DISTRIBUTION AND METABOLISM OF I^{131} LABELED HUMAN SERUM ALBUMIN IN CONGESTIVE HEART FAILURE WITH AND WITHOUT PROTEINURIA

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The role of serum albumin in the causation of cardiac edema has been disputed. Starling (1) postulated that circulatory stasis resulting from cardiac insufficiency is responsible for anoxia and increased permeability of the capillary membrane, the consequence of which is the passage of albumin and water into the extravascular spaces. Although Smirk (2) observed evidence of increased capillary permeability to water and crystalloids in congestive heart failure, Stead and Warren (3) demonstrated, by direct analysis, that the protein concentration of cardiac edema fluid was not elevated above that of the edema fluid produced by tourniquet stasis in normal subjects. Other studies have directed attention to the low serum albumin concentrations occasionally encountered in heart failure (4–7), and have suggested or implied (4, 8) a causal relationship to the pathogenesis of cardiac edema. Abnormalities in liver function tests and morphologic evidence of liver injury in severe congestive failure (9–14) have appeared to strengthen the possibility that hepatic synthesis of albumin may be impaired in this condition. Alternatively, low serum albumin concentrations have been attributed to inadequate dietary protein intake or poor absorption from a congested gastrointestinal tract (15, 16).

Since the plasma concentration of albumin is not an adequate measure of the total quantity of circulating and extravascular albumin stores, it was of interest to measure the amount of total exchangeable albumin in subjects with heart failure employing I^{131} labeled albumin as a tracer. The rate of metabolism of I^{131} labeled albumin was also studied with the aim of evaluating the ability of the subject in heart failure to synthesize albumin. Although there may yet remain some reservations regarding the validity of this tracer in the investigation of serum albumin metabolism, the present study was designed to eliminate several objections posed previously (17). Observations on the effect of proteinuria on albumin distribution and metabolism are also presented.

METHODS

Twenty hospitalized patients were divided into groups according to the lot of I^{131} labeled albumin employed. There were ten patients in heart failure, five of whom had proteinuria, and ten non-cardiac control subjects, two of whom had marked proteinuria. In each of the first three groups there were cardiac and non-cardiac control subjects: Group IV consisted of two non-cardiac control subjects and one patient with myxedema. In addition, the data pertaining to ten subjects without heart failure or proteinuria, who were studied in a manner identical with that in the present study, using lots of I^{131} labeled albumin free from excessive amounts of rapidly degraded components (17), are included as controls. Both control groups included normal volunteers as well as patients with such chronic illnesses as rheumatoid arthritis, uncomplicated duodenal ulcer, and well controlled diabetes mellitus. Diet and therapy were not regulated in any particular fashion for the purpose of this study but were dictated by individual requirements. For the experimental group only patients with markedly advanced and intractable congestive heart failure were chosen.

I^{131} labeled albumin was prepared according to the method of Newerly (18) and contained 1 to 2 iodine atoms per molecule of protein. The method consists essentially in the oxidation of iodide to elemental iodine by the method of Pressman and Eisen (19), extraction of the oxidized iodine with chloroform, and slow addition of the chloroform-iodine solution to an appropriate dilution of pooled human serum albumin. Non-protein bound iodine was removed by dialysis against normal saline solution for 48 hours following which the solutions were sterilized by passage through Seitz filters and cultured before use. The final products contained less than 2 per cent non-protein bound radioactivity as determined by precipitation with 10 per cent cold trichloracetic acid.

Weighed solutions of the tagged albumin containing 85 to 115 μc I^{131} and 1 to 10 mg. protein were administered intravenously. Thyroidal uptake of radioiodine was

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inhibited by the administration of 10 drops of Lugol's solution daily throughout the period of observation. Heparinized blood samples were taken without tourniquet stasis 15 minutes, 2, 6, 24, 30, and 48 hours after injection and at almost daily intervals thereafter for the next 25 to 35 days. Complete urinary collections were made daily. All samples were assayed in a well type scintillation counter with a sensitivity of 1.00×10^6 counts per min. per μc I^{131} above a background of about 200 counts per min. Serum albumin concentrations were determined at weekly intervals by the method of Kingsley (20). Urinary albumin concentrations were determined by the biuret method of Foster, Rick, and Wolfson (21).

The data were analyzed according to methods previously described (17), and only a brief summary is given here for the purpose of orientation. Plasma volume was determined from the I^{131} labeled albumin space of dilution, 15 minutes after administration (22). Total circulating albumin was calculated as the product of serum albumin concentration and plasma volume, and total exchangeable albumin (TEA) was calculated from the total plasma albumin and the relative apparent spaces of distribution at 15 minutes and at distribution equilibrium (Figure 1). When necessitated by incompleteness of urinary collections, appropriate corrections for the apparent spaces of distribution were made as previously described (17). Extravascular albumin was determined from the difference between total exchangeable albumin and total circulating albumin.

In the steady state, total albumin synthesis is equal to the sum of albumin degraded and albumin lost in the urine and was calculated independently from urine and plasma data as previously described (Figures 1, 2) (17):

1) Total albumin synthesis equals the product of total exchangeable albumin and the rate constant of decrease of I^{131} labeled albumin in plasma after distribution equilibrium (Figures 1, 2).

2) Total albumin synthesis equals the product of total exchangeable albumin and the fraction of total iodoalbumin remaining in the body which is excreted daily after distribution equilibrium (Figures 1, 2).

In the subjects with proteinuria, total urinary radioactivity was fractionated by precipitation with cold 10 per cent trichloracetic acid into that derived from albumin metabolism (non-protein bound urinary radioactivity) and that resulting from albuminuria (protein bound urinary radioactivity). The partition of radioactivity between the two fractions was strikingly constant from day to day in individual subjects (Figure 2).

Albumin loss due to proteinuria was calculated from the product of the total exchangeable albumin and the

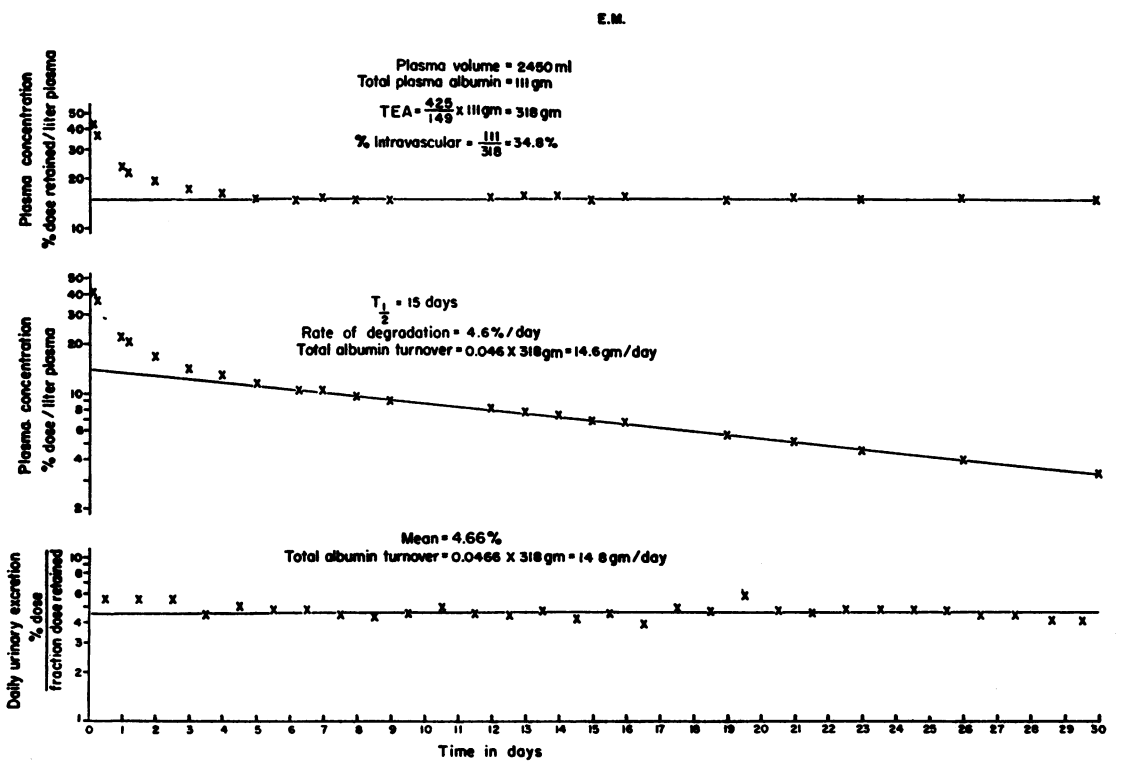

FIG. 1. TYPICAL SET OF CURVES AND CALCULATIONS (A NON-CARDIAC SUBJECT WITHOUT PROTEINURIA)

The mean per cent urinary excretion $\left(\frac{\%\text{ dose}}{\text{fraction dose retained}}\right)$ was obtained from the values between the sixth and thirtieth day inclusive.

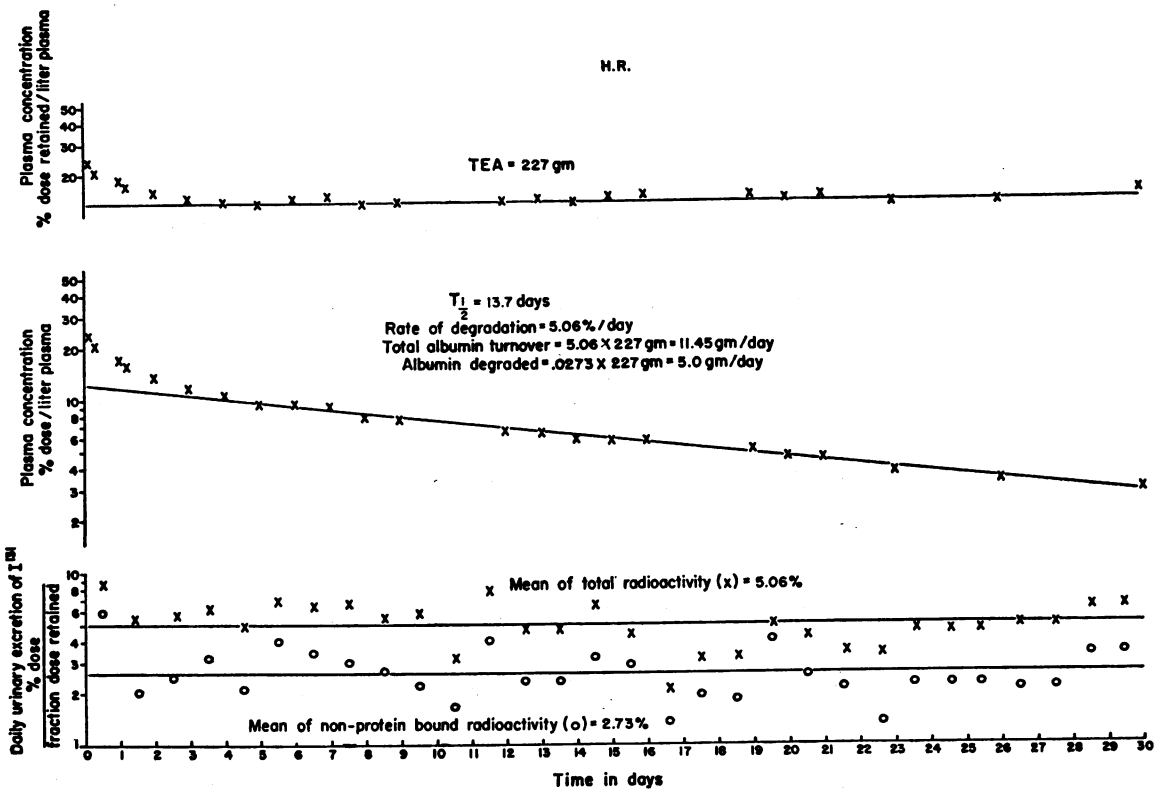

FIG. 2. TYPICAL SET OF CURVES AND CALCULATIONS (A CARDIAC SUBJECT WITH PROTEINURIA)

The mean per cent urinary excretions $\left(\frac{\%\text{ dose}}{\text{fraction dose retained}}\right)$ were obtained from the values between the sixth and thirtieth days inclusive.

fraction of total radioactivity remaining in the body excreted in protein bound form daily. The values obtained by this method agreed reasonably well with those obtained by direct chemical analysis of urinary albumin (Table I). In two subjects with proteinuria, A. M. and M. C., urinary radioactivity data were incomplete and radioactive albumin losses in the urine were calculated from the plasma I^{131} labeled albumin disappearance curves and the fraction of total urinary radioactivity which was precipitable.

RESULTS

Results for individual subjects are given in Table I, and the mean values for different groups are compared in Table II. Serum albumin concentrations varied considerably from patient to patient, but the mean concentration in the cardiac group as a whole was only slightly lower than that of the present non-cardiac group and insignificantly lower than that of the control group studied previously. Cardiac subjects without proteinuria demonstrated no decrease in serum albumin level.

Plasma volumes in the subjects with heart failure were definitely increased over those of both non-cardiac control series, which is in agreement with many previous studies (23–27). Total circulating albumin was therefore actually greater in cardiac subjects without proteinuria (165 gm.) than in control subjects (140 gm.). The mean value for total exchangeable albumin in the non-proteinuric cardiac group (5.45 gm. per Kg. body weight) was higher than in the two control groups (5.07 gm. per Kg. and 5.18 gm. per Kg., respectively). Two of the proteinuric cardiac subjects (H. R. and W. J.) had diminished values for circulating and total exchangeable albumin but the lowest values were obtained in the two patients with glomerular nephritis who were free of heart failure. These findings were not related to differences in the degree of proteinuria.

Average values for the intravascular-extravascular partition of albumin were quite similar in all groups, with the plasma containing about 40

TABLE I

Distribution and degradation of I^{131} labeled human serum albumin in chronic congestive heart failure *

Group	Subject	Diagnosis	Age	Weight Kg.	Mean serum albumin concentration gm./100 ml.	Plasma volume ml.	TEA gm.	TEA gm./Kg.	% Iodoalbumin intravascular	Data from plasma concentration curves: $T_{\frac{1}{2}}$ days	Data from plasma concentration curves: %/day	Data from plasma concentration curves: Total albumin synthesis gm./day	Data from plasma concentration curves: Total albumin synthesis mg./Kg./day	Data from urinary excretion curves: Albumin degradation gm./day	Data from urinary excretion curves: Albumin degradation mg./Kg./day	Data from urinary excretion curves: Albumin lost in urine† gm./day	Data from urinary excretion curves: Albumin lost in urine† mg./Kg./day	Chemically determined proteinuria gm./day
I	C. E.	UHD	47	88.7	4.24	4,000	454	5.13	37.2	20.0	3.46	15.7	177	D.I.				
	C. W.	ASHD	53	70.4	3.95	4,220	442	6.28	37.6	22.0	3.15	13.9	198	11.5	163	1.9	28	(3†)
	J. G.	RHD	61	84.9	4.90	3,870	406	4.78	46.7	17.3	4.00	16.3	191	15.1	178			
	R. H.	P, Control	25	78.5	4.51	3,080	352	4.58	39.6	15.5	4.47	15.7	200	15.2	194			
	E. F.	Conv. malaria, Control	24	76.9	5.18	3,490	490	6.38	36.9	21.5	3.23	15.8	205	15.6	203			
	D. G.	ASPVD, Control	57	75.0	4.90	2,565	383	5.12	32.9	18.2	3.81	14.6	195	14.5	194			
II	H. R.	HHD	58	66.0	3.10	4,260	227	3.44	58.5	13.7	5.06	11.5	174	6.5	97	5.0	77	7.2
	R. J.	ASHD	73	65.8	4.24	3,905	371	5.64	40.2	18.8	3.71	13.8	209	D.I.				
	W. J.	ASHD, Diab M., K.W.	57	76.0	3.20	2,690	315	4.15	29.0	8.3	8.35	26.3	347	12.8	168	11.6	153	9.5
	F. B.	RHD, HSBE	29	65.3	3.71	3,890	291	4.44	49.6	18.0	3.85	11.2	172	9.9	152	1.1	17	1.5
	E. M.	Diab M., Control	43	57.3	4.52	2,450	318	5.54	34.8	15.0	4.60	14.6	255	14.8	258			
	E. W.	Duo. ulcer, Control	43	85.9	4.26	2,900	324	3.77	38.0	12.5	5.53	17.9	207	16.6	194			
III	A. H.	ASHD	58	65.5	3.71	3,860	408	6.20	35.3	20.0	3.46	14.1	216	D.I.				
	E. T.	RHD	65	54.6	3.73	3,080	302	5.53	38.2	18.5	3.74	11.3	207	10.1	184			
	A. M.	RHD	50	54.6	3.65	3,830	343	6.30	40.9	24.0	2.89	9.9	182	D.I.		3.1‖	56	3.0
	A. R.	G-N, Control	37	70.4	1.75	3,580	128	1.82	50.5	5.5	12.55	16.1	229	D.I.		10.1‖	141	7.0
	D. S.	P, Control	41	63.3	4.68	3,250	331	5.20	46.4	18.0	3.85	12.4	197	13.2	208			
IV	M. C.	G-N, PFE	56	46.8	1.76	2,400	98	2.10	43.0	5.9	11.70	11.5	246	7.2	154	4.5	96	4.2
	A. Z.‡	Appendicitis	25	60.5	4.86	2,665	291	4.81	44.6	13.7	5.10	14.9	247	15.2	251			
	M. Co.§	Myxedema, ASHD	64	68.0	3.73	3,120	427	6.43	26.7	33.6	2.06	8.8	129	8.2	121			

* TEA —Total exchangeable albumin.
D.I. —Data insufficient.
UHD —Undiagnosed heart disease.
ASHD—Arteriosclerotic heart disease.
RHD —Rheumatic heart disease.
P —Psychoneurosis.
ASPVD —Arteriosclerotic peripheral vascular disease.
HHD —Hypertensive heart disease.
Diab M.—Diabetes mellitus.
K.W. —Kimmelstiel-Wilson Syndrome.
G-N —Nephrotic phase of glomerular nephritis.
HSBE—Healed subacute bacterial endocarditis.
PFE —Pulmonary fibrosis and emphysema.

† Calculated from protein bound radioactivity in the urine.
‡ Study carried out during convalescence from an attack of acute appendicitis. Appendectomy performed 5 months later.
§ No definite evidence of congestive heart failure.
‖ Determined from precipitable fraction of urinary radioactivity and plasma concentration curve (see Methods).

TABLE II

Mean values for control subjects and subjects with congestive heart failure with and without proteinuria

	Groups	Number of subjects	Mean serum albumin concentration *gm./100 ml.*	Plasma volume *ml./Kg.*	TEA	TEA / *Kg.*	% Iodoalbumin intravascular	$T_{\frac{1}{2}}$ Plasma curve in days	Total albumin synthesis daily *gm.*	Total albumin synthesis daily *mg./Kg.*
I	(a) All controls*	9	4.05	43.8	302	4.37	40.7	14.0	14.8	220
	(b) All cardiacs	10	3.84	55.5	356	5.19	41.3	18.1	14.4	207
II	With proteinuria									
	(a) Controls	2	1.76	51.1	113	1.96	46.8	5.7	13.8	238
	Cardiacs	5	3.52	57.9	323	4.92	43.1	17.2	14.6	214
III	Without proteinuria									
	(a) Controls*	7	4.71	41.4	356	5.07	39.0	16.3	15.1	215
	(b) Previous controls†	10	4.18	47.5	325	5.18	38.9	17.8	13.0	205
	(c) Cardiacs	5	4.16	53.1	389	5.45	39.5	19.0	14.2	200

* Myxedematous subject M. Co. not included.
† See Berson, Yalow, Schreiber, and Post (17).

per cent of the total exchangeable albumin. Significant individual variation was noted in only three subjects. Two cardiac subjects with marked proteinuria (H. R. and W. J.) had 59 per cent and 29 per cent of their albumin stores intravascularly, respectively; the third subject with myxedema but without proteinuria had only 27 per cent circulating in the plasma.

As previously observed (17), at least two rates were involved in the equilibration of plasma iodo-

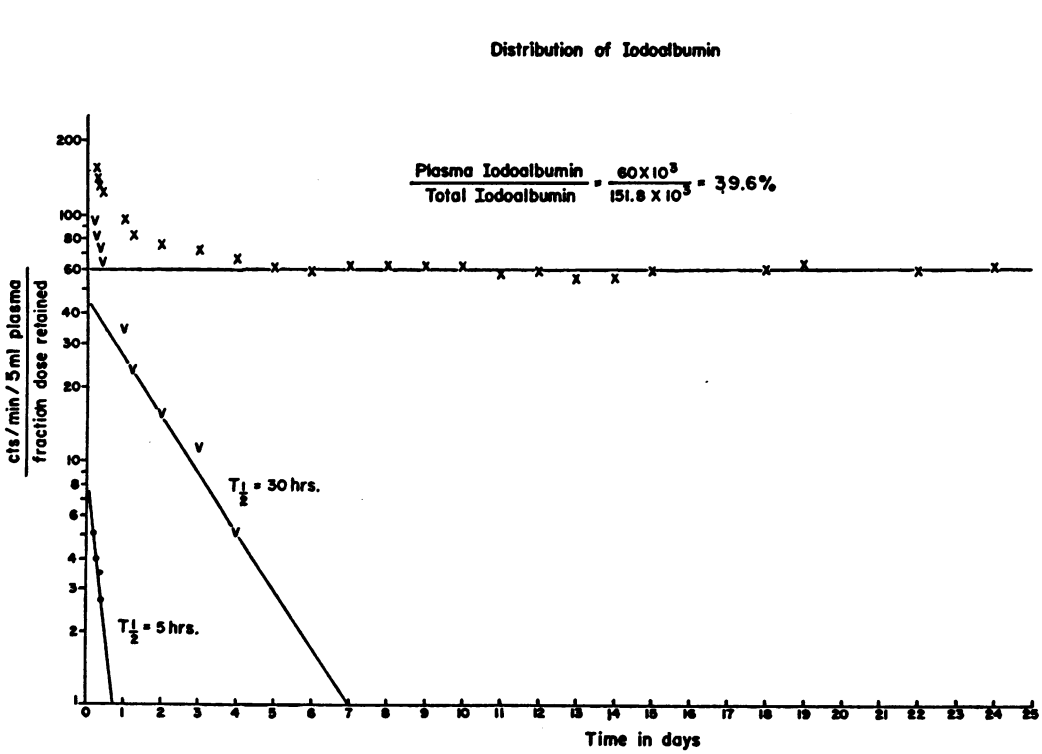

FIG. 3. ANALYSIS OF THE DISTRIBUTION CURVE OF IODOALBUMIN FOR PATIENT R. H., SHOWING 2 COMPONENTS WITH HALF-TIMES OF 5 HOURS AND 30 HOURS, RESPECTIVELY, INDICATING AT LEAST TWO SETS OF EXTRAVASCULAR COMPARTMENTS EQUILIBRATING WITH PLASMA IODOALBUMIN

albumin with extravascular albumin (Figure 3). The mean half times for decrease in the plasma concentration after distribution equilibrium in non-proteinuric subjects were shorter in the control groups ($T_{1/2} = 16.3$ and 17.8 days) than in the cardiac patients ($T_{1/2} = 19.0$ days). However, total exchangeable albumin was somewhat greater in the latter subjects so that the amount of albumin synthesized was approximately the same (Table II). In all cases a single rate of degradation was obtained after distribution equilibrium occurred (Figures 1, 2). In previous studies I^{131} labeled albumin prepared in this manner was also consistent in showing a single rate of degradation (17).

Since serum albumin levels remained essentially constant in all subjects throughout the study, steady state conditions were obtained and the rates of albumin synthesis were calculated according to each of the methods described. The values for synthesis given in Table I were determined from the plasma disappearance curves (method 1) but were generally in excellent agreement with those obtained from the urinary data (method 2); *i.e.*, the sum of albumin degraded and albumin lost via proteinuria as calculated from the urinary data was usually almost identical to that calculated for total synthesis from the plasma data (Table I). Albumin synthesis ranged from 195 mg. per Kg. per day to 255 mg. per Kg. per day in the nine control subjects with a mean of 220 mg. per Kg. per day. The mean value for the seven non-proteinuric patients in this group was 215 mg. per Kg. per day. In the entire group of cardiacs the range was 172 mg. per Kg. per day to 347 mg. per Kg. per day, the mean values for those with and without proteinuria being 214 mg. per Kg. per day and 200 mg. per Kg. per day, respectively. Only one subject (W. J.) with marked proteinuria failed to show a significant diminution in the quantity of albumin degraded in spite of the fact that this subject had the most marked proteinuria of all patients studied (11.6 gm. per day.). All other proteinuric subjects degraded considerably less than normal amounts of albumin so that the sum of degradation plus urinary losses approximated degradation in non-proteinuric subjects (Table I).

The patient with myxedema (M. Co.), who had arteriosclerotic heart disease but no definite evidence of heart failure, had the lowest rate of albumin synthesis (129 mg. per Kg. per day) of all subjects studied, although his total exchangeable albumin was the highest in terms of body weight (6.43 gm. per Kg.).

DISCUSSION

While the use of I^{131} labeled albumin for the investigation of the distribution of endogenous serum albumin is validated by the finding of essentially identical specific activities at distribution equilibrium in several measurable compartments such as plasma, pleural fluid (28), and ascitic fluid (17, 29), caution has been advised in its application to the study of endogenous albumin metabolism (17). Difficulties experienced previously (17) were related to differences in the rates of degradation of lots of iodoalbumin prepared by different methods and the presence of rapidly degraded components in many of the lots. In the present study these difficulties have been circumvented by preparing all batches of iodoalbumin by a method which was previously found to yield material free of significant quantities of rapidly degraded components.[8] As noted above, only a single rate of degradation was found in all the lots employed in the present study. Furthermore, in order to make more valid comparisons between cardiac and control groups, subjects of both groups received material from the same lots. Finally, in normal subjects the differences were slight in the mean values for degradation of iodoalbumin among the various lots of tagged albumin administered. Notwithstanding these attempts at critical control of the experimental cases, there must still remain some reservations in accepting I^{131} tagged albumin as a valid tracer for serum albumin metabolism on the grounds that *in vitro* labelling may produce alterations in the protein, that iodoalbumin is not a normal serum protein, and that the pooled albumin used for iodination is not necessarily a single protein identical to that present in the plasma of all subjects.

However, an observation which emerges from these studies indicates that the I^{131} labeled albumin was not regarded by the body indifferently as a

[8] Obvious alteration of the protein leads to rapid degradation (17, 30).

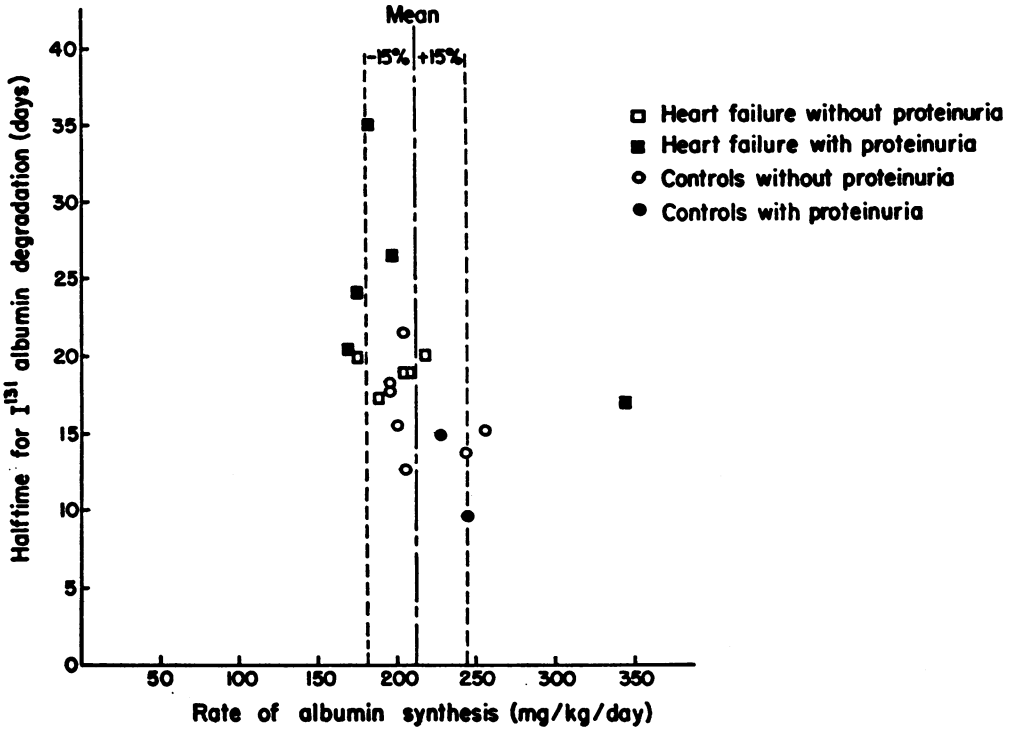

FIGURE 4

Albumin synthesis remains within a narrow range although there are wide variations in the half times for I^{131} albumin degradation.

foreign protein. This observation relates to the widely different half times for albumin degradation[4] obtained in subjects with small albumin pools due to persistent proteinuria (*e.g.*, $T_{1/2} = 9.4$ days in M. C. and 14.7 days in A. R.) and in subjects with large albumin pools (*e.g.*, $T_{1/2} =$ 35.0 days in A. M. and 26.7 days in C. W.). If the metabolism of iodoalbumin were unrelated to that of endogenous albumin it might be expected that the rate of I^{131} labeled albumin degradation would be a random process, independent of abnormalities in the size of the exchangeable albumin pool or of the presence of proteinuria. That the products of such widely different rates of I^{131} labeled albumin metabolism and almost as widely varying total exchangeable pools gave values for degradation which, when added to the urinary losses, resulted in a much narrower range for the rates of synthesis suggests very strongly that metabolism of the iodoalbumin did indeed reflect that of the endogenous serum albumin (Figure 4).

It has been demonstrated (31, 32) that the sodium sulfate fractionation technique may lead to spuriously high values for serum albumin in cases where there is a low serum albumin concentration; hence, values for total exchangeable albumin, total synthesis of albumin, and the amounts of albumin degraded may have been lower than those calculated for the patients with marked hypoalbuminemia. If so, the conclusions regarding a diminished rate of albumin degradation in these cases would be strengthened and even subject W. J. may then have had a normal synthesis and a diminished degradation of albumin. However, since the determination of urinary excretion of albumin by the radioactive iodine method is dependent upon the accuracy of the serum albumin determinations, the agreement between chemical and I^{131} labeled albumin determinations of urinary protein indicates that the serum albumin values were not grossly in error.

[4] These half times can be calculated from Table I, as follows: $\lambda = \frac{\text{grams of albumin degraded per day}}{\text{total exchangeable albumin}}$, where λ is the fraction of the total exchangeable albumin pool degraded per day. Then, $T_{\frac{1}{2}} = \frac{.693}{\lambda}$.

The present studies reveal that there is generally no obvious impairment of albumin synthesis or albumin stores in patients with congestive heart failure. The total exchangeable albumin and the rate of synthesis of albumin were about the same for patients in heart failure and for control patients without proteinuria. Therefore, the fall in serum albumin concentration which may occur in heart failure appears to be related to increase in plasma volume rather than to abnormalities in albumin metabolism.

With one exception (W. J.), a decrease in the amount of albumin degraded appeared to accompany significant albuminuria. Thus, only in the exceptional case (W. J.) was there a successful attempt by the body to compensate for urinary loss by an increase in the rate of albumin synthesis, the mechanism for compensation in the other cases being a diminution in the rate of degradation. It may legitimately be questioned why a reduction in the total exchangeable pool and in the serum albumin concentration develops in proteinuric subjects if there is a compensatory decrease in the rate of albumin degradation and a maintenance of normal levels of albumin production. However, it is common experience that many patients can tolerate a mild degree of proteinuria for prolonged periods without suffering a depletion of their protein stores, while in those with marked chronic proteinuria the limit to which degradation can be inhibited may be insufficient to prevent total albumin removal (degradation plus proteinuria) from exceeding replenishment by synthesis. Thus, protein losses in the patients studied here may well have been more prominent earlier in the course of disease (when serum albumin concentrations were as yet undiminished) and as little as a 10 per cent discrepancy between synthesis and total removal of albumin would have resulted in a 30 per cent deficit in total exchangeable albumin within a couple of months. Furthermore, it is probable that a decrease in albumin degradation is not effected until there has been some reduction in the serum albumin concentration and in the total albumin pool, since such changes would appear to be the most likely stimuli for this compensatory mechanism which acts to conserve the body's albumin stores. At this point the limited capacity of the liver to synthesize albumin may barely maintain the lowered albumin stores against the continued demands of wasting proteinuria.

The amount of albumin normally synthesized by the control subjects in this and previous studies (17) is only about 15 gm. per day. Nephrotic subjects, occasionally reported to excrete as much as 36 to 60 gm. of protein per day (33, 34), must obviously be synthesizing increased amounts of albumin (if such losses are continued over prolonged periods) even if the rate of degradation is markedly diminished. Furthermore, Whipple and Madden (35) believed that dogs provided with adequate dietary protein could increase their rate of protein synthesis when subjected to repeated plasmapheresis. It has been suggested that inability to increase albumin production in the presence of marked protein loss in the urine may be indicative of a defect in the liver's reserve capacity to synthesize albumin (34). If so, this defect was present in all but one (W. J.) of the severely proteinuric subjects studied here. However, further evidence is required to establish the limits to which albumin synthesis can increase in human subjects before it can be concluded that a normal rate of synthesis in the presence of marked proteinuria is evidence of a defect in ability to synthesize albumin. The present report provides insufficient data from which to derive conclusions on this point. However, Gutman (36) has suggested the possibility that a normal rate of synthesis may represent the maximal synthetic capacity in view of the fact that there is no clinical condition (other than dehydration) associated with an abnormally high serum albumin concentration.

SUMMARY AND CONCLUSIONS

1. The distribution and metabolism of I^{131} labeled human serum albumin were studied in 10 cardiac subjects with congestive heart failure and in 10 non-cardiac control subjects. Significant proteinuria was present in 5 of the cardiac and 2 of the non-cardiac patients.

2. Total exchangeable albumin, the intravascular-extravascular partition of exchangeable albumin, and the rate of albumin synthesis were not significantly altered in patients with congestive heart failure.

3. In 6 of 7 patients with proteinuria, the amount of albumin degraded was significantly less

than in non-proteinuric subjects and the amount of protein synthesized was approximately the same as in subjects without proteinuria.

4. A subject with myxedema but without proteinuria had the greatest quantity of exchangeable albumin, the largest extravascular fraction of total exchangeable albumin, and the lowest rate of albumin synthesis.

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