THE EFFECT OF RESTRICTION OF PROTEIN INTAKE ON THE SERUM PROTEIN CONCENTRATION OF THE RAT*

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Diminution of the plasma proteins which has been found to occur so frequently in certain types of Bright's disease and in various states of undernutrition is attributed by most students of the subject either to loss of protein in the urine or to lack of protein in the diet. In some cases it is believed that both loss and lack may play a part. The current views are clearly stated by Peters and Van Slyke (1) in their recent monograph, by McCann (2), and by Peters (3) in a long series of papers one of which deals especially with the status of the blood proteins in nephritis. The studies which have led up to present concepts of hypoproteinemia have been exposed so often in the recent literature that detailed review now would serve no useful purpose. We wish, however, to analyze critically the evidence for the loss and lack theory and to point out, as some writers have already suggested, that additional factors may play a part.

The arguments usually advanced in support of the loss and lack theory are the following.

1. There is an undoubted association between hypoproteinemia and the types of Bright's disease in which large quantities of protein are lost in the urine. This observation, confirmed every day in the clinic, certainly offers an apparent explanation of lowered blood proteins, and as Peters (3) points out "a patient of average size losing as much as 16 grams of protein a day . . . . would excrete all the albumin of his serum in the course of a week or two."

2. The finding of hypoproteinemia without proteinuria in various states of malnutrition together with clinical improvement and rise of blood proteins after the patient has been placed on an adequate diet, high in protein, has been interpreted as indicating that lack of protein intake is the cause of the low blood proteins.

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The subject is reviewed by Weech and Ling (4) who take up the story with the so called war edemas which occurred so frequently in the Central Empires when large groups of people were forced to subsist on inadequate diets low in protein and high in certain types of vegetables. They proceed to discuss cases of nutritional edema (with hypoproteinemia) in the Chinese from the famine districts and they point out that the patients had been subsisting on a diet composed chiefly of wheat, rice, corn, and millet, inadequate in protein as well as in vitamins and salts. Among the many recent papers emphasizing the same point of view may be mentioned those of Peters (5) and his associates. Peters (3) also feels that malnutrition as well as protein loss is an important factor in lowering the blood proteins in Bright's disease.

It should be made clear that those who adhere strictly to the protein lack theory of malnutritional hypoproteinemia attribute the disturbance of the blood proteins specifically to a shortage of protein building stones and not to an interference with the protein-building mechanism. Peters, for example, in commenting on rises of blood protein in malnourished patients after they were placed on a high protein diet says "the gradual rise of the serum proteins which accompanied the protein storage in these cases suggests that the low serum proteins resulted from protein deficiency alone" (5).

3. Experimental Hypoproteinemia in Animals on Low Protein Diets.—Kohman (6) several years ago found that rats placed on a low protein diet with large amounts of carrots became edematous after a number of weeks. While no blood protein determinations were made it seems certain that the edema was associated with hypoproteinemia. In analyzing her experiments Kohman felt that vitamin deficiency, salt effects, and possible toxic substances in carrots were all eliminated as causal factors and that the edema was due purely to lack of protein in the diet. Frisch, Mendel, and Peters (7) conducted similar experiments but on a less extensive scale. They confirmed Kohman's observations and made determinations of blood proteins which were found after a period of weeks to be very low. Shelburne and Egloff (8) report observations on a dog in which the blood proteins fell during a period of 24 days of low protein feeding from 6.8 gm. per cent to 4.7 gm. per cent.

4. Experimental Hypoproteinemia Produced by Withdrawal of Blood Proteins (Plasmapheresis).—Whipple and his associates (9) in connection with studies on the regeneration of blood proteins produced marked hypoproteinemias in dogs by removing large amounts of plasma (several hundred cubic centimeters). Leiter (10) and Barker and Kirk (11) in efforts to produce experimental edema were able to lower the blood proteins of dogs by similar drastic bleedings of 400 to 500 cc. twice daily. Fishberg and Fishberg (12) reduced the plasma proteins in rabbits to about 50 per cent of the normal value by daily bleedings of about 35 cc.

Certainly the array of evidence presented above can leave no doubt in any mind that loss of protein and lack of protein play an important

part in the production of hypoproteinemia. The question may be raised, however, whether such a simple concept fits all the facts of clinical and experimental experience and whether one must not postulate in addition some injury to, defect of, or inadequacy of the blood protein-forming mechanism as an accessory if not a primary difficulty; in point of fact, the evidence for the loss and lack theory when inspected from this angle reveals a number of serious breaches which will next be analyzed.

While lowering of blood proteins, as we pointed out above, has resulted from experimental withdrawals of plasma, the procedure has always been an extreme one. In Leiter's experiments a large portion of the dogs' plasma was abstracted day after day, and Fishberg's rabbits suffered similar losses. Despite these drastic procedures which could hardly be paralleled in man under any natural conditions, the animals exhibited an amazing ability rapidly to regenerate their blood proteins. Whipple's dogs, for example, even when fasted after the bleeding, had built up their proteins to a normal level in 14 days, and Fishberg's Rabbit 4, although deprived of approximately half its blood each day for 18 days, maintained its plasma proteins during the last 10 days of the experiment at a nearly constant level of about 4 gm. per cent. It may be estimated from the protocol that during the 18 day period this rabbit regenerated the total amount of its blood proteins six times. Barnett, Jones, and Cohn (13), in this clinic have also shown that dogs may maintain a normal blood protein level in the face of daily large bleedings, although the procedure in this case was less formidable than in the plasmapheresis experiments of Whipple and of Leiter. It seems clear, then, that protein loss in order to be effective in lowering blood proteins must be extreme unless there is in addition some impediment to the remarkable regenerative mechanism which normally comes promptly into play.

Concerning the experimental results of low protein feeding (including fasting) in animals, the observations of Kohman and of Frisch, Mendel, and Peters have already been mentioned. They ascribed their findings purely to a lack of protein in the diet, but inasmuch as they dealt with young (60 gm.) rats which as a result of the treatment suffered a profound interference with their growth, another interpretation, namely that the protein-building mechanism was disturbed as a result of inanition, would have to be considered. This question is raised because other experiments reported in the literature seem to be at variance with the above observations. There are, for example, a good many papers dealing with the effects of fasting on the blood proteins. Hanson (14), working with rabbits, followed the plasma proteins during alternate periods of feeding and of fasting. The animals went without food for as long as 6 days. In no case was there any drop in the blood proteins. Hanson also reviews the older literature and refers to similar results in fasted dogs. Burkhardt (15) years ago found the total proteins only

slightly depressed in dogs which went without food for 4 days; the globulins were found to be increased at the expense of the albumins. Then there are some irreconcilable contradictions. Shelburne and Egloff's dog (8), for example, suffered a marked lowering of blood proteins within 3 weeks when placed on a low protein diet, whereas Whipple's dogs (9), in spite of an initial large bleeding followed by total fasting, regenerated their plasma proteins to normal within 14 days. The whole subject evidently requires more experiment before final conclusions can be drawn.

We may next return to a consideration of the relation of proteinuria to the low blood proteins of Bright's disease. If this loss of protein in the urine were the major factor one would expect the blood proteins to bear some relation to the degree of such loss and one would also expect the blood proteins to return to a higher level if large quantities of protein were fed. With regard to the first point, Fig. 3 from the paper by Peters, Bruckman, Eisenman, Hald, and Wakeman (3) is of interest. The urine protein in grams per day is plotted against the serum protein per cent, and it appears that there is no exact relation. Some patients with very heavy proteinurias (over 10 gm. per day) had plasma proteins only moderately low (4.5 gm. per cent or over) whereas a good many subjects with urine proteins under 10 gm. had serum proteins under 4 gm. per cent. The extremes were urine protein 1 gm. per day with serum proteins 3+ gm. per cent, and urine proteins 19 gm. per day with serum proteins 4.5 gm. per cent. Furthermore, patients with the most marked proteinurias do not continue to lower the blood proteins indefinitely but they seem to establish them at a more or less constant low level despite the continued loss.

Even more striking are certain observations, such as those of Peters and Bulger (16), on the effect of high protein feeding on the low blood proteins in nephritis. Regardless of the diet and regardless of whether the patient was storing or losing nitrogen the blood proteins in a number of cases remained at a practically constant level. Their Case 2 is a good illustration. During the first period of 17 days the patient had a positive balance of 0.7 gm. protein per day but lost 10.8 gm. of protein per day in the urine. Despite this loss the plasma proteins at the start were 4.03 gm. per cent and on the 12th day 4.20 gm. per cent. During the final 26 day period there was a positive daily balance of 22.7 gm. of protein, whereas 9.3 gm. were lost each day in the urine. The plasma proteins at the beginning of the period were 4.20, at the end 4.34. The patient's weight is not stated but if one estimates his total plasma as 3000 cc. then he had a total of 126 gm. of plasma protein at the start and 130 gm. at the end of the period. Although he had stored 348 gm. of protein only 4 gm. were diverted to the formation of plasma proteins. These considerations make it clear that, in this case at least, the low blood proteins bore no relation to the nitrogen metabolism of the body as a whole but clearly were dependent on some special failure of the blood protein-forming mechanism which may have been exaggerated by the loss of protein in the urine. This specific instance is in accord with general clinical experience and while various writers

stress the benefit of high protein feeding in malnourished nephritics (Peters *et al.*, McCann, etc.) and while such feeding doubtless often improves their general condition it must be admitted that there is no adequate documentary evidence to show that such high feeding of protein specifically raises the blood proteins. Such slight changes as have been described may be found in subjects on a constant and even inadequate diet. Similar considerations apply to instances of hyproproteinemia in malnourished subjects without proteinuria. First of all, only a small percentage of malnourished patients show any marked lowering of the blood proteins and when they do there are usually other obvious factors aside from lack of protein in the diet, such as infection and the generally poor hygienic conditions of famine. Furthermore, the blood proteins may, on occasion, be unaccountably low in people who are not malnourished and who have not suffered from deficiency of protein in the diet. Youmans and Bell (17), for example, report curious instances of seasonal edema with low plasma proteins in subjects who were well nourished and who had no cardiac or renal disease. And, finally, the rise of blood proteins which gradually follows the exhibition of adequate diets in malnourished patients can, perhaps, be as well explained by a restoration of a debilitated blood protein-forming mechanism as a result of elimination of infection and general malnutrition as by a specific effect of protein. Bruckman, D'Esopo, and Peters (5), for example, report the effects of high protein feeding in a malnourished diabetic (their Table 1). Between Sept. 16 and Oct. 22 the serum proteins rose from 5.18 to 5.93, an increase in the total serum proteins of only about 16 gm., although during the period of observation there was a gain in weight of nearly 6 kilos. Clearly simple protein lack was not the cause of the low proteins since we know that a man can readily regenerate 16 gm. of plasma protein in 1 day whereas in this case even though nitrogen was being stored in large amounts it took 36 days to regenerate this amount. The only possible interpretation is that as the general nutrition improved and as she got rid of a severe infection of the hand which was present at the start the protein-forming mechanism gradually recuperated. Even when her condition was at its worst 16 gm. of protein could certainly have been diverted from other body stores to be reformed into blood proteins had the blood protein-regenerating mechanism been functioning properly.

The foregoing analysis forces one to the conclusion that the lack and loss theory is inadequate to explain the observed facts in all instances of hypoproteinemia. In normal animals there exists a tremendous capacity rapidly to regenerate blood proteins from the body stores of protein even during fasting, a capacity which obviously would hardly be taxed in many clinical instances of hypoproteinemia were there not some interference with its normal functioning. The evidence suggests that part at least of the difficulty which leads to lowering of the blood proteins is an impairment of the blood protein-

regenerating mechanism; lack and loss undoubtedly contribute an added burden which, on occasion may be insuperable, but lack and loss clearly fail to explain the whole problem.

It would serve no purpose to hypothecate an indefinite defect of the protein-forming mechanism without proposing some explanation for such a defect. In the clinical cases, apart from protein lack in the diet, deficiencies of the diet in other respects, infection, intoxication from wasting disorders such as cancer, and the hardships and hazards of famine conditions have been outstanding, and they have been stressed by various writers. Any or all of these factors might be imagined to lead (apart from lack of protein *per se*) to an interference with the blood protein-forming mechanism, which perhaps readjusts itself when infection is eliminated and the general nutrition is improved. One must also consider the possibility of some more specific agent which may have a positive inhibiting effect on the blood protein-forming mechanism. In many of the famine cases as well as in experiments the diet has not only been low in protein but there has been an excess of certain vegetables such as turnips (war edema, Shelburne and Egloff's dog), and carrots (Kohman's and Frisch's rats). May these vegetables and perhaps other articles of food contain some agent which is antagonistic to the formation of blood proteins?

Finally, little is known as to the normal rate of blood protein destruction (18). While it is probably very slow as judged by endogenous protein metabolism in general, an abnormal acceleration must be considered as a possible factor in clinical hypoproteinemia.

EXPERIMENTAL

The purpose of the present experiments was to test, definitively if possible, the effect of low protein diet on the serum protein level of rats. The procedure differed from that employed by Kohman (6) and by Frisch, Mendel, and Peters (7) in three principal respects: many more animals were used, they were mature, and carrots, which may be a disturbing factor, were eliminated from the diet.

Material and Methods

Young but mature female white rats were used. They weighed, for the most part, 140 to 190 gm. The stock was that which has been inbred in this laboratory (19) for many years; the animals are vigorous and very satisfactory for observa-

tions of this sort. The stock rats were all on an adequate control diet consisting of corn-starch, casein, lard, cod liver oil, salt mixture, yeast, and alfalfa (19). The methods of caging, feeding, and general care, devised by Addis and his associates and in regular use in this laboratory, were adhered to in every detail (20).

The Experimental Diet.—The experimental diet was adequate in total calories and in salts and vitamins but contained a minimum of protein. It consisted of corn-starch (cooked, dried, and powdered) 74 per cent, salt mixture (Osborne and Mendel) 4 per cent, cod liver oil 10 per cent, yeast (Harris, dried and powdered) 10 per cent, and alfalfa (dried and powdered) 2 per cent. The yeast and alfalfa contain respectively about 2.6 per cent and 0.3 per cent of protein (Addis (19)); hence the diet contained about 3 per cent of protein in contrast to the 17.3 per cent of the stock diet. It was estimated that each rat ate from 6.0 to 8.0 gm. of the food mixture daily with a protein intake of about 0.2 gm. Water was allowed *ad libitum*. That this diet was actually defective in protein from a physiological standpoint is shown by the rapid loss of weight and by the changes in the hair (see below).

Estimation of the Serum Proteins.—It was not considered feasible or desirable to bleed individual rats on successive occasions since the withdrawal of even 1.0 cc. represents a considerable proportion (one-fifth to one-tenth) of the total blood volume, and such a procedure might, in itself in the long run, result in depletion of blood proteins. The rats were therefore bled to death in groups of five or more at various intervals, and determinations were made on the pooled serum. The technique is as follows: Under ether anesthesia the abdomen is opened, the viscera are displaced, and the lip of a graduated centrifuge tube is pressed against the large abdominal vessels which are then severed with small sharp scissors. Blood flows directly into the tube until the animal is exsanguinated. The total volume obtained from each animal is recorded. The tubes are centrifuged and aliquot parts of serum are pooled for protein determinations. Serum rather than plasma was used because of the variable effect of citrate or of oxalate in pulling water out of the red cells (21).

Total proteins were estimated by the method recently described by Barnett, Jones, and Cohn (13). In brief, the proteins of 1 cc. of serum are precipitated by boiling for 20 minutes in 5 cc. of half-normal sodium acetate-acetic acid buffer with a pH of about 4.9. The contents of the tube are transferred to a weighed asbestos filter and washed by suction with distilled water, 95 per cent alcohol, ether, and finally with boiling absolute alcohol. The filter is then dried to constant weight (24 to 48 hours) at 105°C. and the weight of the protein is determined by difference. The reliability of this procedure is discussed in detail in Barnett, Jones, and Cohn's paper. All observations were made in duplicate and discarded unless there was close agreement.

RESULTS

Clinical Course.—The general condition of the animals was excellent throughout the 21 weeks of the experiment. They remained lively

and they ate well. From about the 3rd week on, the hair became discolored, brownish grey, and brittle and it fell out in patches of variable size. No dermatitis developed, however, and the bald areas soon developed an abundant growth of delicate snow-white hair. There were none of the usual evidences of vitamin deficiency; the eyes, feet, and skin remained normal. There was no rhinitis or diarrhea. Only one rat in the whole group of 144 died during the course of the experiment, on the 140th day. No animal developed any signs of edema.

Examination of the organs (macroscopic) at various periods showed a progressive and obvious decrease in size but no apparent pathological lesions. Even after 20 weeks there was still a good deal of mesenteric fat. The muscles and subcutaneous tissues looked dry rather than moist.

The output of protein in the urine was determined in a group of eight rats which had been on the diet for 19 weeks. The method devised by Shevky and Stafford (22) was used. The average protein excretion per 12 hours per 100 sq. cm. of body surface was 0.1827 mg., a figure within the normal range (23).

That lack of protein in fact rendered the diet inadequate for maintenance is clear from the weight curves (Figs. 1 and 2). Fig. 2 shows the average loss at various intervals in terms of percentage of the initial weight which is taken as 100 per cent. With minor variations there was a steady decrease which at 21 weeks amounted to 26+ per cent.

Changes in the Serum Proteins.—As the rats lost weight the total blood volume was diminished. Figs. 3 *a* and 3 *b* show a definite correlation between weight and the total blood obtained by the present method of exsanguination. The serum volumes were not measured accurately so that no figures can be given as to their relation to body weight. It is evident, however, that the total serum proteins were markedly decreased. But the point of importance is not so much the total quantity of protein as the concentration.

Changes in Concentration of the Serum Proteins.—Fig. 4 and Table I show the values for serum protein concentration at various intervals during the course of the experiment. Each estimation was made on the pooled serum of a group of rats. The events can be divided into

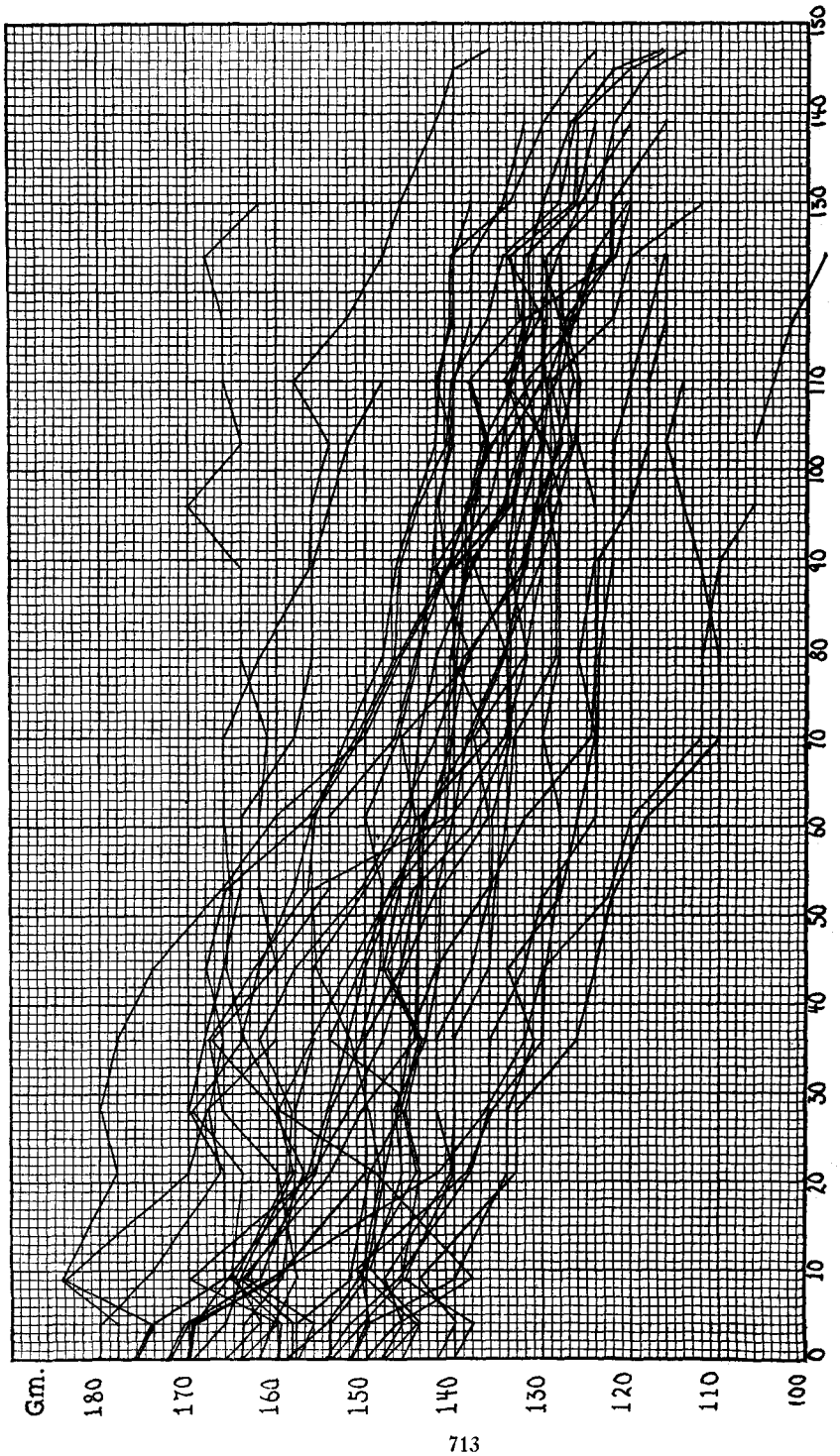

FIG. 1. Individual weight curves of a series of rats on low protein diet.

two stages the first of which one may speak of as the initial drop. We have found when rats are put under a variety of conditions of low protein intake that there is a prompt fall in the concentration of the serum proteins amounting to approximately 10 per cent of the initial value. This fall may be manifest in a day or two or may extend over several days or even a week. The drop is at the expense of the serum albumin; the globulin remains essentially unchanged. Return

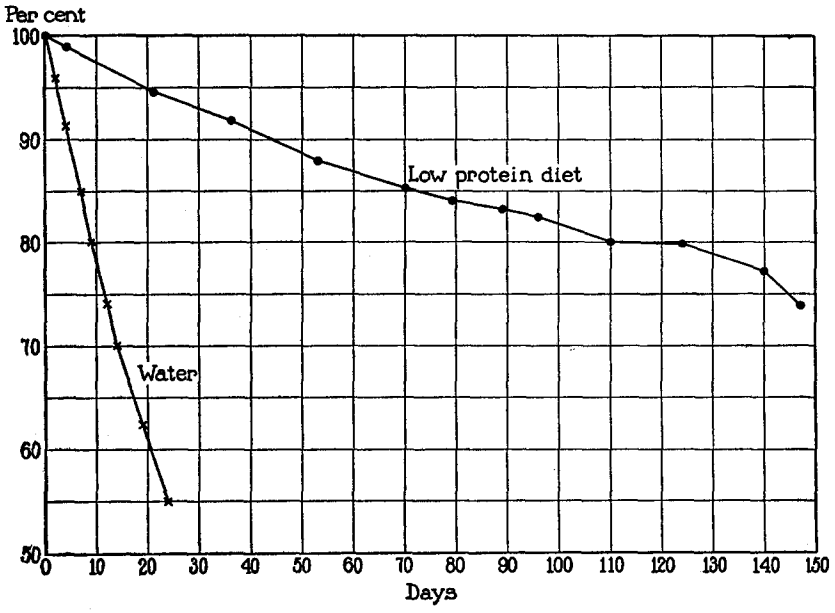

FIG. 2. Composite weight curves of rats on low protein diet and on fasting (water). Loss of weight at various times is expressed in percentage of initial weight (100 per cent).

to a diet containing adequate proteins is followed by an equally prompt restoration of the initial serum protein level. So rapid are these fluctuations and so small their extent, that we are inclined, tentatively at least, to regard them as of physiological and not of pathological significance. This view is reinforced by the stubborn resistance put up by the serum proteins to further depletion at the very time when readily available carbohydrate and fat are being exhausted, and body protein must be drawn upon as a source of energy, as in fasting (see

below). The final explanation is not at hand and the matter requires further study.

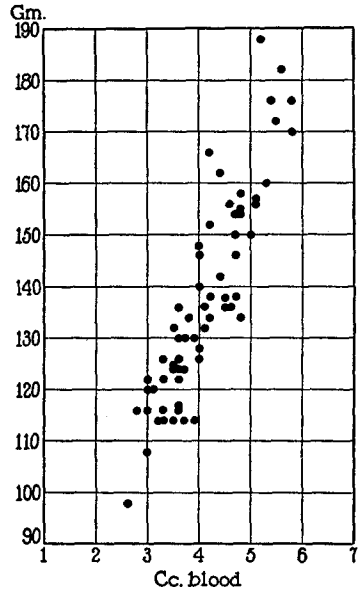

FIG. 3*a*

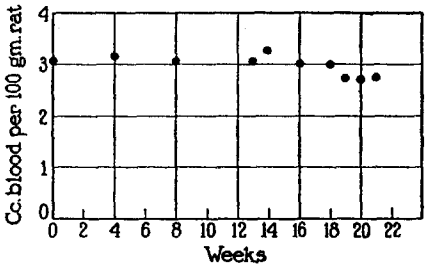

FIG. 3*b*

FIG. 3*a*. Relation of body weight to total blood volume as measured by exsanguination. Each dot refers to a different rat.

FIG. 3*b*. Cubic centimeters of whole blood per 100 gm. of rat. Averages of groups at various intervals.

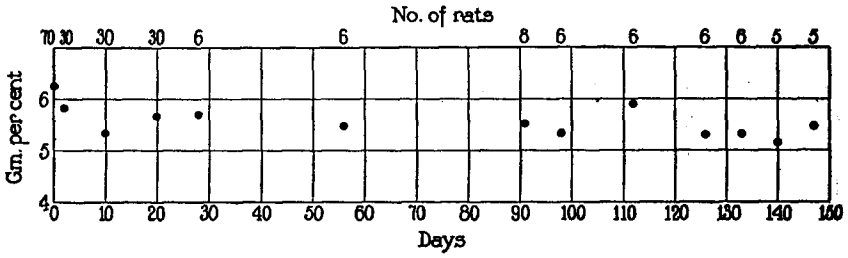

FIG. 4. Serum protein concentration of rats on low protein diet. Each dot gives the value obtained from pooled sera of a number of animals.

At any rate, in the present experiment this initial drop took place. It was evident by the 2nd day and obvious on the 10th day. From

this time on, however, over a period of 20 weeks—an interval which corresponds roughly to 10 years of a man's life (24)—there was no further significant drop in total serum protein. At 10 days a value of 5.35 gm. per cent was obtained, at 147 days a value of 5.47. Looking at it in another way, in order to smooth out minor variations, an average of all values from the 10th to the 70th days was 5.53 gm. per cent; from the 70th day to the end of the experiment 5.44 gm. per cent, a decrease of 1.6 per cent. Such a difference, if significant at all, is obviously negligible compared to decreases of serum proteins to 40 to 60 per cent of normal such as frequently have been found in

TABLE I

Concentration of Serum Proteins of Rats on Low Protein Diet

Length of time on low protein diet	No. of rats (pooled serum)	Serum protein
days		*gm. per cent*
Controls	70	6.25
2	30	5.82
10	30	5.35
20	30	5.68
28	6	5.70
56	6	5.48
91	8	5.52
98	6	5.33
112	6	5.93
126	6	5.30
133	6	5.31
140	5	5.14
147	5	5.47

human nutritional hypoproteinemias. Furthermore, partition of the serum from the last lot of rats, killed on the 147th day, into albumin and globulin showed again that the latter was within normal range and that the albumin was decreased just as was found in the case of the initial drop.

A group of five rats which had been on the low protein diet for 140 days were then placed on the control diet (casein 16 per cent). There was a rapid gain in weight (see Fig. 5) and after 6 days, when the animals were killed, the total serum protein had risen to 6.19 gm. per cent. This rise was found to be due to a restoration of the albumin fraction.

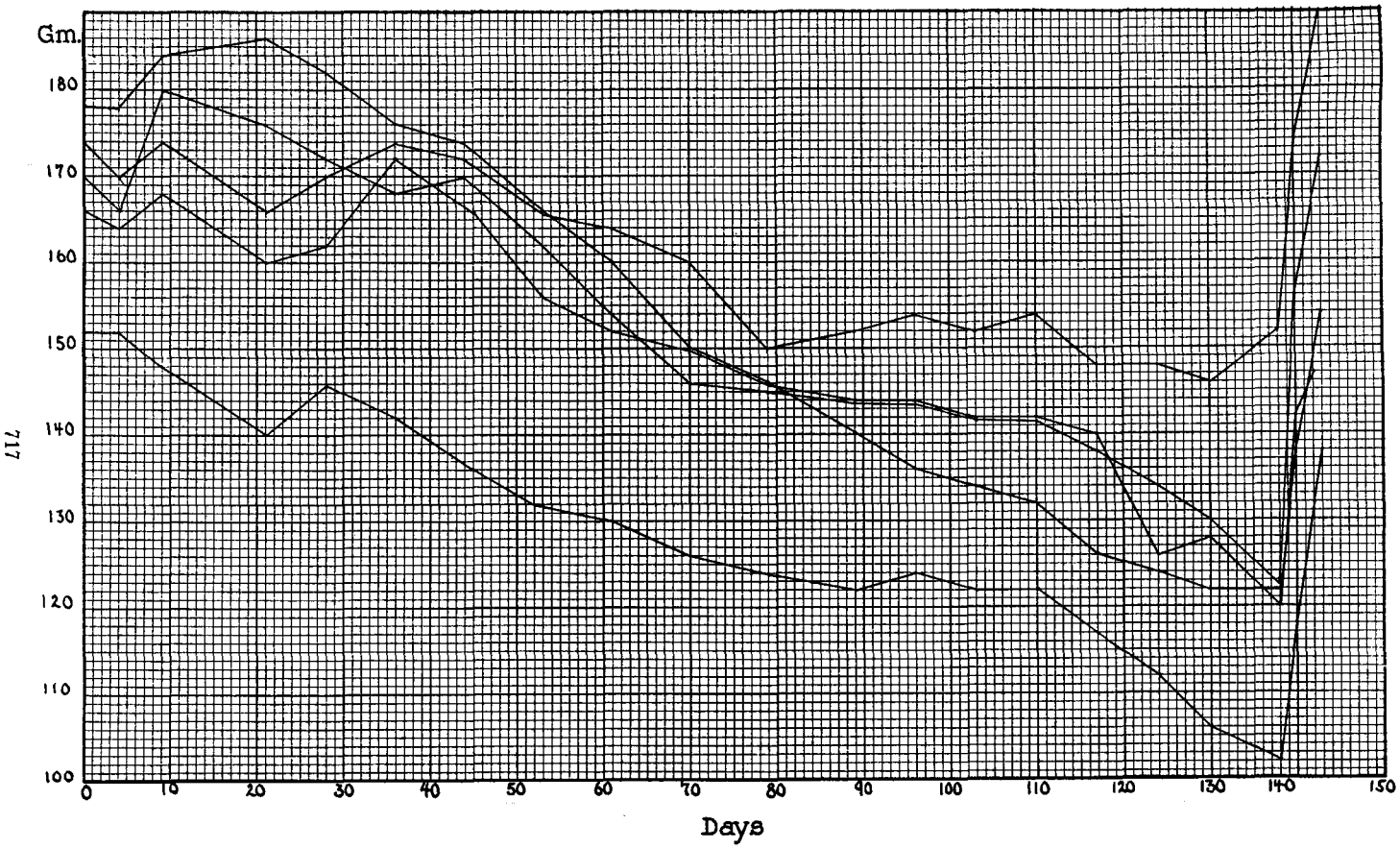

FIG. 5. Weight curves of five rats on low protein diet for 140 days and then on stock diet (casein 16 per cent) for 6 days.

Finally, in order to reduce the problem of low protein intake to the simplest terms, all food was withheld from another group of rats. Water was allowed *ad libitum*. Aside from rapid loss of weight (see Fig. 2) the animals remained in excellent condition for periods of time up to 3 weeks depending upon the extent of the fat stores at the beginning of the fasting period. There were no signs of vitamin deficiency and the most striking finding at autopsy was a decrease in the mesenteric and retroperitoneal fat. Determinations of plasma or of serum proteins were made at various intervals (Fig. 6). Just as with the low protein diet there was an initial drop after which no significant decrease occurred despite the rapid loss of weight. The results are therefore in

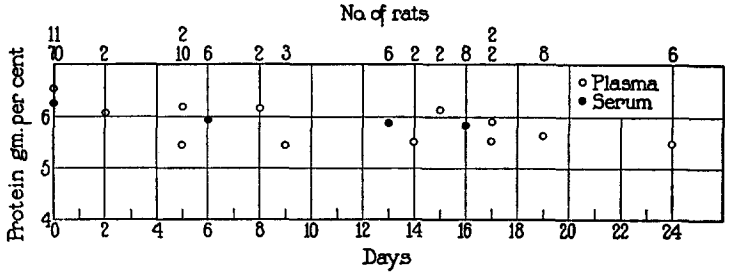

FIG. 6. Concentration of plasma or serum proteins from rats on water alone. Each dot indicates the value obtained from pooled serum or plasma from a number of animals.

harmony with those obtained in the long time low protein diet observations.

DISCUSSION

It has been shown, in brief, that aside from the initial drop, which is probably a physiological phenomenon, a low protein diet fed over a period of 21 weeks led to no significant decrease in concentration of total serum proteins despite marked loss of body weight. The blood volume and the total serum proteins were decreased, roughly, in proportion to weight loss. To what extent the serum proteins are broken down and to what extent body protein is diverted to their restoration so that their concentration can be maintained is not revealed by the present experiments. The exact nature of the mechanism requires special analysis.

Our results do not agree with those of Kohman (6) and of Frisch, Mendel, and Peters (7), who, as we pointed out, obtained a marked lowering of plasma protein concentration with low protein diets. The explanation of this discrepancy lies, perhaps, in the fact that carrots were one of the principal features of the diet used by these investigators; we have found that a diet of carrots alone leads to a definite fall in serum protein concentration (25). In Frisch's experiments, furthermore, the repeated withdrawal of blood for the tests may have played a part in lowering the plasma proteins.

The present observations have, of course, no direct bearing on the nutritional hypoproteinemia of man. They suggest, however, the need of a reassessment of the various possible etiological factors with emphasis on influences other than protein deficiency *per se*. The effects of inadequate caloric intake, vitamin deficiency, excess of certain foods such as carrots and turnips, and infection are especially to be considered.

CONCLUSIONS

Rats placed on a low protein diet for 21 weeks, in spite of marked loss of body weight, showed no significant decrease of serum protein concentration aside from an initial (physiological ?) drop.

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