Relative importance of kidney and liver in synthesis of arginine by the rat

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Experimental Procedure

Male albino rats of the Sprague-Dawley strain weighing approximately 150 g were fed, for 7 days, an 8% casein diet containing, in percent: casein, 8; L-methionine, 0.2; sucrose, 81.3; mineral mixture, 5; vitamin fortification mixture, 0.5; and corn oil, 5. The rats had access to food until the time they were anesthetized prior to surgery. Approximately equal numbers of animals from each treatment were taken for the incorporation studies at 9 AM and 1 PM. The rats were anesthetized with sodium pentobarbital administered intraperitoneally at a rate of 6 mg/100 g body wt. The animals were then opened with a median incision and the liver and/or kidneys were ligated by double ligatures of no. 1 cotton surgical suture. The liver was ligated at the hepatic-portal hiatus while the renal vein and artery of the kidney were ligated.

The extent to which the kidney and liver contribute to the arginine synthesis in the rat was measured by the amount of L-citrulline-ureido-¹⁴C used. Five treatments were used in experiment 1: 1) intact controls which were administered the labeled citrulline 60 and 120 min, respectively, prior to being killed, 2) rats with their kidneys ligated, 3) rats with their livers completely ligated, and 4) rats with both the kidneys and livers completely ligated. At the termination of the incorporation period, the rats were killed and the kidneys, livers, spleen, brain, and a sample of the longissimus dorsi muscle were taken for analyses.

The L-citrulline-ureido-¹⁴C used (New England Nuclear Corp., Boston) had a specific activity of 4.6 mc/mmole and by ion-exchange chromatographic analysis, using a scintillation anthracene flow cell, was 94% pure. The only detectable impurity was radioactive urea which would not present any problems in this study. Ten microcuries of L-citrulline-ureido-¹⁴C in 0.1 ml of saline was injected into the femoral vein of each animal.

It was originally planned that a 2-hr period would be used for the incorporation of the labeled arginine into protein. Since many of the animals with ligated livers or with ligated livers and kidneys died prior to that time, control animals and animals with ligated kidneys were killed at 1 and 2 hr after administration of the isotope. The tissues and organs were removed at the termination of the incorporation period, weighed, and frozen in acetone and Dry Ice. They were then stored at -20 C until analyses were performed. A portion of the tissue samples was homogenized in 9 volumes of cold distilled water. The homogenates were used to determine total nitrogen by the Kjeldahl method and for determination of the radioactivity present in the protein fraction. The protein from duplicate 1-ml samples of homogenate was precipitated with 3 ml of
cold 10% trichloroacetic acid (TCA). The samples were then centrifuged, the supernatant was poured off, and then the precipitates were washed twice by resuspending in 5 mL of 10% TCA followed by centrifugation. After each centrifugation, the supernatant was poured off and the tubes were allowed to drain while inverted on absorbant paper. The precipitated protein was solubilized by the addition of 1 mL of concentrated formic acid. After standing overnight, the tubes were heated to 70 and the solution was transferred quantitatively to scintillation vials by washing with 3- to 6-mL aliquots of XDC scintillation fluid (9). Five percent (w/v) of thixotropic gel powder (Cab-O-Sil, Packard Instrument Company, Inc. La Grange, Ill.) was added to the kidney samples. The samples were counted without and with an internal standard in a liquid scintillation counter.

In experiment 2, the treatments studied were: 1) intact controls, 2) rats with kidneys ligated, and 3) rats with livers ligated. Three rats were used per treatment and all animals were killed and the tissues removed 60 min after isotope administration. The experimental procedure in this experiment was similar to that in the previous study with the following exceptions. The kidneys of the rats with ligated kidneys were removed prior to administration of the isotope. The lungs were removed at autopsy in addition to the tissues removed in experiment 1. The precipitated protein was solubilized with 2 mL of NCS solubilizer (Nuclear-Chicago Corporation, Des Plaines, Ill.) and was counted by liquid scintillation counting in 15 mL of toluene scintillation fluid.

In order to confirm the presence of $^{14}$C in arginine with the protein, a sample of protein which had been isolated from the spleen of 2-hr control rats in experiment 1 was washed 2 times with 10% TCA, then dried with acetone and ether. A sample of the protein was hydrolyzed in a vacuum-evacuated sealed tube with 6 N HCl (1 mL/mg protein) for 22 hr at 110 C. The HCl was then evaporated, distilled water added followed by evaporation, and then the sample was dissolved in 0.25 mL lithium citrate-lithium chloride buffer, pH 2.2. The amino acids present in the hydrolysate were determined using a modified Beckman model 120 B amino acid analyzer which was connected in series (between column and Ninhydrin mixer) to a flow cell of a Packard liquid scintillation spectrometer, model 2002.

**RESULTS**

The validity of the experimental approach of using the total tissue radioactivity as a measure of the extent of conversion of citrulline to arginine was demonstrated by measuring the specific activity of the amino acids of a sample of hydrolyzed proteins. Arginine was the only radioactive amino acid present in the protein as a result of the prior intravenous injection of L-citrulline-ureido-$^{14}$C.

The extent to which the L-citrulline-ureido-$^{14}$C was converted to labeled arginine by control rats and rats with ligated kidneys and/or livers is indicated by the results shown in Tables 1 and 2. Ligation of the kidney resulted in a decrease in the specific activity of skeletal muscle protein to 11-15% of the 1-hr controls, whereas ligation of the liver resulted in a decrease to 32-59% of the 1-hr-control values. The specific activity of skeletal muscle protein was 2.8-3.8 times higher when the liver was ligated than when the kidneys were ligated.

When the kidneys were ligated a decrease to 37-48% of the 1-hr controls was observed in the specific activity of the liver protein; whereas, when the liver was ligated a decrease to only 63-92% of the 1-hr controls occurred in the specific activity of kidney protein; in neither case was the decrease significant. A decrease to 55% of the 1-hr controls was observed in the specific activity of muscle protein (Table 2) when the kidneys were ligated, whereas no decrease was observed when the liver was ligated.

Little or no decrease in specific activity of brain protein was observed when the liver was ligated; whereas the specific activity decreased to 38-54% of the 1-hr controls when the kidneys were ligated.

### Table 1. Radioactively labeled arginine incorporation into tissues of rats injected with $^{14}$C (experiment 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Avg Time of Incorporation, min</th>
<th>Liver dpm/mg protein</th>
<th>Kidney dpm/mg protein</th>
<th>Muscle dpm/mg protein</th>
<th>Brain dpm/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact controls</td>
<td>3</td>
<td>60</td>
<td>155±8 (100)</td>
<td>910±45 (100)</td>
<td>46±5 (100)</td>
<td>109±8 (100)</td>
</tr>
<tr>
<td>Ligated kidneys</td>
<td>5</td>
<td>79</td>
<td>72±18 (48)</td>
<td>13±2.1 (1)</td>
<td>7±3.1 (13)</td>
<td>11±2 (11)</td>
</tr>
<tr>
<td>Ligated livers</td>
<td>5</td>
<td>67</td>
<td>3±0.3 (2)</td>
<td>840±95 (59)</td>
<td>27±3 (59)</td>
<td>151±7 (130)</td>
</tr>
<tr>
<td>Ligated kidneys and livers</td>
<td>5</td>
<td>57</td>
<td>3±0.4 (2)</td>
<td>9±0.5 (1)</td>
<td>3±3.5 (6)</td>
<td>72±13 (66)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values in parentheses indicate the relative change based on the 1-hr control values being 100.

### Table 2. Radioactively labeled arginine incorporation into tissues of rats injected with L-citrulline-ureido-$^{14}$C (experiment 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver dpm/mg protein</th>
<th>Kidney dpm/mg protein</th>
<th>Muscle dpm/mg protein</th>
<th>Brain dpm/mg protein</th>
<th>Lung dpm/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact controls</td>
<td>254±26 (100)</td>
<td>1049±109 (100)</td>
<td>79±9 (100)</td>
<td>144±12 (100)</td>
<td>287±15 (100)</td>
</tr>
<tr>
<td>Ligated kidneys</td>
<td>93±25 (37)</td>
<td>0* (0)</td>
<td>9±3 (11)</td>
<td>35±3 (38)</td>
<td>157±33 (55)</td>
</tr>
<tr>
<td>Ligated livers</td>
<td>1±0.2 (&lt;0.4)</td>
<td>663±185 (65)</td>
<td>25±6 (32)</td>
<td>135±12 (94)</td>
<td>367±38 (120)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Three animals per group. All animals killed at 60 min postadministration of isotope. Values in parentheses indicate the relative change based on the control values being 100. * Kidneys removed prior to isotope administration.

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ARGinine SYNTHESIS BY KIDNEY AND LIVER

DISCUSSION

The results of this study lend strong support to the conclusions of Rogers et al. (10) that the kidney plays a major role in the arginine synthesis of the rat. These workers postulated that the liver produces more citrulline than it can convert to arginine and most of the arginine produced by the liver is converted to urea as a result of the high activity of liver arginase (11, 12). It has been regularly observed that much of the arginine that is produced in the liver is broken down to urea and ornithine as a result of the high activity of liver arginase (11, 12). Rogers et al. (10) suggested that part of the citrulline produced in the liver leaves via the bloodstream and is taken by the kidney where it is converted to arginine. The ability of the kidney to synthesize arginine from citrulline has been reviewed by Ratner (7).

Szepesi and co-workers (13) and Rogers et al. (10) have recently shown that conditions in the kidney are more favorable than those present in liver for arginine synthesis. They have reported that the kidney possesses a lower arginase-to-arginine synthetase ratio and that two-thirds of the kidney arginase is located in the kidney cortex, whereas 85% of the arginase synthetase is present in the medulla; this contributes to a favorable environment in the kidney for arginine synthesis (13). The arginine synthesized in the kidney could be used for protein synthesis or transported via the blood for protein synthesis in other tissues.

The present study demonstrates that ligation of the kidney results in considerably less labeled arginine being incorporated into skeletal muscle than when the liver was ligated. The specific activity of skeletal muscle from rats with ligated livers was on the average 3.3 times higher than when the kidneys were ligated. Likewise, a greater decrease in the specific activity of lung and liver protein was observed when the kidneys were ligated than when observed in lung and kidney protein when the liver was ligated. These results are at point to the greater contribution of kidney as compared to liver in the synthesis of arginine for tissue proteins. A preliminary experiment using L-leucine-3H indicates that the differences observed between the ligation observed in lung and kidney protein when the liver was ligated was on the average 3.3 times higher than when the kidneys were ligated. Likewise, a greater activity of liver arginase (11, 12). It has been regularly observed that much of the arginine that is produced in the liver is broken down to urea and ornithine as a result of the high activity of liver arginase (11, 12). Rogers et al. (10) suggested that part of the citrulline produced in the liver leaves via the bloodstream and is taken by the kidney where it is converted to arginine. The ability of the kidney to synthesize arginine from citrulline has been reviewed by Ratner (7).

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The specific activities of brain are of interest as a result of the observation that ligation both the liver and kidney resulted in a decrease of only 34% whereas skeletal muscle decreased by 94% as compared to the 1-hr controls (Table 1). These results provide supporting evidence to the findings of Ratner et al. (8) that the brain possesses arginosuccinate synthetase and arginosuccinate lyase and that it can synthesize some of the arginine it needs for protein synthesis. The low amount of labeled arginine present in the muscle of the doubly ligated rats suggests, however, that little of the arginine synthesized in the brain is released for use by other tissues.

The limited ability of the kidney to form citrulline from ornithine (4) in contrast with the extensive ability of the liver (6) demonstrated the importance of the liver to the total-body synthesis of citrulline. Recent measurement of citrulline production by perfused rat liver clearly illustrates this point. It has been observed that the capacity of the rat liver for citrulline production approaches that for glucose production (5). The studies of Gornall and Hunter (6) indicated that the conversion of citrulline to arginine was the rate-limiting step in liver. While the rat kidney contains lower activities of all urea cycle enzymes than liver, the arginase-to-arginine synthetase ratio lower than that in liver (10) and the differences in distribution of the enzymes in the medulla and cortex of the kidney (13) create a more favorable environment for arginine synthesis per se than occurs in liver. The specific activities of arginine synthetase in acetone-dried tissue extracts from liver and kidney of the pig and ox have been reported to be similar (9) indicating that the kidney may play an even greater role in the net arginine synthesis of these species than that demonstrated in these studies with the rat.

These findings demonstrate another example of the close synergetic relationship between the liver and kidney. Thus, it appears that the liver produces citrulline at a more rapid rate than arginine and that most of the arginine produced is further converted into urea and ornithine. The kidney, on the other hand, utilizes citrulline produced by the liver for the synthesis of arginine which is mainly used by the kidney and other tissues of the body for protein synthesis.

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