**Short-timed Peroxidase Reaction of Blood Leucocytes of
B₁-avitaminotic Rats ; and Effect of Different
Amounts of Vitamin B₁ on It.**

**163rd Report of the Peroxidase Reaction.
(110th Human Milk Study.)**

By

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INTRODUCTION.

It has been reported in a number¹⁻⁵⁾ of papers from our Laboratory that the prolongation of the normal shortest peroxidase stain-time occurs in B-avitaminosis, in human cases as well as in experimental animals. In 1938, Suzuki and Shiraishi⁶⁾ (Cf. 23rd Hematological Paper) proposed to use the Tohoku Pediatric Method⁷⁾ instead of Sato and Sekiya's peroxidase reaction for the short-timed peroxidase reaction, and stated that the short-timed peroxidase reaction could be shown more precisely by the Tohoku Pediatric Method than by the original method. In a paper of mine, (Cf. 170th Report of the Peroxidase Reaction)⁸⁾ I will further report the practicability of the "new technique" for the short-timed peroxidase reaction and that a prolongation of the shortest peroxidase stain-time of blood leucocytes in the cases of B₁-

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

2) M. Chiba and J. Abe, Tohoku J. Exp. Med., 1932, **19**, 479.

3) T. Suzuki, Tohoku J. Exp. Med., 1934, **23**, 23; 1935, **25**, 23 and 186; 1938, **24**, 417.

4) F. Ohta and T. Suzuki, Tohoku J. Exp. Med., 1937, **31**, 247.

5) S. Shiraishi, Tohoku J. Exp. Med., 1937, **31**, 268; 1938, **32**, 73.

6) T. Suzuki and S. Shiraishi, Tohoku J. Exp. Med., 1938, **34**, 24.

7) A. Sato, T. Suzuki and Ry. Shibata, Tohoku J. Exp. Med., 1934, **24**, 195.

8) S. Watanabe, to be published in a coming issue of this Journal.

avitaminosis was gradually restored to normal on account of an administration of vitamin B₁. It is my present object to investigate, in experimental B₁-avitaminosis, the close relation between the short-timed peroxidase reaction and the effect of vitamin B₁ in different amounts on it.

METHOD OF EXPERIMENT.

1. Feeding.

a. Basal diet. Before the experiment healthy male white rats were fed on the basal diet for from 5 to 7 days. It consisted of polished rice powder 84.4 parts, purified casein 10 parts and Fujimaki's* salt mixture 3.6 parts. All these constituents were auto-claved for two hours and a half at a temperature of 120°C and 2 kgrms. per sq. cm of steam pressure. In addition to autoclaving constituents, ebios** was supplemented in the amount of 2 parts. Moreover, each rat was given 3 drops of a good preparation of cod liver oil† every day.

b. Vitamin B₁-deficient diet. This diet was similiar to the basal diet except for autoclaving ebios (4 hours at a temperature of 120°C).

2. Blood examination.

Blood was obtained by bleeding from the tail every second day and the smear was stained by the Tohoku Pediatric Method. The technique used will be described below :—

On an air-dried fresh blood smear Solution A (10% copper sulfate solution) was poured and thrown‡ away immediately; and then as soon as Solution B (the same as in the original Sato-Sekiya-Suzuki's⁹⁻¹¹⁾ short-timed peroxidase method) was poured on it, the metronome was

* Ry. Fujimaki, Vitamin, 3rd Edition, Tokyo, 1936, 575. Fujimaki's salt mixture has a following composition :

NaCl	6.5
Na ₂ HPO ₄ ·H ₂ O	10.0
K ₂ HPO ₄	30.0
MgSO ₄ ·H ₂ O	13.0
Ca-lactate	37.0
Ammonium-Fe-Citrate	3.0
KI	trace

** Ebios is brewer's yeast.

† Shimoda's cod liver oil was used.

‡ Not quite.

9) A. Sato and S. Sekiya, Tohoku J. Exp. Med., 1926, 7, 111.

10) K. Suzuki, Tohoku J. Exp. Med., 1932, 19, 262.

11) A. Sato and T. Suzuki, Tohoku J. Exp. Med., 1936, 29, 92.

started to count seconds. The normal shortest peroxidase stain-time is 4".

3. Administration of vitamin B₁.

From the commencement of the experiment all the rats were weighed every second day. Since these rats received B₁-deficient diet, body weight had increased gradually in the initial stage, reached the maximum in the middle stage, and then begun to decrease. When the body weight varied from 75 to 80% of that in the commencement of B₁-avitaminotic feeding, vitamin B ("oryzanin"[§]) was given by subcutaneous injection in the following amounts: 1. 0.03 mgrms. to Group 1, 2. 0.01 mgrm. to Group 2, 3. 0.005 mgrms. to Group 3, 4. 0.001 mgrm. to Group 4 and 5. 0.0005 mgrms to Group 5.

RESULT OF EXPERIMENT.

As is shown in Tables 1, 2, 3 and 4, the shortest stain-time for peroxidase reaction was prolonged without exception in the course of B₁-avitaminotic experiments. This change was quite constant. B₁-avitaminotic symptoms such as staggering gait, or paresis or impossibility of gait occurred in a later period of experiment or near death, while a prolongation of the shortest peroxidase stain-time occurred in an early stage of feeding, generally much earlier than any clinical symptom. The maximum time up to the prolongation was 32 days and the minimum 10 days of B₁-deficient feeding. The average time was 20 days. And when a clinical symptom, be it both paresis and impossibility of gait or paresis alone, manifested itself, the prolongation in question was so remarkable as to be more than 9 seconds. Prior to the present experiment, I had already examined infantile cases as well as maternal ones which were in state of B-avitaminosis with respect to the shortest peroxidase stain-time (Cf. 151st Report of the Peroxidase¹²⁾ Reaction), but the maximum prolongation time was 7 seconds (in contrast to normal 4 seconds). In the present experiment I could frequently see such a prolongation as 9 or 10 seconds in the terminal stage. The time of prolongation became longer and longer as B-avitaminotic feeding was continued. And it was 60 days in the maximum and 32 days in the minimum to reach the time 9 or 10 seconds in the terminal stage, in which body weight would decrease by 25-20% compared with that

§ It is a vitamin B preparation by U. Suzuki and contains in 1 c.c. 0.5 mgrms. of B₁ crystals = 333 International Units.

12) S. Watanabe, Tohoku J. Exp. Med., 1941,

TABLE 1.

No. 1.

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "ory-zanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
11. IV	1	48	Basal diet			
13. "	3	47	"		4"	
15. "	5	59	"		4"	
17. "	7	63	"		4"	
19. "	9	77	B ₁ -deficient diet		4"	
21. "	11	73	"		4"	
23. "	13	71	"		4"	
25. "	15	73	"		4"	
27. "	17	77	"		4"	
29. "	19	81	"		5"	
1. V	21	84	"		5"	
3. "	23	55	"		5"	
5. "	25	84	"		6"	
7. "	27	85	"		6"	
9. "	29	86	"		6"	
11. "	31	81	"		7"	
13. "	33	70	"		7"	
15. "	35	65	"		7"	
17. "	37	65	"		8"	Staggering gait
19. "	39	65	"		8"	"
21. "	41	58	"		9"	"
23. "	43	53	"		9"	Paresis
25. "	45	45	"	0.03	9"	Gait impossible
26. "	46			0.03	7"	Slightly recovered
27. "	47	58		0.03	6"	Gait remarkably better
28. "	48			0.03	5"	Paresis almost recovered
29. "	49	56	"	0.03	4"	"
31. "	51	70	"		4"	"

No. 2.

3. IV	1	32	Basal diet			
5. "	3	37	"		4"	
7. "	5	47	"		4"	
9. "	7	44	"		4"	
11. "	9	61	B ₁ -deficient diet		4"	
13. "	11	68	"		4"	
15. "	13	72	"		4"	
17. "	15	77	"		4"	
19. "	17	82	"		5"	
21. "	19	86	"		5"	
23. "	21	91	"		5"	
25. "	23	92	"		6"	
27. "	25	94	"		6"	
29. "	27	88	"		6"	
1. V	29	91	"		6"	
3. "	31	93	"		7"	
5. "	33	97	"		7"	
7. "	35	102	"		7"	Staggering gait
9. "	37	103	"		8"	"
11. "	39	98	"		8"	"

No. 2. (Continued.)

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "ory-zanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
13. "	41	87	B ₁ -deficient diet		8"	Staggering gait
15. "	43	67	"		9"	Paresis
17. "	45	58	"	0.03	9"	Gait impossible
18. "	46		"	0.03	7"	Gait slightly better
19. "	47	59	"	0.03	4"	Almost recovered
20. "	48		"	0.03	4"	"
21. "	49	59	"	0.03	4"	"
22. "	50		"	0.03	4"	"

No. 3.

3. IV	1	35	Basal diet			
5. "	3	42	"		4"	
7. "	5	45	"		4"	
9. "	7	54	"		4"	
11. "	9	60	B ₁ -deficient diet		4"	
13. "	11	66	"		4"	
15. "	13	61	"		4"	
17. "	15	59	"		4"	
19. "	17	68	"		4"	
21. "	19	68	"		5"	
23. "	21	72	"		5"	
25. "	23	76	"		5"	
27. "	25	75	"		6"	
29. "	27	76	"		6"	
1. V	29	70	"		6"	
3. "	31	70	"		6"	
5. "	33	74	"		7"	
7. "	35	66	"		7"	
9. "	37	63	"		8"	Staggering gait
11. "	39	65	"		8"	"
13. "	41	62	"		8"	"
15. "	43	52	"		9"	Paresis
17. "	45	47	"	0.03	9"	Gait impossible
18. "	46		"	0.03	7"	Gait slightly better
19. "	47	56	"	0.03	6"	Paresis remarkably recovered
20. "	48		"	0.03	4"	Gait almost normal
21. "	49	70	"	0.03	4"	"
22. "	50		"	0.03	4"	"

No. 4.

15. VIII	1	60	Basal diet			
17. "	3	72	"		4"	
19. "	5	67	"		4"	
21. "	7	81	B ₁ -deficient diet		4"	
23. "	9	86	"		4"	
25. "	11	72	"		4"	
27. "	13	86	"		4"	
29. "	15	100	"		4"	
31. "	17	102	"		4"	
2. IX	19	107	"		4"	
4. "	21	93	"		5"	

No. 4. (Continued.)

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "oryzanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
6.	"	23	116	B ₁ -deficient diet		5"
8.	"	25	111	"		5"
10.	"	27	115	"		6"
12.	"	29	104	"		6"
14.	"	31	102	"		6"
16.	"	33	102	"		6"
18.	"	35	103	"		7"
20.	"	37	100	"		7"
22.	"	39	90	"		8" ~ Staggering gait
24.	"	41	85	"		8" "
26.	"	43	86	"		8" "
28.	"	45	82	"		9" "
30.	"	47	82	"		9" "
2. X	49	73	"		9"	Paresis
4.	"	51	64	"	0.03	10" Gait impossible
5.	"	52	"	"	0.03	7" Paresis remarkably better
6.	"	53	79	"	0.03	5" Almost recovered
7.	"	54	"	"	0.03	4" Recovered to normal
8.	"	55	82	"	0.03	4"

TABLE 2.

No. 1.

11. IV	1	56	Basal diet		4"	
13. "	3	59	"		4"	
15. "	5	70	"		4"	
17. "	7	71	"		4"	
19. "	9	85	B ₁ -deficient diet		4"	
21. "	11	88	"		4"	
23. "	13	90	"		4"	
25. "	15	87	"		4"	
27. "	17	93	"		4"	
29. "	19	97	"		4"	
1. V	21	97	"		4"	
3. "	23	98	"		4"	
5. "	25	95	"		5"	
7. "	27	106	"		5"	
9. "	29	101	"		6"	
11. "	31	102	"		6"	
13. "	33	100	"		6"	
15. "	35	100	"		6"	
17. "	37	92	"		7"	
19. "	39	91	"		7"	
21. "	41	79	"		8"	Staggering gait
23. "	43	74	"		8"	"
25. "	45	70	"		9"	"
27. "	47	66	"		9"	"
29. "	49	50	"		9"	"
31. "	51	41	"	0.01	10"	Paresis
1. VI	52	"	"	0.01	8"	Slightly better
2. "	53	48	"	0.01	7"	"
3. "	54	"	"	0.01	5"	"
4. "	55	52	"	0.01	4"	Almost recovered

No. 2.

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "ory-zanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
3. IV	1	55	Basal diet		4"	
5. "	3	62	"		4"	
7. "	5	68	"		4"	
9. "	7	70	"		4"	
11. "	9	72	B ₁ -deficient diet		4"	
13. "	11	78	"		4"	
15. "	13	86	"		4"	
17. "	15	90	"		4"	
19. "	17	95	"		4"	
21. "	19	102	"		4"	
23. "	21	100	"		4"	
25. "	23	108	"		5"	
27. "	25	101	"		5"	
29. "	27	97	"		6"	
1. V	29	92	"		6"	
3. "	31	84	"		6"	
5. "	33	85	"		7"	
7. "	35	81	"		7"	
9. "	37	79	"		7"	
11. "	39	70	"		8"	
13. "	41	65	"		8"	
15. "	43	62	"		9"	Staggering gait
17. "	45	59	"		9"	"
19. "	47	56	"	0.01	9"	{ Paresis, gait impossible
20. "	48		"	0.01	8"	Slightly better
21. "	49	63	"	0.01	7"	"
22. "	50		"	0.01	6"	"
23. "	51	65	"	0.01	5"	"
24. "	42		"	0.01	5"	Almost recovered
25. "	53	72	"	0.01	4"	Normal

No. 3.

3. IV	1	48	Basal diet		4"	
5. "	3	54	"		4"	
7. "	5	60	"		4"	
9. "	7	66	"		4"	
11. "	9	74	B ₁ -deficient diet		4"	
13. "	11	78	"		4"	
15. "	13	78	"		4"	
17. "	15	85	"		4"	
19. "	17	81	"		4"	
21. "	19	91	"		4"	
23. "	21	99	"		4"	
25. "	23	100	"		4"	
27. "	25	109	"		4"	
29. "	27	105	"		4"	
1. V	29	112	"		5"	
3. "	31	115	"		6"	
5. "	33	116	"		6"	
7. "	35	108	"		7"	
9. "	37	100	"		7"	
11. "	39	87	"		8"	

No. 3. (Continued.)

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "ory-zanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
13. V	41	73	B ₁ -deficient diet		8"	Staggering gait
15. "	43	64	"		9"	"
17. "	45	61	"		9"	"
19. "	47	58	"	0.01	10"	Paresis
20. "	48		"	0.01	8"	Slight better
21. "	49	59	"	0.01	8"	"
23. "	50		"	0.01	7"	"
24. "	51	64	"	0.01	6"	"
25. "	52		"	0.01	5"	Almost recovered
26. "	53	70	"	0.01	4"	Recovered to normal

No. 4.

15. VII	1.	58	Basal diet		4"	
17. "	3	61	"		4"	
19. "	5	63	"		4"	
21. "	7	70	B ₁ -deficient diet		4"	
23. "	9	72	"		4"	
25. "	11	78	"		4"	
27. "	13	80	"		4"	
29. "	15	83	"		4"	
31. "	17	88	"		4"	
2. VIII	19	95	"		4"	
4. "	21	98	"		4"	
6. "	23	100	"		4"	
8. "	25	92	"		4"	
10. "	27	103	"		4"	
12. "	29	93	"		4"	
14. "	31	84	"		4"	
16. "	33	88	"		4"	
18. "	35	85	"		4"	
20. "	37	81	"		5"	
22. "	39	83	"		5"	
24. "	41	77	"		5"	
26. "	43	77	"		6"	
28. "	45	75	"		6"	
30. "	47	74	"		7"	
1. IX	49	74	"		7"	
3. "	51	66	"		7"	
5. "	53	67	"		8"	
7. "	55	70	"		8"	Staggering gait
9. "	57	72	"		9"	"
11. "	59	70	"		9"	"
13. "	61	78	"		9"	"
15. "	63	62	"		9"	"
17. "	65	58	"		10"	"
19. "	66	55	"	0.01	10"	Paresis
20. "	67		"	0.01	9"	"
21. "	68	59	"	0.01	8"	Slight better
22. "	69		"	0.01	7"	"
23. "	70	63	"	0.01	5"	"
24. "	71		"	0.01	5"	"
25. "	72	75	"	0.01	4"	Almost recovered

No. 5.

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "ory-zanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
15. VIII	1	60	Basal diet		4"	
17. "	3	64	"		4"	
19. "	5	68	"		4"	
21. "	7	69	B ₁ -deficient diet		4"	
23. "	9	75	"		4"	
25. "	11	81	"		4"	
27. "	13	87	"		4"	
29. "	15	71	"		4"	
31. "	17	83	"		4"	
2. IX	19	100	"		4"	
4. "	21	108	"		4"	
6. "	23	104	"		4"	
8. "	25	93	"		4"	
9. "	27	104	"		4"	
10. "	29	90	"		4"	
12. "	31	87	"		4"	
14. "	33	88	"		4"	
16. "	35	87	"		5"	
18. "	37	87	"		5"	
20. "	39	85	"		6"	
22. "	41	80	"		6"	
24. "	43	83	"		7"	
26. "	45	80	"		7"	
28. "	47	79	"		7"	
30. "	49	76	"		8"	
2. X	51	72	"		8"	Staggering gait
4. "	53	68	"		8"	"
6. "	55	63	"		9"	"
8. "	57	57	"		9"	"
10. "	59	55	"	0.01	10"	Paresis
11. "	60		"	0.01	8"	Slightly better
13. IX	61	60	"	0.01	7"	"
14. X	62		"	0.01	7"	"
15. "	63	65	"	0.01	6"	"
16. "	64		"	0.01	5"	Almost recovered
17. "	65	71	"	0.01	4"	

No. 6.

15. VIII	1	60	Basal diet		4"	
17. "	3	70	"		4"	
19. "	5	73	"		4"	
21. "	7	75	B ₁ -deficient diet		4"	
23. "	9	72	"		4"	
25. "	11	87	"		4"	
27. "	13	85	"		4"	
29. "	15	85	"		4"	
31. "	17	91	"		4"	
2. IX	19	100	"		4"	
4. "	21	100	"		4"	
6. "	23	98	"		4"	
8. "	25	105	"		4"	
10. "	27	104	"		4"	
12. "	29	114	"		4"	
14. "	31	96	"		5"	

No. 6. (Continued.)

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "oryzanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
16. IX	33	96	B ₁ -deficient diet		5"	
28. "	35	97	"		6"	
20. "	37	95	"		6"	
22. "	39	95	"		6"	
24. "	41	96	"		7"	
26. "	43	87	"		7"	
28. "	45	82	"		8"	Staggering gait
30. "	47	81	"		8"	"
2. X	49	73	"		9"	"
4. "	50	60	"	0.01	9"	Paresis
5. "	51		"	0.01	7"	Slightly recovered
6. "	52	65	"	0.01	6"	"
7. "	53		"	0.01	5"	"
8. "	54	70	"	0.01	4"	Almost recovered

TABLE 3.

No. 1.

15. VIII	1	60	Basal diet		4"	
17. "	3	69	"		4"	
19. "	5	75	"		4"	
21. "	7	80	B ₁ -deficient diet		4"	
23. "	9	83	"		4"	
25. "	11	80	"		4"	
27. "	13	93	"		4"	
29. "	15	72	"		4"	
31. "	17	75	"		4"	
2. IX	19	78	"		4"	
4. "	21	92	"		4"	
6. "	23	103	"		4"	
8. "	25	104	"		4"	
10. "	27	103	"		4"	
12. "	29	111	"		4"	
14. "	31	100	"		5"	
16. "	33	95	"		6"	
18. "	35	92	"		6"	
20. "	37	86	"		7"	
22. "	39	81	"		8"	Staggering gait
24. "	41	72	"		8"	"
26. "	43	70	"		9"	"
28. "	45	68	"		9"	"
29. "	46	63	"	0.005	10"	Paresis
30. "	47		"	0.005	9"	"
1. X	48	63	"	0.005	8"	Slightly better
2. "	49		"	0.005	8"	"
3. "	50	64	"	0.005	8"	"
4. "	51		"	0.005	7"	"
5. "	52	66	"	0.005	6"	"
6. IX	53		"	0.005	4"	"
7. "	44	69	"	0.005	4"	Almost recovered

No. 2.

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "ory-zanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
15. VIII	1	60	Basal diet		4"	
17. "	3	66	"		4"	
19. "	5	72	"		4"	
21. "	7	80	B ₁ -deficient diet		4"	
23. "	9	81	"		4"	
25. "	11	82	"		4"	
27. "	13	85	"		4"	
29. "	15	88	"		4"	
31. "	17	91	"		4"	
2. IX	19	101	"		4"	
4. "	21	107	"		4"	
6. "	23	108	"		4"	
8. "	25	114	"		4"	
10. "	27	106	"		5"	
12. "	29	100	"		5"	
14. "	31	90	"		6"	
16. "	33	89	"		7"	
18. "	35	85	"		7"	Staggering gait
20. "	37	74	"		8"	"
22. "	39	75	"		8"	"
24. "	41	70	"		9"	"
26. "	43	64	"	0.005	9"	Paresis
27. "	44		"	0.005	8"	"
28. "	45	64	"	0.005	8"	Slightly better
29. "	46		"	0.005	8"	"
30. "	47	66	"	0.005	6"	"
1. X	48		"	0.005	5"	"
2. "	49	69	"	0.005	4"	Ever paretic
3. "	50		"	0.005	4"	"
4. "	51	71	"	0.005	4"	Almost recovered

No. 3.

15. VIII	1	60	Basal diet		4"	
17. "	3	62	"		4"	
19. "	5	66	"		4"	
21. "	7	78	B ₁ -deficient diet		4"	
23. "	9	77	"		4"	
25. "	11	80	"		4"	
27. "	13	85	"		4"	
29. "	15	74	"		4"	
31. "	17	99	"		4"	
2. IX	19	108	"		4"	
4. "	21	111	"		4"	
6. "	23	111	"		4"	
8. "	25	105	"		4"	
10. "	27	112	"		5"	
12. "	29	97	"		5"	
14. "	31	92	"		6"	
16. "	33	94	"		6"	
18. "	35	91	"		6"	
10. "	37	87	"		7"	
12. "	39	78	"		7"	
14. "	41	68	"		8"	Staggering gait
16. "	43	66	"		8"	"

No. 3. (Continued.)

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "oryzanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
28. IX	45	63	B ₁ -deficient diet		9"	Staggering gait
30. "	47	60	"	0.005	9"	Paresis
1. X	48		"	0.005	8"	"
2. "	49	62	"	0.005	7"	Slightly better
3. "	50		"	0.005	7"	"
4. "	51	61	"	0.005	6"	"
5. "	52		"	0.005	5"	"
6. "	53	64	"	0.005	4"	"

No. 4.

15. VIII	1	60	Basal diet		4"	
17. "	3	65	"		4"	
19. "	5	67	"		4"	
21. "	7	69	B ₁ -deficient diet		4"	
23. "	9	76	"		4"	
25. "	11	82	"		4"	
27. "	13	85	"		4"	
29. "	15	88	"		4"	
31. "	17	91	"		4"	
2. IX	19	93	"		4"	
4. "	21	95	"		5"	
6. "	23	97	"		5"	
8. "	25	96	"		6"	
10. "	27	99	"		7"	
12. "	29	93	"		7"	
14. "	31	92	"		7"	
16. "	33	90	"		8"	
18. "	35	86	"		8"	
20. "	37	80	"		8"	
22. "	39	75	"		8"	
24. "	41	69	"		9"	Staggering gait
26. "	43	66	"		9"	"
28. "	45	59	"		9"	"
29. "	46	55	"	0.005	9"	Paresis
30. "	47		"	0.005	8"	"
1. X	48	58	"	0.005	8"	"
2. "	49		"	0.005	6"	Slightly better
3. "	50	60	"	0.005	6"	"
4. "	51		"	0.005	6"	"
5. "	51	60	"	0.005	5"	"
6. "	53	63	"	0.005	4"	"

TABLE 4.

No. 1.

27. V	1	50	Basal diet		4"	
29. "	3	52	"		4"	
31. "	5	55	"		4"	
2. VI	7	60	"		4"	
4. "	9	65	B ₁ -deficient diet		4"	
6. "	11	78	"		4"	
8. "	13	80	"		4"	

No. 1. (Continued.)

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "oryzanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
10. VI	15	85	B ₁ -deficient diet		4"	
12. "	17	95	"		4"	
14. "	19	101	"		4"	
16. "	21	110	"		4"	
18. "	23	113	"		4"	
20. "	25	107	"		5"	
22. "	27	100	"		6"	
24. "	29	100	"		6"	
26. "	31	93	"		7"	
28. "	33	88	"		7"	
30. "	35	82	"		8"	
2. VII	37	78	"		8"	Staggering gait
4. "	39	70	"		8"	"
6. "	41	65	"		9"	"
8. "	43	58	"		9"	"
10. "	45	51	"	0.001	9"	Paresis
11. "	46		"	0.001	8"	"
12. "	47	56	"	0.001	7"	Slightly recovered
13. "	48		"	0.001	7"	"
14. "	49	55	"	0.001	6"	"
15. "	50		"	0.001	6"	Paresis again
16. "	51	50	"	0.001	6"	Peath on the evening

No. 2.

27. V	1	48	Basal diet		4"	
29. "	3	50	"		4"	
31. "	5	53	"		4"	
2. VI	7	58	"		4"	
4. "	9	62	B ₁ -deficient diet		4"	
6. "	11	70	"		4"	
8. "	13	81	"		4"	
10. "	15	85	"		4"	
12. "	17	90	"		4"	
14. "	19	98	"		4"	
16. "	21	103	"		4"	
18. "	23	110	"		4"	
20. "	25	114	"		4"	
22. "	27	108	"		5"	
24. "	29	95	"		5"	
26. "	31	88	"		5"	
28. "	33	81	"		6"	
30. "	35	76	"		6"	
2. VII	37	70	"		7"	
4. "	39	67	"		8"	
6. "	41	61	"		8"	
8. "	43	55	"		9"	Staggering gait
9. "	44	48	"	0.001	9"	"
10. "	45		"	0.001	8"	Paresis, gait impossible
11. "	46	52	"	0.001	8"	Slightly recovered
12. "	47		"	0.001	6"	"
13. "	48	53	"	0.001	6"	"
14. "	49		"	0.001	5"	"
15. "	50	60	"	0.001	4"	Almost recovered
16. "	51		"	0.001	4"	Recovered to normal

No. 3.

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "oryzanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom !
15. VII	1	60	Basal diet		4"	
17. "	3	65	"		4"	
19. "	5	68	"		4"	
21. "	7	70	B ₁ -deficient diet		4"	
23. "	9	80	"		4"	
25. "	11	82	"		4"	
27. "	13	93	"		4"	
29. "	15	88	"		4"	
31. "	17	92	"		4"	
2. VIII	19	97	"		4"	
4. "	21	99	"		4"	
6. "	23	98	"		5"	
8. "	25	100	"		5"	
10. "	27	101	"		5"	
12. "	29	92	"		6"	
14. "	31	92	"		6"	
16. "	33	95	"		7"	
18. "	35	92	"		7"	
20. "	37	87	"		8"	
22. "	39	78	"		9"	
24. "	41	81	"		9"	Staggering gait
26. "	43	79	"		9"	"
28. "	45	76	"		9"	"
30. "	47	73	"		9"	"
31. "	48	56	"	0.001	10"	Paresis
1. IX	49		"	0.001	9"	"
2. "	50	57	"	0.001	8"	Slightly recovered
3. "	51		"	0.001	6"	"
4. "	52	56	"	0.001	5"	"
5. "	53		"	0.001	5"	Almost recovered
6. "	54	70	"	0.001	4"	"

No. 4.

15. VII	1	60	Basal diet		4"	
17. "	3	65	"		4"	
19. "	5	70	"		4"	
21. "	7	79	B ₁ -deficient diet		4"	
23. "	9	78	"		4"	
25. "	11	78	"		4"	
27. "	13	84	"		4"	
29. "	15	90	"		4"	
31. "	17	97	"		4"	
2. VIII	19	100	"		4"	
4. "	21	101	"		4"	
6. "	23	102	"		4"	
8. "	25	96	"		4"	
10. "	27	108	"		4"	
12. "	29	94	"		4"	
14. "	31	95	"		4"	
16. "	33	96	"		4"	
18. "	35	95	"		4"	
20. "	37	93	"		5"	
22. "	39	93	"		5"	
24. "	41	90	"		5"	

No. 4. (Continued.)

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "oryzanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
26. VIII	43	86	B ₁ -deficient diet		6"	
28. "	45	82	"		6"	
30. "	47	79	"		6"	
1. IX	49	70	"		7"	
3. "	51	69	"		8"	Staggering gait
5. "	53	71	"		8"	"
7. "	55	73	"		9"	"
9. "	57	69	"		9"	"
11. "	59	68	"		9"	"
13. "	61	62	"	0.001	10"	Paresis
14. "	62		"	0.001	9"	"
15. "	63	61	"	0.001	8"	"
16. "	64		"	0.001	7"	Slightly better
17. "	65	60	"	0.001	6"	"
18. "	66		"	0.001	6"	"
19. "	67	62	"	0.001	5"	"
20. "	68		"	0.001	5"	"
21. "	69	68	"	0.001	4"	"
22. "	70		"	0.001	4"	Almost recovered

No. 5.

15. VII	1	62	Basal diet		4"	
17. "	3	70	"		4"	
19. "	5	72	"		4"	
21. "	7	74	B ₁ -deficient diet		4"	
23. "	9	83	"		4"	
25. "	11	90	"		4"	
27. "	13	84	"		4"	
29. "	15	95	"		4"	
31. "	17	102	"		4"	
2. VIII	19	106	"		4"	
4. "	21	105	"		4"	
6. "	23	110	"		4"	
8. "	25	103	"		4"	
10. "	27	115	"		4"	
12. "	29	99	"		4"	
14. "	31	96	"		4"	
16. "	33	96	"		4"	
18. "	35	94	"		4"	
20. "	37	90	"		5"	
22. "	39	90	"		5"	
24. "	41	79	"		5"	
26. "	43	75	"		6"	
28. "	45	74	"		7"	
30. "	47	66	"		7"	
1. IX	49	66	"		8"	Staggering gait
3. "	51	69	"		8"	"
5. "	53	68	"		9"	"
7. "	55	60	"		9"	"
9. "	57	63	"		9"	"
11. "	59	58	"	0.001	10"	Paresis
12. "	60		"	0.001	9"	"
13. "	61	58	"	0.001	8"	"
14. "	62		"	0.001	7"	Slightly recovered

No. 5. (Continued.)

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "oryzanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
15. IX	63	60	B ₁ -deficient diet	0.001	7"	Slightly recovered
16. "	64		"	0.001	7"	"
17. "	65	62	"	0.001	6"	"
18. "	66		"	0.001	6"	Ever paretic
19. "	67	65	"	0.001	5"	"
20. "	68		"	0.001	5"	"
21. "	69	71	"	0.001	4"	Almost recovered
22. "	60		"	0.001	4"	"

No. 6.

15. VII	1	55	Basal diet		4"	
17. "	3	67	"		4"	
19. "	5	75	"		4"	
21. "	7	77	B ₁ -deficient diet		4"	
23. "	9	81	"		4"	
25. "	11	89	"		4"	
27. "	13	90	"		4"	
29. "	15	92	"		4"	
31. "	17	96	"		4"	
2. VIII	19	99	"		4"	
4. "	21	100	"		5"	
6. "	23	114	"		5"	
8. "	25	109	"		6"	
10. "	27	98	"		6"	
12. "	29	105	"		6"	
14. "	31	95	"		7"	
16. "	33	96	"		7"	
18. "	35	94	"		8"	
20. "	37	85	"		8"	
22. "	39	81	"		8"	
24. "	41	84	"		8"	Staggering gait
26. "	43	77	"		9"	"
28. "	45	74	"		9"	"
30. "	47	68	"		9"	Paresis
1. IX	49	58	"	0.001	10"	"
2. "	50		"	0.001	9"	Slightly better
3. "	51	60	"	0.001	7"	"
4. "	52		"	0.001	7"	Paresis again
5. "	53	51	"	0.001	7"	Death on the evening

TABLE 5.

No. 1.

3. IV	1	28	Basal diet			
5. "	3	34	"		4"	
7. "	5	39	"		4"	
9. "	7	40	"		4"	
11. "	9	51	B ₁ -deficient diet		4"	
13. "	11	74	"		4"	
15. "	13	73	"		4"	
17. "	15	82	"		4"	
19. "	17	83	"		4"	
21. "	19	96	"		4"	

No. 1. (Continued.)

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "oryzanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
23. IV	21	94	B ₁ -deficient diet		5"	
25. "	23	95	"		5"	
27. "	25	93	"		5"	
29. "	27	92	"		5"	
1. "	29	87	"		6"	
3. "	31	80	"		7"	
5. "	33	73	"		7"	
7. "	35	67	"		8"	
9. "	37	69	"		8"	
11. "	39	65	"		9"	
13. "	41	67	"		9"	
15. "	43	58	"		9"	Paresis
17. "	45	51	"	0.0005	10"	Gait impossible
18. "	46		"	0.0005	9"	"
19. "	47	56	"	0.0005	8"	Gait slightly recovered
20. "	48		"	0.0005	9"	Gait difficult
21. "	49	52	"	0.0005	9"	Paresis again
22. "	50		"		9"	Death on the evening

No. 2.

3. IV	1	35	Basal diet			
5. "	2	39	"		4"	
7. "	5	47	"		4"	
9. "	7	48	"		4"	
11. "	9	50	B ₁ -deficient diet		4"	
13. "	11	59	"		4"	
15. "	13	67	"		4"	
17. "	15	71	"		4"	
19. "	17	79	"		4"	
21. "	19	83	"		4"	
23. "	21	90	"		4"	
25. "	23	96	"		4"	
27. "	25	94	"		5"	
29. "	27	98	"		5"	
1. V	29	102	"		6"	
3. "	31	101	"		6"	
5. "	33	100	"		6"	
7. "	35	104	"		7"	
9. "	37	106	"		7"	
11. "	39	99	"		7"	
13. "	41	97	"		7"	
15. "	43	92	"		8"	Staggering gait
17. "	45	89	"		8"	"
19. "	47	87	"		8"	"
21. "	49	89	"		9"	"
23. "	51	86	"		9"	Gait impossible, paresis
25. "	53	78	"	0.0005	10"	"
26. "	54		"	0.0005	9"	"
27. "	55	91	"	0.0005	9"	"
28. "	56		"	0.0005	10"	"
		76	"	0.0005	10"	Death on the evening

No. 3.

Date	Day of experiment	Body weight (ingrms.)	Manner of nutrition	Injection of "oryzanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
11. IV	1	57	Basal diet			
13. "	3	65	"		4"	
15. "	5	68	"		4"	
17. "	7	69	"		4"	
19. "	9	73	B ₁ -deficient diet		4"	
21. "	11	78	"		4"	
23. "	13	79	"		4"	
25. "	15	80	"		4"	
27. "	17	82	"		4"	
29. "	19	84	"		4"	
1. V	21	84	"		5"	
3. "	23	88	"		5"	
5. "	25	85	"		5"	
7. "	27	83	"		6"	
9. "	29	79	"		6"	
11. "	31	75	"		6"	
13. "	33	77	"		7"	
15. "	35	70	"		7"	
17. "	37	68	"		7"	
19. "	39	70	"		7"	
21. "	41	65	"		8"	
23. "	43	63	"		8"	
25. "	45	59	"		8"	
27. "	47	58	"		8"	
29. "	49	60	"		9"	
31. "	51	58	"		9"	Paresis
2. VI	53	56	"	0.0005	9"	"
3. "	54		"	0.0005	9"	Paresis slightly better
4. "	55	58	"	0.0005	8"	"
5. "	56		"	0.0005	9"	Paresis again
6. "	57	48	"	0.0005	9"	Death on the evening

No. 4.

11. IV	1	56	Basal diet			
13. "	3	63	"		4"	
15. "	5	67	"		4"	
17. "	7	72	"		4"	
19. "	9	68	B ₁ -deficient diet		4"	
21. "	11	70	"		4"	
23. "	13	75	"		4"	
25. "	15	73	"		4"	
27. "	17	79	"		4"	
29. "	19	76	"		4"	
1. V	21	76	"		5"	
3. "	23	82	"		5"	
5. "	25	78	"		6"	
7. "	27	76	"		6"	
9. "	29	75	"		6"	
11. "	31	74	"		7"	
13. "	33	67	"		7"	
15. "	35	65	"		7"	
17. "	37	65	"		7"	
19. "	39	58	"		8"	
21. "	41	50	"		8"	

No. 4. (Continued.)

Date	Day of experiment	Body weight (in grms.)	Manner of nutrition	Injection of "oryzanin" (in mgrms.)	Shortest peroxidase stain-time	Symptom
23. V	43	57	B ₁ -deficient diet		9"	Paresis
25. "	45	46	"	0.0005	9"	"
26. "	46		"	0.0005	8"	Paresis slightly better
27. "	47	52	"	0.0005	8"	"
28. "	48		"	0.0005	9"	Paresis became more severe again
29. "	49	40	"	0.0005	8"	"
30. "	50		"	0.0005	9"	"
31. "	51		"			Death on the morning

just before B-avitaminotic feeding. On an average it was 44 days. At this time, an administration of vitamin B₁ was performed by subcutaneous injection in different amounts. If a large amount of vitamin B₁ (see Comment) was administered, the prolongation would be restored gradually to normal with an increase of body weight and recovery of symptoms. On the contrary, if it was too small, the prolongation would not become shorter at all; paresis would occur again and it could not save the animal from death, even though the administration could immediately shorten the prolongation in a slight degree. We shall take up this point later.

1) Group 1 (Nos. 1, 2, 3 and 4 in Table 1).—After 34 to 36 days 3 cases showed 9 seconds of the shortest stain-time, and after 46 days the remaining 1 case (No. 4) 10 seconds of same. A daily administration of vitamin B (0.03 mgrms of B₁-crystals by subcutaneous injection) restored the prolonged stain-time to normal (4") after 2 to 4 days (with the average time of 3 days) and was further able to rescue those animals from death. The average increase in body weight was 9 grms. after 2 days or 18 grms after 4 days.

2) Group 2 (Nos. 1, 2, 3, 4, 5 and 6 in Table 2).—36 to 60 days after the rats received B₁-deficient diet, 2 cases showed 9 seconds of the shortest stain-time and 4 cases 10 seconds. A daily administration of vitamin B₁ (0.01 mgrm. of B₁-crystals) restored the prolonged stain-time to normal after 4 to 6 days (with an average time of 5 days) and rescued animals from death. An average gain of growth was 5 grms. after 2 days, and 9 grms. after 4 days of the vitamin administration. This increase of body weight is just half that we saw in the animals of Group 1 after the vitamin administration.

3) Group 3 (Nos. 1, 2, 3 and 4 in Table 3).—After 36 to 41 days 3 cases showed 9 seconds of the shortest stain-time and the remaining one case 10 seconds. A daily administration of vitamin B₁ (0.005

mgrms. of B_1 -crystals) restored the prolonged stain-time to normal after 6 to 7 days in 2 cases respectively. With this amount of the vitamin the increase in body weight was not remarkable: In 1 case (No. 11) body weight remained to be the same after 2 days, and in 1 case (no. 14) it rather decreased after 4 days. The average growth was only 4 grms. after 6 days. At any rate, a daily administration of this amount of the vitamin was still sufficient to save animals from death.

4) Group 4 (Nos. 1, 2, 3, 4, 5 and 6 in Table 4).—After 34 to 56 days 2 cases showed 9 seconds of the shortest stain-time, and 4 cases 10 seconds. In this experiment, 2 cases died in spite of a daily administration of vitamin B_1 (0.001 mgrm. of B_1 -crystals). In these cases, a prolongation was not restored to normal until death; besides, paresis occurred again as death drew near. Although body weight increased slightly on vitamin B_1 administration, it decreased again in spite of the continued administration. In the 4 cases which survived due to a daily administration of vitamin B_1 , a prolongation was restored to normal after 6 to 10 days (with an average time longer than 7 days). An increase of body weight was remarkably slight in comparison with that of the rats of Groups 1, 2 and 3, and even after a lapse of 6–8 days they showed a poor increase.

5) Group 5 (Nos. 1, 2, 3 and 4 in Table 5).—After 32 to 46 days, 2 cases showed 9 seconds of the shortest stain-time and 2 cases 10 seconds. Even a daily administration of vitamin B_1 (0.0005 mgrms. of B_1 -crystals) was scarcely able to influence the prolonged stain-time, or a beginning restoration was of only a short duration in spite of the continued vitamin administration, so that a new prolongation ensued again with a renewed occurrence of pareisis. Then they all died after 4 to 5 days. Body weight also decreased remarkably, though a slight increase was seen in the initial stage of the vitamin administration. In short, with 0.0005 mgrms. of the vitamin, it was almost impossible to influence the prolongation of the shortest stain-time to any remarkable extent and to cure B_1 -avitaminotic symptoms, such as paresis and decrease of body weight. It is natural that this amount failed to rescue animals from death.

In order to simplify the above description, Figures 1, 2, 3, 4 and 5 are given. These figures represented one case from each of the 5 groups; body weight was almost equal in these 5 representatives at the beginning of experiment. If we glance at the final stage of experiment, we can understand the difference of the change of the curves due to the difference of the amounts of vitamin B_1 in the different groups.

Fig. 1. Curve showing the shortest peroxidase stain-time of leucocytes and the body weight of No. 4 in Table 1.

Fig. 2. Curve showing the shortest peroxidase stain-time of leucocytes and the body weight of No. 5 in Table 2.

Fig. 3. Curve showing the shortest peroxidase stain-time of leucocytes and the body weight of No. 1 in Table 3.

Fig. 4. Curve showing the shortest peroxidase stain-time of leucocytes and the body weight of No. 3 in Table 4.

Fig. 5. Curve showing the shortest peroxidase stain-time of leucocytes and the body weight of No. 4 in Table 5.

COMMENT.

- The experiment above described has shown the following results. 1. A prolongation of the shortest peroxidase stain-time of B_1 -avitaminotic rats occurs in the early stage and before the manifestation of B_1 -avitaminotic symptoms, becoming much more remarkable in the terminal stage. 2. A daily administration of vitamin B_1 in a proper amount causes the prolongation to become shorter and shorter until the normal shortest stain-time is attained, while, if the amount of vitamin B_1 used is much smaller, the restoration of the prolonged stain-time will occur at a much slower pace, or death will soon follow with a recurring prolongation.

Ohdake and Yamagishi¹³⁾ reported that 0.001 mgrm. of oryzanin hydrochloride was the minimum for maintaining the normal growth of young albino rats. This is interesting, if my own experiment in Group 4 is seen. According to Kagawa, Ohdake and Sawada¹⁴⁾, about 5 weeks after rats weighing 40 grms. received B₁-deficient diet growth ceased and B₁-avitaminotic symptoms occurred. At this time, if the rats were given 0.01–0.005 mgrms. of B₁-crystals, they regained health in several days and then maintained the normal growth. Moreover, if 0.1 mgrm. of B₁-crystals was daily given by subcutaneous injection to the rats with such B₁-avitaminotic symptoms as a loss of weight, anorexia and paresis, the rats would not show symptoms any longer, while these B₁-avitaminotic symptoms developed again with a cessation of the dose. This is the result shown by Kagawa and his co-workers. In my last series of experiment (Cf. Table 5) a much smaller amount of vitamin B₁ than that in the just mentioned work of the three authors was given subcutaneously. The prolongation of the shortest stain-time continued to remain uninfluenced then. In short, rats fed on B-avitaminotic diet *will succumb in spite of a continued administration of vitamin B₁*, if the amount of the vitamin will not influence their prolonged peroxidase stain-time to any extent.

SUMMARY.

1. A prolongation of the shortest peroxidase stain-time is also seen in B₁-avitaminotic rats.
2. The longer the B₁-avitaminotic feeding, the more pronounced the prolongation becomes. It may be prolonged to 9–10 seconds in the terminal stage.
3. A daily administration of 0.03 mgrms. of B₁-crystals restored the prolonged stain-time to normal in 3 days averagely, that of 0.01 mgrm. in 5 days, and that of 0.005 mgrms. in 7 days. A prolongation was restored to normal in time longer than 7 days in the rats which had survived on a daily administration of 0.001 mgrm. of B₁-crystals, while the prolongation was not restored to normal in the rats which had died in spite of an administration of the same dose. In the last series of experiment in which 0.0005 mgrms. of B₁-crystals were used, the prolongation was restored at a much slower pace, in order to re-

13) S. Ohdake and T. Yamagishi, Bulletin of the Agricultural Chemical Society of Japan, 1935, 11, 111; Chiryō Yakuho, 1936, No. 404, 12.

14) S. Kagawa, S. Ohdake and K. Sawada, Iji Koron, 1932, No. 1066, 4.

gain its former figures or even larger figures. At length they died in 5 days averagely without exception.

CONCLUSIONS.

1. A prolongation of the normal shortest peroxidase stain-time of blood leucocytes is a very early sign of avitaminosis B.

2. In an advanced stage of avitaminosis B, a daily administration of 0.001 mgrm. of B₁-crystals will be the necessary minimum for normal growth, and will gradually restore the prolonged stain-time to normal.

3. The larger the amount of the vitamin, the shorter the days of restoration will be. And in parallel with the gradual restoration, an increase of body weight will be seen.