Possible Rate-Limiting Factors in Urea Synthesis by the Perfused Rat Liver[1] (36883)

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There are numerous examples of changes in the activity of enzymes in response to changes in diets and hormones (1). Proportional changes in the activity of urea cycle enzymes versus changes in the amount of urea excreted in urine have been reported, but the maximum change in urea excretion occurs before the maximum change in enzyme activity (2, 3). This delay in change in enzyme activity suggests that the increase in enzyme activity is not the immediate cause for the increased rate of urea synthesis and that the change in enzyme activity may be in response to a change in urea or ammonia production.

In view of interactions between urea cycle intermediates and enzymes, we have examined the relationships between previously reported amounts of argininosuccinate synthetase activity and the rate of synthesis of urea by perfused livers from rats pretreated with hormones and diets.

Materials and Methods. Male Sprague–Dawley rats weighing 140 to 160 g were fed 1 of 3 diets or given hormonal treatments. Diets were fed *ad libitum* and were the following: Control (65% glucose and 25% casein) (4); high protein (90% casein) (4) and protein-free diet (90% glucose) (5). All groups were fed their diet for 5 days prior to perfusion except one group which was fed the protein-free diet for 9 days. The thyroxine (T_4) treated group received 1 mg T_4, dissolved in 1 ml of 0.01 *N* NaOH/rat per day interperitoneally, and the cortisol group received 5 mg of cortisol acetate/rat per day subcutaneously for the 5 days immediately prior to perfusion. During the treatment period, both hormone-treated groups were fed the control diet *ad libitum*. The starved group received the control diet for 3 days before the 72 hr starvation period. Liver perfusion was carried out by the method described by Hems *et al.* (6). Ammonium chloride was added to the perfusion media, to produce a final concentration of 10 m*M*, 37 min after the start of the perfusion. Each group was divided into 2 subgroups, one subgroup was perfused as described above with NH_4Cl, and in the 2nd subgroup ornithine and NH_4Cl were added to the perfusion media. In experiments which included ornithine, it was added 37 min after the start of the perfusion to give a final concentration of 2.7 m*M*. Higher concentrations of ornithine did not further increase the rate of urea synthesis by the control group. The addition of ornithine has been reported to increase the rate of urea synthesis from NH_4Cl in the perfused rat-liver system (6). Ornithine was added to supply an excess of urea-cycle intermediates, which in turn would relieve limitations on the rate of urea synthesis which might be due to an insufficient concentration of urea-cycle intermediates. One-half ml samples of perfusion media were taken at zero time, 30 min and for every 10 min thereafter until completion of the 90 min perfusion period. Samples of perfusion fluid were deproteinized in 2% perchloric acid. The urea (7) and ammonia (8) concentrations in samples of the deproteinized perfusion media were determined.

Results and Discussion. The rates of urea production are reported in Table I. The activity of argininosuccinate synthetase is the

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TABLE I. Rates of Urea Synthesis by Perfused Rat Livers.

		No ornithine added[a]			Ornithine added[a]	
Treatment	n	Urea	p	n	Urea	p
Fed control	8	2.9 ± 0.3		6	7.8 ± 0.2	
Protein-free, 5 day	4	2.7 ± 0.4	>.05	6	7.7 ± 0.5	>.05
9 day	4	0.7 ± 0.2	<.05	4	2.0 ± 0.4	<.05
Starved (72 hr)	3	2.7 ± 0.2	>.05	3	5.9 ± 0.5	<.05
High-protein	6	6.0 ± 0.5	<.05	6	7.5 ± 0.5	>.05
Cortisol	4	7.8 ± 0.4	<.05	4	9.5 ± 1.8	>.05
Thyroxine	14	2.7 ± 0.5	>.05	13	4.0 ± 0.7	<.05

[a] Values are means ± SE of the mean and are expressed on the basis of μmoles of urea synthesized per min per 100 g of body weight.

lowest of the urea-cycle enzymes reported and is regarded as the primary limiting enzyme in the urea cycle (9) and for this reason in our discussion we have chosen to compare the amount of argininosuccinate synthetase activity previously reported (2, 10) with the rate of urea synthesis found in our studies.

Schimke has reported that the argininosuccinate synthetase activity decreases in liver from rats fed a protein-free diet (2) and by 7 days the amount of activity was no longer decreasing. However, after receiving the protein-free diet for 5 days the capacity of the perfused liver to synthesize urea was not appreciably different from the capacity of the control group with or without adding ornithine. By the 7th day hepatic argininosuccinate synthetase activity in the protein-free group was 25% of the control group's activity (2) and urea synthesis by the perfused livers of the 9 day protein-free group, in the presence or absence of ornithine was 25% of the control group's with or without ornithine added. Thus even though the amount of argininosuccinate synthetase activity in the 4 day protein-free group had decreased to less than the enzyme activity of the control group there was an adequate amount of activity to maintain urea synthesis at the control level. Therefore, the decrease in enzyme activity in the 5 day protein free group without any accompanying change in rate of urea synthesis suggests that the argininosuccinate synthetase of the control group was not rate limiting for urea formation.

Rats starved 4 days increase their daily urinary urea output to nearly twice that of fed controls and the argininosuccinate synthetase activity increases to 1.7 times that of the fed control group's (2). But the increase in enzyme activity after 3 days of starvation did not result in an increase in the rate of urea synthesis by the perfused liver and the addition of ornithine resulted in a smaller increase in the rate of urea synthesis than was observed with the lower amount of enzyme activity in the control group. This increase in argininosuccinate synthetase activity but little or no increase in rate of urea synthesis again suggests that the increased enzyme activity was probably not directly related to maximum productive capacity of the urea cycle.

Without the addition of ornithine, perfused livers of the high-protein group synthesized urea at twice the control group's rate and at the same rate as the control group's when ornithine was added. The activity of argininosuccinate synthetase of the high-protein group was reported to be about 2.7 times that of the control group's (10) and in the absence of added ornithine, there was an apparent correlation, between enzyme activity and the rate of urea synthesis, but this correlation disappears when ornithine was added. The livers of mice fed a 70% casein diet were reported to contain 5 to 6 times more ornithine than the livers of mice fed a 15% casein diet (11). If there was an increase in the concentration of ornithine in the liver of the rats in the high-protein group, it would sug-

gest that the urea-cycle intermediates were already in concentrations that permitted near-maximum rates of urea synthesis without the addition of ornithine to the perfusion media and account for the minor increase observed in the rate of urea synthesis when ornithine was added.

Although the high-protein and cortisol-treated groups were reported to have nine times more argininosuccinate synthetase activity than the 5 day protein-free group (10), the maximum rates of urea synthesis obtained in the perfused liver, in the presence of excess ornithine, were not appreciably different in the three groups. If there was a direct relationship between the amount of argininosuccinate synthetase activity as measured *in vitro* and the total amount of enzyme, and if all enzyme present in the control high protein and cortisol groups were active, then it would appear that the argininosuccinate synthetase of the 5 day protein-free group was converting more substrate to product per molecule of enzyme than the enzymes of the control, high protein and cortisol groups.

It was apparent from the results of this study that under certain circumstances the amount of argininosuccinate synthetase activity and concentrations of urea-cycle intermediates were primary controlling factors. This was most evident when high concentrations of ornithine and high argininosuccinate synthetase activity was present, but the rate of urea synthesis was not appreciably different from when the activity of argininosuccinate synthetase was lower, *i.e.*, 5 day protein-free group with ornithine-added versus the cortisol-treated group without ornithine or high-protein group with ornithine.

This study has served to demonstrate that the amount of urea cycle enzyme activity and the concentration of urea cycle intermediates are probably not the sole limiting factors of urea synthesis. The intermitochondrial transport of urea-cycle intermediates may be another limiting factor to consider. However, the role of intermitochondrial transport as a limitation in urea synthesis remains an open question and will be investigated in this laboratory.

Summary. Rates of urea synthesis from ammonium chloride by perfused rat livers, pretreated with different diets and hormones, were measured. Addition of ornithine to the perfusion media increased rates of urea synthesis, but not in proportion to the relative amount of argininosuccinate synthetase activity present. Livers from rats receiving a protein-free diet for 4 days were reported to have about 12% of the argininosuccinate synthetase (E.C. 6.3.4.5.) activity as livers from rats receiving a high-protein diet or rats pretreated with cortisol. However, in the presence of excess ornithine all 3 groups of rats synthesized urea at the same rate.

This investigation suggests that under conditions in which high ammonia concentrations prevailed a factor or factors in addition to urea-cycle intermediates and the activity of urea-cycle enzymes were limiting the rate of urea synthesis, and that there was not a correlation between maximum rate of urea synthesis and the activity of argininosuccinate synthetase.

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