Effect of experimental chronic renal failure upon the production of urea, as measured by the liver arginase activity in rats[1]

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Summary. A significant diminution in liver arginase activity of chronic renal failure uremic rats is described, thus implying that the experimental finding of an increased urea production is not due to a depressed arginase activity.

Studies have demonstrated an increased production of urea in rats with acute renal failure, as measured by the whole body[2-7] and by the hepatic production of urea[8,9]. On the other hand, enzymatic systems have been shown to be altered during acute and chronic renal failure, both in human[10-13] and experimental animals[14,15]. Thus, urea production measured by human red blood cell arginase activity showed a decrease[11]. This investigation was designed to determine whether the increased production of urea could have any relationship with the activity of liver arginase of rats with experimental chronic renal failure.

Material and methods. Experimental procedures: Chronic renal failure (CRF) was produced in 28-week-old male random-bred R/A rats. The method by which the renal failure was obtained has been described elsewhere[16].

Preparation of liver extract: The liver extract was prepared simultaneously and identically in both nephrectomized and sham-operated rats. The whole liver was homogenized at 4 °C for 5 min, using an 'Omni-Mixer' apparatus (Sorvall Company) at a constant speed. The resulting extract was again diluted in distilled, demineralized water, fixing a concentration of dry liver tissue of about 30-60 mg/ml of solvent.

Determination of arginase activity: The arginase activity was determined in duplicate and measured by the amount of urea in mg produced per g of dry tissue/h. The following method was employed: 1 ml of 8 mM/l solution of $MnCl_2$ was added to each of the 3 tubes (reaction, Blank A, Blank B) (table 1). 10 min later the liver extract was added to the reaction and Blank A tubes and water to Blank B, and the tubes were placed back in the shaking water bath for 15 min. This step allowed the activation of the enzyme by the Mn^{++}. The reaction was then started by the addition of 1 ml of 75 mM/l solution of L-arginine, pH 9.0. The incubation of this final mixture at 37 °C was continued for exactly 20 min, when the reaction was stopped by adding to each tube 0.5 ml of 2.5 N HCl. After thoroughly mixing, the tubes were centrifuged, the supernatant was removed and urea measured by the manual method of Marsh and coworkers[17]. The validity of this arginase assay was confirmed by studies in which it was determined that the reaction was linear for 40 min, the concentration of L-arginine employed was not inhibitory and the concentration of manganese and the pH of the reaction mixture were optimal.

Results. Liver extracts for arginase assay were obtained from paired nephrectomized and sham-operated rats divided in 3 groups according to time of CRF development (30, 90 and 120 days after the 2nd nephrectomy). The results are presented in table 2. They have been expressed as ratios as follows: arginase activity of liver extract of nephrectomized rats/arginase activity of liver extract of sham-operated rats. The differences shown in table 2 are significantly different from zero, which means a significant diminution in the arginase activity of chronic renal failure uremic rats at the 30th, 90th and 120th day of evolution ($p = 0.01$). When we pooled the data of the 3 groups (28 pairs), the significance was greater; and when we compared the difference according to the duration of renal failure, it can be observed that there was a significant decrement of arginase activities when the 30th day is compared to the 90th and 120th days evolution, while the 90th is not different from the 120th day, indicating that the defect becomes stable.

Discussion. Studies on urea production have usually been carried out by 1 of the 3 following procedures: a) Calculation of the rate of increment of plasma urea concentration multiplied by the volume of body[2,4-7]. b) Study of the changes in the composition of the solution which perfuses the liver[8]. c) Determination of the rate of urea produced by liver slices in a closed system.

These experiments have demonstrated increased urea production in animals in contrast to observations in humans with acute and chronic renal failure. It is known that the rate of plasma urea concentration increment increases after hemodialysis and decreases as the uremic state becomes worse[18]. It has been pointed out that urea is recovered in the dialysis fluid of patients under hemodialysis, in excess of the increment of plasma urea multiplied by the total body water[19]. As the serum levels of urea were reduced, its formation proceeded at a higher rate, implying that a metabolic blockage had been released. These observations are consistent with the findings of Reynolds and coworkers[11] who determined red blood cell arginase activity in a few cases of patients with chronic renal failure and found a very low activity of this enzyme. It must be cited, however,

Table 1. Standard arginase assay: chronological order of addition of components

Solution		Assay tubes			
Volume	Type	Reaction	Blank A	Blank B	Time of shaking incubation at 37 °C
1 ml	$MnCl_2$ 8 mm/l	01	01	01	10 min
1 ml	Liver extract	02			15 min
1 ml	Boiled liver extract		02		15 min
1 ml	Distilled water			02	15 min
1 ml	L-arginine 75 mm/l in NaCl (pH = 9.0)	03	03	03	20 min
0.5 ml	HCl 2.5N*	04	04	04	

* Stop the reaction, tubes vortexed, spun for 15 sec and urea measured in supernatant.

Table 2. Arginase activities of rats' livers, expressed as differences: Activity of control rat activity of nephrectomized rat (C–E)

No. of pair	Differences according to duration of renal failure 30 days			90 days			120 days		
	C	E	C–E	C	E	C–E	C	E	C–E
01	33	21	+12						
02	24	30	−06						
03	36	26	+10						
04	33	27	+06						
05	36	30	+06						
06	45	24	+21						
07	36	33	+03						
08	30	27	+03						
09	27	30	−03						
10	48	21	+27						
11	33	27	+06						
12	33	24	+09						
13				81	60	+21			
14				75	69	+06			
15				87	70	+17			
16				87	69	+18			
17				93	75	+18			
18				78	72	+06			
19				84	66	+18			
20				90	69	+21			
21				99	66	+33			
22				87	75	+12			
23							63	48	+15
24							54	39	+15
25							60	36	+24
26							63	30	+33
27							69	48	+21
28							63	40	+23
X	34.50	26.66		86.10	70		62.00	40.16	
SD	6.70	3.72		7.07	3.16		4.89	6.99	
t			2.95			6.46			7.98
p			0.01			0.01			0.01

that arginase activity on human erythrocytic membrane fragments was not diminished in uremic patients[12], who had a concomitant decrease in the magnesium dependent, sodium and potassium activated adenosine triphosphatase. Differences between these arginase results could be derived from the methodology employed in the preparation of cell fragments, a procedure which involves a sequence of washing of cell fragments by buffered distilled water. This approach would not interfere with a more stable union between the uremic toxin and the enzyme (ATPase), while it would disrupt a looser bonding of the same toxin to the arginase molecule, residing in the cell membrane. Therefore we suppose that there may be only one toxin, with variations in the strength of its chemical union with 2 specific enzymes. Conversely, it can also be speculated that 2 specific toxins exist, one acting on ATPase and the other on arginase. If arginase activity is really decreased in humans, the excessive recovery of urea in the dialysis bath could be explained, supporting the theory that urea production is blocked during the uremic state, and the blockage is released after dialysis.

Finally the question arises about the toxin or toxins which could block this enzyme. They could, of course, be the products of the reaction, urea and/or ornithine. We have determined the arginase activity, before and after adding fair amounts of urea to the system, and have not observed enzymatic inhibition of arginase. The effect of ornithine and of other toxins retained in renal failure on arginase, requires further investigation.

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