EFFECT OF VITAMIN B₁₂ ON THE INTERMEDIARY METABOLISM OF CHICKS¹

YOSHIKAZU SAHASHI, KIICHI IWAMOTO AND JUNZO HAYASHI

*Laboratory of Biochemistry, Tokyo University of Agriculture, Setagaya,
Tokyo and Shiga Agricultural College, Ritto-cho, Shiga-ken.*

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In 1953, the authors (1) reported that a decrease of thiamine and vitamin B₁₂ was found in the blood as the result of the energy metabolism of cattle, and that the deficiency of the vitamins was completely protected against by a prophylactic injection of the vitamins at the beginning of the experiment. The intimate relation between both vitamins and metabolism was thus recognized for the formation of enzymic system containing thiamine in animal bodies. Vitamin B₁₂ nutrition has since been reported on for a number of different organisms as well as the effects of the vitamin upon the low requirement of various nutrients, but few reports have appeared with reference to the enzyme-forming activity of vitamin B₁₂. The authors have attempted to study the effects of vitamin B₁₂ on the formation of enzymes containing thiamine, nicotinic acid and pantothenic acid, and it was confirmed that vitamin B₁₂ may be closely related to the energy metabolism of the above-mentioned vitamins. (2)

EXPERIMENTAL AND RESULTS

1. *Preparation of Vitamin B₁₂-deficient Chicks* (3).

Fifty-day-old chicks were previously fed on a vitamin B₁₂-deficient diet composed of a mixture of corn meal 37, wheat flour 10, wheat bran 10, rice bran 10, soy bean meal 27, soy bean oil (supplemented with cod-liver oil in the proportion of 14:1) 3, and mineral matter (NaCl, CaCO₃ and bone dust) 3. Chicks were divided into two groups: the 1st group of 5 chicks weighing aver. 265g each were orally administered with 1 γ of vitamin B₁₂, while the 2nd group of 5 chicks served as a control. After 20 days, vitamin B₁₂ in the blood was determined by Euglena method, and the change in the vitamin content of the liver and blood was studied (4). The results are shown in Tables I—II. There was a distinct difference in the vitamin B₁₂ content of the liver, but scarcely any difference, in the vitamin content of the blood was observed in the period of 20 days.

¹ Biochemical Studies on Vitamin B₁₂. VIII.

Table I
The Change in Body Weight of Chicks with Vitamin B₁₂-Deficiency.

Days	Amount of ration	Amount of green leaves	Body wt. of the group treated with vitamin B ₁₂		Body wt. of the control group	
	<i>g/day</i>	<i>g/day</i>	<i>g</i>	% of Increase	<i>g</i>	% of Increase
0	50	20	265	0	276	0
5			325	22.6	341	23.6
10			408	54.0	428	55.1
15	60	25	474	78.9	478	73.2
20			535	101.9	527	90.9

Table II
The Change in Vitamin B₁₂ in the Liver and Blood of Vitamin B₁₂-Deficient Chicks.

Organ	No. of animals	Vitamin B ₁₂ content of the group treated with vitamin B ₁₂	Vitamin B ₁₂ content in the control group
		<i>mγ/g</i>	<i>mγ/g</i>
Liver	1	29.65	16.80
	2	29.80	13.20
	3	29.80	7.95
	4	19.80	13.20
	5	32.00	12.20
	6	29.10	—
	Aver.	28.07	12.03
Blood ^a		<i>mγ/ml</i>	<i>mγ/ml</i>
		2.75	2.58

^a The collected blood of 6 chicks was investigated because the blood of each chick was insufficient.

2. Metabolic State of Pantothenic Acid and Nicotinic Acid in Vitamin B₁₂-deficient Chicks.

Further experiments were carried out with vitamin B₁₂-deficient chicks obtained as mentioned above. The change in pantothenic acid was tested by the microbiological assay of Snell using *Lactobacillus arabinosus* 17-5. The results are given in Table III.

Table III
The Change in Pantothenic Acid in the Liver and Blood.

Organ	No. of animals	Pantothenic acid in the group treated with vitamin B ₁₂				Pantothenic acid in the control group			
		Total	Free	Conj.	Conj./Total	Total	Free	Conj.	Conj./Total
		γ/g	γ/g	γ/g	%	γ/g	γ/g	γ/g	%
Liver	1	35.0	4.0	31.0	88.6	28.0	4.0	24.0	85.7
	2	38.0	4.0	34.0	89.5	42.0	6.0	36.0	85.7
	3	35.0	3.0	32.0	91.4	36.0	6.5	29.5	81.9
	4	34.0	3.5	30.5	89.5	40.5	5.2	35.3	87.2
	5	29.0	3.2	25.8	89.0	42.5	5.0	37.5	88.2
	Aver.	34.2	3.5	30.6	89.6	37.8	5.3	32.4	85.9
Blood ^a		γ/ml	γ/ml	γ/ml	%	γ/ml	γ/ml	γ/ml	%
		0.75	0.62	0.13	17.3	0.66	0.57	0.09	13.6

^a The collected blood of 5 chicks was investigated.

Similar experiments were also performed to study the change in nicotinic acid in the vitamin B₁₂-deficient chicks. Nicotinic acid was determined microbiologically by Snell method using *Lactobacillus arabinosus* (Table IV).

Table IV
The Change in Nicotinic Acid in the Liver and Blood

Organ	No. of animals	Nicotinic acid in the group treated with vitamin B ₁₂				Nicotinic acid in the control group			
		Total	Free	Conj.	Conj./Total	Total	Free	Conj.	Conj./Total
		mg/100g	mg/100g	mg/100g	%	mg/100g	mg/100g	mg/100g	%
Liver	1	20.13	6.50	13.63	67.7	21.00	8.70	12.30	58.6
	2	17.72	5.18	12.54	70.8	26.70	14.55	12.15	45.5
	3	21.78	7.89	13.89	63.8	16.20	6.90	9.30	57.4
	4	17.55	6.22	11.33	64.6	19.58	7.88	11.70	59.7
	5					18.30	5.83	12.47	66.5
	Aver.	19.27	6.44	12.85	66.6	20.36	8.78	11.58	56.9
Blood ^a		mg/ml	mg/ml	mg/ml	%	mg/ml	mg/ml	mg/ml	%
		1.54	0.17	1.37	88.0	1.29	0.30	0.99	77.0

^a The same as described in Table II.

From the above findings, it seems to be probable that some relation of vitamin B₁₂ to pantothenic acid and nicotinic acid may exist for the *in vivo* formation of the conjugated forms of these vitamins.

3. Further Experiments with 15-Day-Old Chicks.

Fifty young chicks were previously fed on a vitamin B₁₂-deficient ration composed of "Awa" (Foxtail millet) 5, corn meal 22, wheat flour 10, wheat bran 10, rice bran 10, soy bean meal 30, polished rice 7, soy bean oil (supplemented with cod-liver oil in the proportion of 15:1) 3, and

mineral matter (NaCl, CaCO₃ and bone dust) 3. After 15 days, the chicks were divided into two groups. The 1st group consisting of 25 chicks weighing aver. 78.7 g each were administered with 1 r of vitamin B₁₂ per day *per os*, while the 2nd group of 25 chicks served as a control. After 45 days of treatment with vitamin B₁₂, the change in the metabolic state of pantothenic acid and nicotinic acid was again tested microbiologically as mentioned above. Results shown in Table V—X indicated a more satisfactory response than in the 1st experiment.

Table V
The Change in Body Weight of Vitamin B₁₂-Deficient Chicks during the Experiment.

The figures indicate the average body weight of 7 chicks.

Days	Group treated with vitamin B ₁₂	Control group
	<i>g</i>	<i>g</i>
0	78.7	83.1
10	127.1	122.3
20	190.9	176.5
30	339.3	305.4
40	424.3	370.7
45	528.6	432.0

Table VI
The Change in Liver Weight of Vitamin B₁₂-Deficient Chicks

Experi- ment	No. of animals	Group treated with vitamin B ₁₂			Vitamin B ₁₂ -deficient group		
		Body wt.	Liver wt.	Liver Body	Body wt.	Liver wt.	Liver Body
		<i>g</i>	<i>g</i>	%	<i>g</i>	<i>g</i>	%
I ^a	1	511	15.9	3.1	517	15.8	3.1
	2	577	16.6	2.9	550	18.8	3.4
	3	534	12.5	2.3	485	17.3	3.6
	4	510	15.6	3.1	547	23.7	4.4
	5	543	15.9	2.9	534	24.2	4.5
	Aver.	535	15.3	2.9	527	20.0	3.8
II ^b	1	530	11.4	2.2	474	13.4	2.8
	2	547	11.6	2.1	407	13.9	3.4
	3	590	12.5	2.1	493	12.7	2.6
	4	530	10.2	1.9	449	12.3	2.7
	5	476	11.3	2.4	329	10.3	3.1
	6	525	12.7	2.4	397	11.5	2.9
	7	502	11.1	2.2	478	12.4	2.6
	Aver.	529	11.5	2.2	432	12.4	2.9

^a Mean value of 70-day-old chicks.

^b Mean value of 60-day-old chicks.

Table VII
The Change in Vitamin B₁₂ in the Liver and
Blood of Vitamin B₁₂-Deficient Chicks

No. of animals	Vitamin B ₁₂ content in the group treated with vitamin B ₁₂		Vitamin B ₁₂ content in the control group	
	Liver	Blood	Liver	Blood
	<i>mγ/g</i>	<i>mγ/ml</i>	<i>mγ/g</i>	<i>mγ/ml</i>
1	21.51	} 3.09 ^a	16.56	} 2.05 ^a
2	19.67		10.30	
3	30.19		12.07	
4	25.50		17.45	
5	18.00	} 2.67 ^b	14.45	} 2.16 ^b
6	26.94		12.52	
7	24.22		14.71	
Aver.	23.72	2.88	14.01	2.11

^a The collected blood of 4 chicks (Nos. 1—4) was investigated.

^b The collected blood of 3 chicks (Nos. 5—7) was investigated.

Table VIII
The Change in Thiamine in the Liver and
Blood of Vitamin B₁₂-Deficient Chicks

Organ	No. of animals	The form of thiamine in the group treated with vitamin B ₁₂			The form of thiamine in the control group		
		Total	Free	Conj. Total	Total	Free	Conj. Total
		<i>γ/100g</i>	<i>γ/100g</i>	%	<i>γ/100g</i>	<i>γ/100g</i>	%
Liver	1	645	109	83.1	440	75	83.0
	2	590	103	82.5	510	89	82.5
	3	548	87	84.1	516	88	82.9
	4	675	113	83.3	532	120	77.4
	5	544	75	86.2	552	118	78.6
	6	606	82	86.5	486	93	80.9
	7	531	73	86.3	505	112	77.8
	Aver.	592	92	84.5	506	99	80.4
Blood		<i>γ/ml</i>	<i>γ/ml</i>	%	<i>γ/ml</i>	<i>γ/ml</i>	%
	1	2.7	1.7	37.0	2.5	2.2	12.0
	2	2.8	2.1	25.0	2.1	1.7	19.0
	Aver.	2.8	1.9	32.1	2.3	2.0	13.0

Table IX
The Change in Pantothenic Acid in the Liver and
Blood of Vitamin B₁₂-Deficient Chicks

Organ	No. of animals	The form of pantothenic acid in the group treated with vitamin B ₁₂			The form of pantothenic acid in the control group		
		Total	Free	Conj./ Total	Total	Free	Conj./ Total
		<i>γ/g</i>	<i>γ/g</i>	%	<i>γ/g</i>	<i>γ/g</i>	%
Liver	1	46.9	3.6	92.3	49.4	5.3	89.3
	2	67.4	7.1	89.5	31.5	4.6	85.4
	3	39.2	4.0	89.8	40.5	9.1	77.5
	4	45.0	5.0	88.9	30.0	3.5	88.3
	5	32.5	3.5	89.2	56.6	8.6	84.8
	6	56.1	5.1	90.9	35.4	4.2	88.1
	7	48.3	4.4	90.9	37.3	5.0	86.6
	Aver.	47.9	4.7	90.2	40.1	5.8	85.5
Blood		<i>γ/ml</i>	<i>γ/ml</i>	%	<i>γ/ml</i>	<i>γ/ml</i>	%
	1	0.94	0.71	25.0	0.94	0.71	25.0
	2	0.94	0.73	22.0	0.94	0.73	22.0
	Aver.	0.94	0.72	23.0	0.94	0.72	23.0

Table X
The Change in Nicotinic Acid in the Liver and
Blood of Vitamin B₁₂-Deficient Chicks

Organ	No. of animals	The form of nicotinic acid in the group treated with vitamin B ₁₂			The form of nicotinic acid in the control group		
		Total	Conj.	Conj./ Total	Total	Conj.	Conj./ Total
		<i>γ/g</i>	<i>γ/g</i>	%	<i>γ/g</i>	<i>γ/g</i>	%
Liver	1	25.2	22.8	90.5	27.9	9.3	33.3
	2	31.8	19.2	60.4	16.5	7.8	47.3
	3	17.4	14.7	84.5	26.7	11.2	41.9
	4	18.6	15.0	80.6	25.1	11.2	44.6
	5	20.3	14.4	70.9	30.0	13.2	44.0
	6	23.4	20.1	85.9	29.4	15.0	51.0
	7	26.4	20.1	76.1	31.8	11.4	35.8
	Aver.	23.3	18.0	77.3	26.8	11.3	42.2
Blood		<i>γ/ml</i>	<i>γ/ml</i>	%	<i>γ/ml</i>	<i>γ/ml</i>	%
	1	1.20	1.11	92.5	1.10	0.53	48.2
	2	1.15	0.88	76.5	1.41	0.71	50.4
	Aver.	1.18	1.00	84.7	1.26	0.62	49.2

SUMMARY

1. The change in vitamin B₁₂ was studied in vitamin B₁₂-deficient chicks, and the remarkable decrease of vitamin B₁₂ was confirmed in the liver, while it was scarcely observed in the blood.

2. The *in vivo* formation of the conjugated forms of thiamine, pantothenic acid and nicotinic acid was repeatedly tested using vitamin B₁₂-

deficient chicks. Vitamin B₁₂-deficient chicks revealed a low content of conjugated vitamins in the liver, but no evidence was ever seen in the chicks treated with vitamin B₁₂. In the blood, a positive change was observed in nicotinic acid, pantothenic acid and thiamine.

3. From these findings, vitamin B₁₂ seems to be of special importance for the formation of coenzymes containing various vitamins. Further enzymic investigation is now in progress.

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