# Spleen and Liver Responses of Protein-depleted Rats to Dietary Egg Protein[1] (37514)

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Protein deficiency has different effects in various body tissues. In the adult rat, altered RNA and protein content of liver cells have reflected marked changes in protein status, while the total DNA (cell number) of the organ remained relatively stable (1–3). In contrast, in the spleen, which is capable of cell division throughout life, protein depletion of adult rats reduced the number of cells but had little effect on the size or nitrogen or RNA concentration of remaining cells (1). The spleen is an important source of antibody after iv injection of antigen (4), and, in the protein-depleted rat with its reduced splenic weight, RNA and DNA, antibody titers were also lowered (1). Repletion of hypoproteinemic rats with adequate amounts of high quality protein led to an increase in output of antibody (5). However, the value of a dietary regimen for growth or recovery of lost weight or for liver repletion has not always been equaled by its ability to support immune response (6–8). Despite the fact that its responses to nutritional variations may differ from those of other tissues, the composition of the spleen has not been examined in relation to diet as systematically as has that of liver. This study was designed to determine the effects on splenic and hepatic nucleic acids of graded levels of a protein of high biological value (egg), fed during repletion of adult protein-calorie-depleted rats.

*Procedure.* Adult male rats of the Wistar strain, weighing 459–516 g, were depleted of protein by feeding a basal diet containing 84% cornstarch, 10% fat, 4% salts and 2% fiber (6) for 26 days. Beginning with the second week of depletion, food intake was reduced to half that consumed *ad libitum* during the first week to accelerate protein depletion. The depleted animals were divided into 4 groups of 20 rats each and fed *ad libitum* a diet in which 0, 3, 6 or 9% protein from hexane extracted whole egg[3] replaced an equal amount of cornstarch in the basal diet. Separate vitamin supplements (6) were given daily throughout the study. At the beginning of repletion, rats were immunized by iv injection of 1.0 ml 2% sheep erythrocytes. Half the rats fed each diet were anesthetized with sodium pentobarbital and killed on Day 5 of repletion, and the others, on Day 6. Blood was drawn from the abdominal aorta or heart, centrifuged in the cold, and the serum was frozen for analysis. Liver and spleen were excised quickly after blood had been collected. The organs were weighed, frozen in liquid nitrogen and stored at −20°. The methods used to determine hepatic and splenic RNA and DNA and serum antibodies have been described previously (1). Differences between group means were tested by multiple *t* tests.

[1] Journal Paper No. J-6847 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 1427. Supported in part by USDA-ARS Grant 12-14-100-9134(61). From a dissertation submitted by the senior author in partial fulfillment of the Ph.D. degree, Iowa State University, Ames, Iowa, 1970. Presented in part at the Federation of the American Societies for Experimental Biology, Atlantic City, N. J., April, 1970; Abst. no. 2567.

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TABLE I. Splenic and Hepatic Weights and Nucleic Acids of Depleted Adult Rats Fed 0–9% Egg Protein for 5 or 6 Days.

| Dietary protein (%) | Period of refeeding (days) | Wt (g) | RNA (mg) | DNA (mg) | RNA/DNA (mg/mg) |
|---|---|---|---|---|---|
| | | | Spleen | | |
| 0 | 5 | 0.60 ± 0.03*[a,b] | 3.02 ± 0.17* | 10.1 ± 0.9* | 0.31 ± 0.02* |
| 3 | 5 | 0.74 ± 0.04# | 4.18 ± 0.25# | 12.3 ± 1.1*# | 0.35 ± 0.03* |
| 6 | 5 | 0.86 ± 0.04# | 4.88 ± 0.32# | 13.8 ± 1.2# | 0.36 ± 0.02* |
| 9 | 5 | 0.82 ± 0.04# | 4.57 ± 0.38# | 14.6 ± 1.7# | 0.33 ± 0.02* |
| 0 | 6 | 0.62 ± 0.03* | 3.23 ± 0.19* | 11.1 ± 1.0* | 0.30 ± 0.02* |
| 3 | 6 | 0.74 ± 0.05# | 4.08 ± 0.32# | 12.9 ± 1.1*# | 0.32 ± 0.01*# |
| 6 | 6 | 0.77 ± 0.04# | 4.53 ± 0.42#¢ | 13.7 ± 1.3*# | 0.34 ± 0.02*# |
| 9 | 6 | 0.86 ± 0.05# | 5.40 ± 0.42¢ | 15.0 ± 1.3# | 0.36 ± 0.02# |
| | | | Liver | | |
| 0 | 5 | 10.50 ± 0.27* | 61.1 ± 1.8* | 33.4 ± 1.5* | 1.81 ± 0.06* |
| 3 | 5 | 13.25 ± 0.50# | 73.6 ± 2.4# | 36.2 ± 2.3*# | 2.10 ± 0.12# |
| 6 | 5 | 14.22 ± 0.62# | 79.6 ± 3.6#¢ | 37.0 ± 1.9*# | 2.24 ± 0.13# |
| 9 | 5 | 13.09 ± 0.59# | 82.3 ± 3.4¢ | 38.6 ± 0.7# | 2.15 ± 0.10# |
| 0 | 6 | 10.07 ± 0.27* | 59.5 ± 1.8* | 30.7 ± 0.9* | 1.94 ± 0.06* |
| 3 | 6 | 13.02 ± 0.83# | 82.9 ± 3.7# | 35.0 ± 1.9*# | 2.38 ± 0.07# |
| 6 | 6 | 13.43 ± 0.47# | 83.1 ± 1.4# | 35.7 ± 1.4# | 2.35 ± 0.09# |
| 9 | 6 | 13.00 ± 0.64# | 92.2 ± 5.0# | 35.5 ± 1.2# | 2.69 ± 0.12¢ |

[a] Each mean represents 8–10 animals.

[b] Mean ± SE; values with same symbol in each refeeding period are not different from one another ($p < 0.05$).

*Results.* During depletion, rats lost 25% of their initial body weight in 26 days. When egg protein was incorporated at 0, 3, 6 or 9% of the diet, gains in body weight were 13, 40, 55 and 60 g after 5 days of unrestricted feeding.

Hepatic weight and total RNA increased significantly when egg protein was incorporated into the repletion diet, compared with values when 0% protein was fed (Table I). Varying dietary egg protein between 3 and 9% did not influence liver weight at 5 or 6 days, but total hepatic RNA tended to increase with increases in dietary protein.

The DNA content of the liver was significantly lower when no egg was fed than when 9% was fed. However, hepatic DNA concentration after 5 or 6 days of repletion was highest in groups fed no protein (about 3.1 mg/g liver vs 2.6–3.1 mg/g in other groups). Others have also observed increased concentrations of hepatic DNA in protein-deficient rats (1–3). An increase in total DNA has also been reported as a result of protein deficiency (1, 3). Because hepatic RNA changed more than DNA, RNA/DNA ratios were greater in rats repleted with 3–9% egg than in those fed 0%. Only after repletion for 6 days did ratios for groups fed 9% exceed those of rats fed 3 or 6%.

Inclusion of protein in the diet significantly increased spleen weights and total splenic RNA after 5 or 6 days of repletion (Table I). Linear regressions of total splenic nucleic acids on egg protein between 0 and 9% of the diet had slopes of 0.21 mg RNA and 0.46 mg DNA per 1% change in protein ($p < 0.05$). Similar splenic RNA concentrations were found among all protein-fed groups on Day 5, but the concentration tended to increase with increasing dietary protein on Day 6. DNA concentrations in spleen were similar for all groups. On Day 5, splenic RNA/DNA ratios were similar, but on Day 6, rats fed the highest intake of protein had a higher ratio than those fed no protein.

The increase in nucleic acids in rats fed

9% protein, compared with those fed 0%, is a convenient index for comparing relative responses of the two tissues. Mean hepatic RNA and DNA were 45 and 16% greater in rats fed 9% egg protein than in those fed 0%; splenic values were 60 and 39% greater with 9% protein. The RNA/DNA ratio was increased by 29% in liver, but only by 13% in spleen when the diet contained 9% protein. The data are consistent with observations in normal and protein-deprived animals which showed cell number to be more stable in liver than in spleen, while cell composition was more nearly constant in spleen (1).

Values for organ weights and nucleic acids were not consistently greater on Day 6 than on Day 5, except perhaps for splenic DNA. Since nonimmune rats were not used in the study, it is not clear if the effect of diet on DNA in spleen was a simple effect of dietary protein or if the nutritional action may have been potentiated by the stimulus of immunization.

Mean antibody titers (measured as hemolysin or agglutinin) did not differ significantly among diet groups on either Day 5 or Day 6, even though splenic RNA increased progressively with protein intake. If RNA is taken as a rough index of protein synthesis, higher intakes of protein may have led to synthesis of protein other than immunoglobulins or affected catabolic rates so that amounts of circulating antibody were kept constant. The condition of the animals at the time of immunization, similar for all animals, may have affected antibody response more than protein intake in the subsequent period, when antibody was measured. Piedad-Pascual *et al.* (8) showed no consistent relationship of serum antibody to percent protein in animals immunized after a week of repletion with 5–10% protein from various sources, even though spleen weights and numbers of antibody-forming cells were related to protein level. Since small changes in serum antibody cannot be detected by common serological methods, negative results cannot be taken as absolute proof that antibody production was unaffected. In cases where the range of protein intakes was greater than the 9% in this study, significant differences in antibody titers have been observed (6).

*Summary.* Protein-calorie-depleted, adult male rats were immunized and fed *ad libitum* 0, 3, 6, or 9% egg protein. Weights and nucleic acids of spleen and liver were determined after 5 or 6 days. Increasing egg protein during repletion from 0 to 9% increased gains in body weight, RNA and DNA of spleen and total hepatic RNA also increased. Hepatic weight and DNA were similar among animals fed 3–9% protein. Serum antibody concentration was not significantly affected by the level of dietary protein.

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Received April 30, 1973. P.S.E.B.M., 1973, Vol. 144.