Mechanism of Hepatic Assimilation of Dipeptides

TRANSPORT VERSUS HYDROLYSIS*

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To investigate dipeptide assimilation by the liver, a series of interrelated experiments were performed in rats. Partial hepatectomy prolonged the plasma half-life (min) of Gly-Ala (3.42 ± 0.22 versus 4.90 ± 0.25, p < 0.05) but had no significant effect on plasma half-life of Gly-Leu, Gly-Pro, or Gly-Sar. We then investigated the rate of disappearance (μmol·(g liver·h)^{-1}) of the above four dipeptides (initial concentration = 1 mM) from the medium during isolated liver perfusion. The order of dipeptide disappearance was: Gly-Leu (8.75 ± 0.65) > Gly-Ala (3.36 ± 0.46) > Gly-Pro (1.29 ± 0.54) > Gly-Sar (0.35 ± 0.12). This order of dipeptide disappearance corresponded exactly to the order of the rates of glycine accumulation in the medium during liver perfusion with the four dipeptides. Addition of glucagon had no effect on the disappearance rate of Gly-Ala from the medium, but reduced accumulation rates of glycine (3.39 ± 0.30 versus 1.42 ± 30, p < 0.01) and alanine (4.42 ± 0.66 versus 1.35 ± 0.39, p < 0.01). Finally, we found that hydrolysis by the liver plasma membranes and/or perfusion medium accounted for disappearance of dipeptides. In conclusion, the liver does not appear to have a transport system for dipeptides, but assimilates dipeptides by extracellular hydrolysis. Hydrolysis is achieved by enzymes either located on the plasma membranes or released from the cytosol. The amino acid residues released as the result of dipeptide hydrolysis are then taken up by the liver.

Dipeptides in the gut lumen produced as the result of protein digestion or direct administration may escape hydrolysis by intestinal mucosa and reach portal blood in intact form (1–3). Whether these dipeptides are assimilated by the liver has not yet been adequately investigated. There is considerable evidence that tissues, such as intestine and kidney, assimilate dipeptides (4–7). The mechanisms involved include (a) dipeptide uptake by peptide carrier systems followed by intracellular hydrolysis by cytoplasmic peptidases and (b) dipeptide hydrolysis by plasma membrane peptidases followed by uptake of amino acid residues by amino acid carrier systems (4–7).

The objective of the present experiment was to investigate hepatic assimilation of dipeptides by three different approaches, one in vivo and the others in vitro. First, we investigated the clearance of dipeptides from plasma in rats when 70% of the liver was removed. Second, we investigated the assimilation of dipeptides in isolated perfused rat liver. Third, we investigated subcellular distribution of peptidase activity in the liver.

The model substrates chosen for these studies were glycyl-L-alanine (Gly-Ala), glycyl-L-leucine (Gly-Leu), glycyl-L-proline (Gly-Pro), and glycylsarcosine (Gly-Sar). Glycyl-dipeptides were chosen since previous studies showed that glycine is generally superior to other amino acids as the N-terminal amino acid residue for decreasing dipeptide hydrolysis in plasma, thereby allowing more substrates to be available for a tissue peptide-transport system (8). Glycylalanine and glycyleucine were selected to determine whether hepatic metabolism of glycyl-dipeptides would differ if they contained gluconeogenic (alanine) or nongluconeogenic (leucine) amino acid residues in the C terminus. The selection of glycylnproline and glycylsarcosine was based on their previous use as model substrates for establishing dipeptide transport in both intestine and kidney (9–12).

EXPERIMENTAL PROCEDURES

Animals—Male Sprague-Dawley rats (Zivic-Miller Laboratories, Allison Park, PA) weighing 250–300 g were used for in vivo and isolated liver perfusion studies and rats weighing 150–200 g were used for hepatic subcellular fractionation studies. For in vivo studies rats had free access to Purina Chow and water until 1 h prior to the experiment. Rats used for isolated liver perfusion and subcellular fractionation studies were fasted overnight.

In Vivo Studies—Rats were anesthetized with ether and weighed. The abdomen was shaved, a 4-cm midline abdominal incision made, and the median and left lateral lobes of the liver were exteriorized. These lobes were ligated with silk sutures as near to the vena cava as possible and then resected. In sham-operated animals the same incision was made, the liver was exteriorized for 2 min but not resected. After the operation the abdominal wall was closed with silk sutures and the animals were given 6 h to recover from surgery, during which time they had free access to water. Then the animals were again anesthetized with ether and the right carotid artery was cannulated with a Teflon catheter (Quik-Cath 20-gauge, Travenol Laboratories, Deerfield, IL) for drawing blood. The catheter was secured with silk sutures and 0.2 ml of saline containing heparin (200 units) was injected.

Then the penis vein was injected with a test solution containing Gly-Sar, Gly-Pro, Gly-Ala, Gly-Leu, or indocyanine green. Dipeptide test solutions contained one of the above dipeptides (150 mM) in 75 mM NaCl. The amount of each dipeptide injected was 0.5 μmol/g, body weight. All dipeptides were purchased from Sigma. Blood was taken via the catheter immediately before and 1, 2, 5, and 10 min after injection, and was immediately centrifuged at 12,800 × g for 1 min in an Eppendorf microcentrifuge. Plasma was removed and deproteinized with an equal volume of 6% sulfosalicylic acid. Sulfosalicylic acid extracts were stored at −20 °C until analysis. For the indocyanine green study, 4 μg of indocyanine green/g, body weight, was injected and blood was taken 1, 5, and 10 min after injection.

Isolated Liver Perfusion—In situ perfusion of isolated livers was carried out by a modification of the method of Hems et al. (13). One hundred ml of perfusion medium (Krebs-Ringer bicarbonate buffer,
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PH 7.4, containing 2 g of bovine serum albumin (Pentex bovine albumin Fraction V, Miles Laboratories, Naperville, IL) and 10 µg of ampicillin) were recirculated at a constant rate of 36 ml/min. The medium was oxygenated by means of a membrane oxygenator using 5-an elastic tubing (inner diameter, 1.67 mm; outer diameter, 1.96 mm; Dow Corning Corp, Midland, MI).

After equilibration for 30 min, the perfusate was replaced with fresh perfusate containing 10 mM pyruvate and 1 mM peptide. The reason for the selection of 1 mM dipeptide concentration was that a previous study (3) had shown this concentration to be within the range of glycol-Dipeptide concentrations found in the mesenteric vein. Perfusion was continued for 60 min. During this time, studies of peptide disappearance and amino acid accumulation as well as viability of the preparation were performed.

Samples for peptide and amino acid analysis (0.5 ml) were taken every 10 min. The samples were immediately deproteinized with an equal volume of 6% sulfosalicylic acid and centrifuged for 1 min at 12,800 × g. After centrifugation for 1 h, liver tissue was freeze-clamped, homogenized with 6% sulfosalicylic acid (100 mg/ml), and centrifuged at 500 × g for 10 min. The supernatant was stored at -20 °C until assayed. Plasma and cytochrome oxidase were assayed with the same method described for plasma.

Results of investigations were performed on dipeptides used in this study. The animals were prepared in the same way for incubation with each peptide. The peptide was assayed at 500 µl of cold saline until they blanched. All subsequent steps were performed continuously (0.7 ml/min) into the medium during perfusion.

Viability of the perfused livers was demonstrated by bile production of 1.52 ± 0.38 µl (g of liver·min)⁻¹, glucose production from pyruvate of 0.51 ± 0.045 µmol·(g of liver·min)⁻¹, and release of enzymes into the medium (e.g., esterases, gluta-mic-oxalo-acetic transaminase). These values represent mean ± S.E. of 25 perfusions and are comparable to those found by others (14, 15).

Additionally, livers were perfused with a mixture of alanine and glycine (1 mM each); uptake of these amino acids was studied for 60 min. In three perfusions alanine and glycine uptake were 4.21 ± 1.72 and 3.76 ± 0.53 µmol·(g of liver·hr)⁻¹, respectively. These data are comparable to the results of Lacy (16) obtained under similar conditions.

Subcellular Fractionation—Rats were killed by decapitation. Livers were excised intact and perfused through the portal vein with ice-cold saline until they blanched. All subsequent steps were performed at 4 °C. Livers were weighed, minced with scissors, homogenized with 10 strokes in a 40-ml Dounce homogenizer with a loose-fitting pestle in 5-fold volume of 0.25 M sucrose, 1 mM MgCl₂, 5 mM Tris-HCl, pH 7.4, and filtered through 4 layers of gauze. This homogenate was centrifuged at 1,500 × g for 10 min. The supernatant was centrifuged for 1 h at 105,000 × g to yield the cytosol. Plasma membranes were prepared by the method of Hubbard et al. (17). Homogenates, cytosol, and plasma membranes were stored in liquid nitrogen until assayed for enzyme activity.

The yield of plasma membranes was 1.12 ± 0.13 mg of protein/g wet weight, liver; enrichment of 5'-nucleotidase was 15-fold that of the homogenate. Contamination with microsomes and mitochondria was assessed by measuring the activity of glucose-6-phosphatase and cytochrome c oxidase, respectively; enrichment of these enzymes was 1- and 0.07-fold compared with the homogenate. Recovery in the plasma membrane fraction of 5'-nucleotidase, glucose-6-phosphatase, and cytochrome c oxidase was 10 ± 1%, 7.4 ± 0.05%, and 0.05 ± 0.05%, respectively, in three preparations. These results are in agreement with previous reports in which plasma membranes were prepared by a similar technique (17).

Peptide Hydrolyase Activity—Plasma of partially hepatectomized and sham-operated rats was assayed for enzyme activity against the dipeptide. The animals were prepared in the same way for the dipeptide injection study, but without receiving the dipeptide. Plasma was separated from blood cells by centrifugation and stored at -20 °C until assayed. Plasma (25 µl) was incubated with 500 µl of 5 mM dipeptide in 50 mM Tris-HCl, pH 8.0, at 37 °C for 21 min. The reaction was stopped by adding 525 µl of 6% sulfosalicylic acid. The supernatant was separated by centrifugation and used for determination of concentration of free constituent amino acids.

Samples of perfusion medium were removed after 60 min of peptide-free perfusion for incubation with each peptide. The peptide hydrolylase activity in the perfusion medium and subcellular fractions was assayed with the same method described for plasma.

Optimal substrate concentration and linearity of the reaction with respect to time and enzyme concentration were established in preliminary experiments. Appropriate blanks were assayed for each substrate and enzyme source. Preliminary experiments showed that perfusion medium lacked peptide hydrolylase activity before the beginning of liver perfusion.

Analysis—Amino acids and dipeptides were analyzed by a Beckman amino acid analyzer (Model 119 CI) or by an HPLC method (18, 19). Indocyanine green concentration in serum was measured with a Zeiss spectrophotometer at 806 nm. Protein concentration was measured by the method of Lowry et al. (20). The activities of 5'-nucleotidase, cytochrome c oxidase, and glucose-6-phosphatase were assayed by methods previously described (21-23).

Calculations and Statistics—The plot of log plasma concentration of dipeptides after intravenous injection versus time yielded a biphasic curve with a steeper slope between 1 and 2 min. A biphasic curve is a common finding in pharmacokinetic studies of substrate elimination from plasma. Such a curve has been explained by a two-compartment model (24). The first part of the curve represents mainly distribution of the substance injected, whereas the second part (β-slope) represents the metabolic clearance (24). Since we were interested in the metabolic clearance, only the terminal linear portion of the curve from 2 to 10 min was used to calculate the half-lives of the dipeptides.

The rates (µmol·(g of liver·hr)⁻¹) of dipeptide disappearance and amino acid accumulation in the isolated liver perfusion medium were calculated by the difference between initial and final concentrations of the substance injected, whereas the second part (β-slope) represents the metabolic clearance.

RESULTS

Effects of Hepatectomy—To assess the role of liver in metabolism of dipeptides in vivo, we investigated the effect of partial hepatectomy on plasma concentrations and half-life of dipeptides. The impairment of hepatic function by 70% hepatectomy was assessed by injection of indocyanine green from plasma. Such a curve has been explained by a two-compartment model (24). The first part of the curve represents mainly distribution of the isolated liver perfusion medium (ml); the product was divided by the wet weight of liver (g). Data are presented as means ± S.E. The statistical analysis of the data was made by analysis of variance and Student's t test. Differences were considered statistically significant if p < 0.05.

Plasma concentrations of dipeptides between 1 and 10 min after the injection of each dipeptide in sham-operated and hepatectomized rats are shown in Fig. 1. Except for Gly-Ala, there was no significant difference between concentrations of dipeptides in plasma of sham-operated and hepatectomized rats at any time interval after the injection. At 5 and 10 min after the injection, the concentration of Gly-Ala was significantly lower in the plasma of hepatectomized rats than in the plasma of sham-operated rats.

The abbreviation used is: HPLC, high performance liquid chromatography.
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Protein concentration was not different between control and hepatectomized rats. Hepatectomy had no significant effect on plasma half-life (min. mean ± S.E. in 5–7 rats) of Gly-Sar (13.53 ± 1.06 versus 13.57 ± 1.58), Gly-Pro (5.57 ± 0.25 versus 6.05 ± 0.36), or Gly-Leu (2.02 ± 0.13 versus 2.33 ± 0.23). However, hepatectomy significantly (p < 0.05) increased the plasma half-life of Gly-Ala (3.42 ± 0.22 versus 4.90 ± 0.35).

To determine whether a selective alteration in plasma peptide hydrolase activity accounted for the increase in plasma half-life of Gly-Ala in partially hepatectomized rats, we measured peptide hydrolase activity against all four dipeptides (Table I). Partial hepatectomy was without a significant effect on plasma peptide hydrolase activity against any of the dipeptides.

After injection of Gly-Leu and Gly-Ala there were large increases in plasma concentrations of glycine and leucine and glycine and alanine, respectively. The plasma levels of these amino acids progressively decreased during the 10-min interval after the injection. Injection of Gly-Pro and Gly-Sar modestly increased plasma concentrations of glycine and proline and glycine and sarcosine, respectively.

To investigate whether hepatectomy affects the assimilation of amino acid residues of dipeptides, we compared plasma concentrations of amino acids during the 10-min interval after dipeptide injection in sham-operated and partially hepatectomized rats. The most pronounced differences between plasma amino acid concentrations of the two groups of rats occurred at 10 min. These data are summarized in Fig. 2. Partial hepatectomy resulted in higher plasma concentrations of both glycine and alanine after the injection of Gly-Ala. The concentration of glycine was also significantly higher in hepatectomized than sham-operated rats after injection of Gly-Leu, whereas there was no difference in leucine concentrations between the two groups. After injection of Gly-Pro and Gly-Sar, no differences were found in the plasma concentrations of constituent amino acids between sham-operated and hepatectomized rats (data not shown).

**Dipeptide Assimilation by Isolated Liver**—The results of in vivo studies in partially hepatectomized rats suggested that liver plays a selective role in assimilation of dipeptides. To investigate this suggestion we determined the rates of disappearance of the four dipeptides from the medium after 60 min of liver perfusion (Fig. 3). Dipeptides disappeared at distinctly different rates. The order of dipeptide disappearance was as follows: Gly-Leu > Gly-Ala > Gly-Pro > Gly-Sar; all differences were statistically significant. The rates of disappearance of Gly-Pro and Gly-Sar were considerably smaller than the rates of disappearance of the other two dipeptides.

**Table I**

*Peptide hydrolase activity in plasma and liver perfusion medium*

Blood was drawn 6 h after sham operation (control) or partial hepatectomy; data are presented as mean ± S.E. in five rats. Plasma protein concentration was not different between control and hepatectomy. Perfusion medium was sampled after 60 min of perfusion with peptide-free medium; data are presented as mean ± S.E. in nine livers. Plasma or perfusion medium (25 μl) was incubated for 20 min at 37°C with 500 μl of 50 mM Tris-HCl, pH 8.0, and 5 mM peptide substrate. Amino acids released by hydrolytic activity were quantitated by HPLC.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Plasma</th>
<th>Liver perfusion medium</th>
</tr>
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<tbody>
<tr>
<td>Gly-Leu</td>
<td>Control: 3.79 ± 0.90</td>
<td>4.14 ± 0.46</td>
</tr>
<tr>
<td>Gly-Ala</td>
<td>7.46 ± 0.84</td>
<td>8.22 ± 1.01</td>
</tr>
<tr>
<td>Gly-Pro</td>
<td>7.69 ± 0.80</td>
<td>8.74 ± 1.07</td>
</tr>
<tr>
<td>Gly-Sar</td>
<td>0.96 ± 0.24</td>
<td>0.65 ± 0.22</td>
</tr>
</tbody>
</table>

To investigate whether dipeptide uptake by hepatocytes accounted for the disappearance of dipeptides from the perfusion medium, we investigated the concentration of Gly-Sar in the liver after 60 min of liver perfusion with this dipeptide. There was accumulation of Gly-Sar in the liver tissue. However, when Gly-Sar concentration in the liver tissue was corrected for the concentration in the extracellular fluid by the method previously described (25), there was no accumulation of Gly-Sar in the intracellular fluid.

To determine whether hydrolysis accounted for the disappearance of dipeptides from the medium we investigated the concentration of Gly-Sar in the intracellular fluid. After injection of Gly-Leu and Gly-Ala there were large increases in plasma concentrations of glycine and leucine and glycine and alanine, respectively. The plasma levels of these amino acids progressively decreased during the 10-min interval after the injection. Injection of Gly-Pro and Gly-Sar modestly increased plasma concentrations of glycine and proline and glycine and sarcosine, respectively.

The addition of Gly-Ala, and particularly Gly-Leu, greatly increased (p < 0.01) the accumulation rate of glycine in the perfusion medium. In contrast, Gly-Pro and Gly-Sar had no
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Rates of amino acid accumulation in liver perfusion medium

Perfusion medium was sampled after 60 min of liver perfusion with the peptide noted. Amino acid concentrations were quantitated using a Beckman amino acid analyzer. Values represent mean ± S.E. of five experiments.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Glycine (µmol·g liver h⁻¹)</th>
<th>Valine (µmol·g liver h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.15 ± 0.21</td>
<td>1.63 ± 0.18</td>
</tr>
<tr>
<td>Gly-Leu</td>
<td>5.24 ± 0.85</td>
<td>1.51 ± 0.28</td>
</tr>
<tr>
<td>Gly-Ala</td>
<td>3.38 ± 0.30</td>
<td>1.41 ± 0.15</td>
</tr>
<tr>
<td>Gly-Pro</td>
<td>1.57 ± 0.22</td>
<td>1.54 ± 0.31</td>
</tr>
<tr>
<td>Gly-Sar</td>
<td>0.87 ± 0.18</td>
<td>1.38 ± 0.06</td>
</tr>
</tbody>
</table>

*Significantly different from none at p < 0.01.

**FIG. 4. Effect of glucagon on dipeptide disappearance and amino acid accumulation in liver perfusion medium.** Glucagon was infused into the perfusion medium (0.7 nmol/min). Gly-Ala was added to an initial concentration of 1 mM. Disappearance of the peptide and accumulation of glycine and alanine were measured after 60 min of perfusion. Values represent mean ± S.E. of four experiments. Solid bars, without glucagon; hatched bars, with glucagon.

**DISCUSSION**

The main objective of the present series of experiments was to determine whether liver assimilates dipeptides. The nutritional and biological significance of these studies is underscored by the fact that dipeptides may enter the portal vein after protein digestion (3), and hereditary and acquired disorders of peptide hydrolysis in tissues may result in dipeptide release to systemic circulation (26, 27).

In our initial experiment we investigated the effect of partial hepatectomy on plasma half-lives of dipeptides. It was assumed that if the liver plays a significant role in metabolism of circulating dipeptides, then partial hepatectomy should prolong the half-lives of dipeptides. This assumption was based on our observation that partial hepatectomy prolonged half-life of indocyanine green and impaired metabolism of glycine and alanine without affecting metabolism of leucine (Fig. 2). The elimination of indocyanine green from plasma is largely a function of liver, and glycine and alanine are sub-

**TABLE III**

Peptide hydrolyase activity in subcellular fractions of rat liver

Homogenates, cytosol, and plasma membranes were prepared as described under "Experimental Procedures." Hydrolyase activity was assayed by incubating a suitably diluted aliquot of each fraction (10–20 μg of protein) with 500 μl of 5 mM peptide substrate, 50 mM Tris-HCl, pH 8.0, for 30 min at 37°C. Amino acids released were quantitated by HPLC. Values represent mean ± S.E. of three different preparations.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Homogenate (µmol protein min⁻¹ mg⁻¹)</th>
<th>Cytosol (µmol protein min⁻¹ mg⁻¹)</th>
<th>Plasma membranes (µmol protein min⁻¹ mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly-Leu</td>
<td>465 ± 58</td>
<td>765 ± 49</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Gly-Ala</td>
<td>157 ± 2</td>
<td>341 ± 22</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>Gly-Pro</td>
<td>33 ± 2</td>
<td>49 ± 3</td>
<td>0</td>
</tr>
<tr>
<td>Gly-Sar</td>
<td>32 ± 8</td>
<td>43 ± 10</td>
<td>0</td>
</tr>
</tbody>
</table>
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strates for hepatic gluconeogenesis. In contrast, metabolism of leucine is largely accomplished in peripheral tissues (28). The result of the experiment on the effect of heptectomy on plasma half-lives of dipeptides suggested that liver plays a selective role in clearance of dipeptides from plasma. Hepatectomy prolonged the plasma half-life of Gly-Ala, but had no significant effect on the half-life of Gly-Leu, Gly-Pro, or Gly-Sar. In view of the presence of hydrolase activity against dipeptides in plasma (29), a simple explanation for this varied effect could have been that heptectomy selectively altered this activity. This explanation was eliminated by lack of any significant effect of heptectomy on plasma hydrolase activity against any of the four dipeptides (Table I).

To investigate whether, indeed, liver plays a selective role in dipeptide clearance, in the next step in our experimental design we investigated assimilation of the above four dipeptides by isolated perfused liver. The results showed that liver assimilates all four dipeptides but with vastly different rates (Fig. 3). In comparison to Gly-Ala and particularly Gly-Leu, the disappearance rates of Gly-Pro and Gly-Sar from the perfusion medium were quite small.

The following lines of evidence suggested that dipeptide disappearance from the perfusion medium occurred largely by hydrolysis rather than by uptake. (a) As noted above, there was very little assimilation of Gly-Pro and Gly-Sar, which are resistant to hydrolysis. (b) The order of rates of accumulation of amino acid residues of dipeptides in the perfusion medium (Table II) corresponded to the order of disappearance of dipeptides (Fig. 3). (c) There was no intracellular accumulation of Gly-Sar in the liver. In the face of large cytosol hydrolase activity (Table III), Gly-Leu and Gly-Ala would not be expected to accumulate intracellularly even if they entered hepatocytes as dipeptides. However, Gly-Sar should have been found in the intracellular fluid if there was appreciable transport of this dipeptide by the liver.

It is possible that the failure to observe any evidence for dipeptide uptake by the isolated perfused liver might have been related to the absence of any hormone in the perfusion medium. To consider this possibility, we investigated the effect of glucagon on the disappearance rate of Gly-Ala. We chose glucagon because previous studies had shown glucagon to be a potent hormone in stimulating amino acid uptake by perfused rat liver (30). Glucagon had no significant effect on the disappearance rate of Gly-Ala, but greatly reduced accumulation of glycine and alanine in the perfusion medium (Fig. 4). The sources of amino acids in the medium during the liver perfusion with dipeptides include amino acids released by the liver and amino acids produced as a result of dipeptide hydrolysis. We found that the presence of dipeptides or glucagon in the perfusion medium had no effect on the release of amino acids by the liver. Therefore, the reduced accumulation of glycine and alanine during the isolated liver perfusion with glucagon appears to be the result of stimulation of hepatic uptake of glycine and alanine released from hydrolysis of Gly-Ala in the medium.

The final step in our present investigation was to determine whether hydrolysis by the liver plasma membrane and the perfusion medium accounted for disappearance of dipeptides. Liver plasma membranes have been previously demonstrated to contain enzymes, including peptidases (31). The results showed membrane-bound hydrolase activity against two of the peptides used, namely, Gly-Leu and Gly-Ala (Table III). No activity was found in the plasma membrane against Gly-Pro and Gly-Sar. In contrast, the perfusion medium contained hydrolase activity against all four dipeptides (Table I). In search of the source of dipeptidase activity against Gly-Pro and Gly-Sar, we investigated dipeptide hydrolysis by the whole liver homogenate and cytosol. The cytosol, unlike the plasma membrane, had hydrolase activity against all four dipeptides. The cytosol accounted for most of the activity found in whole homogenate and the order of activities was the same in homogenate and cytosol. The same order of hydrolase activities was also found in the liver perfusion medium indicating that the hydrolases in the medium were released from the cytosol. The membrane-bound hydrolase activity is not likely to be a contamination by soluble enzymes because (a) it could not be removed by washing the membranes with buffer and (b) the order of hydrolase activities was different from the order in the cytosol. In cytosol, hydrolase activity against Gly-Leu was 2-fold higher than against Gly-Ala, whereas in membranes, activity was highest against Gly-Ala.

In conclusion, liver, unlike intestine (4, 5) and kidney (7), does not appear to have a transport system for dipeptides. This conclusion is based on the hepatic assimilation of selected dipeptides. Since the investigation of all possible dipeptides would have been impractical, some dipeptides had to be selected for our experiments. We chose glycyldipeptides because previous studies (8) had shown that among dipeptides glycyldipeptides are more likely to be substrates for a peptide-transport system in the liver if indeed such a system existed. Nevertheless, a definitive conclusion regarding presence or absence of a peptide-transport system in the liver must await investigation of other dipeptides.

Despite apparent absence of a transport system, the isolated perfused liver assimilates dipeptides by extracellular hydrolysis. In the case of some dipeptides the hydrolysis is effected by cytosolic enzymes released into the perfusion medium. In addition, some dipeptides are hydrolyzed by membrane-bound hydrolases. The amino acid residues released as the result of dipeptide hydrolysis are then transported into the liver by amino acid carriers. Finally, the fact that heptectomy prolonged the plasma half-life of Gly-Ala without affecting that of Gly-Leu may be related to the removal of a higher liver membrane hydrolase activity against Gly-Ala than Gly-Leu. The failure of heptectomy to alter plasma half-lives of Gly-Pro and Gly-Sar is consistent with the isolated liver perfusion data, which showed minimal assimilation of these dipeptides by the liver. The assimilation of these dipeptides appear to be accomplished largely by the kidney (8, 32). Indeed, bilateral nephrectomy greatly prolongs the plasma half-life of Gly-Sar (33).

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REFERENCES
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