STUDIES ON THE METABOLIC INTERRELATIONSHIP BETWEEN BIOTIN AND FOLIC ACID IN RATS

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A significant influence of dietary biotin has been observed in enhancing the blood, liver and fecal levels of unconjugated reduced folate cofactors in folate deficient rats. Total folate levels were, however, not influenced by dietary biotin. The observed decrease of liver folylconjugate synthetase activity in the presence of dietary biotin may plausibly explain the increased tissue levels of unconjugated folates in biotin fed rats.

Various reports (1, 2) on the metabolic interrelationship between biotin and folic acid have confirmed the observation of LUCKEY *et al.* (3), who originally suggested that biotin might influence tissue folate synthesis. Since the earlier suggestion (4) that biotin might influence folates synthesized by intestinal microflora, we have recently demonstrated that in *Escherichia coli*, biotin caused a redistribution of intracellular folate derivatives favouring accumulation of unconjugated methyltetrahydrofolates at the expense of conjugated formyltetrahydrofolates (5). We now present similar conclusions with the whole rat, measuring tissue and excretion levels of simple and conjugated folates under conditions of minimal alteration or loss (6).

Weanling male Wistar strain rats of average initial weight of 50-60 g were housed individually in raised bottom cages. They were divided into the following dietary groups:

- I Diet supplemented with folic acid and biotin
- II Diet deficient in folic acid alone
- III Diet deficient in biotin alone
- IV Diet deficient in biotin and folic acid

The composition of the basal diet was similar to that reported earlier by NORONHA *et al.* (4). Where present, biotin and folic acid were added at 1.0 mg and 3.0 mg, respectively, per kg diet. The animals were fed *ad libitum* for nine weeks and their increase in weight was recorded throughout the experiment.

Prior to sacrifice, urine and feces collections were made in metabolic cages for a 24 hr period in ascorbate (final concentration 1%). These were extracted with 1% ascorbate (stored frozen as a 10% solution adjusted to pH 6.0 with potassium hydroxide) in a boiling water bath for 20 min, centrifuged and the clear supernatant was assayed for folate activity. The liver was excised, frozen in liquid nitrogen and kept at -20°C until used; weighed aliquots were homogenized individually in hot 1% ascorbate, extracted at 100°C for 20 min, spun (at $5,000 \times g$ for 10 min) and the supernatant used for folate assay. To the freshly collected blood from the portal vein was added an equal volume of 1% ascorbate followed by extraction in a boiling water bath for 20 min. The blood-ascorbate mixture, after extraction, was spun and the supernatant made up to the original blood volume with 1% ascorbate and assayed for folate activity.

Folate activity was assayed microbiologically with *Lactobacillus casei* and *Pediococcus cerevisiae* using DL-leucovorin Ca-salt (Lederle) as the standard, with corrections made for the inactive isomer. Samples were assayed before and after digestion with a chicken liver conjugase preparation according to the method of NORONHA and ABOOBAKER (7).

The assay system for measuring liver folylconjugate synthetase was essentially as described by GRIFFIN and BROWN (8) and NAIR and NORONHA (5): 0.1 ml of $10,000 \times g$ (30 min) supernatant from a 1:4 (w/v) homogenate of excised liver in 50 mM phosphate buffer (pH 7.3) was the enzyme source (4.0 mg protein) in a total reaction volume of 2.0 ml. The decrease in *L. casei* assayable free folic acid activity was considered a measure of folylconjugate synthetase activity. Digestion of the reaction mixture with folyl conjugase (7) gave total recovery of the substrate tetrahydrofolic acid.

Table 1. Effect of dietary biotin

			Liver		
Dietary Groups	Folate intake m μ g/rat/day	<i>L. casei</i> activity			
		Conjugase digestion ¹		Conjugated	
		before	after	unconjugated	
		m μ g/g			
I	+Biotin	49,474	3,836	6,452	0.97
	+Folate	$\pm$ 683	$\pm$ 462	$\pm$ 725	$\pm$ 0.38
II	+Biotin	142	632	1,248	1.22
	−Folate	$\pm$ 27	$\pm$ 208	$\pm$ 241	$\pm$ 0.47
III	−Biotin	42,182	4,242	7,777	1.33
	+Folate	$\pm$ 780	$\pm$ 580	$\pm$ 910	$\pm$ 0.65
IV	−Biotin	118	374	1,114	2.52
	−Folate	$\pm$ 21	$\pm$ 33	$\pm$ 108	$\pm$ 0.58

Each value represents the average of sixteen individual observations.

¹ Conjugase digestion converts conjugated derivatives into assayable monoglutamyl forms

² *L. casei* responds to all known natural folate compounds after conjugase digestion while

The average increase in weight of the animals over a period of nine weeks was in decreasing order: Group I—control (250 g) > Group II—folate deficient (240 g) > Group III—biotin deficient (210 g) > Group IV—biotin and folate deficient (138 g).

In folate deficiency there was a marked reduction in the levels of simple (unconjugated) as well as total (unconjugated+conjugated) folates in liver, indicating a significant lowering of the tissue folate stores (Table 1). A superimposed deficiency of biotin (Group IV) resulted in further reductions in the levels of simple folates although there was no reduction in total folates. The ratio of non-methyltetrahydrofolates to methyltetrahydrofolates was also found to be significantly reduced in the presence of dietary biotin during folate deficiency. The observed higher fecal folate excretion compared with the folate intake in the folate deficient groups was due to the synthesis of folates by intestinal microflora at a site from which they were presumably not available for absorption. The lowered ratio of conjugated to unconjugated folates in the fecal excretion of the biotin supplemented group parallels our earlier findings on the role of biotin in effecting a similar redistribution of intracellular folates in the coliform *Escherichia coli* (5).

The observed effect of biotin in effecting a shift of the liver and fecal folate profiles toward simpler methyltetrahydrofolate derivatives is most pronounced in folate deficient animals. In a dietary folate sufficiency (Groups I and III), this effect is not clearly observed and was probably masked by the high folate levels *per se*.

In untabulated observations, folate deficiency reduced the average total blood

on rat liver and fecal folate levels.

folates			Fecal folates			
<i>P. cerevisiae</i> activity			<i>L. casei</i> activity			
Conjugase digestion		Non-methyl THFA ^a	Conjugase digestion		Conjugated	
before	after		before	after	conjugated	unconjugated
m μ g/g		methyl THFA	m μ g excreted/24 hr			
3,237	3,558	1.47	3,090	4,438	0.58	
$\pm$ 423	$\pm$ 319	$\pm$ 0.28	$\pm$ 209	$\pm$ 684	$\pm$ 0.16	
536	1,056	5.87	150	1,285	7.68	
$\pm$ 252	$\pm$ 273	$\pm$ 0.73	$\pm$ 13	$\pm$ 203	$\pm$ 0.49	
3,214	4,073	1.29	2,860	5,264	0.97	
$\pm$ 707	$\pm$ 470	$\pm$ 0.17	$\pm$ 456	$\pm$ 567	$\pm$ 0.21	
327	1,085	24.60	72	1,373	17.52	
$\pm$ 33	$\pm$ 97	$\pm$ 3.70	$\pm$ 12	$\pm$ 72	$\pm$ 1.47	

(17). *L. casei* would, however, also respond partially to pteroyl di- and triglutamates. *P. cerevisiae* does not respond to methyltetrahydrofolates.

folates from 395 m μ g/ml in control rats to 198 m μ g/ml and simple folate levels from 49 m μ g/ml to 14 m μ g/ml. While a superimposed biotin deficiency did not influence the total blood folates (210 m μ g/ml), the simple folate levels were reduced further by 35% (9 m μ g/ml) and the ratio of conjugated folates to unconjugated folates increased from 13 (Group II) to 22 (Group IV). Total urinary folates per rat decreased from 8 μ g/day in control rats to 1.5 μ g/day in folate deficiency; superimposition of biotin deficiency did not cause any significant alteration in urinary folate excretion (1.9 μ g/day). Blood folates were exclusively methyltetrahydrofolates.

The tetrahydrofolate-glutamate conjugating activity in the liver of the biotin-supplemented group was significantly lower than in the biotin-deficient group irrespective of the folate status of the animals. In folate deficiency the average conjugate synthetase activity of liver was found to be 214 (m μ g conjugated folates synthesized/hr/mg protein), which was comparable to the values obtained for control rats (204). In biotin deficiency and with the superimposed folate deficiency these values increased to 535 and 445, respectively. This increase in folylconjugate synthetase may plausibly explain the higher levels of conjugated folates associated with biotin deficiency. This is in conformity with our earlier observation in *Escherichia coli* where folylconjugate synthetase levels are increased in the absence of exogenous biotin (5).

These results indicate that biotin could influence mainly the conversion of conjugated to unconjugated folate derivatives, the total folate levels remaining unaltered. The higher levels of unconjugated methyltetrahydrofolates, which have been found to be poor substrates for conversion into higher polyglutamyl forms (9), together with the observed decrease in the folylconjugate synthetase activity in the presence of biotin, may explain the decreased conjugated folate levels (tissue and excretion) associated with biotin supplementation under conditions of folate insufficiency. The consequent increase observed in the levels of simple folates had been erroneously interpreted by earlier workers (3, 4) in favour of a role for biotin in tissue folate synthesis; conjugated folates were not measured in these earlier studies.

It might be relevant to note here that earlier reports had indicated the presence of polyglutamylfolate derivatives in the liver of rats (10, 11) and monkeys (12). While formylconjugated tetrahydrofolates are more active in the cofactor functions of the vitamin, the simpler methyl derivatives are the preferred storage and transport forms (13-16). Hence biotin could have a role in mobilizing scarce folate stores during folate deficiency.

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