Proceedings of the Iowa Academy of Science

Volume 59 | Annual Issue Article 22

1952

Increasing the Retention of Nitrogen in Albino Rats through Vitamin B12 Administration

Edmund Wei-Kuang Cheng
Iowa State College

Byron H. Thomas
Iowa State College

Increasing the Retention of Nitrogen in Albino Rats through Vitamin B_{12} Administration

By Edmund Wei-kuang Cheng and Byron H. Thomas

Journal Paper No. J-2099 of the Iowa Agricultural Experiment Station. Ames, Iowa. Project 1131.

The growth promoting activity of vitamin B_{12} in rats has been demonstrated by many workers. Earliest among them was Emerson (1949) who showed that the retarding effect of thyroid powder when given to rats was counteracted by oral administration or subcutaneous injection of vitamin B_{12}. Since 1949 many others have demonstrated the beneficial effect of B_{12} in augmenting growth in the young of several mammalian and avian species. Bioassay procedures for the estimation of vitamin B_{12} are based on the capacity of B_{12} to stimulate growth. Cheng and Thomas (1950) showed that not only was growth enhanced but also food efficiency was improved by injecting "protamonized" rats with vitamin B_{12}.

Such findings make it appear highly probable that vitamin B_{12} may play a rôle in the metabolism of protein. Nitrogen balance experiments might yield results which would indicate the validity of such an assumption. Chow and Barrows (1950), however, were not able to demonstrate any increase of nitrogen retention in rats by B_{12} administration. On the other hand, Rupp et al. (1951) showed that the loss of nitrogen was significantly smaller in thyroxine-treated rats receiving B_{12} than in the thyroxine-treated controls. The investigation here reported was carried out to verify one or the other of these contradictary observations, and to obtain additional data showing the relation of vitamin B_{12} to the metabolism of protein.

Procedure

The experimental plan adopted called for demonstrating possible differences in the abilities of weanling male rats to utilize protein of animal or vegetable origin when supplemented or unsupplemented with vitamin B_{12}. This called principally for quantitative measurement of the amount of ingested nitrogen which was retained. The conventional procedure for determining retained nitrogen was used.

Weanling male rats were confined individually to metal cages equipped with feeding and watering devices to permit accurate measurement of ration ingested and collection of urinary and fecal eliminations. All rations and water were fed ad libitum and supplied fresh daily.

All nitrogen balance periods were of four days duration. Total fecal nitrogen eliminated during each period was determined for each rat. Urinary output of nitrogen was determined on a daily basis. All determinations of nitrogen were made according to the micro-Kjeldahl procedure of Pregl (1937).

Either casein or soybean oil meal was the basic proteinaceous ingredient of all rations used in this study. The dry components of all rations were ground finely. All ingredients of each ration were thoroughly mixed in the proportions desired and sufficient ration was made to complete each experiment. The composition of the rations used in the several balance experiments is given in table 1.

Throughout this study, whenever vitamin B_{12} was used it was injected intraperitoneally into each rat at the dosage of one microgram daily in dilute saline solution (Cobione).

Results and Discussion

All together there were eight balance experiments including 26 4-day metabolism periods involving a total of 40 weanling male rats. During all periods animals consumed their rations well and accordingly all gained body weight acceptably (tables 2 thru 7).

The data summarized in table 2 illustrates the futility of not conditioning rats during a pre-experimental period when metabolism periods of relatively short duration are used. Intraperitoneal injections of one microgram daily per rat during period 2 failed to increase the average nitrogen retained during either period 2 or the post-injection period 3. It is not unreasonable to assume the body reserves of B_{12} in these rats were ample enough at the beginning of the experiment to nullify any beneficial effects on growth and nitrogen retention of additional B_{12}.

In view of the foregoing steps were taken to raise the requirement of weanling rats for this vitamin by supplementing the ration with protamone (iodinated casein). There is evidence that the addition of small amounts of thyroid active substances such as dessicated thyroid or iodinated casein will produce hyperthyroid rats. The growth rate of animals so treated is greatly reduced and simultaneously their requirement for certain vitamins, including B_{12}, is raised (Emerson, 1949; Frost et al., 1949; Register et al., 1949). Here then was a procedure whereby the body stores of vitamin B_{12} possibly could be depleted rapidly by the inclusion of protamone in the ration.

Having this objective in mind, ration 1 was modified to include 0.05 per cent protamone (ration 2, table 1). Ration 2 was fed to weanling male rats as in the preceeding test. The data obtained are

Table 1

Composition of rations and vitamin mixture used in nitrogen metabolism experiments.

Ingredient	Ration number and composition (Amount)						Vitamin mixture	
	1	2	3	4	5	6	Ingredient	Amt.
	gm.	gm.	gm.	gm.	gm.	gm.		mg.
§Casein	18	18					Thiamin	0.8
‡Soybean oil meal			60	18	18	18	Riboflavin	0.6
Sucrose	68	68					Niacin	10.0
Dextrose			24	66	66	66	Pyridoxine	0.8
Crisco	9	9	10	10	10	10	Calcium pantothenate	5.0
Cod liver oil	1	1	2	2	2	2	Biotin	0.0004
*Salt mixture	4	4	4	4	4	4	p-amino benzoic acid	5.0
Vitamin mixture	.164	.164	.164	.164	.164	.164	Folic acid	0.2
Protamone		0.50	0.25	0.25	0.25	0.25	Choline	100.0
DL-methionine					0.42		Inositol	40.0
DL-homocystine						0.50	Menadione	1.0
							Total	164.4004

§Vitamin test casein obtained from General Biochemicals, Inc. Chagrin Falls, Ohio.
‡Produced by expeller process.
*Hawk, Oser, Summerson (1947) salt mixture modified by adding 1.5 grams copper sulfate ($CuSO_4 \cdot 5H_2O$) per kilogram.

Table 2

Effect of injecting vitamin B_{12} into weanling male rats on their retention of the nitrogen in casein.

Feeding period and duration		*Av. ration consumed daily per rat	Rat	Initial and final wt.		Daily nitrogen balance data per rat			
							Out-put		
						Intake	Fecal	Urinary	Retained
no.	days	gm.	no. ‡	gm.		mg.	mg.	mg.	mg.
1	4	10.1	1	63	90	251.5	4.6	66.3	180.6
		10.1	2	65	92	251.5	5.7	66.9	178.9
		12.3	3	65	89	243.4	4.9	65.6	172.9
		10.8	Av.	64.3	90.3	248.8	5.1	66.3	177.5
§2	4	12.0	1	90	115	297.9	9.1	101.3	187.5
		11.1	2	92	114	275.8	11.6	93.9	170.3
		10.6	3	89	109	264.0	9.2	93.3	161.5
		11.2	Av.	90.3	112.7	279.2	10.0	96.2	173.1
3	4	12.4	1	115	139	307.5	8.6	126.1	172.8
		11.5	2	114	134	286.4	10.5	119.5	156.4
		12.7	3	109	132	316.3	9.3	118.6	188.1
		12.3	Av.	112.7	135.0	303.4	9.5	121.4	172.4

*Ration 1, Table 1, was fed ad libitum during all periods. There was no transitional feeding period between weaning and beginning of period 1.

‡Identical numbers refer to same animal in all periods.

§One microgram vitamin B_{12} in saline solution (Cobione) injected daily intraperitoneally into each rat.

presented in table 3. The results obtained by including protamone in the ration constituted an improvement over those obtained without it. In the first experiment where protamone was not included the rats failed to show any immediate or delayed increase in average nitrogen retention (177.5, 173.1, 172.4 mg.). However, in the next experiment (table 3), carried out under identical experimental conditions, except for the inclusion of protamone in the ration, injections of B_{12} produced a delayed increase in average nitrogen retention (160.3, 162.0, 195.2 mg.).

These results indicated that immediate response to B_{12} injections, as judged by increased retention of nitrogen, might be obtained if the conditions for B_{12} depletion were made more exhaustive. The finding of Emerson (1949); namely, that the daily requirement of the rat is about 0.125 micrograms, is further evidence in support of drastic exhaustion of B_{12} reserves during the pre-experimental period.

In her study of the growth promoting property of vitamin B_{12} for rats Emerson (1949) obtained good results using rations in which soybean oil meal supplied the protein. Since, in the first place the B_{12} requirement of the rat is exceedingly small; and secondly, casein contains B_{12} and requires exceedingly exhaustive treatment to free it of all B_{12} (Cary and Hartman, 1947); and thirdly, soybean oil meal ordinarily does not contain B_{12}, modification of the Emerson ration was made (table 1, ration 3) and its suitability for nitrogen balance studies tested. Furthermore, the rats which were used in this test were limited to this ration during a 30-day pre-experimental B_{12} depletion period. The data obtained are summarized in table 4. Note that the average nitrogen retained was increased from 94.1 to 172.5 mg. due to B_{12} injections and continued essentially at the same level, 171.0 mg. after injections had ceased. It is quite apparent from this table that the stimulating effects of B_{12} injections are demonstrable immediately in rats provided adequate depletion has been attained.

Rations containing 60 per cent soybean oil meal supply considerably more protein than is ordinarily required by rats for satisfactory growth. Ration 3, table 1 contains approximately 24 per cent protein. At this high level of intake it is reasonable to assume that some of the absorbed amino acids derived from digested soybean protein occurred in excess in the blood of the rats. Charkey et al. (1950) suggested that the building of fixed body tissues would be enhanced by vitamin B_{12} through greater utilization of the amino acids circulating in the blood. Whatever the conditions for demon-

Table 3

Effect of injecting "protamonized" weanling male rats with vitamin B_{12} on their retention of the nitrogen in casein.

Feeding period and duration		*Av. ration consumed daily per rat	Rat	Initial and final wt.		Daily nitrogen balance data per rat			
							Out-put		
						Intake	Fecal	Urinary	Retained
no.	days	gm.	no. ‡	gm.		mg.	mg.	mg.	mg.
		10.5	1	58	77	275.9	12.1	79.7	184.1
1	4	8.3	2	57	71	219.1	8.5	56.2	154.4
		8.2	3	54	70	215.8	8.4	65.0	142.4
		9.0	Av.	56.3	72.7	236.9	9.7	67.0	160.3
		12.1	1	77	91	319.4	16.7	149.6	153.1
§2	4	10.3	2	71	87	271.3	12.7	98.3	160.3
		11.1	3	70	87	291.7	10.4	108.7	172.6
		11.2	Av.	72.7	88.3	294.1	13.3	118.9	162.0
		16.3	1	91	115	431.0	25.9	164.9	240.2
3	4	11.9	2	87	103	314.8	16.9	137.9	160.0
		12.9	3	87	104	341.9	14.1	141.9	185.9
		13.7	Av.	88.3	107.3	362.6	19.0	148.2	195.4

*Ration 2, table 1, was fed ad libitum for three days immediately preceding period 1 and similarly throughout periods, 1, 2 and 3.
‡Identical numbers refer to same animals in all periods.
§One microgram vitamin B_{12} in saline solution (Cobione) injected daily intraperitoneally into each rat.

Table 4

Effect of injecting "protamonized" weanling male rats with vitamin B_{12} on their retention of the nitrogen in soybean oil meal.

Feeding period and duration		Av. ration consumed daily per rat		Rat	Initial and final wt.				Daily nitrogen balance data per rat.							
									Intake		Out-put: Fecal		Out-put: Urinary		Retained	
*no.	days	I	II	I & II §	I		II		I	II	I	II	I	II	I	II
		gm.	gm.	no.	gm.	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
		12.5	18.1	1	114	105	165	169	159	766	40	151	125	499	-6	116
		11.6	15.8	2	99	97	154	150	146	665	35	130	124	482	-13	53
		13.2	12.7	3	116	109	120	121	165	535	44	113	126	397	-5	25
1	4	12.5	15.3	4	104	102	124	129	156	644	39	113	123	447	-6	84
		13.7	14.6	5	103	106	110	124	186	623	45	127	115	392	26	104
		13.4	16.9	6	95	95	109	127	182	722	46	157	106	428	30	137
			17.3	7			119	136		740		137		437		166
			15.4	8			113	122		658		131		459		68
		12.8	15.8	Av.	105.2	102.3	126.8	134.8	165.7	669.1	41.5	132.4	119.8	442.6	4.3	94.1
		15.8	23.1	1	105	111	169	187	198	974	48	191	113	600	37	183
		13.2	22.5	2	97	99	150	176	166	949	38	169	104	609	24	171
‡2	4	15.0	19.9	3	109	110	121	144	188	838	48	168	112	502	28	168
		14.9	19.8	4	102	102	129	150	187	833	45	160	113	538	29	135
		17.4	20.4	5	106	113	124	149	236	872	58	171	118	520	60	181
		15.8	22.1	6	95	97	127	156	215	947	54	176	119	581	42	190
			21.8	7			136	158		933		176		552		205
			21.5	8			122	145		922		174		601		147
		15.4	21.4	Av.	102.3	105.3	134.8	158.1	198.3	908.5	48.5	173.1	113.2	562.9	36.7	172.5

Table 4 (Continued)

Effect of injecting "protamonized" weanling male rats with vitamin B_{12} on their retention of the nitrogen in soybean oil meal.

Feeding period and duration		Av. ration consumed daily per rat		Rat	Initial and final wt.				Daily nitrogen balance data per rat.							
									Intake		Out-put				Retained	
											Fecal		Urinary			
*no.	days	I	II	I & II	I		II		I	II	I	II	I	II	I	II
		gm.	gm.	no. §	gm.	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
		16.7	25.2	1	111	117	187	210	209	1061	48	233	118	698	43	130
		14.4	27.6	2	99	103	176	205	180	1164	44	235	104	697	32	232
		19.0	21.8	3	110	126	144	163	239	921	57	204	124	541	58	176
3	4	14.7	21.7	4	102	107	150	169	185	912	50	174	110	564	25	174
		18.2	21.0	5	113	123	149	162	249	899	63	180	127	571	59	148
		16.0	22.2	6	97	99	156	175	218	950	52	195	126	571	40	184
			21.2	7			158	176		907		174		541		192
			20.9	8			145	160		897		189		577		131
		16.5	22.7	Av.	105.3	112.5	158.1	177.5	213.3	963.3	52.3	199.0	118.2	595.0	42.8	171.0

*I and II designate the groups of rats which received rations 4 and 3, table 1; respectively, (low and high levels of soybean oil meal) during periods 1, 2 and 3. Group I received rations 3 and 4 during the 30 and 3 days; respectively, preceding periods 1, 2, and 3; whereas groups II received ration 3 for 30 days preceding period I.

§Identical numbers refer to same animal in all periods for each level of soybean oil meal.

‡One microgram vitamin B_{12} in saline solution (Cobione) injected daily intraperitoneally into each rat.

strating the immediate effect of B_{12} injections on increasing the retention of nitrogen may be, the results of the last experiment indicate they must have been fulfilled.

Next, an experiment was undertaken to learn whether B_{12} would have a comparable stimulating effect on nitrogen retention when the ration consumed by rats contained only 18 instead of 60 per cent of soybean oil meal. The experimental procedure followed was the same, except for one minor difference, as in the preceding test in which ration 3 (60 per cent soybean oil meal) was used. Experimental period 1 (table 4) was preceded by two adjustment periods; namely, a 30-day depletion period and a 3-day conditioning period during which rations 3 and 4; respectively, (table I) were fed. Ration 4 was fed throughout the experiment.

The effect of B_{12} on protamonized rats restricted to a ration containing only 18 per cent soybean oil meal is summarized by the data presented in table 4. During period 1, before B_{12} was injected, four of the six rats were losing nitrogen from their bodies. The average amount of nitrogen retained daily by all six rats during this period was only 4.3 mg.

Since the nitrogen content of this ration was very low; namely, 1.3 per cent making it equivalent to about 7.4 per cent protein, it was expected that the quantity of nitrogen retained would be small. The effect on amount of nitrogen retained of injecting each rat with one microgram daily of vitamin B_{12} was both immediate and markedly increased. The average change was from 4.3 to 36.7 mg. It is to be noted that the increase continued through the third period during which no vitamin B_{12} was given. These data would seem to indicate that under the rigid conditions of protein exhaustion and B_{12} deficiency imposed by this experiment, injections of B_{12} greatly improved the utilization of protein. Another interpretation, though less plausible, is that vitamin B_{12}, under the conditions of this experiment lowers the rat's requirement for protein.

As has been mentioned above, this 18 per cent soybean oil meal ration contained approximately 7.4 per cent protein. A ration having so little protein is likely to be deficient in one or more essential amino acids. A deficiency of one essential amino acid may limit the availability of others in the synthesis of tissue protein. The beneficial effect of vitamin B_{12} in increasing the nitrogen retention of growing rats might be due to the ability of the vitamin to catalyze the synthesis of some of the deficient amino acids. Both in vivo and vitro experiments furnish evidence indicating the formation of methionine in the presence of vitamin B_{12}. Oginsky (1950) was

able to show that when liver slices from B_{12} supplemented rats were incubated with homocystine and choline or betaine, more methionine was formed than when liver slices from B_{12}-deficient rats were used. Stekol and Weiss (1950) also showed that when rats fed a ration free of labile methyl compounds, but containing homocystine and B_{12}, growth was possible presumably due to the synthesis of methionine. In as much as the physiological units of the protein are a mixture of essential and non-essential amino acids, it is possible that the capacity of vitamin B_{12} to increase the nitrogen retention of the growing rat may reside in its ability to synthesize one or more essential amino acid which are present in limited amounts. In the present experiment, although no specific proof was offered to show any synthesis of methionine or other essential amino acids the increased nitrogen retention obtained following the administration of vitamin B_{12} might possibly be accounted for on the basis of the operation of such a system.

Methionine is one of the essential amino acids that plays an important rôle in the metabolism of the animal body. Although the amount of methionine actually in ration 4 which contained 18 per cent soybean oil meal was not determined, it is a fact that soybean protein contains an unusually small percentage of sulfur-containing amino acids. In view of the foregoing facts and observations it was decided to determine whether vitamin B_{12} administration could increase the nitrogen retention of rats restricted to a methionine deficient ration. First, it was necessary to measure the effect of methionine supplementation on a ration deficient in both methionine and vitamin B_{12}. Suitable male weanling rats were conditioned prior to experimental use similar to the procedure employed in the preceding experiment. The results obtained are summarized in table 5 and are essentially what one would predict. The daily average retention of nitrogen was increased from 11.5 to 66.2 mg. simply by supplementing a methionine-deficient ration with a liberal amount of methionine.

Whether or not supplementation of ration 4 with both methionine and B_{12} would increase the retention of nitrogen beyond that of methionine supplementation remained to be determined. The plan of experiment and a summary of the data collected are presented in table 6. Although these data indicate the B_{12}-treated rats retained more nitrogen than those untreated, the increase was small. It was suggested earlier that B_{12} might play a rôle in the synthesis of methionine in the presence of compounds with labile methyl groups such as choline and betaine.

Table 5

Effect of the supplemental feeding of DL-methionine to "protamonized" male weanling rats on their retention of the nitrogen in soybean oil meal.

Feeding period and duration		Av. ration consumed daily per rat	Rat	Initial and final wt.		Daily nitrogen balance data per rat.			
							Out-put		
						Intake	Fecal	Urinary	Retained
no.	days	gm.	no. §	gm.	gm.	mg.	mg.	mg.	mg.
		12.3	1	93	96	153	40	93	20
		10.0	2	87	87	124	31	91	2
*1	4	12.1	3	97	101	151	41	96	14
		10.7	4	87	90	135	42	83	10
		11.3	Av.	91.0	93.5	140.8	38.5	90.8	11.5
		13.0	1	96	104	170	40	60	70
		13.2	2	87	95	173	43	72	58
2	4	14.7	3	101	106	192	45	69	78
		10.9	4	90	99	142	36	47	59
		13.0	Av.	93.5	101	169.3	41.0	62.0	66.2

*During periods 1 and 2 rations 4 and 5 (methionine) respectively, table 1, were fed ad libitum. Period 1 was preceded by two transitional feeding periods of 30 and 3 days each during which rations 3 and 4, respectively, were fed.

§Identical numbers refer to same animal.

It seems reasonable to assume that when rats are fed a low protein ration in which methionine was deficient they would show an increase in nitrogen retention in the presence of vitamin B_{12} because of the increased availability of methionine. However, when liberal amounts of methionine are used as a supplement to the ration, as was the case in this experiment, it no longer plays the rôle of a limiting factor in the synthesis of tissue protein. If this assumption is correct there should have been no significant increase in nitrogen retention. If the increase in nitrogen retention observed here is considered significant the results would seem to indicate that vitamin B_{12} plays another rôle in addition to the synthesis of methionine. Judging from the data presented in the foregoing the indications are that B_{12} can effect a conversion of homocystine to methionine when B_{12} and homocystine are fed to rats that are limited to a ration deficient in methionine. The acceptability of this hypothesis was investigated in the following experiment. The experimental procedure followed was essentially the same as that used in the preceding experiment. The ration fed was the same except that homocystine replaced methionine (table 1, ration 6). The experimental plan followed and the results obtained are presented in table 7.

Examination of the data in table 7 show that injections of one microgram of vitamin B_{12} daily during period 2 increased the retention of nitrogen in growing rats from 37.0 to 74.8 mg. per day. At the same time there was little change from period to period in the average amount of nitrogen retained by the uninjected group. The increased retention observed may have been due either to the effect of B_{12} on the improvement of the utilization of protein in general, or due to the formation of more methionine from homocystine in particular. The latter supposition is more in line with the current view advanced by Jukes et al. (1950), Stekol and Weiss (1950) and Oginsky (1950) in which it is maintained that vitamin B_{12} is required for the formation of methionine from homocystine. It appears then that in methionine deficient rations vitamin B_{12} and homocystine supplementation enables growing rats to form more methionine. Under these conditions the additional methionine formed is responsible for the increase in growth, or as in the present instance, increase in the retention of nitrogen.

Summary

A series of eight nitrogen balance experiments were carried out to determine whether vitamin B_{12} plays a part in the utilization of protein for the growth of animal tissues. These experiments included 26 metabolism periods of four days duration each involving

Table 6

Effect of injecting "protamonized" weanling male rats with vitamin B_{12} on their retention of the nitrogen in soybean oil meal fortified with DL-methionine.

Feeding period and duration		Av. ration consumed daily per rat		Rat	Initial and final wt.				Daily nitrogen balance data per rat.							
									Intake		Out-put				Retained	
											Fecal		Urinary			
*I	II	I	II	I & II	I		II		I	II	I	II	I	II	I	II
no.	days	gm.	gm.	no. §	gm.	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
		14.7	13.0	1	101	106	96	104	192	170	45	40	69	60	78	70
		10.9	13.2	2	90	99	87	95	142	173	36	43	47	72	56	58
1	4	13.5	12.8	3	115	117	108	113	182	173	37	36	69	79	76	58
		11.0	12.4	4	85	91	94	102	148	167	36	41	57	64	55	62
		12.5	12.9	Av.	97.8	103.3	96.3	103.5	166.0	170.8	38.5	40.0	60.5	68.8	66.2	62.0
		16.4	14.1	1	106	127	104	121	215	185	43	39	69	62	103	84
		11.2	15.0	2	99	105	95	107	147	197	35	48	51	77	61	72
‡2	4	13.8	14.8	3	117	120	113	120	186	199	49	42	85	91	52	66
		10.9	13.8	4	91	96	102	110	147	186	35	39	57	67	55	80
		13.1	14.4	Av.	103.3	112.0	103.5	114.5	173.8	191.8	40.5	42.0	65.5	74.3	67.8	75.5
		17.3	17.8	1	127	132	121	138	226	234	55	55	81	68	90	111
3	4	13.6	14.3	2	105	113	107	116	179	188	42	45	59	75	78	68
		14.3	14.8	3	120	127	120	123	193	199	40	44	92	93	61	62
		10.9	15.3	4	96	99	110	121	147	207	36	42	68	75	43	90
		14.0	15.6	Av.	112.0	117.8	114.5	124.5	186.3	207.0	43.3	46.5	75.0	77.8	68.0	82.8

*The rats comprising groups I and II were fed individually and ad libitum ration 5 (methionine) table 1, during all periods. Period 1 was preceded by two transitional feeding periods of 30 and 7 days each during which rations 3 and 4 respectively, table 1, were fed.

§Identical numbers refer to the same rat in all periods for each group of animals.

‡One microgram of vitamin B_{12} in saline solution (Cobione) was injected daily intraperitoneally into each rat in group II only.

Table 7

Effect of injecting "protamonized" weanling male rats with vitamin B_{12} on their retention of the nitrogen in soybean oil meal fortified with DL-methionine.

Feeding period and duration		Av. ration consumed daily per rat		Rat	Initial and final wt.				Daily nitrogen balance data per rat.							
									Intake		Out-put				Retained	
											Fecal		Urinary			
*I & II		I	II	I & II	I		II		I	II	I	II	I	II	I	II
no.	days	gm.	gm.	no. §	gm.	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
		13.1	12.4	1	111	118	90	103	173	164	37	38	72	77	64	49
1	4	12.3	11.6	2	86	93	100	106	162	152	38	36	72	73	52	43
		12.1	10.6	3	98	100	106	110	159	139	41	36	71	72	47	31
		12.0	10.7	4	87	97	88	92	159	141	25	30	81	86	53	25
		12.4	11.3	Av.	95.5	102.0	96.0	102.8	163.3	149.0	35.3	35.0	74.0	77.0	54.0	37.0
		12.9	16.9	1	118	125	103	118	170	223	34	50	65	87	71	86
		12.5	13.4	2	93	98	106	113	165	176	38	38	68	64	59	74
‡2	4	12.5	13.7	3	100	107	110	120	165	180	44	35	70	81	51	64
		13.2	14.5	4	97	103	92	102	174	192	42	43	67	74	65	75
		12.8	14.6	Av.	102.0	108.3	102.8	113.3	168.5	192.8	39.5	41.5	68.0	76.5	61.5	74.8
		13.6	16.1	1	125	129	118	130	179	212	43	46	66	77	70	89
		12.7	13.1	2	98	109	113	121	167	173	38	36	66	63	63	74
3	4	14.1	14.0	3	107	118	120	130	186	184	48	41	72	82	66	61
		12.2	13.3	4	103	110	102	114	161	179	37	37	63	69	61	73
		13.2	14.1	Av.	108.3	116.5	113.3	123.8	173.3	187.0	41.5	40.0	66.8	72.8	65.0	74.3

*The rats comprising groups I and II were fed individually and ad libitum ration 6 (homocystine), table 1, during all periods. Period 1 was preceded by two transitional feeding periods of 30 and 3 days each during which rations 3 and 4 respectively, table 1, were fed.

§Identical numbers refer to the same rat in all periods for each group of animals.

‡One microgram of vitamin B_{12} in saline solution (Cobione) was injected daily intraperitoneally into each rat in group II only.

40 weanling male rats. In every instance vitamin B_{12} administration was by intraperitoneal injection.

Under rather rigid conditions of B_{12} depletion it was demonstrated readily that B_{12} injection increases the utilization of protein as judged by its capacity to increase nitrogen retention in rats. Also, evidence is presented which indicates that B_{12} aids in the conversion of the amino acid homocystine to methionine. In the case of soybean protein which is low in methionine, vitamin B_{12} helps the animal to more completely utilize protein from this source.

To demonstrate these effects of vitamin B_{12} on the utilization of protein by the rat required rigorous depletion of the body reserves of B_{12} through the combined use of protamone and moderately long pre-experimental periods of B_{12} depletion.

The role of vitamin B_{12} in the metabolism of protein viewed in the light of the present investigation is discussed briefly.

Literature Cited

Cary, C. A., and Hartman, A. M. 1947. Unidentified nutrients. Yearbook of Agriculture, U. S. D. A., 1943-47:779.

Charkey, L. W., Wilgus, H. W., Patton, A. R., and Gassner, F. X., 1950. Vitamin B_{12} in amino acid metabolism. Proc. Soc. Exp. Biol. Med., 73:21.

Cheng, E. W., and Thomas, B. H., 1950. Effect on growth and efficiency of food utilization of quantity of protein and vitamin B_{12} supplementation. Iowa Academy of Science, 57:165.

Chow, B. F., and Barrows, L. 1950. Role of B_{12} on nitrogen retention of rats fed on soybean protein diets at different caloric levels. Fed. Proc., 9:354.

Emerson, G. A., 1949. Growth-promoting activity of vitamin B_{12} in rats receiving thyroid substance. Proc. Soc. Exp. Biol. Med. 70.392.

Frost, D. V., Fricke, H. H., and Spruth, H. C., 1949. Rat growth assay for vitamin B_{12}. Proc. Soc. Exp. Biol. Med. 72:102.

Hawk, P. S., Oser, B. L., and Summerson, W. H., 1947. Practical Physiological Chemistry, 12th Edition, Blakiston Co., Philadelphia.

Jukes, T. H., Stokstad, E. L. R., and Broquist, H. P., 1950. Effect of vitamin B_{12} on the response to homocystine in chicks. Arch. Biochem., 25:453.

Oginsky, E. L., 1950. Vitamin B_{12} and methionine formation. Arch. Biochem., 26:327.

Pregl, F., 1937. Quantitative organic microanalysis. Blaskiston's Son and Co., Inc., Philadelphia.

Register, U. D., Lewis, U. G., Thompson, H. T., and Elvehjem, C. A., 1949. Variations of the vitamin B_{12} content of selected samples of pork and beef muscle. Proc. Soc. Exp. Biol. Med., 70:167.

Rupp, J., Paschkis, K. E., and Cantarow, A., 1951. Influence of vitamin B_{12} and liver extract on nitrogen balance of normal and hyperthyroid rats. Proc. Soc. Exp. Biol. Med., 76:432.

Stekol, J. A., and Weiss, K., 1950. Vitamin B_{12} and growth of rats on diets free of methionine and choline. J. Biol. Chem., 186:343.

IOWA STATE COLLEGE
AMES, IOWA