Dietary Protein and Serum Electrophoretic Patterns of the Adult Rat.* (24802)

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Since the introduction of the moving boundary method of electrophoresis, a number of studies concerned with effects of protein level of the diet on concentration and distribution of serum proteins have been made. Due to volume of serum required, individual measurements have been limited to a great extent to man and the dog(1,2) and studies with rats were of necessity on pooled samples(3,4). Development of filter paper electrophoresis has made possible analyses on individual serum samples from small animals.

In the present investigation, the effects of inanition, acute and chronic protein depletion, and of repletion with several levels of protein on the partition of serum proteins have been determined in adult rats.

Methods. Male, Sprague-Dawley rats were housed singly in suspended type wire cages with wire bottoms and maintained on a diet of Purina Laboratory Chow and tap water, *ad lib.* until initiation of the study. Blood samples were obtained by cardiac puncture under light ether anesthesia. To avoid effects of repeated bleedings(5) each animal was bled only once. *Normal.* The group was composed of animals maintained under conditions described above. *Inanition.* Food was withheld from rats until they had lost 25% of their original weight. Drinking water was available at all times. *Acute depletion.*† The rats were fed the protein-free diet previously described(6) until they had lost 25% of original weight. *Chronic depletion.*† The diet contained 2% casein, 69% starch, 10% vegetable oil, 4% salt mixture (U.S.P. XIV), 14% alphacel (cellulose), 1% cod liver oil, and vitamin diet fortification mixture which was incorporated into the protein-free diet(6). The diet was fed until a 25% weight loss was attained. *Repletion.* Animals were depleted by feeding either the protein-free diet or the 2% casein diet until a 25% weight loss was attained. They were then repleted by feeding either the stock diet of ground Purina Laboratory Chow (25% protein,(7)) or a purified diet. The purified diet† contained 10% vegetable oil, 4% salt mixture (U.S.P. XIV), and the vitamin diet fortification mixture(6). The contents of casein and starch were adjusted to yield the desired protein level. All diets were fed *ad lib.* The rats were bled after they had regained their original weight. *Chemical and hematologic determinations.* Total serum protein, hemoglobin, and hematocrit values were determined by methods previously reported(5). Electrophoretic analyses were performed in a Durrum type, Spinco, Model R apparatus. Duplicate separations on 0.01 ml serum samples were carried out for 16 hours at 5 Ma in veronal buffer, pH 8.6, ionic strength 0.075 on Whatman 3 MM paper strips. The strips were dried in an oven at 115°C for 30 minutes then dyed with bromphenol blue. The relative amounts of protein in the fractions were determined by use of an integrating photometric scanner (Analytrol). Absolute concentrations were estimated by reference to total serum protein levels. Analysis of the data was made by standard statistical procedures, employing the *t* test(8).

Results. General and hematologic data are presented in Table I. The effects of rate of weight loss on plasma volume were demonstrated by the hematocrit data for the depleted groups. The most rapidly depleted group, B, exhibited hemoconcentration, the 2% casein group, D, hemodilution, while the protein-free group, C, had values in the normal range. All repleted groups showed varying de-

* Supported in part by research grant from Nat. Cancer Inst.

† Obtained from Nutritional Biochemicals Corp., Cleveland.

TABLE I. General and Hematologic Data.

Group	No. rats	Diet	Initial wt (g)†	Depletion wt (g)†	Depletion time (days)	Repletion wt (g)†	Repletion time (days)	Hematocrit (%)†	Hemoglobin (g %)†
A. Normal	40	Stock	404 ± 3.0					45 ± .3	14.3 ± .19
Depleted									
B. Inanition	22	None	407 ± 8.5	305 ± 6.2*	7.9			53 ± .8*	16.5 ± .28*
C. Protein-free	47	Protein-free	401 ± 4.9	303 ± 4.0*	19.9			45 ± .4	14.0 ± .22
D. 2% casein	16	2% casein	399 ± 4.8	301 ± 3.4*	61.8			42 ±1.2*	12.3 ± .37*
Repleted		Protein-free:							
E1. 8% casein	19	8% casein	403 ± 5.8	306 ± 4.5*	21.1	400 ± 6.3	16.8	43 ± .5*	13.1 ± .09*
E2. 17% "	18	17% "	399 ± 5.8	304 ± 4.1*	20.6	401 ± 5.6	12.0	43 ± .8*	12.6 ± .13*
E3. 27% "	18	27% "	403 ± 6.6	302 ± 4.8*	21.0	406 ± 4.7	11.5	42 ± .6*	13.3 ± .20*
E4. 40% "	17	40% "	405 ± 3.6	303 ± 3.3*	16.1	400 ± 3.9	8.6	40 ± .4*	12.1 ± .13*
E5. 64% "	14	64% "	406 ± 2.6	309 ± 2.2*	17.0	406 ± 3.3	11.9	43 ± .5*	13.1 ± .21*
F1. Stock	16	Ground stock	407 ± 3.3	308 ± 2.8*	16.8	408 ± 3.5	8.7	41 ± .6*	12.8 ± .23*
F2. Stock	16	2% casein: Ground stock	401 ± 4.3	301 ± 2.6*	58.0	402 ± 4.4	11.9	40 ± .7*	12.0 ± .23*

Statistically significant differences from normal values are indicated: * P = <.01.
† Including the stand. error of the mean.

grees of hemodilution. The observations were in accord with earlier studies(1) which demonstrated that alterations in plasma volume may accompany depletion and repletion. Relative concentration changes of the serum fractions, however, were of such magnitude that only in a few cases were they masked by the fluctuations in plasma volume.

The results of electrophoretic analyses are summarized in Table II. All depletion regimens caused significant decreases in the albumin, α_1- and α_2-globulin fractions. Starvation and acute depletion also elicited significant decreases in concentrations of β-globulin concomitant with increases in the γ-globulin fraction. All the depletion diets, however, had the same relative influence on loss of albumin and serum globulins as shown by the similarity of the A/G ratios.

Repletion with the 8% casein diet following acute depletion restored the albumin, α_2- and β-globulin fractions to normal. A higher level of dietary protein, 17%, was required to restore the α_1-globulins. With the exception of the 8% protein, all other purified diets were not only adequate to restore total serum protein levels to normal, but elicited significant increases. The 17% casein diet was the best balanced for restoration of the serum proteins as measured by maximum increase of serum values over depletion levels. Comparative effects of acute and chronic depletion on capacity to respond to a standard diet are shown by the results of groups F1 and F2. Although the weight loss in each group was identical, the chronically depleted rat was unable to respond to the same extent during repletion. The major divergences were in α_2-globulin and total serum protein values. The increased A/G ratios for groups F1 and F2 suggested that repletion on a diet of natural products (7) promoted regeneration of serum albumin to a greater extent than a purified diet of similar protein content, E3.

The current studies have demonstrated that in the rat, components of the globulin fraction are more affected by protein depletion than the corresponding fractions of man and the dog(1,2). Diets with a protein content of 17% have been found to be adequate for re-

TABLE II. Effects of Protein Depletion and Repletion on the Partition of Serum Proteins in the Adult Rat.

	Group	Total serum protein	Albumin	Globulin α_1	Globulin α_2	Globulin β	Globulin γ	A/G ratio
		(g %)						
A.	Normal	5.8 ±.04	2.0 ±.03	1.5 ±.03	.7 ±.03	1.1 ±.03	.5 ±.02	.53 ±.014
	Depleted							
B.	Inanition	5.1 ±.02	1.7 ±.05†	1.3 ±.04†	.5 ±.03†	.9 ±.03†	.7 ±.03†	.50 ±.017
C.	Protein-free	4.9 ±.06†	1.7 ±.02†	1.2 ±.03†	.5 ±.02†	.9 ±.02†	.6 ±.03*	.53 ±.010
D.	2% casein	4.9 ±.07†	1.7 ±.04†	1.3 ±.03†	.4 ±.02†	1.0 ±.05	.5 ±.04	.53 ±.015
	Repleted							
E1.	8% casein	5.5 ±.07†	2.0 ±.05	1.3 ±.07†	.6 ±.03	1.1 ±.04	.5 ±.02	.57 ±.021
E2.	17% "	6.4 ±.08†	2.2 ±.04†	1.5 ±.06	.8 ±.04	1.3 ±.05†	.6 ±.05	.55 ±.018
E3.	27% "	6.0 ±.06*	2.0 ±.06	1.7 ±.05†	.7 ±.04	1.1 ±.04	.5 ±.03	.50 ±.023
E4.	40% "	6.2 ±.08†	2.2 ±.06†	1.6 ±.06	.7 ±.04	1.2 ±.03	.5 ±.04	.55 ±.020
E5.	64% "	6.1 ±.07†	2.1 ±.07	1.5 ±.05	.8 ±.04	1.2 ±.03	.5 ±.05	.52 ±.029
F1.	Stock	6.1 ±.06†	2.3 ±.06†	1.5 ±.04	.7 ±.04	1.1 ±.04	.5 ±.04	.61 ±.030†
F2.	"	5.8 ±.07	2.3 ±.06†	1.5 ±.06	.5 ±.04†	1.1 ±.05	.4 ±.03*	.66 ±.071†

Findings include stand. error of mean.

Statistically significant differences from normal values are indicated: * P = <.05 >.01. † P = <.01.

storation of the serum proteins in the adult, male rat following acute depletion. It may also be inferred from the data, that the marked increases in the α- and β-globulin concentrations observed in many pathologic conditions are not the result of reduced protein intake. The greater lability of the serum proteins of the adult rat to dietary influences suggests that they might be more sensitive indicators of nutritional states than those of man or the dog(1,2).

Summary. The effects of inanition, acute and chronic protein depletion and of repletion with several levels of protein on distribution of serum proteins in the adult rat have been investigated by filter paper electrophoresis. Decreases in the albumin, α_1-, α_2- and β-globulin fractions were observed following depletion regimens. Purified diets containing 17% or more of casein not only restored all values to normal, but elicited significant increases in total serum protein concentrations. A differential response of the α_2-globulin fraction upon repletion occurred between groups previously subjected to acute and to chronic depletion. Significantly increased A/G ratios were found in groups repleted with a diet of natural products. Rate of depletion was found to exert a pronounced effect on hematocrit values.

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Received February 9, 1959. P.S.E.B.M., 1959, v100.