

METABOLIC FUNCTIONS OF BIOTIN

IV. THE RÔLE OF CARBAMYL-L-GLUTAMIC ACID IN THE SYNTHESIS OF CITRULLINE BY NORMAL AND BIOTIN-DEFICIENT RATS*

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A characteristic consequence of a biotin deficiency in animals is a decreased ability to fix $C^{14}O_2$ into various tissue components including the amino acid arginine (1). Carbon dioxide is incorporated into arginine as a result of the conversion of ornithine to citrulline in the Krebs-Henseleit urea cycle (2). The methods developed by Cohen and Hayano (3, 4) for the synthesis of citrulline from ornithine, glutamate, CO_2 , and ammonia by washed residue of rat liver homogenate have been applied to preparations from biotin-deficient rats (5). A greatly decreased rate of citrulline synthesis was observed in these preparations as compared to those obtained from rats fed an adequate diet.

The specificity of this effect of biotin deficiency is attested to by the finding that essentially normal rates of citrulline synthesis were obtained in liver preparations from rats deficient in riboflavin or vitamin B_6 and that injection of biotin into rats fed the egg white diet results in normal rates of citrulline synthesis 24 hours later (5).

Since Cohen and Grisolia (6, 7) have shown that carbamyl-L-glutamate rather than glutamate is the actual catalytic intermediate in citrulline synthesis from ornithine, it seemed of importance to learn whether the effect of biotin nutriture on citrulline synthesis is manifested at a step prior to or subsequent to the reactions in which carbamyl glutamate participates.

EXPERIMENTAL

The animals used in the experiments were male albino rats of the Sprague-Dawley strain. After weaning at 3 weeks of age they were fed a purified ration containing 18 per cent raw egg white¹ for a period of 7 to 12 weeks. Control animals were pair-fed a similar ration containing casein in place of egg white.

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¹ The complete ration (biotin-deficient Ration A) was described in Paper III (5). The vitamin levels given in that paper are for 10 gm. rather than for 100 gm. as stated.

The twice washed residue of rat liver homogenate was prepared as described by Cohen and Hayano (4) and 0.5 ml. of residue suspension, containing 3 to 4 mg. of N was incubated in Warburg flasks at 37° in air. Unless otherwise indicated, the final substrate concentrations in the flasks were L-ornithine 0.0025 M, fumarate 0.015 M, NH₄Cl 0.005 M, MgSO₄ 0.004 M, adenosinetriphosphate 0.002 M, phosphate buffer, pH 7.15, 0.012 M, NaHCO₃ 0.005 M, and isotonic KCl to bring the final volume to 4 ml. When L-glutamate or carbamyl-L-glutamate was tested, the final concentration was 0.0025 M unless specified as 0.01 M. This mixture differs from that used in the earlier study (5) in that a lower concentration of glutamate is employed and fumarate is added because of its accelerating influence on the synthesis of citrulline in the presence of carbamyl glutamate (6).

After a 20 minute incubation period, perchloric acid was added to stop the reaction and precipitate the protein. The contents of the flasks were centrifuged to remove the insoluble protein and the citrulline in the filtrate was determined colorimetrically by the method of Archibald (8) as employed by Cohen and Hayano (3). The results are expressed as micromoles of citrulline formed per mg. of washed residue nitrogen.

Results

The comparative effect of glutamate and carbamyl glutamate on the synthesis of citrulline by washed residue of liver homogenates from biotin-deficient and control rats during a 20 minute incubation period is summarized in Fig. 1. With glutamate the average amount of citrulline synthesized by fifteen liver preparations from as many biotin-deficient rats was $0.24 \pm 0.13 \mu\text{M}$ per mg. of N.² The corresponding amount for ten pair-fed control animals was $1.38 \pm 0.23 \mu\text{M}$ of citrulline per mg. of N. However, in the presence of carbamyl glutamate the synthesis of citrulline by biotin-deficient liver preparations was the same as that obtained in the control group (1.34 ± 0.31 and $1.35 \pm 0.21 \mu\text{M}$ per mg. of N respectively). Results from stock animals did not differ significantly from those of the pair-fed controls.

Cohen and Grisolia (6) have shown that the initial rate of synthesis of citrulline is increased if the enzyme preparation is preincubated with glutamate or carbamyl-L-glutamate, ammonia, and carbon dioxide for 20 minutes prior to the addition of ornithine. Fig. 2 presents results of similar experiments in which, after 20 minutes preincubation of the reaction mixture, ornithine was tipped in from the side arm and the synthesis of citrulline was allowed to proceed for 10 minutes. Cohen and Grisolia have reported (6) that under these conditions carbamyl glutamate is more ef-

² Standard deviation, $\sigma = \pm \sqrt{\sum nd^2 / (N - 1)}$.

fective than glutamate in supporting citrulline synthesis. In the presence of glutamate, liver preparations from thirteen biotin-deficient rats syn-

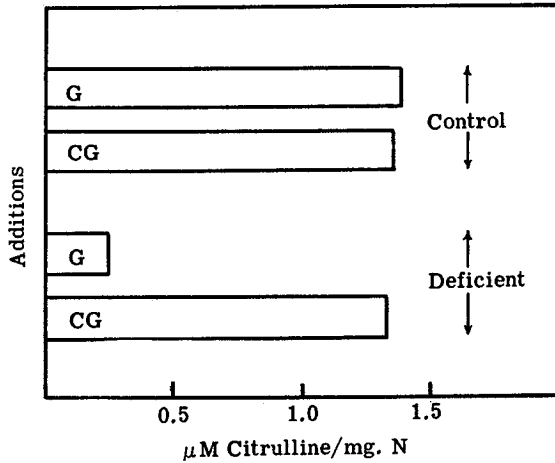


FIG. 1. Synthesis of citrulline from ornithine by washed residue of liver homogenate from biotin-deficient and pair-fed control rats. $G = 0.0025$ M glutamate added to the reaction mixture (see the text); $CG = 0.0025$ M carbamyl-L-glutamate added in place of glutamate. Incubation time, 20 minutes at 37° .

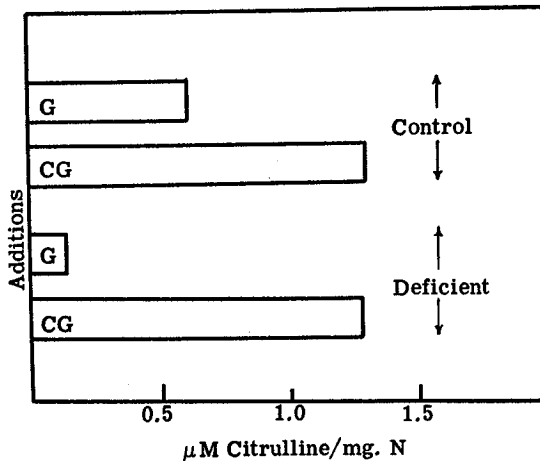


FIG. 2. Synthesis of citrulline from ornithine following a preincubation period. For conditions see the text.

thesized 0.14 ± 0.09 μM of citrulline per mg. of N and the preparations from ten control rats synthesized 0.75 ± 0.20 μM under the same conditions. With carbamyl-L-glutamate the same amounts of citrulline were

synthesized by both tissues (1.29 ± 0.24 and $1.32 \pm 0.37 \mu\text{M}$ respectively). It should be noted here that the over-all synthesis of citrulline by the control tissue was more than twice as great with carbamyl-L-glutamate as with glutamate. This is in agreement with the experimental data of Cohen and Grisolia (6) who found that carbamyl-L-glutamate was 2 to 3 times more active than L-glutamate under these conditions.

These results suggest that tissue from the biotin-deficient rat converts glutamate to a more active form of this compound at a slower rate than

TABLE I

Comparative Effect of Fumarate on Citrulline Synthesis by Liver Preparations from Biotin-Deficient and Pair-Fed Control Rats

The results are expressed as micromoles of citrulline synthesized during a 20 minute incubation period. 0.015 M fumarate present except where otherwise indicated. The data on any given line were collected in a single experimental run and are for pairs of rats having the same food intake.

Biotin-deficient					Control					
0.0025 M glutamate		0.01 M glutamate		0.01 M carbamyl glutamate	0.0025 M glutamate		0.01 M glutamate		0.01 M carbamyl glutamate	
No fumarate		No fumarate			No fumarate		No fumarate			
0.28	0.07	0.67	0.40	1.92						
0.72	0.35	1.68	0.62	2.90	1.07	0.96	1.58	1.28	2.86	
0.51	0.31	0.91		1.70	1.48	1.58	1.82		2.30	
0.17	0.06	0.45	0.10	2.14	1.48	1.71	1.86	1.89	2.14	
0.31	0.15	0.62	0.32	1.81	1.33	1.42	2.02	2.00	2.09	
0.72	0.50	0.94	0.78	1.82	2.07	1.95	2.10		1.97	
0.56	0.31	1.19	0.75	2.28	1.21		1.50		2.46	
Averages	0.47	0.25	0.92	0.50	2.08	1.44	1.52	1.81	1.72	2.30

normal. For comparison, the rates of citrulline synthesis with other derivatives of glutamate were measured. Glutamine was as good as, but no more effective than, glutamate with either biotin-deficient or normal preparations. The hydantoin of glutamic acid (5-propionic hydantoin) was inert in both preparations (*cf.* (6)). When freshly prepared ammonium carbamate was used as the source of ammonia, synthesis of citrulline was equivalent to, but not better than, when NH_4Cl was present.

A search for other variations in experimental conditions which might differentially influence citrulline synthesis by normal and biotin-deficient tissues was made with a separate group of animals. Although fumarate or some other oxidizable substrate is required for optimum citrulline syn-

thesis in the presence of carbamyl glutamate, it was observed that fumarate exerted a definite inhibition on citrulline formation by the biotin-deficient tissue preparations with glutamate (Table I). Oxalacetate and aspartate also inhibited citrulline synthesis by biotin-deficient preparations in the presence of glutamate. These substrates had no effect on the formation of citrulline from ornithine by control liver preparations. α -Ketoglutarate enhanced citrulline formation in the control group but had no effect on the biotin-deficient tissue. Increasing the concentration of glutamate increased citrulline synthesis by both biotin-deficient and normal tissue. With higher glutamate levels the percentage difference between the two types of tissue decreased, but the absolute difference remained essentially unaltered. In these experiments of 20 minutes duration, maximum citrulline synthesis was obtained with about 0.02 M glutamate, considerably less than is required in experiments of 1 hour's duration (3).

DISCUSSION

The data presented in this and Paper III of this series (5) clearly indicate a decreased rate of citrulline formation from ornithine in the presence of glutamate by biotin-deficient rat liver preparations. Since the rate of synthesis in the presence of carbamyl glutamate is the same in the biotin-deficient animals as that in normal animals, it would appear that the lack of biotin results in a decreased rate of conversion of glutamate to carbamyl glutamate.

We have attempted the synthesis of carbamyl glutamate from glutamate by liver preparations in order to examine the site of action of biotin in this reaction. However, no measurable quantity of carbamyl glutamate accumulated in either the control or biotin-deficient animal tissues.

No explanation can be offered, at the present time, for the fact that such compounds as fumarate, oxalacetate, and aspartate inhibit the synthesis of citrulline from ornithine and glutamate in the biotin-deficient rat liver preparations but not in those from normal animals.

We are indebted to Dr. P. P. Cohen and Dr. S. Grisolia for the carbamyl-L-glutamate used in the early part of this study.

SUMMARY

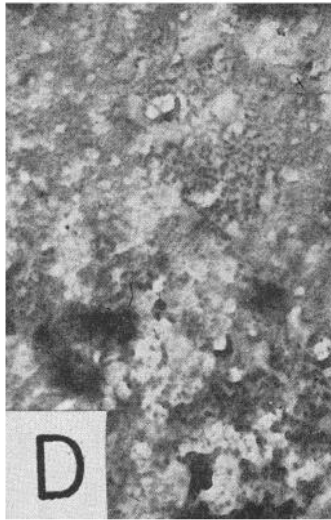
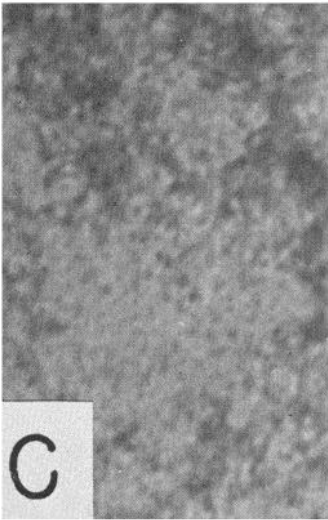
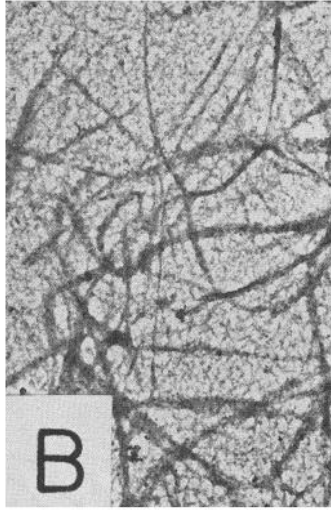
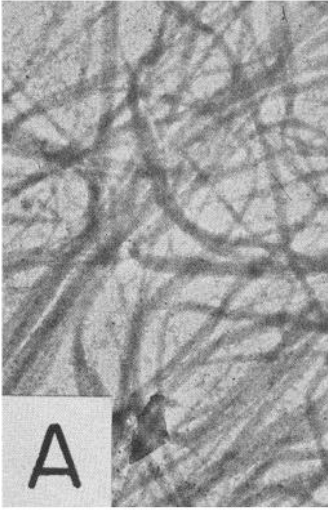
The synthesis of citrulline from ornithine, with L-glutamate as a specific adjuvant, was greatly depressed in washed residue of liver homogenate from biotin-deficient rats as compared to similar preparations from paired control animals. Replacing the glutamate by carbamyl-L-glutamate resulted in equal rates of citrulline synthesis by the biotin-deficient and control preparations.

The decreased citrulline formation by the biotin-deficient preparation in the presence of glutamate was further inhibited by fumarate, oxalacetate, and aspartate. Under the same conditions, these substrates were without effect on citrulline synthesis by liver preparations from the pair-fed control rats.

The results indicate that the influence of biotin on CO₂ fixation into citrulline is at a step prior to that at which carbamyl glutamate functions in the conversion of ornithine to citrulline.

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(Sizer and Wagley: Action of tyrosinase)

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