stain-time.

No.	Name	Age d.=days m.=months y.=years	Diagnosis	Stain- time	Remark
1	K. Mor.	4 d.	Melaena neonatorum + icterus neonatorum	3"	
2	T. Sho.	1 y. 5 m.	Dyspepsia	3"	
3	T. Sat.	1 y. 7 m.	"	3"	
4	Y. Kat.	1 y. 6 m.	"	3"	
5	T. Mut.	1 y. 6 m.	Dyspepsia + thrush	3"	
6	T. Um.	9 y. 3 m.	Beriberi	3"	Has taken vitamin B and "Haiga-mai."
7	T. Wat.	25 y.	"	3"	Has been taking vita- min B.
8	I. Tak.	11 y. 7 m.	Gastritis acuta	3"	
9	M. Jo.	3 y.	" "	3"	
10	Y. Nun.	7 y. 7 m.	" "	3"	
11	K. Oka.	4 y.	Icterus catarrhalis	6"	
				3"	12 days later, jaundice disappeared.
12	I. Sat.	6 y. 9 m.	" "	3"	
13	J. Uch.	50 d.	" "	3"	Jaundice almost disap- peared.
14	I. Hat.	5 y. 5 m.	Angina acuta	3"	
15	C. Ab.	5 y. 5 m.	Rhinopharyngitis acuta	3"	
16	K. Ab.	2 y. 10 m.	Pharyngitis acuta	3"	
17	H. Fuk.	1 y. 4 m.	Rhinopharyngitis acuta	3"	
18	I. Tak.	9 y.	Hypertrophy of tonsil	3"	
19	N. Iwa.	5 y. 8 m.	Bronchitis acuta	3"	
20	S. Ab.	8 y. 2 m.	Periodic vomiting	4"	
				3"	9 days after administra- tion of vitamin B and injection of Yakriton $\frac{1}{2}$ unit once.
21	T. Tak.	9 y. 10 m.	Orthotic albuminuria	3"	
22	T. Ito.	4 m.	Meningitis purulenta	3"	
23	H. Ot.	8 y. 6 m.	Choreic movement + weakness of mind (con- sequence of encephalitis)	5"	Administration of vita- min B and injection of Yakriton 1 unit twice. 9 days after choreic movement decreased.
				3"	
24	M. Mor.	14 y.	Chorea	3"	
25	J. Sai.	2 y. 11 m.	Dyspepsia acuta	3"	
26	E. Ko.	5 y. 11 m.	" "	3"	
27	A. Tak.	3 y. 11 m.	" "	3"	
28	A. Ak.	3 y. 5 m.	" "	3"	
29	K. Rau.	4 y. 10 m.	" "	3"	
30	N. Nag.	4 y. 1 m.	" "	3"	
31	R. Asa.	1 y. 11 m.	" "	3"	
32	C. Kum.	9 y. 1 m.	Latent tuberculosis	3"	
33	Y. Nar.	1 y. 8 m.	Erythema nodosum	3"	
34	H. Shib.	1 y. 10 m.	Lues congenita	3"	
35	T. Tak.	2 y. 11 m.	Eczema	3"	
36	K. Wat.	3 y. 3 m.	Healthy	3"	
37	I. Tak.	7 y. 3 m.	"	3"	

TABLE VIII.

Cases of different diseases including healthy. 2 seconds as the shortest peroxidase stain-time.

No.	Name	Age d.=days m.=months y.=years	Diagnosis	Stain- time	Remark
1	K. Nak.	1 y. 4 m.	Möller-Barlow's disease	2"	
2	F. Suz.	6 m.	Lead-poisoning	2"	
3	K. Tak.	9 m.	Poisoning of human milk	2"	
4	K. Tsu.	1 y. 5 m.	Dyspepsia acuta	2"	
5	Y. Sun.	5 y. 11 m.	" "	2"	
6	M. Tak.	1 y. 3 m.	" "	2"	
7	H. Ao.	1 y. 4 m.	" "	2"	
8	T. Ak.	1 y. 8 m.	Hypotrophy	2"	
9	K. Suz.	13 y.	Appendicitis acuta	2"	
10	T. Suz.	8 y. 11 m.	Stomatitis	2"	
11	H. Hash.	5 y. 4 m.	Ascariasis	2"	
12	E. Ak.	2 y. 10 m.	Obstinate chronic diarrhea	2"	
13	M. Nak.	3 y. 6 m.	Abscess in abdomen	2"	
14	H. Uj.	10 y. 2 m.	Icterus catarrhalis	2"	Has been taking vita- min B.
15	S. Ko.	4 y. 6 m.	False croup	2"	
16	A. Mo.	8 y. 2 m.	Angina	2"	
17	A. Min.	1 y. 2 m.	"	2"	
18	M. Sat.	8 y. 1 m.	Bronchitis acuta	2"	
19	M. Sas.	1 y. 4 m.	" "	2"	
20	R. Hor.	1 y. 7 m.	Bronchopneumonia	2"	
21	K. Suz.	1 y. 8 m.	"	2"	
22	K. Chi.	2 y.	"	4"	Since 1 month ill.
23	M. End.	9 y. 2 m.	Asthma bronchiale	2"	
24	T. Ik.	21 y.	Dysmenorrhoe	2"	
25	Y. Um.	10 y. 11 m.	Nephritis acuta	2"	
26	S. End.	8 y. 2 m.	Chorea minor	2"	
27	K. Tom.	3 y.	Aphasia	2"	
28	S. Mat.	2 y. 5 m.	Idiocy	2"	
29	S. Chi.	9 y. 7 m.	Epilepsia	2"	
30	S. Tak.	1 y. 10 m.	Hydrocephalus	2"	
31	S. Hin.	50 y.	Morbus Weilli	3"	
32	K. Ak.	8 y. 9 m.	Influenza	3"	High fever has conti- nued long days with no appetite.
33	Z. Tak.	9 y. 8 m.	Typhus abdominalis	2"	
34	K. Sas.	12 y. 1 m.	" "	2"	
35	K. Tak.	14 y.	Typhus abdominalis + beriberi	3"	
36	M. Kud.	7 y. 1 m.	Morbilli	2"	
37	K. Tak.	6 y. 11 m.	"	2"	
38	A. Hor.	1 y. 8 m.	"	2"	
39	S. Shi.	13 y. 4 m.	Scarlatina	2"	
40	S. Ob.	1 y. 6 m.	Erythema infectiosum	2"	
41	M. Sug.	8 y.	Diphtheria	2"	
42	S. Kur.	2 y. 11 m.	"	2"	
43	T. Kam.	1 y. 7 m.	Pleuritis tuberculosa	2"	
44	I. Shi.	2 y. 1 m.	" "	2"	
45	Y. Kum.	5 y. 6 m.	" "	2"	
46	Y. Noz.	8 y. 1 m.	Latent tuberculosis	2"	

No.	Name	Age d.=days m.=months y =years	Diagnosis	Stain- time	Remark
47	H. Fuj.	11 y.	Latent tuberculosis	2"	
48	Z. As.	4 y. 1 m.	Tuberculosis of abdomen	2"	
49	Y. Sui.	6 y. 9 m.	Pulmonary tuberculosis	2"	
50	K. Nag.	5 y. 4 m.	Latent tubercnlosis	2"	
51	M. Tan.	12 y. 2 m.	Spondylitis tuberculosa	2"	
52	Y. Suz.	5 y. 2 m.	Latent tuberculosis	2"	
53	K. Ab.	10 y. 10 m.	" "	2"	
54	T. Nuk.	9 y.	" "	2"	
55	Z. Mak.	8 y. 2 m.	Pleuritis tuberculosa + pulmonary tuberculosis	2"	
56	H. Kan.	8 y. 2 m.	Latent tuberculosis	2"	
57	F. Gom.	8 y. 4 m.	Pulmonary and abdomi- nal tuberculosis	2"	
58	K. Tak.	6 y.	Abdominal tuberculosis	2"	
59	T. Um.	10 y.	Spondylitis tuberculosa	2"	
60	S. Chi.	1 y.	Lues congenita + dispepsia	2"	
61	Y. Sek.	2 y. 2 m.	Lues congenita	2"	
62	T. Sat.	9 m.	" "	2"	
63	I. Iak.	1 y. 6 m.	Dyspepsia acuta	2"	
64	A. Ko.	2 y. 10 m.	" "	2"	
65	M. Saw.	2 y. 2 m.	" "	2"	
66	S. Nak.	3 y.	" "	2"	
67	A. Nak.	3 y. 11 m.	" "	2"	
68	M. Kim.	2 y. 2 m.	" "	2"	
69	C. Ish.	8 y. 3 m.	Abscess in abdomen	2"	
70	M. Kat.	12 y.	Healthy	2"	
71	M. Tak.	21 y.	"	2"	
72	S. Shi.	27 y.	"	2"	
73	S. Sat.	8 y. 10 m.	"	2"	
74	T. Sat.	28 y.	"	2"	
75	H. Tan.	20 y.	"	2"	
76	Y. Kan.	6 y. 1 m.	Abdominal pain	2"	
77	H. Sat.	1 y. 2 m.	Tendency of cramp + obstipation	2"	
78	E. Tak.	10 y.	Incontinentia albi	2"	
79	T. Tom.	6 y. 9 m.	Head-ache + anorexia	2"	
80	K. Has.	2 y. 5 m.	Idiopathic cramp	2"	
81	S. Fuj.	5 y. 6 m.	Myelogenic leukemia	2"	
82	R. Suz.	10 y. 3 m.	Lymphatic leukemia	2"	
83	T. Sat.	4 y. 2 m.	Purpura	2"	
84	T. Mom.	10 y. 11 m.	Hemorrhagic diathesis	2"	
85	T. Kiu.	27 y.	Pregnancy	2"	Arakawa's reaction: both sides utterly negativ.
86	S. Sat.	11 y. 3 m.	White hair + nystagmus + excited patellar reflex + small stature.	2"	
87	S. Ui.	11 y. 4 m.	" "	2"	
88	K. Ai.	11 y.	Tumour of brain	2"	
89	K. Wak.	8 y.	Doubt of tumour of brain	2"	

(Table VII), and the "two seconds method" in the other series (Table VIII); so there can be only an indirect comparison, not a direct one.

In the former series with the "three seconds method," there were 35 cases of different diseases, and three cases (Case No. 11, Case No. 20 and Case No. 23 in Table VII) among them that showed a prolongation, suffering evidently from avitaminosis B.

As to healthy individuals, there were 31 cases tested with the "three seconds method." There were two cases with a prolongation (Cf. Table III). One mother was secreting milk utterly negative to the Arakawa's reaction and the other whose infant showed a prolongation (5 seconds) of the shortest peroxidase stain-time.

In the latter series with the "two seconds method," 83 cases of different diseases were put to the test. And there were 4 cases (Case No. 22, Case No. 31, Case No. 32 and Case No. 35 in Table VIII) that showed a prolongation of the shortest peroxidase stain-time; it was evident that all of them suffered from B-vitamin-deficit.

Again 84 apparently healthy individuals were tested with the "two seconds method," and there were 4 cases with a prolongation of the shortest peroxidase stain-time (Cf. Table VI), all of whom were secreting milk utterly negative to the Arakawa's reaction.

It will thus be concluded from either of these two methods that a prolongation of the shortest peroxidase stain-time is a sign of avitaminosis B.

TABLE IX.

Relation between the Age and the Shortest Peroxidase Stain-time (Compiled from Tabs. I, II, III, IV, V and VI of my own experience)

Age m.=months y.=years	Number of tested cases	Prolonged		Not prolonged	
		Number of cases	%	Number of cases	%
1 m.	12	9	75.0	3	25.0
2 m.	41	25	60.9	16	39.1
3 m.	25	9	36.0	16	64.0
4 m.	9	3	33.3	6	66.7
5 m.	10	4	40.0	6	60.0
6 m.	12	2	16.6	10	83.4
7 m.	10	2	20.0	8	80.0
8 m.	9	4	44.4	5	55.6
9 m.	3	1	33.3	2	66.7
10 m.	3	0	0	3	100.0
11 m.	4	1	25.0	3	75.0
12 m.	10	4	40.0	6	60.0
2 y.	23	7	30.4	16	69.6
3 y.	1	0	0	1	100.0
Total	172	71	41.2	101	58.8

TABLE X.

*Relation between the Age and the Shortest Peroxidase Stain-time
(Complied from Tabs. I and II of Chiba)*

Age m.=months y.=years	Number of tested cases	Prolonged		Not prolonged	
		Number of cases	%	Number of cases	%
1 m.	34	14	41.1	20	58.9
2 m.	23	9	39.1	14	60.9
3 m.	13	2	15.3	11	84.7
4 m.	9	3	33.3	6	66.7
5 m.	5	1	20.0	4	80.0
6 m.	14	7	50.0	7	50.0
7 m.	5	1	20.0	4	80.0
8 m.	3	0	0	3	100.0
9 m.	2	2	100.0	0	0
10 m.	6	3	50.0	3	50.0
11 m.	6	4	66.6	2	33.4
12 m.	3	2	66.6	1	33.4
2 y.	46	15	32.6	31	67.4
3 y.	1	0	0	1	100.0
Total	170	63	37.0	107	63.0

Figure II. Relation between the age and the shortest peroxidase stain-time (Complied from Tabs. I and II of Chiba's experience)

We have seen from the paper of Chiba and Abe⁵⁾ that mothers with a deficit in vitamin B can make their sucklings more B-avitaminotic than their own bodies. I have, as already stated, experienced the same thing in my investigation. This fact may be explained from a difference in age. If this way of explanation is applied to infants themselves, there must be some difference concerning the prolongation of the shortest peroxidase stain-time according to the difference of age, namely, according as infants are older or younger. As will be seen from Tab. IX and Fig. I, younger infants are surely more disposed to the prolongation than older ones, though the relation is not so clearly seen from Tab. X and Fig. II, as from Tab. IX and Fig. I. At any rate, it seems that older people are less prone to the alleged prolongation than infants are.

Conclusions.

1. The shortest stain-time for copper peroxidase reaction (of neutrophile leucocytes) has been considered by K. Suzuki to be nor-

mal in human cases, if it is three seconds. But my experience shows that it is two seconds instead of three. In other words, neutrophiles of human blood will begin to take peroxidase stain just perceptibly in two seconds.

2. By this method of short-timed peroxidase reaction a similar conclusion as has been made by Chiba and Abe is attained. In individuals with B-vitamin-deficit or with diseases related to it, a prolongation of the short stain-time will occur; the longest prolongation I have ever experienced is 8 seconds (in contrast to 2 seconds as normal). The prolongation will be made good if vitamin B is administered in a fair amount.

3. The prolongation of the shortest peroxidase stain-time occurs more remarkably in younger individuals. This explains the fact that it is found much more frequent in suckling infants than in their mothers, more frequent in younger infants than in older ones.