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Urea Synthesis in the Intact and in the Isolated Perfused Liver of the Biotin-Deficient Rats.

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ABSTRACT

Biotin-deficient rats were raised on a purified ration containing raw egg white plus avidin. Urea synthesis and excretion were compared between the biotin-deficient and the pair-fed control rats. 24hr-urinary urea excretion and the specific activities of carbamylphosphate synthetase, ornithine transcarbamylase, and arginase in the liver mitochondria fraction were no different between these two groups.

The net urea production in the liver slice and in the isolated perfused liver of the biotin-deficient rats was similar to that of the pair-fed control. Thus the conclusion must be that biotin is not involved in urea biosynthesis in the rat.

INTRODUCTION

Several investigators reported that biotin was involved as a cofactor for CO_2 fixation enzymes such as acetyl coenzyme A carboxylase (Wakil et al, 1960), oxalate transcarboxylase (Swich and Wood, 1960), pyruvate carboxylase(Utter, 1960), ß-methylcrotonyl coenzyme A carboxylase (Knappe et al, 1961), propionyl coenzyme A carboxylase (Kaziro and Ochoa, 1962), and carbamyl-phosphate synthetase(Lynen et al, 1969). In vivo studies, MacLead and Lardy(1949) showed that biotin-deficient liver synthesized citrulline to about one-half of the normal rat tissue and also demonstrated that the biotin-deficient animal had decreased ability to fix $^{14}CO_2$ into arginine. Feldott and Lardy(1951) found that the synthesis of citrulline from ornithine was greatly decreased in a washed residue of liver homogenate from biotin-deficient rats as compared with pair-fed control rats. Grisolia and Cohen(1953) observed that the effect of biotin nutrient on citrulline synthesis was manifested at a step prior to or subsequent to the reaction in which carbamylglutamate participates.

By these facts we assume that biotin could participate in the carbamylphosphate synthesis reaction which is a reaction involved in the Krebs-Henseleit urea biosynthesis cycle. Wellner and Meister(1968) have studied and consider that carbamylphosphate synthetase in E.coli was a biotin enzyme. On the other hand, Huston and P.P Cohen(1969) reported that purified carbamylphosphate synthetase from frog and beef liver mitochondria and from E. coli was not effected by avidin. Peng and Jones (1969) considered that unpurified glutamin utilizing CPS 11 and 35% pure frog liver CPS 1 were not inhibited by avidin. Guthöhrlein and Knappe(1968) investigated rat liver mitochondria CPS was absent of biotin.

Among these controversial findings there are few experiments on urea synthesis in the intact and in the isolated perfused liver of biotin-deficient rats. In biotin-deficient and pair-fed normal control rats we investigated (1) urinary urea excretion and Krebs-Henseleit urea cycle enzymes, carbamylphosp-

Abbreviation: CPS I: ammonia utilizing carbamylphosphate synthetase in mitochondria fraction.
CPS II: glutamin utilizing carbamylphosphate synthetase in cytosol.
OTC: ornithine transcarbamylase.

hate synthetase, ornithine transcarbamylase, and arginase in intact rats, (2) net urea production in the liver slice and in the isolated perfused liver.

METHODS AND MATERIALS

Rats weighing 40-50g were fed a purified ration containing 20% raw egg white(biotin-deficient) for a period of six weeks, and 7-9 days before sacrificing an average of 14.4μg avidin was added to the diet daily. Control animals were pair-fed a similar ration containing a 20 μg of biotin per 100 g of the basal diet (Table 1).

Table 1. Composition of the Basal Diet

Sugar	70.64%
Egg white	20.00%
Salt mixture	4.00%
Corn oil	4.00%
Vitamin B mixture (1)	1.00%
Vitamin A and D mixture (2)	0.25%
Choline chloride	0.10%
Vitamin E acetate	0.01%

(1) Composition of vitamin B mixture (percentage of mixture): Ascorbic acid, 2.50%; thiamin- HCL, 1.00%; Ca-D-pantothenate, 0.40%; riboflavin, 0.15%; pyridoxine-HCL, 0.06%: menadione (VK), 0.50%; folic acid, 0.40%; p-aminobenzoic acid, 0.02%; vitamin B_{12}(0.1% trituration with mannitol), 0.20%; glucose, 93.58%.
* Vitamin B_{12} (cyanocobalamin): 1μg/1g vitamin B complex/100gm of diet.
(2) Composition of A and D(both water dispersible): Vitamin A palmitate, 1.2×10^6 IU; vitamin D_2, 1.2×10^6 IU: and glucose to make up to 150g: 100 g of diet contained vitamin A palmitate 2000 IU: and vitamin D_2 200 IU.
(3) Vitamin E (dl-α-tocopheryl acetate): Vitamin E was premixed with corn oil before it was added to the diet.

At the end of six weeks raised a mean body weight was 157.0g(142.5-192.0) in the control and 151.9g(138.0-178.0) in the biotin-deficient rat. A daily intake of diet during the 24-hour urine collection was 17.2g (16.0-19.0) in the control and 14.4g (11.8-16.9) in the biotin-deficient rat. Portein intake was neglected of the calculation for 24-hour urine urea excretion. 24-hour urines were collected for urea excretion determination for 6-9 days. Liver mitochondria were fractionated by the method of Lawrence (1968) and ammonium sulfate precipitate was used for the enzymes (carbamylphosphate synthetase, ornithine transcarbamylase and arginase) measurement. The activity of CPS was measured by a modified Lawrence technique(1968), OTC by Ceriotti(1966) and arginase by Loeb(1969). Urea was determined by the urease microdiffusion method. Liver slices were made with the Stadie-Riggs tissue microtome and the net urea production in the system was determined by the Hirs and Rittenberg (1950) method. Urea production is expressed in terms of urea produced per 300 cm^2 body surface area of the liver donor.

RESULTS

Urea excretion in the 24-hour urines averaged 508.8 mg in the controls and 486.8 mg in the biotin-deficient rats, respectively(Table 2).

Table 2. 24-hour Urinary Urea Excretion(300 cm^2)

Urea excretion mg/24 hrs	Mean	Range
Control (5)	508.8	400.5—618.3
Biotin-deficient(6)	486.8	349.7—586.1

Range indicated daily variation in 6-9 days
Numer in blanket is number of experiments

The mean value of the enzyme activity was 502.1±40.3, 549.8±77.8 umole per mg of protein in CPS, 85.8±77.9, 86.6±12.3 umole in OTC, 300.8 ±79.6, 280.3±65.7 μmole in arginase in the pair-fed controls and biotin-deficient rat, respectively(Table 3). In the liver slice the net urea production for the 100 mg slice for 100 minutes was 0.463±0.021 and 0.536±0.015 mg in the control and in biotin-deficient liver(Table 4). Although the rate of urea production in the first 45 minutes of the perfusion in the biotin-deficient liver was rather less than that in the control, the urea production for 4 hours was not significantly different, that is 13.0 mg in the control and 14.4 mg in the biotin-deficient rat fed avidin as shown in Table 5 and Figure 1.

Table 3. Comparison of Enzymes Activity between Biotin-deficient and Pair-fed Normal Intact Rats

Enzymes \ Experimental condition	Pair-fed Control (5)	Biotin-deficient (6)
Carbamyl phosphate synthetase (umole/mg of protein)	502.1±*40.3	549.8±*77.8
Ornithine transcarbamylase (umole/mg of protein)	85.8±*7.9	86.6±*12.3
Arginase (μmole/mg of protein)	300.8±*79.6	280.3±*65.7

* ··· indicated standard error
Number in blanket is number of experiments.

Table 4. Urea Production in Liver Slice

A 200mg liver slice, 0.9 μM L-ornithine, 60 μM L-glutamine and 3 ml of Krebs-Ringer Bicarbonate solution (pH 7.4) were in a Warburg's vessele. The vesseles were aerated with 95% O_2 and 5% CO_2 for ten minutes and incubated for 100 minutes at 37°C water bath. The reaction was stopped by 5% zinc sulfate and 0.3 N barium hydroxide. The protein was precipitated. After centrifuging urea content was determined in the supernatant fluid.

Urea produced mg/100 mg/100 min.	Mean	S. E.
Control (4)	0.463	±0.021
Biotin-deficient (4)	0.536	±0.015

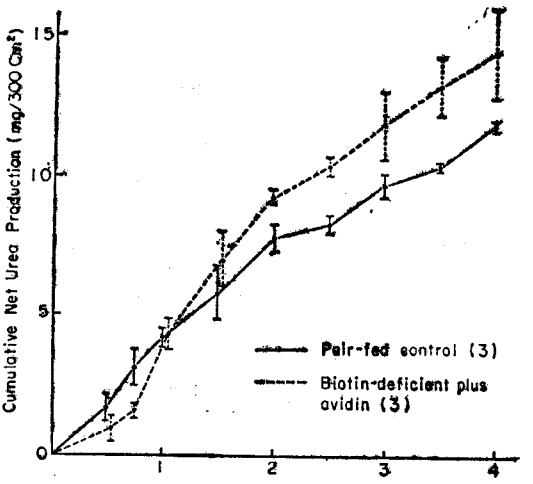
Number in blanket is number of experiments

Table 5. Cumulative Net Urea Production by Isolated Rat Liver Perfused (mg/300 cm²)

Perfused time(min)	Control Rats Mean	Control Rats S. E.	Biotin-def. Rats Mean	Biotin-def. Rats S. E.
0	0	0	0	0
30	1.67	0.56	0.83	0.33
45	3.11	0.71	1.63	0.10
60	4.22	0.39	4.25	0.67
90	5.88	1.11	6.99	1.00
120	7.77	0.44	9.32	0.06
150	8.21	0.33	10.21	0.33
180	9.66	0.44	11.88	1.44
210	10.32	0.10	13.21	1.14
240	11.88	0.10	14.44	1.67

DISCUSSION

Urinary excretion of urea in 24-hours and the enzymes, carbamylphosphate synthetase, ornithine transcarbamylase and arginase in the liver mitochondria fraction in the intact rat were not shown to be significantly different in the control and the biotin-deficient plus avidin fed rats. These results were not consistent in showing the decreased ability to fix CO_2 into arginine in the biotin-deficient rat (MacLeod and Lardy, 1949). However, the enzyme activity of CPS, OTC, and arginase was not different between the two groups. Since CPS activity was almost the same in biotin-deficient rat liver as that in the pair-fed normal, biotin need not to be a cofactor in carbamylphosphate synthesis reaction. Net urea production in the first 45 minutes of perfusion was less in biotin-deficient liver than it was in the control. Avidin is a basic protein which can combine with and inhibit anionic proteins



Hours of Liver Perfusion

Number of experiments is indicated in parentheses

Figure 1. Cumulative Net Urea Production in the Isolated Perfused Rat Liver

L-Glucose(250mg) in 6.0 ml distilled water was infused at a constant rate of 1.2 ml per hour throughout a four perfusion. No exogenous substrate was added. The data plotted represent the average values for 3 experiments for each group with the range of standard error indicated by verical line at each time.

Avidin (0.5 mg=6.5 unit) dissolved in 0.5 ml of distilled water was added to the perfusate at the beginning as a stat dose in the biotin-deficient perfused liver experiments.

(Peng and Jones, 1969). This may be explained by interfering with the extrusion of the preexisting urea in the liver cell by avidin when added as a stat dose in the perfusate. The rate of urea synthesis following 45 minutes of perfusion in biotin-deficient liver was higher than that in the control. This suggests that biotin may not effect the enzymes involved in the Krebs-Henseleit urea cycle as well as carbamylphosphate synthetase. These findings concided with those of the liver slice experiments.

The finding that biotin has no effect on urea biosynthesis in vivo and vitro experiments on rats is well supported by Peng and Jones(1969) who reported that biotin is not a cofactor of carbamylphosphate synthetase.

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