Considerable information concerning the effects of amino acid imbalance has accumulated (1–6), and some of the salient features of this phenomenon have been discussed in a recent review (7). Although efforts are being made (8–10) to explain the underlying mechanism, the physiological or metabolic changes associated with the induction of an amino acid imbalance are still unknown. Recently, Deshpande et al. (11, 12), who maintained weanling rats on a low protein diet which contained fibrin, detected decreases in the percentage of absorbed nitrogen retained, in total nitrogen retention, and in food consumption within 24 hours after induction of an amino acid imbalance. It was suggested that the diminished food intake and the rapid decrease in percentage of absorbed nitrogen retained by the weanling rats might be an indication of their reduced ability to utilize the imbalanced diet. Attempts to equalize the food intake by forcibly feeding the imbalanced diet resulted in the death of animals in 2 to 3 days. It was therefore thought that rats trained to eat only during a single short interval each day might be used in order to equalize food intake and to eliminate the possible nonphysiological conditions of forced feeding (18). It was also thought that this procedure should stimulate increased ingestion of the imbalanced diet and thereby magnify the effects observed earlier. The results of such an investigation are included in the present report. In addition, the effects of amino acid imbalance on the nitrogen balance of protein-depleted adult rats and adult rats trained to eat for only 2 hours each day have been compared. Both ad libitum and pair-fed control animals were used.

EXPERIMENTAL

The animals in all the experiments were male, adult rats of either the Sprague-Dawley or the Holtzman strain. Their weights ranged from 120 to 150 gm. For growth experiments rats were housed in individual, suspended cages. Water was supplied ad libitum in all experiments. The animals in all the experiments were male, adult rats of either the Sprague-Dawley or the Holtzman strain. Their weights ranged from 120 to 150 gm. For growth experiments rats were housed in individual, suspended cages. Water was supplied ad libitum in all experiments.

The basal diet (Diet A) in these experiments was essentially that described earlier (11, 12). It contained, in the percentages indicated; fibrin, 6; salt mixture, 4 (19); corn oil, 5; vitamin supplements; 0.25; choline chloride, 0.15; and dextrin as the carbohydrate source to make up 100 per cent. The imbalanced diet (Diet B) was prepared by incorporating in Diet A, 0.4 per cent of DL-methionine and 0.6 per cent of DL-phenylalanine, which had been shown to produce a severe imbalance in weanling rats (11, 12). In some experiments, the levels of these amino acids were increased to 0.6 and 0.9 per cent respectively. Diet C, designed to correct the imbalance and stimulate growth (13), contained, in addition to the methionine and phenylalanine in the imbalanced diet, 0.2 per cent of L-leucine, 0.2 per cent of DL-isoleucine, 0.4 per cent of DL-valine, and 0.6 per cent of L-histidine·HCl. The protein-free diet was of the same composition as the basal diet except that the fibrin was replaced by dextrin. Diets A, B, and C were made isonitrogenous by the addition of DL-glutamic acid and were fed to the animals under different conditions as described below.

Feeding Ad Libitum—Rats which weighed between 130 and 150 gm. were initially offered the basal diet for 4 to 5 days after which they were sorted into groups (6 animals per group). They were then offered the various experimental diets ad libitum.

Single Daily Feeding (Space-Feeding)—Male rats that weighed approximately 150 gm. were fed the basal diet ad libitum for 7 days. They were then given the same ration for only 2 hours each day during the next 2 weeks. During this training period there was an initial loss in weight, but after a week the animals began to gain weight. At the end of the 2-week training period rats which had gained the same amount of weight and which were consuming the same amount of food were selected for the subsequent experiments. Two groups of rats (6 animals per group) were then placed in metabolism cages and continued on the same regimen for a period of 7 days to allow them to adapt to the new environment. These rats then became the subjects of the growth and nitrogen balance studies; one group was given Diet A and the other was offered Diet B.

Protein Depletion—For this purpose, rats which weighed between 150 and 160 gm. were fed the protein-free diet for 1 week. The animals lost approximately 20 to 25 gm., and only those rats which showed similar weight losses were selected and separated into groups consisting of 6 rats each. The rats were then used for both growth and nitrogen balance studies.

In most of the experiments in regard to nitrogen balance, individual 24-hour collections of urine and feces were made, but in a few experiments urine and feces for a 3-day period were pooled. Spilled food was separated from feces, and the amount of nitrogen in it was determined and subtracted from the gross nitrogen intake. Nitrogen determinations on urine samples were made by the micro-Kjeldahl method. The nitrogen content of the feces and the food was determined by the macro-Kjeldhal procedure.

RESULTS

Values for food consumption and weight gain of rats fed for only 2 hours per day have been plotted in Fig. 1. Although the rats had been trained to eat approximately 12 gm. of diet during
the 2-hour feeding period, those which received the imbalanced diet consumed consistently less than this amount except on the first day. Their rate of gain was also slower than that of the control group.

The weight gains of protein-depleted rats fed the basal diet or the imbalanced diet are presented in Fig. 2. There was a marked retardation of growth when the imbalanced diet (Diet B) was given.

The effects on the growth of adult rats of Diets A, B, and C under different conditions are summarized in Table I. The rate of gain of rats offered the imbalanced diet ad libitum was only 37 per cent of that of the control group. The growth rates were lower when the rats were fed for only 2 hours each day, but the value for the group fed the imbalanced diet was 47 per cent of the control value. In contrast to the relatively low rate of gain of rats which were fed for only 2 hours per day, protein-depleted rats given the balanced diet grew very rapidly. Protein-depleted rats ingesting the imbalanced diet also gained more rapidly, but their rate of gain was still only 39 per cent of that of their control group. Diet C, which was supplemented to correct the imbalance, did not augment the growth of protein-depleted rats as it did that of nondepleted adult rats.

Results which show the influence of amino acid imbalance on the nitrogen balance of adult rats fed under different conditions are presented in Tables II, III and IV. Since there was a decrease in the food intake of both protein-depleted and nondepleted rats when they were offered the imbalanced diet ad libitum, pair-fed control animals were included. The food consumption of rats fed for only 2 hours per day was not appreciably reduced within 24 hours after they eaten the imbalanced diet, but a considerable decrease in food intake was noted on the 2nd day. Therefore, in these experiments too, a pair-fed control group was included.

The nitrogen intake of rats given Diet B ad libitum (Table II) decreased to less than half that of the control group, and the value for the percentage of absorbed nitrogen retained fell to approximately two-thirds of the control value. However, the percentage of absorbed nitrogen retained by pair-fed controls was lower than that of the imbalanced group. When the levels of methionine and phenylalanine were increased to 0.6 and 0.9 per cent, respectively, similar results were obtained. Further nitrogen balance studies revealed that these effects of amino acid imbalance persisted on the 2nd day.

Results presented in Table III show that similar effects of amino acid imbalance on the nitrogen balance were obtained with protein-depleted rats.

The results presented in Table IV, obtained with rats fed for only 2 hours per day, merit special attention. When the difference between the food intake of the control group and that of the imbalanced group was small (1st day), neither the amount of nitrogen excreted nor the percentage of absorbed nitrogen retained was significantly lowered, as was anticipated. On the 2nd day, when the nitrogen intake decreased by 33.8 per cent, the value for the percentage of absorbed nitrogen retained fell. In these experiments, too, the pair-fed control rats retained somewhat less nitrogen than did the group given the imbalanced diet.
TABLE II
Effect of amino acid imbalance on nitrogen balance of nondepleted adult rats (ad libitum feeding)*

<table>
<thead>
<tr>
<th>Diet</th>
<th>Nitrogen intake</th>
<th>Urinary nitrogen</th>
<th>Fecal nitrogen</th>
<th>Nitrogen retained</th>
<th>Change in weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>6% Fibrin (control)</td>
<td>187.3 ± 3.3</td>
<td>32.0 ± 1.9</td>
<td>28.8 ± 1.4</td>
<td>66.4 ± 2.1</td>
<td>3.3 ± 0.6</td>
</tr>
<tr>
<td>6% Fibrin + 0.4% dl-methionine + 0.6% dl-phenylalanine</td>
<td>82.3 ± 4.9</td>
<td>23.7 ± 1.7</td>
<td>21.6 ± 1.5</td>
<td>44.6 ± 1.1</td>
<td>-2.3 ± 1.5</td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>82.3 ± 4.9</td>
<td>27.6 ± 1.6</td>
<td>20.1 ± 1.0</td>
<td>41.5 ± 0.8</td>
<td>-0.3 ± 0.9</td>
</tr>
<tr>
<td>6% Fibrin (control)</td>
<td>150.5 ± 6.7</td>
<td>35.4 ± 0.6</td>
<td>28.2 ± 1.6</td>
<td>56.7 ± 1.6</td>
<td>3.6 ± 1.3</td>
</tr>
<tr>
<td>6% Fibrin + 0.6% dl-methionine + 0.9% dl-phenylalanine</td>
<td>110.6 ± 3.3</td>
<td>29.4 ± 3.0</td>
<td>34.0 ± 2.0</td>
<td>41.5 ± 0.7</td>
<td>-5.0 ± 1.4</td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>110.6 ± 3.3</td>
<td>39.9 ± 1.8</td>
<td>28.3 ± 3.5</td>
<td>37.5 ± 2.6</td>
<td>-4.1 ± 0.2</td>
</tr>
</tbody>
</table>

* Figures represent the mean for 6 rats ± the standard error.

TABLE III
Effect of amino acid imbalance on nitrogen balance of protein-depleted adult rats (ad libitum feeding)*

<table>
<thead>
<tr>
<th>Diet</th>
<th>Nitrogen intake</th>
<th>Urinary nitrogen</th>
<th>Fecal nitrogen</th>
<th>Nitrogen retained</th>
<th>Change in weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>6% Fibrin (control)</td>
<td>483.0 ± 6.0</td>
<td>90.3 ± 5.9</td>
<td>106.8 ± 4.9</td>
<td>59.9 ± 1.1</td>
<td>19.2 ± 3.8</td>
</tr>
<tr>
<td>6% Fibrin + 0.4% dl-methionine + 0.6% dl-phenylalanine</td>
<td>227.8 ± 5.7</td>
<td>69.9 ± 3.7</td>
<td>58.8 ± 5.9</td>
<td>43.7 ± 1.5</td>
<td>3.2 ± 2.1</td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>227.8 ± 5.7</td>
<td>91.9 ± 7.0</td>
<td>57.3 ± 5.9</td>
<td>32.9 ± 5.0</td>
<td>2.3 ± 2.1</td>
</tr>
</tbody>
</table>

* Figures represent the mean for 6 rats ± the standard error.
† 3-Day experimental period.

TABLE IV
Effect of amino acid imbalance on nitrogen balance of space-fed rats*

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Nitrogen intake</th>
<th>Urinary nitrogen</th>
<th>Fecal nitrogen</th>
<th>Nitrogen retained</th>
<th>Change in weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-24 hrs.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6% Fibrin (control)</td>
<td>134.8 ± 1.7</td>
<td>23.5 ± 2.3</td>
<td>20.4 ± 1.9</td>
<td>67.6 ± 2.5</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>6% Fibrin + 0.4% methionine + 0.6% phenylalanine</td>
<td>129.1 ± 1.2</td>
<td>25.0 ± 1.8</td>
<td>23.6 ± 2.2</td>
<td>62.7 ± 0.8</td>
<td>0.8 ± 1.3</td>
</tr>
<tr>
<td>24-48 hrs.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6% Fibrin (control)</td>
<td>138.2 ± 1.3</td>
<td>20.5 ± 2.0</td>
<td>26.2 ± 1.2</td>
<td>65.9 ± 0.9</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>6% Fibrin + 0.4% methionine + 0.6% phenylalanine</td>
<td>91.5 ± 2.6</td>
<td>21.4 ± 1.6</td>
<td>18.6 ± 2.0</td>
<td>53.3 ± 2.3</td>
<td>-2.0 ± 1.5</td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>91.5 ± 2.6</td>
<td>20.8 ± 2.2</td>
<td>17.8 ± 2.0</td>
<td>45.3 ± 2.1</td>
<td>1.0 ± 1.0</td>
</tr>
</tbody>
</table>

* The figures represent the mean for 6 rats ± the standard error.

DISCUSSION

The results of the present investigation of the influence of amino acid imbalance on adult rats support earlier observations on weanling rats (17). Even rats that had been trained to eat for only 2 hours per day were unable to consume as much of the imbalanced diet as of the basal ration; however, the decrease in food intake was smaller than that of animals fed ad libitum. This may be the result of the ability of the trained rats to eat a large amount of food in a short time. On the 1st day of this experiment both groups of rats consumed the same amount of diet, but on the 2nd day the food intake of the group fed the imbalanced diet was less. These results indicate that the decrease in food intake is probably the result of some metabolic disorder consequent to the ingestion of the imbalanced diet rather than to low palatability of the imbalanced diet. Evidence now accumulating in this laboratory further supports this possibility.

The results of the studies of protein-depleted rats indicate that the imbalanced diet is not properly utilized for the synthesis of tissue proteins. Thus, during the 10-day growth period, rats fed the basal diet gained 51.5 gm., whereas those fed the imbalanced diet gained only 20.0 gm. Although the supplements of leucine, isoleucine, valine, and histidine corrected the imbalance, there was no marked stimulation of growth over and above that of the control group. The failure of the additional amino acids to augment growth, as was observed in nondepleted rats, indicates that the amino acids in 6 per cent of fibrin are utilized to the maximal extent by protein-depleted rate. It seems that the
addition of methionine and phenylalanine to a diet which contained 6 per cent of fibrin decreased utilization of those four amino acids. The interrelationship among those amino acids is being investigated further.

Ingestion ad libitum of the imbalanced diet brought about a significant decrease in nitrogen intake and in percentage of absorbed nitrogen retained (Tables II and III). However, the pair-fed control animals in all these experiments (Tables II, III, IV) showed a diminution in the retention of ingested nitrogen as great as or greater than that of the imbalanced groups. It is difficult to separate the effects on nitrogen retention from those on food intake. The rat apparently makes a physiological adjustment within a short time after ingesting this kind of imbalanced diet, as is indicated by the decrease in food intake within 24 hours when the imbalanced diet is substituted for the balanced diet. If it were possible for the rat to utilize a small quantity of imbalanced diet as efficiently as it utilizes a similar small quantity of balanced diet, very little difference between the nitrogen retention of the imbalanced group and that of the control group would be expected under conditions of pair-feeding.

The performance of the pair-fed control groups may be influenced by several factors. Thus, aside from a low calorie intake, which itself may cause reduced nitrogen retention (20), each of these conditions the percentage of absorbed nitrogen retained 6 per cent of fibrin decreased utilization of those four in the work of Geiger (21-23), Cannon (24), and others (25-27).

The critical role of the time factor in the utilization of amino acids has been emphasized in the work of Geiger (21-23), Cannon (24), and others (25-27). Increased catabolism of body proteins would be expected when the pair-fed control animals undergo a relatively long period without food. Such an effect can be observed very clearly during the 1st week when rats are being trained to consume their entire daily ration in 1 to 2 hours (28). In contrast, rats given the imbalanced diet cut over a longer period of time and do not undergo a period of fasting despite their low food intake.

SUMMARY

The influence of amino acid imbalance on growth and nitrogen retention of adult rats has been studied under different conditions of feeding.

Rats trained to cut a large amount of a low protein (6 per cent of fibrin) basal diet in a single, short, daily interval were unable to consume an equivalent amount of the diet after the addition of amino acids which caused an imbalance. The growth of rats on this regimen for 10 days was retarded.

Gain in weight of normal or protein-depleted adult rats given the imbalanced diet brought about a out food. Such an effect can be observed very clearly during the limited daily ration in a short period of time. The critical role of the time factor in the utilization of amino acids has been emphasized in the work of Geiger (21-23), Cannon (24), and others (25-27).

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