

Effect of deoxypyridoxine on signs of pyridoxine deficiency and intestinal 5-hydroxytryptamine

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Many experiments have been devised to study the physiological significance of 5-hydroxytryptamine. To do so, it has often been desirable to deplete organs of the amine, and the Rauwolfia alkaloid reserpine has been commonly used for this purpose. Though reserpine is capable of producing a severe depletion of 5-hydroxytryptamine in brain (Brodie, Pletscher & Shore, 1955) and intestine (Pletscher, Shore & Brodie, 1955; Bülbring & Crema, 1959), amongst other tissues, synthesis of the amine remains unimpaired, with the result that the depletion induced by reserpine is never absolute. To overcome this difficulty it has been necessary to use other methods, including nutritional methods, to prevent synthesis of 5-hydroxytryptamine. Theoretically this can be done in several ways: decarboxylase inhibitors, such as 3,4-dihydroxyphenyl- α -methylalanine (α -methyl-dopa), can be used to interfere with the enzymic conversion of the amino-acid precursor 5-hydroxytryptophan into 5-hydroxytryptamine by 5-hydroxytryptophan decarboxylase; a pyridoxine-deficient diet can be given as the enzyme 5-hydroxytryptophan decarboxylase is pyridoxine-dependent (Buxton & Sinclair, 1956; Buzard & Nytech, 1957), or a tryptophan-free diet can be given.

This paper describes some unsuccessful attempts at preventing 5-hydroxytryptamine synthesis by giving a pyridoxine-deficient diet with deoxypyridoxine. As reported elsewhere (Boullin, 1963, 1964) attempts with a tryptophan-free diet were successful and resulted in apparently complete depletion of 5-hydroxytryptamine in some instances.

EXPERIMENTAL AND RESULTS

Animals. Litter-mate male Sprague-Dawley rats were used (weight 30–60 g). They were segregated into pairs of equal weight ($\pm 10\%$); one rat of each pair was given a pyridoxine-deficient diet *ad lib.* while its litter-mate was pair-fed on an identical diet with the addition of 80 μ g pyridoxine hydrochloride daily.

Pyridoxine-deficient diet. The diet used was essentially that described by Boullin (1961) except that pyridoxine hydrochloride was omitted and thiamine hydrochloride included. Additionally, deoxypyridoxine was given as a dose of 70 or 280 μ g/rat daily, in Expts. 2 and 3 respectively, to both deficient and control animals.

Skin lesions. The presence of typical skin lesions or 'rat acrodynia' (György, 1935; Birch, György & Harris, 1935), together with loss of body-weight, was used as an

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indication of the pyridoxine-deficiency state. The skin lesions were arbitrarily divided into three grades of severity: slight (+), erythema of dorsa of hind paws, spreading to the plantae, with hyperkeratosis and scaling; moderate (++), as above with the addition of swollen digits and mild lesions at mucocutaneous junctions; severe (+++), exacerbation of the aforementioned signs and development of similar lesions on ears and tail.

Intestinal 5-hydroxytryptamine. 5-Hydroxytryptamine was extracted from the mucosal layer of the entire small intestine by the method described by Boullin (1963) and portions of the extract were assayed biologically for 5-hydroxytryptamine activity on a rat stomach preparation by the method of Vane (1957).

Expt 1. In the first experiment thirty rats received the pyridoxine-deficient diet without deoxypyridoxine for 66–81 days (mean 72 days). They gained weight for the first few days, but growth then ceased. The maximal body-weight attained was significantly lower, at the 1% level, than that of the pair-fed controls, even though the total food intake was virtually identical (Table 1).

Table 1. *Effect of deoxypyridoxine on body-weight and food intake of pyridoxine-deficient and control rats*

Expt no.	Deoxy-pyridoxine intake ($\mu\text{g}/\text{day}$)	Body-weight (g)						Mean food intake (g/day) D and C
		Initial		Maximum		Final		
		D	C	D	C	D	C	
1	0	54.6	53.4	86.0*	103.5††	65.5	92.3	4.5
		SE ± 1.71	± 1.45	± 1.77	± 2.22	± 1.25	± 3.64	
2	70	57.1	56.2	75.1†	89.7	60.3	82.1	4.6
		SE ± 1.14	± 1.19	± 2.05	± 2.19	± 2.64	± 2.54	
3	280	52.0	52.7	71.0†	81.4	56.1	79.2	5.7
		SE ± 1.17	± 1.08	± 2.31	± 3.12	± 2.26	± 3.00	

D, pyridoxine-deficient; C, pair-fed litter-mate control.

* Value significantly greater at 1% level than values marked † and significantly less, at 1% level, than value marked ††.

Table 2. *Effect of deoxypyridoxine added to the diet on incidence and severity of signs of pyridoxine deficiency in rats given a pyridoxine-deficient diet*

Deoxypyridoxine intake ($\mu\text{g}/\text{day}$)	No. of animals*	Percentage of rats showing deficiency signs				
		Skin lesions†				Convulsions
		None	+	++	+++	
None	30	10	0	20	70	0
70	16	0	0	31	69	0
280	29	45	10	28	17	3

* All deficient animals duplicated by pair-fed controls.

† For grading of skin lesions see above.

The incidence of skin signs is given in Table 2. Lesions eventually appeared in 90% of the deficient group, though not before at least 1 month. As the pharmacological responses of isolated tissues were normal (Boullin, unpublished observations), and no

convulsions evident, it seemed likely that the deficiency was not particularly severe, so intestinal 5-hydroxytryptamine was not estimated.

Expt 2. For the second experiment 70 $\mu\text{g}/\text{day}$ deoxypyridoxine were included in the diets given to sixteen rats for 36–40 days (mean 38 days). Inclusion of the anti-vitamin resulted in a maximal body-weight that was significantly lower, at the 1% level, than that seen in deficient rats which were not given deoxypyridoxine. However, this difference was not due to a reduction in food intake (Table 1). Deoxypyridoxine had the expected effect of increasing the incidence and severity of skin lesions, so that all rats now showed some degree of acrodynia, though there were still no convulsions. When intestinal 5-hydroxytryptamine was measured in seven deficient and seven control rats, there was no significant difference, at the 5% level, between the values (Table 3).

Table 3. 5-Hydroxytryptamine content of mucosa of the small intestine of pyridoxine-deficient and control rats given deoxypyridoxine

Deoxypyridoxine intake ($\mu\text{g}/\text{day}$)	5-Hydroxytryptamine content ($\mu\text{g}/\text{g}$)		Deoxypyridoxine intake ($\mu\text{g}/\text{day}$)	5-Hydroxytryptamine content ($\mu\text{g}/\text{g}$)	
	D	C		D	C
70	2.04	2.92	280	2.10	2.00
	3.14	2.86		2.50	2.80
	4.24	3.81		3.28	3.76
	1.07	2.46		3.37	3.27
	6.19	6.80		2.02	4.96
	4.29	5.70		2.36	8.45
	6.04	5.38		2.00	2.43
Mean	3.86	4.28	Mean	2.52	3.95
SE	± 0.73	± 0.64	SE	± 0.23	± 0.84
P	> 0.05		P	> 0.05	

D, pyridoxine-deficient; C, pair-fed litter-mate control.

Expt 3. A third experiment was therefore carried out with twenty-nine rats given 280 $\mu\text{g}/\text{day}$ deoxypyridoxine for 26–58 days (mean 32 days). This high level of anti-vitamin resulted in a further, but slight, reduction in the maximal body-weight (Table 1). However, the average daily food intake was higher; there was certainly no evidence of anorexia in these deficient rats.

Regarding the effect of 280 $\mu\text{g}/\text{day}$ deoxypyridoxine on the incidence of skin lesions and intestinal 5-hydroxytryptamine, the results were most unexpected. Instead of an increase in acrodynia, there was in fact a decrease, nearly half the animals being free from signs, and again there was no significant depletion of intestinal 5-hydroxytryptamine in the deficient group compared with the controls (Table 3).

DISCUSSION

Although these results were disappointing inasmuch as there was no depletion of intestinal 5-hydroxytryptamine, their interest, from a nutritional standpoint, lies in this failure and the apparent lack of correlation between the several signs commonly associated with the pyridoxine-deficiency state. Young animals are known to be especially susceptible to lack of pyridoxine, and, as other workers (e.g. Beaton &

McHenry, 1953) have shown that deficiency is aggravated by deoxyripyridoxine, it was most surprising to find that the higher level of deoxyripyridoxine did not exacerbate the signs of deficiency, but rather had the reverse effect. There seems no obvious explanation of this result.

It is quite clear from this and previous work that the effects of pyridoxine deficiency on decarboxylating enzymes depend on the organ and species from which the enzymes are obtained. Blaschko, Carter, O'Brien & Stanley (1948) showed, in rats, that pyridoxine deficiency depressed liver DOPA decarboxylase, and Blaschko *et al.* (1948) and Blaschko, Datta & Harris (1953) found that L-cysteic acid decarboxylase virtually disappeared. Pogrund, Drell & Clark (1961) confirmed this result, but then found that kidney DOPA decarboxylase was but little affected. Furthermore, in pyridoxine-deficient rats bearing a transplantable hepatoma (F-Hep), normal histidine decarboxylase activity was detected in the rapidly growing malignant tissue (Boullin, MacKay, Marshall & Riley, 1961).

Considering the effects of pyridoxine deficiency on tissue 5-hydroxytryptamine levels, the first report (Weissbach, Bogdanski, Redfield & Udenfriend, 1957) was encouraging as there were big reductions in brain, liver, blood and intestinal 5-hydroxytryptamine in chickens. It has not, however, proved possible to obtain a severe depletion in the rat, possibly owing to variations in coenzyme-apoenzyme affinity relationships of the various decarboxylating enzymes between species. Thus Yeh, Solomon & Chow (1959) reported only slight reductions in rat brain, liver and spleen, and the work discussed now indicates that rat intestine is also resistant to depletion.

The results of the small number of experiments given in Table 3 indicate, however, that there was a trend towards lower values on the part of the deficient rats, though this was not statistically significant by the *t* test (Student). Analysis of variance between the deficient groups given 70 and 280 μg deoxyripyridoxine daily showed that the variance of the latter group was significantly less, at the 1% level, than that of the former. This difference appeared to be due to the inability of the deficient rats to attain values comparable to those of their litter-mate controls when the latter were high (e.g. 2.02 compared with 4.96 $\mu\text{g/g}$; 2.36 compared with 8.45 $\mu\text{g/g}$., Table 3). Consequently, though more experiments might have shown a significant depletion of intestinal 5-hydroxytryptamine, it seems improbable that the degree of depletion would have compared with that seen in chickens (Weissbach *et al.* 1957), or in rats given a tryptophan-free diet (Boullin, 1963, 1964).

It is concluded that a pyridoxine-deficient diet, even with addition of deoxyripyridoxine, did not greatly influence production of 5-hydroxytryptamine in rat intestinal mucosa.

SUMMARY

1. Male Sprague-Dawley rats were fed on a pyridoxine-deficient diet alone or with 70 or 280 μg deoxyripyridoxine daily; litter-mate controls were pair-fed on an identical diet with pyridoxine hydrochloride.
2. Of the rats given the deficient diet alone 70% showed severe acrodynia. Intestinal 5-hydroxytryptamine was not estimated.

3. When 70 μg deoxy pyridoxine/day were given there was increased incidence and severity of acrodynia, but there was no significant depletion of the 5-hydroxytryptamine in the mucosa of the small intestine.

4. When 280 μg deoxy pyridoxine were given daily, almost 50% of the animals were free of acrodynia and again there was no significant depletion of intestinal 5-hydroxytryptamine.

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