Albumin Synthesis in Cirrhotic Subjects with Ascites Studied with Carbonate-^{14}C

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ABSTRACT The synthesis of serum albumin was measured in 19 patients with cirrhosis of the liver and ascites. Carbonate-^{14}C was used to label the guanido carbon of arginine in albumin.

18 of the patients had the diagnosis of cirrhosis confirmed by biopsy and/or by the presence of esophageal varices. Seven patients with albumin synthesis rates of 42–105 mg/kg per day demonstrated the lowest serum cholesterol esters and highest serum glutamic oxalacetic transaminase (SGOT) levels, while the seven patients whose albumin synthesis rates ranged from 203–378 mg/kg per day, had significantly higher cholesterol ester levels and significantly lower values for SGOT. The serum albumin levels were equally depressed in all patients.

In patients with cirrhosis and ascites albumin production was found to be normal or elevated in seven of the 19 subjects, and only markedly depressed in seven patients, in spite of the fact that the serum albumin level was depressed in all patients.

INTRODUCTION

In patients with liver disease, the serum albumin concentration is frequently decreased (1–8) and these low albumin levels have been attributed to a decrease synthetic capacity (1). In the decompensated patient with liver disease and ascites, this hypothesis has prompted the therapeutic use of albumin infusions in an attempt to augment the presumed low synthetic rate (1, 2). The supposition of impaired hepatic synthesis of albumin in liver disease is equivocated by the poor correlation between serum albumin concentration and prognosis (3), the failure of albumin infusions to alter the clinical course, and the presence of a normal or increased exchangeable albumin pool in some patients with hypoalbuminemia and ascites (8). The latter observation suggests that in some patients with severe liver disease and ascites albumin synthesis may be increased and the low serum level may reflect dilution and altered distribution.

The introduction of a method which permits the study of albumin production over a period of a few hours has permitted the examination of albumin production in acutely ill patients who cannot be expected to remain in the steady state for more than a few hours to say nothing of the days or weeks required to follow the degradation of exogenously labeled albumin (9–11). This report describes the measurement of albumin synthesis by endogenous labeling of albumin with carbonate-^{14}C in 19 subjects with liver disease and massive ascites associated with alcoholism, and demonstrates that in 7 of the 19 patients albumin production was normal or elevated.

METHODS

Patients. 19 patients hospitalized at the Manhattan Veterans Administration Hospital were studied. All subjects were acutely ill and demonstrated large amounts of ascites and weight gain with chemical evidence of severe hepatic dysfunction. Patients with evidence of active gastrointestinal bleeding were excluded. All patients had a prolonged history of heavy alcoholic intake. The minimum diet consisted of 40 g of protein low in sodium and supplemented by vitamins. Patients were studied after an overnight fast. The clinical and laboratory data are included in Tables I and II.

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TABLE I

Patients and Clinical Course

Group	Patient	Age	Weight	Liver histology	Esophageal varices (esophagosopy)	Course	Clinical diagnosis
		yr	*kg*				
I	Hor.	42	71.3	Cirrhosis	3+	Died after 4 wk	Cirrhosis
	Sha.	42	96.4	—	1+	Alive	Cirrhosis, fatty liver, alcoholic hepatitis
	Ste.	35	84.1	Cirrhosis	3+	Alive	Cirrhosis, fatty liver, alcoholic hepatitis
	Kra.	46	70.5	—	3+	Died after 7 wk	Cirrhosis
	Lav.	39	55.0	Cirrhosis	3+	Died after 3 wk	Cirrhosis, fatty liver, alcoholic hepatitis
	Sie.	46	77.0	—	2+	Alive	Cirrhosis
	Mel.	59	65.9	—	2+	Alive	Cirrhosis
II	Mag.	55	72.7	Cirrhosis	1+	Alive	Cirrhosis
	Pan.	47	55.9	Cirrhosis	4+	Died after 3 wk	Cirrhosis
	Dri.	55	59.1	—	4+	Alive	Cirrhosis
	Wal.	55	65.0	—	2+	Died after 8 months	Cirrhosis
	Qui.	49	75.9	—	3+	Alive	Cirrhosis
III	Cha.	71	75.0	Fatty liver, alcoholic hepatitis	0	Alive	Fatty liver, alcoholic hepatitis
	Mat.	47	67.3	Cirrhosis, fatty liver, alcoholic hepatitis	0	Alive	Cirrhosis, fatty liver, alcoholic hepatitis
	Mar.	46	77.7	Cirrhosis	0	Died after 5 months	Cirrhosis
	Kam.	47	72.0	—	2+	Alive	Cirrhosis, alcoholic hepatitis
	Sat.	57	96.0	Cirrhosis	3+	Alive	Cirrhosis
	Pop.	45	82.0	—	3+	Died after 6 months	Cirrhosis, alcoholic hepatitis
	Bur.	54	78.0	—	3+	Alive	Cirrhosis

Protocol. Hepatic albumin production was studied by means of the carbonate-^{14}C method introduced by Swick, Reeve, Pearson, and Martz and McFarlane (9–11). After a 12 hr fast, 3–5 μc human serum albumin-^{131}I and 200 μc carbonate-^{14}C were injected intravenously. 10 ml of heparinized blood was drawn at 10–15 min and 200 ml of blood drawn at 4–5 hr. The patients were then fed. The overnight fast was repeated, and the following morning approximately 18 hr after the first study, 15 μc urea-^{14}C was injected. Five to six blood samples were obtained at 45 min intervals commencing at 1.5 hr after the injection. Urine was collected with an indwelling catheter.

Methods. The plasma volume was determined from the space of distribution of albumin-^{131}I at 10–15 min and the net loss of albumin from the circulation during the measuring period from the change in plasma ^{131}I activity in the 15 min to 4–5 hr plasma sample. After the injection of carbonate-^{14}C the specific activity of the carbon of urea and the guanido carbon of albumin were determined from urea and albumin isolated at the 4–5 hr bleeding. The fractional rate of urea synthesis was calculated from a semilogarithmic plot of urea-^{14}C specific activity against time, after urea-^{14}C injection. The specific activity of the urea carbon after the carbonate-^{14}C injection was calculated back to zero time using the fractional rate determined from the plasma specific activity curve after the urea-^{14}C injection (11).

Determination of urea carbon specific activity. The

TABLE II

Albumin Synthesis in Patients with Cirrhosis and Ascites

Group	Patient	Albumin synthesis		Serum		Total bilirubin	Alkaline phosphatase	Prothrombin time*	Free cholesterol	Cholesterol esters	SGOT
				Albumin	Globulin						
		mg/kg per day	*g/day*	*g/100 ml*		*mg/100 ml*	*K.-A. units‡*	*sec long*	*mg/100 ml*	*mg/100 ml*	*Sigma units*
I	Hor.	42	3.0	1.7	3.5	9.8	27	9	60	80	80
	Sha.	63	6.1	2.3	3.6	24.0	17	7	128	72	216
	Ste.	80	6.7	1.6	4.5	24.0	47	3	120	30	216
	Kra.	82	5.8	1.5	4.8	4.7	17	6	44	70	51
	Lav.	84	4.6	2.1	2.6	19.0	41	9	150	40	166
	Sie.	100	7.7	1.5	6.4	3.0	11	7	45	73	—
	Mel.	105	6.9	1.5	5.3	3.4	19	5	76	119	55
	Mean			1.7	4.4	12.5	26	6.6	89	69	147
	±SEM			0.1	0.5	3.7	4.9	0.8	17	10	28
II	Mag.	136	9.9	2.4	4.0	0.2	18	4	31	96	35
	Pan.	149	8.3	1.7	5.1	3.2	22	3	—	—	60
	Dri.	150	8.9	2.6	5.8	0.9	22	0	42	71	23
	Wal.	160	10.4	1.8	3.9	1.9	9	3	40	111	38
	Qui.	167	14.1	1.9	3.3	2.4	10	6	44	86	80
III	Cha.	203	15.2	3.0	2.3	0.9	8	2	92	88	30
	Mat.	243	16.4	1.9	4.0	1.9	32	4	71	106	132
	Mar.	262	20.4	2.2	3.8	1.4	30	3	60	180	20
	Kam.	264	15.0	1.7	4.8	11.0	11	11	48	124	120
	Sat.	290	12.6	2.7	5.0	0.5	14	0	25	105	20
	Pop.	370	30.3	2.1	5.1	20.2	30	3	115	80	80
	Bur.	378	29.5	1.4	7.4	0.8	13	2	33	117	50
	Mean			2.1	4.6	5.2	20	3.6	63	114	65
	±SEM			0.2	0.6	2.8	3.8	1.2	12	12	18
	P value			N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	<0.01	<0.05

±SEM, Standard error of the mean.
N.S., not significant.
* Prothrombin time expressed in sec prolonged.
‡ King-Armstrong units.

plasma samples were treated with tungstic acid and heated at 100° C to drive off ^{14}C-CO_2. An aliquot was taken for nitrogen analysis by incubating with urease according to the method of Conway and Byrne (12). Ammonia was released with 45% K_2CO_3, trapped in 2 N H_2SO_4, and assayed with Nesslers reagent. A second aliquot was incubated with urease and the CO_2 released with H_3PO_4 and trapped in phenethylamine which was subsequently dissolved in a solution of 0.7% Butyl-PBD [2-(4-*tert*-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole] in toluene-methanol (1:1) and counted in an ambient temperature liquid scintillation counter (Nuclear-Chicago Corporation, Des Plaines, Ill.).

Determination of albumin guanido carbon specific activity. Albumin was isolated from the last sample of blood by precipitation with 10% trichloracetic acid (TCA). The precipitate was washed with 5% TCA and then extracted with 95% ethanol–1% TCA. Three parts of diethyl ether were added to the acid alcohol extract to phecipitate albumin. The albumin was dissolved in dilute alkali and dialyzed overnight, in the cold, against distilled water.

The dialyzed contents were centrifuged and the supernatant precipitated with TCA at a final concentration of 5%, reextracted with alcohol-TCA and again precipitated with 3 volumes of ether. The precipitate was dissolved in dilute alkali and dialyzed against cold distilled water. A sample was concentrated to 6% protein concentration and qualitatively examined by cellulose acetate electrophoresis and agar-gel immunoelectrophoresis to guard against the use of a preparation which contained obvious serum protein contaminants (13). This separation technique was tested with rabbit plasma from a rabbit given carbonate-^{14}C. The specific activity of the guanido carbon of rabbit albumin isolated as above was within 6% (14) of the specific activity of the guanido carbon of albumin isolated with sheep anti–rabbit albumin, and within 12% of the preparation isolated from Geon resin–block electrophoresis. These studies were performed with rabbit plasma because of the limitations imposed by the volume of human plasma required for these simultaneous studies. The protein was hydrolyzed with 6 N HCl, neutralized, and passed through a resin column according to the method of McFarlane and treated consecutively with arginase and urease (11). Nitrogen and ^{14}C-CO_2 were determined as above. Total protein was determined with a biuret reagent (15) and protein partition with a microelectrophoresis unit (Kern Instrument, Aarau, Switzerland) (16).

Hepatic function was evaluated in each patient at the time of the study by means of the prothrombin time (17), cholesterol and cholesterol esters (18), bilirubin (19), alkaline phosphatase (20), and serum glutamic oxalacetic transaminase (SGOT) (21). After the acute phase of the disease, 3–10 wk later, endoscopy was performed in 17 patients, and the presence and degree of esophageal varices were evaluated according to the method of Palmer and

Boyce (22). Four of the seven patients who expired had autopsy examination, and in five other patients hepatic biopsies were performed when the patients were stable in terms of weight and when the ascites had essentially cleared.

RESULTS

All 19 patients were acutely ill with liver disease. 7 of the 19 succumbed to their disease 1–13 months after the study. To confirm the presence of cirrhosis, we re-evaluated each patient during convalescence. Hepatic biopsies or autopsy specimens were obtained in nine of the 19 patients, and in eight of these nine the findings were characteristic of cirrhosis. In patient Cha., only fatty infiltration was noted. Esophageal varices were demonstrated in 17 patients during convalescence. Thus eighteen patients had cirrhosis documented histologically or hemodynamically. Patient Cha. was presumed to suffer from acute alcoholic hepatitis and fatty liver.

The metabolic data on the 19 patients are shown in Table II. The absolute synthesis rate was below 105 mg/kg per day in seven patients and between 203–378 mg/kg per day in the seven patients with the highest synthetic rate. These two groups of patients were compared in terms of liver function studies to determine if a correlation existed.

There was no relation between the high and low synthetic groups and the serum protein levels. The albumin levels were depressed and the globulin levels elevated to the same degree in both groups.

The bilirubin level in the low albumin producers was more than twice that found in the group with the highest rate of albumin production, but the range of values was wide and the mean values did not differ significantly. No difference was noted in the values for alkaline phosphatase averaging 20 and 26 King-Armstrong units in the high and low albumin producers, respectively.

The prolongation in the prothrombin time averaged 6.6 sec in the group with low albumin production and 3.6 sec in the high group. While these values are not statistically different, only patient Kam. had a significantly prolonged value in the high albumin synthetic group.

The cholesterol esters averaged 69 ±10 mg, 43% of the total cholesterol in the low albumin producing group and increased to 114 ±12 mg/100 ml, 64% of the total cholesterol in the high producing group ($P < 0.01$). A similar high degree of significance was found when the values for SGOT were compared. In the low production group the mean value was 147 ±28 and in the high group this had fallen to 65 ±18 ($P < 0.05$) (Sigma units with normal values of 0–40) (21).

The plasma volumes were elevated in 17 of the 19 patients averaging 55 ml/kg with a range of 38–84 ml/kg per wet wt. The serum urea nitrogen level averaged 17 mg/100 ml on the 1st day of the study and was not significantly different on the 2nd day in any of the 19 patients. Occasional urine specimens did contain protein but not on the days of the study.

DISCUSSION

The use of the carbonate-^{14}C method has made possible the study of hepatic protein synthesis directly and over a short period of time. It has been shown that the injection of carbonate-^{14}C will label the guanido carbon of arginine and hence the carbon of urea derived from this pool will have the same specific activity as the immediate precursor amino acid arginine. Swick, Reeve, et al., and McFarlane (9–11) described the method based on this immediate product-precursor relationship. Studies of albumin metabolism have been reported in humans and in rabbits in steady states where the over-all absolute catabolic rate determined with albumin-^{131}I and the short term synthesis rate with carbonate-^{14}C have agreed remarkedly well considering the different aspects of metabolism measured with these two tools (11, 23–25).

Tavill, Craigie, and Rosenoer (24) have recently reviewed this technique. It is necessary to assume that urea and albumin are synthesized from the same pool of arginine and that the rates of synthesis of urea and albumin remain stable one to the other for the period of study. Further, the ^{14}C label must, as nearly as possible, act as a pulse label so that the liver is rapidly freed of labeled precursor permitting the fall in plasma specific activity after distribution to reflect the continued synthesis of cold urea. Evidence to support these assumptions inherent in the method has been presented and reviewed previously (14, 24–27).

McFarlane (11) has shown that in the human the curves of endogenously produced urea-^{14}C and exogenously administered urea-^{13}C are different reflecting the continued labeling of urea and protein within the liver. This potential problem was obviated in the present study by administering urea-^{14}C to measure the fractional rate of urea synthesis.

This present study incorporates two variations of the previously described techniques for measuring albumin synthesis (9–11). The use of the Conway microdiffusion method permits the rapid examination of many samples and is simple and accurate (12). In acutely ill cirrhotic patients the low rate of urea production makes it difficult to estimate the fractional rate of urea synthesis from the initial 200 μc injection of carbonate-^{14}C. The subsequent injection of 15 μc of urea-^{14}C provides enough activity to measure the fractional rate of urea synthesis from the plasma specific activity curve. However, it is assumed that the rate of urea synthesis remains unchanged during the 24 hr interval. Studies in fasted and fed animals whose individual rates of urea synthesis varied from 200 to 450%/day have shown that these fractional rates

determined by endogenous and exogenous urea-^{14}C specific activity curves agree very well (14). In one patient in this study where it was possible to determine the fractional rates by the two methods, the agreement was nearly perfect.

The patients with cirrhosis who have been studied with albumin-^{131}I have, for the most part, been those who exhibit stable conditions without marked changes in clinical status so that metabolic studies could be conducted over a period of days or weeks (2–7). The patients in the present study were acutely ill, all with the onset of ascites a few weeks before admission. Even though the serum albumin level was depressed in all the subjects, albumin synthesis was normal or elevated in over 50% of the patients and markedly depressed only in 37% (seven patients). This observation would be more striking if the results were not expressed in terms of wet weight which includes the mass of ascites. Employing a dry weight obtained just before discharge, the absolute values would be increased by some 10% (0-19%) reflecting the loss in fluid and a gain in tissue mass.

Although most studies of albumin metabolism in cirrhosis of the liver have shown a low fractional rate of degradation and a diminished absolute quantity of albumin degraded per day, there have been many instances where the albumin pool size has been increased (2–7).

When the quantity of albumin removed by paracentesis was taken into consideration or when the albumin pool size in cirrhotic subjects with ascites was measured evidence has been accumulated to suggest that synthesis may be active (2–7) even though the fractional degradative rates are low.

Thus, in the reported studies there are patients who probably have absolute rates of albumin production which are not depressed and pool sizes that are not decreased in the presence of decreased plasma albumin concentration. In an animal study reported by Levin and Jeffay where cirrhosis was produced by a dietary deficiency albumin production was not found to be impaired (28).

The results of the present study demonstrate that in patients with liver disease and the recent accumulation of ascites, albumin synthesis need not be impaired. No direct relation to the serum albumin level was seen. In hypergammaglobulinemia or in the presence of circulating dextran low albumin levels per se were not a stimulus to albumin synthesis (29). In the present study the patients with the lowest levels of albumin production also had the lowest levels of cholesterol esters, the highest SGOT levels, and a trend toward the more prolonged prothrombin times. These abnormalities are most frequently seen with liver cell damage, whereas elevation of the bilirubin and alkaline phosphatase levels may occur in the absence of other types of hepatocellular dysfunction. It is not surprising, therefore, that no correlation was found with albumin synthesis and these two aspects of hepatic function.

There are other factors in the cirrhotic subjects which may influence protein synthesis directly or indirectly. Alcohol per se has been shown to cause ultrastructural changes within the liver cell and some alteration in leucine-^{14}C incorporation into hepatically made proteins in the perfused liver system (30, 31). Specific toxic effects of alcohol, therefore, probably played some role in depressing and altering albumin production since all the patients were studied within days of admission. Nitrogen and amino acid intake and amino acid levels within the cell are important (32–36). Studies with albumin-^{131}I indicate a marked decrease in synthesis in patients on deficient caloric diets. Using the carbonate method a short-term fast rapidly lowers albumin synthesis. Prefeeding can restore the synthetic capacity within 24 hr (36). A specific stimulatory ability has been found to reside in tryptophan. When this amino acid is force-fed 10–30 min before sacrifice in fasted mice an enhanced microsomal incorporating ability has been found and a change in ribosomal profile from the monosome to the polysomal type has been seen (34). While the over-all concentration of plasma amino acids has been reported as normal in patients with alcoholic hepatitis, an altered plasma amino acid pattern has been noted. However, data on intracellular levels are not available. Also, tryptophan was not measured (37).

Albumin production could be influenced by other factors which are probably altered in cirrhosis. Change in colloid osmotic pressure, pressure alterations within the liver, thyroid and cortisone metabolism, as well as changes in the nutritional state, all could influence albumin synthesis (29, 32, 33, 38–44).

While the mechanisms regulating albumin synthesis remain obscure, adequate nutrition appears to be vital. The influence of the heavy alcoholic intake and very poor nutrition in these patients before hospitalization cannot be evaluated in this series since studies were not carried out before admission. However, these factors did not impair the albumin synthesizing potential in at least half of our cirrhotic ascitic subjects.

The serum albumin level, which represents the net result of degradation, synthesis, and distribution is neither a good index of hepatic albumin synthesizing capacity nor the over-all functional capacity of the liver in cirrhosis. Clinically the low serum albumin level has been interpreted as representing altered or damaged hepatic production. The results of this study illustrate that even though the serum albumin level is low in patients with cirrhosis and ascites and evidence of severe liver disease, albumin synthesis may be increased. Many factors are present all tending to lower albumin production in hepatic cirrhosis without demanding that

the hepatocyte be damaged in its albumin producing capacity. The use of the carbonate technique offers great promise in elucidating some of the interwoven factors regulating protein synthesis by permitting the study of albumin synthetic rates during brief periods.

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