Effects of Protein Depletion and Repletion on Serum Electrophoretic Patterns of Adult Rats.* (25362)

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A number of studies concerned with effects of nutritional states on serum proteins of several species have been reported(1,2). Serial determinations of serum proteins during protein depletion and repletion, however, have been limited to a great extent to man and the dog, and there is some evidence of species differences in their response(2). In the present study, partition of serum proteins has been determined by filter paper electrophoresis in groups of adult rats sacrificed at intervals during protein depletion and repletion.

Methods. Male, Sprague-Dawley rats were housed singly in suspended type wire cages with wire bottoms and maintained on a diet of Purina Lab. Chow and tap water, *ad lib.* until initiation of the study. Blood samples were obtained by exsanguination from the heart under ether anesthesia. *Normal.* Two normal groups were employed. Group A1 served as base line control and A2 as age and weight control. They were maintained under conditions described above. *Depletion.* The animals were fed protein-free diet†(3) until the desired weight loss was achieved. Groups of rats were sacrificed after losing 10, 25, 33 and 40% of original weight. *Repletion.* Purina Lab. Chow with protein content of approximately 25% was fed *ad lib.* to animals with 25% weight loss. In an earlier study(4), the diet was adequate for weight and serum protein repletion. Groups of rats were sacrificed after regaining 35, 70 and 100% of their lost weight. One group, C4, was allowed to survive 4 weeks following weight repletion. *Chemical and hematologic analyses.* Total serum protein, hemoglobin and hematocrit values were determined by methods previously reported(3). Electrophoretic analyses were carried out by procedures previously reported (5) except that a current of 6 ma was employed. Use of a slightly higher amperage resulted in better resolution of the albumin and α_1-globulin fractions. Analysis of the data was made by standard statistical procedures employing the t test(6).

Results. General and hematologic data are summarized in Table I. Plasma volume as indicated by hematocrit values, fluctuated during protein depletion and was significantly increased in repletion. Relative concentration changes of serum fractions, however, were not significantly affected. Hemoglobin was depleted and restored at a slower rate than the serum proteins. Four weeks following repletion (C4), hemoglobin and hematocrit values were in the normal range. Body weight curves during protein depletion and repletion are presented in Fig. 1. The curves were characterized by marked inflections following dietary change.

Results of electrophoretic analyses are summarized in Figs. 2, 3, and 4. Feeding a protein-free diet for one week (B1) caused highly significant decreases in total protein, albumin and β-globulin concentrations. Moderate depletion (B2) elicited significant decreases in total protein, albumin and the α-globulins

TABLE I. General and Hematologic Data.

Group	No. rats	Hemoglobin (g %)*	Hematocrit (%)*
A1 Normal (400 g)	14	14.1 ± .10	44 ± .4
A2 " (460 g)	14	13.8 ± .20	46 ± .5†
B1 10% depleted	12	14.7 ± .15†	49 ± .3†
B2 25% "	12	14.1 ± .40	43 ± .4
B3 33% "	9	11.4 ± .36†	36 ±1.4†
B4 40% "	9	11.8 ± .33†	42 ±1.7
C1 35% repleted	12	11.8 ± .24†	38 ± .6†
C2 70% "	12	11.8 ± .30†	37 ± .5†
C3 100% "	12	11.9 ± .24†	38 ± .6†
C4 4 wk post repletion	26	14.0 ± .20	45 ± .5

* Including stand. error of mean.

Statistically significant differences from baseline values (A1) are indicated:
† P = <.01.

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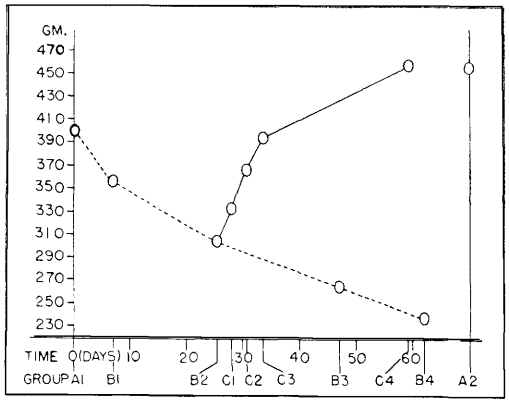
† Obtained from Nutritional Biochemicals Corp., Cleveland.



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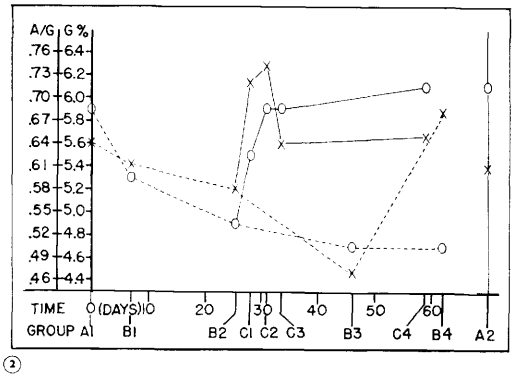

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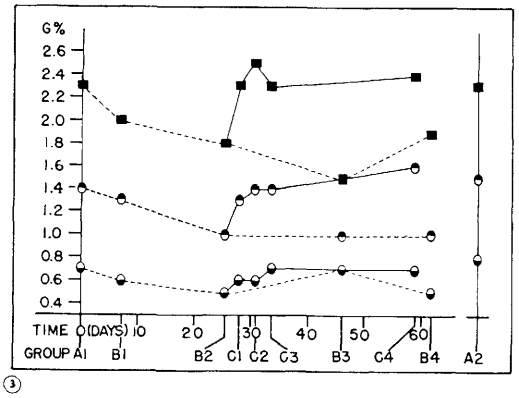

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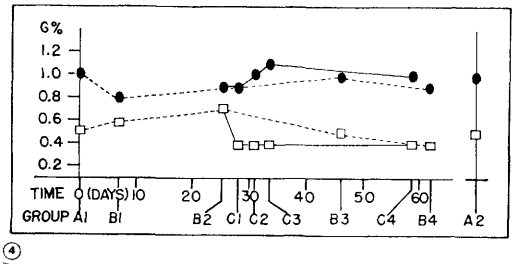

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FIG. 1. Effects of protein depletion and repletion on body wt. Dashed and solid lines indicate when protein-free and stock diets, respectively, were fed.

FIG. 2-4. Effects of protein depletion and repletion on serum protein concentrations. Dashed and solid lines indicate when protein-free and stock diets, respectively, were fed. Plotted values are not corrected for experimental hematocrit. 2. ○, total protein; ×, A/G ratio. 3. ■, albumin; circle (solid top), α_1-globulin; circle (solid bottom), α_2-globulin. 4. ●, β-globulin; □, γ-globulin.

concomitant with a significant increase in γ-globulin. Further depletion was characterized by divergent responses in subfractions of serum. The greatest changes were in the albumin fraction which resulted in marked alterations in A/G ratios (*cf.* B3, B4). At end of the depletion phase (B4), decreases in total protein, albumin, α_1 and α_2-globulin values were significant at the 1% level; those for β- and γ-globulins at the 5% level.

Rapid increases occurred in major serum components upon introduction of the stock diet (C1). Total protein, albumin, α_1–, α_2– and β-globulin concentrations were restored to their normal range before weight repletion (C2). At weight repletion (C3) and 4 weeks following repletion (C4), the differences between γ-globulin values for the experimental and control groups (A1, A2) were significant at the 5% level. Comparison of the 2 normal groups (A1, A2) showed no significant differences in distribution of serum proteins with increasing age and weight.

Summary. Distribution of serum proteins has been determined by filter paper electrophoresis in groups of adult rats sacrificed at intervals during protein depletion and repletion. In the early stages of protein depletion and repletion, body weight changes were paralleled to a considerable extent by similar alterations in serum protein levels. Such correlation was not apparent during later stages. The albumin and α_1-globulin fractions were most sensitive to nutritional influences. The γ-globulin fraction deviated from the general pattern of response.

The technical assistance of Dorothy Roberts is gratefully acknowledged.

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Tolerance of the Profoundly Hypothermic Dog to Complete Circulatory Standstill.* (25363)

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Introduction of a clinical method of inducing and reverting hypothermia(1) has made determination of safe limits of complete circulatory standstill at 10°C or lower of practical importance. Experiments outlined below represent our efforts to define these safe limits.

Methods. Healthy mongrel dogs were anesthetized with intravenous sodium pentobarbital. Extracorporeal circulation and hypothermia were carried out by a pump-oxygenator-heat-exchanger device already described (1). Just before perfusion, 28-30 mg/kg of quinidine hydrochloride was given intravenously. The mid-esophageal temperature, as measured by a thermistor, was rapidly reduced to between 5°C and 9°C. At this point circulatory standstill was permitted by stopping the pump. Temperature drifts during cessation of circulation were prevented by external application of ice. The chest was opened only when electrical defibrillation of the heart was needed.

Experimental design. The period of complete circulatory standstill and the number of dogs in each period were as follows: 30 min., 2 dogs; 60 min., 12 dogs; 90 min., 6 dogs; 120 min., 2 dogs; 180 min., 2 dogs. Surviving animals were followed for at least 7 days and observed closely for central nervous system changes.

Results. The 14 dogs in the 30-minute and 60-minute groups all survived 7 days or longer and were completely normal. In the 90-minute group, 4 survived and were normal; but 2 of the survivors developed ventricular fibrillation that had to be corrected by electrical defibrillation. The remaining 2 in this group, as well as the 4 dogs in the 120-minute and 180-minute groups, died from irreversible ventricular fibrillation that developed in the rewarming period.

Discussion. Temperatures below 10°C induced and reverted with an extracorporeal system, permit 60 minutes of safe complete circulatory standstill in dogs. Periods of 90 minutes are less well tolerated, as shown by occurrence of ventricular fibrillation. This arrhythmia was irreversibe in 4 dogs in circulatory standstill for more than 90 minutes, as well as the 2 in the 90-minute group. Development of ventricular fibrillation may indicate that rate of metaboism in the heart, even though greatly reduced by the cold and the asystole, is still sufficient to cause irreversible changes if oxygen deprivation is continued beyond 60 minutes.

Summary. Periods of 60 minutes of complete circulatory standstill were tolerated in 10 dogs at temperatures of 10°C or lower.

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