16250

Effects of Protein and Fluid Consumption Upon Plasma Volume and Circulating Protein in the Rat.

RICHARD W. LIPPMAN.* (Introduced by T. Addis.)
From the Department of Medicine, Stanford University School of Medicine, San Francisco, Calif.

In studying rats under experimental conditions, changes in the concentration of serum constituents were noted. It was considered possible that such changes might be the consequence of changes in blood and plasma volume, induced by alterations in the consumption of food and fluid, rather than the consequence of a direct metabolic effect. This study was undertaken in order to determine whether the conditions of our experiments had produced such alterations in blood and plasma volumes.

Methods. A total of 101 normal male rats were used, ranging in weight from 148 to 196 g. The rats were taken from stock, where they had received a diet containing 17% of protein, and water as desired. They were placed in individual cages and given experimental diets for a specified time. At the end of this time, blood and plasma volume determinations were made by the method previously reported.[1] The serum from each experimental group of 8 to 10 rats was pooled in aliquot portions, and the total serum protein was determined by a modification of Kingsley's biuret method.[2] Exact composition of the diets is given in Table I. Total circulating protein was calculated from the measured data.

The rats received unrestricted amounts of the 3% and 77% protein diets and water. With the exception of the total starvation group, each group received an adequate number of calories.

Results. Detailed results are given in Table II, and compared with normal control values previously established for rats of the same weight taken directly from stock diet.[3]

None of the protein-deficient diets, whether of high or low fluid content, induced any appreciable change in plasma volume at the end of 7 days, although the total blood volume was increased about 10% when compared with normal for the final weight.

The fluid diet with added salt appeared to increase both total blood volume and plasma volume about 10% at the end of 17 hours. The 77% protein diet produced a similar change at the end of 7 days.

Neither the fluid diet without salt, nor total starvation for 17 hours altered the total blood or plasma volume.

None of the 17-hour experiments showed any significant change in total circulating serum protein or total serum protein concentration. However, all the protein-deficient diets reduced the total circulating serum protein by 15-20% in 7 days, with an almost commensurate reduction in the total serum protein concentration. The 77% protein diet did not produce any significant change in total circulating serum protein or in the total serum protein concentration during the period studied.

Discussion. Although there have been many studies of the effect of diet upon total serum protein concentration, very few have correlated such effects with changes in the blood and plasma volumes, or have studied volume changes alone.

Boycott and Chisolm[4] found no change in blood volume, but a fall in plasma volume,

* The author gratefully acknowledges the technical assistance of Helen J. Ureen and Way Lew. This work was aided by a grant from the U. S. Public Health Service. Hemoglobin solution was provided by Sharp and Dohme, Inc., Philadelphia, Pa. Dr. Lippman is now at the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, Calif.

1 Lippman, R. W., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 188.

2 Kingsley, G. R., *J. Biol. Chem.*, 1939, **131**, 197.

3 Lippman, R. W., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 193.

TABLE I.
Percentage Composition of Diets.

Constituent	Stock diet (17% prot.)	3% protein	77% protein	10% dext.	10% dext. with salt
Bone ash	1.5				
Soy bean meal	5.7				
Wheat	73.4				
Corn starch	4.0	74.0			
Casein	9.7		74.0		
Yeast		10.0	10.0		
Sardine oil	3.2	10.0	10.0		
Alfalfa	2.0	2.0	2.0		
Salt mixture	0.5	4.0	4.0		
NaCl					0.4
Vit. B complex				1.0	1.0
Dextrose				10.0	10.0
Water				89.0	88.6

TABLE II.
Effect of Various Regimens on Blood and Plasma Volume and Serum Protein.
Mean Results.

Diet	Total starv.	10% dext.	10% dext. with salt	10% dext.	10% dext. with salt	3% prot.	77% prot.
Duration	17 hr	17 hr	17 hr	7 days	7 days	7 days	7 days
No. rats	9	16	20	8	8	20	20
Initial wt, g				151	153	172	172
Final wt, g	147	173	163	119	125	155	185
Blood vol., cc	11.29	11.91	12.56	9.67	10.01	11.57	13.68
% normal	109 ± 1.4	99 ± 2.1	112 ± 1.1	110 ± 2.3	110 ± 2.3	107 ± 2.6	110 ± 2.3
Plasma vol., cc	6.22	6.66	7.14	4.90	5.35	6.10	7.52
% normal	103 ± 1.8	100 ± 2.6	111 ± 1.4	96 ± 3.4	103 ± 3.8	99 ± 2.5	106 ± 2.3
Total serum protein, g %	5.33	5.42	5.28	4.28	4.48	4.70	5.62
% normal	105	102	101	89	92	92	102
Total circ. protein, mg	332	361	377	210	240	287	423
% normal	102	96	106	81	87	84	106

after 9 to 11 days on a protein-free diet. Frisch, Mendel and Peters[5] produced marked depression of serum protein concentrations by using a carrot diet for 18 weeks. Bloomfield,[6] on the contrary, found that an otherwise balanced 3% protein diet did not produce much lowering of serum protein concentration at the end of 21 weeks.

Metcoff, Favour and Stare[7] studied protein deficiencies in the rat, and their conditions for "acute protein deficiency" closely resembled our 3% protein diet for 7 days. They found, under these conditions, a decrease in the circulating total protein per unit surface area, and in the plasma volume per unit surface area.

The apparent increase that we found in the cell volume of rats with protein deficiency is probably due to the stability of the erythrocyte mass compared with the lability of plasma volume and body weight. In one week, the erythrocyte mass formed at the starting weight would not have changed appreciably, while the plasma volume and body weight had diminished, so that the erythrocyte mass was relatively increased.

We have avoided using the relation of variables to body surface area in these comparisons because of the pitfalls that have

[4] Boycott, A. E., and Chisolm, R. A., *J. Path. and Bact.*, 1911, **16**, 263.

[5] Frisch, R. A., Mendel, L. B., and Peters, J. P., *J. Biol. Chem.*, 1929, **84**, 167.

[6] Bloomfield, A. L., *J. Exp. Med.*, 1933, **57**, 705.

[7] Metcoff, J., Favour, C. B., and Stare, F. J., *J. Clin. Invest.*, 1945, **24**, 82.

been suggested previously.[3] The situation would be additionally complicated by the fact that body surface formulae are derived from rats on stock diet. It is known that, after starvation or over-feeding, the composition of the body changes with respect to the basic constituents such as protein.[8] With such changes, the relation between body weight and surface area may change. Therefore, we have compared our results in each group with rats on stock diet at the same weight.

[8] Addis, T., *Proc. 6th Pacific Science Congress*, 1939, **6**, 677.

Summary. 1. Drastic changes in protein, fluid, and salt consumption do not produce striking changes in blood and plasma volumes of the rat, over the periods studied. 2. Increased fluid consumption produced an increase of about 10% in total blood volume and plasma volume at the end of 17 hours, only when salt consumption was simultaneously increased. 3. A high protein diet increased blood and plasma volumes slightly at the end of 7 days, but did not appreciably alter the serum protein concentration. 4. All protein-deficient diets reduced the total circulating protein by 15-20% at the end of 7 days.

16251

Effect of Lipotropic Agents in a Diet Containing Pure Amino Acids in Place of Protein.

CATHERINE S. ROSE, THOMAS E. MACHELLA, AND PAUL GYÖRGY.*
From the Nutritional Service of the Department of Pediatrics and the Gastro-Intestinal Section of the Medical Clinic, School of Medicine, University of Pennsylvania, Philadelphia.

In an experiment where an amino acid mixture replaced casein in a diet deficient in lipotropic factors, Rose, Machella, and György[1] found no appreciable effect of methionine fed to rats at a level of 50 mg per day or equivalent to about 0.8% of the diet. It was not immediately possible to extend this work but the need to determine whether more methionine would be effective or whether some other factor was involved was recognized. In the present study the effect of methionine in the original and in larger dosage was compared with that of choline.

Experimental. The experimental diet and the procedure followed were as previously described. Male albino rats, weighing 150 to 165 g were divided into 4 groups of which one received basal diet alone, the others receiving in addition respectively, 50 mg of methionine, 100 mg of methionine and 25 mg of choline chloride per day in aqueous solution. For about three-fourths of the animals we followed our usual procedure of giving the supplement in a Caster Cup. Since some of the rats disliked the solution of methionine a stomach tube was used for supplementing those animals which started later in the experiment (equal numbers of all groups). The animals were sacrificed at the end of 3 weeks and the liver fat determined.

Results and Discussion. The experimental data are summarized in Table I. As previously observed[1] the basal diet was quite inadequate in maintaining the weight of the animals and addition of the sulfur containing amino acid neither improved the nutritive value of the ration in this regard to any great extent, nor encouraged appetite. Choline was equally without effect on these factors. Food intake was remarkably constant in all of the groups but much lower than in the previous experiment. The average consumption was 4.1 g per day as compared with 5.9 g in the earlier study.

In the control animals the low food intake and weight loss did not prevent the development of fatty livers, an average value of 20.4% being found. A greater effect of methionine was observed in this experiment than in that previously reported. There was

* The authors are grateful to Miss Caroline Perna for technical assistance.

[1] Rose, C. S., Machella, T. E., and György, P., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 352.