THE EFFECT OF FASTING ON THE SERUM PROTEIN CONCENTRATION OF THE RAT*

With Special Reference to the Question of the Existence of an Immediately Utilizable Circulating Protein Fraction

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Though apparently made untenable by more modern theories concerning the formation and intermediary metabolism of proteins by way of the amino acids, their so called building stones, the hypothesis of a "circulating protein" which could be absorbed, carried to the tissues and there used directly, is one which has proved attractive to a number of investigators ever since it was first enunciated by Voit (1). This pioneer investigator drew his conclusions from the results of experiments published in 1866 (2) in which he showed that the amount of nitrogen excreted by a starving dog during the first few days of fasting was directly dependent on the level of previous protein ingestion. Voit cited experiments by Landois (3), who found that when blood was injected, the serum proteins were readily burned and the nitrogen excreted in the urine, while the presumably "organized" protein of the red blood cells was much more slowly destroyed. Forster (4) found that if serum alone was injected, its protein was rapidly destroyed.

All of this work was done before the masterly researches of Kossel, Hofmeister and Emil Fischer had elucidated the essential nature of proteins as complexes of amino acids, in the light of which the interpretation of Voit is by no means necessary to explain his results. Meanwhile further study has shown the allegedly "unorganized" protein of blood plasma to be a complex of separate substances of a fairly regular, though somewhat variable pattern and with definite functions of its own entirely unconnected with protein transport. One of the most important of these functions was enunciated by Starling (5), who in the latter part of the 19th century published evidence that tissue fluid regulation was due to a balance between the colloidal osmotic pressure of the plasma proteins and the hydrostatic pressure of the blood flow in the capillaries. Since Starling's paper, the attention of investigators of the blood proteins has been centered largely on various aspects of this problem, with valuable additions to existing knowledge of edema in nephritis, malnutrition and various other diseases. Too many excellent reviews of this

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aspect of the problem (6) are available to make it necessary to discuss it further here.

In a recent paper Whipple and his coworkers (7) found that dogs receiving only sugar by mouth could be maintained practically in nitrogen equilibrium by means of intravenous injection of normal dog plasma. Plasma protein fed by mouth showed the same general reaction but the urinary nitrogen excretion was slightly higher. These writers interpret this as meaning that the injected protein is utilized a little more completely for forming new body protein, and suggest that the difference may be due to deaminization of the protein given by mouth. They feel that the injected protein must be utilized by the body, and that if this can happen in this emergency it may be suspected that normally there is a certain amount of give and take between body protein and plasma protein.

One need not question the authors' general conclusion that the body can use material coming from one body protein to fabricate badly needed protein material of different character, to point out, first, that the work presented throws no light on the method by which the transfer took place, and second, the fact that the utilization of a surplus quantity of *foreign* protein is not evidence that the organism's own plasma protein, present in only normal amounts, will be similarly used.

Bloomfield (8) recently reported studies on the effect of long continued low protein diet on the plasma protein level. Working with the white rat, he found that on a diet adequate in calories, minerals and vitamins, but containing only minimal amounts of protein, there was a small but definite drop in the serum protein level, occurring within the first week, with no further drop even when the low protein diet was continued for 21 weeks. Similar results were obtained when the animals were fasted for 3 weeks except for water.

In summary, it appears clear that animals receiving a diet inadequate in protein show a small initial drop in the level of the blood proteins. It also is evident that proteins injected into the blood stream are available in the metabolism of the recipient, and that such injected protein is perhaps more efficient even than the same protein fed by mouth. No data are available, however, to explain the mechanism of either the maintenance of the blood proteins following the small initial drop or the utilization of ingested protein.

Bloomfield's work was done on pooled sera, and therefore the factor of individual variation was not studied. Also, his data for the first few days of the experiments, which is apparently the critical time, were not as complete as appeared desirable. It was, therefore, determined to extend the tests in order to analyze more accurately what happens to the plasma proteins during the period immediately following the elimination of protein from the diet. It was felt that

such an experiment would provide conditions more closely approaching normal without such complicating factors as plethora, extraordinarily high plasma protein concentrations and the possible abnormal effects of a strange protein in the circulation.

EXPERIMENTAL

Healthy female rats of as nearly uniform size as possible were selected and divided into groups of five or ten. All had been on the laboratory stock diet (9) for their entire lives and were in an excellent state of nutrition. The animals were exsanguinated from the abdominal vessels as detailed by Bloomfield (8). The following observations were made: weight of animal; blood volume (as determined by exsanguination); total serum protein concentration. It was at first hoped to determine not only total proteins but to partition the albumin and globulin in individual rats. However, this proved impractical because of the small size of the blood samples obtained, particularly in the second series of experiments with young animals. Therefore separate groups of rats were killed and their sera pooled for the determination of the albumin-globulin ratios.

Two series of experiments were made. In both series the animals received nothing by mouth except water, which was freely available. In the first, large mature rats which had been discarded from the breeding stock of the laboratory were used. These animals averaged well over 200 gm. in weight at the beginning of the experiment. In this series a group of five animals on stock diet served as controls. Other groups of five or ten animals were killed after fasts of 1, 2, 3, 4, 5, 7, 9, 12 and 15 days. In the second series, young virgin females approximately 80 days old and weighing from 110 to 140 gm. were used. Groups of five were killed after fasts of 2, 3, 5, 7 and 9 days. The experiment had to be terminated at this time because of the death of the remaining animals. Whereas the old rats remained in excellent condition except for emaciation after a 15 day fast, the young animals looked decidedly ill after fasting 5 days and began to die on the 7th day.

Fasting, rather than low protein diet, was chosen as the condition of the experiment in order to make the protein deficiency as acute as possible and to obviate the protein-sparing action of carbohydrate.

Serum proteins were determined gravimetrically by the method of Barnett, Jones and Cohn (10). Separate determinations were made on the serum of each rat. Serum was chosen rather than plasma to avoid the variable withdrawal of water from the erythrocytes by oxalate or citrate, in accordance with the suggestion of Bloomfield. In determining the albumin-globulin ratios, globulin was precipitated by half saturation with ammonium sulfate in the usual manner, followed by gravimetric determination of the albumin in the filtrate by the same method as used for total proteins.

RESULTS

Details of the experimental results are presented in the accompanying charts and tables. Figs. 1 and 2 show the individual total serum protein concentrations. Fig. 3 portrays the average total protein concentration in both series. Table I presents a summary of the data obtained in the series of young rats.

Study of these data confirms Bloomfield's finding of a small initial drop in the concentration of serum protein. This drop in the old

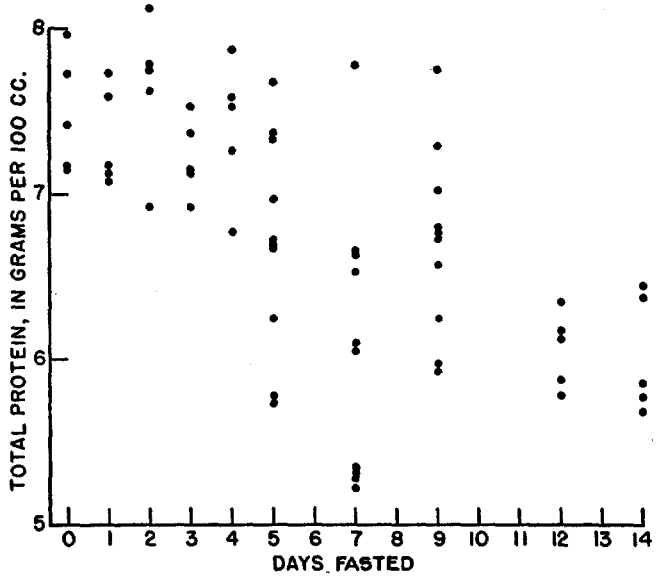

FIG. 1. Serum protein concentration in individual large mature female rats receiving only water. Each dot represents one animal.

rats is not obvious until the 5th day of fasting, but then it is very definite (see Fig. 3). It is also clear that no subsequent drop occurs up to 15 days of fasting. If the protein concentrations for the first 4 days, and then those of the succeeding periods, are averaged in order to smooth out minor variations, a drop of from an average of 7.65 to 6.60 is found. That is, there is a fall of about 14 per cent in the total protein. Similar results are obtained with the series of young animals except that here the initial level of the proteins was considerably lower, the drop is of less magnitude—from an average of

6.39 to 6.01, or about 7 per cent—and the fall is perceptible on the 3rd day.

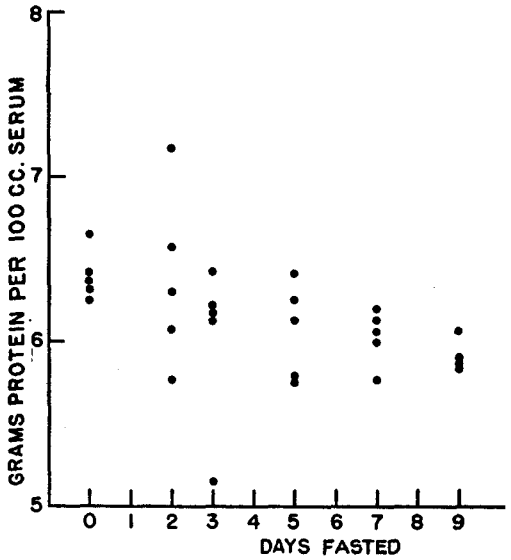

FIG. 2. Same as Fig. 1, but showing the values obtained with young virgin female rats.

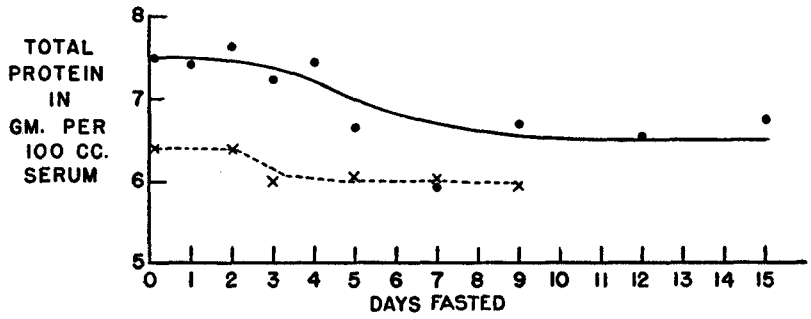

FIG. 3. Average values for the serum protein concentration. Each value on the chart represents the average of the corresponding individual values as shown in Figs. 1 and 2. Dots and solid line represent the values in the mature animals; crosses and broken line those of the young rats.

A glance at Figs. 1 and 2, however, makes clear at once that there are marked individual variations from the trends shown in the aver-

ages. In the old rats, for example, individuals maintain levels above 7.5 gm. of total protein per 100 cc. of serum for as long as 9 days of

TABLE I

Summary of Averaged Values Obtained in Observations on the Series of Young Rats

Experimental group	Original weight	Weight when killed	Per cent of original weight when killed	Blood volume	Total protein
	gm.	*gm.*		*cc.*	*gm. per 100 cc.*
Controls	128.8	128.8	100.0	4.6	6.40
Fasted 2 days	126.8	114.2	90.0	3.9	6.38
" 3 "	116.8	102.8	88.0	3.9	6.02
" 5 "	120.4	95.4	79.2	—	6.07
" 7 "	124.4	93.6	75.2	3.1	6.01
" 9 "	118.4	80.0	67.6	2.4	5.94

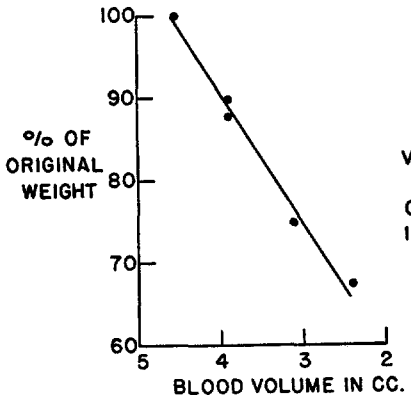

FIG. 4 *a*

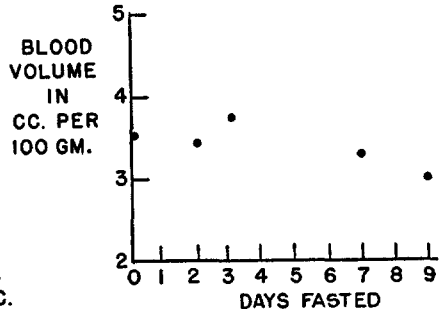

FIG. 4 *b*

FIG. 4 *a*. The relation of blood volume to weight loss. The values plotted are averages for all the animals in each group, as shown in Table I. Blood volume is to be understood as that quantity of blood obtained by exsanguinating the animal as completely as possible from the great vessels of the abdomen, with no attempt to wash out final traces of blood.

FIG. 4 *b*. The plotted values are averages of the individual groups, as in Fig. 4 *a*.

fasting. This individual resistance seems of theoretical importance and will be referred to again.

Figs. 4 *a* and 4 *b* show the relationship between body weight and blood volume as measured by exsanguination. As is well known, the method of determining the total volume of blood by simply exsanguinating the animal is not entirely accurate. The relationships shown in the charts, however, appear so clear that it was felt that the results at least approximated the true state of affairs, and the data are therefore presented. Although the blood volume is directly related to the weight of the body, the serum protein concentration shows no correlation whatever with size or weight. The figures from several of the experiments, presented in detail in Table II, show this.

TABLE II

Details of Some of the Experiments on Individual Rats of the Mature Series, to Show Lack of Correlation between Body Weight and Serum Protein Concentration

Weight of rat	Total serum protein	Weight of rat	Total serum protein
gm.	*gm. per 100 cc.*	*gm.*	*gm. per 100 cc.*
Controls		Fasted 3 days	
244	7.74	246	7.36
236	7.26	220	7.54
224	7.42	218	7.15
218	7.96	204	6.93
192	7.25	202	7.13
Fasted 5 days		Fasted 9 days	
258	6.70	202	5.96
242	7.39	200	7.75
220	6.67	194	7.32
206	6.97	194	6.56
196	7.38	188	5.95
158	5.78	184	6.69
152	7.68	182	6.81
150	6.66	162	7.02
146	6.24	158	6.74
140	5.77	134	6.26

It is fairly well established that the albumin fraction of the serum proteins is the more labile, and its concentration is altered more readily in various disease states than that of the globulin fraction, though this is by no means an invariable rule. However, from the results shown in Table III it seems clear that the fall of concentra-

tion of plasma protein in the fasting rat is due to a decrease in the albumin fraction with the globulin changed little or not at all.

DISCUSSION

What light do these results throw on the hypothesis of a circulating fraction of protein utilizable by the tissues and used by the cells of the body in preference to the more resistant organized tissue protein, or in the words of the more modern champions of the same hypothesis, on the question of give and take between body protein and plasma protein?

At first glance it might seem that these experiments offer evidence in favor of such a hypothesis. The definite decreases shown in Fig.

TABLE III

Albumin-Globulin Ratios on Pooled Sera from Fasting Rats

Experimental group	No. of rats in group	Total protein	Albumin	A/G ratio
		gm. per 100 cc.	*gm. per 100 cc.*	
Controls	4	6.90	3.15	0.84
Fasted 6 days	4	6.95	2.00	0.40
" 10 "	3	6.12	2.50	0.69
" 14 "	4	6.14	2.25	0.58

3 might be interpreted as due to the sudden giving up of some special fraction of labile protein to the tissues following exhaustion of their own reserves. A study of the results in individual cases, however, shows that this explanation is not the probable one. Although in the old rats the average drop occurs at about the 5th day, some individuals maintain a normal concentration of plasma protein for as long as 9 days. It is difficult to conceive of these individuals as having sufficient nitrogenous reserves somewhere in their tissues to last 9 days before it becomes necessary to call on an easily available protein constantly at hand in the blood stream.

The relationship of the blood volume to weight in the fasting animals also appears to be pertinent. It is clear that as body weight decreases the blood also decreases. This can only mean that a

certain portion of all of the blood proteins is destroyed to provide fuel and nitrogen for essential vital tissues. The concentration of proteins in the diminished volume of serum is not increased, but on the contrary shows the early decreases noted and then maintains itself stubbornly for long periods.

It would seem more logical to suppose that the early drop in the more labile albumin fraction of the blood plasma represents the same destruction that results in decrease in size of any tissue during fasting, and that the later maintenance of the concentration of the plasma proteins is a part of this same conservation of tissue whose function is essential to life. Physiologists have long been familiar with this conservation of tissue in the case of such organs as heart and brain, which maintain their integrity at the expense of muscle and other less vital tissue. From the point of view of normal function, the plasma protein is assuredly an essential tissue.

No evidence is at hand regarding the process by which the concentration of protein in the plasma is maintained following the initial drop. It appears from the present experiments that age is one factor. Mature rats maintain the plasma proteins somewhat more tenaciously than young, active growing animals. Another very definite factor is that complex of unknown characteristics which is summarized as individual resistance. Beyond this one cannot at present go.

SUMMARY AND CONCLUSIONS

1. Experiments were carried out to test the hypothesis that there exists a special circulating fraction of plasma protein available for use by the tissues.

2. The changes in serum protein concentration after varying periods of fasting were followed in large numbers of individual rats.

3. Previous reports from this laboratory of a small initial drop in the total protein concentration of the serum, with subsequent maintenance of the serum protein at the new level are confirmed.

4. Evidence is offered that this initial fall involves chiefly or solely the albumin fraction.

5. The mechanism responsible for the observed initial drop and subsequent maintenance of the protein is not exactly known, but two definite factors are age and individual resistance.

6. It is concluded that no satisfactory evidence is available to support the hypothesis of a directly utilizable protein fraction in the blood.

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