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AMINO ACID IMBALANCE

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Amino Acid Imbalance*

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The term 'amino acid imbalance' can be used in different ways. It can be used to describe any fairly drastic change in the pattern of amino acids in a diet, particularly one that leads to adverse effects. Used in this way it is non-specific and its historical significance is lost. We use the term in a more restricted way to describe dietary amino acid patterns resembling those originally referred to as amino acid imbalances by Elvehjem & Krehl (1955).

The observations they made were similar to those of Morrison, Reynolds & Harper (1960) presented in Table 1. They used a basal diet that was low in protein (9% of casein supplemented with sulphur-containing amino acids) and devoid of nicotinic acid. If this diet is supplemented with nicotinic acid, it is primarily deficient in threonine, but when nicotinic acid is omitted, tryptophan becomes the most limiting amino acid. The growth of rats fed on the basal diet supplemented with gelatin or threonine is less than that of a control group receiving no supplement. The growth retardation is prevented by further supplements of either nicotinic acid or tryptophan (Morrison *et al.* 1960). Addition of 6% of the tryptophan-deficient protein, gelatin, results in a quite drastic change in the dietary pattern of amino acids, but addition of threonine (in some experiments as little as 0·1% of L-threonine) changes the pattern very little; yet, both of these treatments cause retardation of growth.

Originally amino acid imbalance was thought to be a highly specific condition that involved tryptophan as a precursor of nicotinic acid. Salmon (1954) later showed that the growth of rats receiving nicotinic acid was depressed if the amount of gelatin in the diet was increased. Similar changes in the amino acid pattern of a variety of diets were subsequently shown to retard the growth of several species of animals (Harper, 1958). It therefore appeared that the phenomenon of amino acid imbalance was a general one involving primarily amino acid interrelationships.

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Table 1. *Effect of nicotinic acid and tryptophan on growth of rats fed on diets containing 8% casein and gelatin or threonine*

Additions to 8% casein diet				Weight gain (g/2 weeks)
Gelatin (%)	DL-Threonine (%)	Nicotinic acid (mg/100 g)	L-Tryptophan (%)	
—	—	—	—	15
6·0	—	—	—	5
6·0	—	2·5	—	27
6·0	—	—	0·1	32
—	0·36	—	—	4
—	0·36	2·5	—	35
—	0·36	—	0·1	32

Adapted from Morrison & Harper (1960). All diets contained 0·3% DL-methionine.

Table 2. *Effect of lysine and threonine supplements on the growth of rats fed on a diet containing 90% rice*

Supplements		Weight gain (g/5 weeks)
L-Lysine hydrochloride (%)	DL-Threonine (%)	
—	—	57
0·1	—	78
0·1	0·1	112
0·1	0·2	138
0·1	0·3	114
0·2	0·3	151
0·2	0·1	136
0·25	0·1	152
0·3	0·1	131
0·3	0·2	154

After Rosenberg *et al.* (1959).

The observations fell into two categories. The first is illustrated in Table 2, which shows that, when the lysine content of a rice diet is held constant and the threonine content is increased stepwise, a point is reached at which the growth of rats fed on the threonine-supplemented rice is retarded unless the lysine content of the diet is also increased. Similarly if threonine content is held constant and that of lysine is increased, a point is reached at which growth is retarded unless more threonine is added (Rosenberg, Culik & Eckert, 1959). These growth retardations resulted from only small alterations in the amino acid pattern of the diet. We should like to emphasize that the growth retardation was caused by a supplement of the amino acid that was the second most limiting for growth. This relationship has been observed frequently but, if several amino acids are about equally limiting in the diet, less specificity is observed and several different supplements may cause retardation of growth (Harper, 1958; Kumta, Elias & Harper, 1961).

The second category is illustrated in Table 3. In these examples the growth of rats fed on a diet that was low in histidine was retarded when an amino acid mixture devoid of histidine was included in the diet, and the growth of rats fed on a diet that

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was low in threonine was retarded when an amino acid mixture devoid of threonine was included in the diet. Growth retardation resulted when the concentrations of all of the indispensable amino acids except one were increased in the diets. A small supplement of the one not provided in the mixture prevented the growth retardation.

Table 3. *Effect of amino acid imbalance on weight gain of rats*

Diet	Weight gain (g/2 weeks)
6% fibrin	33
6% fibrin + aa mixture − histidine	2
6% fibrin + aa mixture + histidine	33
6% casein	18
6% casein + aa mixture − threonine	10
6% casein + aa mixture + threonine	21

aa, amino acid. Adapted from Harper (1959) and Kumta & Harper (1960).

Table 4. *Effect on the weight gain of rats of an amino acid imbalance due to addition of methionine and phenylalanine to a diet having fibrin as the protein*

Addition to 6% fibrin diet	Weight gain (g/2 weeks)
None	32
0·2% DL-methionine, 0·3% DL-phenylalanine	20
0·2% DL-methionine, 0·3% DL-phenylalanine; 0·1% L-leucine, 0·1% DL-isoleucine, 0·15% DL-valine and 0·05% L-histidine hydrochloride	45

One particular imbalance, that we shall refer to later, is illustrated in Table 4. It is an example of a somewhat more complex relationship in which 6% of fibrin, a well-balanced protein, was used as the protein source in the basal diet. Addition of methionine and phenylalanine to this diet caused an imbalance that was corrected only by adding four amino acids—histidine, leucine, isoleucine and valine (Harper, 1958). Actually this still fits the general pattern if the added amino acids are looked on as two groups, the last four as the most limiting amino acid, and the first two as the second most limiting.

Two important points in relation to amino acid imbalances are: first, that both the control diet and the 'imbalanced' diet (i.e. the diet containing additional amounts of amino acids which cause growth retardation) have exactly the same content of the amino acids that limit growth; and, secondly, that the concentration of the limiting amino acid or acids must be increased in the imbalanced diet to prevent growth retardation. The imbalanced diet supplemented in this way we call a 'corrected' diet. We shall not attempt a more succinct definition of amino acid imbalance (it has been done before by Harper, 1958, 1959, 1964*a*, to the satisfaction at least of ourselves and our close colleagues), but when we use the term, it will be restricted to conditions resembling those illustrated in Tables 2–4.

What does this restriction accomplish? We think that it effects a convenient separation between amino acid imbalances and conditions that we prefer to call amino acid antagonisms and toxicities.

The category of antagonism is exemplified by leucine, isoleucine and valine interrelationships which have been studied in some detail (Spolter & Harper, 1961; Rogers, Spolter & Harper, 1962) and probably by lysine–arginine interrelationships (Jones, 1964; Lewis, 1965; Smith & Lewis, 1964). The basis for making a distinction between imbalances and antagonisms is illustrated in Table 5. Inclusion of 3–5% of

Table 5. *Effects of supplements of various amino acids given to rats on the growth-retarding action of leucine*

Diet			Weight gain
Casein (%)	L-Leucine (%)	Amino acid supplement	(g/2 weeks)
9	—	—	33
9	3·0	—	9
9	—	0·9% DL-threonine	44
9	3·0	0·9% DL-threonine	8
9	—	0·5% DL-isoleucine	31
9	3·0	0·5% DL-isoleucine	23
9	3·0	1·2% DL-isoleucine + 1·2% DL-valine	30

Adapted from Harper, Benton & Elvehjem (1955) and Benton, Harper, Spivey & Elvehjem (1956).

L-leucine in a diet containing 9% of casein supplemented with methionine retards the growth of rats. A supplement of threonine, the limiting amino acid in the control diet, does not prevent or alleviate the growth retardation; however, supplements of isoleucine and valine, neither of which is as limiting as threonine in this diet, do. The lack of response to a supplement of the limiting amino acid distinguishes this condition from an imbalance, and because of the structural similarities among leucine, isoleucine and valine, we have called it an amino acid antagonism. Although, in analogy to observations made on micro-organisms, the term may imply competition for transport or for some enzyme system, we have no evidence that this is true. The term as we use it should not be taken to imply anything about the mechanism responsible for the growth depression.

The category of toxicity is a loose one. A dietary excess of tyrosine (3% or more) causes severe eye and paw lesions in the young growing rat (Schweizer, 1947). Excess methionine (2% or more) will completely inhibit growth and at higher levels will cause atrophy of cells in some organs (Earle, Smull & Victor, 1942*a*,*b*). Phenylalanine in excess affects brain function (Waisman & Harlow, 1965). These are clearly toxic effects. Many other individual amino acids in excess will retard growth (Sauberlich, 1961). How many of these effects can legitimately be attributed to toxicity is a moot question. Nevertheless, we have grouped them as toxic effects, a completely non-specific term, until we have some basis for separating them into other categories.

It might be noted that the growth depressions attributed to toxic effects of disproportionately large amounts of individual amino acids are usually less severe if

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the diet is concomitantly supplemented with more of the limiting amino acid or acids or with additional protein—a distinct resemblance to an amino acid imbalance. However, if the individual amino acid and the dietary level of protein are increased proportionately, growth depressions attributable to toxic effects still occur, whereas those attributable to imbalances are alleviated (Harper, 1964*b*).

From here on we shall discuss only amino acid imbalances which, as indicated above, are recognized by the growth depressions they cause, growth depressions that are prevented by increasing the concentration of the limiting amino acid in the imbalanced diet. As shown in Table 6, when a mixture of amino acids causing an imbalance is added to a low-protein diet the amount of the limiting amino acid needed to support a given rate of growth is increased (Kumta & Harper, 1960). A similar phenomenon occurs with increasing intakes of poorly balanced proteins (Salmon, 1954). In Table 7 is shown the effect of increasing increments of wheat gluten on the requirement of the rat for lysine (Munaver & Harper, 1959).

Table 6. *Influence of amino acid imbalance on intake of the limiting amino acid by rats*

Fibrin in diet (%)	Supplement	Weight gain (g/2 weeks)	Histidine intake (g/2 weeks)
6	—	33	2·0
6	aa mixture − histidine	2	0·6
6	aa mixture + histidine	33	2·4

aa, amino acid. Adapted from Kumta & Harper (1960).

Table 7. *Effect of the wheat gluten content of the diet on the lysine requirement for maximum growth of rats*

Wheat gluten (%)	Total lysine (%)	Weight gain (g/2 weeks)	Lysine intake (g/2 weeks)
30	0·9	73	1·3
30	1·0	83	1·4
47	1·05	71	1·4
47	1·10	79	1·5
53	1·05	70	1·4
53	1·15	76	1·6
53	1·20	79	1·6

Adapted from Munaver & Harper (1959).

As growth is retarded by an amino acid imbalance and as a supplement of the limiting amino acid must be added to the imbalanced diet to prevent the growth retardation, it is a logical inference that an amino acid imbalance reduces the efficiency of utilization of the limiting amino acid (Salmon, 1958). It might further be inferred that an amino acid imbalance would reduce efficiency of nitrogen utilization. When this was measured, however, results of the type shown in Table 8 were obtained, indicating that the amino acid imbalance depressed nitrogen retention no more than did equivalent restriction of food intake (Kumta, Harper & Elvehjem,

Table 8. *Effect of amino acid imbalance and pair-feeding on nitrogen balance of protein-depleted adult rats*

Diet	3-day nitrogen intake (mg)	Nitrogen retained (%)
6% fibrin* *ad lib.*	483	60
6% fibrin + 0·6% DL-methionine + 0·9% DL-phenylalanine *ad lib.*	228	44
6% fibrin* pair-fed	228	33

Adapted from Kumta *et al.* (1958).
*Glutamic acid added to make all diets isonitrogenous.

1958). This suggested that in the rat, at least, depression of food intake occurred so rapidly after ingestion of an imbalanced diet that measurements of nitrogen balance would give no information about the basis for the adverse effects. It also raised some question about the validity of the hypothesis than an amino acid imbalance resulted in an increase in the rate of katabolism of the limiting amino acid. Further, it became evident that, in order to explain the effects of an amino acid imbalance, it would be necessary to know what changes occurred during the interval before food intake fell.

To determine how rapidly an amino acid imbalance depressed food intake, rats were depleted of protein, kept without food overnight, and then allowed to eat

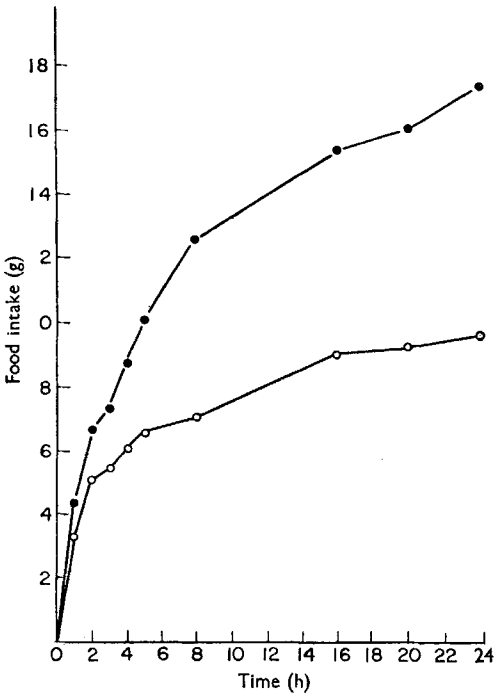

Fig. 1. Food intake patterns over 24 h of rats fed on control (6% casein), •—•, or imbalanced (6% casein + amino acid mixture − threonine), o—o, diets.

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ad lib. (Harper & Kumta, 1959). Food intake was measured at short intervals, and within 4–6 h a depression in food intake could be detected (Fig. 1). This indicated that, whatever the effect of an amino acid imbalance was, it occurred so rapidly that the depressed food intake could be looked on almost as a primary effect, and the retarded growth could be attributed to depressed food intake. The appetite depression occurs much more rapidly than with most deficiencies in which low food intake is apparently the result of impaired metabolic function and can usually be regarded as an effect of impaired growth rather than the cause of it. The only deficiency causing such a rapid fall in food intake is an amino acid deficiency—presumably because the body cannot store amino acids—as shown by studies of delayed amino acid supplementation in which a few hours delay results in greatly impaired efficiency of amino acid utilization (Elman, 1939; Geiger, 1947; Cannon, Steffee, Frazier, Rowley & Stepto, 1947). To attribute the effect of an amino acid imbalance solely to an amino acid deficiency when both the control and the imbalanced diets contain exactly the same concentration of the amino acid that is limiting growth represents a failure to recognize the nature of the problem.

The problem of explaining the adverse effects of amino acid imbalances then became one of explaining the depression of food intake. Was it just a matter of palatability? This was unlikely, because rats ingested the control and the imbalanced diets at the same rapid rate for several hours before a depression in the food intake of the group given the imbalanced diet could be detected. This pattern implied that something happened within those few hours to inhibit food intake. Also, and perhaps more convincing, an amino acid that caused a depression in food intake when added to one diet, prevented the depression of food intake when added to another. This is illustrated in Table 2. To explain this as an effect of altered palatability, it is necessary to assume that a small amount of threonine can improve the palatability of one diet and reduce that of another differing from the first by only 0·1% of lysine hydrochloride.

Various other possibilities were entertained in an effort to account for depressed food intake. Stomach emptying was measured and found not to be delayed by an amino acid imbalance (Kumta & Harper, 1961). Amino acid imbalances could be demonstrated in experiments in which the dietary protein was replaced completely by amino acids, so impaired protein digestion was unlikely as a cause of the depressed food intake (Henderson, Koeppe & Zimmerman, 1953). Urinary excretion of amino acids by rats given imbalanced diets was either not increased or enhanced very slightly (Sauberlich & Salmon, 1955), so it seemed improbable that amino acid imbalances caused excessive losses of amino acids in urine. Studies of blood urea gave little support to the idea that enhanced oxidation of amino acids occurred during the period immediately after ingestion of an imbalanced meal (Kumta & Harper, 1961). In fact, the only positive evidence of some physiological or metabolic change occurring as a result of ingestion of a meal in which an amino acid imbalance had been created was the consistent finding that the plasma concentration of the amino acid that was limiting in the diet fell markedly within a few hours (Fig. 2) (Kumta & Harper, 1962; Sanahuja & Harper, 1963*a*). This type of plasma pattern

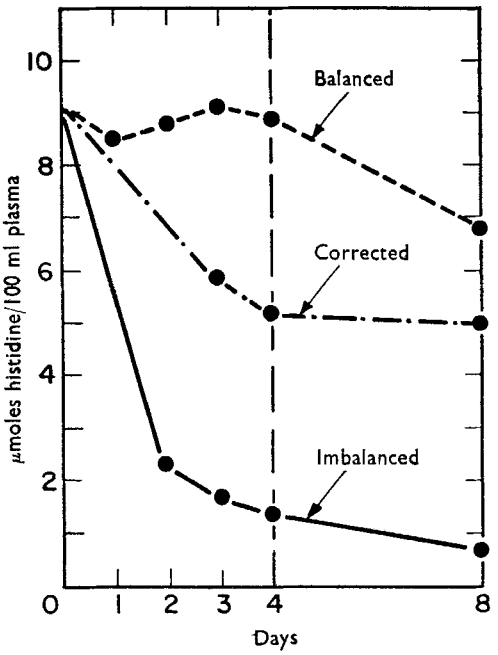

Fig. 2. Plasma histidine concentration of rats fed on balanced (6% fibrin); imbalanced (6% fibrin + amino acid mixture − histidine); or corrected (6% fibrin + amino acid mixture + histidine) diets.

resembles very much that seen in animals fed on diets severely deficient in a single amino acid (Longenecker & Hause, 1959; McLaughlan & Morrison, 1965).

Another gross effect of an amino acid imbalance was observed at this time. When rats were given a choice of two diets, a protein-free diet and a diet in which an amino acid imbalance had been created, within a short time they were selecting the protein-free diet that would not support life and were rejecting the imbalanced diet that would not only support life but would also support growth (Sanahuja & Harper, 1962, 1963*b*). In one experiment some animals continued to eat the protein-free diet for over 30 days until they died, even though they had the imbalanced diet before them at all times (P. M-B. Leung, unpublished findings).

A summary of some of the effects observed is shown in Fig. 3 (taken from Mr P. M-B. Leung's PhD thesis in preparation). Rats were given a choice between a protein-free diet and one of a series of diets containing increasing quantities of an amino acid mixture devoid of threonine. The amount of protein-free diet they consumed increased as the quantity of the amino acid mixture devoid of threonine used to create imbalance in the other diets was increased. Concomitant with this, the rats ate less of the imbalanced diet. As threonine was added back to the most drastically rejected of the imbalanced diets, increased quantities of it were consumed with each increasing increment of threonine. Ultimately a point was reached at which the protein-free diet was completely rejected, and only the corrected diet was eaten.

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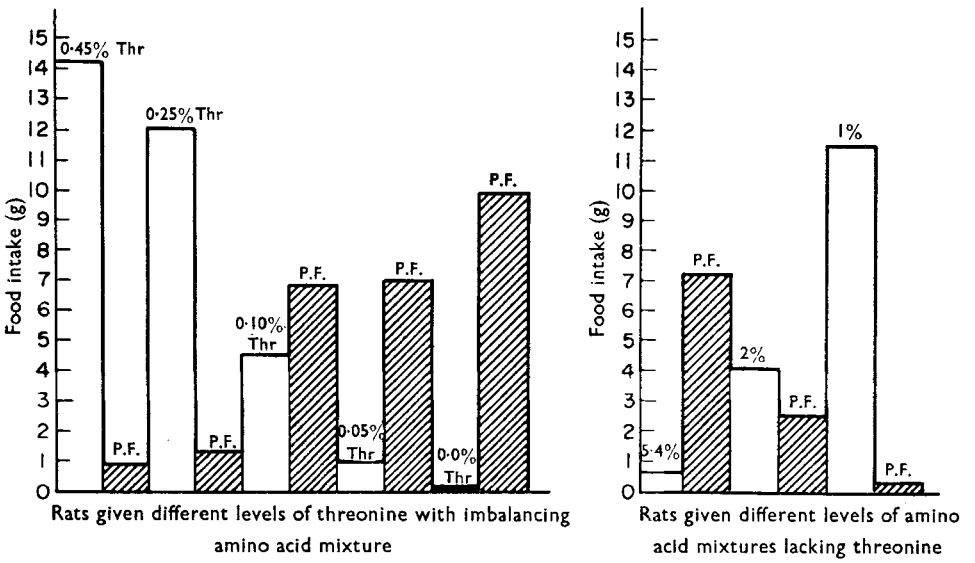

Fig. 3. Diet selection by rats offered a choice of a protein-free diet or one of several imbalanced diets (6% casein + amino acid mixture − threonine). Right: Effect of increasing amounts of amino acid mixture − threonine on selection of protein-free diet (P.F.). Left: Effect of increasing amounts of threonine in imbalanced diet on selection of protein-free diet (P.F.).

It is known that rats given a diet devoid of one amino acid will eat less of it than they will of a protein-free diet (Frazier, Wissler, Steffee, Woolridge & Cannon, 1947; Greenstein & Winitz, 1961). It is also known that if rats are force-fed on a diet that is devoid of one amino acid they will develop pathological lesions and survive only for a short time; whereas, if they are allowed to eat the same diet *ad lib.* their food intake will fall, no pathological lesions will develop, and they will survive longer (Sidransky & Farber, 1958; Sidransky & Baba, 1960).

The similarities between the responses of rats fed on a diet devoid of a single amino acid and the responses of rats fed on a diet in which an amino acid imbalance had been created led us to think that the depression in food intake caused by an amino acid imbalance might be a protective response. Our thought was that ingestion of the imbalanced diet resulted in a signal being sent to an appetite-regulating centre which resembled the signal sent when a much more severely deficient diet was ingested. The only physiological response which is seen consistently before the fall in food intake is the fall in the concentration of the limiting amino acid in the plasma. The resulting amino acid pattern in the plasma then resembles that seen when a more deficient diet is ingested (Longenecker & Hause, 1959; McLaughlan & Morrison, 1965). It is not clear if or how the plasma amino acid pattern results in a signal to reduce food intake. It is, of course, quite possible that the plasma amino acid pattern is merely a reflection of a much more substantial change that occurs elsewhere, say, for example, at the site of protein synthesis in the muscle or some other organ, and that the signal is transmitted by some nervous or hormonal mechanism. This, however, is a realm for speculation.

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If it is accepted that the depressed food intake is responsible for the growth depression caused by an amino acid imbalance and that there is a link between depressed food intake and altered plasma amino acid pattern, it then becomes necessary to explain why the concentration of the limiting amino acid in plasma falls if there is not some reduction in the efficiency of amino acid utilization or some enhanced oxidation of the limiting amino acid as a result of an amino acid imbalance. Some experiments in which the metabolism of the limiting amino acid was followed by the use of isotopes have given some clues about this (A. Yoshida & P. M-B. Leung, unpublished findings). The procedure consisted of giving rats a single meal of either a control or an imbalanced diet containing a tracer dose of uniformly ^{14}C-labelled limiting amino acid (either threonine or histidine) and studying the fate of the radioisotope. Fig. 4 shows the cumulative curve for $^{14}CO_2$ expired. The curve

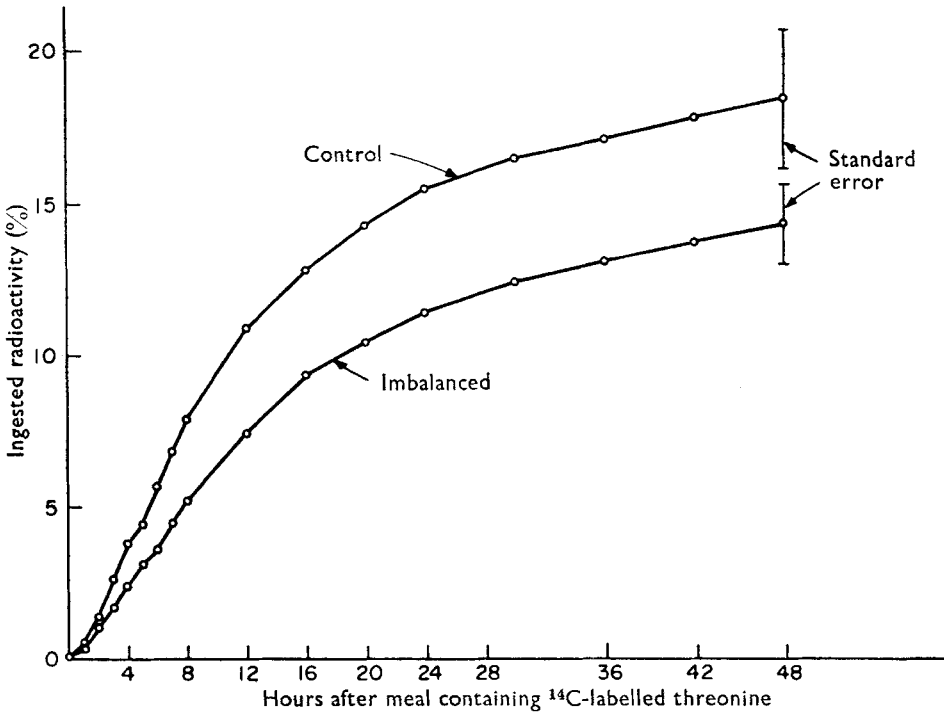

Fig. 4. Cumulative percentage of $^{14}CO_2$ expired by rats fed on control or imbalanced (6% casein + amino acid mixture − threonine) diets containing uniformly ^{14}C-labelled threonine.

for the control group is actually higher than that for the imbalanced group, indicating that oxidation of the limiting amino acid was not enhanced by the amino acid imbalance.

Values for the disappearance of ^{14}C from the gastro-intestinal tract are shown in Table 9. These indicate that the amino acids added to create the imbalance did not interfere with the absorption of the limiting amino acid.

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Table 9. *Absorption by the rat from the digestive tract of uniformly ^{14}C-labelled threonine after eating a threonine-imbalanced diet*

	Radioactivity absorbed from tract*	
Hours after feeding	Control (%)	Imbalanced (%)
3·5	65 ± 6	59 ± 3
8	97 ± 1	96 ± 1

*Mean values with their standard errors for six rats.

Table 10. *Distribution of radioactivity, as a percentage of that ingested, after feeding rats on basal or imbalanced diets containing uniformly ^{14}C-labelled threonine*

	Control, 6% casein	Imbalanced, 6% casein, 10% amino acid mixture − threonine
Expired CO_2	18·4	14·3
Urine	2·1	2·2
Faeces	1·6	1·2
Carcass	70·1	74·6
Liver	5·9	7·2
Total	98·1	99·5

Each rat was given 7 g diet containing 8 μc ^{14}C-labelled threonine at the beginning of experiment and the same amount of the same diet 24 h later. The experiment lasted for 48 h.

In Table 10 is shown a summary of the results of the isotope study. There was no evidence of enhanced excretion of ^{14}C from the limiting amino acid in either urine or faeces. There was, however, evidence of greater retention in the carcass and more specifically in the liver. The higher amount of radioactivity in the liver was found mainly in the liver protein fraction (Table 11). Only one of several values was statistically significantly greater than the control value, but the trend was the same throughout. Incorporation into muscle was about the same for both control and imbalance groups (Table 12).

In relation to these observations Tarver (1963) has postulated that an unbalanced mixture of amino acids should increase the efficiency of incorporation into proteins of the one in short supply. Sidransky & Farber (1958) have shown that, in rats fed on a diet completely devoid of threonine, incorporation of a tracer dose of a

Table 11. *Effect of amino acid imbalance on incorporation of uniformly ^{14}C-labelled threonine or histidine into liver protein of rats*

Hours after feeding	Diet	Total radioactivity* (% of dose)	Specific activity* (disintegration/min mg)
8	Control	4·4 ± 0·2	926 ± 38
8	Threonine-imbalanced	5·1 ± 0·4	989 ± 41
48	Control	5·0 ± 0·3	667 ± 41
48	Threonine-imbalanced	6·2 ± 0·5	788 ± 49
8	Control	10·3 ± 0·5	1638 ± 69
8	Histidine-imbalanced	15·9 ± 0·4	2402 ± 115

*Mean values with their standard errors.

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Table 12. *Effect of amino acid imbalance on incorporation by rats of uniformly ^{14}C-labelled threonine or histidine into muscle protein 8 h after feeding*

Diet	Radioactivity: Trichloroacetic acid soluble† (disintegration/min mg muscle)	Radioactivity: Protein† (disintegration/min mg)
Control	17·9±0·1*	221±5·5
Threonine-imbalanced	7·5±0·1	230±5·9
Control	36·9±1·4*	177±8·9
Histidine-imbalanced	12·6±0·5	175±9·0

*$P<0.01$.

†Mean values with their standard errors.

labelled amino acid into liver protein was increased and total liver protein content was also elevated above the control value.

From the results obtained so far, we have developed a hypothesis concerning the sequence of events leading to the altered plasma amino acid pattern of rats ingesting an imbalanced diet. The dietary protein is apparently digested normally and the amino acids from the meal are absorbed efficiently. This results in a surplus of all but one of the indispensable amino acids in the portal blood flowing to the liver. The surplus of amino acids stimulates synthesis or suppresses breakdown of protein so that more of the limiting amino acid is retained in the liver in the imbalanced than in the control group. The supply of the limiting amino acid for peripheral tissues is thereby reduced but not to a level that will depress protein synthesis. Muscle protein continues to be synthesized at a normal rate, so the eventual result is that the free amino acid patterns of both muscle and plasma become severely unbalanced, the patterns resembling those produced by ingestion of a severely deficient diet. Some of these changes are monitored by an appetite-regulating centre and food intake is depressed. As a consequence of the lower food intake, the supply of energy and amino acids is reduced and growth is retarded.

This hypothesis would account for the unexpectedly large depressions of growth and food intake caused by relatively small supplements of the second most limiting amino acid or acids. It has always been very difficult to envisage how supplements of 1% or less of one or a few amino acids that cannot be used for protein synthesis could cause any untoward effect in an animal when the body has the capacity to oxidize 20–30% of protein in excess of its needs without difficulty (Harper, 1958). If, however, these amino acids, being the second most limiting for protein synthesis, exert a mass-action effect resulting in highly efficient utilization of the most limiting amino acid and its depletion from certain critical body pools, the effects observed would not seem nearly so incomprehensible.

If this hypothesis is correct, it should be possible to show that the extra amount of the limiting amino acid incorporated into tissues will account for the fall in its concentration in blood plasma and muscle. The plasma threonine pool is small relative to the total body pool of free threonine (Table 13) and the total body pool is minute relative to the total amount of threonine in body proteins. A fall of 40 μmoles

Table 13. *Free threonine content of tissues of rats 5 h after eating a threonine-imbalanced diet*

	Control	Imbalanced
	(μmoles/100 g body-weight)	
Plasma	1·1	0·9
Muscle	72·0	25·7
Liver	1·6	4·7
Intestine	2·8	3·7

in the body pool of free threonine as observed in the experiment reported in Table 13 could be accounted for by an increase in body proteins of only 100 mg. In some experiments an increase in the imbalance group of about half this amount or more has been observed in the liver alone and it has not been statistically significant. The changes observed in the concentration of free threonine in body fluids could therefore be accounted for by changes in incorporation and in total body protein content that are within the limits of variability among the individual animals of a group; therefore, incontrovertible evidence for this aspect of the hypothesis is difficult to obtain.

Nevertheless, the studies of the various organ and tissue pools have revealed two interesting points. These are illustrated in the aminograms in Fig. 5. All of the indispensable amino acids were present in intestinal tissues from rats fed on the imbalanced diet in higher concentration than in intestinal tissues from controls. This would be expected for all but threonine, as they were added to produce the imbalance. Threonine was less elevated than the others. The high value for threonine is further evidence that threonine absorption was not impaired.

The aminograms for liver resembled those for intestine but the increases in the imbalanced group were much greater. The values for the imbalanced group were much higher 5 h after the meal than at 3 or 8 h. Despite evidence of increased threonine incorporation into liver proteins, the free threonine concentration in liver was elevated rather than depressed as a result of ingestion of the imbalanced diet. This indicates that threonine was being taken up efficiently by the liver and is of particular interest in relation to the observation that incorporation of ^{14}C from threonine and total liver protein content were elevated in rats fed on the imbalanced diet.

The aminograms for plasma show that threonine concentration in the imbalanced group was depressed below the control value, whereas that of most of the other indispensable amino acids was elevated. The depression in threonine in this experiment was not as great as that seen in histidine in experiments on an imbalance involving histidine or as great as that observed for threonine in a comparable study by J. C. Sanahuja (1965, personal communication) in which a different type of diet was used. However, in muscle from the imbalanced group threonine concentration was depressed much more than it was in plasma and also much more relative to the control value. Histidine concentration was also depressed in muscle, possibly because it was the second limiting amino acid in the diet.

The other ramification of this hypothesis (and actually some of the observations came before rather than after its development) is that, if food intake can be maintained in rats fed on a diet in which there is an amino acid imbalance, their growth

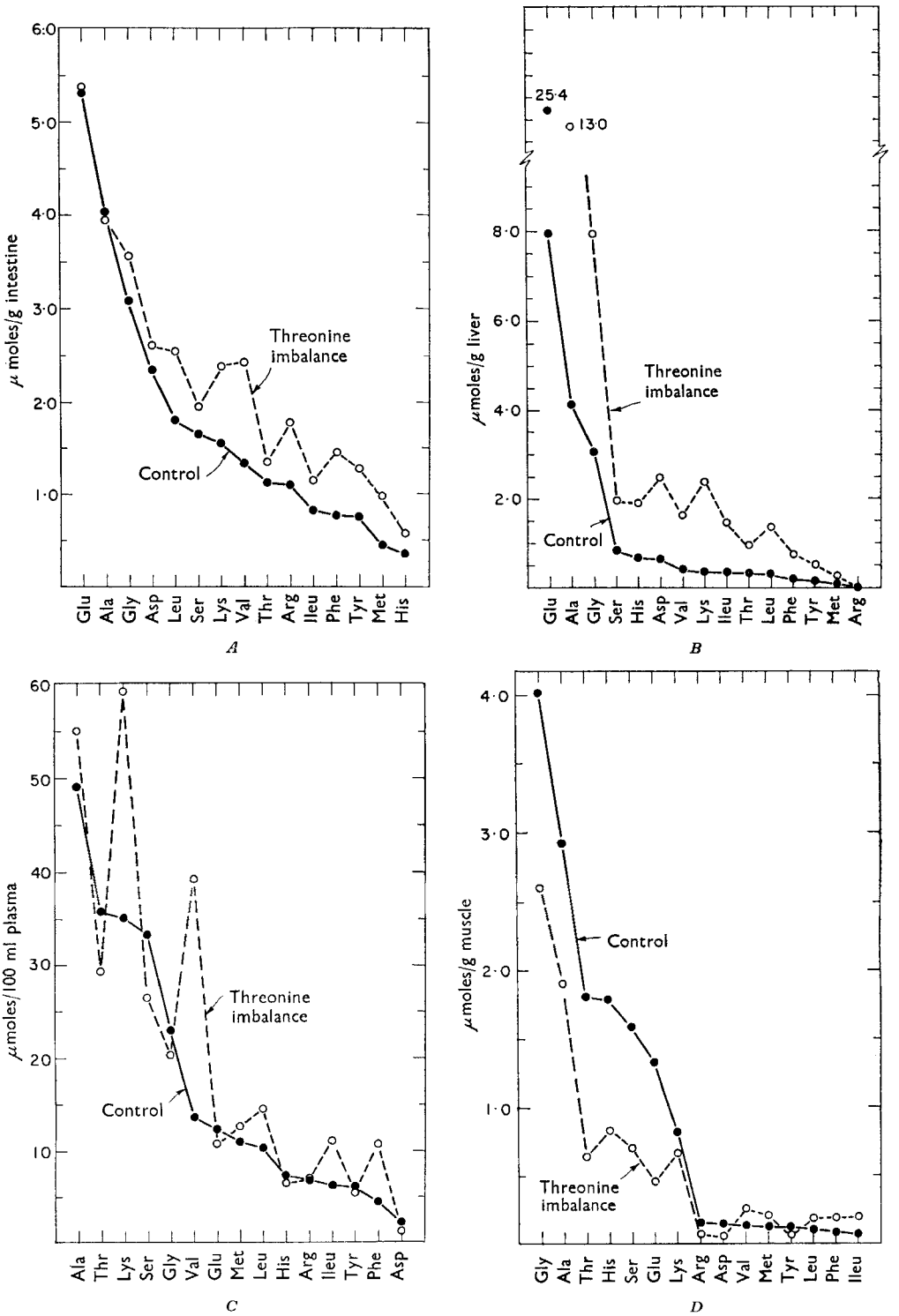

Fig. 5. Aminograms of free amino acids in plasma and tissues of rats fed on control or threonine-imbalanced (6% casein+amino acid mixture−threonine) diets (5 h after the meal). *A*, intestinal tissues; *B*, liver; *C*, plasma; *D*, muscle.

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should not be retarded and no adverse effects should be observed. This has been tested in several ways: by using insulin injection to stimulate food intake (Kumta & Harper, 1962); by adjustment of the protein : calorie ratio of the diet (Fisher & Shapiro, 1961); by exposure of the animals to a cold environment (Klain, Vaughn & Vaughn, 1962; Klain & Vaughn, 1963); by use of cortisol injection (Leung, Rogers & Harper, 1964); and by force-feeding (Leung *et al.* 1964). All of these procedures have resulted in improved growth, and growth equivalent to that of control animals similarly treated.

In Fig. 6 are shown some of our unpublished observations on rats fed on severely imbalanced diets in a cold environment. It is worthy of note that when food intake was stimulated in this way the group fed on the imbalanced diet in fact grew a little more rapidly than the control group, an indication that utilization of the limiting amino acid may actually be improved by the imbalancing amino acid mixture. Similar results were obtained with protein-depleted rats when they were given equal quantities of a control diet or a diet imbalanced with respect to histidine during the repletion period (Sanahuja & Harper, 1962).

The observations on rats kept in a cold environment (Klain *et al.* 1962; Klain & Vaughn, 1963) suggest that the usual appetite-depressing effect of an amino acid imbalance cannot be demonstrated when calorie requirement is so greatly increased. Nevertheless, when very large amounts (10–15%) of an amino acid mixture devoid

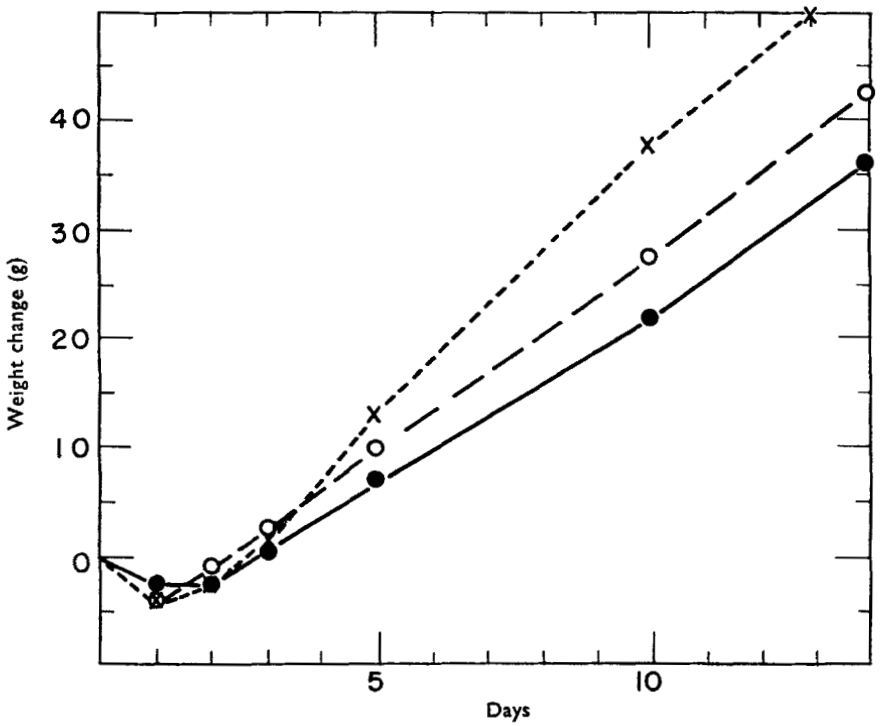

Fig. 6. Effect of cold environment (7°) on growth of rats fed on imbalanced diets: ●—●, control diet, 5% casein; ○--○, imbalanced diet, 5% casein+amino acid mixture−histidine; ×---×, corrected diet, 5% casein+5% amino acid mixture+histidine.

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of histidine were included in a low-protein diet, the usual depression in food intake and growth rate brought about by an amino acid imbalance could be demonstrated in the cold (Fig. 7).

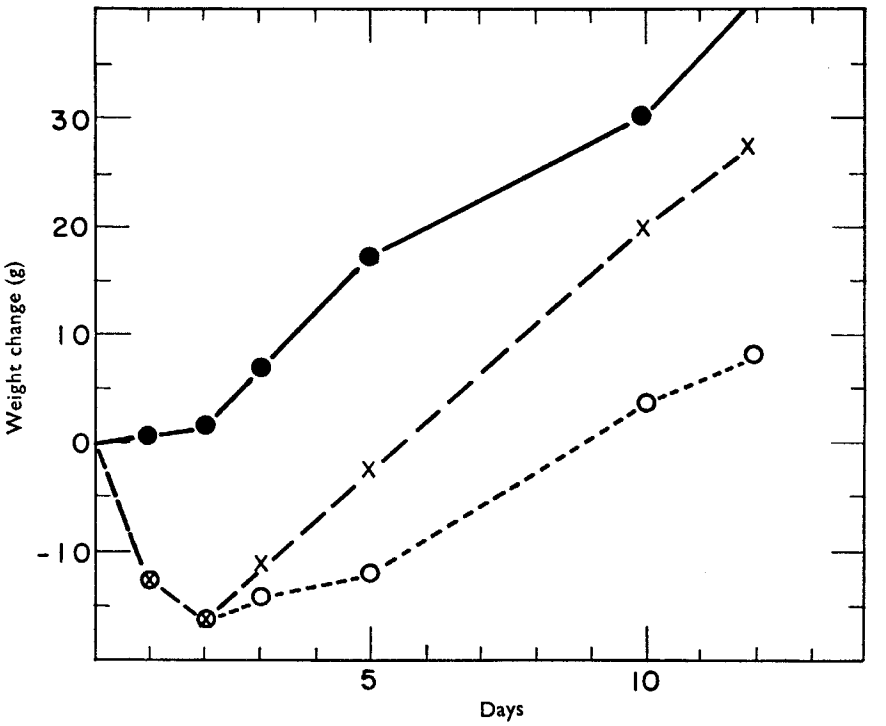

Fig. 7. Depression of growth of rats at 7° by severely imbalanced diets. ●—●, control diet, 5% casein; ×--×, 5% casein+10% amino acid mixture−histidine; ○- - -○, 5% casein+15% amino acid mixture−histidine.

This brings us back to the question of the effect of an amino acid imbalance on the efficiency of utilization of the limiting amino acid. When efficiency of utilization of the limiting amino acid is calculated, as shown in Tables 6 and 7, and for similar examples from some other studies (Salmon, 1954; Fisher & Shapiro, 1961) it is evident that gain per unit of ingested amino acid for animals given the corrected diets is lower than that for animals given control diets supporting equivalent growth. This we think is not because the imbalancing amino acid mixture causes an increase in the rate of katabolism of the limiting amino acid but because the concentration of the limiting amino acid must be increased in a diet containing a surplus of all but one of the indispensable amino acids in order to stimulate food intake and that any extra amount of the limiting amino acid beyond that required for protein synthesis is merely katabolized normally, as is any other surplus. Low efficiency of nitrogen utilization has been observed with rats given a corrected diet (Deshpande, Harper & Elvehjem, 1958).

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Taken altogether, observations on the effects of an amino acid imbalance have led us to the conclusion that the imbalancing amino acid mixture results in an abnormal pattern of amino acids in certain body fluid compartments by causing a quantitative shift in normal pathways of amino acid metabolism, probably by stimulating incorporation of amino acids into tissues that are actively synthesizing protein. The homoeostatic capacity of the body to restore the free amino acid pattern of the blood, muscle, and possibly those of some other organs or tissues, to normal is exceeded and, in response to the abnormal amino acid pattern which resembles that of animals fed on a much more deficient diet, food intake is depressed, and hence growth is retarded.

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The balancing of amino acid mixtures and proteins

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In discussing imbalance or unbalance it is desirable to know the composition of the ideal mixture of amino acids which might be described as constituting perfect balance. The work reported here was directed towards correlating nutritive value (biologically determined) of proteins and amino acid mixtures of known composition with their amino acid make-up. The results may be applied to answer whether deviations from the ideal composition create an imbalance, and whether the relation between chemical score and biological value would be upset by such an imbalance.

The results reported in this paper have not been presented before except at a special meeting of the Committee on Protein Malnutrition (Bender, 1961).

The ideal mixture, i.e. perfect balance, is defined as that which has a biological value of 100 when given to the rat at 1·6% nitrogen level.

Methods

Net protein utilization was measured by the carcass analysis method (Miller & Bender, 1955); digestibility was measured in the same experiment and biological value (BV) was calculated from net protein utilization (NPU) divided by digestibility (D).

In the amino acid diets the L-forms of histidine, lysine and leucine were used, and the DL-forms of the other amino acids at double the level of the L-isomer required, except for methionine of which the D-form is fully utilized (Berg, 1959). Non-essential amino acids were supplied as a mixture of equal parts of glycine, arginine, alanine and glutamate to avoid an excess of any one amino acid. The unusable D-forms of the essential amino acids were included with the non-essential nitrogen for the purposes of calculation.

Evaluation of egg protein

In calculating chemical scores, Block & Mitchell (1946–7) and subsequent workers used egg protein as the standard, regarding this as the best available approach to the perfect protein. Apart from the question of the accuracy of the analysis (Bender, 1954) errors could arise if egg protein contains one or more essential amino acids in quantities greater than required by the rat. Such a surplus would not be revealed by a biological assay but would cause an error in estimating chemical score. If the essential amino acid in question were present in egg in amount greater than 100% of the rat's needs then the chemical score would be undervalued.