It was shown by Seth and Luck (1) that the intestinal absorption of glycine and alanine by rabbits was followed by pronounced aminoacidemia. Other amino acids when administered in similar amounts provoked smaller increases in the amino nitrogen content of the blood. Glutamic acid and aspartic acid were absorbed with but a slight aminoacidemia.

The question then arose as to whether these striking differences in behavior were due to equally profound differences in the rates of absorption from the alimentary canal, in the rates of diffusion into liver, muscle, and other tissues, and in the rates of metabolism of the amino acids therein. It may be recalled that Bang (2) demonstrated marked inequalities in the absorption of various amino acids by isolated fragments of liver suspended in solutions of these substances. A not unlikely explanation of the results of Seth and Luck would seem to be that the glycine-alanine aminoacidemia was due to slow diffusion of the substances into liver and muscle, or a low rate of oxidation of the metabolites, or both. In contrast, the failure of glutamic and aspartic acids to provoke an appreciable increase in the amino acid content of the blood might be attributed to their rapid absorption therefrom.

According to such a theory it might be expected that the ingestion of glycine and alanine would cause but a small increase in the amino acid content of liver and muscle, or a slow conversion of the amino nitrogen to urea. On the other hand glutamic acid might be expected to increase more markedly the amino acid content of liver and muscle or to lead to more rapid urea formation than is observed with glycine and alanine. Finally one may advance the view that the differences in the degree of aminoacidemia may be due in part to specific differences in the excretion of amino acids by the kidney.
In this paper a report is given of changes in the amino acid content of blood, liver, and muscle following the oral administration of amino acids. The object of the work was to determine the relationship if any which existed between the degree of aminoacidemia obtained and the concentration increase of the amino acids in liver and muscle. The findings proved to be of a somewhat unexpected nature.

Experimental.

Male albino rats of 160 to 220 gm. in weight (except in Experiments 55 and 58) were employed throughout. The actual weights of the animals in gm. on the day of experiment were as follows: Experiment 19, 160, 164, 180, 164; Experiment 21, 188, 206, 188, 196; Experiment 23, 200, 180, 188, 180; Experiment 25, 198, 220, 188, 190; Experiment 27, 210 to 220; Experiment 29, 194, 190, 198, 199; Experiment 31, 210 to 218; Experiment 33, 190 to 210; Experiment 35, 200 to 220; Experiment 39, 200 to 220; Experiment 41, 190 to 210; Experiment 44, 160 to 170; Experiment 48, 200 to 210; Experiment 50, 180 to 200; Experiment 52, 155 to 160; Experiment 55, 120 to 151; Experiment 58, 128 to 147.

The animals were maintained on a standard diet and fasted for 24 hours preceding an experiment. The animals were used in groups of four or five. The experimental material was administered by stomach tube (a No. 8 French catheter was used) after which the animals were killed at intervals of approximately 0, 0.8, 2, 4, and 6 hours from the time of administration. The rats were killed by stunning, and rapid incision of the thorax. The blood which drained away was collected in a crucible containing powdered potassium oxalate. It was found necessary to stun the animal by a sharp blow in the mid-cervical region. If the blow were received on the head, profuse bleeding took place through the nose. Such blood was rejected in view of the possibility that it might have come in contact with the mouth which would probably contain drippings from the stomach tube. If the blow were received in the thoracic region, intravascular blood clotting was observed to proceed with such rapidity that only a drop or two of semiclotted blood would drain from the incision.
from the hind limbs were promptly excised and frozen by immersion in liquid air. The amino nitrogen contained in these samples was determined by the method described in the preceding paper (3). The amino nitrogen content of the blood was determined by the method of Folin (4).

The dicarboxylic acid fraction of hydrolyzed caseinogen, the monoamino monocarboxylic acid fraction of hydrolyzed caseinogen, totally hydrolyzed egg albumin, $dl$-aspartic acid, $d$-glutamic acid, glycine, and $dl$-alanine, were used. The last four were obtained from the Eastman Kodak Company. From acid-hydrolyzed caseinogen the monoamino monocarboxylic acids were obtained by the butyl alcohol extraction method of Dakin (5), and the dicarboxylic acids by precipitation of the calcium salts (6) from the non-extractable portion. The residue represented the hexone base fraction. The sulfuric acid-barium hydroxide method was employed in hydrolysis of the egg albumin.

Each rat received 3 cc. of an aqueous, neutralized, solution of the experimental substance equivalent in concentration to 0.2, 0.3, or 0.4 gm. of amino nitrogen per kilo.

It will be seen that by this procedure any questionable secondary effects resulting from prolonged anesthesia and the use of surgical methods are avoided. The animal is in a normal state throughout the experiment. The metabolism of the amino acids proceeds normally in every tissue. Within 2 minutes of the killing of the animal the samples are frozen and postmortem autolytic changes are prevented.

It is also apparent that the method of group experimentation permits one to make a number of successive analyses at suitably spaced intervals of time. This cannot be done by the single animal method without employing anesthetics and resorting to troublesome if not questionable operative means. It is evident moreover if smooth continuous curves be obtained by the series method when time is plotted against the tissue concentration of the metabolite, that each animal serves as a confirmatory check against its neighbors in the series. It is almost unnecessary to point out that the use of small animals is no mean consideration when costly amino acids are to be administered.
Amino N, mg. per 100 cc. blood.

CHART 1A.

Amino N, mg. per 100 gm. liver.

CHART 1B.

Amino N, mg. per 100 gm. muscle.

CHART 1C.

CHART 1. Experiment 19, water; Experiment 23, glycine; Experiment 27, dicarboxylic acids (□——□□); Experiment 31, hexone bases (X——X); Experiment 35, monoamino acids (·—···); Experiment 39, alanine (X——X).
Amino N, mg. per 100 cc. blood.

Chart 2 A.

Amino N, mg. per 100 gm. muscle.

Chart 2 B.

Amino N, mg. per 100 gm. liver.

Chart 2 C.

Chart 2. Experiment 21, water; Experiment 25, glycine; Experiment 29, dicarboxylic acids; Experiment 33, hexone bases; Experiment 41, alanine; Experiment 44, totally hydrolyzed albumin; Experiment 48, alanine.
Metabolism of Amino Acids

Chart 3. Experiment 50, totally hydrolyzed albumin; Experiment 52, aspartic acid; Experiment 55, monoamino acids; Experiment 58, glutamic acid. 
Results.

Amino Acid Content of Blood, Liver, and Muscle.—The accompanying curves are self-explanatory. Charts 1 A, 1 B, 1 C represent the results of experiments in which the substances were administered in quantities of 0.20 gm. of amino nitrogen per kilo.

### TABLE I.

Ammonia Content of Liver and Muscle.

<table>
<thead>
<tr>
<th>Experimental substance</th>
<th>Tissue</th>
<th>Ammonia N, mg. per 100 gm. tissue.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time after administration.</td>
<td>0 hr.</td>
</tr>
<tr>
<td>Water</td>
<td>Muscle.</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>Liver.</td>
<td>18.5</td>
</tr>
<tr>
<td>Water</td>
<td>Muscle.</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td>Liver.</td>
<td>17.4</td>
</tr>
<tr>
<td>Glycine, 0.10 gm. N per kilo.</td>
<td>Muscle.</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>Liver.</td>
<td>19.5</td>
</tr>
<tr>
<td>Glycine, 0.38 gm. N per kilo.</td>
<td>Muscle.</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>Liver.</td>
<td></td>
</tr>
<tr>
<td>Dicarboxylic acid fraction, 0.20 gm. N per kilo.</td>
<td>Muscle.</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>Liver.</td>
<td>18.7</td>
</tr>
<tr>
<td>Dicarboxylic acid fraction, 0.40 gm. N per kilo.</td>
<td>Muscle.</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>Liver.</td>
<td>16.3</td>
</tr>
<tr>
<td>Hexone base fraction, 0.20 gm. N per kilo.</td>
<td>Muscle.</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>Liver.</td>
<td>14.3</td>
</tr>
<tr>
<td>Hexone base fraction, 0.80 gm. N per kilo.</td>
<td>Muscle.</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>Liver.</td>
<td>18.4</td>
</tr>
<tr>
<td>Monoamino acid fraction, 0.20 gm. N per kilo.</td>
<td>Muscle.</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>Liver.</td>
<td>16.9</td>
</tr>
<tr>
<td>Alanine, 0.20 gm. N per kilo.</td>
<td>Muscle.</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>Liver.</td>
<td>17.1</td>
</tr>
<tr>
<td>Alanine, 0.40 gm. N per kilo.</td>
<td>Muscle.</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>Liver.</td>
<td>17.0</td>
</tr>
</tbody>
</table>
Metabolism of Amino Acids

of rats. In Charts 2 A, 2 B, 2 C the quantities were 0.40 gm. of amino nitrogen, and in Charts 3 A, 3 B, 3 C, 0.30 gm. of amino nitrogen per kilo. There are, however, the following exceptions:

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>gm. per kg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>0.19, amino N</td>
</tr>
<tr>
<td>25</td>
<td>0.38, &quot; &quot;</td>
</tr>
<tr>
<td>33</td>
<td>0.80, total &quot;</td>
</tr>
<tr>
<td>44</td>
<td>0.47, amino &quot;</td>
</tr>
</tbody>
</table>

Ammonia Content of Liver and Muscle.—It proved convenient in some of the experiments to determine the amounts of ammonia evolved during the period of boiling in alkaline solution required by this analytical method. The ammonia was collected in dilute sulfuric acid and estimated by Nesslerizing. The results are presented in Table I.

The ammonia content of liver and muscle showed no appreciable change or but slight increases following the absorption of amino acids. The absolute ammonia values were invariably greater in liver than in muscle, being 14.3 to 22.5 mg. per cent in liver and 10.8 to 15.0 mg. per cent in muscle. These values agree approximately with those reported by others (7). We are not satisfied, however, that the methods employed in this work or in that of any others known to the writer, give ammonia values of much significance. It is probable (cf. Gad-Andresen, 1919; Warburg et al., 1924; Meyerhof, 1925; Grafe, 1925) that the same scrupulous care needs to be exercised in such estimations as is known to be indispensable in determining the ammonia content of blood.

DISCUSSION.

The most striking result of these experiments is to be seen in the absence of any appreciable increase in the amino nitrogen content of muscle following the oral administration of amino acids.

To this generalization, glycine alone is exceptional. Following the oral administration of this substance the amino nitrogen content of muscle increases slowly and uniformly, this increase proceeding at an unchanged rate after the maximum increases in the blood and liver have been observed (Charts 2 A, 2 B, 2 C). Whatever additional significance these relative rates of change may have, certain it is that the accumulation of glycine in muscle
proceeds much more slowly than it does in the liver. This is
doubtless accountable in part by the method of administration,
—all of the absorbed amino acids having to pass through the liver
before entering the systemic circulation. It is not even im-
probable that the pronounced difference in the behavior of the
whole group of amino acids in liver and muscle is to be explained
similarly. We are examining this point experimentally by ad-
ministering these substances in other ways.

It is perhaps well to point out that the amino nitrogen increase
in muscle is not determined by the level of amino acids in blood.
This follows from the results obtained with alanine which caused
an equally marked aminoacidemia but no apparent increase in
muscle amino nitrogen. Nor is there any relationship between the
amino acid increase in liver and the behavior in muscle. Totally
hydrolyzed egg albumin increased the amino nitrogen content of
the liver almost as greatly as did glycine, but resulted in no appreci-
ciable change in the amino acid content of muscle (Charts 2 B,
2 C). The behavior of glycine in muscle is apparently specific for
that amino acid. It cannot be considered a representative mem-
ber of the products of protein hydrolysis and the deduction of
generalizations in protein metabolism from results obtained pri-
marily if not solely with glycine is to be cautioned against.

The remarkable differences in behavior of glycine and alanine
were quite unexpected. In view of their neighborly relationship
in a homologous series and the similar increases in the amino
nitrogen content of blood following their oral administration, it
was considered likely that they would induce the same measure
of change in liver and muscle. It is to be observed, however, that
while glycine induced a marked increase in the amino nitrogen
content of the liver, alanine caused but a slight and transient
increase. The contrast in their behavior in muscle has already
been pointed out. It is not likely, moreover, that results materi-
ally different would have attended the use of d-alanine instead
of the racemic mixture. For though it might be supposed that
d-alanine would be metabolized more or less rapidly than l-alanine,
it is very improbable that any pronounced increase in liver and
muscle amino nitrogen caused by one isomer would be completely
nullified by the other. It is to be observed in this connection that
the naturally occurring isomer of glutamic acid behaved quali-
tatively in muscle like the racemic alanine (Charts 2 C, 3 C).
It will have been noticed that though in muscle none of the amino acids except glycine increased the amino nitrogen content of that tissue, most of them elevated in some measure the amino nitrogen content of the liver. This in itself throws little light on the relative dominance of the roles of liver and muscle in protein metabolism. As has already been mentioned, the mode of administration of the experimental material almost certainly determines in part the muscle-liver picture. It might appear permissible, moreover, to regard muscle as being so efficient in the metabolism of absorbed amino acids that it succeeds in maintaining its amino nitrogen content at the normal level. This sort of explanation is discredited by the vast amount of evidence, much of it of great weight, concerning the locus of amino acid catabolism. Most of this regards but poorly the urea-forming power of muscle. As for the anabolic change, the rapid synthesis of protein from the absorbed material, it is difficult to see how this could proceed from a single amino acid. Finally, a quite improbable explanation would be to regard muscle as being impermeable to all amino acids but glycine. One would then be driven to exercise unusual ingenuity to account for the formation of the muscle proteins.

It is somewhat premature to advance an explanation for the profound inequalities in the rates of increase of the various amino acids in blood and liver. Additional information must first be had concerning the excretion of these substances by the kidney, their rates of oxidation, and the differences if any in their rates of absorption from the alimentary canal. With respect to the last mentioned point we have reason to believe that glycine, alanine, glutamic acid, and aspartic acid are absorbed at much the same rate from an isolated intestinal loop of the dog (1). It will also be noticed that all of the amino acid preparations employed in this work are readily soluble in water,—a consideration which is pertinent to the question of their absorption.

A discussion of these experiments cannot be complete without reference to the closely related work of others.

It appears as a first consideration that the amino acid content of animal tissues fluctuates normally within rather narrow limits. Fasting appears to be without effect, as demonstrated by Van Slyke and Meyer (8) on dogs and by Mitchell, Nevens, and Kendall (9) on the entire carcasses of rats after fasting for 19 to
26 hours. We too have never observed any change in the amino acid content of the whole animal or in the muscles and livers of rats as a result of fasting 1 or 2 days. Buglia and Costantino (10) in a few experiments on dogs observed slightly higher values for the non-urea, non-protein nitrogen of muscle after fasting periods of 12 to 25 days. It does not necessarily follow, however, that the amino acids were similarly increased in quantity. The analytical method employed by these investigators (desiccation of the samples at 70-80° and formal titration of the extract) is also open to criticism.

Neither do high nor low protein diets appear to alter very markedly the concentration of amino acids in tissues. Mitchell, Nevens, and Kendall report experiments of 11 to 48 days duration in which rats were maintained on nitrogen-free diets without

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Amino N, mg. per 100 gm. tissue.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle.</td>
</tr>
<tr>
<td>1</td>
<td>78.9</td>
</tr>
<tr>
<td>2</td>
<td>76.0</td>
</tr>
<tr>
<td>3</td>
<td>72.6</td>
</tr>
</tbody>
</table>

change in the amino acid content of the animals. Kiech (unpublished data) in this laboratory has observed that high and low protein diets administered to rats for 2 day periods are productive of small but certain differences. Thus twenty-six rats which had been on a starch-butter diet for 2 days contained an average of 47.4 mg. of amino nitrogen per 100 gm. of tissue. All values fell between 40.2 and 51.8. Similarly nine rats on a high protein diet (87 per cent caseinogen) for 2 days gave corresponding values of 50.6 (47.1 to 53.5).

There is, however, an indication as shown in Table II that some factors presumably of dietary origin may influence profoundly the amino acid content of liver and muscle. The group of rats reported in Table II was of the same age, size, and sex as the animals used in all the other experiments. They had been fasted for 24 hours before analysis. They came from another colony and
were related as second or third cousins to one section of our own stock. They had, however, been maintained since weaning on a very different and much more varied basal diet. This group, the only foreign one examined, has been the only one to give abnormal basal values. Mitchell (7) has also reported on adult rats which gave, so it seems to us, unusually high amino acid values.

The amino acid content of muscle is increased following hepatectomy (11).

Apart from changes induced by the ingestion of proteins or their products of hydrolysis, the amino acid content of blood is maintained at quite a constant level. In acute yellow atrophy of the liver (12), myelogenous leucemia (13–15), possibly in pernicious anemia (16), in polycytemia (17), and in hydrazine poisoning (18), the amino acid content of the blood is greater than normal. It is also probable that in disturbances of carbohydrate metabolism of pancreatic origin (13, 19), the amino acid content of the blood is altered.

Finally reference may be made to other experiments on the effect of protein or amino acid administration on the amino acid content of muscle and liver. The feeding of large quantities of meat to dogs was found by Wishart (20) to be without influence on the non-urea, non-protein nitrogen of the muscle. This agrees with our own observations on the fate of ingested amino acids. In the well known experiments of Van Slyke and Meyer (21), alanine and hydrolyzed caseinogen were injected intravenously in dogs. Cathcart (22) administered glycine to dogs by the same means. Although the results of these experiments are in agreement with our own in so far as they indicate changes of greater magnitude and rapidity in the amino acid content of liver than in muscle, they differ with respect to the absolute increases observed. This is, however, to be expected in view of the different mode of administration of the experimental materials, and the important differences in subsequent treatment of the animal and analysis of the tissue samples.

The interesting experiments of Lombroso, Artom, Paterni, and Luchetti (23) on the entrance of amino acids into the perfused liver, muscle, and kidney are hard to interpret because of the conflicting results obtained with defibrinated blood and Ringer's solution respectively.
Experiments similar in method are now in progress to study the entrance of the amino acids into liver and muscle after subcutaneous injection, and to determine the differences, if any, in their rates of oxidation, in vivo.

Part of this work was done in the Biochemical Laboratory of the University of Toronto.

SUMMARY.

1. When amino acids are administered to rats, per os, and in equimolecular amounts, increases of varying magnitude are observed in the amino acid content of liver, but no appreciable change, except with glycine, is observed in the amino acid content of muscle.

2. Although glycine and alanine increased in the same measure the amino acid content of the systemic blood, the former provoked a great increase in the amino nitrogen content of liver, while the latter caused no significant change.

3. In most cases no appreciable changes were observed in the ammonia content of liver and muscle.

BIBLIOGRAPHY.

17. Luck, J. M., unpublished data.
THE METABOLISM OF AMINO ACIDS
James Murray Luck


Access the most updated version of this article at http://www.jbc.org/content/77/1/13.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/77/1/13.citation.full.html #ref-list-1