

Antipyridoxine Activity of Methoxypyridoxine in the Chick.

WALTHER H. OTT. (Introduced by H. Molitor.)

From the Merck Institute for Therapeutic Research, Rahway, N.J.

Desoxy pyridoxine (2,4-dimethyl-3-hydroxy-5-hydroxymethylpyridine), an analogue of pyridoxine, has been shown to be a strong inhibitor of pyridoxine in chicks¹ and to exhibit a similar but less potent effect in rats.² Further studies on additional analogues of this vitamin have led to the discovery that 2-methyl-3-hydroxy-4-methoxymethyl-5-hydroxymethylpyridine (methoxypyridoxine) also has pronounced antipyridoxine activity in chicks. In rats, however, this methoxypyridoxine has some vitamin activity,³ presumably due to the ability of this species to convert part of the compound to pyridoxine.⁴

The procedure employed in studies on the effect of methoxypyridoxine in chicks was similar to that described previously.¹ To begin an assay, eight-day-old chicks weighing approximately 60 g after maintenance for 5 days on a purified diet (Table I) deficient in pyridoxine were distributed into groups balanced in regard to body weight. The chicks were dosed orally with the test substances on the first, third, fifth and seventh days of the assay period. Body weights were recorded on these days as well as on the ninth (last) day of the test. The pyridoxine-deficient diet was fed to all groups during the assay period.

The curve of response (weight gain *vs.* log dose) was established for each assay by using 3 or more of the groups of chicks on dosages in the range from 5 to 50 micrograms pyri-

TABLE I.
Pyridoxine-deficient Diet for Chicks.

	g
Dextrose (cerelose)	51.5
Casein (vitamin free)	25.0
Salts IV ⁵	5.0
Cellulose (ruffex)	5.0
Calcium gluconate	2.5
Glycine	2.0
Liver extract L*	2.0
KH ₂ PO ₄	1.0
Wheat Germ Oil	4.5
400 D fish liver oil	0.5
Arginine	0.5
Cystine	0.2
Choline	0.2
Inositol	0.1
p-Aminobenzoic acid	0.03
Niacin	0.01
Calcium pantothenate	0.004
Riboflavin	0.002
Thiamine	0.002
Menadione	0.0004
Biotin	0.00004
Total	100.05

* Wilson & Co., Inc., Chicago, Ill.

doxine per dose. The growth response of each group receiving a combination (premixed solution) of methoxypyridoxine and pyridoxine was compared with the curve of response for pyridoxine to determine the net pyridoxine activity of the combination. The inhibition ratio between analogue and vitamin was then calculated from the amount of methoxypyridoxine administered and the apparent loss in pyridoxine activity.

A single dose of 200 μ g of methoxypyridoxine killed all the chicks in the group within 48 hours after administration (Table II). Even quantities as low as 15 μ g per chick were fatal in the absence of pyridoxine. This toxic effect was prevented by simultaneous administration of an equal amount of pyridoxine, thus indicating that methoxypyridoxine had antipyridoxine activity. The bioassays (Table II) showed that approximately 4 parts by weight of methoxypyridoxine hy-

¹ Ott, W. H., *Proc. Soc. Exp. Biol. and Med.*, 1946, **61**, 125.

² Emerson, G. A., unpublished data.

³ Unna, K., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 122.

⁴ Porter, C. C., Clark, I., and Silber, R. H., *J. Biol. Chem.*, 1947, **167**, 573.

⁵ Hegsted, D. M., Mills, R. C., Evehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **138**, 459.

TABLE II.
Antipyridoxine Activity of Methoxypyridoxine in 8-day Curative Assays with Chicks Receiving a Pyridoxine-deficient Diet.

Exp.	Oral Supplement*		Survival of chicks, alive/total	Wt gain per chick, g	Loss in pyridoxine activity, μ g	Ratio of inhibition, analogue : vitamin
	Pyridoxine hydrochloride, μ g	Methoxypyridoxine hydrochloride, μ g				
1	0	200	0/6	†		
	0	0	4/7	11.4		
	20	0	7/7	50.9		
2	0	15	0/7	‡		
	0	0	4/7	10.0		
	7.5	0	2/7	34.7		
	15	0	7/7	38.8		
	50	0	7/7	46.7		
	15	15	7/7	43.3	0	—
	15	30	5/7	33.5	8	4:1
	15	45	5/7	28.1	11	4:1
3	20	40	1/7	—	20	2:1
	20	60	5/7	20.8	16	4:1
	20	80	5/7	22.9	15	5:1
	0	0	6/7	14.5		
	5	0	14/14	24.9		
	10	0	13/14	31.6		
	20	0	6/7	37.1		
	40	0	7/7	56.2		
4	60	60	7/7	36.6	24	2.5:1
	60	120	3/7	26.0	42	3:1
	60	240	6/7	17.5	50	5:1
	60	360	3/7	8.7	55	6.5:1
	0	0	3/7	4.0		
	10	0	14/14	17.7		
	40	0	14/14	38.6		

* Amount given per chick on each of 4 successive alternate days.

† All dead after 1st dose.

‡ 2 dead after 1st dose, 3 dead after 2nd dose, 2 dead after 3rd dose.

drochloride counteracted the biological activity of one part by weight of pyridoxine hydrochloride. In the chick, therefore, methoxypyridoxine is approximately as powerful an inhibitor of pyridoxine as is desoxypyridoxine.¹

The inhibition ratio of 2:1 reported for desoxypyridoxine¹ is not significantly different from the inhibition ratio of 4:1 estimated above for the methoxy analogue of pyridoxine. Nevertheless, there seems to be a difference in the antivitamin action of the two analogues. Although both compounds appear to compete with pyridoxine, it has been observed on the basis of limited tests that the effects of lethal doses of desoxypyridoxine were relatively easily counteracted by administration of pyridoxine even after a con-

siderable length of time. On the other hand, when a lethal dose of methoxypyridoxine had been given, subsequent administration of pyridoxine was generally ineffective in preventing the death of the chicks. These and other observations on comparative antipyridoxine activity in the chick are being investigated further.

Summary. 2-Methyl-3-hydroxy-4-methoxymethyl-5-hydroxymethylpyridine (methoxypyridoxine) has been shown to have the same order of antipyridoxine activity as desoxypyridoxine in chicks. In these experiments, approximately four moles of methoxypyridoxine counteracted the vitamin activity of one mole of pyridoxine when suboptimal or optimal amounts of the vitamin were given to pyridoxine-deficient chicks.