It is 12 years since Krehl and coworkers (1, 2) reported that the growth of young rats fed on low protein diets deficient in niacin and tryptophan was retarded when a tryptophan-deficient protein supplement such as gelatin or zein was added to the diet, and that the growth retardation was prevented if additional tryptophan was provided. In the interval, amino acid imbalances involving practically all of the essential amino acids have been demonstrated in several species of animals raised on variously supplemented diets containing cereals or purified proteins (3-7). Growth retardations have generally served as a means of detecting amino acid imbalances; however, in some instances other metabolic defects such as an accumulation of fat in the liver (8, 9) and changes in the activities of certain liver enzyme systems (10, 11) were found.

Observations on the relationship between amino acid imbalance and liver fat deposition suggest that the proportions of amino acids required for certain specific physiological processes may differ from those required for growth. When the rate of gain is increased by providing the amino acid most limiting for growth, more of the other amino acids are probably used for tissue protein synthesis and less may be available for specific functions such as the control of liver fat deposition (9). With regard to the growth-retarding effect of amino acid imbalance, it seems likely that, when additional amino acids which cannot be used for tissue protein synthesis are excreted, there may also be some increase in the loss of the amino acid which is most limiting for growth (12); such a loss would depress the growth rate. It seems likely that a general increase in amino acid catabolism might result from the creation of an amino acid imbalance which retards growth.

The present investigation on the effect of amino acid imbalance on nitrogen retention in rats fed a low protein diet containing 6 per cent of fibrin...
was undertaken in order to extend the previous observations. Various imbalances which can be created by using such a dietary regimen and the effect of these amino acids on growth and on liver fat deposition have been reported in the preceding paper (7).

EXPERIMENTAL

The basal diet used in these experiments was the same as that described in the preceding paper (7). Weanling rats of the Sprague-Dawley strain, weighing 40 to 50 gm., were used. They were kept in metabolism cages and fed ad libitum. Urine and feces were collected during the periods specified in the text and a record of food consumption and weight gain was kept.

The diets were designated as A, B, and C. Diet A was the basal, containing 6 per cent of fibrin unsupplemented. Diet B also contained 6 per cent of fibrin, but 0.4 per cent of \textit{m}-methionine and 0.6 per cent of DL-phenylalanine were added to produce a severe imbalance. Diet C was prepared by adding to the basal diet the following: DL-methionine, 0.4; DL-phenylalanine, 0.6; L-histidine \( \cdot \) HCl, 0.2; L-leucine, 0.4; DL-isoleucine, 0.4; and DL-valine, 0.6 per cent. This mixture prevented the imbalance and stimulated growth.

Two groups of rats (six animals per group) were observed simultaneously. Group 1 was fed on Diet A for a preliminary period of 5 days to allow the animals to adapt to the diet. Collections of urine and feces were made for the next 3 days. Then, after a 4 day interval, collections were again made for another 3 day period. After this second collection period, the rats were fed Diet B which produced a severe imbalance (7). Again urine and feces were collected daily for 3 days and, after an interval of 4 days, pooled collections were made for another 3 day period. Subsequently the animals were fed Diet C which prevented the imbalance and stimulated growth (7). As before, individual 24 hour collections of urine and feces were made for 3 days and, after 4 days, pooled collections were made for another 3 day period.

The second group of rats was started on Diet B which produced an imbalance and, after two 3 day collections had been made, they were placed on Diet C. After 10 days on this diet, during which time collections were made as for the previous group, the animals were again placed on Diet B. Collections were repeated on this diet as before.

The urine samples were collected in bottles to which a few ml. of dilute sulfuric acid were added. The samples were stored in a refrigerator until the determinations of nitrogen were made.

The feces were collected on a fine mesh wire screen at the bottom of the cage and were also stored in a refrigerator.
Nitrogen determinations were made by a Kjeldahl method with copper sulfate as the catalyst.

Results

The average nitrogen intake calculated from the food consumption, the nitrogen retention, and the growth of the two groups are shown in Figs. 1 and 2. It will be seen from Fig. 1 that, when on the 15th day (Point B) methionine and phenylalanine were added to the 6 per cent fibrin diet, there was a sudden drop in food consumption for 2 days as shown in the graph for nitrogen intake. This had a corresponding effect on the total nitrogen retention and on growth. The percentage of ingested nitrogen retained dropped from 76 to 59, while total nitrogen retention fell from 55 to 25 mg. per day. When, at Point C, Diet C was given, there was a sudden increase in food consumption from 6 to 9 gm. per day. The total nitrogen retention increased from 40 to 65 mg. per day, but there was little change in the percentage of ingested nitrogen that was retained. The average gain in weight during this period was 3 gm. per day as against 0.6 gm. per day during the previous regimen.

Reference to Fig. 2 shows a similar trend for both nitrogen retention and growth. At Point C (Fig. 2), Diet B was replaced by Diet C which resulted in increased food consumption and an increased rate of growth. Both the total and the per cent nitrogen retention increased for the 1st day, but on the 2nd day, since there was a drop in food consumption, there was a dip in the total nitrogen retention. At Point B₁ when Diet B was substituted for Diet C, food intake, total nitrogen retention, and the percentage of ingested nitrogen retained all decreased.

Discussion

Results presented in this paper show that the addition of amino acids which create an imbalance leads to reductions in food intake, in total nitrogen retention, and in the percentage of ingested nitrogen retained. The fall in nitrogen intake was 42.7 per cent, while the fall in total nitrogen retention was 53.5 per cent within 24 hours. This suggests that the decreased food intake is a reflection of a decreased ability of the rat to use nitrogen for body protein synthesis.

In another experiment one group of rats was fed ad libitum on Diet A while a similar quantity of Diet B was forcibly fed to a second group to determine whether the decrease in nitrogen retention observed in rats fed on Diet B could be magnified by preventing the decrease in food intake. It was observed that most of the rats in the forcibly fed group died within 2 or 3 days, which further suggested that the rapid fall in the food consump-
tion of rats fed the imbalanced diet *ad libitum* was due to their inability to metabolize this diet in an efficient manner.

The subsequent increase in the consumption of the imbalanced diet indicates an ability of the animal to adapt to this diet. At Point C, al-
though the percentage of ingested nitrogen retained was practically the same for Diets B and C, the total nitrogen retention showed a sudden rise because of the extra food consumed. These observations might be explained on the basis of the relative proportions of the various amino acids.
that are supplied in the different diets. Such a picture is presented schematically in Fig. 3.

The solid bars represent the proportions in which the essential amino acids are required by the young rat based on the requirement for tryptophan as unity, as calculated from Rose's data (13). The open and the shaded bars represent the proportions of the same amino acids, with tryptophan as unity, in the 6 per cent fibrin diet (Diet A) and in the diet containing 6 per cent of fibrin plus six amino acids (Diet C), respectively. Since the actual amount of tryptophan required by the growing rat and

![Fig. 3. Comparison of relative amino acid requirements of the rat with relative proportions of amino acids in Diets A and C.](image)

that present in the two diets is the same (0.2 per cent of the diet), the figure represents not only the proportions but also the actual amounts.

The proportions supplied by 6 per cent of fibrin show deficiencies of all amino acids except tryptophan and arginine and growth is limited by the most deficient amino acids, leucine, isoleucine, valine, and histidine, all of which are limiting to the same extent (7). When methionine and phenylalanine are added, the deficiencies of the most limiting amino acids become more severe, but this effect is overcome when leucine, isoleucine, valine, and histidine are added. However, when the six amino acids are provided, growth is still below normal and lysine still meets only a portion of the requirement. Therefore, an excess of some of the additional amino acids in Diet C would not be used for protein synthesis; thus the per cent nitrogen retention might be lower with Diet C than with the basal diet. This indicates that, although the rate of growth is determined mainly by the absolute amount of the limiting amino acid ingested, efficiency of utiliza-
tion of nitrogen is determined by the relative proportions of the various amino acids.

Salmon (12) and Sauberlich and Salmon (14) have suggested that, when the surplus amino acids which cannot be utilized for tissue protein synthesis are excreted, there is some loss of the most limiting amino acid. This explanation finds some support in the observations of Sauberlich et al. (15) and Schweigert (16), who reported a significantly higher percentage excretion of ingested amino acids from incomplete proteins than from complete proteins. Beyer et al. (17) and Kamin and Handler (18) infused leucine into the blood of a dog and observed that this increased the level of isoleucine excreted in the urine. This increased loss of amino acids in urine may reflect an attempt by the animal to eliminate excess amino acids. However, the losses were not great enough to account for the low rate of growth (14). The present investigations suggest that, immediately after the ingestion of the imbalanced diet, amino acids are more rapidly catabolized, resulting in increased nitrogen excretion, which in turn leads to a rapid drop in the food consumption. There then appears to be an improvement in the nitrogen utilization with the lower food intake. Therefore, it seems important to study the effect of an amino acid imbalance on nitrogen metabolism during this transition period.

SUMMARY

Retention of ingested nitrogen in rats fed a 6 per cent fibrin diet supplemented with amino acids has been studied.

When the 6 per cent fibrin diet was fed without any supplement, 76 per cent of the nitrogen was retained.

Addition of methionine and phenylalanine, which created an imbalance, caused a reduction in food consumption, in total nitrogen retention, and in the per cent of ingested nitrogen retained.

On adding four more amino acids to overcome the imbalance, food intake was increased, total nitrogen retention increased, and there was a gradual rise in the percentage of ingested nitrogen retained.

The results show that, when an amino acid imbalance is created, the animal’s appetite is less, as is also its capacity to retain ingested nitrogen. These two changes result in severe growth depression.

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