

Direct Measurement of the Rates of Synthesis of Plasma Proteins in Control Subjects and Patients with Gastrointestinal Protein Loss

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ABSTRACT The guanido carbon of hepatic arginine is the common precursor of urea and of the arginine of plasma proteins synthesized in the liver. It is possible to measure the momentary synthetic rates of plasma proteins by "pulse labeling" this arginine pool with bicarbonate- ^{14}C . In the current study, this method has been adapted in order to use urinary urea data and was applied to control subjects and patients with gastrointestinal protein loss. The assumptions required for this determination are discussed.

There was close agreement between albumin synthetic rates measured by this method and albumin catabolic rates derived from simultaneous albumin- ^{131}I studies, supporting the validity of the method and suggesting that there is relatively little fluctuation in the rate of albumin synthesis from time to time. The albumin synthetic rates in six control subjects averaged 5.8 mg/kg per hr, while those of five patients with gastrointestinal protein loss averaged 7.2 mg/kg per hr. Thus in these patients, there was relatively little acceleration of albumin synthesis in response to continued loss of plasma proteins into the gastrointestinal tract.

Fibrinogen synthetic rates averaged 1.9 mg/kg per hr in five control subjects and 3.2 mg/kg per hr in five patients with gastrointestinal protein

loss. Transferrin synthetic rates exhibited considerable individual variation in both groups and averaged 0.24 mg/kg per hr in four control subjects and 0.31 mg/kg per hr in five patients with gastrointestinal protein loss.

The method employed in this study offers several advantages in studying plasma protein metabolism. It provides a direct measurement of protein synthesis, applicable to several proteins simultaneously, does not require a long-term steady state in the metabolism of the proteins, and is capable of measuring short-term fluctuations in synthetic rates. Therefore, this approach is applicable to the investigation of the physiological factors controlling the rates of synthesis for plasma proteins.

INTRODUCTION

The physiological factors which control the rates of synthesis of plasma proteins are not well understood. The effects of variations in plasma protein concentration and of continued loss of protein into the urine or gastrointestinal tract are among the factors which remain incompletely defined, largely due to limitations of methodology.

The approaches to the problem of measuring plasma protein synthetic rates have been either (a) to measure the rate of catabolism of an isotopically labeled plasma protein and infer synthetic rates by assuming steady-state conditions in which synthetic and catabolic rates are equal, or (b) to deduce synthetic rates from the incorporation of

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Received for publication 15 March 1967 and in revised form 7 August 1967.

isotope from a precursor into the protein being studied.

Most of our current information about the metabolism of plasma proteins has been provided by labeling them with radioiodine. With present methods of labeling and of analysis, it is possible to quantitate the rate of catabolism of many different proteins. However, there are several limitations to this approach. First, most of these analyses require maintenance of steady-state conditions with regard to total quantity of the protein, its distribution, and its rate of synthesis. Second, this approach cannot detect short-term fluctuations in the rate of synthesis. Third, analysis of this type of data becomes complex when the method is applied to proteins with rapid renewal rates, where the survival of the protein is short relative to the rates of protein distribution and of clearance of the freed radioiodide. Fourth, only two proteins may be studied at the same time with the available isotopes ^{131}I and ^{125}I . Fifth, relatively long periods of observation may be required.

Methods which compute synthetic rates from the incorporation of radioactive precursors are in theory superior for the determination of synthetic rates. However, with these methods, it is necessary to assess the specific activity of the precursor pool during the period of synthesis and to correct for any continuing incorporation of precursor into the protein. This approach was used by Weissman, Tschudy, Bacchus, and Eubanks (1) to determine the rate of synthesis of albumin after administration of glycine- ^{14}C by measuring the specific activity of albumin, glycine, and urinary hippuric acid. More recently, McFarlane (2, 3) and Reeve, Pearson, and Martz (4) have proposed adapting the method of Swick (5) and using the relationship between hepatic arginine and urea for the measurement of synthetic rates of plasma proteins. This method depends on the fact that the guanido carbon of hepatic arginine is the common precursor for both urea and the arginine guanido carbon of liver-made plasma proteins (Fig. 1). This being true, the absolute rate of synthesis of any protein derived from this same precursor pool can be determined by measuring the relative rates of synthesis of protein and urea, and the absolute rate of urea production.

In the current study, a modification of the method of McFarlane (2, 3) using urinary urea

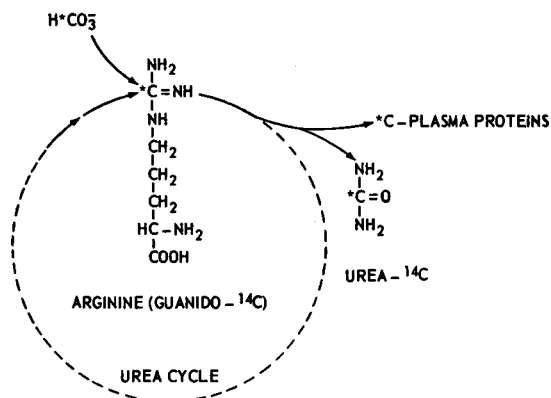


FIGURE 1 The relationships between bicarbonate, hepatic arginine, urea, and plasma proteins. The method of measuring protein synthetic rates is based upon these relationships.

specific activity was employed to obtain simultaneous measurements of the synthetic rates for albumin, fibrinogen, and transferrin. The synthetic rates for albumin determined using this method were compared to those estimated from simultaneous studies with iodinated albumin. These studies were performed in control subjects and in patients with protein-losing gastroenteropathy, in whom the effects of continued loss of plasma proteins and reduced protein concentration could be evaluated.

METHODS

Patients

The studies were performed in patients with gastrointestinal protein loss and in control subjects with normal rates of albumin catabolism. Table I presents a summary of clinical data on these patients. The patients with gastrointestinal protein loss included four patients with intestinal lymphangiectasia and one with regional enteritis. Intestinal protein loss was demonstrated with ^{51}Cr -labeled albumin (6). Control subjects included four patients with amyotrophic lateral sclerosis, one with chronic myelogenous leukemia, and two with chronic lymphocytic leukemia. An additional patient with carcinoma of the lung was studied during a period in which his serum albumin concentration was falling. With this exception, serum albumin concentrations determined from three to five times during the period of study were constant for each patient.

Preparation of labeled albumin

The albumin used for the turnover studies in the patients with gastrointestinal protein loss was Cohn fraction V.¹ The albumin used in each of the control subjects

¹ Cutter Laboratories, Berkeley, Calif.

TABLE I
Clinical Data

Initials	Age	Sex	Wt	Diagnosis	Total serum protein	% injected albumin- ⁵¹ Cr in 4 day feces*
			kg		g/100 ml	
Patients with gastrointestinal protein loss						
J.T.	41	M	52.2	Intestinal lymphangiectasia	3.8	19.6
N.D.	37	M	59.4	Intestinal lymphangiectasia	4.1	14.7
B.D.	27	F	56.1	Intestinal lymphangiectasia	4.3	5.1
G.L.	43	F	60.4	Intestinal lymphangiectasia	4.1	12.7
T.G.	35	F	45.3	Regional enteritis	4.3	11.3
Control subjects						
C.E.B.	52	M	84.6	Amyotrophic lateral sclerosis	6.4	—
R.M.	47	M	70.0	Amyotrophic lateral sclerosis	6.4	—
W.M.	62	M	92.2	Chronic myelogenous leukemia	8.0	—
J.G.	46	M	90.0	Amyotrophic lateral sclerosis	6.2	—
J.M.G.	46	M	63.2	Amyotrophic lateral sclerosis	6.5	—
J.L.	62	M	65.3	Chronic lymphocytic leukemia	7.0	—
G.G.	78	M	71.9	Chronic lymphocytic leukemia	6.4	—
Patient with falling serum albumin (non-steady state)						
J.H.	49	M	52.7	Carcinoma of lung	5.9	—

* Range in 60 control subjects: 0.0-0.7%.

was obtained by block electrophoresis of the patient's own serum. Electrophoresis was carried out for 18 hr in sodium barbital buffer pH 8.6 with polyvinyl chloride particles² and polyvinyl chloride-polyvinyl acetate copolymer³ as supporting medium. The albumin region was eluted with saline, and each preparation was demonstrated to be pure by Ouchterlony double-diffusion analysis using a rabbit antiserum to whole human serum. The albumin was then labeled with ¹²⁵I using the iodine monochloride method of McFarlane (7). All preparations of labeled proteins were calculated to have an average of less than one atom of iodine per molecule of protein, and greater than 99% of the radioactivity in the final product was precipitable with phosphotungstic acid. Normal human albumin was added to each preparation to minimize damage to the protein by irradiation, and the mixture was sterilized by filtration.

Protocol of study

Each patient was hospitalized at the National Cancer Institute during the study. Beginning 2 days before the injection of isotope, we administered 10 drops of Lugol's solution three times daily to prevent thyroidal uptake of released ¹²⁵I. During the 5 days before injection of isotope, each patient was given a constant diet containing at least 70 g of protein daily, and at least 2500 ml of oral fluids. Total serum protein (8), serum albumin (9),

fibrinogen (10), transferrin (11), and blood urea nitrogen were measured during this period and at intervals during the 2 wk after injection of isotopes to verify that each patient was in a steady state with regard to these parameters. Each patient was given a light, fat-free breakfast 2 hr before the administration of isotopes. 100 µc of sodium bicarbonate-¹⁴C and 15-30 µc of albumin-¹²⁵I were administered simultaneously by intravenous infusion. 2 ml of plasma samples were obtained at 10 min and 2, 5, 8, and 20 hr after injection of isotope, and then daily for 7-21 days. These samples were counted with appropriate ¹²⁵I standards in an automatic gamma ray well-type scintillation counter using a thallium activated sodium iodide crystal. 250 ml of plasma was obtained by plasmapheresis 3 hr after the injection of isotope. Separation of albumin, fibrinogen, and transferrin from this plasma was accomplished as outlined below. In some instances, 50 ml of heparinized blood was obtained at 2, 5, 8, 21, and 45 hr, and 14 or 15 days, and albumin was purified for ¹⁴C assay. Urine collections were obtained over progressively increasing intervals as follows: 1.5, 1.5, 3, 3, 6, 6, 12, 12, and 24 hr, and daily thereafter. Aliquots of this urine were analyzed for ¹²⁵I content as outlined above and for urea and urea-¹⁴C content as described below.

Isolation of ¹⁴C-labeled proteins

Albumin was prepared as outlined above by block electrophoresis, except that 25 ml of plasma was concentrated to 8 ml before application to the block. All preparations were demonstrated to be immunochemically pure albumin.

² Geon resin, The B. F. Goodrich Company, Niagara Falls, N. Y.

³ Pevikon Superfosfat, Fabrika, Aktiebolog, Stockholm, Sweden.

The remainder of the 3 hr plasmapheresis sample was used for separation of fibrinogen and transferrin. In order to follow the location and quantity of transferrin, we preincubated the sample with 5–25 μc of ^{59}Fe (specific activity 15 $\mu\text{c}/\mu\text{mole}$). The plasma sample was brought up to 23% saturation in ammonium sulfate to precipitate fibrinogen, while transferrin and most of the other plasma proteins remained in the supernatant. The precipitate was collected by centrifugation at 8500 g and redissolved in saline at 0°C. The solution was reprecipitated at 23% ammonium sulfate saturation and redissolved in saline. The fibrinogen solution was brought to the original volume of the plasma sample, and a fibrin clot was formed by the addition of 500 U (NIH) of bovine thrombin. The clot was allowed to retract for 4 hr and then separated by centrifugation at 8500 g . It was washed with water to remove any contaminating serum proteins and analyzed for ^{14}C content as described later.

Transferrin was prepared from the supernatant of the original 23% ammonium sulfate precipitation by an adaptation of the technique of Nagler, Kockwa, and Wasserman (12) as follows. The supernatant was dialyzed on a rocking dialyzer against six to eight changes of 0.005 M potassium phosphate buffer pH 8. It was then applied to a 4.8 × 40-cm column of *O*-(diethylaminoethyl)cellulose (DEAE-cellulose) equilibrated with the same buffer. Transferrin adheres to the cellulose under these conditions, while the bulk of the gamma globulins do not. After the plasma sample passed into the column, 0.005 M phosphate buffer was allowed to pass through until no protein was detectable in the effluent. At this time, a linear gradient was set up with 4 liters each of 0.005 M potassium phosphate pH 8 (starting buffer) and 0.075 M potassium phosphate pH 8 (limit buffer). Under these conditions, transferrin was eluted as the first protein peak in the effluent, free of albumin. The transferrin fractions were pooled, dialyzed against six to eight changes of 0.01 M potassium phosphate buffer pH 6.3 and passed over a 2 × 25-cm column of *O*-(carboxymethyl) cellulose (CM-cellulose) equilibrated with the same buffer. Most of the contaminating gamma globulin adhered to the CM-cellulose under these conditions, whereas the transferrin did

not. 30–40% of the initial ^{59}Fe activity was recovered in the final product. Transferrin was evaluated for purity by immunochemical techniques. By Ouchterlony analysis, the preparations were found to have two proteins present which reacted with rabbit anti-whole human serum. These were transferrin and IgG. The quantities of each were determined with the immunodiffusion technique of Fahey and McKelvey (13). Transferrin preparations isolated in this manner were found to contain 95–99% transferrin.

Measurement of specific activity of urinary urea and the guanido carbon of plasma proteins

The method used has been described by McFarlane (2) and depends on the conversion of urea carbon to carbon dioxide by urease⁴ and the subsequent collection of carbon dioxide in a calibrated manometer. Reagent blanks were prepared with distilled water instead of urine and run with each group of samples. The Tungstic acid-citric acid mixture used by McFarlane was found to be a source of spurious carbon and was replaced by 3 ml of 4 M H_3PO_4 . The carbon dioxide was condensed into phenethylamine in vacuo at the temperature of liquid nitrogen, transferred to a counting vial with the addition of 2,5-diphenyloxazole (PPO) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) as described by Woeller (14), and then counted in a liquid scintillation spectrometer (Packard Tri-Carb model 500B). The yield of carbon from standard urea solutions was 94–98%.

The procedure used to measure the specific activity of guanido carbon of the proteins was also described by McFarlane (2) and consists of hydrolysis of the protein followed by reaction with arginase⁵ to convert the guanido group of arginine to urea. The urea was subsequently processed as outlined above.

Calculation of albumin- ^{131}I data

The turnover of iodinated albumin was analyzed according to modification of the method of Pearson, Veall, and Vetter (15), as summarized in the following equations:

$$\begin{aligned} \text{(a) Plasma volume (ml/kg)} &= \frac{\text{radioactivity administered}}{\text{radioactivity/ml of plasma at 10 min} \times \text{body weight (kg)}}; \\ \text{(b) Total circulating albumin} &= \text{plasma volume} \times \text{plasma albumin concentration}; \\ \text{(c) Fraction of circulating albumin catabolized/day} &= \frac{\text{radioactivity excreted in 24 hr}}{\text{plasma volume} \times \text{mean circulating radioactivity/ml during that day}} \\ \text{(d) Albumin catabolic rate (mg/kg per hr)} &= \frac{\text{total circulating albumin} \times \text{fraction of circulating albumin catabolized/day}}{24} \end{aligned}$$

This fraction was determined for each day and the mean value for all 24-hr periods after day 3 was used in the following calculation:

⁴ Type V, Sigma Chemical Company, St. Louis, Mo.

⁵ Worthington Biochemical Corporation, Freehold, N. J.

When the data were analyzed instead by the model of Matthews (16), the results were not significantly different from those obtained by the above method.

Calculation of ^{14}C Carbon data

Calculation of the ^{14}C Carbon data depends on the relationship illustrated in Fig. 1 and referred to in the introduction. The specific activity of carbon incorporated into newly synthesized urea and into the arginine guanido carbon of newly synthesized proteins will be the same. Thus,

$$\frac{\text{total dpm in protein GC}}{\text{mg of protein GC synthesized during } t^1} = \frac{\text{total dpm in urea C}}{\text{mg of urea C synthesized during } t^1} \quad (1)$$

where t^1 = time period during which isotope is incorporated into protein or urea, mg of protein GC = mg of arginine guanido carbon in the protein, mg urea C = mg of urea carbon, and total dpm = total radioactivity (disintegrations per minute) incorporated. This relationship holds for any period of time during which the ratio of the rates of synthesis of urea and the protein remain constant.

Of the urea synthesized at any moment, a fraction, α , will ultimately appear in the urine. If α is constant throughout the period t^1 , both the numerator and denominator of the right-hand side of equation 1 may be multiplied by α , converting them to terms related to urinary urea. The radioactivity of the urinary urea ($\alpha \times \text{dpm in urea C}$, see equation 1) is directly measurable. However, one cannot identify and quantitate that portion of excreted urea which was synthesized during the time t^1 ($\alpha \times \text{mg of urea C}$, see equation 1). Therefore, it must be assumed that the over-all rate of urinary urea excretion measured shortly after t^1 is a reliable estimate of that amount of urea produced during t^1 which is eventually excreted. This estimate will be valid unless there are major short-term fluctuations in the rates of urea production or of urea excretion. Using this estimate, and an hour as the unit of time,

$$\frac{\text{dpm in protein GC}}{\text{mg protein GC synthesized/hr}} = \frac{\text{total dpm in urinary urea}}{\text{mg urinary urea C/hr}} \quad (2)$$

where mg of urinary urea C/hr refers to the rate of urea excretion measured shortly after t^1 . The numerator of the right-hand side of equation 2 may be rewritten: $\int d(\text{dpm in urinary urea}) dt/dt$. In the present studies, the rate of urinary urea excretion, i.e. the denominator of the right side of equation 2, remained essentially constant during the 24-48 hr period in which data were obtained to calculate the above integral. Under this circumstance, the value for this excretion rate may be enclosed under the integral sign, and equation 2 may be rewritten:

$$\frac{\text{dpm in protein GC}}{\text{mg protein GC synthesized/hr}} = \int_0^\infty u(t) dt \quad (3)$$

where $u(t)$ = dpm/mg urea C at time t , measured in hours after isotope injection.

Rearranging the left-hand side in terms of the substances measured and multiplying the numerator and denominator by mg protein/mg protein GC gives:

$$\frac{\frac{\text{dpm}}{\text{mg protein GC}} \times \frac{1}{F} \times \frac{\text{mg protein}}{\text{ml plasma}}}{\text{mg protein synthesized/kg per hr}} \times \frac{\text{plasma volume (ml/kg)}}{\text{mg protein synthesized/kg per hr}} = \int_0^\infty u(t) dt \quad (4)$$

where F = fraction of newly synthesized protein which is intravascular at the time of measurement of dpm/mg protein GC. In these studies, the plasma sample was taken 3 hr after injection, and F was assumed to be 0.90, according to McFarlane (2).

Rearranging further gives:

$$\text{mg protein synthesized/kg per hr} = \frac{\frac{\text{dpm}}{\text{mg protein GC}} \times \frac{\text{mg protein}}{\text{ml plasma}} \times \text{plasma volume (ml/kg)}}{F \times \int_0^\infty u(t) dt} \quad (5)$$

Dividing each side by the quantity of total circulating protein and converting to percentage, gives the following:

$$\text{Per cent of intravascular pool of protein renewed/hr} = \frac{\frac{\text{dpm}}{\text{mg protein GC}} \times 100}{F \times \int_0^\infty u(t) dt} \quad (6)$$

Equations 5 and 6 are thus used to determine the synthetic rates and the fractional renewal rates of the proteins studied.

RESULTS

In the control subjects, albumin- ^{131}I was catabolized at an average rate of 0.39 per cent of the intravascular pool per hour, or 6.1 mg/kg per hr (Table II). The fractional catabolic rate for albumin was markedly increased in patients with gastrointestinal protein loss, averaging 1.18% of the intravascular pool per hour. The absolute catabolic rate in these patients averaged 8.7 mg/kg per hr.

The specific activity of the albumin guanido carbon rose rapidly in the first 2 hr after injection of bicarbonate- ^{14}C and reached a maximum at 3 hr. After this time, the loss of labeled albumin from the plasma compartment by distribution and catabolism exceeded that of any further delivery of labeled albumin from the synthetic site, and the decline in ^{14}C specific activity paralleled that of the injected albumin- ^{131}I . The specific activity

TABLE II
Albumin Synthesis and Catabolism

Patient	Catabolism: albumin ¹³¹ I						Synthesis: ¹⁴ carbon			
	Serum albumin concentration	Plasma volume	Survival t _{1/2}	% Plasma pool catabolized/hr	Catabolic rate	Urea excretion rate	Guanido carbon	$\int_0^{\infty} u(t) dt^*$	% Plasma pool synthesized/hr	Synthetic rate
Gastrointestinal protein loss										
J.T.	1.9	37	4.5	1.50	10	110	313	21,400	1.46	10.0
N.B.	1.5	38	4.4	1.75	10	202	157	14,200	1.10	6.3
B.D.	2.5	38	8.2	1.00	9.5	127	228	27,700	0.82	7.8
G.L.	1.7	43	9.6	0.79	5.8	120	119	14,600	0.82	6.0
T.G.	1.8	53	6.6	0.85	8.1	130	159	25,100	0.63	6.0
Mean	1.9	42	6.7	1.18	8.7	138	195	20,600	0.97	7.2
Control subjects										
C.E.B.	3.5	40	21.0	0.42	5.9	166	82	19,400	0.42	5.9
R.M.	3.5	36	18.8	0.44	5.7	160	93	19,600	0.48	6.2
W.M.	4.0	41	16.0	0.46	7.4	150	63	13,600	0.46	7.4
J.G.	4.1	35	16.4	0.37	5.5	92.5	55	17,500	0.32	4.8
J.L.	4.2	51	20.6	0.29	6.1	144	60	22,400	0.27	5.7
G.G.	3.6	46	24.9	0.36	6.1	113	40	13,300	0.30	5.1
Mean	3.8	41	19.6	0.39	6.1	138	67	17,600	0.37	5.8
Non-steady state‡										
J.H.	2.8§	55	13.7	0.52	7.8	87.4	48	26,600	0.18	2.7

* $u(t)$ = dpm/mg urea carbon at time t , measured in hours after isotope injection.

† Adjusted to "initial" value by dividing by $F = 0.90$ (see text).

‡ The serum albumin of patient J.H. fell from 3.2 to 2.4 g/100 ml during 15 days of observation. His synthetic rate measurements were made when the albumin was 2.8 g/100 ml.

of urinary urea also reached its peak within 2–3 hr, and thereafter it declined predominantly as a single exponential function (Fig. 2). The specific activity of CO₂ in the expired air determined as previously described (17) declined rapidly (Fig. 3), reaching one-tenth of the initial specific activity in 3 hr.

Table II lists the synthetic rates for albumin as determined by ¹⁴C incorporation, and those estimated indirectly from the catabolic rates measured simultaneously with albumin-¹³¹I. The estimates of albumin synthesis obtained by the two methods agreed closely in both control subjects and patients with gastrointestinal protein loss. The mean difference between the two estimates did not differ significantly from zero in either of the two groups of patients ($P > 0.10$).

These results obtained under steady-state conditions were in contrast to those obtained in patient J.H. In this subject, the serum albumin concentration fell from 3.2 to 2.4 g/100 ml during the 15 days of the study indicating a catabolic rate greater than the synthetic rate. The measured

catabolic rate (albumin-¹³¹I) was 7.8 mg/kg per hr, nearly threefold greater than the synthetic rate of 2.7 mg/kg per hr measured with the ¹⁴carbon

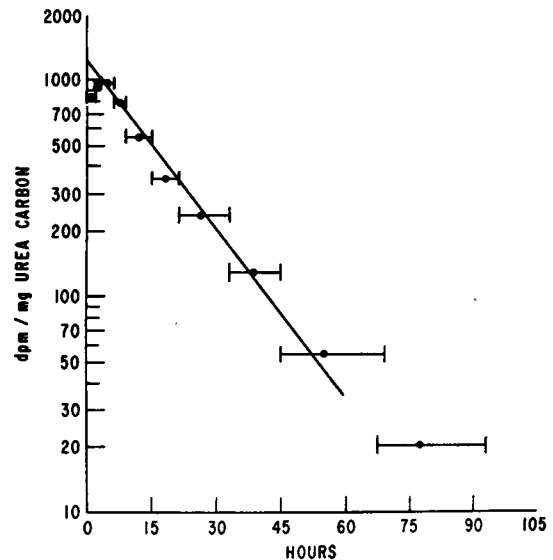


FIGURE 2 The specific activity of urinary urea reaches a maximum within 2–3 hr and then falls as a single exponential function.

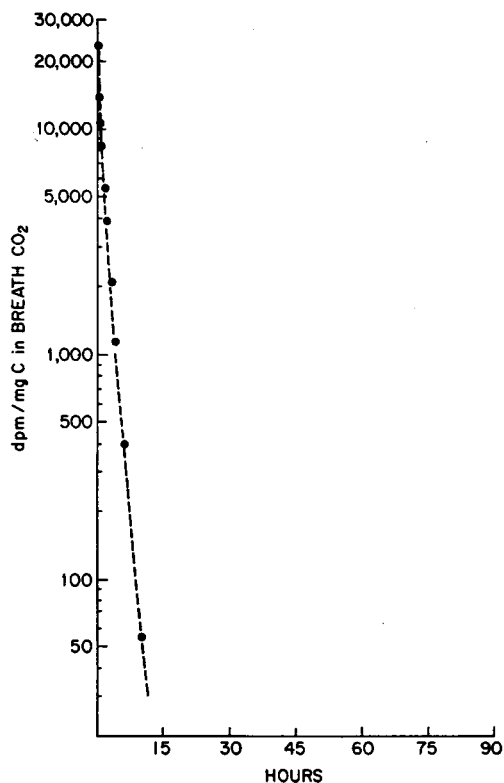


FIGURE 3 The specific activity of CO₂ in the breath declines rapidly after intravenous injection of ¹⁴C-labeled bicarbonate.

method. This illustrates the value of the method for the direct measurement of the synthetic rate since an iodinated protein turnover, as usually performed, cannot be used to estimate the synthetic rate in non-steady state conditions.

The rates of albumin synthesis in the seven control subjects averaged 5.8 mg/kg per hr and varied between 4.8 and 7.4 mg/kg per hr. In the patients with gastrointestinal protein loss, the albumin synthetic rates were higher, averaging 7.2 mg/kg per hr and varying from 6.0 to 10.0 mg/kg per hr. This difference was statistically significant ($P < 0.05$) using Student's *t* test. The increase in the fractional synthetic rates in the patients with gastrointestinal protein loss was more striking, averaging 0.97% of the plasma pool per hour, compared to an average of 0.37% of the plasma pool per hour in the control subjects.

Fibrinogen synthetic rates were somewhat greater in the five patients with gastrointestinal protein loss than in the five control subjects, averaging 3.2 and 1.9 mg/kg per hr, respectively

(Table III). There was some variation between the individual values in both groups, and the difference between groups was significant at the 10% but not at the 5% level ($0.05 < P < 0.10$).

Variation was also observed in the calculated synthetic rates of transferrin, especially in the four control subjects (Table IV). One control subject, W.M. failed to incorporate radioactivity into circulating transferrin during the 3 hr period of observation, even though he simultaneously synthesized albumin and fibrinogen at normal rates and had a normal serum concentration of transferrin. In the patients with gastrointestinal protein loss, the variation observed in the synthetic rates of transferrin was similar to the variation observed in the synthetic rates of fibrinogen.

DISCUSSION

The results of the present study must be considered in the light of the assumptions and limitations of the methods used. The assumptions involved in the use of ¹³¹I-labeled proteins in the determination of catabolic rates have been discussed in detail previously (18). However, the assumptions of the method used for determination of synthetic rates will be reviewed, as well as the measurements necessary to perform the calculations. The following are the assumptions inherent in this method.

Methodological assumptions

(1) ¹⁴C-labeled bicarbonate must have the characteristics of a "pulse label" with little or no persistent incorporation of radioactivity into the urea or the arginine guanido carbon of plasma proteins. If persistent incorporation were to occur, the spuriously high ¹⁴C activity in urinary urea would result in underestimation of the synthetic rates. The rapid decline in specific activity of the expired carbon dioxide, the decline of the urinary urea specific activity as a single exponential, and the parallel decline of ¹³¹I and ¹⁴C specific activities in plasma albumin, obtained both in this study and in preceding ones (2), provide evidence that this requirement is fulfilled.

(2) Both the plasma proteins being studied and urea must be synthesized from the same labeled pool of liver arginine (Fig. 1), and this must be the only significant synthetic site for these substances. It has been shown that albumin and fibrin-

TABLE III
Fibrinogen Synthesis

Patient and diagnosis	Plasma fibrinogen concentration	Total circulating fibrinogen	Guanido carbon	$\int_0^{\infty} u(t)dt^*$	% Plasma pool synthesized/hr	Synthetic rate
	mg/100 ml	mg/kg	dpm/mg†			
Gastrointestinal protein loss						
J.T.	505	190	260	21,400	1.2	2.3
N.B.	420	160	350	14,200	2.5	4.0
B.D.	315	120	380	27,700	1.4	1.7
G.L.	420	180	330	14,600	2.3	4.1
T.G.	550	290	350	25,100	1.4	4.1
Mean	442	188	332	20,600	1.8	3.2
Control subjects						
C.E.B.	465	190	130	19,400	0.67	1.3
R.M.	660	240	150	19,600	0.76	1.8
W.M.	590	240	110	13,600	0.81	1.9
J.G.	420	150	400	17,500	2.30	3.4
J.M.G.	410	180	200	33,000	0.60	1.1
Mean	509	200	198	20,600	1.03	1.9
Non steady state						
J.H.	740	410	110	26,600	0.41	1.7

* $u(t)$ = dpm/mg urea carbon at time t , measured in hours after isotope injection.

† Adjusted to "initial" value by dividing by $F = 0.90$ (see text).

TABLE IV
Transferrin Synthesis

Patient and diagnosis	Total serum iron-binding concentration	Total circulating transferrin	Guanido carbon	$\int_0^{\infty} u(t)dt^*$	% Plasma pool synthesized/hr	Synthetic rate
	μg/100 ml	mg/kg	dpm/mg†			
Gastrointestinal protein loss						
J.T.	235	69	120	21,400	0.56	0.39
N.B.	184	57	81	14,200	0.58	0.33
B.D.	206	64	98	27,700	0.35	0.22
G.L.	195	67	99	14,600	0.68	0.45
T.G.	200	85	49	25,100	0.20	0.17
Mean	204	68	89	20,600	0.47	0.31
Control subjects						
C.E.B.	214	69	130	19,400	0.67	0.46
R.M.	198	57	38	19,600	0.19	0.11
W.M.	309	100	0	13,600	0	0
J.G.	197	55	130	17,500	0.74	0.41
Mean	230	70	74	17,500	0.40	0.25

* $u(t)$ = dpm/mg urea carbon at time t , measured in hours after isotope injection.

† Adjusted to "initial" value by dividing by $F = 0.90$ (see text).

ogen are synthesized predominantly or totally by the liver (19, 20), and evidence obtained in hepatectomized dogs (20) strongly suggests that transferrin is also synthesized predominantly by the liver. Evidence from experiments with hepatectomized dogs also indicates that at least 95% of urea also originates in the liver (21).

(3) As discussed earlier, calculations of protein synthetic rates are based upon urinary urea specific activity curves. This method of calculation is valid unless there are major short-term fluctuations in rates of urea synthesis or excretion. Within these limitations, this method of analysis provides a major advantage because one does not have to recover all of the newly synthesized urea, only the portion which is excreted into the urine. In the current study, the available evidence indicated that if changes in urea metabolism occurred, they were insufficient to affect the above relationship: (a) plasma urea concentration (BUN) remained constant throughout the period of study in all patients; (b) urea excretion rates (milligram urea C per hour) showed no significant changes during the period of study; and (c) radioactive urea excretion rates (disintegration per minute excreted per hour) declined as a single exponential, paralleling the urea specific activity curves (disintegrations per minute per milligram urea, Fig. 2) in each patient.

(4) As with all other isotopic studies, it is assumed that radioactive molecules are metabolized in the same manner as their nonradioactive counterparts. In this instance, labeling takes place as an endogenous biosynthetic process, so that potential damage to the protein by purification and labeling with radioactive iodine is eliminated.

Experimental measurements

Based on the foregoing assumptions, the following determinations are required to calculate the synthetic rates for plasma proteins after the injection of bicarbonate- ^{14}C : (a) plasma concentration of the protein being studied; (b) plasma volume; (c) the specific activity of arginine guanido carbon of the protein; (d) the fraction of newly synthesized protein which is within the plasma compartment at the time the protein specific activity is measured; and (e) urinary urea specific activity during the period of the study. It should be noted that there are several determi-

nations which do not have to be made for this calculation. These include: the other pathways and products of labeled carbonate and arginine, the total body pool of urea, and the quantity of arginine present in the protein being studied.

Potential sources of experimental error

In practice, several possible sources of experimental error in these determinations may be considered. The potential errors related to the measurement of plasma protein concentrations and plasma volume are primarily technical.

The carbon and ^{14}C carbon measurements present some special problems. In order to obtain the specific activity of the guanido carbon of a particular protein, the preparation of protein analyzed must be pure. The magnitude of error due to a contaminating protein depends on the specific activity of the contaminant as well as its relative amount.

An error may also arise from the introduction into the manometer of carbon (as carbon dioxide) which is not derived from the urea or protein digestion. Therefore, it is necessary to process blanks with all sample runs to quantitate the amount of spurious carbon that might be introduced from particular batch of reagent. It is also desirable to have as much protein and urea to analyze as possible in order to minimize this error. Since arginine guanido carbon comprises only about 0.5% of most proteins, rather large quantities of purified protein are required for analysis. The urea determinations in the present study were performed with urea containing from 3 to 15 mg of carbon, and the protein determinations with sufficient protein to yield from 0.3 to 2.8 mg of carbon as carbon dioxide. The blank averaged 0.018 mg of carbon with a standard deviation of 0.008 mg of carbon.

Another potential problem lies in determining the fraction of newly synthesized protein which is within the plasma compartment at the time the specific activity of the protein is measured. Not all of the newly synthesized protein is delivered to the plasma at the same time, and as the later portions are being delivered, the earlier portions are beginning to undergo distribution and catabolism. The protein samples in the current study were obtained 3 hr after the injection of the bicarbonate- ^{14}C . McFarlane has presented evidence indicating that approximately 90% of the newly synthesized

albumin and fibrinogen are found in the plasma at this time (2), and this figure was used in the current study. Potential errors due to biological variation in the rates of distribution and catabolism are small, probably not exceeding 5–10%. With proteins for which the rate of delivery of newly synthesized protein to the plasma has not been determined, the potential error in determining this fraction may be greater.

Interpretation of results

Two separate parameters are used to describe the results in this study, and it is important to keep in mind the distinction between them. Each parameter is applicable to both synthesis and catabolism of proteins. The first is the fraction (or per cent) of the intravascular pool of protein catabolized or synthesized per unit time, and is referred to as the fractional catabolic rate or the fractional synthetic rate. The second is the product of the first parameter and the total circulating pool of protein, and measures the mass of protein synthesized or catabolized per unit time. This parameter is expressed here in milligrams per kilogram body weight per hour, and is referred to as the synthetic rate or the catabolic rate of the protein. Thus, two subjects might have markedly different fractional catabolic rates (per cent of plasma pool per hour), but their catabolic and synthetic rates (milligram per kilogram body weight per hour) may be the same if they have differences in the size of their intravascular pool of protein.

The mean of albumin synthetic rates estimated using the bicarbonate- ^{14}C method was 5.8 mg/kg per hr (139 mg/kg per day) in the control subjects. This value is in close agreement with the estimates of Wilkinson and Mendenhall (22) and Takeda and Reeve (23) derived from catabolic rates of iodinated albumin, but is 30–70% lower than the values obtained by other workers using iodinated albumin (24–28). In most of these latter studies, the albumin survival half-times were significantly shorter than those observed in the present study, and the albumin used in these earlier studies may have been damaged during purification and labeling.

In the present study, there was a close similarity between the average catabolic rate (albumin- ^{125}I) and the momentary synthetic rate (bicarbonate- ^{14}C) of albumin, both in control subjects and in

the patients with gastrointestinal protein loss. This suggests that relatively little fluctuation occurs in the rate of synthesis of albumin from moment to moment in either group, and provides further evidence that the subjects studied were in a steady state of albumin metabolism. The presence of the steady state was confirmed in each of the subjects by the unchanging serum albumin throughout the course of the study. In addition, each of the patients with gastrointestinal protein loss had had no significant change in serum albumin in multiple determinations performed over the previous 1–10 yr. Four of the five patients had also had previous albumin- ^{125}I turnover studies with estimates of albumin metabolism that were essentially the same as those obtained in the present study; three of these, B.D., N.B., and J.T., were included in a previously published report (29).

In the current study, albumin catabolic rates (milligram per kilogram body weight per hour) measured with albumin- ^{125}I averaged 43% higher in patients with gastrointestinal protein loss than in controls, a value nearly identical with the 41% increase observed in the adults with gastrointestinal protein loss in the previous study (29). The increase in albumin synthetic rates (milligram per kilogram body weight per hour) measured by ^{14}C carbon incorporation was quite comparable, averaging 24% in the patients with gastrointestinal protein loss. This increase in the albumin synthetic rates was statistically significant, but must be considered relatively small when compared to the threefold increase in the fractional catabolic rates (per cent of plasma pool per hour) in these subjects. A similarly limited increase in albumin synthesis is seen in subjects with hypoalbuminemia secondary to nephrosis (30–32). Thus, the profound hypoalbuminemia which may occur in these and other subjects with accelerated albumin catabolism is readily understood. It represents an altered equilibrium in which the fractional catabolic rate is markedly increased, and the size of the plasma pool of protein is markedly diminished; thus their product, the catabolic rate or the equivalent synthetic rate, is normal or only moderately increased. In this system, the increase in fractional catabolic rate is the primary event, the limitation in albumin synthesis may be looked upon as a modifying condition, and hypoalbuminemia as the result. The failure to increase albumin synthesis further in

response to accelerated catabolism and reduced concentration suggest that in these patients, albumin is synthesized by the liver at nearly its maximal capacity.

For fibrinogen, the mean of fractional synthetic rates in control subjects was 1.0% of the plasma pool per hour. This agrees well with average fractional catabolic rates of 1.0%, 0.90%, and 1.3%/hr derived from the iodinated fibrinogen turnover studies of McFarlane, Todd, and Cromwell (33) Amris and Amris (34), and Takeda (35), respectively. Fibrinogen synthetic rates were more variable than albumin synthetic rates in both groups of subjects. Patients with gastrointestinal protein loss produced an average of 70% more fibrinogen per kilogram body weight than did control subjects, but because of the variability from individual to individual, this difference was not statistically significant at the 5% level. Serum fibrinogen levels were only slightly (13%) lower in the patients with gastrointestinal protein loss. The increase in synthetic rate of fibrinogen in these subjects is adequate to account for the near normal serum levels without invoking compensatory decreases in extraintestinal catabolism.

Marked variation was observed in the calculated synthetic rates for transferrin. Such variation might represent true variation in the rates of synthesis among patients, fluctuations in synthetic rates from time to time in each patient, or undetected and variable errors in the determination. Among the potential errors previously discussed, one which may be pertinent is the possibility of variation in the rate of delivery of newly synthesized transferrin to the plasma, since this has not been directly measured. Further work will be required to differentiate these possibilities. The fractional synthetic rates observed here varied from 0 to 0.74% of the plasma pool per hour and averaged 0.40%. Fractional catabolic rates derived from iodinated transferrin studies generally have been somewhat higher. The study of Cromwell (36) showed an average catabolic rate of 0.62% of the plasma pool/hr, that of Awai and Brown 0.67%/hr (37), Jarnum and Lassen 0.77%/hr (38), and Katz 0.95%/hr (39).

The study performed in patient J.H. demonstrates the use of the method to measure protein synthesis in the non-steady state. This patient had widespread carcinoma of the lung and died a few

weeks after the study. His serum albumin fell from 3.2 to 2.4 g/100 ml during the 15 days of the observation. The albumin-¹³¹I study performed during this time demonstrated a somewhat accelerated rate of catabolism, with a survival half-time of 13.7 days and an average catabolic rate of 7.8 mg/kg per hr. At the same time, his rate of albumin synthesis was only one-third as much, 2.7 mg/kg per hr.

The methodology employed in this study provides a potent tool in the investigation of the metabolism of plasma proteins. It is limited to the investigation of proteins made in the liver and requires rather large quantities of purified protein. However, it provides an opportunity to study several proteins simultaneously and to evaluate the short-term effects of drugs, infusions, acute illness, and other physiological factors upon protein synthesis.

REFERENCES

1. Weissman, S., D. P. Tschudy, H. Bacchus, and M. Eubanks. 1961. Use of precursor product relationships in determining serum albumin half-life. *J. Lab. Clin. Med.* **57**: 136.
2. McFarlane, A. S. 1963. Measurement of synthesis rates of liver-produced plasma proteins. *Biochem. J.* **89**: 277.
3. McFarlane, A. S., L. Irons, A. Koj, and E. Regoezci. 1965. The measurement of synthesis rates of albumin and fibrinogen in rabbits. *Biochem. J.* **95**: 536.
4. Reeve, E. B., J. R. Pearson, and D. C. Martz. 1963. Plasma protein synthesis in the liver: method for measurement of albumin formation *in vivo*. *Science*. **139**: 914.
5. Swick, R. W. 1958. Measurement of protein turnover in rat liver. *J. Biol. Chem.* **231**: 751.
6. Waldmann, T. A. 1961. Gastrointestinal protein loss demonstrated by ⁵¹Cr-labeled albumin. *Lancet*. **2**: 121.
7. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature*. **182**: 53.
8. Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177**: 751.
9. Rutstein, D. D., E. F. Ingenito, and W. E. Reynolds. 1954. The determination of albumin in human blood plasma and serum. A method based on the interaction of albumin with an anionic dye 2-(4' hydroxybenzeneazo) benzoic acid. *J. Clin. Invest.* **33**: 211.
10. Ratnoff, O. D., and C. Menzie. 1955. A new method for the determination of fibrinogen in small samples of plasma. *J. Lab. Clin. Med.* **37**: 316.
11. Ramsay, W. N. M. 1957. The determination of the total iron-binding capacity of serum. *Clin. Chem. Acta.* **2**: 221.

12. Nagler, A. L., C. Kockwa, and L. R. Wasserman. 1962. Improved isolation of purified siderophilin from individual sera. *Proc. Soc. Exptl. Biol. Med.* 111: 746.
13. Fahey, J. L., and E. M. McKelvey. 1965. Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.* 94: 84.
14. Woeller, F. H. 1961. Liquid scintillation counting of $C^{14}O_2$ with phenethylamine. *Anal. Biochem.* 2: 508.
15. Pearson, J. D., N. Veall, and H. Vetter. 1958. A practical method for plasma albumin turnover studies. *Strahlentherapie Sonderbaende.* 38: 290.
16. Matthews, C. M. E. 1957. The theory of tracer experiments with I^{131} -labeled plasma proteins. *Phys. Med. Biol.* 2: 36.
17. Berlin, N. I., B. M. Tolbert, and J. H. Lawrence. 1951. Studies in glycine-2- C^{14} metabolism in man I: The pulmonary excretion of $C^{14}O_2$. *J. Clin. Invest.* 30: 73.
18. Freeman, T., and C. M. E. Matthews. 1958. Analysis of the behavior of I^{131} -albumin in the normal subject and the nephrotic patient. *Strahlentherapie Sonderbaende.* 38: 283.
19. Miller, L. L., and W. F. Bale. 1954. Synthesis of all plasma protein fractions except gamma globulins by the liver. *J. Exptl. Med.* 99: 125.
20. Weissman, S. M., R. D. Wochner, F. X. Mullins, A. Wynngate, and T. A. Waldmann. 1966. Synthesis of plasma proteins by hepatectomized dogs. *Am. J. Physiol.* 210: 128.
21. Craigie, A., V. M. Rosenoer, C. Shaldon, and A. S. Tavaill. 1965. Extrahepatic urea synthesis in the dog. *Biochem. J.* 97: 31. (Abstr.)
22. Wilkinson, P., and C. L. Mendenhall. 1963. Serum albumin turnover in normal subjects and patients with cirrhosis measured by I^{131} -labeled human albumin. *Clin. Sci.* 25: 281.
23. Takeda, Y., and E. B. Reeve. 1963. Studies of the metabolism and distribution of albumin with autologous I^{131} -albumin in healthy men. *J. Lab. Clin. Med.* 61: 183.
24. Sterling, K. 1951. The turnover rate of serum albumin in man as measured by I^{131} -tagged albumin. *J. Clin. Invest.* 30: 1228.
25. Bauman, A., M. A. Rothschild, R. S. Yalow, and S. A. Berson. 1955. Distribution and metabolism of I^{131} -labeled human serum albumin in congestive heart failure with and without proteinuria. *J. Clin. Invest.* 34: 1359.
26. Steinfeld, J. 1960. Difference in daily albumin synthesis between normal men and women as measured with I^{131} -labeled albumin. *J. Lab. Clin. Med.* 55: 904.
27. Jarnum, S., and M. Schwartz. 1960. Bestemmelse of albuminomsaetningen med I^{131} maerket albumin. *Nord. Med.* 63: 708.
28. Beeken, W. L., W. Volwiller, P. D. Goldsworthy, L. E. Garby, W. E. Reynolds, R. Stogsdill, and R. S. Stemler. 1962. Studies of I^{131} -albumin catabolism and distribution in normal young male adults. *J. Clin. Invest.* 41: 1312.
29. Waldmann, T. A., J. L. Steinfeld, T. F. Dutcher, J. D. Davidson, and R. S. Gordon, Jr. 1961. The role of the gastrointestinal system in "idiopathic hypoproteinemia." *Gastroenterology.* 41: 197.
30. Gitlin, D., C. A. Janeway, and L. E. Farr. 1956. Studies on the metabolism of plasma proteins in the nephrotic syndrome I. Albumin, globulin and iron-binding globulin. *J. Clin. Invest.* 35: 44.
31. Kaitz, A. L. 1959. Albumin metabolism in nephrotic adults. *J. Lab. Clin. Med.* 53: 186.
32. Freeman, T., and S. Cohen. 1960. Plasma protein metabolism in the nephrotic syndrome. In *Protides of the Biological Fluids*. H. Peeters, editor. Proceedings of the 8th colloquium. Bruges. Elsevier, Amsterdam. 230.
33. McFarlane, A. S., D. Todd, and S. Cromwell. 1964. Fibrinogen catabolism in humans. *Clin. Sci.* 26: 415.
34. Amris, A., and C. J. Amris. 1964. Turnover and distribution of I^{131} -iodine labeled human fibrinogen. *Thromb. Diath. Haemorrhag.* 11: 404.
35. Takeda, Y. 1966. Studies of the metabolism and distribution of fibrinogen in healthy men with autologous I^{131} -labeled fibrinogen. *J. Clin. Invest.* 45: 103.
36. Cromwell, S. 1964. The metabolism of transferrin. In *Protides of the Biological Fluids*. H. Peeters, editor. Proceedings of the 11th colloquium. Bruges 1963. Elsevier, Amsterdam. 484.
37. Awai, M., and E. B. Brown. 1963. Studies of the metabolism of I^{131} -labeled human transferrin. *J. Lab. Clin. Med.* 61: 363.
38. Jarnum, S., and N. A. Lassen. 1961. Albumin and transferrin metabolism in infections and toxic diseases. *Scand. J. Clin. Lab. Invest.* 13: 357.
39. Katz, J. H. 1961. Iron and protein kinetics studied by means of doubly-labeled human crystalline transferrin. *J. Clin. Invest.* 40: 2143.