# Physiological consequences of feeding to rat a browned synthetic amino acid-sugar mixture (Maillard reaction)

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SUMMARY

An amino acid mixture was stored for 30 days at 37°C in the presence of excess glucose and 16% water (w/w) for browning.

A mixture of identical composition was kept in the freezer (-20°C) to be used as control.

Ion exchange chromatography of unhydrolyzed extract of the control and browned mixture indicated appreciable losses for all amino acids of the browned mixture except cystine.

Greater losses were observed for tryptophan 100%, histidine 76%, arginine 63%, and serine 51%.

For the biological studies the browned amino acid-sugar mixture both unsupplemented and supplemented with the amino acids lost during browning were used. The diet containing the unsupplemented browned mixture promoted no growth of the weanling rats, but it permitted the rats to maintain the initial body weight. Supplementation restored the growth to only about 65% of the control. Analysis of protein, nucleic acids and lipids in freeze-dried livers of these rats showed: (a)smaller total amount of protein, RNA, and DNA in the rats receiving the unsupplemented mixture; (b) no significant differences were found between the group on the control and on the brown supplemented diet.

In the blood serum of rats receiving the browned mixture the concentration of all amino acids increased markedly, particularly threonine and serine (20 fold). The high concentration of serine and threonine in the blood serum of rats on brown unsupplemented diet, however, was decreased considerably by supplementation. The liver polyribosome pattern of rats on brown unsupplemented diet altered markedly. This effect was greater after two days feeding than after 8 days, and could be completely reversed by supplementation.

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## INTRODUCTION

The Maillard reaction, a condensation between the amino groups of amino acids and the carbonyl group of reducing sugars such as glucose, is catalyzed by mild temperatures even in relatively dry mixtures (1, 2). The resulting glucose-amino acid complexes are known to be stabilized by isomerization of the glucose moiety to form a fructose-amino acid (Amadori rearrangement) compound, which can easily be isolated by colum chromatography (3). During this process, the mixture usually turns brown due to simultaneous formation of brown substances called melanoidins.

Since the Maillard reaction is likely to occur in many foods during storage (non-enzymatic browning) it is of interest to study the nutritional and physiological significance of feeding a severely browned diet.

The biological availabilities of methionine and glycine from their corresponding fructose derivatives have been determined for the microorganism *L. mesenteroides* P-60 and for the rat (4, 5). The microorganism was able to utilize 80% of the methionine in the complex, but rat was unable to recover the essential amino-acid. In the case of glycine, the Maillard reaction had no detectable effect on the availability of nitrogen to the rat but inhibited the growth of *L. mesenteroides* by 40%. The authors concluded that fructose complexes of both essential and nonessential amino acids can be formed, that make the essential form of nitrogen and carbon unavailable to the rat. Recently we have found that some of the nitrogen present in fructose-tryptophan is only sparingly available to the rat in the form of essential amino acid, and this marginal availability has been estimated to be about 5%, when the concentration of the complex in the diet was raised to contain bound-tryptophan equivalent to five times the free amino acid required for maximum rate of growth (6).

In the same study we also reported that the absorption rate of radioactivity from the small intestine is much lower for fructose-$^{14}$C-L-tryptophan than for free$^{14}$C-L-tryptophan. This was interpreted to be caused by either a low affinity

of the complex for the absorption site or by the need of a time lag before the free amino acid can be generated and absorbed.

While something is known about the biological effects of individual fructose-amino acid complexes, nothing is known about the consequences of feeding rats with a complex mixture of amino acids wich had been browned. We prepared diets containing these mixtures, determined the amino acid losses chromatographically, and then, we fed growing rats for approximately three weeks with the unsupplemented and supplemented diets. The purpose was to test the biological availability of the entire set of amino acids after browning, to find the extent to which one can correct for any growth depression by supplementation of the diet with free amino acids, and to observe some of the early physiological effects of both the brown unsupplemented and the brown supplemented mixtures on rats.

## MATERIALS AND METHODS

### *Preparation of the three diets*

Three lots of 2.242 kg of mixture were prepared containing 370g of a synthetic amino acid mixture suitable for growing rats (Nutritional Biochemicals Corp.), 1248g of dextrin and 624g of glucose. The composition of the synthetic amino acid mixture is specified in Table 1A.

The mixtures were mechanically homogenized while the water content was made up to 16% (w/w) with tap water.

One lot was stored at -20°C for'control, and the other two were incubated for non-enzymatic browning at 37°C in sealed glass containers.

After 30 days of incubation at 37°C all the three samples were stored in the freezer (-20°C), and subsequently analyzed chromatographically (Technicon Autoanalyzer). For the analysis the amino acids were extracted with water and directly analyzed, without previous hydrolysis to avoid further degradation of the mixture.

Those amino acids that were destroyed or bound during browning according to the amino acid analysis, were added back

to supplemented one of the brown mixtures to give a free amino acid composition identical to that of the control (Tables 1, 1A and 2).

To each of the three lots namely, the control, the brown unsupplemented and the brown supplemented mixtures, were added the missing ingredients to prepare the diets of Table 1.

TABLE 1
APPROXIMATE COMPOSITION OF UNBROWNED AND BROWNED SYNTHETIC AMINO ACID DIETS

| Ingredients (g) | Control I | Browned 1 Unsupplemented II | Browned 1 Supplemented III |
|---|---|---|---|
| Amino acid mixture (NBC) [2] | 370.0 | 370.0 | 370.0 |
| Dextrin | 1248.0 | 1248.0 | 1248.0 |
| Glucose | 624.0 | 624.0 | 624.0 |
| Corn oil | 266.0 | 266.0 | 266.0 |
| Salt mixture (NBC) | 133.0 | 133.0 | 133.0 |
| Vitamin mixture (NBC) | 13.0 | 13.0 | 13.0 |
| Amino acids supplemented [2] essential | | | 82.4 |
| non-essential | | | 38.0 |
| weight totals | 2654.0 | 2654.0 | 2774.4 |
| Nitrogen % | 1.90 | 1.94 | 2.50 |

1. The amino acids bound or destroyed during browning were disregarded in the calculation of the brown diets.
2. Table 1A.

## TABLE 1A
## COMPOSITION OF THE NBC AMINO ACID MIXTURE AND THE SUPPLEMENTATION MIXTURE

| Amino Acids (g) | NBC mixture* | Supplementation mixture |
|---|---|---|
| L- Lysine | 38.22 | 21.48 |
| L- Theonine | 21.76 | 8.68 |
| L- Methionine | 21.76 | 7.90 |
| L- Tryptophan | 4.64 | 4.64 |
| L- Valine | 21.76 | 2.67 |
| L- Isoleucine | 21.76 | 2.18 |
| L- Leucine | 29.46 | 2.68 |
| L- Phenylalanine | 30.78 | 6.74 |
| L- Arginine | 29.72 | 18.78 |
| L- Histidine | 8.76 | 6.63 |
| L- Tyrosine | 9.29 | 0.85 |
| L- Aspartic acid | 9.29 | 3.41 |
| L- Proline | 9.29 | 1.62 |
| L- Alanine | 9.29 | 2.36 |
| L- Serine | 9.29 | 4.75 |
| L- Glutamic acid | 9.29 | 2.22 |
| Glycine | 61.84 | 22.80 |
| L- Cystine | 9.29 | 0.00 |
| L- Asparagine | 15.92 | |

* NBC Diets Manual, page 14- Based on: Rogers R. Q., Harper, A. E., J. Nutr., 87, 267 (1965).

TABLE 2

AMINO ACID ANALYSIS OF A FREE AMINO ACID-CARBOHYDRATE MIXTURE SUBJECT TO BROWNING FOR 30 DAYS AT 37°C (g/100g mixtures).

Amino Acid Analyzer

| Amino Acids | Control | Brown Mixture | % Loss |
|---|---|---|---|
| Lysine | 1.14 | 0.50 | 56.20 |
| Arginine | 1.06 | 0.39 | 63.20 |
| Histidine | 0.34 | 0.08 | 75.70 |
| Threonine | 1.58 | 0.95 | 39.90 |
| Methionine | 0.86 | 0.55 | 36.30 |
| Tryptophan | 0.20 | 0.00 | 100.00 |
| Valine | 0.87 | 0.76 | 12.30 |
| Isoleucine | 0.90 | 0.81 | 10.00 |
| Leucine | 1.23 | 1.12 | 9.10 |
| Phenylalanine | 1.30 | 1.02 | 21.90 |
| Tyrosine | 0.31 | 0.28 | 9.20 |
| Cystine | 0.27 | 0.27 | 0.00 |
| Aspartic Acid | 0.39 | 0.25 | 36.70 |
| Proline | 0.47 | 0.39 | 17.40 |
| Glycine | 1.80 | 1.14 | 37.00 |
| Alanine | 0.32 | 0.24 | 25.40 |
| Serine | 0.49 | 0.24 | 51.10 |
| Glutamic Acid | 0.31 | 0.24 | 23.90 |

*The Biological Assay*

Thirty weanling rats (Sprague Dawley type) were distributed into three equal groups caged individually. Each group was fed *ad libitum* for a period of approximately three weeks during which the food intake was determined daily and body weight changes, every other day.

*The Analysis of blood and liver*

At the end of the feeding period, five rats from each group were sacrificed by decapitation and exanguinated into heparinized tubes: The livers were frozen in liquid nitrogen and stored in a freezer. The blood cells were separated from the plas-

ma by centrifugation. The plasma was then treated with 30% trichloroacetic acid (TCA) to give a final 10% concentration. The precipitate was extracted once more with 10% TCA solution and the supernatant were combined. The TCA was extracted from the combined supernatant with ethyl ether. For the amino acid analysis the sample was lyophilized and its concentration adjusted for the Technicon Autoanalyzer.

The frozen livers were lyophilized to less than 1% moisture and pool-ground in a mortar with a pestle.

To determine the general composition of the livers, lipids were extracted by the method of Folch (7) and RNA, protein and DNA by the method of Schmidt-Tannhauser (8) as recommended by Hutchisson and Munro (9).

The lipid-free insolubles of the liver were hydrolyzed in 0,3 N KOH at 37°C for one hour, and the RNA determined in the 5% perchloric acid (PCA) soluble fraction by its absorbance at 260nm. The hyperchromicity observed in an RNA standard treated in identical manner was 14%. The PCA pellet was redispersed in $H_2O$ and then made 0,5 N NaOH to directly determine DNA by the Ceriotti method (10). Protein was determined by Kjeldahl nitrogen analysis on the lipid-free insoluble (nucleic acid N was discounted).

The polysomes from fresh liver tissue were prepared according to the method of Wettstein *et al* (11). Grinding of the minced tissue was done in a size 24 tissue grinder (886000-9018, Kontes Gass-Vineland, N. J.).

The polysome patterns were obtained by reading the linear gradients at 254nm in an ISCO continuous flow spectrofotometer.

## RESULTS AND DISCUSSION

The effect of the brown amino acid diet on the rate of growth was higly significant. The group of rats fed with the unsupplemented brown diet (Group II) virtually had no weight gain throughout the twenty two day period, while those fed with the brown supplemented diet (Group III) did not grow as fast as the control, group I (Fig. 1). The average nitrogen efficiency ratio (NER) taken at the end of the feeding period on seven of the ten rats of each group was

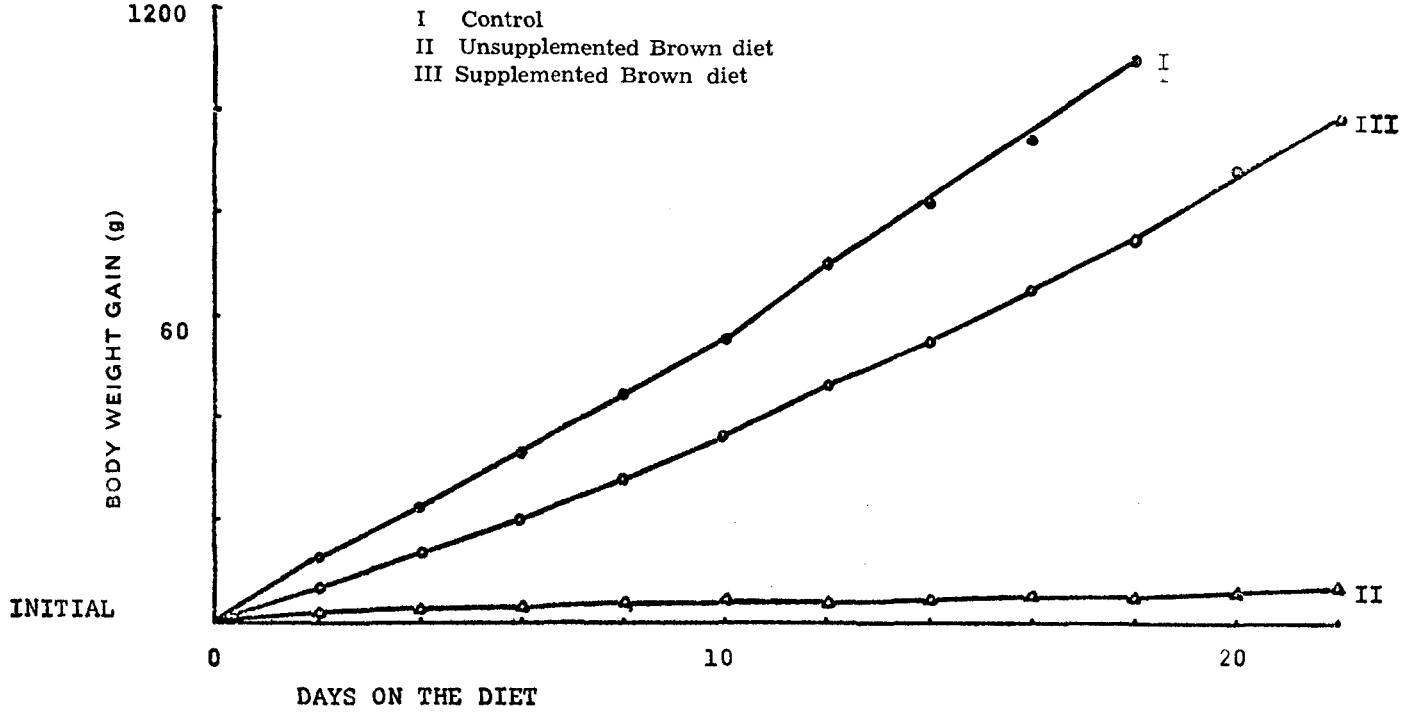


**Figure 1: Growth curves of weanling Sprague Dawley male rats on synthetic amino acid diets.**

22,73 ± 2.00 for group I, 3.77 ± 1.21 for Group II, and 14.20 ± 2.00 for Group III. The analysis of variance for the NER data by the F-test indicated that the differences between the treatments were highly significant ($p<0,001$).

Comparison of the plasma amino acid patterns revealed an increase in the concentrations of leucine, valine, proline, and threonine in the rats receiving the brown unsupplemented diet that was associated with the inability of the diet to support growth. By and large, the concentration of threonine was the highest; approximately twenty-fold in group II (Table 3).

The effect of the unsupplemented brown diet on the pattern of total liver polysomes was an increase in the proportion of 80S monomers, dimers, and oligomers. It was also noted that after two days the 80S peak was higher than eight days after starting the experimental feeding (Fig. 2). No significant difference was found by this method between the patterns of polysomes from Group I and Group III.

The size and weight of the livers in Group II were reduced to about one third of those in Group I. Accordingly, the proportions of liver DNA and RNA were higher in Group II. The RNA and protein-to-DNA ratios remained practically unaffected by the brown supplemented diet, while the lipid-to-DNA ratio was lowest for this group (Table 4). Other physiological consequences in the groups receiving the brown diets were a persistent diarrhea and hipertrophy of the large intestine particularly of the *caecum* (Plate I).

These data indicate that a free amino acid mixture with a good biological value does not support growth in rats after "dry" incubation with an excess of glucose at 37°C for one month (Maillard reaction). The lower NER's of the Maillard-reacted mixtures suggest: 1) that both essential and non-essential amino acids may be chemically altered to the extent that they are no longer available as a normal source of nitrogen to the rat; 2) that essential amino acids become largely unavailable causing a major decrease in the efficiency of the whole diet; 3) that compounds formed during the process of browning of the amino acid-sugar mixture might be toxic to the animals, what was also suggested by the persistent diarrhea and enlargement of the *caecum* of the rats on the brown

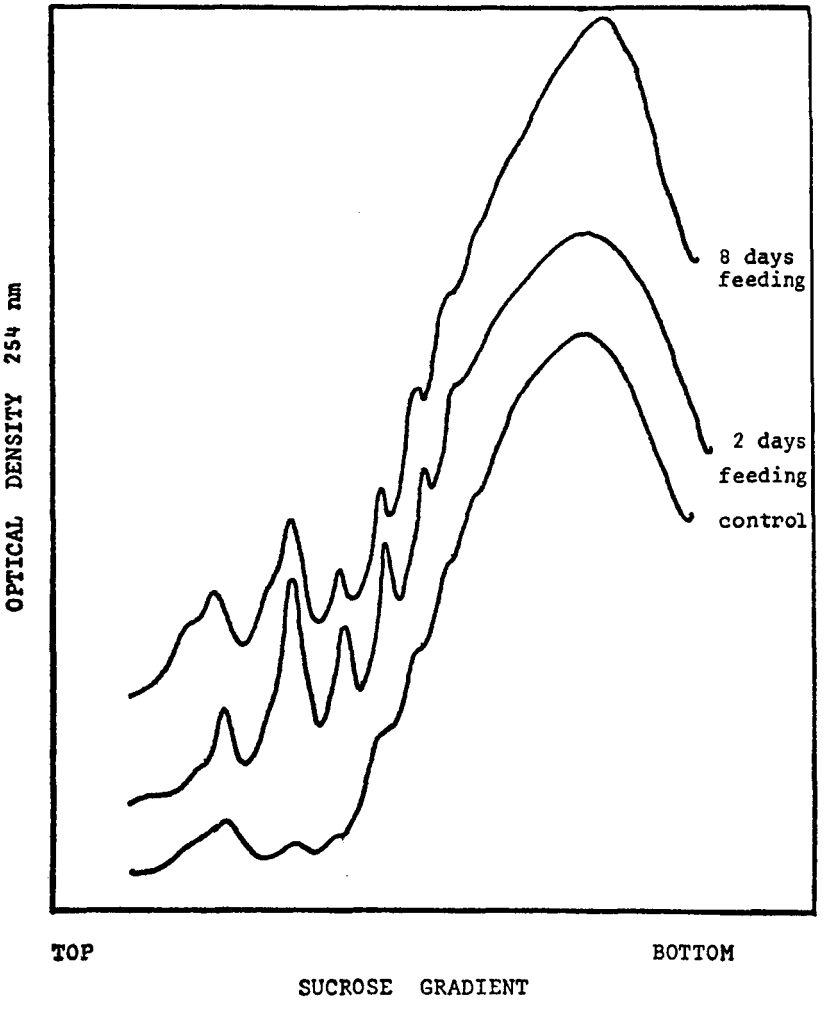


Figure 2: Effect of a browned synthetic amino acid diet on the liver polyribosome pattern of young rats fed "ad libitum".

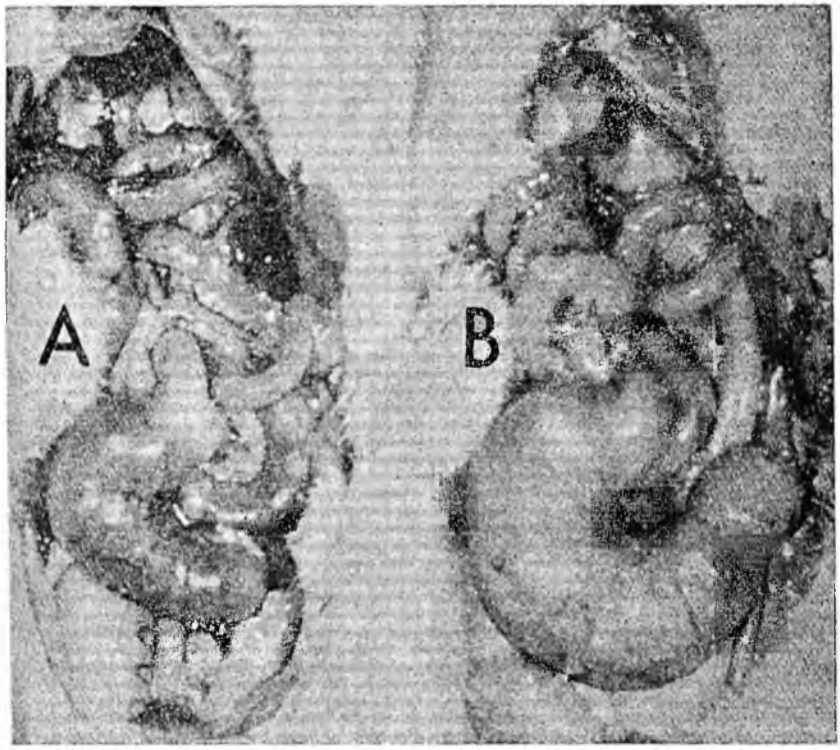


Plate I: Difference in the caecum size of rats receiving brown diet (B) and control diet (A).

TABLE 3
AMINO ACID PATTERNS IN THE BLOOD PLASMA OF RATS FED APPROXIMATELY 3 WEEKS WITH SYNTHETIC AMINO ACID DIET

| Amino acids (ug/ml plasma) | Group I | Group II | Group III |
|---|---|---|---|
| Aspartic acid | 11.0 | 28.5 | 69.4 |
| Threonine | 10.5 | 295.8 | 166.1 |
| Serine | 44.7 | 105.1 | 73.4 |
| Glutamic acid | 11.0 | 29.9 | 26.5 |
| Proline | 11.9 | 26.3 | 20.5 |
| Glycine | 68.4 | 77.2 | 125.3 |
| Alanine | 22.2 | 43.5 | 60.2 |
| Valine | 7.8 | 24.5 | 16.7 |
| Cysteine | 2.0 | 1.7 | 3.9 |
| Methionine | 1.1 | 1.5 | trace |
| Isoleucine | 4.4 | 11.6 | 11.6 |
| Leucine | 5.0 | 22.1 | 14.7 |
| Tyrosine | 11.8 | 16.2 | 15.9 |
| Phenylalanine | 29.4 | 24.6 | 17.1 |
| Lysine | 57.9 | 80.9 | 91.4 |
| Histidine | 5.2 | 13.6 | 18.0 |
| Arginine | 14.2 | 29.2 | 29.9 |
| Ornithine | 8.3 | 19.8 | 16.3 |
| Ratio EAA/NEAA | 0.7 | 1.5 | 0.9 |

I Unbrowned diet (control).
II Browned, unsupplemented.
III Browned, supplemented with all amino acids lost.

**TABLE 4**
**AVERAGE COMPOSITION OF RAT LIVERS AFTER THREE WEEKS ON SYNTHETIC AMINO ACID DIETS**

| Group | Pool size (g) | DNA % | RNA % | Protein % | Lipid % | RNA / DNA | Protein / DNA | Lipid / DNA |
|---|---|---|---|---|---|---|---|---|
| I | 9.79 | 1.60 | 3.48 | 62.9 | 19.8 | 2.18 | 39.4 | 12.4 |
| II | 3.24 | 2.10 | 4.28 | 60.5 | 21.3 | 2.03 | 28.7 | 10.0 |
| III | 10.32 | 1.62 | 3.70 | 65.0 | 15.3 | 2.28 | 40.0 | 9.4 |

1. I. non-browned synthetic amino acid diet (contol).
   II. browned amino acid diet unsupplemented for the amino acids lost.
   III. browned amino acid diet supplemented with all amino acids lost.
2. Each pool contained the lyophylized (<1% moisture) livers of five equally old rats.

diets, and; 4) that supplementation of the brown diet does not restore all the lost efficiency. As confirmed by both amino acid analysis and rat assay, the unsupplemented brown mixture is strongly unbalanced, lacking mainly tryptophan, histidine and lysine.

The nutritional consequences of browning an entire mixture of amino acids are consistent with previous observations which suggest that the absorption and utilization of the fructoseamino complexes are impeded in some manner. Low absorption rates of radioactivity were reported to occur after feeding rats with the sugar complexes of U-$^{14}$C-L-leucine and 3-$^{14}$C-L-tryptophan. Complete unavailability of methionine to the rat was reported to occur from fructose-methionine (4).

In addition to the deficiency of essential amino-acids, the brown diet could have lost efficiency even further due to the presence of 5-hydroxymethyl furfural, a heat degradation product of glucose (12).

Moreover, degradation products of the initial amino acid-sugar complexes are known to form during browning (1) which could be physiologically detrimental to the rat. After the brown diet was supplemented with all the amino acids lost, the efficiency of the diet was only 63% of the control diet.

From the changes observed in the amino acid patterns, the following can be suggested: 1) the utilization of plasma amino acids was largely decreased in the group fed the brown unsupplemented diet; 2) possibly not only the incorporation of free amino acids into protein was affected, but the catabolic rates of some amino acids particularly serine and threonine could have been decreased, and; 3) supplementation of the diet increased the utilization of the blood amino acids.

It was not explained how threonine and serine accumulated to such high levels in the plasma of rats fed the brown unsupplemented diet. Whatever the machanism may be, the condition of unbalance introduced during browning does not seem to the sole reason for the accumulation of threonine and serine.

Although the proportions of DNA, RNA and lipids were largest in the livers of Group II, the total amounts of DNA, RNA, protein and lipids were lowest as expected from the

small weight increase of these animals. The lower content of liver DNA in Group II may be accounted for, first by a curtailment of cell division and DNA synthesis similar to that caused by a caloric restriction of fasting (13, 14) and secondly, by a possible elimination process of DNA associated with the organ (15).

Apart from the relative decrease of liver fats, the brown supplemented diet had little or no effect on either the size of the organ or in the proportions of the other fractions of the liver.

The increase in the monosome and oligosome peaks of the polysome patterns upon essential amino acid starvation or fasting has been reported by several authors (13, 16). The observation that the brown unsupplemented mixture induced dissembling of polysomes, while no appreciable change was effected by the brown supplemented mixture, was consistent with body growth and protein content of the livers in these groups of rats.


## RESUMEN

**Consecuencias fisiológicas de la alimentación de ratas con una mezcla *marrón* de aminoácidos sintéticos y azúcar (reacción de Maillard)**

Se almacenó una mezcla de aminoácidos durante 20 días a 37° en presencia de un exceso de glucosa y 16% de agua (p/p) para provocar el oscurecimiento (reacción de Mailiard). Otra mezcla igual fue conservada a -20°.

Por cromatografía en cambiadores iónicos de extractos no hidrolizados se demostró la pérdida de considerables cantidades de aminoácidos en la mezcla oscura con excepción de la cistina. Las pérdidas de triptofano llegaron al 100%, de histidina al 76%, arginina al 63% y serina al 51%.

Para las pruebas biológicas se usaron las mezclas no suplementadas y suplementadas con los aminoácidos perdidos durante el oscurecimiento. La dieta no suplementada de la mezcla oscura no provocó ningún crecimiento en ratas de destete, pero estos mantuvieron su peso. La suplementación causó crecimiento de aprox. 65% de los controles. Los análisis de los hígados liofilizados de estos animales mostraron un contenido bajo en proteínas, RNA y DNA en las ratas que consumieron la dieta no suplementada, mientras que no existían diferencias significativas entre los grupos control y el que recibió la dieta suplementada.

Aumentó la concentración de todos los aminoácidos especialmente de la de treonina y serina (20x) en el suero sanguíneo de las ratas alimentadas con la mezcla oscura. Esta concentración elevada de serina y treonina se redujo considerablemente por la suplementación.

**El aspecto de los poliribosomas hepáticos estaba muy cambiado en las ratas que comieron la mezcla oscura. Este efecto era más pronunciado después de 8 días en la dieta y desapareció completamente con la suplementación.**

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