7964 C

Effect of a Low Calorie, Low Protein Diet on Blood Proteins.

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The function of the labile protein of the blood is almost as poorly understood in the rat where it has to some extent been studied as in man where its workings are largely a matter of conjecture. Bloomfield,[1] and Torbert[2] have shown that starved rats lose a small percentage of plasma protein in from 4 to 6 days, and then stay constantly at the reduced level until death. This easily lost protein, largely albumin, is the labile fraction.

To study the same problem in man 26 normal subjects were placed for 2 days on a diet containing only 500 calories per day, and no protein. Fluids were not restricted. The protein content of the blood serum, taken in the fasting state, was determined before and after the experimental period by the method of Barnett, Jones and Cohn.[3]

Surprisingly enough, instead of falling, the total serum protein concentration rose during the test. The average value for the 26 subjects at the start was 7.40 gm. of protein per 100 cc. of serum. At the end it was 7.74 gm. per 100 cc. The individual values are shown in Table I.

To see whether or not the increased protein concentration was due to concentration of the blood, blood volume estimations were made on 9 of the subjects before and after the test diet, using the method of Keith, Rowntree and Geraghty.[4] The plasma volume fell consistently, the average at the start being 2,366 cc., and at the end being 2,058 cc., showing that the blood was being concentrated. However, the total protein of the plasma, as obtained from the product of the concentration and the volume, fell slightly, the average before being 170.7 gm., and afterwards 156.5 gm. This indicated that in spite of the increased protein concentration a small amount of protein was being lost.

An average of 4 pounds of body weight was lost during the 2 days of the test. This was apparently due to the combination of

1 Bloomfield, Arthur L., unpublished data.

2 Torbert, Harold C., in press.

3 Barnett, C. W., Jones, R. B., and Cohn, R. B., *J. Exp. Med.*, 1932, **55**, 683.

4 Keith, Rowntree and Geraghty, *Arch. Int. Med.*, 1915, **16**, 547.

TABLE I.
Protein Concentration and Blood Volume before and after 2 days of protein starvation.

Start 7.68 g/100cc.	Finish 7.42 g/100cc.	Plasma Volume Start	Plasma Volume Finish	Total Plasma Protein Start	Total Plasma Protein Finish
8.23	8.29				
7.99	7.94				
6.88	7.27				
7.18	7.60				
8.03	8.45				
6.82	7.38				
7.11	7.81				
7.57	7.55				
7.14	7.40				
7.74	8.03				
7.39	8.10				
7.96	8.44				
7.43	8.39				
8.15	8.10				
7.53	7.71				
6.28	6.53				
7.25	8.11	2400 cc.	2055 cc.	174.0 gm.	166.6 gm.
7.02	7.60	4180	3340	293.3	253.6
7.43	8.02	2370	2277	176.2	182.5
7.00	7.15	2995	2220	210.0	158.0
6.51	6.90	2145	1978	139.8	136.5
7.15	7.42	2220	2180	158.0	161.5
8.40	8.13	1900	1605	159.5	130.5
7.74	8.41	1544	1353	119.4	113.8
6.90	7.00	1542	1511	106.3	105.7
Av. 7.40	7.74	2366	2058	170.7	156.5

lack of food intake, loss of water, and perhaps some tissue destruction. The subjects were as a rule hungry throughout the experiment, at times to the point of nausea, and they drank little water unless forced.

It was apparent, therefore, that 2 days of protein starvation in man concentrated the blood as evidenced by increased protein concentration and lessened volume, and that it slightly lowered the total protein content.

In order to investigate the factors which caused the blood concentration several further procedures were carried out.

That activity was not involved was shown by the fact that of the 26 subjects 10 were in bed for several days before and during the test; 8 were very active in their duties as house officers throughout the test; and 8 changed from some sort of activity to bed rest for the experiment. The rise was practically uniform in all these groups.

Since it is the blood proteins of small molecular size that exert the highest osmotic pressure, a decrease of these proteins greatly reduces the ability of the blood to hold water. It seemed that a small amount of this fraction might have been used, and so have reduced the oncotic pressure of the serum out of proportion to its weight, with resultant concentration. The oncotic pressures were measured in 3 subjects by Dr. Garnett Cheney who found that the serum oncotic pressure did not change significantly during the 2 days of the test. The average oncotic pressure at the start of the diet was 300 mm. of water, and at the end was 296 mm. of water. A slight increase of oncotic pressure would have been expected with the more concentrated plasma unless some protein were lost, and so this may be further evidence of protein loss.

However, it could not be shown by fractionating the proteins that there had been any changes in the relative concentrations of any of the proteins. Dr. Eloise Jameson separated the pooled sera from 7 subjects into the various protein fractions. The concentration curves including the smallest molecule fractions were identical before and after the experiment. Individual protein fraction loss, therefore, as a mechanism for lowering osmotic pressure and thus concentrating the blood seemed to be of no importance.

The test diet contained only one gram of salt per day, but salt deprivation was not responsible for the blood concentration, since when 7.5 gm. of salt per day were added to the diet, the usual fall of blood volume was noted in 2 subjects. The average plasma volume before the experiment was 2,112 cc., and afterwards 1,931 cc. In 2 other subjects who were given diets of 2,400 calories, but salt free, the fall in volume did not occur. The average before was 2,413 cc. and afterwards 2,405 cc.

It was presumed that the low food, and particularly the low fluid, intake might be important. In 2 subjects, therefore, fluids were forced to 3,000 cc. and the changes in blood volume found to be diminished. The average volume before the test was 2,205 cc. and after it 2,155 cc. Dehydration is therefore an important factor in the blood concentration.

That the blood should become concentrated during a time when the diet approaches starvation, unless large amounts of fluids are taken, is credible. Why the total amount of protein should decrease and yet retain its previous character absolutely, however, is less apparent, especially since it is well known that patients with edema due to low plasma protein have a reduction in albumin out of proportion to that of globulin. It must be presumed that this change of total plasma protein indicates a diminution of all tissue proteins to a level commensurate with the true decrease in body weight. This is analogous to the natural changes of growth, or those of a wasting disease in which body weight, and therefore its corollary, blood volume, changes and the total protein changes correspondingly. The 2 days of protein deficiency apparently represent these slow and more or less natural changes in an accelerated form and are insufficient to cause alteration in the relationships of the blood protein fractions, which longer starvation would probably expose. It would seem that the loss of body weight is probably due in part to the tissue destruction which reduces total serum protein, but principally to dehydration, since a high fluid intake minimizes it.

Conclusions. 1. Due principally to low fluid intake, the blood is concentrated and the serum protein concentration slightly less than proportionately increased by a low calorie diet containing no protein.

2. Two days of protein deprivation is an insufficient time to make any change in the character of the blood proteins, but lowers the total blood protein definitely.

7965 C

Stimulation of Adrenal Medulla by Irradiated Insulin.*

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Davis, Luck, and Miller[1] showed that insulin on long exposure to soft X-rays of high intensity loses its characteristic ability to lower the blood-sugar content and, in massive doses, may even produce a slight degree of hyperglycemia. A substantial portion of its phosphate-lowering activity is retained, as well as part of its amino-acid-lowering activity. It was suggested, by way of explanation, that the irradiated insulin, though otherwise inactivated, retains its power to stimulate the adrenal medulla, thus causing the slight hyperglycemia, hypophosphatemia, and hypoaminoacidemia actually observed.

To test this hypothesis we have administered insulin, 7 to 10 units per kilo, irradiated with soft X-rays for 3 hours at an intensity of 3,400 Roentgen units per second, to adreno-demedullated rabbits. Six animals were employed, and in no case was a lowering in blood

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[1] Davis, B. L., Jr., Luck, J. M., and Miller, A. G., *Biochem. J.*, 1933, **27**, 1643.