DEPLETION OF RESERVE PROTEIN FROM EXTRAVASCULAR EXTRACELLULAR FLUID

C^{14} Labeling of Plasma Proteins in Dogs after Plasmapheresis*

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Lymph and other extravascular, extracellular fluids have been shown in relatively recent years to contain, under normal conditions, a mass of protein approximately equal in magnitude to the total circulating plasma proteins (5, 6, 10). The present study focuses interest on the reserve function of the interstitial fluid proteins in dogs rendered protein-deficient by plasmapheresis and a very low protein diet. Details dealing with plasma protein turnover and tissue exchange in these depleted animals are discussed in the accompanying paper (12) in relation to similar data from other experiments.

It has been found during depletion that the extravascular, extracellular proteins constitute a ready though limited source of reserve protein for the circulating plasma. A shift in the ratio of protein, in the 2 compartments of the exchangeable pool due to depletion, suggests that rapid changes in plasma protein concentration may result from modification of the ratio in either direction by other as yet undetermined factors.

Methods

Three female dogs and 1 male dog, all healthy mongrels weighing from 9 to 10 kilograms were used for protein depletion in this study. A very low protein diet (10) composed of carbohydrate, fat, vitamins, and minerals containing 0.12 per cent N was fed daily in amounts calculated to supply 100 calories per kilogram of initial body weight. Plasmapheresis, a procedure involving bleeding with return of red blood cells suspended in 0.9 per cent NaCl (1), was carried out approximately 3 times each week. This procedure was designed merely to

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lower the plasma protein concentration to between 4.0 and 4.5 gm. per 100 ml. and to maintain this level for the desired length of time.

Following these periods of depletion, pertinent details of which are shown in Table I, each animal received an intravenous injection of C^{14}-labeled plasma, obtained from a donor dog whose plasma protein had been labeled by the prior feeding of DL-lysine-6-C^{14}. The fluid volume, plasma protein content and C^{14} activity of the plasma injected into each animal are shown in Table II. The experiments were then continued for a 7 day period with blood samples being collected 3 times on the 1st day, and daily thereafter. Nitrogen and C^{14} determinations of total plasma protein, albumin, and globulin were made on each of these samples. Carbon dioxide from the air expired during 1 hour periods was collected in 20 per cent potassium hydroxide, 3 times on the 1st day and once on subsequent days for measurement of C^{14} content. Nitrogen and C^{14} were also determined in aliquots of urine excreted during each 24 hour period. On the 7th day all dogs were killed, 3 by viviperfusion under light ether anesthesia and 1 by intravenous injection of a few milliliters of chloroform. Complete autopsies were performed. Samples of tissues were rapidly frozen, then lyophilized and analyzed for nitrogen and C^{14} content. Additional aliquots of dried, ground lung, spleen, liver, kidney, and skeletal muscle were extracted with acetone and trichloracetic acid in a Waring blendor and the residues were analyzed for nitrogen and C^{14}. All chemical procedures and those pertaining to measurement of C^{14} have been described in previous publications (4, 10, 11).

TABLE I

Details of Protein Depletion

Dog No.	Days of depletion	Blood removed	Plasma protein concentration						Red cell hematocrit value	
			Total protein		Albumin		Globulin			
			Initial	Final	Initial	Final	Initial	Final	Initial	Final
		ml.	*gm. %*	*gm. %*	*gm. %*	*gm. %*	*gm. %*	*gm. %*	*per cent*	*per cent*
51-171	56	1928	6.1	4.5	2.8	0.8	3.3	3.7	50	45
54-36	53	1202	5.4	4.0	1.6	1.5	3.8	2.4	54	44
52-132	36	1221	6.0	4.1	3.3	1.2	2.7	2.9	50	44
53-154	26	1365	6.1	4.4	3.3	1.2	2.8	3.2	48	46

Two normal dogs placed on the low protein diet 4 days before intravenous injection of C^{14}-labeled plasma served as controls.

EXPERIMENTAL OBSERVATIONS

Fig. A illustrates the rates of decline of C^{14} activity from the total plasma proteins of the 4 depleted dogs and 2 control dogs. In all the curves illustrated the usual rapid initial drop in isotope concentration is followed by a slower rate of decline. The most noteworthy feature of these curves is that the concentration of C^{14} falls an average of only 30 per cent compared to a drop of over 50 per cent in the controls, during the early rapid phase. A somewhat accelerated turnover of plasma protein in the dogs depleted by reduced protein intake and plasmapheresis is also indicated by the steeper slopes of the slow phase of the experimental curves. Actual half-times of total plasma protein were 7.8 days

TABLE II

Details of Plasma Protein Labeling

Dog No.	Volume of plasma injected	Plasma protein injected	C^{14} activity injected	Initial per cent C^{14} dose per gm.*			C^{14} albumin/globulin ratio
				Total protein	Albumin	Globulin	
	ml.	*gm.*	*μc.*				
51-171	80	4.0	1.56	5.3	24.0	6.6	0.5
54-36	125	5.2	1.14	3.9	—	—	—
52-132	142	6.7	1.34	3.8	9.5	6.5	1.1
53-154	133	7.3	1.32	4.0	6.5	10.0	1.2

* Per cent of C^{14} in respective protein fraction of donor plasma.

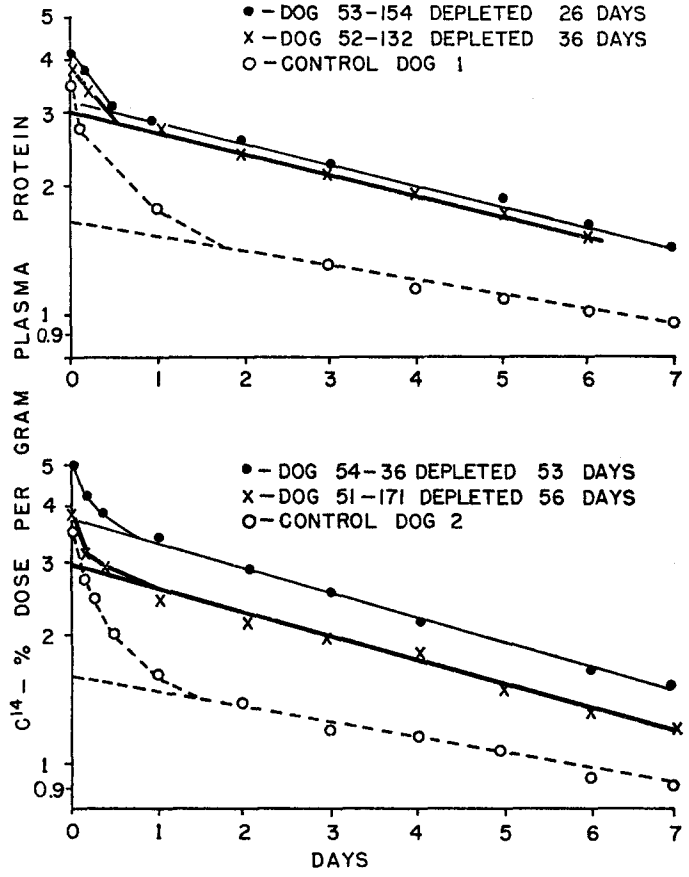

FIG. A. Decline in C^{14} activity of total plasma proteins following intravenous injection of labeled plasma to 2 normal and 4 protein-depleted dogs receiving an essentially non-protein diet. The much less marked and more rapid mixing phase in the depleted dogs is readily apparent, while the somewhat steeper slope of the later, slow phase of the curves in these animals indicates a more rapid metabolic turnover of plasma protein.

for the 2 control dogs, 5.2 and 5.4 days, respectively, for the 2 dogs subjected to longer and 6.4 days for both dogs subjected to shorter periods of depletion.

As pointed out by Sterling (6) and illustrated in Fig. A, extrapolation of the straight portion of the curve back to zero time, gives a value indicating the theoretical concentration of isotope which would have resulted if immediate complete mixing with all protein in both intra- and extra-vascular portions of the exchangeable pool were possible. The amount of protein in the extravascular, extracellular fluids is then determined by subtracting the grams of circulating protein from that in the total pool.

The result of applying this type of analysis to the data obtained in the present experiments is shown in Table III.

TABLE III

Effects of Protein Depletion on Total Plasma Protein Pool

Dog No.	Days of depletion	Plasma protein				Plasma protein lost from total pool	Plasma protein removed by plasmapheresis
		Before depletion		After depletion			
		Total in circulating plasma	Total in extravascular extracellular fluid	Total in circulating plasma	Total in extravascular extracellular fluid		
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control 1	0	29.0	29.8	—	—	0	0
Control 2	0	28.6	28.0	—	—	0	0
51-171	56	24.5	24.5	15.9	6.3	26.8	52
54-36	53	22.8	22.8	19.0	8.9	17.7	29
52-132	36	27.6	27.6	20.0	8.7	26.5	34
53-154	26	25.6	25.6	17.8	6.6	26.8	34

Equal amounts of protein were assumed to be present in the extravascular, extracellular fluid before depletion, this assumption being based on the findings in the 2 control dogs, in other animals studied in this laboratory and reports in the literature (5–7). Values shown in Table III for the amounts of protein in the 2 compartments of the total plasma protein pool after periods of depletion were obtained from the data shown in Fig. A. Corrections for plasma protein injected were made so as to indicate the status at the time of C^{14} labeling. It is readily apparent, Table III, that as a result of depletion there has been a much greater loss of protein from the extravascular portion of the exchangeable pool than from that within the circulating plasma. The figures shown indicate a maximum loss of circulating plasma protein of 35 per cent (dog 51-171) compared to losses of interstitial fluid protein ranging from 61 to 74 per cent. Expressed in another way this means that while the protein in the total exchangeable pool was reduced by approximately 50 per cent in each experiment

this was largely at the expense of the extravascular component which fell from an initial 50 to a final 30 per cent of the total.

The last 2 columns of Table III show for each experiment the amount of protein lost from the total exchangeable pool during the depletion period and the amounts of plasma protein removed by plasmapheresis. The extra amount removed over and above that lost from the pool was derived presumably from tissue sources.

Table IV summarizes the over-all nitrogen balance observed during the periods of depletion for each animal. The sole dietary nitrogen intake was in yeast powder added as a source of B vitamins. This accounted for 0.12 per

TABLE IV

Nitrogen Balance during Depletion Period

Dog No.	Days of depletion	Weight		Total nitrogen intake diet	Total nitrogen output		Net nitrogen loss		Nitrogen in lost body weight‡
		Initial	Loss		Urine and feces*	Plasma protein N removed by bleeding	Total	From tissues	
		kg.		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
51-171	56	9.3	1.9	12	57	8	53	49	46
54-36	53	8.9	1.0	11	42	5	36	33	24
52-132	36	10.6	0.6	9	25	5	21	17	14
53-154	26	10.0	0.7	6	25	5	24	20	17

* Estimated as 0.1 gm. N per day.

‡ Estimate based on 15 per cent protein content.

cent of the total diet weight and provided approximately 0.023 gm. of nitrogen per kilogram of body weight per day. On the debit side is the nitrogen excreted in urine and feces plus that removed by plasmapheresis as plasma protein. A further slight deficit due to the mild anemia that developed is not included. All nitrogen lost during depletion came either from the exchangeable pool (Table III), or from tissue sources. Values for tissue nitrogen loss are shown in the second to last column, Table IV, and closely approximate those in the final column, representing estimates of nitrogen in the lost body weight for each dog.

DISCUSSION

In this type of experiment, with a moderate degree of plasma protein depletion maintained at a fairly constant level for periods ranging from 2 to 6 weeks, considerably more plasma protein must be removed than can be accounted for by the drop in total circulating levels. An appreciable quantity of this extra or "reserve" protein is derived from the proteins of the extravascular, extracellular fluid. The remainder, greater in amount in the longer term experi-

ments, is roughly proportional to loss of body weight and is apparently supplied directly or indirectly from tissue sources. It is possible that unequal reduction in the masses of protein in the plasma and extravascular fluid pool may be related to the technique of plasmapheresis, in which only 1 of 2 balanced compartments is being tapped, rather than by depletion *per se*. However, an analysis of previously published data dealing with experimental ascites (2, 3) and pregnancy (8) as well as several unpublished experiments with starvation, suggests that a proportionately greater reduction in extravascular, extracellular protein occurs under these conditions also.

Since the 2 major compartments of the total exchangeable pool of plasma protein are in a state of fairly ready equilibrium, the extravascular, extracellular component would appear to provide a major immediate source of reserve protein for the circulation or a first line of defense.

Limitation of protein loss from the total pool as well as the degree of shift in relative amounts of protein in the 2 compartments is indicated by the fact that changes of almost identical magnitude were observed in all animals irrespective of the duration of depletion. Apparently beyond this limit tissue sources are called on to support minimum levels required for the metabolic and osmotic functions of the plasma proteins. This is somewhat comparable to the raiding of body tissue protein to form plasma protein in doubly depleted dogs (9).

The disturbed plasma to interstitial fluid protein ratio, from a normal 1 to 1 to over 2 to 1, following depletion suggests that similar or reverse shifts may be involved in other conditions associated with relatively rapid changes in plasma protein concentration.

SUMMARY

During protein depletion produced by plasmapheresis and a very low protein diet there is a proportionately greater decrease in extravascular, extracellular fluid protein than in plasma protein.

A shift in the normal ratio of protein in these 2 compartments, approximately 1 to 1 in the dog, to over 2 to 1 as a result of depletion indicates an important, labile source of reserve protein for the plasma in the interstitial fluids.

This reserve source is limited since a maximum drop of 50 per cent in the total exchangeable pool and of 75 per cent in the extravascular, extracellular protein occurred after both shorter and longer periods of depletion. Under the rigid conditions of these experiments additional plasma protein removal was associated with loss of weight despite adequate caloric intake.

Investigation of the status of the interstitial fluid proteins in other conditions associated with disturbed protein metabolism seems warranted.

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