AMINO ACID BALANCE AND IMBALANCE

I. DIETARY LEVEL OF PROTEIN AND AMINO ACID IMBALANCE

A. E. HARPER
Department of Biochemistry, University of Wisconsin, Madison

(Received for publication November 20, 1958)

The concept of amino acid balance is founded on a knowledge of the relationship between the amino acid composition of a protein and its biological value. A protein that provides amino acids in roughly the proportions in which they are required by the body is termed a balanced protein and has a high biological value; a protein that is low in one or more of the indispensable amino acids is termed an unbalanced protein and has a lower biological value. The more unbalanced a protein is, the lower the efficiency with which it is used and the greater the amount needed in a diet to satisfy the amino acid requirements (Block and Mitchell, '46-'47; Oser, '51; Almquist, '53; Mitchell, '54; Flodin, '53, '57).

The term amino acid imbalance has arisen from studies in which adverse effects, beyond the expected fall in the efficiency of protein utilization, have been observed when the protein of a diet, usually one low in protein, has been thrown out of balance by the addition of amino acids or a quantity of an unbalanced protein. In order to reverse these adverse effects, such as retarded growth or an accumulation of liver fat, a supplement of the amino acid that is most limiting in the diet must be provided. Thus, an amino acid imbalance,
besides causing a fall in the efficiency of nitrogen utilization, also causes an increase, specifically, in the need for the most limiting amino acid, (Harper, '58; Salmon, '58).

Although Salmon ('54) has studied the effect of dietary additions of gelatin on the tryptophan requirement there have been relatively few studies of a quantitative nature on the effects of amino acid imbalances. In fact, much of our information about such imbalances has been obtained from studies designed for other purposes.

There is even some difference of opinion about the use of the term amino acid imbalance (Harper, '58; Salmon, '58). For the present, an amino acid imbalance will be defined as any change in the proportions of the amino acids in a diet that result in an adverse effect which can be prevented by supplementing the diet with a relatively small amount of the most limiting amino acid or acids. This leaves open the question of whether there are different types of amino acid imbalances, i.e., whether imbalances caused by adding relatively small amounts of one or two amino acids to a diet (Hankes et al., '49; Deshpande et al., '58a) are identical with those produced by adding a relatively large quantity of a protein or of an amino acid mixture lacking a single amino acid (Salmon, '54; Deshpande et al., '55, '58a, b; Sauberlich, '56; Harper, '59). It excludes, however, those conditions described as antagonisms and toxicities (Harper, '58), in which adverse effects are caused by the addition of a fairly large excess of a single amino acid, and which are not known to be prevented by a relatively small supplement of the amino acid that is most limiting for growth.

The objectives of the investigation to be reported in this and in subsequent papers in this series are: one, to obtain both qualitative and quantitative information about the effects of changes in amino acid balance in an effort to provide a link between the observations on the relationship between the amino acid composition of a protein and its biological value and the observations on alterations in the proportions of amino acids in a diet that lead to amino acid imbalances;
and, two, to extend the previous observations on the physiological and metabolic effects of amino acid imbalances (Sauberlich and Salmon, '55; Deshpande et al., '58b; Kumta et al., '58).

The original purpose of the experiments reported in this paper was to study quantitatively the effect of the dietary level of protein on the severity of an amino acid imbalance. As a result of the initial experiments an hypothesis was developed which appeared to explain why dietary additions that caused quite severe imbalances in low protein diets were almost without effect when the protein level was sufficiently high to satisfy the amino acid requirements of the experimental subjects. The hypothesis was based on the fact that the growth response to a given increment of the amino acid that is most limiting in a diet diminishes as the growth rate approaches a maximum (fig. 2). From this it followed that if the requirement for the limiting amino acid were increased by a constant amount when a quantity of an amino acid mixture causing an imbalance was added to a diet, then the growth-retarding effect of such an addition should diminish as the dietary level of protein approached adequacy. The hypothesis could be tested experimentally by determining the growth rates of groups of animals ingesting diets containing: (1) a constant level of balanced protein but increasing increments of an unbalanced protein; (2) a constant level of an unbalanced protein or amino acid mixture but increasing increments of balanced protein; (3) either of the above with increments of the amino acid most limiting for growth. The results of such experiments appeared to support the hypothesis.

EXPERIMENTAL

Male weanling rats of the Sprague-Dawley strain, 21 days old and weighing from 40 to 50 gm were fed the basal diet for three days. They were then separated into groups of 5 rats each and were maintained in individual suspended cages with ½ in. mesh screen bottoms. The average initial
weights for the groups within each experiment did not differ by more than 1 gm. The rats were fed ad libitum and were weighed at least twice weekly during the two-week experimental periods.

The percentage composition of the basal diet was as follows: casein, 6.0; gelatinized corn starch, 83.6; corn oil, 4.5; mineral mixture, 5.0; choline chloride 0.15; fat-soluble vitamin mixture in corn oil, 0.5; and water soluble vitamin mixture in sucrose, 0.25. The mineral mixture was devised in collaboration with Dr. R. E. Boldt on the basis of a review of the rat requirements by Cuthbertson ('57). It had the following percentage composition: CaCO₃, 29.29; Ca HPO₄ · 2H₂O, 0.43; KH₂PO₄, 34.31; NaCl, 25.06; MgSO₄ · 7H₂O, 9.98; Fe(C₆H₅O₇) · 6H₂O, 0.623; CuSO₄ · 5H₂O, 0.156; MnSO₄ · H₂O, 0.121; ZnCl₂, 0.02; KI, 0.0005; (NH₄)₆MoO₇ · 4H₂O, 0.0025. The water-soluble vitamin mixture provided in milligrams per 100 gm of diet: thiamine · HCl, 0.5; riboflavin, 0.5; nicotinic acid, 2.5; calcium pantothenate, 2.0; pyridoxine · HCl, 0.25; vitamin K (menadione), 0.05; biotin, 0.01; folic acid, 0.02; vitamin B₁₂, 0.002; inositol, 10.0; and ascorbic acid, 5.0. The fat-soluble vitamin mixture provided per 100 gm of diet: α-tocopherol, 10.0 mg; vitamin A, 400 I.U.; and vitamin D, 200 I.U. The ascorbic acid was included in the vitamin mixture to minimize the destruction of thiamine (Kandutsch and Baumann, '53) and all rations were refrigerated. Changes in the protein level and all additions of amino acids, as indicated in the tables of results, were compensated for by adjusting the percentage of carbohydrate.

RESULTS

In order to determine the extent to which the severity of an amino acid imbalance was influenced by the level of the protein causing the imbalance, various levels of gelatin were added to diets containing either 6 or 8% of casein supplemented with 0.3% of DL-methionine. This procedure is known to produce an imbalance involving tryptophan even if the
supply of niacin is adequate (Salmon, '54). As is shown in table 1, the growth of rats fed a diet containing 6% of casein supplemented with methionine was stimulated by the addition of threonine. Gelatin, which contains threonine but not tryptophan, stimulated growth when added at a level of only 3%; however, when increments greater than 3% of gelatin were added, the rate of gain fell off until, with the addition of 12% of gelatin the rate of gain was only 13 gm in two weeks, considerably less than that of the group fed only the

TABLE 1

Effect of gelatin level on rate of gain of rats fed on diets containing 6 or 8% of casein supplemented with 0.3% of DL-methionine

<table>
<thead>
<tr>
<th>Gelatin</th>
<th>DL-threonine</th>
<th>DL-tryptophan</th>
<th>6% casein g/m/2 wks.</th>
<th>8% casein g/m/2 wks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>22 ± 3 †</td>
<td>53 ± 4 †</td>
</tr>
<tr>
<td>0.2</td>
<td>—</td>
<td>—</td>
<td>43 ± 3</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>—</td>
<td>31 ± 4</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>—</td>
<td>23 ± 3</td>
<td>52 ± 3</td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>—</td>
<td>20 ± 2</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>12</td>
<td>—</td>
<td>0.2</td>
<td>13 ± 2</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>12</td>
<td>—</td>
<td>0.2</td>
<td>51 ± 4</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>—</td>
<td>0.2</td>
<td>—</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>15</td>
<td>—</td>
<td>0.2</td>
<td>—</td>
<td>77 ± 5</td>
</tr>
</tbody>
</table>

† Standard error of the mean.

basal diet. The addition of tryptophan, the most limiting amino acid, not only prevented the growth retardation but stimulated growth above that obtained with the basal diet. A similar trend was seen when the diet contained 8% of casein, but the values in each case were higher.

The influence of the level of dietary protein on the imbalance caused by adding an amino acid mixture lacking threonine to diets containing casein supplemented with methionine is shown in figure 1. The amino acid mixture contained 3.45% of amino acids, equivalent to the amounts of the L-isomers of these indispensable amino acids in 6% of casein. This mixture, which induces an amino acid imbalance that
is readily corrected by a supplement of threonine (Harper, '59), caused the greatest depression in growth rate when the diet contained 6% of casein. The depression was less when the casein content of the diet was dropped to 4% and also when it was increased above 6%. Very little growth depression was observed when the diet contained 15% of casein.

![Graph](https://example.com/graph.png)

**Fig. 1** Effect of an amino acid mixture lacking threonine on the rate of gain of rats fed on diets containing different levels of casein supplemented with 0.3% of DL-methionine. --- no addition; —— amino acid mixture lacking threonine. The amino acid mixture provided in per cent of the diet: DL-tryptophan, 0.15; L-leucine, 0.54; DL-isoleucine, 0.8; DL-valine, 0.82; L-histidine-HCl, 0.22; DL-phenylalanine, 0.34; L-lysine-HCl, 0.58.

The next experiment provided information about the effect of 4% of the amino acid mixture lacking threonine on the need for threonine by rats fed a diet containing 6% of casein supplemented with 0.3% of DL-methionine. The results are presented in table 2. The amino acid mixture lacking threonine depressed the growth rate, as before, and somewhere between 0.025 and 0.05% of L-threonine had to be
AMINO ACID IMBALANCE

added to overcome the growth depression. These results suggest that this percentage of the threonine in the original diet was made unavailable to the animal because of the imbalance. Increments of threonine greater than this stimulated growth above that of the control group indicating that threonine was by far the most limiting amino acid in this diet.

TABLE 2
Effect of 4% of an amino acid mixture lacking threonine on the need for threonine by rats fed a diet containing 6% of casein supplemented with 0.3% of DL-methionine

<table>
<thead>
<tr>
<th>Casein</th>
<th>A. A. mix</th>
<th>L-threonine</th>
<th>WEIGHT GAIN (gm/2 wks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>—</td>
<td>18 ± 3*</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>—</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.025</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.05</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.075</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.1</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.15</td>
<td>51 ± 4</td>
</tr>
</tbody>
</table>

* For the composition of the amino acid mixture see figure 1. The quantity of each of the amino acids was increased proportionally.

The results presented in figure 1 indicate that the magnitude of the growth depression caused by the addition of an unbalanced amino acid mixture depends upon the level of protein in the diet. This suggests that the magnitude of the effect can be related to the standard growth response curve obtained when gain in weight is plotted against the response to increasing increments of protein or of the limiting amino acid in the diet. Also, from the results in table 2, it is evident that close to an additional 0.05% of L-threonine was required in the diet to overcome the growth retardation caused by the amino acid mixture lacking threonine or, as was concluded from these results, this percentage of the threonine in the original diet was made unavailable to the animal when the amino acid imbalance was created. If an amino acid
mixture causing an imbalance increases the need of the animal for the limiting amino acid by a constant percentage regardless of the original level of protein in the diet, then, although the magnitude of the growth depression caused by adding a stated amount of an unbalanced amino acid mixture to a diet would depend upon the adequacy of the diet, the growth depression should be prevented by the same level of the limiting amino acid in each case.

This is illustrated by figure 2 which has been constructed from data obtained by Armstrong ('54) in an investigation of the phenylalanine requirement of the rat. It also indicates the basis for the final experiments in this study. The cross-hatched areas indicate that an amino acid mixture which increased the requirement for the limiting amino acid by 0.1% would cause a much greater growth depression when added to a diet supporting a rate of gain that fell on the steeper part of the curve than it would if it were added to a diet that was nearly adequate (upper part of the growth response curve) or was severely inadequate (lower part of the growth response curve).

The final experiments were conducted to determine how closely experimental results would fit this hypothesis. In order to increase the severity of the imbalance, and on the basis of the results obtained in experiment 1, the amount of the amino acid mixture lacking threonine that was added to the diet was increased to 6%; however, the relative proportion of each of the amino acids in the mixture remained unaltered (see fig. 1). The results of the experiment are presented in table 3. Three levels of casein, supplemented with 0.3% of DL-methionine in each case, were used in this experiment and for the sake of economy DL-threonine (a 50-50 mixture of D-allo-threonine and L-threonine) was substituted for L-threonine. With each level of casein, 4, 7 and 10%, the amino acid mixture lacking threonine caused a growth depression. The magnitude of the growth depression was greatest when the diet contained 7% of casein and was less if the diet contained either 4 or 10% of casein. Regardless
of the level of casein the growth depression was not prevented by the addition of 0.05% of DL-threonine but was completely prevented by an addition of 0.1%. These results are what would have been expected on a theoretical basis.

Fig. 2 Growth response curve. Gain in weight plotted against phenylalanine content of diet; taken from data of Armstrong ('54). Cross-hatched areas indicate the effect on the rate of gain of removing 0.1% of L-phenylalanine from the diet.
Although the magnitude of the growth depression was greatest when the diet contained 7% of casein, i.e., when the diet supported about half the maximum rate of gain, it is of interest to look at the figures expressed on a percentage basis.

**Table 3**

*Effect of 6% of an amino acid mixture lacking threonine on the need for threonine by rats fed on diets containing various levels of casein supplemented with 0.3% of DL-methionine*

<table>
<thead>
<tr>
<th>Casein %</th>
<th>A. A. mix %</th>
<th>DL-threonine%</th>
<th>Weight gain gm/2 wks</th>
<th>% of control growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>9 ± 1*</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>0.05</td>
<td>1</td>
<td>5 ± 1</td>
<td>56</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>9 ± 1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>41 ± 2</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.05</td>
<td>27 ± 2</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.1</td>
<td>41 ± 4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>73 ± 4</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.05</td>
<td>65 ± 4</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.1</td>
<td>76 ± 5</td>
<td>104</td>
<td></td>
</tr>
</tbody>
</table>

*For the composition of the amino acid mixture see figure 1. The quantity of each of the amino acids was increased proportionally.

*Standard error of the mean.

When the diet contained 4% of casein, the amino acid mixture lacking threonine depressed the growth rate to 11% of that of the control group; with 7% of casein in the diet, the value was 46%; and with 10% of casein, 83%. Thus, the effect of the imbalance was most severe when the diet contained only 4% of protein.

**Discussion**

The procedure used in these experiments to induce an amino acid imbalance consisted of adding a fairly large quantity of an amino acid mixture or of a protein lacking a single amino acid to a diet that contained an inadequate amount of protein. This has proven to be an effective and
consistent method of creating amino acid imbalances (Salmon, '54; Deshpande et al., '55, '58a,b; Säuberlich, '55; Harper, '59). There are, however, some cases in which a relatively small addition of amino acids, usually the amino acid that is second most limiting in the diet, causes an unexpectedly severe imbalance (Hankes et al., '49; Henderson et al., '53; Deshpande et al., '58a). The question therefore arises as to whether both of these situations are examples of a general phenomenon or whether they represent two different conditions. Although some preliminary observations suggest that there are differences between them a definitive answer must await the completion of further experiments.

These observations also pose a question concerning the method of determining amino acid requirements. The usual procedure is to provide all of the amino acids except one in excess and then to measure the response to increasing increments of that one. This is very similar to the procedure that was used to create amino acid imbalances in this study. It would seem advisable for the amino acid balance to be maintained as closely as possible to the ideal if minimum values for amino acid requirements are to be obtained.

Also, in the studies in which amino acid requirements have been shown to increase with increasing levels of protein, the procedure used is similar to that used to induce amino acid imbalances. Therefore, there is also need for reinvestigation of the influence of protein level on amino acid requirements. An investigation of this subject, using wheat gluten as the source of protein, will shortly be reported.

Finally it seems clear that the magnitude of the growth depression caused by adding to a diet a mixture of amino acids that creates an imbalance depends upon the rate of gain supported by the original diet. When the diet provides all of the indispensable amino acids in quantities that exceed the

---

*Kumta and Harper, unpublished results.

*Munaver and Harper, unpublished results.
requirements the diet may be thrown considerably out of balance without causing an adverse effect (fig. 1). This raises the question of the relationship between the amino acid balance of a protein and its nutritive value. Some proteins are so much out of balance (such proteins as zein, gelatin and hemoglobin which lack an amino acid), that they cannot satisfy the amino acid requirements of an animal under any circumstances. We do not, however, know at what degree of unbalance it becomes impossible to satisfy the requirements of an animal by increasing the level of protein in the diet, i.e., the stage at which amino acid supplementation becomes mandatory. This can be answered empirically by determining the effects on the growth rate of adding different levels of an amino acid mixture lacking a single acid to diets containing various levels of protein. The results of such a study could be used to calculate the point at which we pass from the stage of a simple deficiency to that of an amino acid imbalance.

SUMMARY

A protein lacking tryptophan (gelatin) or an amino acid mixture lacking threonine has been used to create amino acid imbalances involving tryptophan or threonine in diets containing casein supplemented with methionine.

The magnitude of the growth depression caused by the addition of gelatin increased as the level of gelatin was increased.

The magnitude of the growth depression caused by the amino acid mixture lacking threonine was greatest when the diet contained 6% of casein and was less if the level of casein in the diet was lower or higher.

The magnitude of the growth depression caused by a constant amount of the amino acid mixture lacking threonine was shown to be related to the rate of growth supported by the original diet. However, the increase in the level of threonine required to prevent the growth depression appeared to be unaffected by the level of protein in the original diet.
ACKNOWLEDGMENT

The author wishes to acknowledge that these experiments were carried out with the cheerful and willing assistance of Miss Deanne Chapman.

LITERATURE CITED

——— 1958b Amino acid imbalance and nitrogen retention. Ibid., 230: 335.

