NUTRITIONAL EDEMA IN THE DOG

I. Development of Hypoproteinemia on a Diet Deficient in Protein

By A. A. WEECH, M.D., E. GOETTSCH, M.D., and E. B. REEVES

(From the Department of Diseases of Children of Columbia University College of Physicians and Surgeons, New York)

(Received for publication, December 15, 1934)

Two years ago in a paper from this laboratory (1) some observations were presented on the development of nutritional edema in the dog. The observations were based on experiments with seven animals which developed hypoproteinemia and edema after subsisting for variable times on a diet low in protein. Since the time of that publication the experimentally induced edema has been utilized as a means of studying particular aspects of the edema problem (2) and a considerable array of additional data has been accumulated. In the light of this further experience it appears desirable to describe in more detail the events which have been observed. The present communication will deal with the behavior of the serum and tissue proteins during the experiments.

Methods

Animals.—25 dogs of mongrel breeds used in these experiments varied in weight from 13 to 22 kilos and averaged 17 kilos. Young adult dogs were selected by preference; their ages, not definitely known, were estimated to be between 1 and 3 years.

Diet.—The first eight dogs in the series were maintained either on a synthetic diet or a natural food diet, both of which have been described previously (1). For the remaining seventeen dogs the composition of the natural food diet was modified by removing the butter fat and supplying its nutritional equivalent with lard and cod liver oil. The composition of the modified diet follows:

	gm.
Carrots	300
Rice	35
Lard	40

	gm.
Cod liver oil	10
Sugar	115
Salt mixture	5

The carrots and rice were cooked together for 20 minutes in a small amount of water; the lard, cod liver oil, sugar, and salt were added after cooking but while the mixture was still hot. Finally water was added until the mixture weighed 900 gm. This amount represented the quantity offered to each dog daily irrespective of its size. It furnished 1,200 calories as calculated from the tables of Atwater and Bryant (3) and by our own analysis contained 1.23 gm. nitrogen.

The salt mixture was similar to that employed by Gamble, Putnam, and McKhann (4) except that it contained sodium chloride and a larger amount of iron. Its composition follows:

	gm.
Calcium carbonate ($CaCO_3$)	26.0
Magnesium carbonate, basic ($3MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$)	6.1
Potassium chloride (KCl)	22.4
Sodium chloride ($NaCl$)	16.8
Sodium acid phosphate ($NaH_2PO_4 \cdot H_2O$)	20.6
Sodium carbonate (Na_2CO_3)	4.1
Ferric ammonium citrate ($Fe_2(NH_4)_2(C_6H_5O_7)_2 \cdot 3H_2O$)	4.0

Additions to and subtractions from the salt mixture were made from time to time with some dogs and will be described as the occasion arises. During all experiments no restriction was placed on the intake of water.

Metabolism Observations.—During the experiments the dogs were kept in metabolism cages and their natural activity was therefore restricted. The type of cage permitted complete collection of stool and urine. Stools were transferred daily to a container kept at refrigeration temperature and were covered with sufficient distilled water to prevent desiccation. At the end of each period, usually 7 days, the total collection with added water was weighed, and then thoroughly stirred with a mechanical mixer for approximately ½ hour. Weighed aliquots from the resulting homogeneous mass were used for analysis. Urine was collected in bottles containing a small amount of toluol and the daily volume carefully measured. The urines from each metabolism period were usually combined before analysis although in some instances the daily voidings were analyzed separately. At the end of each period the cages were washed thoroughly with approximately 2 liters of distilled water and the washings saved for analysis. The intake of the elements studied was calculated from the known composition of the diet and the weighed quantity of food consumed each day. Only distilled water was offered for drinking during periods of metabolism study.

Blood Samples.—Blood for analysis was obtained at intervals of from 1 to 3 weeks by puncture of the femoral artery. In some experiments the analyses were made on serum and in some on plasma in which coagulation had been prevented by the addition of heparin, 1 mg. for each cc. of blood. The samples were not protected with oil against loss of CO_2 as it was not possible to demonstrate significant change in the blood protein when this precaution was taken.

Analytical Procedure.—Methods for the determination of nitrogen and of the serum or plasma proteins have been described previously (1). The micro Kjeldahl apparatus for distillation of ammonia in steam, which we have used, is similar to that described by Peters and Van Slyke (5) and by these authors is attributed to Goebel. A calculation of the accuracy, or better the reproducibility, of analytical results for protein obtained by this method has been made from data secured in duplicate determinations on 152 samples of serum.[1] The calculation for total protein expressed as grams per 100 cc. shows a probable error of measurement (PE_M) of 0.077 for a single determination and a PE_M of 0.054 for analyses which represent the average of duplicate determinations. All analyses in this investigation were performed in duplicate. The result means that the chances are even (50–50) that a given analytical figure will not differ by more than 0.054 gm. from the theoretical average of an infinity of determinations on the same sample. Furthermore, the chances are 993 in 1,000, *i.e.* practically certain, that the figure will not differ from the true average by more than four times this amount or 0.22 gm. The calculation in the case of albumin reveals a PE_M of 0.069 gm. per cent for a single determination and a PE_M of 0.050 gm. per cent for analyses which represent the average of duplicate determinations.

Blood Proteins of Normal Dogs

An examination of the data from these and other experiments discloses 57 analyses of the serum or plasma proteins in normal dogs. The average results with accompanying statistical data are presented in Table I. In general the figures for total protein agree with those presented by other authors although there is considerable discrepancy in the albumin and globulin fractions; that is, our series has yielded lower levels for albumin and higher levels for globulin. In 35 normal dogs Hurwitz and Whipple (6) found, by a microrefractometric method, concentrations of albumin between 3.5 and 6.0 gm. per cent and an average concentration of 4.6 gm. per cent. In this series the average albumin concentration was 3.3 gm. per cent and the individual findings were between 2.3 and 4.3 gm. per cent. Similarly Hurwitz and Whipple reported globulin variations between 0.4 and 2.8 gm. per cent and an average of 1.5 gm. per cent, whereas in our series

[1] The methods of statistical analysis employed in this paper were taken from: Garrett, H. E., Statistics in psychology and education, New York, Longmans, Green and Co., 1926.

of 38 serum analyses the range was from 1.8 to 5.4 gm. per cent and the average value was 2.7 gm. per cent. The values reported in 1883 by Burckhardt (7), who used an antiquated and cumbersome method of analysis, digress even further in the direction of high levels for albumin and low levels for globulin. It is possible that difference in diet and previous illness may be factors in explaining the discrepancies. However, they probably result chiefly from difference in analytical procedure.

The statistical data in Table I show that globulin exhibits a wider range of normal variation than albumin. Indeed the variations en-

TABLE I

Proteins in Serum and Plasma of Normal Dogs

	Average per 100 cc.	Standard deviation of distribution	Standard error of average	Coefficient of variation
	Serum (38 dogs)			
	gm.			
Albumin	3.26	0.48	0.08	14.8
Globulin	2.72	0.76	0.12	27.8
Total protein	5.98	0.67	0.11	11.3
	Plasma (19 dogs)			
Albumin	3.38	0.38	0.09	11.2
Globulin	2.98	0.55	0.13	18.4
Total protein	6.36	0.71	0.16	11.2

countered in the globulin analyses are greater than those recorded for total protein. The fact suggests that albumin and globulin are not completely independent variables but that there is a certain tendency for low albumin to be associated with high globulin and *vice versa*. To evaluate the possible relationship we have determined the coefficient of correlation between albumin and globulin in the 38 analyses of serum and found it to be −0.49.[2] The probable error of the coefficient

[2] A negative correlation coefficient between two variables indicates that the relation is inverse, that is, that a large degree of one is associated with a low degree of the other and *vice versa*.

is 0.08. This means that the chances are even that the true coefficient falls within the limits -0.49 ± 0.08 and that the chances are 9,999 in 10,000 that it has at least a negative value. It is therefore certain that a negative correlation does exist between the albumin and globulin of normal dogs but with the data available it is not possible to prove a high correlation.

The averages given in Table I also show that the globulin of plasma is slightly higher than the globulin of serum, the difference being 0.26 gm. per cent. The number of animals is not sufficient to prove that the difference is significant, although it is apparent that a real difference must exist from the circumstance that fibrinogen and serum globulin are included together when plasma is analyzed by the method used in this investigation. In subsequent portions of this paper in which the trend of the serum proteins will be pictured from average results in a group of animals,, we have diminished the values for globulin and total protein by 0.3 gm. per cent when plasma rather than serum was analyzed. This means of adjusting plasma and serum analyses to a common basis is of course only approximate but because of the relatively small size of the fibrinogen fraction it cannot introduce significant errors. The analyses were about equally divided between serum and plasma.

Effect of Inadequate Dietary Protein on Serum Proteins

The protocols disclose 21 animals in which diet was the only significant factor in the production of hypoproteinemia for a period of 50 days. In several other experiments initial periods of plasmapheresis render the data unsuited for studying the uncomplicated effect of diet. With some of the 21 animals the effect could be followed for a longer time; with eight dogs through the 80th day and with three dogs for a hundred days or longer. Altogether 150 determinations of albumin, globulin, and total protein are available for analysis. The results for albumin and total protein are presented graphically in Chart 1. In order to construct a composite curve from the results, the serum proteins were calculated by interpolation for each dog at 10 day intervals throughout the period of observation. The average values at these intervals together with their standard deviations are presented in Chart 2. Both charts indicate the downward trend of albumin and total protein with continuance of the diet. It is seen more clearly in Chart 2 that the decline is most rapid during the early days on the diet, that it becomes progressively slower during subsequent periods, and that terminally the rate of fall is again accelerated. The terminal rapid fall is seen better in the separate protocols and is somewhat obscured in the curves of averages because of its occurrence at differerent times in different animals.

Chart 2 also shows that average globulin concentration is almost unaffected by the diet. The terminal values are very slightly, and not

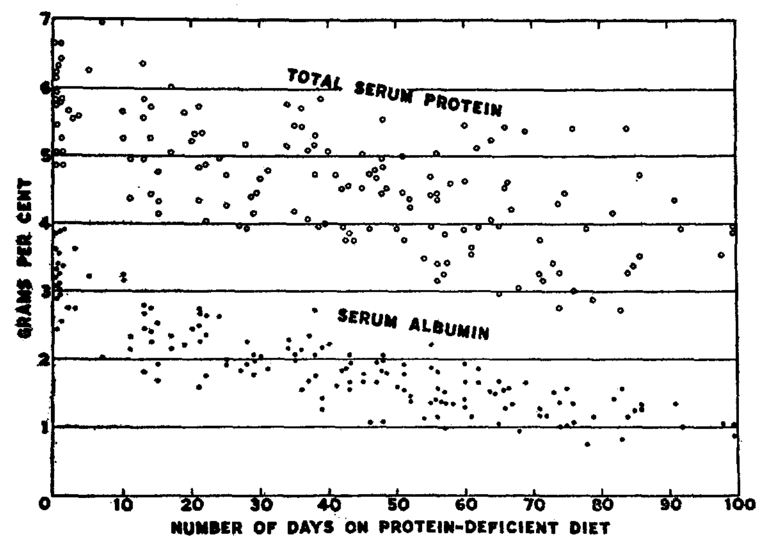

CHART 1. The results of 150 determinations of the albumin and total protein of serum in 21 dogs which were maintained on the protein-deficient diet.

significantly, higher than the initial. In a previous communication (1) we reported a tendency for globulin to rise in association with the fall in albumin. This was indeed true in a number of experiments in most of which the initial values for globulin were rather low. In other experiments, however, the initial levels were higher and the diet resulted in a decline rather than a rise. The circumstance has led us to inquire whether maintenance on the diet may not lead to greater uniformity in globulin concentrations than are encountered in normal animals. Table II presents calculations in which the possibility is examined. Both from the standard deviations and the coefficients of variation it is seen that the globulin concentrations do tend to become less scattered and more concentrated about their averages for as long as 50 days. Thereafter the scattering again increases.

Effect of Diet on Nitrogen Balance

The protocols disclose five animals in which the nitrogen balance was followed through a total of 42 metabolism periods (approximately

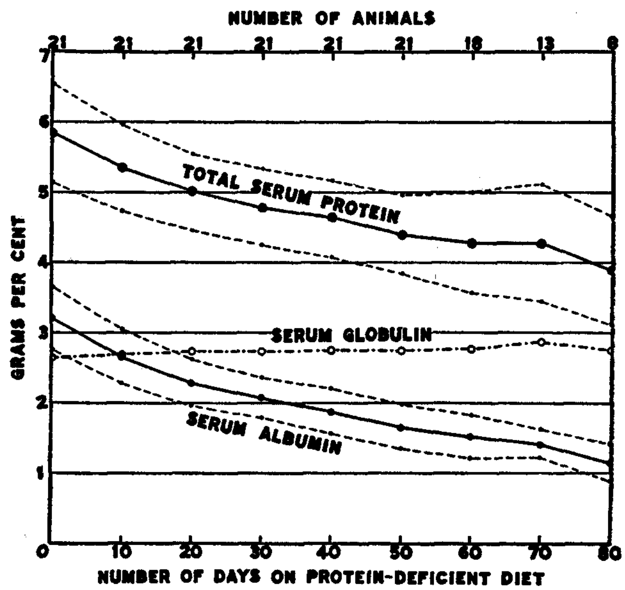

CHART 2. The effect of the protein-deficient diet on the albumin, globulin, and total protein of serum. The curves, which represent average values, were prepared from the data in Chart 1. The dotted lines above and below the unbroken lines for albumin and total protein are placed at a distance of one standard deviation from the average values. They mark out the range within which 68 per cent of the observations may be expected to fall.

294 days). In every period the excretion of nitrogen was greater than the intake. The quantities of nitrogen lost are shown in Table III. With two animals, in which the record of nitrogen balance is complete for the entire experimental period, there is seen to be a tendency toward a decrease in the amount of nitrogen excreted with continuance

of the diet. These animals lost an average of 1.77 gm. nitrogen daily during the first 2 weeks of maintenance on the diet and an average of only 0.85 gm. during the last 2 weeks. A similar progressive diminution in the nitrogen loss has been described by Liu and his collaborators (8) among patients who were subsisting on low protein diets similar to those prevalent in the famine districts of China. The adaptive ability of the body in adjusting its metabolic processes so as to spare protein is further seen from the circumstance (Table III) that the decrease in the amount of nitrogen lost has taken place in spite of a marked reduction in the quantity of food consumed. The table does reveal a rise in the loss of nitrogen with each sharp drop in the intake of food

TABLE II

Course of the Serum Globulin in 21 Dogs Maintained on Protein-Deficient Diet

Time maintained on defective diet	Average per 100 cc.	Standard deviation of distribution	Coefficient of variation
days	*gm.*		
Start	2.65	0.77	29.1
10	2.69	0.63	23.3
20	2.72	0.52	19.2
30	2.72	0.50	18.4
40	2.75	0.49	17.7
50	2.74	0.46	16.9
60	2.78	0.52	18.6
70	2.88	0.67	23.4
80	2.74	0.70	25.7

but the rises are not sufficient to compensate for the general progressive decline in nitrogen excretion. The average daily loss of nitrogen for all of the animals studied was 1.15 gm. It is evident that the figure would be greater if more animals had been studied during the initial weeks of maintenance on the diet.

Relation between Negative Nitrogen Balance and Decline in Serum Albumin

The data presented in the preceding sections permit an estimate of the relationship between loss of albumin from the circulation and loss of nitrogen from the body as a whole. The average initial weight of the five dogs whose nitrogen balance was studied was 16.8 kilos. If the average volume of the plasma is taken to be 50 cc. per kilo, and the average concentration of albumin in the serum is 3.2 gm. per cent, it follows that the average circulating albumin with these animals was initially 27 gm. After 30 days of maintenance on the diet, when the

TABLE III

Nitrogen Balance and Food Consumption in Five Dogs Maintained on Protein-Deficient Diet

Time on diet	Dog 8-06 15.45 kg.		Dog 8-4a 15.55 kg.		Dog 8-38 20.60 kg.		Dog 8-4b 18.00 kg.		Dog 2-3 14.15 kg.	
	Food* consumed per day per kg.	Daily† loss of nitrogen	Food consumed per day per kg.	Daily loss of nitrogen	Food consumed per day per kg.	Daily loss of introgen	Food consumed per day per kg.	Daily loss of nitrogen	Food consumed per day per kg.	Daily loss of nitrogen
days	*calories*	*gm.*	*calories*	*gm.*	*calories*	*gm.*	*calories*	*gm.*	*calories*	*gm.*
1–8	77.7	1.76	77.2	2.02	58.3	—	66.7	—	84.8	—
8–15	77.7	1.52	77.2	1.77	58.3	—	66.7	—	84.8	—
15–22	77.7	1.42	77.2	1.59	58.3	—	66.7	—	84.8	—
22–29	77.7	1.39	77.2	0.85	58.3	—	66.7	—	84.8	—
29–36	77.7	1.42	64.8	1.06	58.3	—	66.7	—	84.8	—
36–43	67.2	0.92	72.4	0.97	55.0	1.49	66.7	—	84.8	0.40
43–50	34.7	1.36	54.4	1.10	36.0	1.79	66.7	—	84.8	0.70
50–57	38.9	1.34	57.8	0.88	39.3	1.76	60.5	—	84.8	0.80
57–64	38.9	0.97	51.7	1.07	27.5	1.48	32.5	—	82.8	0.54
64–71	38.9	1.02	37.2	1.13	29.2	1.38	33.4	0.77	55.8	0.81
71–78	32.2	1.08	24.8	0.77	29.2	0.42	31.4	0.96	33.6	1.04
78–85	38.9	0.90	18.6	0.79	23.2	1.28	19.5	1.60	28.8	1.22
85–92	38.9	0.94								
Average......		1.23		1.17		1.37		1.11		0.79

* The food consumption per kilo was calculated on the basis of body weight at the start of each experiment.

† Loss of nitrogen represents the excess of excretion (nitrogen in urine plus nitrogen in stool plus nitrogen in washings from cage) over intake (nitrogen in diet).

concentration had been reduced to 2.1 gm. per cent, the total albumin in the circulation was 18 gm. This means a loss of 9 gm. albumin or 1.4 gm. nitrogen from the circulation. During the same 30 days the dog lost at least 1.15 gm. nitrogen daily from its entire body, or a total of 35 gm. It is, therefore, seen that the loss of nitrogen from the circulation was only 4 per cent of the loss from the organism as a whole. A similar calculation based on the first 60 days of maintenance on the diet shows only 3 per cent of the total nitrogen loss accounted for by the decline in albumin in the serum. For purposes of simplicity we have omitted from the calculations consideration of the decline in plasma volume which has been reported to accompany plasma albumin deficits. In our experience the decline averages around 20 per cent of the initial volume and the error introduced by simplifying the calculation would seem to be compensated by the low figure used for total

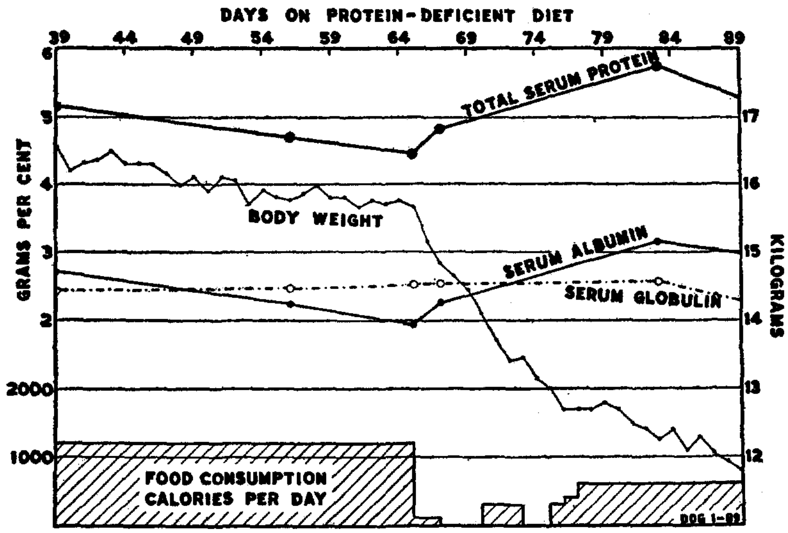

CHART 3. Spontaneous regeneration of the albumin of serum associated with partial fasting and rapid loss of weight.

daily nitrogen loss. At all events it is desired merely to stress the discrepancy which exists between serum loss of nitrogen and total loss of nitrogen, a discrepancy which must remain after refinements of computation.

Spontaneous Regeneration of Plasma Protein at Expense of Tissue Protein

In the majority of experiments, as has been described, depletion of the tissue proteins has been accompanied by gradually declining plasma proteins. One episode, however, has demonstrated that these processes need not always go hand in hand. The details of the episode are shown in Chart 3.

During the first 64 days of maintenance on the diet the dog reacted similarly to other animals and the serum albumin was gradually depleted to a level of 1.95 gm. per cent. On the 65th day, simultaneously with the appearance of a mild edema about the Achilles tendons, the dog suddenly refused to eat. The anorexia was not associated with fever or other evidence of infection. For about 10 days fasting was complete except for small amounts of sugar and ample amounts of salt and

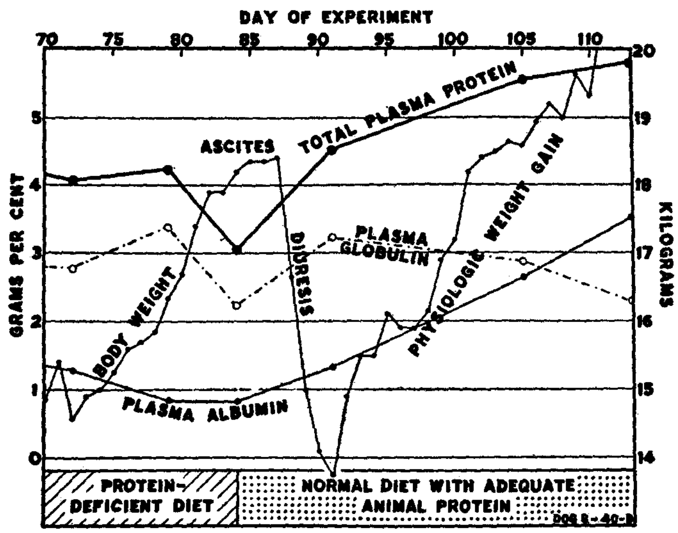

CHART 4. Recovery from edema and restoration of the serum proteins to normal following the administration of a diet containing adequate protein.

water which were given by gavage. Finally after the 76th day smaller amounts of the previous diet were administered by force. During the interval of approximate fasting the animal lost about 3 kilos. A small portion of the loss may have resulted from the elimination of interstitial fluid but the greater part must have represented consumption of tissue for energy needs. There was certainly a considerable loss of nitrogen. Under the circumstances the course of the serum proteins was astonishing. The albumin rose to a level of 3.17 gm. per cent, a value within the range of normal, and the globulin remained constant at a normal level. During this period there was no evidence of dehydration and in fact the change in the serum proteins which involved a shift in the albumin:globulin ratio, could not have resulted from blood concentration alone.

We have been forced to believe that the serum albumin in this animal was regenerated at the expense of catabolized tissue protein. The belief is strengthened by the subsequent course of events, when the dog began to eat voluntarily, and the serum albumin again declined, not acutely, but gradually, as during the early days of the experiment.

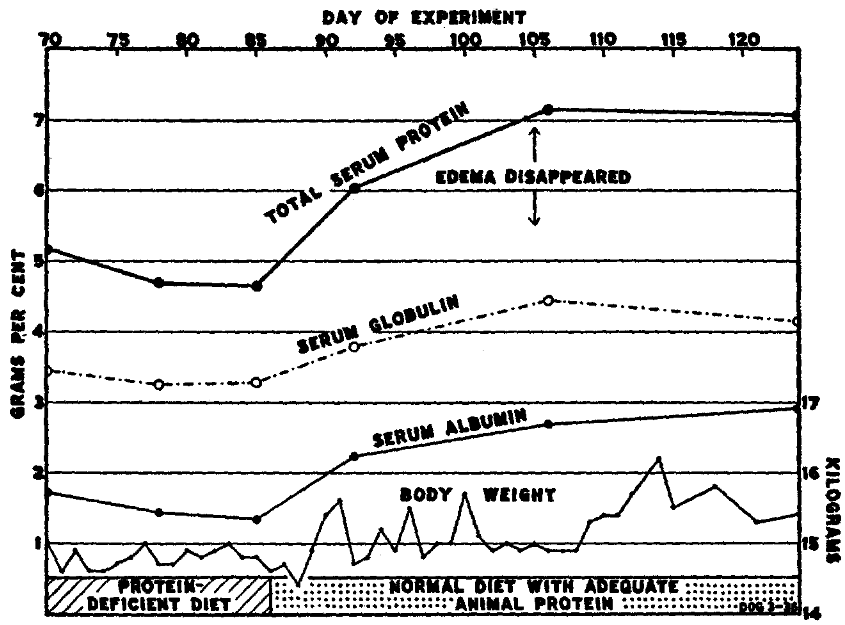

CHART 5. Replenishment of the serum proteins from feeding a diet adequate in protein in an animal in which recovery was very slow. At the start of the experiment the albumin concentration was 3.9 gm. per cent. The lowest level recorded was 1.4 gm. per cent on the 85th day. After 38 days of adequate feeding it had again risen to 2.9 gm. per cent.

Serum Proteins during Recovery

If the process of serum protein depletion is interrupted by administering a diet containing adequate protein, it is usually possible to bring about a return to normal health. If the protein is fed before marked weakness and loss of vitality have ensued, the rise in the serum albumin may be astonishingly rapid. In one experiment on a diet of milk and meat the albumin rose from 1.1 to 2.8 gm. per cent in 10 days. In a few animals the attempt to evoke recovery was not made until the physical condition was critical and it seemed that life could not be prolonged on the low protein diet for more than a few days. Due to weakness and severe anorexia these animals may require much careful nursing before health is restored. We have usually begun by administering daily milk gavages and have added casein, lean meat, or liver to a diet composed chiefly of bread and vegetables in attempts both to provide protein and to stimulate appetite. Under this treatment an immediate rise in both the albumin and globulin fractions of serum has always been observed. The rise need not, however, be associated immediately with improvement in the physical condition and some dogs have died at this stage. In others restoration of vitality and return of the serum proteins to normal levels occurs but may be an extremely slow process. Chart 4 illustrates the phase of recovery in an average animal. In this experiment the serum proteins had returned to normal levels after 29 days on a diet containing added milk, meat, and liver consecutively. Chart 5 outlines an experiment in which restoration of the normal blood protein pattern was greatly delayed. With this animal feedings by gavage were necessary for many days and even after 38 days of adequate protein intake, the period embraced by the chart, the serum proteins were still appreciably removed from the previous level of health.

Charts 4 and 5 both show that the feeding of adequate protein is followed immediately by a rise in serum globulin as well as in albumin. The regeneration of globulin often leads to concentrations which are above normal. Subsequently as the albumin continues to rise the globulin again sinks until the normal ratio is reestablished. This experience has been a general one. The course of events is quite analogous to that described by Weech and Ling (9) in human beings during recovery from the hypoproteinemia of famine edema.

Our experiments have yielded no clue as to which of the several dietary proteins produced the most rapid regeneration of serum protein. It is generally agreed that animal protein is more suitable than vegetable protein and for this reason we have employed protein of animal origin only. Between the several proteins there has been little to choose. We have felt that progress was more satisfactory when the individual animal's whims of appetite were catered to than when persistently trying the effect of this or that type of protein. In a subsequent paper it will be shown that dogs deprived of protein for long periods present histologic and symptomatic evidence of liver damage. It is possible that replenishment of the serum proteins is determined not only by adequate diet but also by the time required for restoration to anatomic and physiologic normality of those organs which are concerned in the synthesis of serum protein.

DISCUSSION

In a recent publication Bloomfield (10) has brought together a number of reasons for suggesting that loss of protein in the urine and lack of protein in the diet are not in themselves sufficient to account for the phenomenon of hypoproteinemia. This he refers to as the inadequacy of "the loss and lack theory." More recently Holman, Mahoney, and Whipple (11) have suggested the existence of a "dynamic equilibrium between tissue protein and plasma protein" which is governed by "the physiological needs of the moment." Inasmuch as an understanding of the nature of an equilibrium would probably go far toward explaining the inadequacies of the loss and lack theory, it appears worth while to summarize the evidence for and against the equilibrium concept.

A. In favor of the existence of an equilibrium between tissue protein and plasma protein, the following facts may be cited:

1. The studies of nitrogen balance reported in this paper show that the depletion of the plasma protein in dogs which results from maintenance on a low protein diet is accompanied by a much larger loss of protein from the tissues.

2. The common forms of hypoproteinemia in the human being are accompanied by malnutrition and asthenia which are probably indicative of tissue protein deficit. The amount of nitrogen retained during recovery from human nutritional edema far exceeds the quantity required to restore the plasma proteins to normal levels (8, 12). One of the patients studied by Schittenhelm and Schlecht retained 16 gm. out of 21 gm. of nitrogen ingested in a single day.

3. In plasmapheresis experiments sudden withdrawal of plasma protein from the animal is followed by the regeneration of a new

supply even though the animal be fasting (13). With repeated plasmapheresis over a number of days the reduction in plasma protein is small in comparison with the volume of blood which must be exchanged. In one experiment in this laboratory conducted over a period of 12 days it was estimated that each 4 gm. of albumin withdrawn from the circulation effected a reduction of only 1 gm. in the circulating amount and that 7.5 gm. of globulin were removed for each gram of reduction in this fraction. Moreover, the attained degree of hypoproteinemia was temporary and followed by further regeneration when the bleedings were stopped.

4. The nitrogen requirement of the tissues can be met by suitable amounts of plasma protein injected intravenously. With such injections Holman, Mahoney, and Whipple (11) have shown that dogs receiving only sugar by mouth can be maintained "practically in nitrogen equilibrium." Large amounts of injected protein do not produce abnormally high concentrations in the recipient, the protein being removed apparently to supply tissue needs.

5. Analogies exist which show that under stress there may be an interchange of protein-building material between other tissues in the body. Davis and Whipple (14) demonstrated that dogs which exhibit hepatic necrosis from poisoning with chloroform can regenerate the liver back to normal on an intake of sugar alone.

B. In conflict with the concept of an equilibrium between tissue protein and plasma protein, the following facts may be cited:

1. The experiment illustrated in Chart 3 shows that tissue protein and plasma protein do not always move in a parallel manner and that occasionally the plasma protein may rise while the tissue protein falls in a way which appears to be at complete variance with the existence of an equilibrium between the two.

2. In these experiments there has been no apparent relationship between the original state of nutrition and muscular development of dogs and the rapidity with which they developed hypoproteinemia. When the experimental program was started it was supposed that edema levels might be reached more quickly if the initial nutritional status was poor. The supposition was in error. The results are more satisfactory when normally nourished vigorous animals are chosen. In the experiment referred to in the preceding paragraph the rate of

decline in the plasma protein following the episode of spontaneous regeneration was not more rapid than is observed in normal animals *de novo*.

3. During recovery from nutritional edema, both in man and in the dog (15), the plasma proteins may regenerate to normal levels in less time than is required for the restoration of normal nutrition.

4. Although experience with human beings shows that plasma protein deficits are generally associated with malnutrition, the reverse is not true and severely malnourished individuals are frequently encountered in whom the plasma proteins have a normal level.

5. Complete fasting is usually not attended by significant reduction in the concentration of protein in the plasma (13, 16). The explanation, that fasting does lead to a loss of protein from the circulation but that concentration is maintained at the expense of reduction in blood volume, is not satisfying as it is difficult to think of an equilibrium between tissue protein and plasma protein which depends upon total quantities rather than upon concentrations.

6. Bloomfield (10, 17) has shown that rats maintained on various synthetic diets of low protein content or on a diet composed of dry carrot powder do not exhibit progressive hypoproteinemia, whereas on a diet of fresh carrots the phenomenon does ensue. In both groups loss of weight was interpreted as evidence that lack of protein had rendered the diets inadequate for maintenance. Bloomfield suggested that difference in water content of the diets might be the determining factor. In any case the experiments indicate again that loss of tissue protein and loss of plasma protein do not always go hand in hand. Because of the importance of Bloomfield's observations it is to be hoped that figures showing the nitrogen balance will eventually be supplied to provide final proof of loss of protein, as opposed to fat and possibly glycogen, from the body. We may note in passing that progressive hypoproteinemia has been observed in this laboratory to develop in dogs maintained on a synthetic diet somewhat similar to that employed by Bloomfield.

In the present state of knowledge it is impossible to reconcile all of the facts presented above. It has seemed desirable to list them merely because in so doing issues are rendered apparent which may become the starting point for new experiments. It is clear that the possibility of exchange of substance between tissue protein and plasma protein does exist and that under appropriate stress either protein may act as a restorative for the other. It is not clear, however, that the exchange results from a direct equilibrium between the two proteins. Under circumstances which are not understood, tissue protein alone may suffer when the nitrogen balance is negative and when both proteins have been depleted tissue protein may suddenly, and for totally obscure reasons, suffer additional losses which permit the replenishment of plasma protein. Because in many instances a restorative action of this nature is in abeyance, it is not justifiable to postulate injury of the blood protein-forming mechanism. The mechanism may fail simply for lack of raw material which must be supplied from other sources.

In spite of many points the nature of which remains obscure, the data presented in this paper support the contention of Bloomfield that the loss and lack theory alone is an inadequate explanation of hypoproteinemia. We have hoped that some of the data might carry therapeutic implications. So long as the theory is believed there can be little justification for attempting to restore the plasma proteins by other means than the feeding of adequate protein. The physician, and particularly the pediatrician, knows how difficult such feeding may be in the face of severe anorexia and when attempts to force the diet regularly lead to vomiting. At the present time it is not unjustifiable to hope that a way will be found for stimulating an internal readjustment to provide temporary relief, a way which will depend upon the potential ability of the tissues to provide sufficient protein for the needs of the plasma.

SUMMARY

1. The concentration of protein in the serum and plasma of normal dogs is given. Analyses of serum from 38 animals yielded the following averages and standard deviations, (*a*) for albumin: 3.26 ± 0.48 gm. per cent, (*b*) for globulin: 2.72 ± 0.76 gm. per cent, and (*c*) for total protein: 5.98 ± 0.67 gm. per cent. Analyses of plasma from 19 animals showed, (*a*) for albumin: 3.38 ± 0.38 gm. per cent, (*b*) for globulin: 2.98 ± 0.55 gm. per cent, and (*c*) for total protein: 6.36 ± 0.71 gm. per cent.

2. A diet for dogs is described, the feeding of which results in a progressive decline in the concentration of protein in the serum. A composite curve constructed from the findings with 21 animals discloses a rapid initial fall and a slower subsequent decrease in albumin and total protein and an approximately constant level for globulin. The course of the globulin curve was subject to wide variation in separate experiments, both increases and decreases being recorded.

3. With five dogs the nitrogen balance was followed through a total of 42 metabolism periods of approximately 7 days each. The average daily loss of nitrogen was 1.15 gm. Approximate calculations disclose that only 3 or 4 per cent of the nitrogen eliminated is accounted for by the decline in circulating protein, the remainder being represented by loss from the tissues.

4. An episode is described with one dog when, during a period of self-imposed fasting, the serum albumin regenerated to a normal level, apparently at the expense of catabolized tissue protein.

5. The course of serum proteins is described during the recovery which follows interruption of the low protein diet and return to a régime of adequate feeding.

6. A discussion is given of the relationship between tissue proteins and plasma proteins. The data permit one to entertain the hope that a way will be found for stimulating an internal readjustment to provide temporary relief from hypoproteinemia, a way which will depend upon the potential ability of the tissues to provide sufficient protein for the needs of the plasma.

BIBLIOGRAPHY

1. Weech, A. A., Snelling, C. E., and Goettsch, E., *J. Clin. Inv.*, 1933, **12,** 193.
2. Weech, A. A., Goettsch, E., and Reeves, E. B., *J. Clin. Inv.*, 1933, **12,** 217, 1021; *J. Exp. Med.*, 1934, **60,** 63.
3. Atwater, W. O., and Bryant, A. P., *U. S. Dept. Agric.*, *Office Exp. Stations*, *Bull. 28*, 1906.
4. Gamble, J. L., Putnam, M. C., and McKhann, C. F., *Am. J. Physiol.*, 1929, **88,** 571.
5. Peters, J. P., and Van Slyke, D. D., Quantitative clinical chemistry, Volume II, Methods, Baltimore, The Williams & Wilkins Co., 1932, 531.
6. Hurwitz, S. H., and Whipple, G. H., *J. Exp. Med.*, 1917, **25,** 231.
7. Burckhardt, A. E., *Arch. exp. Path. u. Pharmakol.*, 1883, **16,** 322.

8. Liu, S. H., Chu, H. I., Wang, S. H., and Chung, H. L., *Chinese J. Physiol.*, 1932, **6,** 73.
9. Weech, A. A., and Ling, S. M., *J. Clin. Inv.*, 1931, **10,** 869.
10. Bloomfield, A. L., *J. Exp. Med.*, 1933, **57,** 705.
11. Holman, R. L., Mahoney, E. B., and Whipple, G. H., *J. Exp. Med.*, 1934, **59,** 269.
12. Schittenhelm, A., and Schlecht, H., *Z. ges. exp. Med.*, 1919, **9,** 75.
13. Kerr, W. J., Hurwitz, S. H., and Whipple, G. H., *Am. J. Physiol.*, 1918, **47,** 356.
14. Davis, N. C., and Whipple, G. H., *Arch. Int. Med.*, 1919, **23,** 612.
15. Unpublished observations by the authors.
16. Hanson, S., *J. Immunol.*, 1918, **3,** 67.
17. Bloomfield, A. L., *J. Exp. Med.*, 1934, **59,** 687.