Note

Effect of Severity of Protein Depletion on the Subsequent Hepatic "Catch-up" Growth in Young Adult Rats

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It is well known that when young protein-depleted rats which have been maintained on a protein-free (PFD) or a low protein diet are refed a diet containing a sufficient amount of protein (a control diet), their body and liver weights increase more rapidly for a while than do those of rats maintained on a control diet throughout, and their growth rates become normal thereafter.1 ~7) To our knowledge, however, there is no study concerning whether the degree of this rapid increase in body and liver weights might be varied by the severity of protein depletion of animals and which protein level in the protein-refeeding diet may be the most effective in producing a maximum increase in body and liver weights. The present experiments were, therefore, undertaken to solve the two problems presented above using young adult rats depleted of protein by feeding a PFD.

Male Wistar strain rats, weighing approximately 120 g initially, were given a diet containing 20% casein (20% CD) until their average body weights reached about 180 g. In Experiment 1 the animals were divided into five groups of seven to nine animals each and given the PFD for 1, 2, 4 or 17 days and then four animals from each group were refed the 20% CD for about 17 hr (PF1, PF2, PF4, PF8, PF17, PF1-RF, PF2-RF, PF4-RF, PF8-RF and PF17-RF groups respectively). In Experiment 2 the animals were given the PFD for 4 days and divided into five groups of four to five animals each and four groups of them refed diets containing 10%, 20%, 40% or 60% casein for about 17 hours (PF4, 10% C, 20% C, 40% C and 60% C groups respectively). The animals were housed in individual cages with wire bottoms in a room at a temperature of 22 ± 1°C and a relative humidity of 55 ~60% with a controlled lighting schedule (light from 06:00 to 18:00 hours). diets and water were available ad libitum. The composition of the PFD was as follows: corn starch (84%), soybean oil (5.0%), cellulose powder (5.0%), Harper's mineral mixture (5.0%),8) Harper's vitamin mixture (0.85%),8) choline chloride (0.15%), a-tocopheryl-acetate (100 mg/kg diet), ergocalciferol (2,000 I.U./kg diet), retinyl palmitate (4,000 I.U./kg diet). In casein-containing diets, corn starch was replaced by the same amount of casein. Dietary changes from the 20% CD to the PFD and from the PFD to the casein containing diets were carried out at 5:00 ~6:00 PM.

The animals were weighed and sacrificed at 10:00 ~11:00 A.M. and their livers quickly removed, chilled in ice, weighed, cut into pieces with scissors and homogenized in three volumes of a 0.27 m sucrose solution containing 2 mM Tris-HCl (pH 7.6) in a Potter-Elvehjem homogenizer fitted with a Teflon pestle. Nucleic acids were extracted with perchloric acid as described by Kimberg et al.9) DNA, RNA and protein were determined by the methods of Burton,10) Mejbaum11) and Lowry et al.,12) respectively. Statistical significance was examined by Student's t-test.

Experiment 1. The upper column of Fig. 1 shows the body weight gain and food intake. It can be noted that since the rats consume nearly all of their daily intakes at night,13 ~15) their body weights are usually higher in the morning and lower in the evening. In the present experiments (1 and 2) 93.7% (for the 20% CD), 88.4% and 87.3% (for the PFD on the 1st day and the 2nd day after dietary alteration respectively) of the total daily intakes were eaten on the average between 5:00 ~6:00 P.M. and 10:00 ~11:00 A.M. on the next day and the body weights were 3 to 6 g more in most rats at 10:00 ~11:00 A.M. than at 5:00 ~6:00 P.M. on the same day. The body weight gains shown in Fig. 1 (the body weight difference between 5:00 ~6:00 P.M. and 10:00 ~11:00 A.M. on the next day) are, therefore, estimated to be 3 to 6 g more than the "true" daily body weight gains.

The body weight gains of rats from PF2-RF, PF4-RF, PF8-RF and PF17-RF groups increased significantly compared with those of the normally growing rats given the 20% CD throughout. From the shape of the curve of the body weight gain, we can say that the greatest body weight gain was obtained when the rats were fed the PFD for 4 to 8 days and refed the 20% CD. The intake of the 20% CD was greater in the groups protein-depleted less severely, but when expressed per 100 g of body weight, the values from every protein-depleted group were almost the same and not different from that of the normally growing group. The body weight gain: food intake ratios were 0.59 (PF1-RF), 0.79 (PF2-RF), 1.01 (PF4-RF), 1.05 (PF8-RF), 0.91 (PF17-RF) and 0.62 (normally growing group). Rats of the PF4-RF and PF8-RF groups showed the highest ratios. So the difference in the body weight gain was not

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Fig. 1. Body Weight Gain (A), Food Intake (B), and Changes in Liver Weight and Its Protein, RNA and DNA Contents (C) after Refeeding a 20% Casein Diet to Rats Previously Protein-depleted at Various Levels. Results are given as mean ± SEM. The values indicated as a) are from 31 normally growing rats given only the 20% casein diet throughout; their initial body weight at 5:00~6:00 P.M. was 174.8±0.5g and their final body weight at 10:00~11:00 A.M. on the next day was 186.2±0.7g. The figures in parentheses refer to percentages of the values of the protein-refed groups. Mean values of the corresponding protein-depleted groups are taken as 100%. O, values of protein-depleted groups i.e. PF1, 2, 4, 8 and 17 groups; ●, values of protein-refed groups i.e. PF1-RF group, only an insignificant increase in body weight gain and no increase in the body weight gain: fold intake ratio over control values were observed. It appears that rats protein-deprived for only one day cannot develop a sufficient “amino acid sparing metabolic state,” considering that adaptations to the protein deficient diets usually take more than several days to reach a maximum level.16,23)

The lower column of Fig. 1. shows the changes in liver weight, and its protein, RNA and DNA contents in rats fed the PFD for 1 to 17 days and then the 20%-CD for about 17hr. Liver weight and its protein and RNA contents during the PFD feeding continued to decrease. Liver DNA content did not change during the PFD feeding. Refeeding of the 20%-CD to protein-depleted rats caused a rapid increase in the liver weight and in its protein and RNA contents significantly except for the liver weight of the PF1-RF group. Per cent gains in the liver weight and protein content after refeeding were greater in the PF8-RF and PF17-RF groups than in other groups. Per cent gain in the liver RNA content was maximal in the PF17-RF group. It may not reach maximum within the present feeding period of the PFD. So an even higher value for it might be obtained if the rats were fed the PFD for a longer period. The extents to which those hepatic parameters increased on refeeding after the PFD are comparable to those reported by Enwonwu et al.5) except the value of the liver weight gain, Rozovski et al.,7) Horie et al.18) and Williams,30* although the former three authors used a low protein diet instead of a PFD. The liver DNA content showed a slight increase significantly in the PF2-RF group and insignificantly in the PF1-RF, PF4-RF and PF17-RF groups.

Experiment 2. The upper column of Fig. 2 shows body weight gain and food intake after refeeding diets containing various levels of casein to rats fed the protein-free diet for 4 days. The body weight gain during refeeding was derived from the difference in the food intake, but derived from the difference in the efficiency of food utilization.

It is known that rats adapted to a PFD or a low-protein diet develop an “amino acid sparing state” in the liver, i.e. the elevation of activities of amino acid activating enzymes,16~19) and a reduction in the activities of urea cycle enzymes20~23) and amino acid degrading enzymes.24~27) This together with the reduction of substrate and activator (N-acetyl-glutamate)28) for urea cycle enzymes results in diminished urinary nitrogen output.29) In the earliest protein-repletion period this metabolic state is known to continue for a while, i.e. there is a lag phase before the activities of enzymes mentioned above and urinary nitrogen output return to their original levels (those before depletion).18~20) This lag might be one of the major causes of elevated efficiency of nitrogen retention as body protein during “catch-up” growth. Therefore the difference in the extent of the sparing of amino acid nitrogen would be the reason for the difference in the efficiency of food utilization. In rats of the PF1-RF group, only an insignificant increase in body weight gain and no increase in the body weight gain: fold intake ratio over control values were observed. It appears that rats protein-deprived for only one day cannot develop a sufficient “amino acid sparing metabolic state,” considering that adaptations to the protein deficient diets usually take more than several days to reach a maximum level.16,23)
Protein Refeeding and Hepatic “Catch-up” Growth

Casein % in diets

Fig. 2. Body Weight Gain (A), Food Intake (B), and Changes in Liver Weight and Its Protein, RNA and DNA Contents (C) after Refeeding Diets Containing Various Levels of Casein to Rats Fed a Protein-free Diet for 4 Days.

Results are given as mean±SEM (for (A) and (B) as absolute values, and for (C) as percentages of the mean values from the PF4 group (●)). 1, 2, values not sharing a common subscript number are significantly different (p<0.05).

The greatest in the 20%C group. The value from the 10%C group was the smallest and almost the same as the estimated control value (11 g, the value from normally growing rats of almost the same body weight, fed the 20% casein diet throughout). The greatest food intake was observed in the 20%C group, but the value was not significantly different from that of the 10%C group and almost the same as the estimated control value (20 g). In the 40%C and 60%C groups food intake was significantly less than that in the 20%C group. The body weight gain: food intake ratios were 0.56 (10%C), 0.88 (20%C), 0.89 (40%C) and 1.15 (60%). The value from the 10%C group was almost the same as the estimated control value (0.55=11 g/20 g).

The lower column of Fig. 2 shows the changes in liver weight, and its protein, RNA and DNA contents after refeeding as percentages of the values of the group not refeed, i.e. the PF4 group. Liver weight gain of the 20%C group was the greatest. Liver weight of the other groups also increased but insignificantly. Greater increases in liver protein and RNA contents were observed in the 20%, 40%, and 60% groups than in the 10% group. The liver DNA content did not change significantly in any group.

Although the food intake of the 10% group was greater than those of the 40% and 60% groups, the gains in body weight and liver protein and RNA contents in the former group were smaller than those in the later groups. A casein level of 10% in the diet was not sufficient for “catch-up” growth.

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