FREE AMINO ACIDS IN PLASMA AND MUSCLE FOLLOWING TOTAL REMOVAL OF THE LIVER*

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In 1924 and 1926 Bollman, Mann, and Magath showed that when the liver is removed the formation of urea ceases (1) and the concentration of free amino acids gradually increases in the plasma, tissues, and urine of the dog (1, 2). Techniques are now available for the determination of the individual amino acids which might be involved. By means of microbiologic or chemical techniques Svec (3) has found that five amino acids, tryptophan, lysine, glutamic acid, alanine, and glycine, increase in the plasma of the dog after hepatectomy, while two, methionine and histidine, decrease. By means of paper chromatography we have found an increase in the same five amino acids studied by Svec and also in ten additional amino acids or derivatives in the plasma of the dehepatized dog. We have also noted some changes in the concentration of amino acids in muscle, but in general these changes in muscle were much less distinct than in the plasma when studied with this technique.

EXPERIMENTAL

Studies were made of eleven dogs, weighing from 12 to 20 kilos, which were dehepatized by the three-stage technique of Bollman, Mann, and Magath (1). The dogs recovered from the ether anesthesia and were up and about during most or all of the period studied. They were given procaine penicillin in oil and were maintained by continuous intravenous injection of a solution of glucose at the rate of from 0.1 to 0.3 gm. per kilo per hour. Samples of blood and of muscle from the adductor group were removed at the time of hepatectomy. At the end of the experiment, 17 to 31 hours after hepatectomy, blood was taken and the contralateral muscle was removed for analysis.

Studies were also made of three control dogs which were treated as nearly as possible like the dehepatized dogs except that total hepatectomy was not performed. Samples of blood and muscle and a lobe of liver were removed. Each control dog was then given a continuous injection of a solution of glucose at the same rate and for the same period of time.

* Read at the meeting of the American Chemical Society, Chicago, September 3-8, 1950.
as the dehepatized dogs, at the end of which time final samples of blood and muscle were also taken.

The plasma was separated by centrifugation, and the protein was removed by dialysis in Visking cellulose sausage casing immersed in 2 volumes of water. The muscles were frozen immediately with solid carbon dioxide, then crushed later between chilled steel blocks. The tissue was transferred to the cellophane tubes with 2.5 volumes of water, and the tubes were immersed in 2.5 volumes of water (4). The tubes were revolved on a ball mill frame for 4 hours in the cold room and then allowed to stand overnight. 15 ml. aliquots of the dialysates were desalted in 15 to 20 minutes by the method of Consden, Gordon, and Martin (5) and were concentrated in vacuo to a small volume. Measurements were made, by the gasometric ninhydrin method of Hamilton and Van Slyke (6), of the concentration of free amino acids in desalted dialysates and in picric acid extracts of the original plasma. These showed that the method of preparation of the plasma did not entail an appreciable loss in total amount of the free amino acids.

Stein and Moore\(^1\) have found large losses of arginine (7) and slight losses of some other amino acids after electrolytic desalting for periods of approximately 2\(\frac{1}{2}\) to 3\(\frac{1}{2}\) hours. In a 30 minute period of desalting they found 21 per cent of the arginine converted to ornithine. Our values for concentration of free amino acids of muscle were 10 to 15 per cent higher in desalted dialysates than in picric acid extracts, probably owing to liberation of some amino acids during dialysis or desalting.

Two-dimensional paper chromatograms (8) were developed by the ascending technique (9) with paper cylinders of Whatman filter paper No. 1 or No. 4 standing in Petri dishes in 20 gallon stone crocks. The sheets were first developed in a 75 per cent solution of phenol, purified according to the method of Draper and Pollard (10) and containing sodium cyanide, and in an ammoniacal atmosphere. The sheets were dried in the early experiments partly at room temperature, then at 80° for 10 to 15 minutes; in later experiments they were dried overnight at room temperature (11, 12). They were then developed in a 65 per cent solution of lutidine in an atmosphere of diethylamine. The sheets were dried and sprayed with a 0.25 per cent solution of ninhydrin in saturated butanol containing 1 per cent glacial acetic acid.

\textit{Results}

Paper chromatograms of plasma removed before and after hepatectomy show clearly that many amino acids or derivatives increase in concent-

\(^1\) We are grateful to Dr. Stein and Dr. Moore for the opportunity of seeing their manuscript prior to publication.
tration in the dehepatized dog. The characteristic pattern of amino acids of plasma appears to be maintained, with a general intensification of each spot, indicative of an increased concentration of each amino acid, as shown in Fig. 1. In this typical experiment, phenylalanine and tyrosine were distinctly visible as grayish green spots in the final sample of plasma, but these were not visible in a 0.2 ml. aliquot of the control plasma. The yellow spot of proline, which does not photograph well, was also visible after hepatectomy but not before. Spots representing valine, lysine, and arginine, alanine, threonine, taurine, glutamine, glycine, serine, and glutamic acid were all more prominent in the plasma after hepatectomy than before it. Glutamine, which occurs in the greatest abundance in the normal plasma, made the most prominent spot in the chromatogram of the plasma of the dehepatized dog.

The increase of phenylalanine and tyrosine was found in all eleven dehepatized dogs studied; increases in the other amino acids were found in from five to ten of these dogs (Table I). Actually increases in all, or all but one, of these fourteen amino acids were found in from five to ten of
the dogs. This improvement in the number of intensified spots seen probably reflects improvements in the technique of making the chromatograms rather than differences in the dog. An increase in histidine, which was found by Svec to increase after hepatectomy, was also noted by us when 0.4 ml. samples of plasma were used for the chromatograms.

**Table I**

*Plasma Amino Acids Definitely Increased after Hepatectomy*

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>No. of dogs</th>
<th>Amino acid</th>
<th>No. of dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>11</td>
<td>Alanine</td>
<td>10</td>
</tr>
<tr>
<td>Leucine</td>
<td>8</td>
<td>Threonine</td>
<td>5</td>
</tr>
<tr>
<td>Valine</td>
<td>8</td>
<td>Glutamine</td>
<td>9</td>
</tr>
<tr>
<td>Proline</td>
<td>10</td>
<td>Taurine</td>
<td>7</td>
</tr>
<tr>
<td>Arginine</td>
<td>10</td>
<td>Glycine</td>
<td>9</td>
</tr>
<tr>
<td>Lysine</td>
<td>10</td>
<td>Serine</td>
<td>10</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>11</td>
<td>Glutamic acid</td>
<td>10</td>
</tr>
</tbody>
</table>

* Paper chromatograms were made from the plasma of eleven dogs in this series 17 to 31 hours after total removal of the liver. The concentration of none of these amino acids appeared decreased. It is also probable that technical difficulties were responsible for our failure to observe increases in all cases.

**Table II**

*Plasma Total \(\alpha\)-Amino Nitrogen*

<table>
<thead>
<tr>
<th>Before hepatectomy</th>
<th>After hepatectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg. per 100 ml.</td>
<td>hrc.</td>
</tr>
<tr>
<td>3.8</td>
<td>27</td>
</tr>
<tr>
<td>6.2</td>
<td>21</td>
</tr>
<tr>
<td>3.1</td>
<td>25</td>
</tr>
</tbody>
</table>

Controls

| 3.7 | 27 | 3.2 |
| 5.5 | 22 | 4.8 |
| 3.6 | 25 | 3.2 |

No increase in concentration of the amino acids was noted in three dogs before and after the control operation.

Determinations of the total \(\alpha\)-amino nitrogen of the plasma by the ninhydrin method of Hamilton and Van Slyke were made on three de-hepatized and three control dogs (Table II). A 2- to 3-fold increase was found after hepatectomy in the total concentration of amino acids in the plasma, but no increase was found in the controls, from which only one lobe of the liver had been removed. Likewise glutamine determination
by the method of Hamilton (13) showed values of approximately 1 mg. of glutamine α-amino nitrogen per ml. in the control plasma, with in-

TABLE III
Effect of Intravenous Injection of Hydrolyzed Protein on Level of Amino Acids*

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Sample No.</th>
<th>Total α-amino N mg. per 100 ml.</th>
<th>Glutamine α-amino N mg. per 100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>3.24</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.14</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.04</td>
<td>1.32</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>4.08</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.67</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.94</td>
<td>1.74</td>
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<tr>
<td>3</td>
<td>1</td>
<td>3.94</td>
<td>1.77</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.81</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.54</td>
<td></td>
</tr>
</tbody>
</table>

* A casein hydrolysate, free of glutamine, was given at a rate of 50 mg. of nitrogen per kilo per hour for 2 hours. Sample 1, before injection; Sample 2, at the end of injection; Sample 3, 80 to 90 minutes after the end of injection.

Fig. 2. Chromatograms of muscle taken before and 25 hours after heptectomy. Aliquots corresponding to 200 mg. of muscle were applied at spot X. Spots, in addition to those noted from plasma, are 15, carnosine; 16, aspartic acid; 17, unidentified.

creases to as high as 4.6 and 5.4 mg. after heptectomy. Thus the most prominent spot on the chromatogram represented about 40 per cent of the total α-amino nitrogen.

Two normal dogs and one nephrectomized dog were injected with an
Acid hydrolysate of casein \(^2\) free of glutamine to produce an increase in the amino acid content of the plasma of similar magnitude to that found after hepatectomy. Under these conditions there was no increase in the glutamine content of the plasma (Table III).

Chromatograms of muscle before and after hepatectomy showed much less contrast than was found in plasma (Fig. 2). The aliquots used represented 200 mg. of muscle for ease of comparison with the 200 \(\mu l\). aliquots of plasma. Since determination of free \(\alpha\)-amino nitrogen of muscle by the ninhydrin manometric method shows values 6 times that of plasma, the chromatograms are much more intense. The most distinct increases after hepatectomy were noted in phenylalanine and tyrosine. Phenylalanine increased in all nine dehepatized dogs from which muscle biopsies were taken; tyrosine increased in eight of these; and leucine and valine appeared to increase in seven dogs. The glutamine and taurine spots were each considerably larger after total hepatectomy than before it in three of these dogs. However, it is apparent that much more certainty is involved in a gross observation of a change from a barely visible to a distinctly visible spot, as with phenylalanine or tyrosine, than of changes in very large spots, as with taurine or glutamine. Changes of physiologic importance may be occurring regularly in more amino acids than phenylalanine, tyrosine, leucine, and valine, but they have not been determined with any degree of certainty.

**Comment**

The pattern of distribution of free amino acids in the plasma, as shown by paper chromatography, is quite distinct from that of the muscle in the normal dog, and this distinction is maintained even when the concentration of amino acids in plasma increases 2- to 3-fold within 24 hours after total hepatectomy. This suggests that amino acids from extrahepatic tissue, most of which is muscle, are liberated into the plasma after hepatectomy in about the same proportion as before. Since fourteen amino acids and taurine were observed to increase in concentration after hepatectomy, the liver probably normally removes all of these from the blood, and at least at a rate comparable to the rate of increase found in the absence of the liver. Since glutamine normally occurs in larger quantities than any other one amino acid in blood, it appears to be the amino acid which the liver normally takes from the blood in largest quantity.

Glutamine is an amino acid which is known to be involved in the production of urea. Its presence in liver slices is essential for the conversion of ornithine to citrulline, as shown by Cohen and Grisolia (14) and others; it accelerates the conversion of citrulline to arginine in such preparations.

\(^2\) Elamine, Interchemical Corporation, Union, New Jersey.
either directly or indirectly, as proposed by Ratner and Pappas (15). It increases the output of urea in urine when fed to guinea pigs and dogs, and to a much greater extent than most other amino acids, as shown by Leuthardt and Glasson (16) and Kamin and Handler (17). Furthermore, 62 per cent of the amide nitrogen of the glutamine administered is excreted within 24 hours as urea by the rat, as shown by Berenbom and White (18).

Muscle contains a large amount of glutamine (19), and it seems probable that the increased glutamine content of the plasma following removal of the liver represents the diffusion of this substance from the muscle rather than an extrahepatic conversion of other amino acids to glutamine. Injections of amino acids into animals with a functioning liver does not produce any increase in the glutamine content of the plasma.

The increase of the amino acids which occurs following removal of the liver appears to be that portion liberated from protein which would be converted in the liver to urea, glucose, or ketone bodies, none of which are formed in appreciable amounts in the absence of the liver. A portion of the accumulated amino acids also represents some which would be resynthesized to protein in the liver were it present. These experiments provide no satisfactory data as to the amount of extrahepatic breakdown and resynthesis of protein except to indicate that excess of amino acids continues to escape from the extrahepatic tissues. The mixture of amino acids which reaches the blood appears to be very similar to the mixture present in the blood under usual fasting conditions. No single amino acid accumulates at the expense of other amino acids of the mixture. If interconversion of amino acids occurs in the absence of the liver, such interconversion reaches the same equilibrium relations as are found in normal plasma.

SUMMARY

Paper chromatography was used to study the distribution of free amino acids in the plasma before and after total removal of the liver. The characteristic chromatogram of the amino acids of the plasma of the normal dog was maintained after hepatectomy, but each spot was intensified, indicating an increase in concentration. All fifteen amino acids or derivatives observed were found to increase, and further studies may show changes in more. This suggests that the liver normally removes all of these amino acids from the blood and at least at a rate comparable to that of the increase found after removal of the liver. Glutamine, the most abundant amino acid in the plasma, is probably normally removed by the liver in greatest quantity. The mixture of amino acids which enters the blood after hepatectomy appears to be similar in composition to that found in normal blood.
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