

## Vitamin B<sub>6</sub> Levels in Rat Tissues

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The level of total vitamin B<sub>6</sub> in the livers of deficient and control rats with and without the administration of the antivitamin, deoxypyridoxin, has been described in previous reports from this laboratory (Beaton, Smith & McHenry, 1953; Sheppard & McHenry, 1946). The level of the vitamin in other tissues of normal rats has already been reported by others (Williams, Eakin & McMahan, 1941). Stoerk (1949-50) has suggested that the administration of deoxypyridoxin prevents reduction in the vitamin B<sub>6</sub> of the livers of deprived animals; details of his experimental procedures were not given.

In this paper, observations are reported on the administration of deoxypyridoxin to control and vitamin B<sub>6</sub>-deprived rats, on the relation of the vitamin B<sub>6</sub> levels in three organs to the absence or presence of dietary pyridoxin, on time studies of the developing vitamin B<sub>6</sub> deficiency in the rat, and on the comparison of body-weight increases with the level of vitamin B<sub>6</sub> in the liver.

### EXPERIMENTAL AND RESULTS

#### *Methods*

Wistar strain albino rats were employed and were housed in individual, screen-bottomed cages. The 20% casein, 20% maize oil, vitamin B<sub>6</sub>-free basal diet described previously (Beaton, Beare, White & McHenry, 1953) was provided throughout these studies. Water was provided *ad lib*. In some experiments, as indicated, deoxypyridoxin hydrochloride was provided to each animal at a level of 100 µg/day in the food; each control animal was provided with 50 µg/day of pyridoxin hydrochloride in the food.

At the conclusion of the experimental periods, the animals were fasted for 18-24 h as indicated and anaesthetized by the intraperitoneal injection of 2% butylone in saline. Organs were removed, weighed, pooled for groups and homogenized in a Waring blender or, for small samples, in the blender described by Campbell & Davidson (1949). Carcasses were pooled for groups, quick-frozen in liquid air and passed through a power grinder. For each pooled tissue triplicate samples were prepared by acid hydrolysis for the determination of vitamin B<sub>6</sub> by the method of Atkin, Schultz, Williams & Frey (1943). In later experiments, as indicated, the hydrochloric-acid hydrolysis procedure described by Rabinowitz & Snell (1947) was employed in place of the sulphuric-acid hydrolysis procedure described by Atkin *et al.* (1943); it was found that both procedures were equally satisfactory for recovery of added vitamin B<sub>6</sub> compounds, but the hydrochloric acid seemed to give higher values for tissue vitamin B<sub>6</sub> than did the sulphuric acid. It was also found that the addition of

deoxypyridoxin hydrochloride to liver-tissue samples before hydrolysis did not affect the growth of *Saccharomyces carlsbergensis*. All results are expressed as vitamin B<sub>6</sub> equivalent to pyridoxin (free base). The figures represent the means of nine determinations on each of triplicate samples of each pooled tissue. Concentrations are expressed in terms of the wet tissue weight.

#### *Administration of deoxypyridoxin*

Thirty-five male rats were divided into four groups similar in number and initial average body-weight of 108 g. All groups were deprived of dietary vitamin B<sub>6</sub> for a period of 16 days. During the next 12 days, groups B and D received a supplement

Table 1. *The effect of deoxypyridoxin on the rat's hepatic vitamin B<sub>6</sub>. Mean values for groups of nine rats*

Group	Treatment*	Liver weight (g)	Vitamin B <sub>6</sub>	
			Concentration (μg/g)	Content (μg)
A	-B <sub>6</sub> +DB <sub>6</sub> †	4.8	6.3	30
B	+B <sub>6</sub> +DB <sub>6</sub> †	5.1	9.6	48
C	-B <sub>6</sub>	5.1	6.3	32
D	+B <sub>6</sub>	4.8	11.0	52

\* See text, this page.

† DB<sub>6</sub> signifies the administration of deoxypyridoxin.

of pyridoxin hydrochloride. Groups A and B received deoxypyridoxin hydrochloride throughout the experiment. All groups were pair-fed with group A, having an average daily food consumption of 9.0 g/rat during the first 16 days, and 5.4 g/rat during the remaining 12 days. Acrodynia was well developed in groups A and B by the 16th day of experimental feeding and was relieved in group B after 5 days of pyridoxin-hydrochloride administration; acrodynia was not evident at any time in groups C or D, which did not receive deoxypyridoxin hydrochloride. After a fast of 18 h, the animals were killed on the 28th day of the experiment. Livers were removed, pooled by groups, homogenized and hydrolysed with hydrochloric acid. The analytical results are reported in Table 1; body-weight changes are shown in Fig. 1.

#### *Vitamin B<sub>6</sub> levels during development of deficiencies*

*Without the administration of deoxypyridoxin hydrochloride.* Eighty-six rats were divided into nine groups similar in number, sex distribution and initial average body-weight of 106 g. Four groups were deprived of all dietary vitamin B<sub>6</sub>; another four groups were given a supplement of pyridoxin hydrochloride and pair-fed with their corresponding deprived groups. The remaining group was fasted for 24 h and killed as an initial base-line control; thereafter, one control and one deprived group were killed, after a fast of 24 h, at the end of each of 1, 2, 4 and 6 weeks of experimental feeding. Liver and carcass samples were hydrolysed with hydrochloric acid before microbiological assay. Acrodynia was apparent in only two of ten rats after 6 weeks of vitamin restriction. Experimental results are reported in Table 2.

With the administration of deoxypyridoxin hydrochloride. Fifty rats were divided into five groups equal in number, sex distribution and initial average body-weight of 115 g. One group was fasted for 17 h and killed as an initial base-line control. Deoxypyridoxin hydrochloride was added to the basal diet of all groups, two of which were also provided with pyridoxin hydrochloride and pair-fed with their corresponding deprived

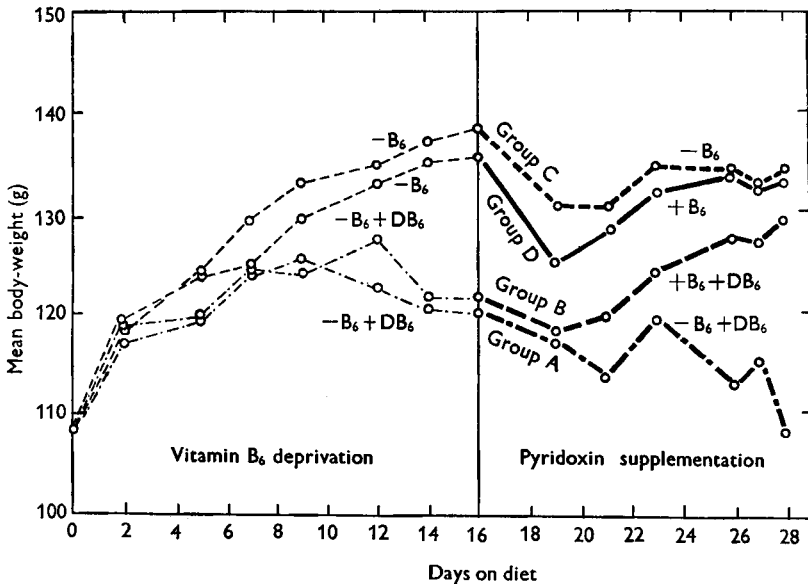


Fig. 1. The effect of deoxypyridoxin administration and resupplementation with pyridoxin on the mean body-weight of groups of nine vitamin B<sub>6</sub>-deprived rats (see p. 358).

groups. One control and one deprived group were killed, after a 17 h fast, at the end of each of 1 and 3 weeks of experimental feeding. In this experiment, liver- and carcass-tissue samples were hydrolysed with sulphuric acid before microbiological assay. Acrodynia was evident in the deprived animals within 2 weeks of experimental feeding. The experimental results of this study are reported in Table 2.

#### *Vitamin B<sub>6</sub> levels in heart, kidney and liver tissues of control and deprived animals*

Thirty rats were divided into three groups equal in number, sex distribution and initial average body-weight of 112 g. All rats were provided with the basal diet and deoxypyridoxin hydrochloride; two groups were further provided with pyridoxin hydrochloride, one control group being pair-fed with the deprived group, the other being fed *ad lib*. Acrodynia was evident in the deprived group by the 18th day of experimental feeding. On the 21st day, the animals were killed after an 18 h fast and the livers, kidneys and hearts removed, pooled separately by groups and analysed after hydrolysis with hydrochloric acid. The experimental results are shown in Table 3.

Table 2. Alterations in mean body and liver weights and tissue levels of vitamin B<sub>6</sub> in developing vitamin B<sub>6</sub> deficiency with and without deoxypyridoxin administration in groups of nine or ten rats

Group*	Experi- mental period (weeks)	Daily food consumption (g/rat)	Body- weight gain (g)	Liver weight (g)	Concentration			Content		
					Carcass (µg/g)	Liver (µg/g)	Liver (µg)	Carcass (µg)	Liver (µg)	Ratio, carcass : liver
Initial	0	—	—	—	Without deoxypyridoxin					
-B <sub>6</sub>	1	12.1	18	5.5	2.7	8.1	44	260	44	5.9
+B <sub>6</sub>	1	12.1	26	6.2	1.8	5.0	31	190	31	6.3
-B <sub>6</sub>	2	13.0	21	6.6	2.6	5.9	37	290	37	7.9
+B <sub>6</sub>	2	13.0	52	8.1	1.6	4.2	28	180	28	6.5
-B <sub>6</sub>	4	10.5	38	6.8	2.6	5.9	47	370	47	7.9
+B <sub>6</sub>	4	10.5	85	8.1	1.3	3.7	25	150	25	6.0
-B <sub>6</sub>	6	10.1	38	7.1	2.5	6.5	52	430	52	8.3
+B <sub>6</sub>	6	10.1	99	8.3	1.3	3.8	26	160	26	6.2
					2.8	7.0	58	530	58	9.2
Initial	0	—	—	—	With deoxypyridoxin					
-B <sub>6</sub> + DB <sub>6</sub> †	1	13.1	11	5.8	2.5	9.1	53	270	53	5.2
+B <sub>6</sub> + DB <sub>6</sub>	1	13.1	32	6.4	2.0	5.8	37	240	37	6.4
-B <sub>6</sub> + DB <sub>6</sub>	3	10.5	13	7.6	2.5	6.3	53	350	53	6.5
+B <sub>6</sub> + DB <sub>6</sub>	3	10.5	55	9.0	1.6	4.3	33	190	33	6.0
					2.7	6.7	60	430	60	7.3

\* For description of treatment see p. 358.

† DB<sub>6</sub> signifies the administration of deoxypyridoxin.

Table 3. Effect of vitamin B<sub>6</sub> nutrition on the levels of vitamin B<sub>6</sub> in three organs of the rat. Mean values for groups of ten rats

Group*	Body- weight gain (g)	Organ weight			Concentration			Content		
		Liver (g)	Kidneys (g)	Heart (g)	Liver (µg/g)	Kidneys (µg/g)	Heart (µg/g)	Liver (µg)	Kidneys (µg)	Heart (µg)
-B <sub>6</sub>	3	5.7	1.3	0.53	5.3	3.4	3.5	30	4.6	1.9
+B <sub>6</sub> , pair-fed	38	5.7	1.2	0.61	9.3	6.9	4.7	53	8.4	2.9
+B <sub>6</sub> , fed <i>ad lib.</i>	73	8.3	1.7	0.89	7.7	6.6	4.6	64	11.0	4.1

\* For description of treatment see p. 359.

*Pyridoxin hydrochloride administration, liver vitamin B<sub>6</sub> and average body-weight gains*

Seventy-two male rats were divided into six groups equal in number and initial average body-weight of 124 g. All groups were fed the basal diet *ad lib*. A solution of pyridoxin hydrochloride, 3 mg/100 ml. physiological saline, was administered by single daily subcutaneous injections to five groups of rats. The amounts injected were

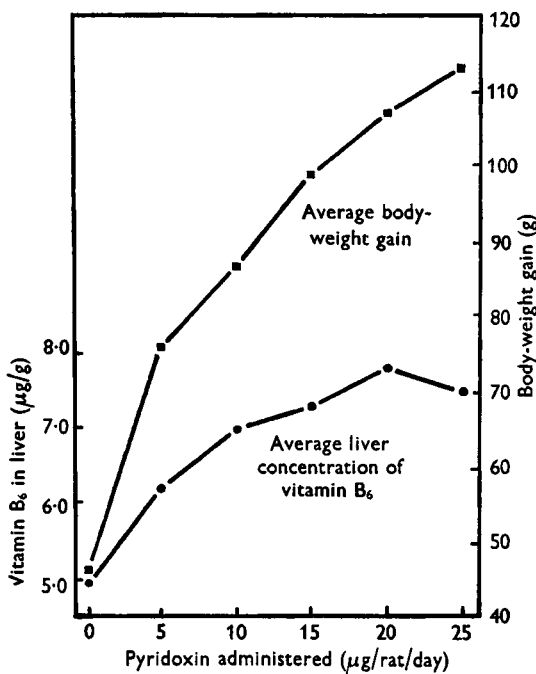


Fig. 2. Comparison of mean body-weight gains and concentration of vitamin B<sub>6</sub> in the livers of groups of twelve rats maintained on a vitamin B<sub>6</sub>-free basal diet and given single daily subcutaneous injections of pyridoxin.

such as to supply to all of the rats in each of five groups 5, 10, 15, 20 or 25  $\mu\text{g}$  pyridoxin (free base) respectively per rat per day for 21 days. The rats in the sixth group, receiving no pyridoxin, were injected with saline. All rats were fasted on the 21st day of the experiment for 18 h and killed and the livers were removed. After hydrochloric-acid hydrolysis, vitamin B<sub>6</sub> determinations were carried out on the separately pooled liver samples. Average body-weight changes and concentrations of vitamin B<sub>6</sub> in the livers of the various groups are shown in Fig. 2.

## DISCUSSION

Deoxypyridoxin administration had no effect on the recovery of rats from an acute vitamin B<sub>6</sub> deficiency, as evidenced by body-weight gains and the alleviation of acrodynia. Administration of the antivitamin did not significantly alter the concentration or content of vitamin B<sub>6</sub> in the livers of deprived or control groups.

Umbreit & Waddell (1949) have suggested that deoxypyridoxin acts by replacing vitamin B<sub>6</sub> in certain enzyme systems. Stoerk (1950) has indicated that deoxypyridoxin administration causes an elevation of the vitamin B<sub>6</sub> concentration in the livers of deprived rats, which could be due to this blocking action. However, such a hypothesis is not supported by the results reported here.

During a simple deprivation of dietary vitamin B<sub>6</sub> without the use of deoxypyridoxin, the vitamin concentration decreased at a relatively steady rate for the first 4 weeks and then appeared to become steady at a minimal level in both liver and extrahepatic tissues. No acrodynia developed in these animals until 2 weeks after the minimal level of vitamin concentration had been attained in the tissue. The changes in vitamin B<sub>6</sub> concentration were similar whether the antivitamin was administered or not, but when deoxypyridoxin was present the deficiency signs appeared soon after a low level of tissue vitamin B<sub>6</sub> had been attained. When the antivitamin was not supplied, there was an appreciable delay in the appearance of acrodynia, even though a similar minimal concentration of vitamin B<sub>6</sub> was attained after approximately the same period of deprivation. Umbreit & Waddell (1949) have suggested that deoxypyridoxin can act only in the presence of an insufficiency of vitamin B<sub>6</sub>, which may explain these findings.

It will be noted that the ratio of the carcass content of vitamin B<sub>6</sub> to that of the liver was remarkably constant in deprived rats, whereas it increased in control rats. This finding suggests that the vitamin is stored to a large extent in extrahepatic tissue and can be mobilized to the liver during vitamin restriction. Though the liver probably contains more vitamin B<sub>6</sub> than any other specific tissue, it should be noted that even the deficient animal contained six times as much vitamin B<sub>6</sub> in the extrahepatic tissues as was present in the liver.

A study of the vitamin B<sub>6</sub> levels in carcass, heart, kidney and liver tissues suggested that the kidney loses the vitamin during deprivation to a greater extent than do the other two organs. However, the total amount of the vitamin in the kidney or in the heart is much less than the amount in the liver. It is likely that the liver-tissue concentration of vitamin B<sub>6</sub> gives a good indication of the status of vitamin B<sub>6</sub> nutriture. This was further validated by the results of the time studies, in which it was observed in vitamin B<sub>6</sub>-deprived rats that decreases in the level of the vitamin in the liver paralleled those in the extrahepatic tissues.

When six dosage levels of pyridoxin were administered to rats by subcutaneous injections, the concentration of the vitamin in the liver showed a fairly constant level at an intake between 15 and 25  $\mu\text{g}$  pyridoxin/rat/day, while the average body-weight gains showed no sign of becoming steady at an intake of even 25  $\mu\text{g}$  pyridoxin/rat/day. It would appear that saturation of liver tissue with vitamin B<sub>6</sub> occurs at a lower level of pyridoxin administration than that required for maximal body-weight increase in the rat. Since the time studies showed a parallelism between concentrations of the vitamin in extrahepatic and liver tissues, it is probable that the extrahepatic tissues were saturated at this same level of pyridoxin administration. This finding is in contradiction to the popular belief that the intake of a vitamin sufficient to saturate the tissues is likely to provide maximal physiological effects.

## SUMMARY

1. The administration of deoxypyridoxin hydrochloride did not significantly alter the concentration or content of vitamin B<sub>6</sub> in the livers of vitamin B<sub>6</sub>-deprived or control rats, nor did it impair the recovery of rats from an acute vitamin B<sub>6</sub> deficiency.
2. The antivitamin did hasten the onset of deficiency signs after a low level of the vitamin in the tissues had been attained.
3. Evidence presented suggests that mobilization of vitamin B<sub>6</sub> from extrahepatic tissue to the liver can occur during deprivation of dietary vitamin B<sub>6</sub>.
4. The liver was found to be more satisfactory than heart or kidney tissue for assessing the effects of vitamin B<sub>6</sub> nutrition on the storage of the vitamin in the rat.
5. Saturation of liver tissue with vitamin B<sub>6</sub> occurred with a lower level of pyridoxin intake than was sufficient to give maximal increase in body-weight.

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