have been well shown in such preparations. Red cells, however, appear badly distorted in the larger vessels although they are rather well preserved in the capillaries and arterioles. This deleterious effect is probably not of osmotic origin as it occurs when iso-osmotic solutions of hydroxybenzene-formol are used as well as when .85% NaCl is added to such solutions. Another undesirable feature of these fixatives is that when tissue blocks remain in them sufficiently long to allow penetration and fixation throughout the block, the cells near the surface are over fixed. This over fixed zone is, however, not very thick when the tissue has been fixed 6 to 8 hours and does not, as a rule, interfere with the study of the tissue.

Summary. Methods for the fixation of the cell cytoplasm employing pyrogallol or resorcinol in neutralized formaldehyde solution are outlined. Tissue pieces fixed with these solutions have, in general, shown few signs of distortion, and the protein cytoplasmic elements of the cells have appeared well preserved, such structures as mitochondria, secretion granules, and some of the specific types of granulation being well maintained. The advantages of these methods lie in the simplicity and rapidity of the procedures, the ease with which the tissue can be sectioned at a thickness of 1 to 2 micra, and the generally satisfactory results obtained.

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Antipyridoxine Activity of 2, 4-Dimethyl-3-Hydroxy-5-Hydroxymethyl-pyridine in the Chick.

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In the course of investigations on the vitamin activity of pyridoxine analogs, it was discovered that 2,4-dimethyl-3-hydroxy-5-hydroxymethylpyridine had very strong antipyridoxine properties. Growth and survival of pyridoxine-deficient chicks in curative assays were used as the criteria of response. This substance, a desoxypyridoxine, had been studied in pyridoxine-deficient rats by Möller[1] and associates and by Unna,[2] both of whom found no pyridoxine activity. No indication of antipyridoxine activity was reported by these investigators.

Day-old female single comb White Leghorn chicks were fed a commercial starting ration for 3 days and then were given a purified diet (Table I) deficient in pyridoxine. After 6 days on this deficient diet, when many chicks were beginning to lose weight, the chicks were divided into groups of 7 selected in such a way that all groups were as nearly alike as possible in weights of the individual chicks. The test substance was given in single oral doses to each chick on the 11th day of age and on the following 3 alternate days. Three or more dose levels of pyridoxine in the range from 0 to 24 μg per dose were given in each assay to establish the standard curve of response for that assay. New standard solutions of pyridoxine and of desoxypyridoxine were prepared in water for each assay and kept under toluene in a refrigerator for the 6-day dosing period. The chicks were weighed before each dose and on the second day after the last dose was given. The pyridoxine-deficient diet was fed to all groups during the assay period.

Two 100 μg doses of desoxypyridoxine were fatal to pyridoxine-deficient chicks even when each dose was preceded by a 16 μg dose of pyridoxine (Table II). Furthermore, a level of the analog as low as 16 μg per

[1] Möller, E. F., Zima, O., Jung, F., and Moll, Th., *Naturwiss.*, 1939, **27**, 228.

[2] Unna, K., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 122.

TABLE I.
Pyridoxine-Deficient Diet for Chicks.

Dextrose (cerelose)	46.5
Casein (vit.-free)	25.0
Gelatin	10.0
Calcium gluconate	5.0
Salts IV[3]	5.0
KH_2PO_4	1.0
Liver extract "L"*	2.0
Wheat germ oil	4.5
400D fish liver oil	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.

[3] Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **138**, 459.

dose also proved fatal to the birds. On the other hand, pyridoxine-deficient chicks which received no supplement suffered less than 60% mortality in the same assays.

That the action of the analog was probably due to interference with pyridoxine activity rather than to some other type of toxicity was indicated by a test on chicks receiving a commercial starting ration. Dose levels of 50, 100, and 200 μg were given to groups of 3 six-day-old chicks each and repeated on the two following alternate days. All chicks survived, and no retardation of growth was observed. These chicks, with normal body stores of pyridoxine and an adequate supply of the vitamin in the commercial starting ration, were able to tolerate total dosages of at least 600 μg of the analog, a quantity more than 6 times as great as the lethal dose to more than half the pyridoxine-deficient chicks.

On the assumption that desoxypyridoxine acted as an antivitamin, further assays were conducted with pyridoxine-deficient chicks on the pyridoxine-deficient diet to estimate the amount of the analog needed to counteract a given amount of pyridoxine (Table III). In these tests the dose of pyridoxine always preceded the dose of the analog by 15 to 30 minutes.

The growth response of each group receiving both compounds was compared with the standard curve of response from graded levels of pyridoxine, and the net pyridoxine activity of each combination was estimated. Subtraction of the observed pyridoxine activity from that theoretically expected on the basis of the level of pyridoxine administered gave the apparent loss in pyridoxine activity occasioned by the dose of desoxypyridoxine. From these last 2 values it can be seen that about 2 parts of the analog counteracted the biological

TABLE II.
Activity of Desoxypyridoxine and Pyridoxine in 9-day Curative Tests with Chicks Receiving a Diet Deficient in Pyridoxine.

Oral supplement*			
Pyridoxine hydrochloride μg	Desoxypyridoxine hydrochloride μg	Survival of chicks, alive/total	Wt gain per chick, g
—	100	0/7	(5 dead after 1st dose) (2 " " 2nd ")
—	—	6/7	3.8
16	—	7/7	36.6
16	100	0/7	(4 dead after 1st dose) (3 " " 2nd ")
—	—	4/7	0.7
16	—	7/7	33.0
—	16	0/6	(3 dead after 1st dose) (2 " " 2nd ") (1 " " 3rd ")
—	—	3/7	—7.4
16	—	6/6	32.0

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

TABLE III.
Antipyridoxine Activity of Desoxypyridoxine as Measured in 9-day Curative Assays with Chicks Receiving a Pyridoxine-Deficient Diet.

Oral supplement*		Survival of chicks, alive/total	Wt gain per chick, g	Loss in pyridoxine activity, μg	Ratio of inhibition, analog : vit.
Pyridoxine hydrochloride, μg	Desoxypyridoxine hydrochloride μg				
16	32	2/7	15.5	11	3:1
16	16	6/7	20.5	7	2:1
16	8	7/7	18.8	8	1:1
4	—	6/7	14.5		
8	—	6/7	19.5		
16	—	7/7	28.0		
16	16	5/7	23.4	3	5:1
24	16	7/7	30.2	8	2:1
35	50	6/7	21.4	23	2:1
—	—	3/7	7.2		
8	—	6/7	16.1		
12	—	7/7	19.6		
16	—	6/6	32.0		

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

activity of one part of pyridoxine (Table III).

Previously reported B vitamin antagonists are relatively much weaker antivitamins. For example, only one molecule of thiamine was required to overcome the antithiamine activity of 40 molecules of pyrithiamine in mice,[4] and a similar ratio was found between riboflavin and isoriboflavin rats.[5] It therefore appears that desoxypyridoxine is the most potent of the B vitamin inhibitors as yet discovered.

Summary. Bioassays with chicks have shown that 2,4-dimethyl-3-hydroxy-5-hydroxymethylpyridine is a very potent inhibitor of pyridoxine. Under the conditions of these experiments, two molecules of the inhibitor were sufficient to offset the vitamin activity of one molecule of pyridoxine. This ratio was found to hold for suboptimal and optimal amounts of pyridoxine given to pyridoxine-deficient chicks.

[4] Woolley, D. W., and White, A. G. C., *J. Biol. Chem.*, 1943, **149**, 285.

[5] Emerson, G. A., and Tishler, M., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 184.

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Adrenolytic and Sympatholytic Actions of Priscol (benzyl-imidazoline)

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Experimentally employed adrenolytic agents include ergotoxine, ergotamine tartrate, yohimbine hydrochloride, various esters of yohimbic acid and the Fourneau Compounds, F933 and F899. In some respects these agents seem to be sympatholytic as well as adrenolytic. It has been reported[1] that Priscol exerts this dual effect.

The adrenolytic properties of priscol have

[1] Meier, R., and Meyer, R. Th., *Schweiz. Med. Wschr.*, 1941, **71**, 1266.