The Effect of Vitamin B₁₂ and Biotin on the Metabolism of Vitamin B₁₂ in Biotin-Deficient Rats

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1. The effect of the administration of vitamin B₁₂ and biotin on the metabolic pattern of vitamin B₁₂ in biotin-deficient rats was studied. 2. No significant changes in the absorption and excretion of orally administered [⁵⁸Co]vitamin B₁₂ were noted either in vitamin B₁₂-treated and or in biotin-fed rats. A significant decrease of the uptake of orally given [⁵⁸Co]vitamin B₁₂ was observed in the liver and kidneys of biotin-treated rats, whereas an increase of uptake in the kidneys of vitamin B₁₂-treated rats was noted as compared with biotin-deficient animals. 3. No significant difference in the excretion of radioactivity was noted between biotin deficient and biotin-fed rats when [⁵⁸Co]vitamin B₁₂ was administered by injection. A small decrease was observed in vitamin B₁₂-treated rats. The retention of injected [⁵⁸Co]vitamin B₁₂ by major organs of biotin-treated rats was lower than that of biotin-deficient rats. A lower content of [⁵⁸Co]vitamin B₁₂ was also detected in the organs, with the exception of the kidneys, of vitamin B₁₂-treated rats. 4. These results are discussed in terms of an interrelationship between biotin and vitamin B₁₂.

Interrelationships between biotin and vitamin B₁₂ have been observed by Marchetti & Testoni (1964). The administration of vitamin B₁₂ to biotin-deficient rats exerts a protective effect against biotin deficiency that results both in better growth and in a delay of deficiency symptoms. Moreover, vitamin B₁₂ supplementation may influence biotin-dependent enzymic activities, e.g. liver ornithine-carbamoyltransferase and aspartate-carbamoyltransferase activities.

In the present paper the effect of the administration of vitamin B₁₂ on the absorption, excretion, retention and distribution among organs of [⁵⁸Co]-vitamin B₁₂ in biotin-deficient rats is reported. The results of comparative studies on the effect of supplementation with biotin are also given; previous studies have shown that biotin deficiency can induce changes in the metabolic pattern of vitamin B₁₂ in the rat (Puddu & Marchetti, 1964).

MATERIALS AND METHODS

Treatment of animals. Weanling male rats of Wistar strain were divided into three groups. The first received a biotin-free diet (Marchetti, Pasquali & Landi, 1965) of the following composition: vitamin-free casein, 20%; sucrose, 59%; salt mixture IV, 5%; groundnut oil, 5%; raw dried egg white, 11%; vitamin supplements, 1%. The second was given the same diet, but the raw dried egg white was replaced by egg white autoclaved at 120° for 15 min. and dried, and biotin

(100 µg./kg.) was added. The third group received the biotin-free diet, supplemented with 100 µg. of vitamin B₁₂/kg. of diet.

All animals were given water *ad libitum*. The first group developed severe biotin deficiency after 60 days.

Determination of vitamin B₁₂ in liver. For the determination of vitamin B₁₂ in liver, six rats of each group were killed and the livers were removed immediately, homogenized and incubated with papain at 60° for 1 hr. The total vitamin B₁₂ activity and the alkali-stable vitamin B₁₂ activity were estimated by the U.S. Pharmacopeia (1955) method, with *Lactobacillus leichmannii* A.T.C.C. 7830 as the test organism and 'B₁₂ assay medium Difco' as the assay medium.

Measurement of [⁵⁸Co]vitamin B₁₂. [⁵⁸Co]Vitamin B₁₂ (obtained from The Radiochemical Centre, Amersham, Bucks.) with a specific activity of 1 µC/µg. was either injected subcutaneously in 0.9% NaCl solution or administered in aqueous solution by stomach tube. The amount of radioactivity used was 0.2 µC (representing only 200 mµg. of vitamin B₁₂). After administration, the rats were placed individually in metabolism cages, and urine and faeces were collected separately for 4 days. The animals were then killed and the liver, kidneys, gastrointestinal tract (from the cardia to the end of the colon), heart and spleen were saved for radioactivity measurements. The tissues were dissolved in alkali, whereas the faeces were softened with 50% (v/v) H₂SO₄. For all samples 4 ml. was counted, with standards of comparable geometry in a low-background γ-scintillation counter with a thallium-activated sodium iodide crystal installed in a well-type sample holder (Tracerlab model SC57/A).

Table 1. *Effect of the administration of biotin and vitamin B₁₂ on the vitamin B₁₂ content of the liver of the biotin-deficient rat*

Experimental details are given in the text. The results are expressed as means $\pm$ S.E.M., with the numbers of animals in parentheses.

Group	Treatment	Wt. gain in 60 days (g.)	Liver vitamin B ₁₂ (μ g./g. of tissue)
1	Biotin-deficient	86 $\pm$ 9.7 (18)	81.1 $\pm$ 6.5 (6)
2	Biotin-treated	182 $\pm$ 6.1 (18)	47.9 $\pm$ 6.2 (6)
3	Biotin-deficient vitamin B ₁₂ -treated	157 $\pm$ 9.1 (18)	166.9 $\pm$ 8.2 (6)

Table 2. *Effect of the administration of biotin and vitamin B₁₂ on the absorption of orally fed [⁵⁸Co]vitamin B₁₂ in the rat*

Experimental details are given in the text. The absorption was calculated by subtracting faecal radioactivity from the oral dose (200 μ g.). The results are expressed as means $\pm$ S.E.M. of six determinations on different animals.

Group	Treatment	Radioactivity (as μ g. of vitamin B ₁₂)		Absorption (as μ g. of vitamin B ₁₂)
		Urine	Faeces	
1	Biotin-deficient	2.1 $\pm$ 0.1	121.4 $\pm$ 4.5	78.6 $\pm$ 2.9
2	Biotin-treated	3.0 $\pm$ 0.5	117.3 $\pm$ 7.7	82.7 $\pm$ 5.3
3	Biotin-deficient vitamin B ₁₂ -treated	3.9 $\pm$ 0.1	132.4 $\pm$ 17.0	67.6 $\pm$ 8.7

Significance of results. The results were analysed statistically by Fisher's *t* test, a difference between two mean values being regarded as significant when *P* was no greater than 0.05.

RESULTS

Liver biological activity of vitamin B₁₂. The results in Table 1 show a markedly higher weight gain in the vitamin B₁₂-treated rats (group 3) than that in the biotin-deficient rats (group 1) (*P* < 0.001), although slightly lower than that in the animals given biotin (group 2). Moreover, the vitamin B₁₂ content of the liver of rats receiving a biotin-free diet is higher (*P* < 0.05) than that of biotin-treated rats, but lower than that of vitamin B₁₂-treated rats (*P* < 0.001).

Absorption and organ uptake of orally administered [⁵⁸Co]vitamin B₁₂. Table 2 shows that no significant variation in the absorption of orally administered [⁵⁸Co]vitamin B₁₂ was observed between biotin-deficient rats and those receiving biotin. The absorption by biotin-deficient vitamin B₁₂-supplemented rats was not significantly lower than that of biotin-deficient animals.

The uptake of orally given [⁵⁸Co]vitamin B₁₂ by various organs is shown in Table 3. No difference in the organ uptake was observed between biotin-deficient and biotin-supplemented rats if the results were expressed on the basis of the weight of the whole organ. On the other hand, if the results were expressed per unit weight of organ, a significant

decrease of vitamin B₁₂ uptake was observed in the liver (*P* < 0.02) and kidneys (*P* < 0.02) of biotin-treated rats as compared with biotin-deficient rats.

In rats given the biotin-deficient vitamin B₁₂-supplemented diet the [⁵⁸Co]vitamin B₁₂ content of the kidneys showed an increase as compared with biotin-deficient rats, whether expressed per unit weight of the organ or on the basis of the weight of the whole organ (*P* < 0.02).

In contrast with the kidneys, radioactivity taken up by the spleen and the heart was less in vitamin B₁₂-supplemented rats (*P* < 0.01 and *P* < 0.05 respectively), whereas no difference between the two groups was apparent in the liver and gastrointestinal tract if the results were expressed either per unit weight of the organ or per whole organ.

Excretion and retention of injected [⁵⁸Co]vitamin B₁₂. No significant differences were observed in radioactivity in the urine and faeces between biotin-deficient and biotin-treated rats. A small decrease of [⁵⁸Co]vitamin B₁₂ excretion was noted in rats receiving the biotin-deficient diet supplemented with vitamin B₁₂ as compared with biotin-deficient rats (*P* < 0.01) (Table 4). The retention of [⁵⁸Co]-vitamin B₁₂ by the organs (liver, *P* < 0.001; kidneys, *P* < 0.001; gastrointestinal tract, *P* < 0.01; spleen, *P* < 0.001; heart, *P* < 0.02) of biotin-treated rats, whether expressed per unit weight of the organ or per whole organ, was lower than that of biotin-deficient animals. The liver (*P* < 0.001), gastro-

Table 3. *Effect of the administration of biotin and vitamin B₁₂ on the organ distribution of [⁵⁸Co]vitamin B₁₂, administered either orally or subcutaneously, in the biotin-deficient rat*

Experimental details are given in the text. The results are expressed as means $\pm$ S.E.M. of six determinations on different animals.

Tissue	Route of administration	Organ uptake of radioactivity (as m μ g. of vitamin B ₁₂)			
		Biotin-deficient rat		Biotin-treated rat	
		Total	Per 10g.	Total	Per 10g.
Liver	Oral	9.4 $\pm$ 1.2	16.9 $\pm$ 2.7	8.7 $\pm$ 0.9	9.4 $\pm$ 0.9
	Injected	20.3 $\pm$ 1.1	29.7 $\pm$ 1.5	13.1 $\pm$ 0.7	13.1 $\pm$ 0.9
Kidneys	Oral	12.0 $\pm$ 1.6	69.0 $\pm$ 9.2	9.2 $\pm$ 1.0	39.8 $\pm$ 4.0
	Injected	30.7 $\pm$ 1.6	184.6 $\pm$ 11.4	20.1 $\pm$ 1.9	78.3 $\pm$ 6.6
Gastrointestinal tract	Oral	13.4 $\pm$ 1.4	18.0 $\pm$ 1.7	11.6 $\pm$ 1.2	12.6 $\pm$ 2.0
	Injected	24.5 $\pm$ 1.0	33.9 $\pm$ 2.4	17.0 $\pm$ 1.8	21.6 $\pm$ 2.6
Spleen	Oral	0.76 $\pm$ 0.05	13.2 $\pm$ 1.1	1.2 $\pm$ 0.1	12.2 $\pm$ 0.9
	Injected	1.9 $\pm$ 0.2	29.6 $\pm$ 1.3	2.1 $\pm$ 0.05	19.2 $\pm$ 0.1
Heart	Oral	0.62 $\pm$ 0.06	12.7 $\pm$ 2.2	0.75 $\pm$ 0.05	8.6 $\pm$ 0.5
	Injected	1.1 $\pm$ 0.01	20.6 $\pm$ 0.6	1.3 $\pm$ 0.04	13.8 $\pm$ 0.4
		Biotin-deficient vitamin B ₁₂ -treated rat		Total	
				5.9 $\pm$ 1.2	12.3 $\pm$ 1.6
				8.0 $\pm$ 0.8	12.8 $\pm$ 3.3
				17.1 $\pm$ 1.3	101.8 $\pm$ 23.4
				86.1 $\pm$ 3.7	524.5 $\pm$ 51.2
				9.9 $\pm$ 2.2	15.4 $\pm$ 3.8
				12.1 $\pm$ 0.5	21.0 $\pm$ 0.9
				0.47 $\pm$ 0.15	6.0 $\pm$ 1.9
				0.58 $\pm$ 0.01	11.6 $\pm$ 1.5
				0.36 $\pm$ 0.1	5.8 $\pm$ 1.3
				0.61 $\pm$ 0.01	11.2 $\pm$ 0.4

intestinal tract ($P < 0.001$), spleen ($P < 0.01$) and heart ($P < 0.01$) of biotin-deficient vitamin B₁₂-treated rats retained less radioactivity as compared with biotin-deficient rats, whereas the kidneys accumulated more [⁵⁸Co]vitamin B₁₂ (Table 3), whether the results were expressed either per unit weight of the organ or per whole organ ($P < 0.001$).

DISCUSSION

The administration either of vitamin B₁₂ or of biotin to biotin-deficient rats results in significant changes in the metabolism of [⁵⁸Co]vitamin B₁₂, administered either orally or subcutaneously. These results could be interpreted as demonstrating further interrelationships between biotin and vitamin B₁₂.

Biotin-deficient vitamin B₁₂-treated rats retained less [⁵⁸Co]vitamin B₁₂ in some organs studied, but the renal uptake was greater than that in biotin-deficient rats. The decreased uptake of radioactivity by the organs of vitamin B₁₂-treated rats may be due in part to isotope dilution and to flushing. On the other hand, the increased retention of [⁵⁸Co]vitamin B₁₂ by the kidney may be related to the role played by this organ in the storage and turnover of vitamin B₁₂, as postulated by Harte, Chow & Barrows (1953). In fact, it has been demonstrated (Okuda, 1962) that, unlike other organs, the kidney has a large capacity for storing vitamin B₁₂ absorbed in excess of the immediate need and that it releases it later. Such accumulation and release do not occur in rats that have not received vitamin B₁₂ in sufficient amounts. The changes in the metabolic pattern of vitamin B₁₂ observed in biotin-deficient rats as compared with those supplemented with vitamin B₁₂ are similar to the changes observed in rats maintained on vitamin B₁₂-free diets as compared with rats that have been supplied with the vitamin (Sauberlich, 1959; Okuda, 1962).

Since it has been demonstrated that the biotin-deficient diet cannot be considered a diet deficient both in vitamin B₁₂ and biotin (Marchetti & Testoni, 1964), then, as a result of the experiments presented above, it seems that biotin deficiency may also induce a conditioned deficiency of vitamin B₁₂, e.g. an impaired utilization of this vitamin. Other studies on the relationships between biotin and vitamin B₁₂ (Marchetti & Testoni, 1964; Puddu & Marchetti, 1964) agree with this hypothesis. In fact, it was found that the biotin-deficient rats have a high microbiological activity of vitamin B₁₂ in the liver and yet require more vitamin B₁₂; this is suggested by the favourable effect on the growth rate and deficiency symptoms exerted by the administration of a large quantity of vitamin B₁₂ and also by the higher retention of [⁵⁸Co]vitamin B₁₂ in the storage organs of biotin-deficient rats.

Table 4. *Effect of the administration of biotin and vitamin B₁₂ on the excretion of injected [⁵⁸Co]vitamin B₁₂ in the biotin-deficient rat*

Experimental details are given in the text. The results are expressed as the means $\pm$ s.e.m. of six determinations on different animals.

Group	Treatment	Radioactivity (as m μ g. of vitamin B ₁₂)		Total excretion in 4 days (as m μ g. of vitamin B ₁₂)
		In urine	In faeces	
1	Biotin-deficient	21.2 $\pm$ 0.6	19.5 $\pm$ 1.0	40.6 $\pm$ 1.5
2	Biotin-treated	17.2 $\pm$ 1.8	16.4 $\pm$ 0.5	33.6 $\pm$ 2.2
3	Biotin-deficient vitamin B ₁₂ -treated	16.2 $\pm$ 1.4	9.0 $\pm$ 1.2	25.2 $\pm$ 2.6

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