AMINO ACID ABSORPTION AND UTILIZATION IN THE CHICK

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(Received for publication, December 27, 1943)

The rates of absorption of amino acids from the gastrointestinal tract have been reported for the rat (1) and the levels of blood amino nitrogen during amino acid absorption have been reported for the rat, dog, and rabbit (2-7), but no studies of this nature have been reported for the chick. In view of the known differences between avian and mammalian nitrogen metabolism (8), it seemed desirable to study amino acid absorption and utilization in the chick. It is the purpose of this paper to present data concerning amino acid absorption in the chick and changes in blood amino nitrogen during the amino acid absorption. These data are compared with those for mammals which have been reported in the literature.

EXPERIMENTAL

The rates of absorption of amino acids from the gastrointestinal tract of the chick were determined by the Cori technique (9, 10). All of the experiments were conducted with single comb white Leghorn chicks which had been previously fed a normal chick diet. The chicks were fasted for 48 hours before the amino acid administration, but were allowed water ad libitum. The majority of the chicks weighed between 150 and 250 gm. at the end of the 48 hour fast.

The amino acids used were commercial preparations. With the exception of tyrosine, all of the amino acids were administered in solutions containing 12 to 31 mg. of amino nitrogen per cc. To establish uniform conditions, an attempt was made to administer the amino acids after half neutralization with sodium hydroxide. When this was impossible, the amino acid was administered in a more soluble form, either as the complete sodium salt, or as the hydrochloride. Tyrosine, which was not soluble enough to give the desired concentration even as the sodium salt, was administered as a well shaken suspension. The concentration of each solution was determined by both micro-Kjeldahl and amino nitrogen analyses. The amino acids in solution were administered directly into the crop by means of a calibrated syringe to which a piece of No. 6 catheter tubing was

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1 The amino acids were purchased with funds donated by the Nutrition Foundation, Inc.
attached. The tyrosine suspension was administered to the crop by means of a serological pipette which was washed out after the administration to determine the tyrosine remaining on it. During the absorption period, the vent was clamped with a paper clamp (Hunt clip No. 0) to prevent any excretion.

To determine whether material injected into the crop passed quickly into the absorbing portions of the gastrointestinal tract, a solution of methylene blue was administered to two fasted chicks. The first chick was killed after 5 minutes and the methylene blue was found in the crop, proventriculus, and gizzard. The second chick was killed after 10 minutes and the methylene blue was found as far as half-way along the small intestine. Henry et al. (11) found that in a fasted chick the crop discharges material into the gizzard almost immediately.

All values of the absorption rate were obtained for a 3 hour absorption period. At the end of 3 hours the chick was killed by dislocating the neck, and the esophagus was tied near the pharynx. The entire gastrointestinal tract was then removed and frozen in a refrigerator. Upon removal from the refrigerator, the exterior of the gastrointestinal tract was carefully washed with warm water and the mesentery was removed. The tract was then opened, washed four times with distilled water, and the volume made up to 500 cc. An aliquot of this solution was diluted with an equal volume of 10 per cent trichloroacetic acid to precipitate the proteins, which were then removed by filtration.

Amino nitrogen was determined in the filtrate by the method of Folin (12) as modified by Danielson (13). The method was further modified by making all of the determinations in 1.66 per cent trichloroacetic acid. Before procedure with the standard method, each sample was neutralized to the phenolphthalein end-point with sodium hydroxide. All of the readings were made between 20 and 22 hours after addition of the sodium β-naphthoquinone-4-sulfonate reagent and 8 minutes after the bleaching reagents. A Cenco photometer with Cenco Filter 2 (green) was used for making the readings. A standard curve with the amino nitrogen concentration plotted against its equivalent photometer reading was used in converting the photometer reading to amino nitrogen values.

Weighed samples of the amino acids used in the study were analyzed by both micro-Kjeldahl and amino nitrogen methods. The values obtained for amino nitrogen were used in calculating the absorption rate in terms of the particular amino acid studied.

To eliminate the turbidity which occurred when large amounts of tryptophane were present, the solutions were filtered just before the photometer readings were made, and a separate standard curve was prepared for tryptophane with filtered solutions. The amino nitrogen of the contents
of the gastrointestinal tract of chicks with and without the administration of tryptophane was calculated as tryptophane from the standard curve for this amino acid. The tryptophane absorption rate was determined for three chicks by this modification of the Folin method.

In order to find the normal amino nitrogen content of the gastrointestinal tract of fasted chicks, distilled water instead of amino acid solution was given to twenty-three chicks during the course of the study. The chicks were killed 3 hours after the administration of 1 cc. of water per 100 gm. of body weight. The residual amino nitrogen values varied between 4.66 and 10.04 mg. per 100 gm. of body weight. The mean value of 7.43 ± 1.65 mg. of amino nitrogen per 100 gm. of body weight was used for all calculations of absorption rate.

To compare the amino nitrogen method of determining the amino acid with a specific amino acid method, the absorption rate of methionine was determined by measuring the unabsorbed methionine by the amino nitrogen method previously described, and by the specific methionine method of McCarthy and Sullivan (14). Methionine determinations on the contents of the gastrointestinal tract of fasted chicks showed that no measurable amount of methionine was present. The specific methionine method eliminates the use of a figure for residual amino nitrogen and should give a truer absorption value for an individual chick. The average methionine absorption value obtained by the amino nitrogen method was 45.6 mg. per 100 gm. of body weight per hour, compared with the value of 42.6 mg. obtained by the specific methionine method. The rate of absorption, even as determined by the specific methionine method, showed considerable variation among chicks. For the whole series, the two methods of determining the rate of methionine absorption showed satisfactory agreement.

Blood samples were obtained from chicks immediately before and 1 hour after the administration of amino acid. 1 cc. samples were taken by heart puncture, with potassium oxalate as an anticoagulant, and blood filtrates were prepared with trichloroacetic acid as a precipitant. Amino nitrogen was determined on these blood filtrates by the same method as was employed on the gastrointestinal tract contents. Taking both initial and 1 hour blood samples from each chick enabled a more accurate comparison of changes in the blood amino nitrogen after the administration of different amino acids than would have been possible if only one sample had been taken. A preliminary experiment showed 1 hour to be the time interval during which blood amino nitrogen increased most after amino acid administration. Initial and 1 hour blood samples were also taken from the chicks to which water was administered. The 1 hour value varied between 79 and 101 per cent of the initial value, and its mean was 88.6 ± 5.5 per cent of the initial value.
Intact protein was studied from the standpoint of changes in the blood amino nitrogen over a 5 hour period. The effect of hydrolysis of protein was investigated by comparing solutions of intact casein and gelatin with acid hydrolysates of these proteins. The method of Caldwell and Rose (15) was used in preparing the protein hydrolysates.

Results

The data obtained for the absorption rates and blood amino nitrogen for the amino acids and amino acid mixtures used are summarized in Table I. The absorption rate for amino acid (Column 1) is the value obtained

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Salt</th>
<th>No. of chicks</th>
<th>Absorption rate (quantity per 100 gm. body weight per hr.)</th>
<th>Blood amino N as per cent of initial</th>
<th>Concentration of solution administered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amino acid (1) Amino acid (2) Amino acid (3)</td>
<td></td>
<td>mg. amino N per cc.</td>
</tr>
<tr>
<td>d,l-Alanine</td>
<td>Half Na</td>
<td>7</td>
<td>6.02 mg. 43.0 mg. 0.486 mg. 121 mg. 12.4 mg.</td>
<td>31 mg.</td>
<td></td>
</tr>
<tr>
<td>l(+)-Arginine</td>
<td>HCl*</td>
<td>8</td>
<td>1.54 mg. 27.0 mg. 0.155 mg. 95 mg. 5.8 mg.</td>
<td>12 mg.</td>
<td></td>
</tr>
<tr>
<td>l(+)-Aspartic acid</td>
<td>Half Na</td>
<td>7</td>
<td>2.11 mg. 37.6 mg. 0.216 mg. 95 mg. 7.1 mg.</td>
<td>30 mg.</td>
<td></td>
</tr>
<tr>
<td>l(-)-Cystine</td>
<td>Na</td>
<td>12</td>
<td>5.03 mg. 47.6 mg. 0.198 mg. 101 mg. 4.3 mg.</td>
<td>26 mg.</td>
<td></td>
</tr>
<tr>
<td>l(+)-Glutamic acid</td>
<td>Half Na</td>
<td>9</td>
<td>3.58 mg. 45.5 mg. 0.309 mg. 96 mg. 7.4 mg.</td>
<td>27 mg.</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>&quot;</td>
<td>6</td>
<td>2.44 mg. 30.9 mg. 0.210 mg. 95 mg. 6.2 mg.</td>
<td>39 mg.</td>
<td></td>
</tr>
<tr>
<td>l(-)-Histidine</td>
<td>HCl</td>
<td>10</td>
<td>2.51 mg. 42.3 mg. 0.273 mg. 103 mg. 8.8 mg.</td>
<td>20 mg.</td>
<td></td>
</tr>
<tr>
<td>l(-)-Leucine</td>
<td>Na</td>
<td>5</td>
<td>3.64 mg. 37.2 mg. 0.284 mg. 118 mg. 4.6 mg.</td>
<td>22 mg.</td>
<td></td>
</tr>
<tr>
<td>d,l-Lysine</td>
<td>Half Na</td>
<td>6</td>
<td>4.95 mg. 23.7 mg. 0.182 mg. 111 mg. 7.6 mg.</td>
<td>30 mg.</td>
<td></td>
</tr>
<tr>
<td>d,l-Methionine</td>
<td>Na</td>
<td>7</td>
<td>3.82 mg. 45.6 mg. 0.306 mg. 120 mg. 11.2 mg.</td>
<td>22 mg.</td>
<td></td>
</tr>
<tr>
<td>d,l-Phenylalanine</td>
<td>&quot;</td>
<td>7</td>
<td>4.72 mg. 56.2 mg. 0.340 mg. 102 mg. 4.9 mg.</td>
<td>24 mg.</td>
<td></td>
</tr>
<tr>
<td>l(-)-Proline</td>
<td>Half Na</td>
<td>10</td>
<td>5.50 mg. 49.8 mg. 0.433 mg. 110 mg. 14.8 mg.</td>
<td>25 mg.</td>
<td></td>
</tr>
<tr>
<td>d,l-Threonine</td>
<td>&quot;</td>
<td>9</td>
<td>4.05 mg. 36.5 mg. 0.306 mg. 109 mg. 9.6 mg.</td>
<td>29 mg.</td>
<td></td>
</tr>
<tr>
<td>d,l-Tryptophane</td>
<td>Na</td>
<td>3</td>
<td>1.49 mg. 21.8 mg. 0.107 mg. 95 mg. 3.2 mg.</td>
<td>20 mg.</td>
<td></td>
</tr>
<tr>
<td>l(-)-Tyrosine</td>
<td>&quot;</td>
<td>6</td>
<td>2.50 mg. 36.3 mg. 0.200 mg. 93 mg. 6.3 mg.</td>
<td>14 mg.</td>
<td></td>
</tr>
<tr>
<td>Glycine + d,l-alanine</td>
<td>Half Na</td>
<td>7</td>
<td>7.42 mg. 48.1 mg. 0.525 mg. 117 mg. 12.9 mg.</td>
<td>31 mg.</td>
<td></td>
</tr>
</tbody>
</table>

* Monohydrochloride.
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in terms of amino nitrogen by the modified Folin method. Column 2 shows the absorption rate for the free amino acid, calculated from Column 1 and the correction for the particular amino acid. In Column 3 the absorption rate is expressed as mM of the amino acid.

The data indicate that all of the amino acids apparently were readily absorbed from the gastrointestinal tract. The variation among individual chicks in the study of a single amino acid was appreciable in many cases; hence close comparison of the absorption rates is not justified. In an attempt to determine whether the level of amino acid administration affected the rate at which it was absorbed, glycine, glutamic acid, tyrosine, and arginine were administered at two levels. Glycine gave practically the same absorption rate at a high level of administration as it did at the usual level. Tyrosine and arginine gave higher absorption values with larger amounts administered, but glutamic acid gave a lower value. The variability was high in all cases and it is doubtful whether any significance can be attached to these comparisons.

To facilitate the comparison of the absorption rate of one amino acid with others, the millimolar absorption rate was plotted against the apparent molal volume. The values of apparent molal volume used were taken from the report of Cohn et al. (16), their observed values being used when given; when these were not available, the volumes were calculated according to their methods. When the amino acids were administered at more than one level, data for the level nearest 30 mg. of amino nitrogen per cc. were used. The plot of these data (Fig. 1) shows a general tendency for the molal absorption rate of the amino acid to increase as its apparent molal volume decreases. The correlation coefficient of this relationship is $-0.81 \pm 0.092$, which indicates that the relationship is significant.

The level of blood amino nitrogen found 1 hour after amino acid administration is dependent upon the amount of amino acid in the blood and the ability of the particular amino acid to give color with the Folin reagent. After water administration, the blood amino nitrogen is 89 per cent of the initial value; hence subtracting 89 from the percentage values for the amino acids (Table I) gives the relative increase of blood amino nitrogen caused by the amino acid studied. When this value is expressed as weight of the amino acid and divided by the molecular weight, the relative molar increase of the specific amino acid in the blood stream is obtained. In Fig. 2, this amino acid increase in the blood is plotted against the millimolar absorption rate from the gastrointestinal tract.

If all amino acids were removed from the blood stream at the same rate, it would be expected that a direct relationship would exist between the relative molar blood increase and the millimolar rate of absorption of the amino acid. The deviations from the direct relationship indicate that some amino acids are removed from the blood stream more rapidly than others.
From Fig. 2 it appears that glutamic acid, aspartic acid, tyrosine, and phenylalanine are rapidly removed from the blood stream, while methionine and leucine are removed from the blood stream more slowly than the other amino acids studied.

![Graph showing the relationship between millimolar absorption rate from the gastrointestinal tract and apparent molal volume.]

Fig. 1. The relation between the millimolar absorption rate of an amino acid from the gastrointestinal tract of the chick and the apparent molal volume of the amino acid.

The data for blood amino nitrogen for the studies with proteins and protein hydrolysates are summarized by Fig. 3. The blood amino nitrogen reached a higher level after the administration of a protein hydrolysate than after intact protein. Gelatin caused a greater increase in blood amino nitrogen than casein when the intact and hydrolyzed forms of the two proteins were compared.
Fig. 2. The relation between the relative molar increase of an amino acid in the blood and the millimolar rate of absorption of the amino acid from the gastrointestinal tract of the chick.

Fig. 3. Curves showing the blood amino nitrogen level in chicks after the oral administration of gelatin, gelatin hydrolysate, casein, casein hydrolysate, and water. Each plotted point represents an average value for three chicks for casein, casein hydrolysate, and gelatin hydrolysate; seven chicks for gelatin; and six chicks for water.
As a basis for discussion of the blood amino nitrogen for the chick, any 1 hour value which is greater than 89 per cent of the initial value is considered an increase in blood amino nitrogen. Water administration gave 1 hour values which were 89 per cent of the initial value.

In chicks, as in dogs, rabbits, and rats (24), glycine and alanine caused the greatest increase (129 or 121 per cent and 121 per cent respectively of the initial) in the blood amino nitrogen after oral administration. Leucine, which was reported by Seth and Luck (2) to give a moderate increase in rabbits, caused a relatively great increase (118 per cent of the initial) in the blood amino nitrogen of chicks. Histidine caused a moderate increase in the rabbit (2) and hexone bases caused a moderate increase in rats (4). Histidine also caused a moderate increase (103 per cent of the initial) in the chick. Aspartic acid and glutamic acid (2, 3) gave slight increases in the blood amino nitrogen in dogs and rabbits, and similar results (93 and 95 or 96 per cent respectively of the initial) were obtained with chicks.

In the rabbit, the blood amino nitrogen increased only slightly after phenylalanine and tyrosine administration (5). In this respect these amino acids more closely resemble the dicarboxylic acids than glycine and alanine. A greater increase was observed after phenylalanine than after tyrosine administration. Similar differences are indicated in the chick (102 and 88 or 93 per cent respectively of the initial).

In the rabbit, the blood amino nitrogen increased moderately after lysine ingestion (3) and in the dog (6) the “blood amino nitrogen increased markedly and remained high for some time.” Lysine caused a moderate increase (111 per cent of the initial) in the blood amino nitrogen in chicks.

Seth and Luck (2) obtained only a slight increase in the blood amino nitrogen level after cystine administration to dogs and rabbits. Lewis and Brown (7) reported that the cystine content of plasma increased “greatly” after cystine administration to rabbits, but no values were given to indicate the extent of the increase. Chicks show a slight (101 per cent of the initial) increase in blood amino nitrogen after cystine ingestion.

Seth and Luck (2), in one experiment, obtained practically no increase in the blood amino nitrogen of the rabbit after tryptophane administration. The chick shows a slight increase with tryptophane (95 per cent of the initial).

Despite the known differences in nitrogen metabolism which exist between the chick and other species, no marked differences between species may be seen in a comparison of the blood amino nitrogen levels after various amino acids are administered orally.

The comparisons made above between the levels of amino nitrogen in chick and mammalian blood after the ingestion of various amino acids are
of value only in comparing the metabolism of various species. To make
the level of blood amino nitrogen a better index of the ability of the animal
to utilize an amino acid, the amino nitrogen level may be calculated as the
amino acid and may be compared with the absorption rate (Fig. 2). The
fact that the correlation of molar increase of blood amino acid and molar
absorption rate from the gastrointestinal tract is of border line significance
indicates that there are differences in the rates of removal of amino acids
from the blood stream. Methionine and leucine appear to be removed from
the blood stream more slowly than the remainder of the amino acids.
Tyrosine, phenylalanine, aspartic acid, and glutamic acid seem to be
rapidly removed from the blood stream.

The absorption values for alanine, leucine, lysine, and methionine in the
chick are very similar to comparable values for the rat reported in the
literature (9, 17–19). The cystine absorption value for the chick com-
pares more favorably with the value for the rat reported by Sullivan and
Hess (20) than with the value reported by Wilson (21). Glutamic acid
and histidine values for the chick are considerably lower than those re-
ported for the rat (17, 19), while glycine is slightly lower (9, 17, 22). Al-
though the tryptophane absorption value for the chick is inconclusive, it is
greatly below the value of 62.9 mg. per 100 gm. of body weight per hour as
reported by Berg and Bauguess (23).

The fact that phlorhizin inhibits carbohydrate absorption and does not
inhibit amino acid absorption (22) suggests that different mechanisms are
involved. Carbohydrate absorption probably proceeds after preliminary
phosphorylation, a process which can be inhibited by phlorhizin (24).
Hober (25) concluded that amino acid absorption is too fast to be explained
by diffusion. Hober and Hober (26) suggested that a cellular mechanism
is involved in the preferential absorption of amino acids. Amino acids
exhibited irregular behavior and there seemed to be no relation between
size of molecule and the rate of absorption. However, this work with rat
intestinal loops did not compare amino acids among themselves. Bolton
and Wright (27) analyzed blood and lymph from various vessels in the cat
and found that absorption of amino acids from the intestine follows the
law of diffusion. Lathe (28) concluded from his work with intestinal loops
in dogs that the rate of intestinal absorption of amino acids decreases with
an increase in their molecular size.

Mehl and Schmidt (29) found that the diffusion coefficient of amino acids
in aqueous solution is related to the size and shape of their molecules, rather
than to their molecular weights. As was shown in Fig. 1, the rate of ab-
sorption of an amino acid from the intestinal tract of the chick varies in-
versely with the apparent molal volume of the amino acid. These data
would indicate that amino acid absorption in the chick is a function of rate
of diffusion of the amino acid and is not controlled by any cellular mechanism. If different amino acids were absorbed by different mechanisms, a considerable increase in the absorption rate would be expected when mixtures of amino acids were administered. Such was not the case with a mixture of glycine and alanine. The casein hydrolysate was absorbed only slightly faster than single amino acids.

Hydrolysis of protein before oral administration apparently allows a more rapid absorption of the constituent amino acids. The possibility exists that absorption of amino acid complexes takes place after protein administration, and that these complexes are relatively poorly detected by the blood amino nitrogen method. The fact that gelatin caused a greater rise in blood amino nitrogen than did casein may be due to the differences in amino acid composition. Gelatin contains more glycine, alanine, and hydroxyproline and less tyrosine, glutamic acid, and hydroxyglutamic acid than does casein (30). Glycine and alanine have been shown to cause large increases in blood amino nitrogen, while tyrosine and glutamic acid cause small or no increases in blood amino nitrogen.

SUMMARY

1. The rate of absorption of amino acids from the gastrointestinal tract of a chick varies inversely with the apparent molal volume of the amino acid.

2. In general, the blood amino nitrogen of a chick increases after the absorption of amino acids from the gastrointestinal tract in proportion to the amount of amino nitrogen absorbed. Methionine and leucine caused disproportionately high levels of blood amino nitrogen, while aspartic acid, glutamic acid, tyrosine, and phenylalanine caused disproportionately low levels of blood amino nitrogen after their oral administration.

3. The relative increase in blood amino nitrogen of a chick after the oral administration of various amino acids is similar to that observed in mammals.

4. The oral administration of a hydrolyzed protein causes a greater increase in the blood amino nitrogen of a chick than does the administration of intact protein.

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