PARTICIPATION OF VITAMIN B₆ IN THE BIOSYNTHESIS OF COENZYME A

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It has been demonstrated by Lipmann and others (1, 2) that coenzyme A (CoA) is a derivative of pantothenic acid, and that in animal and plant cells pantothenic acid exists mostly as CoA. Pantothenic acid is therefore synthesized into the coenzyme within the body for manifesting enzymic action. But the process for biosynthesis into a coenzyme has not yet been clarified. However, a part of this mechanism has been made clear by Lipmann (3), Baddiley (4), Levintow (5), and Hoagland and Novelli (6), and the participation of vitamin B₆ (VB₆) has been suggested. In order to explain the relationship between VB₆ and CoA, we undertook the following experiments.

EXPERIMENTAL

Rats deprived of VB₆ were bred according to the method of Stöerk *et al.* (7) modified in this laboratory, and at the end of two months of feeding a deficient diet the CoA content of the liver was estimated by the method of Kaplan and Lipmann (8). VB₆-avitaminosis was clearly demonstrated by the decrease in body weight, dermatitis, atrophy of the thymus and spleen, increase in urinary excretion of xanthurenic acid after test doses of tryptophan, and recovery experiments.

Table I shows the decrease in liver CoA of VB₆-deprived rat. Since this finding suggest the participation of the vitamin in the biosynthesis of

TABLE I
Effects of VB₆ on Liver CoA
(Units/g wet liver tissue)

No.	Normal	Deficient
1	133	92
2	123	92
3	108	92
4	108	103
5	103	46
Average	115	85

CoA, an attempt was made to clarify the site of the participation of the vitamin. The enzymes fractionally prepared from the liver of rats and pigs according to the method of Hoagland and Novelli (6) were employed, and experiments were made to determine whether VB₆ is involved in the synthesis of (a) pantothenate $\rightarrow$ pantetheine (first incubation) or in (b) pantetheine $\rightarrow$ CoA (second incubation).

TABLE II
Pyridoxal-phosphate Requirements for Pantetheine Synthesis

First incubation	CoA
	<i>Units</i>
Enzyme + ATP + pantothenate + cysteine	10.9
Enzyme + ATP + pantothenate + <i>N</i> -pyridoxal-L-cysteine (13 γ)	0
Enzyme + ATP + pantothenate + pyridoxal-phosphate (10 γ)	13.9
Enzyme + ATP + pantothenate + pyridoxal-phosphate (25 γ)	24.4
Enzyme + ATP + pantothenate + pyridoxal-phosphate (50 γ)	13.5
Enzyme + ATP + cysteine + pyridoxal-phosphate (10 γ)	0

0.4 ml of rat supernatant fraction was treated with Dowex and dialyzed for 4 hours. It was incubated with 10 μ M ATP (adenosine-triphosphate), 0.05 μ M pantothenate, 0.05 μ M cysteine, 5 μ M Mg⁺⁺, 50 μ M Tris buffer, pH 7.7, in a volume of 1 ml, at 37° for 30 minutes. After the second incubation, CoA was determined by the Kaplan and Lipmann method.

TABLE III
Influence of Pyridoxal-phosphate for CoA Synthesis from Pantetheine

Second incubation	CoA
	<i>Units</i>
Enzyme + ATP + first incubation supernatant fraction	10.9
Enzyme + ATP + first incubation supernatant fraction + pyridoxal-phosphate (10 γ)	10.9

0.2 ml of the 0–38 per cent-saturated ammonium sulfate fraction from hog liver supernatant fraction was incubated with 10 μ M ATP, 5 μ M Mg⁺⁺, 10 μ M cysteine, 100 μ M Tris buffer, pH 8.0, and a 0.5 ml aliquot of the first incubation supernatant fraction in a volume of 1 ml, at 37° for 6 minutes. A properly diluted aliquot was analyzed for CoA by the Kaplan and Lipmann method.

From Table II and III pyridoxal-phosphate appears to be involved in the first incubation, that is, in the synthesis of pantetheine from pantothenate, and not in the synthesis of CoA from pantetheine.

In the case of the first incubation, cysteine was replaced by synthesized *N*-pyridoxal-L-cysteine, but neither transformation nor condensation reactions resulted between pantothenate and *N*-pyridoxal-L-cysteine.

Based on the enzymic action of pyridoxal-phosphate in the decarboxyla-

tion of amino acids (9), VB₆ is assumed to be involved in the decarboxylation of pantothenyl-cysteine to pantetheine.

SUMMARY

1. A marked decrease in liver CoA was observed in rats maintained on VB₆-deficient diets.

2. The enzymes fractionally prepared from the liver of rats and pigs according to the method of Hoagland and Novelli were employed for the biosynthesis of CoA, and VB₆ appears to be involved in the synthesis of pantetheine from pantothenate.

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