UREA SYNTHESIS IN THE ISOLATED PERFUSED RAT LIVER

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Received October 27, 1960

Recently we have reported data on the production of urea in the perfused rat liver using both endogenous and exogenous amino acids as nitrogen sources (Burke and Miller, 1956, 1959A, 1959B, 1960). While continuing studies related to a comparison of amino acid metabolism in normal livers and in livers undergoing carcinogenesis we have accumulated considerable quantitative information on the utilization of specific amino acids for urea synthesis in this system.

Following the early work of Krebs (1933) a number of investigators have measured urea formation by the use of washed rat liver slices. The only extensive data on urea production from a number of specific amino acids seems to be that of Kamin and Handler (1951) who used intact dogs for their studies.

We have used the technique of isolated rat liver perfusion in these investigations. We have discussed previously the superiority of this preparation as compared with the liver slice

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1 These investigations have been supported by research grants from the Williams-Waterman Fund, The American Cancer Society and the U.S. Public Health Service (National Cancer Institute).

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technique for metabolic studies and have demonstrated that the functional capacity of the perfused liver simulates that of the intact animal (Miller, Burke and Haft, 1956). In the studies reported here we have added 1-1/2 mM of L-amino acid to 100 ml. of blood perfusate with the exception of the experiments involving L-arginine. In this case 3/4 mM were used as the supplement since 2 mM of nitrogen from the arginine will be utilized for each millimol of urea produced from this amino acid. In all other respects the experiments and methods were as described previously (Burke and Miller, 1956).

The rats used were adult, male, 200-400 gram Wistars obtained commercially[3]. Urea nitrogen was determined by the Conway micro-diffusion method (Conway and O'Malley, 1946). Determinations using this micro-diffusion method were made in experiments involving glutamine, asparagine or ammonia, with and without added urease and urea values were corrected for the presence of ammonia in the blood samples. This was found to be necessary only when ammonia was added.

The amounts of urea nitrogen produced from each amino acid tested during four-hour perfusion are indicated in Table I. Values for excess urea nitrogen production as presented in the table were calculated by correcting for the amounts of urea nitrogen which would have been produced by comparable rats from the endogenously available amino acids. These calculations have been discussed in detail previously (Burke and Miller, 1959A). Also indicated in Table I are the relative rates of urea formation found by Kamin and Handler (1951) in dogs and by Krebs (1933) for rat liver slices. Strictly quantitative comparisons between our data and those of these investigators are difficult

[3] Obtained from: Hemlock Hollow Farm, 414 Black Oak Road, Wayne, New Jersey.

Table 1
AVERAGE UREA NITROGEN PRODUCED FROM L-AMINO ACIDS

(A) (B) Amino Acid Added	Number Expts.	Total Urea Nitrogen (mgms)	Urea Nitrogen (mgms)/300 gms. Body Wt.	Urea Nitrogen (mgms)/10 Liver	Excess Urea Nitrogen (mgms)	A	B
1. L-Arginine	2	33.7	29.1	40.6	14.6	2	
2. L-Glutamine	7	30.4	26.5	32.0	12.3	1	
3. L-Histidine	3	30.1	25.1	25.3	11.4	4	5
4. Glycine	2	26.3	24.6	28.9	9.8	3	3
5. L-Asparagine	2	26.0	25.2	24.5	10.1	3	2
6. L-Lysine	3	23.9	21.6	25.4	7.0	7	8
7. L-Citrulline	3	20.7	18.7	21.6	4.1		
8. Ammonia	3	20.3	19.6	17.8	4.5		
9. L-Alanine	2	16.3	15.7	18.6	0.7	7	1
10. L-Leucine	3	13.5	12.5	15.2	None	6	6
11. L-Aspartic Acid	2	15.6	12.0	15.0	None	8	7
12. L-Glutamic Acid	3	10.9	10.8	13.0	None	8	7
13. L-Methionine	2	11.0	10.3	12.4	None	9	
14. L-Valine	2	12.7	9.1	10.9	None		

(A) Amino acids ranked according to rate of urea nitrogen production, data of Kamin & Handler (1951)
(B) Amino acids ranked according to rate of urea nitrogen production, data of Krebs (1933).

to make for individual amino acids and have not been included.

Discussion. These studies provide a basis for comparing the relative contribution of a number of specific amino acids to urea synthesis. No series of generalizations about these results need be made. A few comments may be worthwhile, however. The nitrogen from all amino acids is not quantitatively converted to urea nitrogen nor would we expect it to be. Previous studies have indicated the high capacity of the perfused liver for liver and plasma protein synthesis, a process which would decrease the availability of amino acids for catabolic reactions (Miller, Bly, Watson and Bale, 1951). We do not suggest that those amino acids which are relatively poor sources of urea nitrogen are being utilized more extensively for protein synthesis but this is certainly a possibility. For example, note might be made of the high proportion of glutamic and aspartic acids in plasma proteins and the low yield of urea nitrogen from these amino acids. This may be an indication that they are preferentially utilized for synthetic reactions. Low permeability of the acids has been suggested as an explanation for the low yield of urea nitrogen from these amino acids (Kamin and Handler, 1951). However, we have shown that the perfused liver is capable of oxidizing extensively small amounts of L-glutamic acid - U-C^{14} (Miller, Burke and Haft, 1956). This observation is not compatible with a low permeability of the liver cell to this amino acid.

The important role of the liver, not only in metabolic and biosynthetic processes, but also as a regulatory organ, controlling the availability of substrate for biochemical activities in extra-hepatic tissues may be considered in relationship to the high yield of urea nitrogen from several of

the amino acids which we have studied. Histidine, glycine, lysine and arginine for example, which are extensively deaminated, are present in significant proportions in plasma gamma globulins. The plasma gamma globulin fraction has not been shown to be synthesized extensively by the liver, but apparently by plasma cells and possibly by other reticulo-endothlial cells; however the liver may exercise a regulatory function on gamma globulin by restricting the levels of these amino acids in the peripheral circulation at times when extensive gamma globulin synthesis is not occurring.

Comment might be made also on the relatively poor utilization of methionine for urea synthesis as reported here and as found by Kamin and Handler (1951). This may be indicative of its utilization in a large number of metabolic processes occurring in the liver. Dietary methionine supplementation has been shown to play an unexplained role favoring the formation of plasma albumin (Allison, 1956). Since the liver can be shown to be the primary source of plasma albumin (Miller, Bly, Watson and Bale, 1951), the participation of methionine in this process might explain the failure of the liver to deaminate this amino acid. Of considerable interest in the metabolism of methionine is the fact that both plasma albumin and gamma globulin are extremely low in methionine content.

We hope to extend these studies to investigate the questions raised and to gain further insight into the mechanism involved in hepatic nitrogen metabolism.

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