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Effect of calories restriction and protein deficiency on protein metabolism in rats

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With 1 figure and 2 tables

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Severe degrees of malnutrition may result from unbalanced or inadequate food intake either because of improper feeding habits or because of insufficiency of the diet. Reporters in the literature (*Falli,* 1961; *Platt,* 1962; *Dean,* 1965, and *Jelliffe,* 1966) concluded that Kwashiorkor is mainly the result of protein deficiency in the diet, while marasmus is primarily the result of inadequate intake of balanced diet, a state which is very similar to starvation.

An important function of the liver under various conditions of dietary stress is to help the animal metabolism to adapt the changes by providing the necessary metabolites from alternate sources. *Ganong* (1969) reported that most of the protein that burns during starvation comes from the liver. Therefore, liver was chosen for this study for being sensitive to the protein supply.

The aim of this work is to investigate the metabolism of liver protein under protein-energy deficiency. These studies include the effects of calories of protein deficiency on the liver contents of proteins, free amino acids, urea and uric acid of rats. The rate of incorporation of 1-C^{14} glycine into liver proteins was also studied as well.

Materials and methods

1. Experimental animals

The effects of calories or protein deficiency on protein metabolism were investigated on white albino rats (local strain), weighing 35–40 g which were grouped into 3 groups, 8 rats each. The first group was fed on complete normal diet (ad-libitum-fed rats) and were used as controls. The second group received a restricted amount of normal diet (pair-fed animals), while the third group was fed on protein-free diet (protein-deficient animals). Water was given at all time in excess.

Feeding of animals lasted for 24 days, and the amount of food consumed was determined every morning. At the end of the experimental period the animals were killed by decapitation. Livers were removed, weighed and homogenized in cold distilled water to attain 1% of the homogenate.

2. Diet preparation

The nitrogen-free diet was prepared according to *Boas, Fixen* and *Jackson* (1932) and had the following composition in gram: Corn starch, 73.2; sucrose, 9; animal lard, 10; cod liver oil, 2; salt mixture, 5; and calcium carbonate, 0.8.

The salt mixture used was that of *Hubbel, Mendel* and *Waksman* (1937). To the mixed diet 0.25 g % of vitamins mixture was added (*Brown & Sturtevant*, 1949). Vitamins A, D, E, and K were used in traces. The total calories provided by this diet equals to 456 kcal./100 g.

The control diet was as the protein-depleted ones, except that 20 % of the corn starch was replaced with casein. The total calories provided by this diet equals to 462 kcal./100 g.

Chemical estimations

Total liver proteins were determined by the method of *Lowry* et al. (1951). Liver-free amino acids were estimated as described by *Strikland* et al. (1961). Urea concentration was assessed by the diacetyl monoxime reagent as described by *Natelson* (1957). Uric acid was estimated by the method of *Carawary* (1963).

Measurement of the incorporation of l-C^{14} glycine[1]) into liver proteins

At the end of the experiment (24 days), animals were injected via i.p. route with 20 μCi of l-C^{14} glycine (specific activity > 40 mCi/mmol). 90 minutes later animals were sacrificed by decapitation. Livers were dissected out and washed with sterile saline solution. Liver proteins were extracted, and the radioactivity in 1 ml of the extract was measured as described by the method of *Anne* and *Robinson* (1963) in 12 ml of Insta-Gel (Universal liquid scintillation cocktail [Packard]).

Results

Results pertaining to table 1 and figure 1a indicate that the weight of the control animals was increased by 60.5 %, whereas only 37.7 % increase was recorded in pair-fed rats. On the other hand, animals fed on protein-deficient diet lost 21.3 % of its weight at the end of the experiment.

Results of table 2 can be represented as follows:

1. The contents of liver proteins decreased significantly by 6 % and 28 % in pair-fed and protein-deficient rats respectively.

Table 1. Effect of diets on food consumption and body weight gain of rats.

Animals groups	food consumed in g/day mean ± S. E.	protein intake in g/day mean ± S. E.	caloric intake in Cal/day mean ± S. E.	weight change/total experimental period
1 *Ad-libitum* fed control rats (range)	17.25 ± 1.12 (10.3–23.3)	3.53 ± 0.22 (2.5–4.66)	73.75 ± 4.72 (53.2–97.8)	+ 33
2 Pair-fed rats (range)	11.07 ± 0.52 (7.3–12.0)	1.96 ± 0.06 (1.46–2.16)	41.33 ± 1.38 (30.78–45.0)	+ 20
P	< 0.001	< 0.001	< 0.001	
3 Protein deficient rats (range)	9.08 ± 0.57 (6.6–10.2)	–	39.4 ± 1.35 (33.6–42.0)	– 10
P	< 0.001		< 0.001	

[1]) Radioactive glycine was purchased from the Radiochemical Centre, Amersham, Bucks., U.K.

2. The incorporation of l-C^{14} glycine into liver proteins increased by 74 % and 60 % in animals maintained on pair-fed or protein-deficient diets respectively.
3. In pair-fed rats no significant difference was noticed in the liver contents of urea and free amino acids. Nevertheless, animal fed on protein-deficient diet showed a significant increase in the concentrations of urea and free amino acids.
4. No significant change was noticed in the concentration of liver uric acid in either pair-fed or protein-deficient rats.

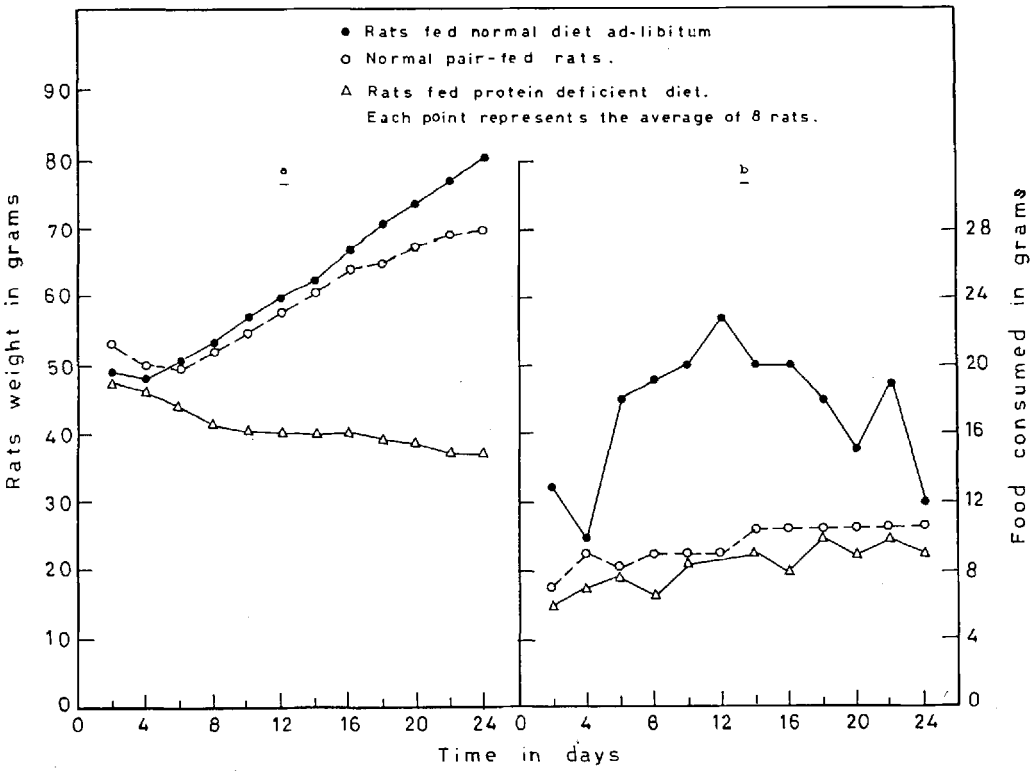

Fig 1a Growth curve of experimental rats

Fig 1b Food consumed of experimental rats

Discussion

Luean and *Joseph* (1975) stated that growth in young animals is by far a more sensitive indicator of protein status than the measurement of tissue protein concentrations, and still much more sensitive than changes in gross composition of blood such as hemoglobin concentration, hematocrit and plasma proteins. In the present work, the results given in figure 1 demonstrate that animals fed on control diets had a steady normal growth curve. Food consumption of rats fed such diet rises to reach a maximum after 12 days and then fall. On the contrary, the rats fed protein-deficient diet gradually lost weight and the pattern of food consumption was

flactuated. These results are in good agreement with those reported by *Luean* and *Joseph* (1975). Rats which received a protein-free diet showed reduction in the amount of food intake compared with control animals (diet *ad libitum*). This finding is in line with the substantiated reports given by *Beaton* (1968) who claimed that animals fed low protein diets have markedly reduced food intake and appetite failure. *Meyer* and *Hargus* (1959) hypothesized that animals fed a low protein diet ate little because their food intake was limited by an excess of energy intake in relation to protein intake. Analysis of the results relating weight gain to calories restriction and protein deficiency showed that although the level of calories intake had some effect upon the rate of weight gain, that of protein was far more important. So calories intake seems to be a limiting factor determining the rate of growth (*Ashwarth* et al. 1968).

The relative importance of anabolic and catabolic processes in rats could be changed by feeding them a low or high protein diet (*Yeh* et al. 1974). The two processes are at equilibrium in the nutritional condition in which the rats were fed a diet containing approximately 10% protein of good quality. This equilibrium shifted towards anabolism when the rats ate a diet containing less protein, and the balance shifted towards catabolism when they ate a diet having a higher level of protein (*Yeh* et al. 1974). These results are in consistency with our observations on pair-fed animals where the rate of incorporation of l-C^{14} glycine in liver proteins was significantly increased. *Yeh* et al. (1974) suggested that shift was achieved by changing the activities of amino acids, catabolic enzymes, and enzymes which promoted protein synthesis. Regression to results relating weight gain to the incorporation of l-C^{14} glycine indicated that although the amino acid incorporation into the liver protein was increased, the rats failed to grow on the protein-deficient diet. This indicates that the liver-labile proteins must attain a high level before nutrients can be diverted to growth (*Luean* and *Joseph*, 1975).

In addition to the observed increase in the incorporation of l-C^{14} glycine, the lack of calories or deficiency of proteins produced a markedly different change in free amino-acid levels. In the pair-fed rats the total free amino acids were maintained at levels close to those found in the control animals. This may be due to the contribution of non-essential amino acids (*Luean* and *Joseph* 1975). On the other hand, in the protein-deficient rats free amino acids showed a highly significant increase ($p < 0.001$) which may be resulted from the rapid destruction of the protein. The loss of total liver proteins in the pair-fed animals averaged 6 % as compared with 28 % in rats that received protein-deficient diet.

The observed increase in the glycine incorporation into liver protein and the increase in the level of total free amino acids in the protein-deficient rats is not surprising. These results implicate that the rate of synthesis of a protein is probably independent to its rate of catabolism and the latter might not be affected with the nutritional state of the animals (*Waterlow* and *Wills*, 1960). In the protein-deficient rats, the protein will continue to be catabolized as long as the amino-acids supply is deficient. As a result, synthesis will lag behind the breakdown, and a new state of equilibrium might be achieved with reduction in the total amount of protein present (*Waterlow* and *Wills*, 1960).

Table 2. Effect of diets on the total proteins, incorporation of l-C^{14} glycine, free amino acids, urea and uric acid in rat liver.

Rat groups	Total protein mg/100 g tissue (mean ± S. E.)	Radioactivity in liver protein hydrolyzate cpm/g × 10^3 (mean ± S. E.)	Free amino acids mg/100 g tissue (mean ± S. E.)	Urea mg/100 g tissue (mean ± S. E.)	Uric acid mg/100 g tissue (mean ± S. E.)
1 *Ad-libitum* fed control rats	18.39 ± 0.23	2.41 ± 0.4	928.84 ± 75.56	104.25 ± 7,95	4.65 ± 0.2
(range)	(17.0–19.6)	(1.84–2.93)	(830.11–1276.31)	(90.32–119.53)	(4.27–5.10)
2 Pair-fed rats	16.54 ± 0.43	4.20 ± 0.81	908.74 ± 41.13	94.25 ± 9.25	6.3 ± 0.64
(range)	(15.20–18.31)	(3.01–5.21)	(722.32–1101.53)	(73.74–118.52)	(4.82–7.4)
P	< 0.05	< 0.001	N. S.	N. S.	N. S.
3 Protein-deficient rats	13.21 ± 1.3	3.86 ± 1.45	1445.66 ± 185.75	211.33 ± 19.69	4.23 ± 0
(range)	(11.4–15.11)	(2.23–5.16)	(1190.0–1720.1)	(192.36–250.47)	(4.23–4.23)
P	< 0.001	< 0.02	< 0.001	< 0.001	N. S.

In the present work, the liver content of urea in rats receiving pair-fed diet showed no significant change, while that of protein-deficient animals was significantly increased ($p < 0.001$). These results indicate that the amount of energy available from dietary carbohydrates (protein-deficient diet) was not enough to compensate for the lack of protein, hence the difference in nitrogen loss between pair-fed rats and protein-deficient ones (*Peter* et al. 1976) could be explained. The observed increase in the contents of urea in liver of protein-deficient rats may be attributed to the deficiency of amino acids necessary for the synthesis of particular protein, and the amino acids, which results from the breakdown of protein, were deaminated, and their nitrogen was excreted as urea. This explanation is in good agreement with that given by *Ganong* (1969).

The data presented in table 2 indicate that neither calories restriction nor protein deficiency changes appreciably the concentration of liver uric acid. The possible explanation of this result is that the activity of uric-acid-synthesizing enzyme was not decreased despite that there is a decrease in the actual amount of liver proteins. It seems more probable that under normal conditions the liver maintains physiologically active concentrations of numerous enzyme systems that are not in immediate use or need (*Peter* et al., 1976). Under conditions of dietary deprivation, active concentrations of only those enzyme systems for which there is an immediate need are maintained.

Summary

The changes induced by the deficiency of calories or nitrogen on the protein metabolism in rats were investigated. Animals were fed either a restricted normal diet, a protein-deficient diet or control diet *ad libitum*. Rats receiving protein-free diet failed to grow, while the growth of animals given restricted diet was less than those fed *ad libitum*. Despite that, the dietary deficiency of either calories or proteins caused the loss of protein and increased the incorporation of 1-C^{14} glycine into liver proteins. The contents of liver-free amino acids and urea were significantly increased only in the protein-deficient rats.

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