Effect of Amino Acid Imbalance on the Fate of the Limiting Amino Acid [1]

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ABSTRACT The fate of the most limiting amino acid (i.e., tracer quantities of threonine-U-^{14}C or histidine-U-^{14}C) was studied in rats fed a single meal of a low protein diet in which an amino acid imbalance had been created by the addition of an amino acid mixture devoid of threonine or histidine. The amino acid imbalances did not increase the rate of oxidation of labeled amino acids as indicated by measurement of radioactivity in expired carbon dioxide; they did not result in enhanced excretion of radioactivity in urine or feces; nor did they result in enhanced incorporation of radioactivity into liver glycogen or fat. The accumulated evidence indicated that amino acid incorporation into liver proteins was enhanced by the amino acid imbalances. Incorporation into other tissue proteins was not depressed, and total retention of label in carcass was slightly greater as a result of the imbalance. On the basis of these and other observations, a hypothesis was formulated to explain the effects of amino acid imbalances. It is suggested that an imbalance leads to more efficient incorporation of the growth-limiting amino acid into tissues with the result that its concentration in blood plasma decreases within a few hours after ingestion of the imbalanced meal. This phenomenon, which resembles the response of an animal to a severely deficient diet — a protective response — results in a signal to an appetite-regulating center indicating that the diet is much more deficient than it actually is. Food intake is subsequently depressed and the food intake depression results in retarded growth.

Rats fed a low protein diet to which has been added a mixture of all but one of the indispensable amino acids gain less weight than control rats fed the low protein diet alone. The growth retardation is attributed to an amino acid imbalance and can be prevented by a small supplement of the amino acid missing from the mixture. Although considerable information about the nutritional effects of amino acid imbalances has accumulated (1), the metabolic events responsible for these effects are not known.

Ten years ago, Salmon (2, 3) postulated that the addition of an incomplete mixture of amino acids to a low protein diet might increase the catabolism of all amino acids and, hence, result in increased destruction of the amino acid in shortest supply in the diet (limiting amino acid). To test this hypothesis which would explain the growth-depressing effect of an amino acid imbalance, 2 groups of workers studied the metabolism of isotopically labeled tryptophan in rats fed a low protein diet lacking niacin and to which threonine had been added to create an amino acid imbalance. Florentino and Pearson (4) obtained results supporting the hypothesis, whereas Wilson et al. (5) found no indication of increased catabolism of tryptophan, the limiting amino acid in the diet used. The rats used in these experiments had been fed the imbalanced diet for a week or more and showed a depressed rate of growth. As the growth depression is mainly the result of low food intake (6–9), it is difficult in experiments conducted under such conditions to distinguish between metabolic changes due directly to the amino acid imbalance and those due to low food intake. The initial metabolic effects of an amino acid imbalance should be detectable before, or at least at the same time as, the food intake of the experimental animals is affected; hence experiments of relatively short duration appear to be needed to resolve the dilemma posed by these disparate results.

Received for publication January 22, 1966.

[1] This investigation was supported in part by Public Health Service Research Grant no. AM-05718 from the National Institute of Arthritis and Metabolic Diseases and in part by a grant from the Nutrition Foundation, Inc., New York.

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A decrease in the concentration of the limiting amino acid in blood plasma is one of the earliest biochemical changes observed in rats fed an amino acid-imbalanced diet (10, 11), and the possibility of a close relationship between the regulation of food intake and plasma amino acid pattern has been suggested (9, 12, 13). Increased catabolism of the limiting amino acid could account for this type of change in the plasma amino acid pattern. In addition, the extra amino acids added to create an imbalance might interfere with absorption of the limiting amino acid from the gastrointestinal tract, with uptake of the limiting amino acid from body fluids into tissues, or with synthesis of protein in the tissues.

In an effort to determine the metabolic basis for the gross effects of an amino acid imbalance and for the reduction in the concentration of the most limiting amino acid in blood plasma, the metabolic fate of ^{14}C-labeled limiting amino acids was studied in rats immediately after they had ingested equal quantities of a balanced or an imbalanced diet.

EXPERIMENTAL PROCEDURE

Young adult male rats of the Sprague-Dawley strain were trained to consume about 10 g of a 15% casein agar-gel diet within 2 hours by feeding them for only 2 to 3 hours daily for about 3 weeks.

Two amino acid imbalances were studied: a threonine imbalance, and a histidine imbalance. These imbalances were produced by adding a mixture of indispensable amino acids lacking either threonine or histidine to a low protein basal diet. The basal diet used in the study of the threonine imbalance contained: (in per cent) casein, 6; L-methionine, 0.3; vitamin mixture in sucrose (14), 0.5; salt mixture (14), 5; corn oil, 5; choline·Cl, 0.2; sucrose, 28; and dextrin, 55. To prepare the threonine-imbalanced diet, 10% of the carbohydrate in the basal diet was replaced by an amino acid mixture lacking threonine. The amounts of individual amino acids added were as follows: (in per cent) L-leucine, 1.67; L-isoleucine, 1.26; L-valine, 1.26; L-histidine·HCl, 0.73; L-phenylalanine, 1.67; L-lysine·HCl, 2.60; L-tryptophan, 0.35; and L-methionine, 0.47. The basal diet used in the study of the histidine imbalance was similar except that the dietary level of casein was reduced to 5%, and 0.2% of L-threonine was added to ensure that this amino acid would not be limiting. To create a histidine imbalance, 5.4% of an amino acid mixture lacking histidine was added to the basal diet in place of carbohydrate. The amounts of individual amino acids added were as follows: (in per cent) L-methionine, 0.3; L-phenylalanine, 0.9; L-leucine, 0.9; L-isoleucine, 0.6; L-valine, 0.6; L-lysine·HCl, 0.9; L-arginine·HCl, 0.6; L-threonine, 0.45; and L-tryptophan, 0.15.

These amino acid mixtures were known from previous work (1, 9, 15,) to cause amino acid imbalances that resulted in growth depressions in rats fed ad libitum. The growth depression caused by the amino acid mixture lacking histidine is prevented by a supplement of histidine; that caused by the amino acid mixture lacking threonine is prevented by a supplement of threonine.

Each diet was dispersed in an equal amount of hot water containing 3% of agar and upon cooling the mixture set as a gel. Threonine-U-^{14}C (6.2 μg) was incorporated into the threonine-imbalanced diet and histidine-U-^{14}C (5.4 μg) was incorporated into the histidine-imbalanced diet such that each rat ingested about 8.3 μc. The quantities of the limiting amino acids added were insufficient to affect growth. Although the diets contained about 50% of water, food intakes are reported on a dry-weight basis. The control diets in these studies contained about half as much nitrogen as the experimental diets; hence, in essence, what is being studied is the effect of a surplus of all of the indispensable amino acids except one on the metabolic fate of the indispensable amino acid that is limiting for growth. The control and experimental diets contained the same amount of the limiting amino acid.

For the collection of carbon dioxide, animals were placed in glass metabolism cages through which dry, carbon dioxide-free air was passed. The expired carbon dioxide was collected in columns containing a mixture of ethanolamine and ethyleneglycol monomethylether (1:2, v/v), and the radioactivity was determined according to the method of Jeffay and Alvarez (16).

All of the samples were counted in a Packard liquid scintillation spectrometer. An internal standard of ^{14}C-toluene was used to determine the disintegrations per minute (dpm).

Spilled food was suspended in 1 N HCl, heated overnight, filtered and an aliquot was used for the determination of radioactivity with aqueous scintillation solution I (17) after neutralizing and decolorizing a 2-ml aliquot with 0.2 ml of 30% hydrogen peroxide for 4 to 6 hours at 100°. Recoveries of radioactivity from protein-^{14}C and threonine-^{14}C using this procedure were 97% and 85%, respectively. Urine was diluted to constant volume, filtered and a 2-ml aliquot was also decolorized and counted as described above.

Feces were homogenized in a mortar with water, made to volume and centrifuged, and 2 ml of the supernatant fluid was counted as described for urine.

In some experiments 0.1 ml of blood was collected hourly from the tail vein, mixed with 5 ml of cold 5% trichloroacetic acid and centrifuged, and 2 ml of the supernatant fluid was used for the determination of radioactivity.

Before the rats were killed, they were anesthetized with ether; blood was taken by heart puncture. Then the gastrointestinal tracts, livers, kidneys and left gastrocnemius muscles were taken out rapidly, frozen, and stored at −29° until analysis was performed. The remainder of each carcass was dissolved in about 600 ml of NaOH in methanol (160 g/liter) and heated at 95° for 2 to 4 hours. The solution was diluted to 1 liter with water and an aliquot taken, neutralized, and aqueous scintillation solution added and the sample counted.

Gastrointestinal contents were washed out with water, dissolved in dilute alkali, neutralized and centrifuged; an aliquot was decolorized and counted as described above. Portions of liver and kidney homogenates were diluted with 2 N NaOH, heated, then neutralized and the radioactivity of the whole tissue was determined as described above. Tissues were homogenized in water, and acid-soluble and protein fractions were obtained acccording to the method of Hutchinson and Munro (18). Protein fractions were dissolved in 2 N NaOH, and a 1-ml aliquot containing about 10 mg of protein was transferred to a counting vial and partially hydrolyzed by heating at 95° for 24 hours. The solution was then neutralized with HCl and mixed with the aqueous scintillation solution and counted. The addition of 1 to 2 ml of absolute ethanol to this counting mixture was necessary to prevent the separation of the aqueous phase from the organic solvent phase. The ethanol also minimized the formation of precipitates.

Another aliquot of the protein solution in 2 N NaOH was used for the determination of nitrogen by the Kjeldahl method. From these data the specific activity of the protein was calculated. For some samples, isolated protein was suspended in methanol, and a portion of it was transferred to a tared counting vial, and the weight determined directly. The nitrogen content of tissue homogenates was also determined by the Kjeldahl method. In some of the samples radioactivity of liver glycogen and liver lipids was measured. To prepare the glycogen a portion of fresh liver was dissolved in 30% KOH, and glycogen was precipitated with ethanol; the precipitate was dissolved in 5% trichloroacetic acid, and the contaminating protein was removed by centrifugation. Glycogen was again precipitated with ethanol, hydrolyzed with dilute HCl, neutralized and counted in aqueous scintillation solution. Glycogen content was determined by a colorimetric method using phenol and sulfuric acid (19). Liver lipids were extracted with diethylether from the dried, powdered, liver homogenates. The lipids were dissolved in petroleum ether, washed with distilled water and counted in toluene containing 0.4% 2,5-diphenyloxazole (PPO).

Uniformly labeled L-threonine and L-histidine were purchased from the New England Nuclear Corporation which had tested them for purity by chromatography in 4 solvent systems and guaranteed impurities of less than 2%. They were used as received. Later, both amino acids were tested for purity in our laboratory by placing a sample in a Technicon Amino Acid Analyzer and passing the effluent from the column through the flow cell of a Nuclear Chicago scintillation spectrometer. For labeled threonine 96 to 98% of the radioactivity

coincided with the standard threonine. For the labeled histidine 75% of the radioactivity coincided with the standard histidine. The rest of the radioactivity was scattered along the chromatogram in small peaks which were apparently breakdown products of histidine. Histidine in very small quantities is known to be light-sensitive. Not more than 4% of the contamination corresponded with peaks for known standard amino acids. All results are based on the amounts of radioactivity absorbed by individual rats.

RESULTS

Shown in figure 1 is the percentage of radioactivity expired as carbon dioxide by animals fed 7 g of either the control diet (4 rats) or the threonine-imbalanced diet (6 rats) containing threonine-U-^{14}C. Carbon dioxide was collected hourly for the first 8 hours; then at 4-hour intervals to the end of 24 hours, and finally at 6-hour intervals until 48 hours. At 24 hours the animals were again fed 7 g of their respective diets, but without threonine-U-^{14}C. Animals fed the control diet expired somewhat more radioactivity in CO_2 than did those of the imbalanced group over the 48 hours of the experimental period, but the difference between the 2 groups was not statistically significant. During the second 24 hours the curves for cumulative radioactivity in CO_2 expired by the 2 groups were almost parallel.

The amounts of radioactivity excreted in the urine and feces of the imbalanced group did not exceed those for the control group (table 1) so that, overall, no more ^{14}C was eliminated by the threonine-imbalanced group than by the control group. The livers of rats fed the threonine-imbalanced diet contained more radioactivity from threonine-U-^{14}C than did those of the control group, and more ^{14}C was also retained in the carcasses of rats fed the imbalanced diet.

To test the effect of dietary amino acid imbalance on the absorption of the first-limiting amino acid, gastrointestinal contents were collected and their radioactivity determined at 3.5 and 8 hours after feeding control and imbalanced diets containing ^{14}C-labeled threonine or histidine. Animals were fed 3 g of diet in the experiment on the histidine imbalance. Absorption was calculated as the difference between the amount of radioactivity ingested and that recovered from the gastrointestinal tract (table 2). By 3.5 hours after the feeding period 65% and 59% of the ingested radioactivity had been absorbed from the intestinal tracts of the control and threonine-imbalanced groups, respectively. The difference between the 2 groups was not statistically significant. By 8 hours 97% of the ingested radioactivity had been absorbed by the animals of the control group and 95% by those of the imbalanced group.

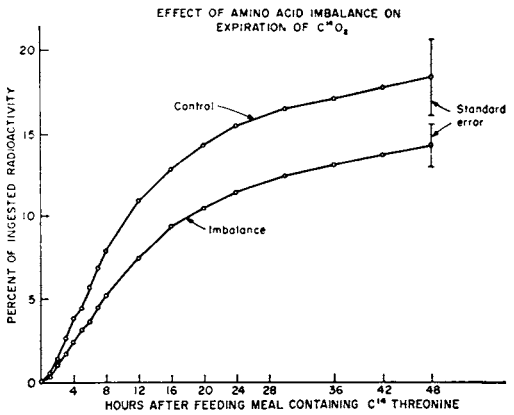

Fig. 1 Expiration of $^{14}CO_2$ after feeding diets containing threonine-U-^{14}C. Rats were fed 7 g of either the control diet or threonine-imbalanced diet containing threonine-U-^{14}C. After 24 hours, each rat was fed 7 g of the same diet without threonine-U-^{14}C. Each point represents the average of 4 rats for the control group and 6 rats for the imbalanced group. Standard errors of the mean are shown at 48 hours.

TABLE 1

Distribution of radioactivity at 48 hours after feeding diets containing threonine-U-^{14}C [1]

	% of ingested ^{14}C	
	Control	Imbalanced
	%	%
Expired CO_2	18.4 ±2.3 [2]	14.3 ±1.3
Urine	2.08±0.25	2.20±0.17
Feces [3]	1.65±0.17	1.25±0.20
Liver	5.87±0.29	7.24±0.26
Carcass	70.1 ±1.87	74.6 ±2.37
Recovery	98.1 ±1.27	99.5 ±2.16

[1] Rats were fed 7 g of either the control or threonine-imbalanced diet containing 8.3 μc of threonine-U-^{14}C. After 24 hours, animals were fed 7 g of the same diet without threonine-U-^{14}C.
[2] Mean ± SE of mean.
[3] Water-soluble portion of feces.

TABLE 2

Percentage absorption [1] of threonine-U-^{14}C or histidine-U-^{14}C after feeding the threonine- or histidine-imbalanced diet

Hours after feeding diet	Threonine-U-^{14}C		Histidine-U-^{14}C	
	Control	Imbalanced	Control	Imbalanced
3.5	64.6 ± 5.79 [2]	58.9 ± 2.84	—	—
8	97.4 ± 0.72	95.7 ± 0.83	92.1 ± 1.89	89.3 ± 1.55

[1] % absorption = $\frac{\text{radioactivity ingested} - \text{radioactivity in g.i. contents}}{\text{radioactivity ingested}} \times 100$.

[2] Mean ± SE of mean.

In the experiment on histidine imbalance 92% of the ingested radioactivity was absorbed within 8 hours by animals fed the control diet and 89% by those fed the imbalanced diet. Thus, amino acid mixtures lacking either threonine or histidine did not appear to affect the extent of absorption, respectively, of threonine and histidine, the limiting amino acids.

A statistically significant difference ($P < 0.05$) was observed between the amounts of radioactivity remaining in the TCA-soluble fraction of blood from control and imbalanced groups 8 hours after the feeding period (fig. 2). A time study was carried out only for the threonine imbalance. In one experiment blood was collected from each group of rats at 0.5, 1.5, 2.5 and 3.5 hours; in another experiment at 8 hours. In the experiment on histidine imbalance, blood was collected only at 8 hours. After feeding the diets containing threonine-^{14}C, the TCA-soluble fraction of blood from animals fed the threonine-imbalanced diet contained less radioactivity at 3.5 hours, and by 8 hours the difference was highly significant. A significant difference was also observed at 8 hours between the experimental and control groups in the study of histidine imbalance. Thus, absorbed threonine-U-^{14}C and histidine-U-^{14}C disappeared more rapidly from the peripheral circulation when animals were fed the threonine- or histidine-imbalanced diets.

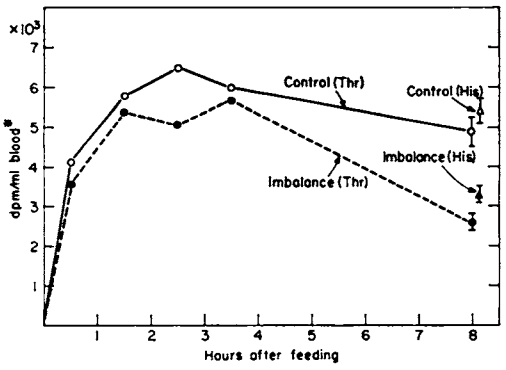

Fig. 2 Effect of amino acid imbalance on disappearance of radioactivity from the acid-soluble fraction of blood. Plasma was used in the histidine study. Differences at 8 hours were significant in both experiments ($P < 0.05$).

The values for total protein content of the livers of animals fed the threonine- and histidine-imbalanced diets were slightly higher than those for their respective controls, but the differences were not significant (table 3.). Values for the percentage of absorbed radioactivity incorporated into liver were significantly higher for both threonine- and histidine-imbalanced groups than for the respective control groups, except for the experiment terminated at 3.5 hours (table 4). The greatest difference was observed in the experiment on histidine imbalance in which the livers of the control group contained 11.2% of absorbed radioactivity, whereas those of the imbalanced group contained 16.1%. Values for the percentage of ingested radioactivity in the TCA-soluble fraction of liver were similar for the control and the threonine-imbalanced groups; but in the experiment on histidine imbalance, the value of 1.56% for the control group was distinctly higher than that of 0.87% for the imbalanced group.

The percentage of ingested radioactivity associated with the total liver protein was significantly higher for the histidine-imbalanced group as also was the specific activity of the liver protein. Similar results were obtained in the experiment on threonine imbalance, but the differences were smaller, and no difference was observed at

TABLE 3

Composition of liver

Hours after feeding ^{14}C-containing diets	Type of diet	Body wt	Liver wt	Total protein	
		g	*g*	*mg/liver*	%
		Threonine imbalance			
3.5	Control	196 ± 10.0 [1]	6.1 ± 0.2	1250 ± 78	20.6 ± 0.8
	Imbalanced	201 ± 7.6	6.7 ± 0.4	1396 ± 60	21.0 ± 0.5
8	Control	129 ± 2.4	4.5 ± 0.1	814 ± 19	18.2 ± 0.5
	Imbalanced	132 ± 1.5	4.6 ± 0.2	892 ± 40	19.4 ± 0.7
48	Control	235 ± 2.8	5.6 ± 0.3	1172 ± 89	20.0 ± 0.8
	Imbalanced	230 ± 4.8	6.6 ± 0.3	1256 ± 59	18.8 ± 0.5
		Histidine imbalance			
8	Control	137.8 ± 5.9	6.3 ± 0.16	909 ± 28	14.4 ± 0.33
	Imbalanced	142.2 ± 6.6	6.3 ± 0.35	949 ± 48	15.1 ± 0.17

[1] Mean ± SE of mean.

TABLE 4

Distribution of radioactivity in liver

Hours after feeding ^{14}C-containing diet	Type of diet	% of absorbed ^{14}C			Specific activity of protein [1]
		Liver	Acid-soluble fraction	Protein	
		%	%	%	
		Threonine imbalance			
3.5	Control	5.73 ± 0.34 [2]	1.02 ± 0.08	3.78 ± 0.34	323 ± 27
	Imbalanced	5.84 ± 0.48	1.23 ± 0.46	3.83 ± 0.42	291 ± 25
8	Control	5.64 ± 0.26 [a]	0.85 ± 0.11	4.39 ± 0.17	926 ± 38
	Imbalanced	6.29 ± 0.10	0.77 ± 0.14	5.14 ± 0.37	989 ± 41
48	Control	5.87 ± 0.29 [a]	0.30 ± 0.03	5.04 ± 0.31	667 ± 41
	Imbalanced	7.24 ± 0.26	0.36 ± 0.03	6.18 ± 0.48	788 ± 49
		Histidine imbalance			
8	Control	11.2 ± 0.64 [b]	1.56 ± 0.18 [b]	10.3 ± 0.45 [b]	1638 ± 69 [b]
	Imbalanced	16.1 ± 0.62	0.87 ± 0.076	15.9 ± 0.44	2402 ± 115

[1] Specific activity = dpm/mg liver protein × $\frac{\text{mean dpm absorbed of control and imbalanced groups}}{\text{dpm absorbed for each rat}}$.

[2] Mean ± SE of mean.

[a] Difference between control and imbalance is statistically significant ($P < 0.05$).

[b] Difference between control and imbalance is statistically significant ($P < 0.01$).

the 3.5 hour interval. The radioactivity of liver glycogen and liver lipids from rats used in the study of histidine imbalance was measured. Liver glycogen and liver lipids from the control group contained 0.15% and 0.073%, respectively, of absorbed radioactivity and values for the histidine-imbalanced group were, respectively, 0.12% and 0.055%. Differences between the control and experimental groups were not statistically significant.

Radioactivity per unit weight of muscle tended to be low for the imbalanced groups up to 8 hours (table 5). In the histidine imbalance experiment the value of 59.2 dpm/mg muscle for the control group was significantly higher than that of 42.6 dpm /mg muscle for the imbalance group. The decrease appeared to be due to a decrease in the amount of radioactivity in the TCA-soluble fraction of the muscle since the specific activity of muscle protein at 8 hours

TABLE 5

Incorporation of threonine-^{14}C or histidine-^{14}C into muscle

Hours after feeding diets	Type of diet	Radioactivity		
		Whole muscle	TCA-soluble fraction	Muscle protein
		dpm/mg muscle	*dpm/mg muscle*	*dpm/mg protein*
		Threonine imbalance		
3.5	Control	28.3 ± 5.1 [1]	—	25.8 ± 2.9
	Imbalanced	20.7 ± 1.1	—	36.7 ± 1.3
8	Control	49.2 ± 2.6	17.9 ± 0.09 [a]	221 ± 5.5
	Imbalanced	44.8 ± 1.5	7.5 ± 0.07	230 ± 5.9
48	Control	38.8 ± 3.3	—	—
	Imbalanced	45.8 ± 3.2	—	—
		Histidine imbalance		
8	Control	59.2 ± 3.7 [a]	36.9 ± 1.4 [a]	177 ± 8.9
	Imbalanced	42.6 ± 2.2	12.6 ± 0.53	175 ± 9.0

[1] Mean ± SE of mean.
[a] Difference between 2 groups is significant ($P < 0.01$).

TABLE 6

Incorporation of histidine-U-^{14}C or threonine-U-^{14}C into kidney and intestine

Hours after feeding diet	Type of diet	Kidney			Intestine
		Wet wt	Acid-soluble fraction	Protein	Protein
		g	*dpm/mg tissue*	*dpm/mg protein*	*dpm/mg protein*
		Threonine imbalance			
8	Control	0.84 ± 0.03 [1]	49.0 ± 1.6 [a]	846 ± 29	2226 ± 156
	Imbalanced	0.85 ± 0.02	40.4 ± 1.1	787 ± 23	2376 ± 247
		Histidine imbalance			
8	Control	1.04 ± 0.04	55.9 ± 1.8	1192 ± 33 [a]	2412 ± 399
	Imbalanced	1.07 ± 0.05	59.0 ± 2.5	1588 ± 86	2724 ± 120

[1] Mean ± SE of mean.
[a] Difference between 2 groups is statistically significant ($P < 0.01$).

was the same for the control and imbalance groups in both the threonine imbalance and histidine imbalance studies. At 3.5 hours in the threonine study, however, the value for the imbalance group was higher.

Analyses of kidney and intestine were carried out 8 hours after feeding diets containing either threonine-U-^{14}C or histidine-U-^{14}C (table 6). In the threonine-imbalance study the value for radioactivity in the TCA-soluble fraction and in the protein of kidney was lower for the imbalanced group than for the control; but in the histidine-imbalance study the values for the imbalance group were higher than the control values. The specific activity of kidney protein from the histidine-imbalanced group was significantly higher than that for the control ($P < 0.01$). The specific activity of intestinal protein tended to be higher in the imbalanced groups in both studies, but in neither study were the differences statistically significant (table 6).

DISCUSSION

From these and previous results we have developed a hypothesis concerning the sequence of events which leads to depressed growth and food intake in rats ingesting a diet in which an amino acid imbalance has been created. The imbalanced meal is evidently digested and absorbed normally, and this results in the flow of a surplus of all but one of the indispensable amino acids to the liver where protein synthesis is stimulated. More of the amino acid in short

supply is incorporated into liver proteins and hence the supply for peripheral tissues is reduced. It is not, however, reduced to the point where protein synthesis is suppressed by low substrate concentration because incorporation into muscle proteins apparently continues at the same rate as in the control animals (table 5). This eventually results in the muscle and plasma free amino acid patterns becoming severely unbalanced, and the patterns then resemble those resulting from ingestion of a severely deficient diet; a homeostatic mechanism is triggered and food intake is depressed. As a consequence of this depression of food intake, the supply of amino acids for protein synthesis is reduced, and the growth of the animal is retarded.

Incontrovertible evidence for the validity of some aspects of this hypothesis has been difficult to obtain. However, some additional indirect evidence from other sources tends to support it, and on the basis of evidence obtained so far, some alternative hypotheses can be eliminated.

The observations on the metabolism of threonine-U-^{14}C (fig. 1 and table 1) indicate that an amino acid imbalance created by adding a surplus of all but one of the indispensable amino acids to a low protein diet does not increase the catabolism of the limiting amino acid as suggested earlier (2, 3,). Actually, the amounts of ^{14}C in carbon dioxide, glycogen, lipids, urine and feces were somewhat lower for the group fed the imbalanced diet. The results of carcass and liver analyses confirmed that most of the ^{14}C had been retained in the body. If, as proposed above, a greater amount of the most limiting amino acid is removed from the circulation because of increased incorporation into proteins, a reduction in the amounts of radioactivity in carbon dioxide, urine, feces, glycogen and fat would be expected. In a study of the effect of an amino acid imbalance involving histidine on the oxidation of histidine-U-^{14}C, less radioactivity was found in CO_2 expired by rats fed the imbalanced diet.[3]

Competition for transport sites has been suggested as a possible explanation of the effects of amino acid imbalance (20). The rates of absorption of threonine-U-^{14}C and histidine-U-^{14}C from the intestine were not lower for the imbalanced groups than for the control groups (table 2). An amino acid imbalance, therefore, does not appear to affect significantly gastrointestinal absorption of the limiting amino acid. This may well be due to the large capacity of the intestine to absorb amino acids (21). Evidence that competition among amino acids during transport into cells does not occur as a result of feeding an imbalanced diet has been presented recently (22).

The observations on the incorporation of ^{14}C-labeled amino acids indicated that the amount of radioactivity incorporated into liver proteins of rats fed the imbalanced diets was greater than for controls, but the differences were not consistently significant. It takes only a small increase in body proteins to account on a quantitative basis for the decrease in the concentration of the growth-limiting amino acid in both muscle and plasma. In the first place the plasma pool represents only a small portion of the total amount of amino acids in the body (22). A small increase in incorporation into the proteins or pools of one or more large organs or tissues or a small decrease in the rate of protein breakdown could cause a substantial change in the amount of an amino acid circulating in blood plasma. An estimate of the total decrease in plasma and muscle free threonine made in another experiment amounted to 37 μmoles (22). A 12% increase (about 100 mg) in liver proteins or a 0.5% increase in total body proteins would account for a decrease of this size. Such changes could well be within the limits of accuracy of measurements made on intact animals owing to the variability among individuals. The development of techniques for the continuous monitoring of amino acid pools and amino acid incorporation into various tissues and organs in the living animal pose almost insuperable problems. The present experiments provide values only at specified time intervals and it must be assumed that the results indicate the true course of events.

In relation to the present results, Sidransky et al. (23–26) observed that liver protein content of rats force-fed a purified diet devoid of threonine was greater than that

[3] Dakshinamurti, K., and A. E. Harper, unpublished results.

of a control group, although muscle protein content was lower. Sanahuja (27) has recently reported higher protein content of the livers of rats fed an imbalanced diet. Sidransky has also reported that liver RNA increased in rats force-fed a diet devoid of threonine and that incroporation of ^{14}C-labeled amino acids into liver protein was enhanced in this condition (25, 26, 28).

At present it is not clear whether protein synthesis may be slightly enhanced in organs other than the liver after ingestion of an amino acid-imbalanced diet. The specific activity of muscle protein was almost the same for the control and imbalanced groups in both experiments despite the lower activity of the muscle TCA-soluble fraction for the imbalanced group. This would indicate that muscle protein synthesis is continuing steadily. The specific activity of kidney protein 8 hours after feeding the diet containing ^{14}C-threonine was slightly less for the imbalanced group, although the specific activity of kidney was clearly higher 8 hours after animals had ingested the histidine-imbalanced diet containing ^{14}C-histidine. The incorporation of the limiting amino acid into intestinal protein was also slightly higher. No evidence was obtained of a substantial depression of incorporation of labeled amino acid, and even if incorporation is unaffected in most organs and accelerated in only a few, the plasma concentration of the most limiting amino acid would decrease.

Quite apart from enhanced incorporation, a lowering of the plama concentration of the limiting amino acid could occur after ingestion of an imbalanced diet, if the imbalanced amino acid mixture decreased the rate of breakdown of body proteins. This mechanism is important in relation to metabolic adaptations of mammalian enzymes (29, 30). In the present study, such an effect could not be distinguished from increased protein synthesis.

It must be kept in mind that despite the apparent increase in protein synthesis after feeding a meal of an imbalanced diet, within a short time (a few hours) an amino acid imbalance results in depressed food intake, depressed growth and, therefore, in reduced overall protein synthesis. Henderson et al. (31) suggested that the increased niacin or tryptophan requirement of rats fed a low protein diet lacking niacin and supplemented with tryptophan might be due to accelerated protein synthesis. This would deplete the supply of tryptophan available for niacin synthesis and lead to the development of niacin deficiency. The above situation is a special case of amino acid imbalance in which an interrelationship with a vitamin is involved, but there is considerable similarity to the imbalances discussed in this paper. Henderson's idea was not generally accepted because the postulation of enhanced protein synthesis appeared to be incompatible with the observed reduction in the growth of rats fed the imbalanced diet.

If food intake can be maintained as by insulin injection (32), exposure to a cold environment (33), cortisol injection,[4] or forced-feeding,[5] no adverse effects of an amino acid imbalance are observed. When rats were fed an imbalanced diet after being protein-depleted, they gained weight a little more rapidly for a few days than rats similarly treated but fed the control diet (12). Also when food intake was stimulated by cold exposure in our laboratory, rats fed imbalanced diets gained somewhat more than those fed the control diet (15). Amino acid imbalances are frequently observed when relatively small amounts (less than 1%) of the second most limiting amino acid or acids are added to a diet. The effect is out of proportion to what would be anticipated. Such quantities are far below those tolerated readily if the amino acid is not second most limiting. If enhanced incorporation of the limiting amino acid is the basis for the changes in blood amino acid pattern as a result of feeding an imbalanced diet, then simply by mass action, provision of an excess of the second most limiting amino acid should lead to more efficient utilization of the most limiting one.

All of these observations indicate that, provided food intake can be maintained, the limiting amino acid in an imbalanced diet is used as efficiently, if not more efficiently, than the same amino acid in an appropriate control diet. These observations,

[4] Leung, P. M-B., Q. R. Rogers and A. E. Harper 1964 Effect of amino acid imbalance on food intake and preference. Federation Proc., 23: 185 (abstract).
[5] See footnote 4.

thus, lend support to the idea that the alteration in blood amino acid pattern as a result of feeding an imbalanced diet is the result of more, rather than less, efficient utilization of the limiting amino acid. The link between the alteration in blood amino acid pattern and depressed food intake is not clear, but it is known that the blood change resembles that observed in animals fed a much more deficient diet, a diet that causes a severe depression in food intake and that, when force-fed, causes the development of pathologic lesions (23–26). Thus, the response to an imbalanced diet appears to be a protective response to a signal that normally arises only when a much more severely deficient diet is fed. Rats given a choice between an imbalanced diet and a protein-free diet will select the protein-free diet (12, 34) which will not support life but will restore the blood amino acid pattern to normal and reject the imbalanced diet which will support growth. These observations suggest that the changed blood amino acid pattern serves as a signal that activates an appetite-regulating mechanism or that the pattern is an indirect reflection of some more subtle change, possibly at sites of protein synthesis, that serves as a signal.

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