min B_6 has been used to study the urinary excretion of the vitamin in the rat. At high dose levels (10 mg per kg and above) 50 to 70% of the vitamin is excreted by both normal and deficient rats. The vitamin is rapidly and completely absorbed and is rapidly excreted. At low levels (2 mg per kg) the data are qualitative, but normal rats appear to excrete a higher percentage of the ingested vitamin than B_6-deficient rats.

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Antidermatitic Effect of Vitamin B_6 Analogues.

KLAUS UNNA. (Introduced by H. Molitor.)

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The following report presents data on the vitamin activity of 10 pyridine compounds closely related to vitamin B_6 These substances were synthesized in the Research Laboratories of Merck & Co., Inc., in connection with studies on crystalline vitamin B_6.

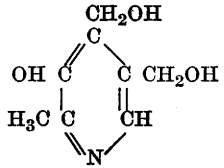
The vitamin B_6 activity of these compounds was determined by the single dose curative assay on rats, first proposed by Moll.[1] In this procedure 21-day-old rats were placed on a synthetic diet consisting of cornstarch 68%, casein 18%, Crisco 8%, salt mixture No. 1 (U.S.P. XI) 4%, cod liver oil 2%, and supplemented with 40 micrograms each of thiamin chloride and riboflavin per rat per day. After 30 days on this diet, the animals reached stationary weights and the first symptoms of dermatitis appeared. Within 7 to 10 weeks, dermatitis was fully developed in 35% of the animals. By this procedure it has been shown[2] that a single dose of 100 micrograms of vitamin B_6 cures 100% of the deficient animals within 14 days, and that a dose of 50 micrograms produces complete cures in 75% of the animals. Lower doses fail to produce complete cures, but signs of partial healing were obtained regularly with 25 micrograms and in some instances with a single dose of 15 micrograms.

In the present study 5 to 6 depleted animals were used for each dose level. Their weight and symptoms were recorded over a period of 14 days. The results obtained with the different pyridine derivatives, as

1 Moll, Th., and Schnittspahn, M., *E. Merck's Jahresberichte*, 1938, **52**, 10.

2 Reedman, E. J., Sampson, W. L., and Unna, K., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 112.

TABLE I.
Antidermatitic Effect of Vitamin B_6 Derivatives.

	Doses in mg						
	0.05	0.1	0.25	0.5	1.0	2.0	2.5
 2-methyl-3-hydroxy-4,5-bis (hydroxymethyl)-pyridine, Vitamin B_6	+	+					
Derivatives of 2-methyl-pyridine:							
I 3-hydroxy-4,5-bis(acetoxy-methyl)	+	+					
II 3-acetoxy-4,5-bis(acetoxy-methyl)[3]	+	+					
III 3-methoxy-4,5-bis(hydroxy-methyl)[4,5]				0	(+)		+
IV 3-hydroxy-4-methoxymethyl-5-hydroxymethyl		(+)	(+)	+			
V 3-hydroxy-4-ethoxymethyl-5-hydroxymethyl[6]		(+)	(+)	+			
VI 3-hydroxy-4,5-(epoxydimethyl)[7]		0	0	0			(+)
VII 3-hydroxy-4-methyl-5-hydroxy-methyl[8]				0	0		0
VIII 3-hydroxy-4,5-dimethyl							0
IX 3-aminoHCl-4-hydroxymethyl-5-aminomethylHCl[7]					0	0	
X 3-aminoHCl-4-ethoxymethyl-5-aminomethylHCl[7]					0	0	

+ Complete cure of at least 75% of the animals within 14 days.
(+) Partial cure accompanied by some gain in weight.
0 No curative effect and no gain in weight.

compared with the crystalline synthetic vitamin, are recorded in Table I.

The di- and triacetyl compounds (I, II) were found equally potent and of the same activity as the vitamin when equimolicular amounts were fed. The activity of these acetyl derivatives may be explained on the basis of liberation of the free vitamin by hydrolysis.

Methylation of the phenolic hydroxyl group of vitamin B_6 (III) decreased the activity to 2% of that of the vitamin. When one of

[3] Kuhn, R., and Wendt, G., *Ber.*, 1938, **71**, 780.

[4] Stiller, E. T., Keresztesy, J. C., and Stevens, J. R., *J. Chem. Soc.*, 1939, **61**, 1237.

[5] Kuhn, R., and Wendt, G., *Ber.*, 1938, **71**, 1534.

[6] Harris, S. A., and Folkers, K., *J. Am. Chem. Soc.*, 1939, **61**, 1245.

[7] Harris S. A., and Folkers, K., *J. Am. Chem. Soc.*, 1939, **61**, 3307.

[8] Harris, S. A., and Folkers, K., *J. Am. Chem. Soc.*, 1939, **61**, 1242.

the 2 hydroxymethyl groups was methylated (IV), a similar but less appreciable loss of activity was found. No difference in potency was observed between the methyl ether (IV) and the ethyl ether of the vitamin (V), but further reduction in activity resulted from the formation of an inner ether between the two hydroxyl groups in the 4 and 5 positions (VI). A derivative of VI, the lactone of 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxy pyridine,[8] has been tested in this laboratory and found to be inactive at a dose level of 1 milligram.

When either one (VII) or both hydroxymethyl groups (VIII) were replaced by a methyl group, no activity could be found at 2.5 mg. However, compound VII has been reported[9] to promote bacterial growth at a concentration 50 times higher than that of the vitamin, but to be inactive in deficient rats at a dose of 1 mg.

Introduction of amino groups in positions 3 and 5 (IX, X) resulted in inactive compounds.

Appreciation is expressed to Dr. S. A. Harris for furnishing the pyridine derivatives and to Miss J. Dawson for valuable technical assistance.

Summary. Acetylation does not diminish the antidermatitic effect of vitamin B_6. Methylation or ethylation of one of the hydroxymethyl groups diminishes the vitamin activity considerably but less than the methylation of the phenolic hydroxyl group. Replacement of one or more hydroxymethyl groups by methyl or amino groups destroys the vitamin activity.

[9] Möller, Zima, Jung and Moll, *Naturwissenschaften*, 1939, **27**, 288.