REVISTA ESPAÑOLA DE FISIOLOGIA. 26. 239-244. 1970

Protein Metabolism in Growing Rats. III. Effects of an Early Severe Protein Deprivation and its Alleviation by Feeding a Low Protein Diet *

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(Received on December 15, 1969)

A. PESTAÑA. *Protein Metabolism in Growing Rats. III. Effects of an Early Severe Protein Deprivation and its Alleviation by Feeding a Low Protein Diet.* R. esp. Fisiol., 26, 239-244, 1970.

Several parameters representative of protein metabolism have been studied in protein depleted rats and after protein repletion with a 4 % casein diet.

Feeding the rats with the protein-free diet during 4 weeks after weaning, decreased serum protein levels (albumin and beta globulin) and nitrogen content in viscera and eviscerated carcass. Liver arginase activity decreased rapidly during the first two weeks on protein-free diet, but slightly increased during the following two weeks. The protein-free diet also prevented the normal increase in gamma globulins and serum complement levels which otherwise occur after weaning.

Feeding the 4 % casein diet for two weeks, to rats previously protein depleted for two weeks after weaning prevented further decrease and, in certain cases, restored these parameters up to the levels found in weanling animals.

In the previous paper of this series (2, 3), data on nutritional parameters of rats fed on diets containing 4 % and 20 % casein, up to 90 days after weaning, were presented. The most striking conclusion from this study was the remarkable ability of the rat to thrive on a long lasting low protein diet. This led me to study those parameters in young rats maintained on a protein free diet from weaning, and to check the ability of a low protein diet (4 % casein) to reverse the effects of this acute protein deprivation.

Materials and Methods

Male Wistar rats 21 days old, weighing 50-60 g, were caged individually and supplied with food and water *ad libitum*. The protein free diet was the same as the 4 % casein diet, previously described (2), but in this case replacing the casein by sucrose. For the repletion studies, rats fed on the protein free diet for two weeks after weaning, were changed to the 4 % casein diet for an additional period of two weeks.

* This work was supported in part by a fellowship from the Ministery of Education and Science.

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At the end of the appropriate periods, the rats were killed by decapitation, bled and eviscerated. A minimal portion of the livers (usually 0.1-0.2 g) was removed for arginase determination. The eviscerated carcass and viscera were processed for nitrogen determination. The analysis of the nitrogen content, liver arginase activity, hematocrit values, serum complement levels, and serum protein and electrophoretic patterns, were performed as previously described (2 and 3). An arginase unit corresponds to the amount which forms 1 mg of urea nitrogen per gram of liver under the assay conditions (10 minutes at 37° C).

Results and Discussion

Changes in body weight are shown in Table I. There was a progressive weight loss, which amounted 37 % of the initial body weight at the end of the 4 week period on protein-free diet. Protein repletion resulted in a net gain in body weight of 6 g.

Body composition (Table I), as percent of protein, water and fat content in the eviscerated carcass, remained essentially unchanged during the protein deprivation. Those results indicated that body weight losses are dependent on a uniform loss in body constituents, giving thus support to the suggested occurrence of a homeostasis for body composition (6). From the estimated nitrogen content, the losses in body proteins during the 4 weeks on protein-free diet, can be estimated to amount to 2.5 g (about 5 % of the initial body weight).

Nitrogen losses in the eviscerated carcass proceeded smoothly during the protein deprivation (Fig. 1). On the contrary, more than 90 % of the nitrogen lost by viscera occurred during the first week on protein-free diet. According to ADDIS (1) liver nitrogen accounts for the greater part of the nitrogen lost by viscera during protein depletion.

In the repletion with the 4 % casein diet, 50 % of the nitrogen losses were restored (Table I). Viscera gained nitrogen more rapidly than carcass (Fig. 1). At the end of the repletion period 65 % of the nitrogen lost by viscera had been restored whereas in the eviscerated carcass only a 29 % was restored.

Table I. *Body composition of protein depleted and repleted rats.* Values are followed by the standard error of the mean. Figures in brackets correspond to the number of animals per group.

Experimental groups	Body weight at killing g	Body nitrogen g	Water %	Protein %	Fat %
Weaning (7)	54.3 ± 2.2 [a]	1.28 ± 0.05	71.1 ± 0.4	15.9 ± 0.2	9.8 ± 0.5
Protein-free diet [b]					
1 week (7)	44.9 ± 0.4	1.15 ± 0.02	69.9 ± 0.6	17.1 ± 0.2	9.8 ± 0.4
2 weeks (7)	40.0 ± 0.9	1.05 ± 0.05	68.6 ± 0.5	17.8 ± 0.3	10.5 ± 0.4
3 weeks (8)	38.0 ± 0.6	1.01 ± 0.03	67.6 ± 0.5	18.1 ± 0.3	11.2 ± 0.5
4 weeks (8)	35.8 ± 0.2	0.93 ± 0.01	69.8 ± 0.8	17.7 ± 0.2	9.7 ± 0.8
4 % Casein diet [c]					
2 weeks (8)	46.7 ± 1.2	1.16 ± 0.04	67.4 ± 0.9	16.3 ± 0.5	13.1 ± 0.7

[a] Standard error of the mean. [b] Given after weaning for the indicated periods. [c] Rats fed the protein-free diet for the two first weeks after weaning, were changed to the 4 % casein diet.

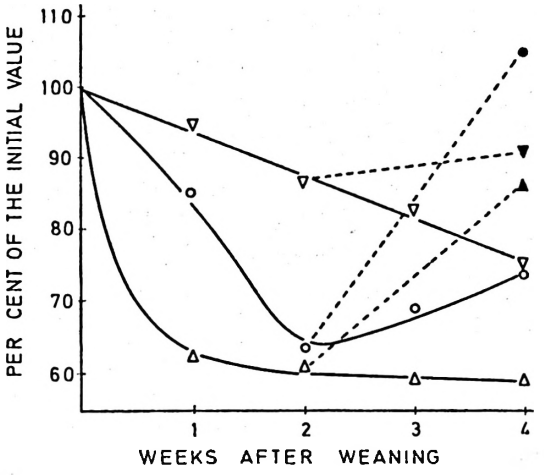

Fig. 1. *Body nitrogen losses and arginase activity.*
Changes in nitrogen content of viscera (△), eviscerated carcass (▽) and liver arginase activity (○) in rats fed the protein-free diet for several time periods after weaning. Solid symbols correspond to animals changed to the 4 % casein diet. Each point corresponds to the average value of 7-8 experimental animals.

Liver arginase. Changes in liver argimase are also show in Fig. 1 as percent of the weaning values (19.9 ± 0.9 units). During the first two weeks on protein-free diet, there was a rapid fall in arginase levels up to 40 % of the initial values. This decrease is in agreement with the results obtained in adult rats (7) and would perhaps be dependent on the high carbohydrate content of the diet (5). During the following two weeks, arginase activity increased slightly, probably as result of the increasing demands for ureogenesis. In fact, liver arginase activity was found to correlate fairly well with nitrogen losses (2). After the two week-period of protein repletion, liver arginase activity raised up to the weaning values.

Hematocrit values are given in Table II. There was a progressive increase in hematocrit value up to the end of the second week on the protein-free diet. This increase is similar to the pattern previously reported to occur in normal (stock diet) fed rats or in rats fed on a 20 % casein diet from weaning (3). During the last two weeks of protein deprivation, the hematocrit fell to values a 20 % less than those found in the two weeks depleted animals. Protein repletion with the 4 % casein diet resulted in hematocrit values within the range found at the beginning of the repletion period.

Serum proteins. Total serum proteins, albumin and beta globulin (the two major components of serum proteins), decreased along the protein depletion period down to levels about 50 % of the initial values (Figs. 2, 3). Those changes do not seem to be dependent on hemodilution (de-

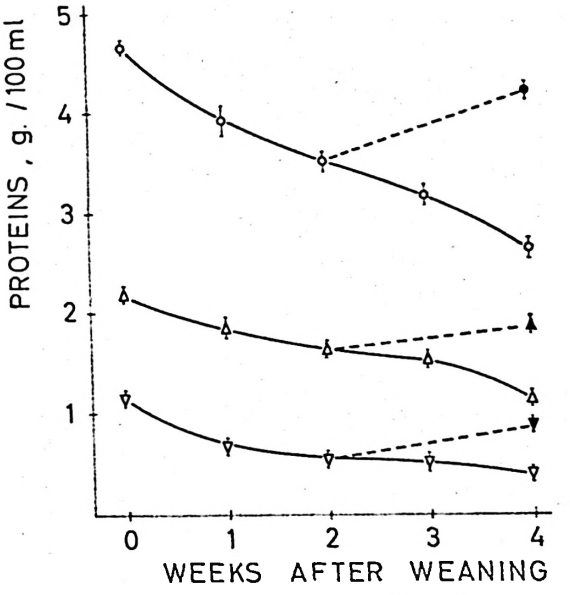

Fig. 2. *Serum proteins in protein depleted and repleted rats.*
Total serum proteins (○), albumin (△) and beta globulin (▽) in rats fed with protein-free diet. Solid symbols stand for the animals protein repleted by feeding the 4 % casein diet. Each point is the mean of 7-8 experimental animals; vertical bars indicate the standard error of the mean.

creased hematocrit value) since at the end of the second week on protein-free diet, total serum proteins, albumin and beta globulin had decreased in a 25, 27 and 50 per cent respectively, whereas hematocrit value increased to about a 30 percent during the same period. Refeeding with the 4 % casein diet caused an increase of 20 % for total serum proteins, 12% for albumin and 51 % for beta globulin above the initial values (2 weeks on protein-free diet). No differences in hematocrit value were found between both groups (see above).

The changes in alpha 1 and 2 serum globulin appeared closely related to those in hematocrit, suggesting that no net change in the concentration of these proteins had occurred as the result of the protein deprivation. Alpha 3 globulin levels seemed also as being independent on dietary proteins, as previously reported (3).

Gamma globulins and serum complement levels. Diminished resistance to infection is one of the most deleterious effects of severe protein malnutrition (4). In this connection, the changes in gamma globulins and serum complement levels found in this study should be emphasized.

In the normal (stock diet) fed animals and in rats fed on a 20 % casein diet, serum complement and gamma globulins levels increased progressively from weaning, up to values 350 % and 100 % respectively higher in the three months old animal (3). However, feeding the protein-free diet resulted in a slight decrease in both proteins (Fig. 3 and Table II), more marked in the case of the gamma globulins. In both cases, protein repletion with the low casein diet prevented further decrease.

Taking into account the changes occurred in hematocrit values, the results found in this study indicated that feeding a protein-free diet from weaning can block the increase in serum complement and gamma globulin levels which normally takes place in growing rats. Protein repletion with the 4 % casein diet seems unable to reverse this stagnation, at least within the period of time studied.

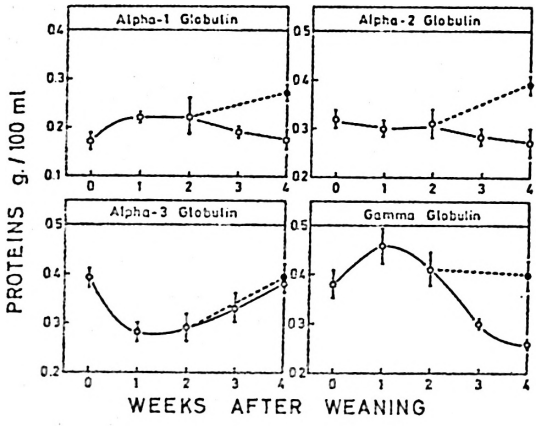

Fig. 3. *Serum globulins in protein depleted and repleted rats.*
Open circles stand for animals fed on the protein-free diet. Solid circles correspond to rat refed with the 4 % casein diet. Each point is the mean of 7-8 animals; vertical bars indicate the standard error of the mean.

Table II. *Serum complement levels and hematocrit value of rats fed the protein-free diet for several time periods, and in rats protein-repleted by the 4 % casein diet.*
Values are followed by the standard error of the mean. Figures in brackets correspond to the number of animals per group.

Experimental group	Serum complement $C'H_{50}$ units	Hematocrit value %
Weaning (7)	33.4 ± 0.8	31.1 ± 0.9
Protein-free		
1 week (7)	29.7 ± 2.2	38.8 ± 0.5
2 weeks (7)	26.8 ± 1.9	42.5 ± 0.7
3 weeks (8)	24.9 ± 1.7	36.8 ± 0.5
4 weeks (8)	21.8 ± 1.6	33.2 ± 0.8
4 % Casein diet		
2 weeks (8)	26.9 ± 0.9	38.1 ± 0.5

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