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Research Article

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Albumin and γ-Globulin Tracer Studies in Protein Depletion States *

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The use of radioactively labeled plasma proteins has contributed to an understanding of the body's adaptation to protein deficiency (1). Such deficiency can occur naturally, i.e., as a result of inadequate dietary protein, or in disease states through abnormal protein loss, e.g., in the nephrotic syndrome or protein-losing enteropathy. Experimental protein depletion can be induced by deprivation of dietary protein or by withdrawal of protein from the plasma by the technique of plasmapheresis.

The present paper is concerned largely with the behavior of labeled plasma albumin in humans with naturally occurring protein deficiency and in mild states of protein depletion induced by dietary protein deprivation; some studies with labeled γ-globulin are included. In addition, protein depletion was induced experimentally in rabbits by restriction of dietary protein and by plasmapheresis. This work was designed primarily to investigate early changes that might occur in mild or subclinical states of protein deficiency.

Methods

Human studies

Subjects. Normal healthy ambulant male volunteers were selected; as far as was reasonably possible, liver disease, kidney disease, and any abnormality of protein metabolism were excluded. A control series was conducted on selected hospital patients to provide "normal" data; these patients were studied during the late convalescent period after mild episodes of myocardial infarction or while receiving therapy for uncomplicated peptic ulceration. In addition a group of patients was selected purely on the basis of low serum protein concentrations. Three suffered from cirrhosis of the liver, but in the remainder hypoalbuminemia was unexplained; in none of this last group was there abnormal fecal or urinary loss of protein, edema, or ascites, and there was no clinical or biochemical evidence of liver disease. Protein malnutrition was thought to be the cause of their low serum albumin levels.

Design of study. The normal controls and hypoalbuminemic patients were given ^{131}I-labeled plasma albumin intravenously, and regular plasma and urinary samples were analyzed for a period of 7 to 10 days. In 18 subjects studies were conducted in a metabolic ward under full metabolic study conditions. In 14 of these (study A) an initial test was performed after a period of equilibration on a normal hospital diet containing 70 g protein per day; the test was repeated after 3 to 6 weeks of low-protein diet (isocaloric, containing 10 g protein per day) and after a similar period of high-protein feeding (isocaloric, containing 150 g protein per day). In six of these subjects simultaneous combined studies were made with albumin-^{131}I and γ-globulin-^{125}I.

In a further four subjects (study B) a single dose of albumin-^{131}I was given intravenously. The initial diet contained 70 g protein per day; after 7 to 10 days the diet was changed to one containing 15 g protein per day, and thereafter, at weekly intervals, the protein content was increased stepwise to the initial level; these diets were all isocaloric. The fate and distribution of the injected albumin-^{131}I were followed throughout this period.

Methods. Pure albumin was prepared initially by fractionation of human plasma through a carboxymethylcellulose column with an acetate buffer (2). Subsequently a modification of the acid-alcohol extraction technique was used (3). Pure γ-globulin was prepared by fractionation through a DEAE-cellulose column with a phosphate buffer (4).

Iodination with ^{131}I and ^{125}I was achieved by use of the potassium iodide/iodate method (5), as ^{131}I was more readily available in thiosulfate solution. In general, iodination was at least 30%. Free iodine was removed by passage through an anion exchange column; trichloroacetic acid precipitation showed not less than 98% of the radioactivity to be protein bound. Carrier albumin or γ-globulin was added to reduce radiation damage, and the whole was Seitz-filtered to render it sterile. The

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TABLE I

Plasma albumin concentration and pool size, catabolic and "synthesis plus transfer" rates in 41 control and 18 hypoproteinemic subjects (means ± standard error)

	Serum albumin concentration		Plasma volume	Plasma albumin pool	Catabolic rate		Synthesis + transfer rate	Weight
	Range	Mean						
	g/100 ml		*ml/kg*	*g/kg*	*Fractional % IVP*/ day*	*mg/kg/ day*	*mg/kg/ day*	*kg*
Control 41 subjects	3.60–5.04	4.12 ±0.07	40.0 ± 0.7	1.64 ±0.04	9.0 ±0.3	148 ± 5	153 ± 5	61.0
Hypoproteinemia 18 subjects	1.91–3.40	2.59 ±0.15	46.8 ± 1.9	1.19 ±0.07	7.2 ±0.5	87 ± 7	105 ± 9	59.7

* IVP = intravascular pool.

final product was always used within 24 to 48 hours of preparation.

The purity of preparation was checked before and after iodination by paper and cellulose-acetate electrophoresis and ultraviolet absorption determination. When these labeled compounds were used, there was no early rise in urinary excretion, such as might indicate components capable of rapid degradation.

For additional plasma volume determinations albumin-^{132}I was used; ^{132}I was eluted daily from a tellurium-^{132}I column, and iodination was performed by the above technique. No significant contamination with ^{131}I was found in these preparations.

In all subjects the thyroidal uptake of ^{131}I released during catabolism was blocked by the administration of Lugol's iodine, 10 drops three times daily, or sodium iodide, 10 mg three times daily, for 24 to 48 hours before injection of the labeled sample and throughout the test period.

For single studies of 7 to 10 days, 10 to 15 μc albumin-^{131}I was administered via an antecubital vein; the same dose of γ-globulin-^{125}I was used for combined studies. For prolonged studies on the four subjects in study B 80 to 100 μc albumin-^{131}I was administered.

In all cases, blood was withdrawn 10 minutes after injection for initial determination of plasma volume; thereafter, samples were removed at 3 hours and daily for the duration of the test. Twenty-four-hour urinary samples were collected throughout. Initially fecal samples were assayed for radioactivity; since these failed to show significant excretion of the label, this practice was subsequently abandoned. In the long-term studies plasma

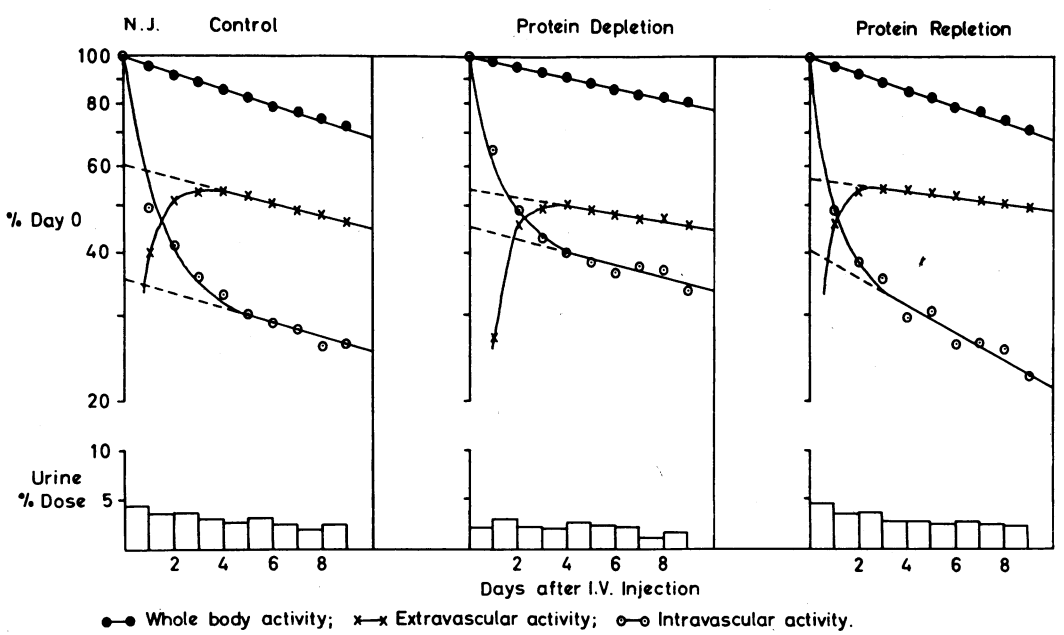

FIG. 1. GRAPH OF WHOLE BODY, INTRAVASCULAR, EXTRAVASCULAR, AND URINARY RADIOACTIVITY DURING CONTROL, PROTEIN DEPLETION, AND PROTEIN REPLETION PERIODS ON A REPRESENTATIVE SUBJECT (STUDY A).

TABLE II

*Albumin pool sizes and ratios and serum concentration in 14 subjects before and after protein depletion and repletion (means ± standard error)**

	g	g/kg
Intravascular pool size		
Control diet	a 102 ± 4	1.71 ± 0.07 c
After protein depletion	89 ± 3	1.58 ± 0.05
After protein repletion	a 107 ± 5	1.79 ± 0.08 b
Extravascular pool size		
Control diet	b 160 ± 10	2.68 ± 0.16 b
After protein depletion	130 ± 8	2.29 ± 0.11
After protein repletion	a 158 ± 6	2.66 ± 0.10 b
	Extra-/intravascular ratio	Serum albumin concentration g/100 ml
Control diet	d 1.60 ± 0.07	4.18 ± 0.12 b
After protein depletion	1.46 ± 0.07	3.89 ± 0.10
After protein repletion	d 1.47 ± 0.05	4.32 ± 0.12 a

* p values: a = 0.0025 to 0.01; b = 0.01 to 0.02; c = 0.02 to 0.10; d > 0.10.

TABLE III

*Gamma-globulin pool sizes and ratios and serum concentration in six subjects before and after protein depletion and repletion (means ± standard error)**

	g	g/kg
Intravascular pool size		
Control diet	45 ± 5	0.77 ± 0.09
After protein depletion	37 ± 3	0.54 ± 0.06
After protein repletion	38 ± 7	0.61 ± 0.13
Extravascular pool size		
Control diet	43 ± 8	0.73 ± 0.13
After protein depletion	33 ± 5	0.56 ± 0.09
After protein repletion	28 ± 5	0.46 ± 0.10
	Extra-/intravascular ratio	Serum globulin concentration g/100 ml
Control diet	0.92 ± 0.11	1.79 ± 0.39
After protein depletion	0.86 ± 0.09	1.73 ± 0.28
After protein repletion	0.76 ± 0.06	1.56 ± 0.59

* No statistically significant differences were observed with dietary change.

volume was determined three times during each week, and mean values were used in calculations. The "equilibrium time" method (5, 6) was used for determination of intra- and extravascular pool size in those cases where a "steady state" could reasonably be held to exist. Catabolic rates were derived from urinary excretion of radioactive iodine as a function of plasma specific activity (5–9). "Synthesis plus transfer" rate was derived according to Matthews (10), where "transfer" refers to net movement of protein from the extra- to the intravascular space per day.

Stable albumin and total protein were measured in duplicate on daily plasma samples by the biuret method (11); stable γ-globulin was determined by cellulose-acetate electrophoresis. All radioactive samples were assayed in an Ecko model N664A well-type scintillation counter. Separation of ^{125}I and ^{131}I was achieved by appropriate voltage discrimination, so that the contribution of each isotope could be determined. Plasma samples taken on the day of plasma volume measurement contained both ^{132}I and ^{131}I; these samples were assayed a second time after the ^{132}I had decayed to negligible activity.

Rabbit studies

Male rabbits weighing approximately 3 kg and obtained from a single source were studied for about 4 weeks after administration of an intravenous injection of 15 μc of rabbit albumin-^{131}I. In the initial period (7 to 10 days) all animals were on a normal diet (15% protein per 100 g food).[1] In the next phase of 7 to 10 days one group received a low-protein diet (5% protein per 100 g),[1] and the other was subjected to daily plasmapheresis while on a normal diet. All animals were studied in a third "recovery" phase. Rabbits were kept in separate cages and weighed daily. Daily food was also weighed, and drinking water containing 0.005% sodium iodide was administered throughout each experiment, starting 48 hours before.

Pure rabbit albumin was prepared, iodinated with ^{131}I, and tested by the methods used for human albumin. Initial plasma volumes were determined from blood samples

[1] Prepared in pellet form by Vereeniging Milling Co., Cape Town, South Africa.

TABLE IV

*Catabolic rates for albumin and γ-globulin after protein depletion and repletion (means ± standard error)**

	g/day	g/kg/day	Fractional % IVP/day
Albumin			
Control diet	a 8.9 ± 0.5	0.151 ± 0.009 a	8.8 ± 0.5 a
After protein depletion	5.7 ± 0.3	0.103 + 0.006	6.5 ± 0.4
After protein repletion	a 8.6 ± 0.5	0.146 ± 0.008 a	8.2 ± 0.5 b
Globulin			
Control diet	c 5.7 ± 0.7	0.095 ± 0.011 c	10.8 ± 1.0 c
After protein depletion	5.0 ± 0.6	0.085 ± 0.011	11.3 ± 1.8
After protein repletion	c 4.6 ± 0.6	0.075 ± 0.011 c	11.0 ± 1.6 c

* p values: a < 0.001; b < 0.0025; c = NS.

TABLE V

*"Synthesis plus transfer" rates for albumin and γ-globulin after protein depletion and repletion (means ± standard deviation)**

	g/day	g/kg/day
Albumin		
Control diet	a {8.9 ± 0.4	0.150 ± 0.009 } a
After protein depletion	a {5.8 ± 0.3	0.103 ± 0.006 } a
After protein repletion	a {9.0 ± 0.4	0.152 ± 0.008 } a
Globulin		
Control diet	b {6.1 ± 0.9	0.102 ± 0.014 } b
After protein depletion	b {5.0 ± 0.6	0.085 ± 0.011 } b
After protein repletion	b {4.7 ± 0.7	0.076 ± 0.013 } b

* p values: a < 0.001; b = NS.

taken 5 minutes after injection; thereafter daily plasma volumes were estimated with rabbit serum-^{132}I. Rabbits were counted daily in a ring counter of 6 Geiger-Muller tubes; in initial experiments whole body activity assayed in this manner had been shown not to differ significantly from the figure derived from serial subtraction of cumulative daily excretion of ^{131}I.

Plasmapheresis was performed under sterile conditions by withdrawal of 40 to 45 ml blood from a marginal ear vein. After centrifugation and removal of plasma, the red cells were suspended in saline and reinjected. The interval between withdrawal and reinjection was approximately 30 minutes.

Data were analyzed by the techniques referred to above. During plasmapheresis periods a correction was applied for the radioactivity removed in the plasma, before urinary loss was derived.

Results

Table I shows the results obtained in control subjects and hypoalbuminemic patients. The catabolic rate (absolute and fractional) in hypoalbuminemic subjects was significantly lower than in controls.

Figure 1 shows graphed data on a representative subject in study A. It illustrates the order of change found at each stage, i.e., after 3- to 6-week periods of low- and high-protein diets.

Table II shows the mean figures for albumin pool sizes in the 14 subjects in this study. Both intra- and extravascular albumin pools were reduced after low-protein feeding; these changes were rapidly reversed by high-protein feeding. Plasma albumin levels fell and rose in a similar manner. The extravascular/intravascular ratio of albumin fell slightly after low-protein feeding and rose after repletion; this alteration in ratio was not highly significant ($p \simeq 0.1$).

Table III shows γ-globulin pool sizes in six subjects who received both albumin and globulin tracers. No significant differences were shown between total, extra-, or intravascular pool sizes after dietary manipulation.

Catabolic rates for albumin and γ-globulin are shown in Table IV. Values are expressed in terms of absolute amounts catabolized per day, as well as fractional rates (percentage of intravascular pool per day). Significant reduction of albumin catabolism was found to follow low-protein feeding, with reversal towards normal levels after repletion; this change was reflected in both absolute and fractional catabolic rates. The γ-globulin rates showed no significant change throughout the study.

Table V presents the figures derived for synthesis plus transfer rate. The rate for albumin fell after low-protein feeding and rose after refeeding (parallel to the changes in catabolic rate). Gamma-globulin rates showed no significant change.

Table VI shows mean albumin catabolic rates, plasma albumin concentrations and pool sizes, and synthesis plus transfer rates in the four subjects studied on weekly changes of protein intake (study B). In each case the catabolic rate reached its

TABLE VI

Study B: albumin data on varying protein diets (mean values in four patients)

Period	Protein intake	Plasma albumin concentration	Intravascular pool	Catabolic rate ± SE		Synthesis + transfer rate	Weight
	g/day	g/100 ml	g/kg	mg/kg/day	% IVP/day	mg/kg/day	kg
I	73	4.23	1.83	173 ± 5	9.8 ± 0.6	174	60.6
II	15	4.13	1.77	160 ± 5	9.3 ± 0.5	163	60.4
III	27	4.07	1.74	143 ± 3	8.5 ± 0.4	142	59.9
IV	43	4.17	1.81	164 ± 5	9.4 ± 0.5	165	59.9
V	58	4.27	1.91	178 ± 4	9.8 ± 0.5	177	60.3
VI	72	4.30	1.83	185 ± 5	10.4 ± 0.5	191	60.5

TABLE VII

Rabbit albumin studies: low-protein feeding and plasmapheresis

Rabbit no.	Plasma albumin concentration			Plasma albumin pool			Catabolic rate						Synthesis plus transfer rate			Weight		
	A*	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
	g/100 ml			*g/kg*			*mg/kg/day*			*% IVP/day*			*mg/kg/day*			*kg*		
Low-protein diet																		
A1	3.31	3.06	3.40	1.22	1.24	1.33	303	267	336	24.9	20.9	24.7	271	260	336	3.1	3.0	3.1
D1	4.04	4.33		1.99	1.90		960	380		48.4	20.1		960	400		3.0	2.9	
D2	3.99	3.96		1.63	1.59		700	490		43.2	30.6		710	500		2.8	3.0	
E4	4.00	4.12		2.09	1.71		485	376		23.2	21.9		479	376		3.4	3.3	
G1	3.65	3.64	3.32	1.47	1.39	1.44	637	407	600	43.1	29.5	40.6	630	438	569	3.0	2.9	2.6
L3	3.38	3.50	3.41	1.71	1.63		905	465		52.1	28.6		886	474		2.2	2.3	
L4	3.86	3.77	3.66	1.75	1.60		738	454		42.7	28.4		731	458		2.6	2.6	
Mean	3.75	3.77		1.69	1.58		675	406		39.7	25.7		667	415		2.9	2.9	
p<	NS†			NS			0.0125			0.01			0.025					
Plasmapheresis																		
F1	3.21	3.29	3.75	1.47	1.37	1.46	222	170	218	15.1	8.1	15.0	230	381	214	2.8	2.8	2.8
F2	3.85	3.62	3.81	1.94	1.73	1.67	412	327	384	21.2	20.0	22.9	412	689	384	2.5	2.4	2.5
H3	3.53	2.81	3.57	1.39	1.22	1.39	578	397	520	43.7	32.6	37.3	578	823	530	3.2	3.0	3.0
J3	3.55	3.26	3.10	1.71	1.47	1.41	580	335	364	33.1	21.3	25.1	588	688	344	2.5	2.6	2.5
O4	3.63	3.12	3.10	1.70	1.48	1.31	544	270	342	32.4	18.1	25.9	538	661	360	3.2	3.2	3.3
Mean	3.55	3.22	3.47	1.64	1.45	1.45	467	300	366	29.1	20.0	25.2	469	648	366	2.8	2.8	2.8
p	0.05	NS		0.10	NS		0.05	NS		0.10	NS		0.05	0.01				

* A = control period; B = low-protein diet or plasmapheresis period; C = "recovery" period.
† Refers to the difference between the two mean values listed immediately above.

lowest point in the third week of study and gradually increased as the protein intake was augmented. Despite the very low protein intake of period II, the fractional catabolic rate did not alter significantly; the fall in this measurement during period III, however, was significant ($0.05 > p > 0.025$). This change was found in the absence of significant alteration in plasma albumin concentration or mass and was reflected in measurements of both absolute and fractional catabolic rate. Synthesis plus transfer rates followed the same pattern as catabolic rates.

Table VII shows the over-all results of the rabbit experiments. Seven rabbits were studied during low-protein feeding. In all cases there was a marked fall in albumin catabolic rate, both absolute and fractional. Synthesis plus transfer rate altered significantly in the same direction. In only two rabbits was it possible to complete the recovery phase studies: reversion towards normal was noted in both.

Plasmapheresis was performed in five rabbits. Again a marked fall in catabolic rate was observed, but in contrast to the rabbits fed low-protein diets, a substantial increase in synthesis plus transfer rate was noted. Studies after plasmapheresis showed a return towards control values.

Discussion

Albumin and γ-globulin pool sizes. The exposure of subjects to low-protein diets might be expected to lead to a reduction in total body albumin mass. The extent of this reduction and its partition between intra- and extravascular compartments is difficult to determine where steady-state conditions are not known to exist. The determination of intravascular pool size depends upon measurement of plasma volume and albumin concentration; within the limits of technical accuracy, this is valid even where dynamic equilibrium does not exist. Much more difficulty obtains in the interpretation of extravascular and total body albumin mass measurements. The equilibrium time method, which we have used, is not valid where conditions are "unsteady" (10, 12). Within each week of measurement in study A, there was no significant change in plasma albumin concentration or mass or in body weight, but the over-all design of the investigation makes unstable conditions possible, and constancy of these measurements does not necessarily indicate equilibrium. For this reason the findings with regard to extravascular or total body albumin mass must be accepted with some reserve.

In study A a distinct fall in intravascular albumin pool followed low-protein feeding, a change that was reversed by high-protein diet. At the same time the extravascular pool appeared to fall and then increase. Total body albumin was found to fall by an average of 16% after low-protein feeding.

Payne and Done, using tritiated albumin in pigs, found a preponderant fall in extravascular albumin pool size after low-protein diets (13). Yuile and his associates (14), Wasserman, Joseph, and Mayerson (15), and Matthews (10), on the basis of plasmapheresis and bleeding in experimental animals, produced excellent evidence to favor the hypothesis that the intravascular albumin mass is kept constant by transfer of albumin from the extravascular pool. Cohen and Hansen (16) reached the same conclusion after measurement on infants with kwashiorkor before and after protein feeding. Gitlin and Janeway (17), studying the behavior of rabbit antibodies, demonstrated the rapid movement of preformed plasma proteins from the extravascular pool into the circulation when the latter compartment was depleted of a specific protein. Despite limited statistical significance, our finding of a fall in extra-/intravascular ratio lends support to this general thesis.

If this is so, it is possible that a degree of depletion of body albumin stores can occur before it is reflected by lowering of serum albumin concentration. In some individuals in our study A a distinct reduction in extravascular albumin was thought to exist without change in plasma albumin concentration [(18) and further observations on additional subjects in this study].

In general, from this and other studies (19–21) it seems that beyond a certain point of depletion the plasma albumin level provides a reasonable index of the degree of protein depletion in any individual, although it is possible that, in the early stages of depletion at least, this concentration is maintained at a normal level by transfer of albumin from the extravascular pool.

In our subjects studied with γ-globulin-^{125}I, no significant changes were found in pool size measurement. There was, however, a progressive fall in plasma γ-globulin pool size and concentration throughout the study, even after protein repletion. The initial γ-globulin concentrations were high and might reflect response to chronic exposure to environmental influences before hospital admission; after several months of "protection" within the hospital, these levels dropped to those more commonly found. The situation might be analogous to the findings of Cohen, McGregor, and Carrington (22), whose Gambian subjects exposed to malaria in West Africa showed higher γ-globulin levels than did a control group in the United Kingdom.

Little information is available about γ-globulin metabolism in protein malnutrition. Weech, Goettsch, and Reeve (23) and Zeldis, Alling, McCoord, and Kulka (24) showed that low-protein feeding resulted in a lowered serum albumin concentration, but there was no corresponding fall in globulin concentration. It was suggested that plasma globulin might enjoy a prior demand on the total available pool of body protein materials. In kwashiorkor serum albumin levels are invariably lowered, whereas globulin, especially γ-globulin, levels are normal or, often, elevated (16, 21, 25, 26). Cohen and Hansen (16) considered that the distribution of γ-globulin was not affected by the state of malnutrition in their kwashiorkor studies.

In study B, because of the weekly change in conditions, an unsteady state was highly probable, and no attempt was made to determine total or extravascular albumin pool sizes. Plasma albumin concentrations and mass failed to show significant changes, a finding that is of interest in view of the changes in catabolic rate to be discussed later.

Albumin and γ-globulin catabolism. Measurement of the catabolic rate of a labeled protein by expressing urinary radioactivity in terms of plasma specific activity is regarded as valid even where pool masses are varying (5–9). The method could be misleading if there was significant loss of unmeasured radioactivity in the feces. The possibility of such fecal loss in young children with kwashiorkor has been debated (16, 27, 28), but in general, the degree of loss does not appear sufficient to affect conclusions about catabolic rates (16). In our adult studies fecal radioactivity was negligible, and measurement of feces was later abandoned.

Children with kwashiorkor show a considerable reduction in albumin catabolic rate (16, 29–32), which returns to normal after recovery. In our adult studies (A and B) we found consistently

reduced rates after low-protein feeding, with reversal to normal after repletion; this change affected fractional, as well as absolute, catabolic rates. The same phenomenon has been observed in rats on a protein-free diet (33) and in our rabbits (Table VII). It is apparent that these rabbits showed unusually high catabolic rates, even in the control period; for several reasons denaturation of the labeled albumin product is not considered likely, and a difference in strain, diet, or other environmental factors may be responsible. Since each rabbit acted as its own control before and after the experimental period and since the changes induced by low-protein feeding and plasmapheresis were both large and clearly divergent, we feel that the high catabolic rates do not affect the conclusions drawn from the experiment.

Waterlow (30) has questioned whether changes in protein metabolism vary with the level of protein intake at the time of the test or with the state of depletion; he concluded that both factors play a part in determining albumin catabolism. Reduction of dietary protein is obviously not the only cause of low albumin catabolic rates, since they are found in human subjects with proteinuria (34) or cirrhosis of the liver (7) as well as in experimental animals subjected to plasmapheresis, where ad libitum feeding is permitted [(10) and our own study]. In our series of hypoalbuminemic subjects (Table I) low catabolic rates were also found without regard to the dietary intake of protein. A fall in albumin catabolism, as suggested by Bauman, Rothschild, Yalow, and Berson (34) and Friedberg (35) appears to be more closely related to reduction in the albumin pool than to protein intake. Our study B provides further evidence that this is so, as these subjects showed the lowest catabolic rates in the third week, when the dietary protein intake, although low, was actually higher than in the second week; this seems to reflect the continued fall in albumin pool size. The whole process of reduction of catabolism appears to provide a relatively prompt and efficient means of conserving albumin in the face of protein depletion or deprivation.

In a series of papers based on experimental reduction and expansion of the albumin pool size Rothschild and his colleagues (36–39) concluded that the quantity of albumin degraded was related to albumin concentration or pool size. It would be of interest to know whether a fall in albumin catabolic rate is brought about by reduction of the serum albumin concentration or by contraction of either the intra- or extravascular pool. In our study A several individual subjects maintained their serum albumin concentrations and pool sizes at control levels during protein deprivation, yet catabolic rates fell significantly. In study B the serum albumin mass and concentration remained relatively constant during the early weeks, whereas catabolism was reduced. In our rabbits on low-protein diets there was no significant change in serum albumin level or pool size, yet, again, catabolic rates fell considerably. These facts do not necessarily preclude the primary role of the intravascular pool in the determination of catabolic rates. It is possible that an immediate effect of reduced protein intake is reduced albumin synthesis, which would tend to diminish the intravascular pool. This tendency could be met by a lowering of catabolic rate and, at the same time, increased transfer of albumin from the extravascular pool—measures that would protect the intravascular pool. For a while, at least, the serum albumin concentration or mass could be maintained at normal levels, but a lowered catabolic rate would reflect the threatening depletion.

Gamma-globulin catabolic rates in six subjects in study A did not alter significantly. Although this conforms to the experience of others (16, 40), it should be noted that Freeman and Gordon (33) found a drop in catabolism in their protein-deprived rats. The relative constancy of the fractional catabolic rate for γ-globulin in our study A contrasts strikingly with the behavior of simultaneously administered albumin.

Albumin and γ-globulin synthesis. In contrast to the measurement of catabolic rates, direct methods for the determination of synthesis rates for albumin and γ-globulin have not yet been applied to problems of protein depletion. In this study the over-all figure derived for synthesis plus transfer fell and rose in parallel with catabolism when dietary protein was varied. Theoretically, the fall on low-protein diet could be due entirely to diminished net transfer from the extravascular pool. Our evidence, as well as that of others cited earlier, indicates that the opposite effect obtains in protein depletion, i.e., net transfer from the extravascular pool is actually increased. If this is so,

the fall in true synthesis rate for albumin is even more significant than our figures suggest.

Cohen and Hansen (16) and Purves and Hansen (31) reported low albumin synthesis rates in children with kwashiorkor studied on low-protein diets and concluded that recovery was accompanied by a considerable increase in synthesis. Picou and Waterlow (29), studying kwashiorkor, reported it as "probable that the rate of synthesis was reduced during the development of depletion," i.e., during the exposure to low-protein intake. Freeman and Gordon (33) found the synthesis plus transfer rate for albumin to fall in rats on a protein-free diet. On the basis of these and our own studies, it seems likely that a genuine fall in albumin synthesis occurs when dietary protein is restricted.

In our six subjects in study A no significant variation was found in γ-globulin synthesis. Cohen and Hansen (16) were also unable to correlate changes in globulin synthesis with changes in diet and showed that protein-deficient children were capable of producing large amounts of globulin, even though they were incapable of synthesizing albumin. They suggested that γ-globulin–forming cells might have priority claims on available amino acids. Freeman and Gordon (33), on the other hand, found γ-globulin production to be depressed in their protein-deprived rats; this difference may be due to the fact that their exclusion of protein from the diet was far more rigid.

Our rabbits exposed to low-protein diets showed the same fall in albumin synthesis plus transfer rate. In plasmapheresis experiments, on the other hand, we confirmed the finding of Matthews (10) that this rate was greatly increased. Matthews (12) subsequently used an analogue computer to compare her experimental results with various theoretical possibilities and concluded that the most important compensating change for the loss of plasma protein was an increase in synthesis rate, as she found only a slight fall in catabolic rate. In our experiments the increase in synthesis plus transfer rate was approximately that same as the fall in catabolic rate, suggesting that both compensatory adaptations participated equally.

Urinary loss of protein, as in the nephrotic syndrome, is in some ways analogous to experimental removal by plasmapheresis. Bauman and coworkers (34) did not find increased albumin synthesis in a series of patients with albuminuria and regarded decreased catabolism as the major compensatory reaction to the urinary loss. In contrast, Blahd, Fields, and Goldman (41) found increased albumin production in the nephrotic syndrome when dietary protein intake was high. These findings may correspond to our plasmapheresis experiments with ad libitum feeding and suggest that albumin synthesis will increase when protein is lost from the body, provided dietary nitrogen is adequate. In their series of experiments Rothschild and his colleagues (36–39) concluded that synthesis of albumin was regulated by the colloidal osmotic pressure of plasma, not by the albumin concentration. This problem may not finally be answered until a direct method of measuring albumin synthesis is applied.

On the basis of our own and other studies discussed above, it is possible to formulate a hypothesis regarding the body's adaptation to protein depletion, whether this results from dietary insufficiency or loss or removal of protein. An early effect of dietary restriction is reduced albumin synthesis; if catabolism continues at a normal rate, diminution of the intravascular albumin pool will after some time result. Two adaptive measures ensue; more albumin is transferred from the extravascular pool, and less albumin is catabolized. These two measures would tend to maintain the size of the intravascular pool at the expense of the extravascular. Initially this might succeed, so that a normal intravascular pool might be found with a reduced catabolic rate. In this event the fractional catabolic rate will also be lowered. As deprivation continues, this compensation fails, and diminution of both intra- and extravascular pools will occur; because of the reduced intravascular pool, the fractional catabolic rate may now return to normal levels. Thus the absolute catabolic rate will always be low, but the fractional rate will depend on the extent and duration of protein depletion. It is generally believed that catabolism of albumin is a first-order process (42); this relationship might be masked in some cases of depletion by the presence of accessory compensating reactions.

When depletion results from loss or removal of body protein, an immediate reduction in intravascular albumin pool ensues. The same two adaptive measures come into force, i.e., reduced catabo-

lism and increased transfer from the extravascular pool. In this case, provided dietary protein is adequate, a third compensatory mechanism operates, viz., increased synthesis.

Summary

Albumin-^{131}I and γ-globulin-^{125}I were administered to human volunteers under conditions of varying dietary protein. Protein depletion was produced in rabbits by low-protein feeding and by plasmapheresis. Low-protein diets in humans appeared to lead to decreased albumin catabolism, a reduction in intravascular albumin pool, and probable increased transfer of albumin from the extravascular pool; synthesis of albumin was probably reduced. Gamma-globulin metabolism did not appear to alter significantly under these conditions.

Rabbits fed low-protein diets behaved in the same way as humans, but plasmapheresis was found to cause increased synthesis of albumin provided ad libitum feeding was permitted.

On the basis of these investigations, a hypothesis is proposed to account for the changes that ensue when protein depletion is produced by dietary deprivation or plasmapheresis.

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