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Metabolic Interrelationships Between Vitamin B_{12} and Pantothenic Acid in the Rat[1]

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Interrelationships between pantothenic acid and vitamin B_{12} in the nutrition of different species of animals have been reported (Evans et al., '51; Yacowitz et al., '51; Welch and Couch, '54; Balhoun and Phillips, '57). Boxer et al. ('53) observed a fivefold increase in the coenzyme A concentration of liver in vitamin B_{12}-deficient chicks. Further studies with rats, although confirming the earlier observations, revealed that increases were also observable in the kidney, although not in the brain (Boxer et al., '55). It was also evident that the increase was due neither to a decreased destruction of coenzyme A in the deficient tissues, nor to a shift in the ratio of the oxidized to the active reduced form of coenzyme A (Boxer et al., '55). Similar observations have since been reported by others (Sanguinetti et al., '56; Wong and Schweigert, '56) and it has been suggested that since the vitamin B_{12}-deficient animal cannot utilize carbohydrate efficiently (Ling and Chow, '54), the increase in liver coenzyme A may be a physiological adaptive mechanism that increases energy production by providing more two carbon fragments from fatty acid oxidation. A similar deranged carbohydrate metabolism exists in diabetic animals and the impaired energy production appears to be offset by an elevation of liver coenzyme A stores.[2] Cold stress observed to produce a vitamin B_{12} deficiency (Ershoff, '53) also causes an increase in coenzyme A levels (Campbell et al., '60).

An increase in liver concentration of vitamin B_{12} in pantothenic acid deficiency, first reported by Radhakrishnamurty and Sarma ('57), and confirmed by several others (Okuda, '57; Moruzzi et al., '58; Aiyar et al., '59) is also attended by increases in serum vitamin B_{12} and in urinary excretion of vitamin B_{12}.[3]

The reported increase in betaine-homocysteine transmethylase activity in pantothenic acid deficiency (Ericson and Harper, '55) and a decrease of the same in vitamin B_{12} deficiency (Oginsky, '50; Williams et al., '53; Mistry et al., '55; Ericson et al., '56) lends further support to the reciprocal nature of the relationship existing between the two vitamins.

In view of the reported metabolic relationships between pantothenic acid and methionine (Ludovici et al., '51; Dinning et al., '54, '55), vitamin B_{12} and methionine (Stekol and Weiss, '50; Bennett, '50; Fox et al., '59; Moruzzi et al., '60) and between the two vitamins themselves, it was thought worthwhile to study the metabolism of coenzyme A and certain related sulfhydryl compounds, in an attempt to elucidate the mechanism of the increased hepatic coenzyme A concentration in vitamin B_{12} deficiency.

Observations on the changes in tissue levels of coenzyme A, glutathione and total soluble sulfhydryl in simple deficiencies of vitamin B_{12} produced by feeding either a high vegetable protein diet or a purified casein ration devoid of the vitamin, and on the kinetics of *in vivo* biosynthesis of coenzyme A in rats with a single deficiency of vitamin B_{12} and with double deficiencies of vitamin B_{12} and pantothenic acid, from intraperitoneally administered precursors are presented and discussed.

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[2] Tompkins, G. Quoted as personal communication, in Novelli, G. D. 1957 Ann. Rev. Biochem., *26*: 249.

[3] Aiyar, A. S., and A. Sreenivasan, unpublished observation.

EXPERIMENTAL

Induction of deficiencies. (a) A simple vitamin B_{12} deficiency was produced by feeding weanling male rats (Wistar strain), weighing 45 to 50 gm, either a maize-groundnut meal diet (Fatterpaker et al., '59), or a purified 10% casein ration (Fatterpaker et al., '60) devoid of vitamin B_{12} for 8 weeks.

Control groups were also maintained with a supplement of vitamin B_{12} (200 μg/kg) to the respective basal diets.

(b) A double deficiency of pantothenic acid and vitamin B_{12} was produced in one group of rats by feeding the purified casein ration devoid of both calcium pantothenate and vitamin B_{12} for 8 weeks, by which time, the animals exhibited severe symptoms of deficiences of both vitamins.

Four animals each from the groups fed the vitamin B_{12}-deficient and vitamin B_{12}-replete casein rations, were given a supplement of L-cysteine hydrochloride (0.1 gm per kg of diet), throughout the experimental period of 8 weeks.

Administration of vitamin B_{12} to deficient rats. To 8 rats made deficient in vitamin B_{12} by maintenance with the basal purified casein ration, vitamin B_{12} (10 μg) was administered intraperitoneally and the animals were sacrificed at intervals of zero, 4, 8, 16 and 48 hours from the time of administration.

In vivo biosynthesis of coenzyme A in vitamin B_{12}-deficient rats. Vitamin B_{12}-deficient and vitamin B_{12}-supplemented rats were injected intraperitoneally with 10 mg each of calcium pantothenate and cysteine hydrochloride and were sacrificed at intervals of zero and 8 hours. Intraperitoneal injection of vitamin B_{12} (10 μg) was given to one group of deficient rats three hours prior to administration of the precursors.

In vivo biosynthesis of coenzyme A in rats deficient in pantothenic acid and vitamin B_{12}. To rats deficient in both the vitamins, L-cysteine hydrochloride (10 mg) was injected with or without prior administration (three hours prior) of either calcium pantothenate (10 mg) or vitamin B_{12} (10 μg) and the animals were sacrificed 8 hours later.

Determinations. The animals were sacrificed at the end of the experimental periods by decapitation, and the livers, after perfusion with isotonic saline, were excised and chilled in cracked ice. The livers were accurately weighed and made into 10% homogenates in isotonic sucrose (0.25 M) using a Potter-Elvehjem type glass homogenizer fitted with a Teflon pestle.

Total soluble sulfhydryl (Grunert and Phillips, '51) and methionine (Horn et al., '46) were determined colorimetrically essentially as described by the authors and glutathione was determined by the procedure outlined by Kasbekar and Sreenivasan ('59).

TABLE 1

Changes in coenzyme A and related sulfhydryl compounds in rat liver in vitamin B_{12} deficiency[1]

Group	Vitamin B_{12}	Total soluble sulfhydryl	Glutathione	Total methionine	Pantothenic acid	Coenzyme A
	mμg	*mg*	*mg*	*mg*	*μg*	*units*
Maize-groundnut meal diet	47 ± 7[2]	1.02 ± 0.11	0.90 ± 0.08	5.07 ± 0.13	131 ± 16	165 ± 21
Maize-groundnut meal diet + vitamin B_{12}	92 ± 19	1.28 ± 0.03	1.15 ± 0.14	5.21 ± 0.01	81 ± 13	94 ± 14
10% Casein diet	49 ± 4	1.14 ± 0.07	0.88 ± 0.03	4.98 ± 0.09	120 ± 12	151 ± 14
10% Casein diet + vitamin B_{12}	87 ± 9	1.26 ± 0.14	0.99 ± 0.07	5.26 ± 0.11	66 ± 19	87 ± 9

[1] Weanling male rats (45 to 50 gm) were reared with either a maize-groundnut meal diet or a purified casein ration deficient in vitamin B_{12}. Where indicated, vitamin B_{12} was supplemented at 200 μg/kg. Determinations were as detailed in text.

[2] Results are averages of 4 independent determinations ± standard error of the mean and are expressed per gram of fresh liver.

Vitamin B_{12} and pantothenic acid were assayed microbiologically using *Euglena gracilis* (Hoff-Jorgensen, '54) and *Lactobacillus arabinosus* (Skeggs and Wright, '44), respectively, as the test organism.

Coenzyme A was assayed using the acetylating enzyme from pigeon liver by the method of Kaplan and Lipman ('48).

RESULTS AND DISCUSSION

Observations on changes in the hepatic stores of coenzyme A and related sulfhydryl compounds in vitamin B_{12} deficiency are presented in table 1. Vitamin B_{12} deficiency produced by feeding either the high vegetable protein diet or the purified casein ration resulted in a similar increase in coenzyme A concentration of liver, attended by a decrease in total soluble sulfhydryl, glutathione and methionine.

Cysteine supplementation of the 10% casein diet, which is low in methionine, resulted in further increases in the coenzyme A levels with insignificant changes in total soluble sulfhydryl and glutathione in the vitamin B_{12}-deficient group. In the vitamin B_{12}-supplemented group, however, the increases in total soluble sulfhydryl and glutathione were more than the increase shown in coenzyme A (table 2).

In table 3 are presented data on the effects of administration of a single dose of vitamin B_{12} intraperitoneally to deficient animals. Significant increases in total soluble sulfhydryl, glutathione and methionine were observed by the end of 8 hours after administration of the vitamin with practically no change in the coenzyme A level. By the end of 16 hours coenzyme A level showed a decline and was consider-

TABLE 2

Changes in liver stores of coenzyme A due to L-cysteine supplementation of the diet[1]

Group	Total soluble sulfhydryl	Glutathione	Total methionine	Total pantothenic acid	Coenzyme A
	mg	*mg*	*mg*	*µg*	*units*
10% Casein diet	1.14 ± 0.07[2]	0.88 ± 0.06	4.98 ± 0.07	120 ± 12	151 ± 16
10% Casein diet + L-cysteine	1.21 ± 0.03	1.07 ± 0.07	5.23 ± 0.09	143 ± 7	179 ± 7
10% Casein diet + vitamin B_{12}	1.26 ± 0.02	0.99 ± 0.07	5.26 ± 0.02	66 ± 11	87 ± 13
10% Casein diet + vitamin B_{12} + L-cysteine	1.30 ± 0.04	1.19 ± 0.11	5.29 ± 0.10	87 ± 13	99 ± 19

[1] L-Cysteine (100 mg/kg diet) was supplemented to both the vitamin B_{12}-deficient and vitamin B_{12}-replete casein diets throughout the experimental period of 8 weeks.

[2] Results are averages of 4 independent determinations ± standard error of the mean and are expressed per gram of fresh-weight liver.

TABLE 3

Effect of administration of vitamin B_{12} to deficient rats on liver levels of coenzyme A[1]

Hours after administration	Total soluble sulfhydryl	Glutathione	Total methionine	Coenzyme A
	mg	*mg*	*mg*	*units*
0	1.14 ± 0.07[2]	0.88 ± 0.10	4.98 ± 0.07	151 ± 16
4	1.21 ± 0.01	0.97 ± 0.02	5.11 ± 0.11	148 ± 11
8	1.24 ± 0.03	1.03 ± 0.05	5.14 ± 0.09	147 ± 12
16	1.29 ± 0.08	1.14 ± 0.09	5.10 ± 0.02	139 ± 19
48	1.29 ± 0.03	1.14 ± 0.02	5.17 ± 0.09	113 ± 15

[1] Vitamin B_{12} (10 µg/rat) was administered intraperitoneally to the deficient animals and sacrificed at intervals of zero, 4, 8, 16 and 48 hours.

[2] Results are averages for duplicate samples of liver for each of the groups of 8 rats ± standard error of the mean, and are expressed as per gram of fresh-weight liver.

TABLE 4

Biosynthesis of coenzyme A in vivo in vitamin B_{12}-deficient rats[1]

Hours after administration	Total soluble sulfhydryl	Glutathione	Total methionine	Coenzyme A
	mg	*mg*	*mg*	*units*
		10% Casein diet		
0	1.14 ± 0.03[2]	0.88 ± 0.07	4.98 ± 0.09	151 ± 9
8	1.19 ± 0.06	0.97 ± 0.03	4.96 ± 0.03	191 ± 17
8[3]	1.27 ± 0.02	1.08 ± 0.10	5.13 ± 0.02	164 ± 13
		10% Casein diet + vitamin B_{12}		
0	1.26 ± 0.10	0.99 ± 0.02	5.26 ± 0.06	87 ± 10
8	1.29 ± 0.09	1.17 ± 0.07	5.29 ± 0.04	101 ± 12

[1] Calcium pantothenate (10 mg) and cysteine hydrochloride (10 mg) were administered intraperitoneally and the animals sacrificed at intervals of zero and 8 hours.

[2] Results are averages of 4 independent determinations ± standard error of the mean and are expressed per gram of fresh weight liver.

[3] Vitamin B_{12} (10 μg) injected intraperitoneally three hours prior to administration of the precursors.

ably reduced by 48 hours, whereas glutathione and methionine show a gradual rise.

Data on the *in vivo* biosynthesis of coenzyme A from intraperitoneally administered precursors in vitamin B_{12}-deficient and vitamin B_{12}-supplemented rats are presented in table 4. The biosynthesis of the coenzyme occurred more in the deficient group than in the supplemented group in which the synthesis of glutathione and total soluble sulfhydryl appeared greatly enhanced. Administration of vitamin B_{12} three hours prior to administration of L-cysteine hydrochloride and calcium D-pantothenate to the vitamin B_{12}-deficient animal resulted in reduction in the biosynthesis of coenzyme A with attendant increases in the synthesis of glutathione, total sulfhydryl and methionine.

A combined deficiency of vitamin B_{12} and pantothenic acid resulted in low hepatic coenzyme A levels and slightly decreased total soluble sulfhydryl and glutathione levels (table 5). Administration of L-cysteine hydrochloride led to a slight increase in the levels of total soluble sulfhydryl. Prior (three hours) administration of calcium pantothenate effected increased synthesis of coenzyme A, and of vitamin B_{12} favored increased synthesis of total soluble sulfhydryl and glutathione.

The results point to an increased channeling of cysteine into coenzyme A rather

TABLE 5

Biosynthesis of coenzyme A in vivo in rats deficient in pantothenic acid and vitamin B_{12}[1]

Compounds administered			Total soluble sulfhydryl	Glutathione	Total methionine	Coenzyme A
Vitamin B_{12}[2]	Calcium pantothenate[2]	L-Cysteine[3]				
			mg	*mg*	*mg*	*units*
—	—	—	0.93 ± 0.03[4]	0.81 ± 0.09	4.77 ± 0.09	94 ± 11
—	—	+	1.21 ± 0.04	0.84 ± 0.03	4.73 ± 0.01	91 ± 13
—	+	+	1.07 ± 0.08	0.86 ± 0.06	4.73 ± 0.07	137 ± 18
+	—	+	1.31 ± 0.06	1.11 ± 0.03	5.01 ± 0.10	99 ± 7

[1] Weanling rats were maintained with a purified 10% casein ration devoid of both vitamin B_{12} and pantothenic acid for 8 weeks.

[2] Where indicated vitamin B_{12} (10 μg) and calcium pantothenate (10 mg) were administered parenterally three hours prior to L-cysteine hydrochloride.

[3] L-Cysteine hydrochloride (10 mg/rat) was administered intraperitoneally and the animals sacrificed 8 hours later.

[4] Results are averages of 4 independent determinations ± standard error of the mean and are expressed per gram of fresh weight liver.

than into glutathione or methionine in the vitamin B_{12}-deficient rat, possibly due to the reported participation of vitamin B_{12} in the biosynthesis of glutathione (Kasbekar et al., '59) and in the formation of methionine (Oginsky, '50) from cysteine through homocysteine. The increase in coenzyme A is possibly a metabolic adaptation necessitated by the impaired carbohydrate metabolism, for more effective concentration of the coenzyme to participate in fatty acid oxidation, in the vitamin B_{12}-deficient animal (Wong and Schweigert '56).

SUMMARY

1. The elevation in hepatic coenzyme A in the vitamin B_{12}-deficient rat was attended by decreases in total soluble sulfhydryl, glutathione and total methionine. The changes were reversed and the levels returned to almost normal values within 48 hours after administration of a single dose of vitamin B_{12}.

2. Supplementation of a low-methionine diet with L-cysteine hydrochloride resulted in increases in liver stores of coenzyme A, total soluble sulfhydryl, glutathione and methionine, the rise in coenzyme A level being more in the vitamin B_{12}-deficient rat than in the supplemented one.

3. The vitamin B_{12}-deficient animal showed greater *in vivo* synthesis of coenzyme A from intraperitoneally administered precursors, than the vitamin-supplemented animal. Prior administration of vitamin B_{12} to the deficient animal decreased the coenzyme A synthesis.

4. Administration of L-cysteine hydrochloride to rats deficient in both pantothenic acid and vitamin B_{12} was without appreciable effect on the liver levels of coenzyme A, total soluble sulfhydryl, glutathione and methionine. Prior administration of pantothenic acid or of vitamin B_{12} favored increased synthesis of coenzyme A or of total soluble sulfhydryl, glutathione and methionine, respectively.

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