JCI The Journal of Clinical Investigation

# Short-term measurement of catabolic rates using iodine-labeled plasma proteins

A. S. McFarlane, A. Koj

*J Clin Invest.* 1970;49(10):1903-1911. https://doi.org/10.1172/JCI106409.

**Research Article**

Fractional catabolic rates of iodine-labeled plasma albumins and fibrinogens have been measured in experiments of a few hours duration in adult rabbits. The method used which is based on release of labeled iodide from the protein gives accurate estimates of catabolic rates (expressed as fractions of the plasma protein pool catabolized per day) after 36 hr without the need for urine collections. At earlier times and using undenatured albumin, fractional catabolic rates increased steadily from approximately 10% per day in the first few hours to a constant 20-25% per day after 24 hr. Fibrinogen values which started at 15% plateaued after 24 hr at 30-35% per day. The fractional synthesis rate of albumin, measured with $^{14}C$-labeled carbonate in the first 6 hr when the short-term fractional catabolic rate was 10.2% agreed with the 36 hr plateau value of 29% per day. The presence of traces of denatured or polymerized proteins was revealed by high initial fractional catabolic rates, and in a few experiments biological screening in another animal was not fully effective in removing them. On the other hand, some labeled albumins showed no traces of denatured protein even without biological screening. Fibrinogen polymer was catabolized rapidly and behaved like denatured or incipiently clotted fibrinogen. Irradiation of albumin produced a marked increase in early fractional catabolic rates, and some proteins […]



# Short-Term Measurement of Catabolic Rates Using Iodine-Labeled Plasma Proteins

A. S. McFarlane and A. Koj

*From the National Institute for Medical Research, Mill Hill, London, England*

Abstract Fractional catabolic rates of iodine-labeled plasma albumins and fibrinogens have been measured in experiments of a few hours duration in adult rabbits. The method used which is based on release of labeled iodide from the protein gives accurate estimates of catabolic rates (expressed as fractions of the plasma protein pool catabolized per day) after 36 hr without the need for urine collections. At earlier times and using undenatured albumin, fractional catabolic rates increased steadily from approximately 10% per day in the first few hours to a constant 20–25% per day after 24 hr. Fibrinogen values which started at 15% plateaued after 24 hr at 30–35% per day. The fractional synthesis rate of albumin, measured with $^{14}$C-labeled carbonate in the first 6 hr when the short-term fractional catabolic rate was 10.2% agreed with the 36 hr plateau value of 29% per day. The presence of traces of denatured or polymerized proteins was revealed by high initial fractional catabolic rates, and in a few experiments biological screening in another animal was not fully effective in removing them. On the other hand, some labeled albumins showed no traces of denatured protein even without biological screening. Fibrinogen polymer was catabolized rapidly and behaved like denatured or incipiently clotted fibrinogen. Irradiation of albumin produced a marked increase in early fractional catabolic rates, and some proteins after prolonged storage and labeling behaved similarly. Alternative theories to explain the low fractional catabolic rates in the first 24 hr are considered. Since radioautographic experiments failed to provide evidence for retention of labeled proteins or their nondiffusible breakdown products inside catabolic cells, preference is given to the view that catabolism occurs in a pool of significant protein content which is either a subunit of the extravascular pool or is independent and sandwiched between the plasma and extravascular pools. Earlier hypotheses concerning small catabolic pools in which protein specific activities approximate closely to plasma values at the same time are considered to be no longer tenable.

Dr. Koj's present address is the Department of Biochemistry and Biophysics, Jagiellonian University, Grodzka 53, Cracow, Poland.

*Received for publication 5 March 1970 and in revised form 22 June 1970.*

## INTRODUCTION

Fractional catabolic rates of plasma proteins are usually calculated by dividing iodide activities in 24-hr urines by mean protein-bound activities integrated in the plasma over the same interval of time (1). Because iodide excretion rates fluctuate, results vary ±30% about the mean and have to be averaged over several days during which time urine must be collected quantitatively. It has been suggested that measurements of nonprotein-bound activity in the body water (or plasma) might be used to calculate fibrinogen catabolic rates (2), and Donato, Vitek, Bianchi, and Federighi (3) describe an elegant mathematical procedure for doing this in the case of albumin after injecting alternatively labeled iodide. As some members of the same group showed (4), a procedure of this kind becomes mandatory when patients with severely impaired renal function are being investigated.

Even when iodide excretion rates are normal it is still advisable to inject labeled iodide when measuring protein catabolic rates over intervals of only a few hours. Only in this way, as will be shown, can the awkward problem of multiple catheterizations of experimental animals to collect urine quantitatively at predetermined times be circumvented, and in addition the injection of independently labeled iodide facilitates the measurement of the iodide space and thus of activity retained in the animal. Attempts to determine fractional catabolic rates of plasma albumin and fibrinogen in rabbits in short intervals of time are described below.

## METHODS

*Protein for labeling. Albumins* were prepared from heparinized rabbit plasma by adding 2 volumes of 0.90% sodium chloride solution and 3 volumes of ammonium sulfate solution saturated at 37°C. The precipitate was centrifuged off and discarded, and the clear supernatant was adjusted to pH 4.6 by adding 0.1 N sulfuric acid. The albumin precipitate was then suspended in a volume of water equal to the initial plasma volume and dialyzed for 24 hr against running tap water and for another 48 hr against a large volume of distilled water. The small precipitate was centrifuged off, and the clear supernatant was dispensed in 10-mg lots (about 0.25 ml) for storage at −10°C. Polyacrylamide electrophoresis showed most of the albumin to contain traces of $\alpha$- and $\beta$-globulins. These could be removed by further CM-Sephadex treatment, but doing so had little or no effect on the catabolic information obtained with the parent labeled albumin in rabbits.

*Fibrinogens* were prepared also by fractional ammonium sulfate precipitation as described (2). At first, steps were not taken to get rid of fibrinogen polymer (or "cryofibrinogen"), but as the need for this became increasingly evident, protein precipitated from the plasma up to 18% saturation with ammonium sulfate was discarded, and only the 18–25% precipitate was harvested and further purified (5). As explained below, this had an important effect on catabolic results obtained with the labeled protein.

*Iodine labeling.* Albumin and fibrinogen were labeled using iodine-monochloride, taking the recognized precautions to avoid denaturation (6). Most albumin preparations contained 0.1–0.2% of nonprecipitable activity, the corresponding figures for fibrinogen being 0.2–0.4%.

*Measuring nonprotein activities.* 0.05 ml of 0.1 N NaI was added to 1 ml of plasma followed by 3 ml of the PTA reagent (5% phosphotungstic acid in 2 N HCl). After thorough mixing, the protein precipitate was removed by centrifugation, and the supernatant was filtered through Whatman No. 1 paper. 2 ml of the filtrate was then counted, and the $^{131}I$ count was doubled to give the iodide activity in 1 ml of body water. $^{125}I$ counts were multiplied by 2.40 to allow for the absorption of the softer radiation of this isotope by the phosphotungstate in the supernatant. This factor was arrived at in a preliminary experiment by adding trace volumes of iodide-$^{131}I$ and -$^{125}I$ to 1 ml lots of rabbit plasma and by deproteinizing each, as described, with 3 ml PTA reagent. In 2 ml of the clear supernatants, 41.7% of the $^{125}I$ activity and over 49% of the $^{131}I$ activity were measured. The $^{125}I$ recovery becomes 100% when multiplied by 2.40. When nonprotein activities were measured in an aliquot of the iodine-labeled protein prepared for injection, bovine albumin was added as carrier before mixing with PTA. The nonprecipitable activities expressed as percent of the total count in the sample are referred to below as "PTA values."

*Measurement of radioactivities.* $^{131}I$ and $^{125}I$ activities were determined simultaneously in a well-type scintillation counter with twin pulse-height analyzers and scalers enabling each isotope to be counted with maximum efficiency and minimum crossover into the other channel. $^{132}I$ activity was measured in the $^{131}I$ channel, and the sample was recounted 24–48 hr later to obtain the $^{131}I$ count alone. Corrections for decay of $^{132}I$ were based on actual counts on a $^{132}I$ standard.

Total body radioactivities of $^{131}I$ were measured with a ring of six Geiger tubes (7).

*Experimental procedure.* Sandylop or New Zealand White rabbits in metabolic cages were supplied with drinking water containing 0.005% sodium iodide and 0.45% sodium chloride, starting at least 48 hr before the experiment. When labeled iodide was given, it was injected subcutaneously followed 40–50 min later by the $^{131}I$-labeled protein given intravenously. When mixtures of $^{131}I$-labeled albumin and $^{125}I$-labeled fibrinogen were used, the parameters of iodide excretion and distribution were measured with $^{132}I$-labeled iodide, while in other cases $^{125}I$-labeled iodide was used. Blood samples from the marginal ear vein were taken into heparinized test tubes 5 min after injection of the labeled proteins and thereafter at approximately hourly intervals up to 6 hr. Aliquots (0.05–0.10 ml) of plasma were diluted to 1 ml with 0.9% NaCl solution for protein counting, while 1 ml of plasma was deproteinized with PTA reagent, as described above, to determine free iodide activities.

Plasma volumes were calculated from the degree of dilution of injected labeled proteins using the activity per milliliter of the first plasma sample taken at 5 min. Activities at zero time obtained by extrapolating the plasma activity curves to $t_0$ were approximately 1% higher than the 5-min values. The volume of distribution of iodide was estimated in the same way, but using the intercept at $t_0$ of the curve of plasma iodide activities per milliliter measured over 8 hr after the injection of alternatively labeled iodide. The slope of the terminal exponential also provided the fractional elimination rate of iodide from the body water, assuming the pool size to be constant.

*Excreted iodide activities.* These were calculated by multiplying the area under a linear plot of plasma iodide activity with time by the $k$ value or slope of injected iodide. The procedure is virtually the same as that used to estimate losses of labeled urea by the socalled "area method," described in detail elsewhere (8). When iodide activities per milliliter of water were plotted, the result had to be multiplied by the volume of the iodide space to give total iodide activity "lost" or excreted in a given interval. Values so obtained agreed well with activities recovered in the urine, where in a few cases the animals were catheterized and urine collected. More exact analytical procedures for making the above calculations involve deconvolution analysis and require computer facilities (4), and these had to be resorted to in a few cases when the elimination curve of injected iodide proved to be biphasic in character. To minimize the incidence of this kind of complication, injections of unlabeled iodide were avoided during an experiment, and no kind of treatment was given that might impair renal function.

*Mean protein activities in the plasma.* These were calculated from integrated areas under the linear plot of protein activities with time. This procedure is essential in dealing with protein activities in the first few samples; but after 24 hr, when protein activities in the plasma fell only slowly, arithmetic mean values were adequate.

*Calculating protein catabolic rates.* The fractional catabolic rate (FCR) of a protein is usually defined as the fraction of protein in the plasma that is catabolized per day. Total iodide activity released from a labeled protein is used as a measure of protein catabolized, being expressed as a fraction of the mean protein-bound activity in the plasma integrated over the corresponding 24 hr interval. After an initial 24–48 hr period during which iodide activity accumulates in the body water, urine activities are assumed to equal iodide activities released by catabolism during the collection interval. Smoothed FCR time curves of metabolically homogeneous proteins such as albumins have been

shown to take the form of straight lines parallel to the time axis (9), and this has been cited as evidence in favor of the catabolic process occurring predominantly in, or in metabolic proximity to, the plasma (10). For present purposes this constant value will be referred to as the "long-term FCR (urine)."

Alternatively, the half-life of total body radioactivities was measured and related to the FCR by means of the equation

$$FCR = \frac{0.693 \times 100}{t_{\frac{1}{2}} \text{ in days} \times f} \quad (1)$$

where f is the fraction of the particular protein present in the plasma. In this case also, the values obtained are essentially long-term ones since smoothed curves of total body radioactivities are required over several days, and protein distributions (or f values) cannot be measured over shorter intervals.

*Short-term catabolic rates.* These were measured over intervals of, at most, a few hours during which, in addition to activity excreted in the urine, iodide activity that had accumulated in the body water was measured. When the interval was timed to begin with the injection of the labeled protein the formula used was:

$$FCR = \frac{\text{Iodide activity/ml plasma at t} \times \text{iodide space} + \text{activity excreted in t}}{\text{Mean protein activity/ml plasma during interval t} \times \text{plasma volume}} \times 24 \times 100. \quad (2)$$

At later times, up to 24–48 hr, the iodide activity per milliliter plasma at t was replaced in equation 2, with the increment of plasma iodide activity during the interval chosen. When the equilibrium was established between the rate of iodide release by catabolism and its rate of excretion, the formula then becomes

$$FCR = \text{PTA value} \times k \times F \quad (3)$$

where $k$ is the iodide turnover rate expressed as a fraction of the body pool of iodide renewed per day, and F is the ratio of the iodide space to the plasma volume.

## RESULTS

Equilibration of the injected labeled iodide usually occurred inside 30 min, and iodide space values for 14 rabbits varied between 28 and 38% of the body weight with a mean of 33%. The ratios of iodide space to plasma volume were between 8 and 10. Half-lives of iodide showed major individual variations (3–24 hr), and in the same rabbit the half-life could change by as much as 50% within a day for no obvious reason.

Injections of $^{125}$I-labeled iodide were repeated 2–5 days after the initial injection in most experiments to measure iodide turnover rates coincidental with measurements of PTA values. FCR's calculated according to equation 3 were in reasonable agreement with long-term FCR's (urine) averaged over 6 days.

When short-term measurements of FCR were attempted, closely similar results were obtained with different labeled albumins in seven experiments using adult normal rabbits; and Fig. 1 shows typical values for plasma protein-bound and protein-free activities in samples taken at 100-min intervals, and a final one at 24 hr (upper and lower curves). Excreted iodide activities per milliliter of body water were estimated by the area method, multiplied by the plasma volume, and combined with measured plasma iodide activities to give curves of iodide activities (retained + excreted) which would have been measured in the plasma if no excretion had occurred. FCR's given in the caption were calculated by equation 2 for consecutive 100-min intervals and for various intervals starting from the time of injection. It can be seen that FCR's increased progressively in the seven experiments from 11% in the first few hours to plateau values of 25% per day after 24 hr.

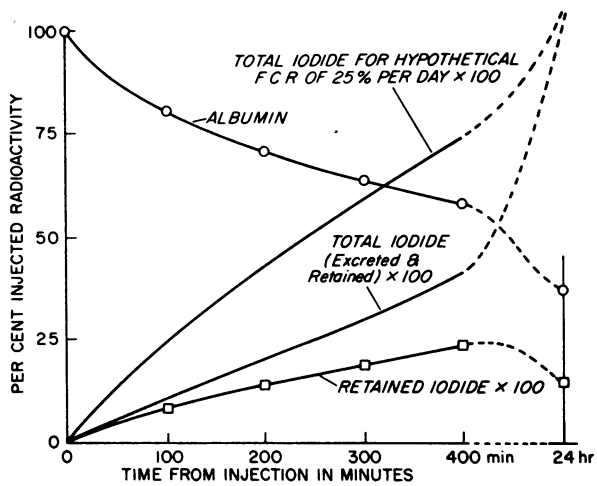


FIGURE 1 This summarizes the results of seven experiments with labeled albumin in normal rabbits in which individual values varied by ±10%. Iodide activities excreted in a given interval and expressed per milliliter of body water were calculated from the area under the iodide curve and the $k$ iodide. When the fraction of this activity in the plasma water was added to the measured activity in the plasma at the end of the interval, hypothetical total plasma iodide activities resulted which would have been obtained if no excretion had occurred. FCR's increased with time from 11% per day in the first 100 min to a plateau of 25% per day after 24 hr.

| Interval (min) | 0–100 | 100–200 | 200–300 | 300–400 | 0–200 | 0–300 | 0–400 |
|---|---|---|---|---|---|---|---|
| FCR (%/day) | 11.0 | 12.8 | 14.3 | 18.7 | 12.0 | 12.7 | 14.0 |

For comparison the figure includes a hypothetical curve of total iodide activities which would have been measured if catabolism had proceeded at a constant rate of 25% per day without excretion of iodide.

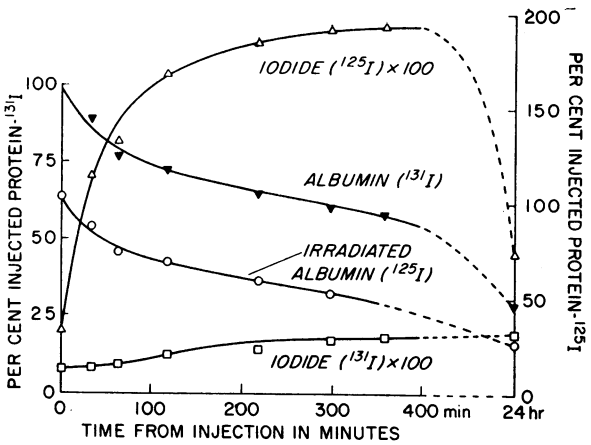


FIGURE 2 This shows protein-bound and "iodide" activities in the plasma of a rabbit at various times after receiving a mixed injection of $^{131}$I-labeled albumin and the same albumin irradiated at 12,000 rpm for 17 hr and then labeled with $^{125}$I.

| | $^{131}$I | | $^{125}$I | |
|---|---|---|---|---|
| Interval (min) | 0–180 | 0–360 | 0–180 | 0–360 |
| FCR (%/day) | 8.4 | 13.1 | 166 | 101 |

In two additional experiments, FCR's were approximately constant at 22% per day from the time of injection; and in two more, the rates in the first 6 hr were higher (45–70% per day) and subsequently declined to normal values. The possibility could not be excluded that labeled albumins used in some of these experiments contained traces of rapidly catabolized denatured protein. Unfortunately, no surplus labeled albumins were available for biological screening tests which might logically have followed, and instead the following experiments which bear in a less direct way on the denaturation possibility were undertaken.

*Effects of irradiation.* 20 mg of rabbit albumin in solution was irradiated in a glass vial by a $^{60}$Co source for 17 hr at 12,000 roentgens/hr. The albumin was then labeled with $^{125}$I and mixed with the unirradiated albumin labeled with $^{131}$I, and the mixture was injected intravenously. $^{132}$I-labeled iodide was used to measure the iodide space and the half-life of iodide in the body water, thus enabling the FCR's of both albumins to be calculated. It is clear that, although the two proteins were eliminated from the plasma at approximately similar rates, the catabolic rate of the irradiated one was much greater in the first few hours (Fig. 2). In a similar experiment with $^{125}$I-labeled albumin which was irradiated for 5½ hr at 12,000 r/hr, PTA's were abnormally high at first, but the area under the "bulge" in the $^{125}$I-labeled iodide curve was much reduced.

*Screened vs. unscreened albumin.* In another experiment, the behavior of screened and unscreened labeled albumins was compared. Approximately 4 mCi of $^{131}$I-labeled rabbit albumin was injected into a 2 kg rabbit, and the animal was bled after 17 hr. To reduce the free iodide counts in 5 ml of screened plasma containing approximately 60 μCi, this was passed through a short ion-exchange resin column (Deacidite in the chloride form) and injected into a 3 kg rabbit along with $^{125}$I-labeled iodide.

An aliquot of the same rabbit albumin preparation was labeled with $^{125}$I and injected into a second rabbit along with some of the $^{131}$I-labeled plasma recovered at 17 hr from the screening animal. The accumulation of $^{131}$I-labeled iodide in the body water of this rabbit, as reflected by PTA values, was almost identical with that in the rabbit which received the same screened $^{131}$I-labeled albumin along with $^{125}$I-labeled iodide (Fig. 3). FCR's for the screened albumin in both animals were 10% in the first 5 hr, 14–16% at 5–10 hr, and constant at 20–25% after 36 hr.

Thus the experiment failed to demonstrate any effect of screening, and it must be concluded that the unscreened protein was completely free of rapidly catabolized denatured material. The most convincing proof of this was a close identity of PTA values for both albumins in successive plasma samples.

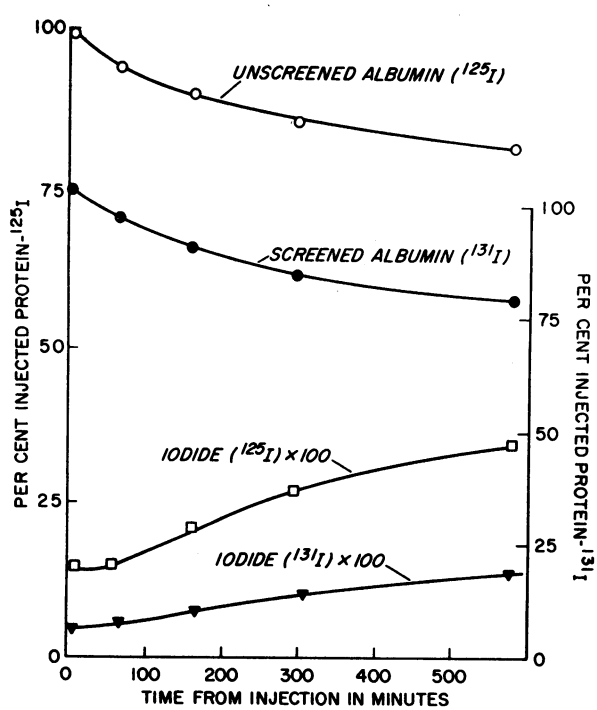


FIGURE 3 This shows protein-bound and iodide activities in the plasma of a rabbit at various times up to 10 hr after receiving a mixture of $^{125}$I-labeled albumin and the same albumin labeled with $^{131}$I and "screened" for 17 hr in another animal.

*Screened vs. unscreened fibrinogen.* To try to shed more light on the phenomenon of FCR's increasing with time after the injection of a labeled protein, a series of experiments with labeled rabbit fibrinogen was undertaken, the general plan being essentially the same as for the earlier ones with albumin. Preliminary results from several experiments suggested that this protein differed from albumin in the rabbit in having a constant FCR of 30–35% per day from as early as 3 hr after injecting the labeled protein. The possibility that this was an accidental result due to small amounts of rapidly catabolized denatured protein in the labeled preparation seemed remote since the labeled fibrinogens used were 85–95% clottable. However, in the fourth and subsequent experiments, iodide was released by catabolism at a much slower rate in the first few hours than later, and this coincided with the change in the technique of preparing fibrinogens mentioned under methods.

McFarlane showed that labeled rabbit fibrinogen polymer which was fully clottable was catabolized much more rapidly than the monomer in the same animal (5) and to a considerable extent could be separated from it by precipitation at 18% with ammonium sulfate saturation. After this step was taken to reject the polymer in rabbit and human fibrinogens, the labeled proteins consistently gave FCR's that increased with time in the first 48 hr analogous to the situation already described with albumin and also to that in an animal that received labeled iodine and 6 hr screened rabbit gamma globulin. However, although fibrinogens prepared in this way consistently contained greatly reduced amounts of rapidly catabolized protein, they were not entirely free of it. This emerged when screened and unscreened labeled fibrinogens were compared in the same animal with the results that are shown in Fig. 4. Although the levels of iodide activities were quite low in the first 100 min, levels of iodide from the unscreened protein later increased more rapidly.

In the above experiment, the plasma from the screening animal after 17 hr had a PTA of 2.35% which was reduced to 0.80% by a single passage through an ion-exchange column. An aliquot of the high PTA plasma was also injected, along with $^{125}$I-labeled iodide, into a second animal. Most of the high PTA value (0.40%) measured initially in the plasma of this animal could be explained by the injection of 2.35% of the total injected radioactivity as "iodide" into an iodide space that was 8 times the plasma volume. Assuming this $^{131}$I-labeled iodide to be excreted or otherwise eliminated from the body water of the animal at the same rate as the simultaneously injected $^{125}$I-labeled iodide, it became possible to calculate the contribution of injected nonprecipitable $^{131}$I activity to total nonprecipitable $^{131}$I activity in plasma samples taken at later times. The curve of net $^{131}$I activities attributable to catabolism alone was so nearly

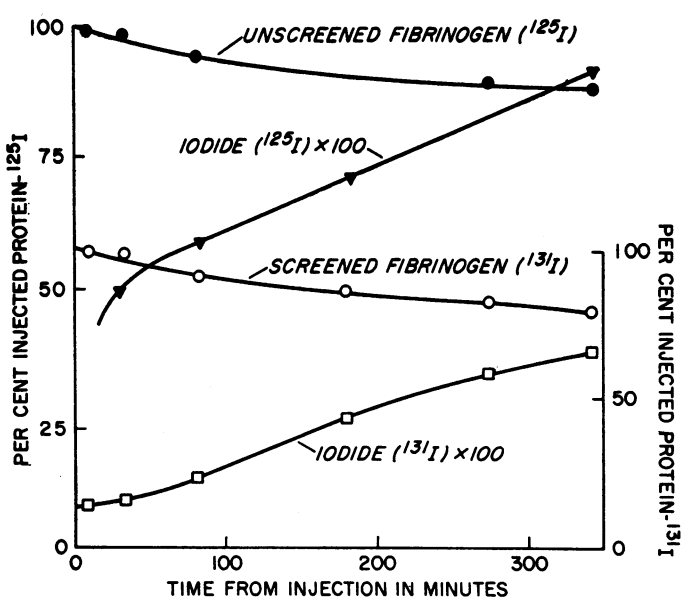


FIGURE 4 This shows protein-bound and iodide activities in the plasma of a rabbit in the first 6 hr after receiving a mixture of $^{125}$I-labeled fibrinogen and the same protein labeled with $^{131}$I and screened for 17 hr in another animal. Note the more rapid accumulation of iodide activity in the animal receiving the unscreened protein.

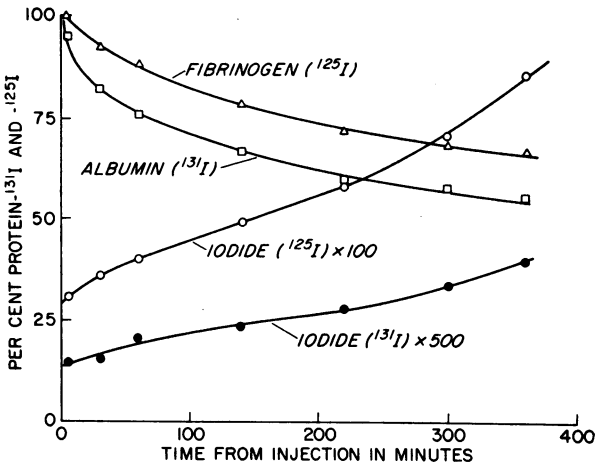


FIGURE 5 Shows fractional levels of iodide activity in the plasma in the first 6 hr after injecting albumin-$^{131}$I and fibrinogen-$^{125}$I into a rabbit. Part of the more rapid accumulation of iodide-$^{125}$I arises from the fact that the fibrinogen was unscreened.

identical with the corresponding $^{131}$I-labeled iodide curve in Fig. 4 as to justify the conclusion that resin treatment of the plasma from the screening animal had had no deleterious effect on the fibrinogen in it.

*Albumin vs. fibrinogen in the same animal.* An experiment was also carried out to compare the behavior of $^{125}$I-labeled fibrinogen and $^{131}$I-labeled albumin simultaneously in the same animal. The results are shown in Fig. 5, where the slopes of the respective iodide curves and the individual PTA values are typical of unscreened labeled proteins in regular use in this laboratory.

In another similar experiment, the $^{131}$I-labeled fibrinogen began to clot as soon as the $^{125}$I-labeled albumin was mixed with it and with $^{132}$I-labeled iodide. The translucent clot was quickly removed by centrifugation, and the residual mixture was injected without apparent ill effects to the animal. PTA's increased rapidly to 3.5% at 5 hr, fractional catabolism measured in the first 3 hr being at a rate of 138% per day and falling to 49% in the next 3 hr period.

The FCR (urine) calculated from 24-hr urinary activities augmented or reduced by changes in the pool of $^{131}$I-labeled iodide activity in the body water in the corresponding intervals were 63.7% in the first 24 hr and 35% in the second. Since the animal still had 59% of the injected $^{125}$I-labeled protein in the plasma at 24 hr compared with a normal value of 65%, and since the rate of elimination in the next few days was within normal limits, only a small per cent of the injected protein could have been incipiently coagulated, the remainder behaving, from a catabolic point of view, like normal fibrinogen. This was not altogether unexpected since the period during which greatly accelerated fractional catabolism was observed extended only over the first few hours.

The above behavior contrasts with that seen on several occasions when albumins or fibrinogens that were taken from prolonged storage or after deliberate mishandling were labeled and then injected. In these experiments also, abnormally high PTA's were measured in the first few plasma samples; but these values increased only slowly or even decreased over the first 24 hr. These labeled proteins were characterized by having abnormally high PTA values before injection which could not be reduced by further ion exchange resin treatments, and an additional peculiarity was that losses of iodide from the body water in the animal experiment as calculated by the area method were higher than activities recovered in the urine in the corresponding interval. One explanation of the latter behavior is that a labeled material was present in the plasma—possibly a nondiffusable breakdown product of the injected protein—that was not precipitable with PTA and was not excretable by the kidneys. The situation is reminiscent of that seen when the normally very low rate of dissociation of the iodide label from a protein during dialysis was observed to accelerate abruptly after contact with phosphotungstic or other acids (11).

*Fractional synthesis rates.* These were measured by the carbonate-$^{14}$C method (12) in a rabbit at the same time as it received iodine-labeled albumin and fibrinogen for short-term FCR measurements. Iodide-$^{132}$I was injected subcutaneously, and urea-$^{13}$C was injected intravenously followed 40 min later by the intravenous injection of 200 $\mu$Ci carbonate-$^{14}$C, 30 $\mu$Ci albumin-$^{131}$I, and 60 $\mu$Ci fibrinogen-$^{125}$I. Blood samples were taken up to 6 hr, and plasma samples were counted for protein-bound iodine activities, protein-free activities ($^{132}$I, $^{131}$I, and $^{125}$I), urea-$^{13}$C enrichment, urea-$^{14}$C specific activities, and 6-arginine-$^{14}$C specific activities in albumin and fibrinogen. FCR's of both proteins were calculated from equation 2, while fractional synthesis rates (FSR's) were calculated as described for the carbonate method. A FCR of 10.2% per day was obtained for albumin in the interval 0–6 hr compared with a FSR of 28.8% per day. The fibrinogen used for labeling with $^{125}$I was prepared before the introduction of the polymer-rejection step (5), and its FCR in 0–6 hr was 34.3% compared with a FSR of 37.4% per day.

## DISCUSSION

Any scheme for measuring protein FCR's over short intervals of time must presume that information will be available about the size and turnover of the iodide pool. With this information and using conventional mathematical procedures it is possible to calculate total iodide released by catabolism, i.e., both excreted and retained

in the body water. It was, therefore, clearly advantageous in the present work to inject alternatively labeled iodide along with the labeled protein, as others have done (3, 4). After 24–36 hr, when an equilibrium had been established between rates of iodide released and excreted, results of short-term measurements of FCR, which can be obtained in an experiment lasting only a few hours, appear however to be no more accurate than those of conventional measurements based on activities excreted in 24-hr intervals. This can be attributed to an essentially erratic quality in iodide excretion in rabbits, affecting both the daily output of activity in the urine and the accuracy of measurement of $k$ values.

To determine FCR's before equilibrium is attained it is essential to measure increments of iodide activity in the body water and to combine these with excreted activities. The very low and rapidly increasing FCR's observed when this was done could result from a defect in the basic principle of the short-term method or may have biological implications concerning the mechanism and site of catabolism of plasma proteins. The first three of the following four factors that might have been wholly or partly responsible had to be rejected for reasons which are given. (*a*) The first factor was the slow rate of penetration of labeled protein into catabolic cells and slow release of labeled iodide from these cells into the plasma or body water. This explanation is unlikely to be true since labeled iodide liberated by catabolism of fibrinogen or albumin begins to appear almost immediately in the plasma. (*b*) The second was an expanded iodide space. The iodide space measured with injected $^{125}$I- or $^{132}$I-labeled iodide, which is almost entirely represented by extracellular water, could be smaller than the space occupied by iodide released intracellularly from the catabolized protein. No independent evidence is available to suggest that monoiodo- and diiodo-tyrosine or other intermediates or iodide accumulate appreciably inside catabolic cells. On the assumption that these cells have a threshold iodide concentration, an attempt was made to presaturate them with inactive iodide by injecting a rabbit with large amounts of albumin iodinated with cold iodine ($^{127}$I) and 12 hr later giving the rabbit $^{131}$I-labeled albumin. Early fractional catabolic rates, however, were still low. (*c*) A third possibility was the direct excretion of early breakdown products. If labeled albumin or fibrinogen was catabolized in the first few hours in the kidney, the iodide released could conceivably be excreted directly into the urine without mixing with the body pool of iodide. If this were to happen, retained iodide activities would be low but correspondingly higher than theoretical amounts that would be found in urine collections; this was not so. Indeed, the reverse was occasionally observed, but this was only featured by a few proteins which for obscure reasons had high and irreducible PTA values before injection. (*d*) The fourth factor was catabolism occurring in a compartment of finite size in reversible communication with the plasma. Most observers agree that such a compartment must exist, outstanding questions being concerned with its size, situation, and the extent to which mixing is complete within it. Of several possible arrangements shown in Fig. 6, C is incompatible with the observation that protein catabolized in unit time became a constant fraction of the intravascular pool within 36 hr, i.e. in advance of equilibrium being established between extra- and intravascular pools as evidenced by a constant fractional elimination rate of albumin from the plasma. To avoid this inconsistency, the plasma in alternative arrangements A and B must be assumed to be in more rapid exchange with the catabolic compartment than with the whole of the extravascular pool. Although scheme $B_2$ is illustrated in the figure as being based on unidirectional protein exchange between compartments, this is not an essential requirement, nor is it essential to the other schemes that protein transfers should occur exclusively via bidirectional pathways as illustrated.

In the numerous experiments in which FCR's of albumin of 11% per day were measured in the first

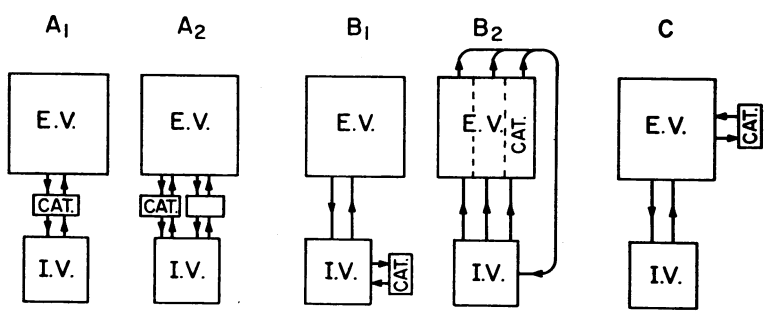


FIGURE 6 Schematic representation of alternative catabolic pool arrangements (see Discussion). E.V., extravascular compartment; I.V., intravascular compartment; CAT., catabolic compartment.

100 min, these increased steadily over the next 24 hr to plateau values of approximately 25% per day (cf. Fig. 1). The hypothetical curve of total iodide activities which is also shown in this figure was arrived at by assuming that iodide was being released from zero time at a constant rate of 25% of the protein-bound activity in the plasma per day and retained in the iodide pool.

In the first 100 min interval the mean concentration of labeled protein in the plasma was 90.6% of the initial value, and of the 9.4% that had escaped only 0.75% of the injected dose had reappeared as iodide, leaving 8.65% presumably still present as labeled protein. Based on the B schemes and assuming that all this protein was retained in the catabolic compartment during the first 100 min, the observed liberation of iodide corresponds with 125% of the albumin in this compartment being catabolized per day. Since this FCR when referred to the plasma compartment must equal 25% per day, the catabolic compartment must have a maximum albumin content equivalent to one-fifth that in the plasma.

The same form of calculation applied to the interval 100–200 min gave a 70% per day turnover rate for the catabolic compartment, suggesting that the compartment must be larger than one-fifth of the plasma. This could be anticipated since the observed rate of loss of albumin from the plasma is greater in the first 100 min than subsequently, whereas iodide activities increase at an approximately constant rate. The initial accelerated loss of labeled albumin from the plasma is believed to be due to plasma albumin equilibrating with extravascular albumin in visceral lymphatics (6) making it tempting to assume that catabolism occurs preferentially at this particular lymphatic site, but there is little or no evidence to support this.

In view of the difficulty of visualizing an anatomical site for a catabolic compartment as large as 20% of the plasma in separate communication with it, the two A schemes and $B_2$ are preferred to $B_1$. Unfortunately, with the A schemes it is not possible to arrive at even approximate limiting values for the turnover rate and size of the catabolic compartments, as has been attempted for the B ones, from measurements made only on plasma samples. It appears, however, that the observed shapes of the early part of iodide curves could be explained by a catabolic compartment of the type illustrated in $A_2$, i.e. one in parallel with a larger non-catabolic one and both communicating with the plasma and extravascular pools. The catabolic one in this case could be considerably smaller in size than 20% of the plasma in which event it would need to turn over proportionately faster than 125% per day. Arrangement $A_3$ can be regarded as a variant of $A_1$ with incomplete mixing in the catabolic compartment, a site for which might be in perivascular lymphatic spaces or in and between endothelial cells lining the capillaries. The essential feature of the A schemes is that catabolism occurs in a compartment of finite size that is metabolically sandwiched between two larger reservoirs, the plasma and extravascular lymph spaces, and such a compartment might be part of more general arrangements for the transcapillary transport of plasma proteins. In view of the paucity of evidence, the authors can only give a personal preference which is for schemes $A_3$ or $B_2$.

Lewallen, Berman, and Rall (13) consider mathematically a six-pool model for albumin distribution and catabolism based on plasma and urine measurements over a study period of 20 days in myxedematous and euthyroid patients injected with albumin-$^{131}$I. Three of the pools are ancillary to a primary three-compartment model which receives generalized treatment, and they conclude that, whereas a parallel arrangement of the three primary compartments is feasible, a series one is not, i.e. one in which one of the two extravascular compartments communicates with the plasma through the other one. The same type of reasoning might enable models $A_1$ and $A_2$ to be excluded from further consideration, but unfortunately the essential data on plasma and urine activities were not obtained in our experiments. Lewallen et al. (13) also concluded that their more rapidly exchanging extravascular compartment must be the principal site of iodoalbumin catabolism.

The various hypotheses (*a–d*) can be roughly divided into those postulating a catabolic pool of significant size on the one hand, and local delaying or concentrating effects on the other. To see whether any such effects occur, a series of radioautographic experiments was carried out on rats in which the animal's vascular system was carefully perfused to wash out labeled plasma proteins 4 hr after injecting large activities in the form of $^{125}$I-labeled rat albumin. A systematic radioautographic examination of the tissues showed intense labeling of lymph especially in perivascular areas, but no signs of labeling in what might be suspected to be catabolic cells. It is unlikely that labeled albumin retained inside such cells was missed unless the tissues concerned happened to be very extensive. The possibility that labeled iodide may have accumulated inside catabolic cells cannot be excluded, however, since this could have been washed out during fixation procedures.

The general conclusions from this investigation are both encouraging and disappointing. On the one hand, a faint picture of the catabolic compartment begins to emerge; a protein pool somewhere in the animal first has to equilibrate with labeled protein before labeled

iodide is released at its full rate. On the other hand, our main objective in undertaking the work, which was to elaborate a short-term method of measuring FCR's, was only partly achieved. It must be concluded that until the pool from which protein molecules are withdrawn to be catabolized has been defined more clearly and more is known about the nature of the catabolic process itself, it is unlikely that meaningful measurements of FCR can be made within 36 hr of injecting an iodine-labeled protein. The rates of iodide release observed within this time can only be compared in an empirical way with those from a normal animal or patient receiving an undenatured version of the same protein. Such a procedure has been shown, nevertheless, to be a most sensitive one for use in diagnosing the presence of small proportions of denatured proteins in an injection and in bringing to light the almost total absence of these in some iodine-labeled plasma protein preparations.

## ACKNOWLEDGMENTS

We are greatly indebted to Dr. K. Peters for placing at our disposal the data from one of his fibrinogen experiments, to Dr. E. Regoeczi for supplying samples of high- and low-solubility rabbit fibrinogens, and to Dr. G. W. Bisset for help with the viviperfusion of rats. Mrs. Wendy Hayward provided expert technical assistance throughout the whole of the work, and we also gratefully acknowledge the help of Mr. G. Dickinson and Mr. J. Heather with $^{13}C$ and $^{14}C$ assays.

## REFERENCES

1. McFarlane, A. S. 1957. The behavior of $I^{131}$-labeled plasma proteins in vivo. *Ann. N. Y. Acad. Sci.* **70**: 19.
2. McFarlane, A. S. 1963. In vivo behavior of $I^{131}$-fibrinogen. *J. Clin. Invest.* **42**: 346.
3. Donato, L., F. Vitek, R. Bianchi, and G. Federighi. 1967. A double tracer method for metabolic studies with iodinated proteins or polypeptides in presence of a relatively slow excretion of iodine. *J. Nucl. Biol. Med.* **11**: 1.
4. Vitek, F., R. Bianchi, and G. Federighi. 1967. The study of albumin metabolism in patients with impaired renal function. *J. Nucl. Biol. Med.* **11**: 139.
5. McFarlane, A. S. 1969. The role of fibrinogen in the management of deep vein thrombosis. *Proc. Roy. Soc. Med.* **62**: 1127.
6. McFarlane, A. S. 1964. Metabolism of plasma proteins. *In* Mammalian Protein Metabolism. H. N. Munro, and J. B. Allison, editors. Academic Press, Inc. New York. **1**: 298.
7. Campbell, R. M., D. P. Cuthbertson, C. M. E. Matthews, and A. S. McFarlane. 1956. Behavior of $C^{14}$ and $^{131}I$-labeled plasma proteins in the rat. *Int. J. Appl. Radiat. Isotop.* **1**: 66.
8. Koj, A., and A. S. McFarlane. 1968. Effect of endotoxin on plasma albumin and fibrinogen synthesis rates in rabbits as measured by the [$^{14}C$] carbonate method. *Biochem. J.* **108**: 137.
9. Cohen, S., T. Freeman, and A. S. McFarlane. 1961. Metabolism of $^{131}I$-labeled human albumin. *Clin. Sci.* **20**: 161.
10. McFarlane, A. S. 1963. Catabolism of plasma proteins. *Lancet.* **i**: 131.
11. McFarlane, A. S. 1969. *In* Physiology and Pathophysiology of Plasma Protein Metabolism. G. Birke, R. Norberg, and L. O. Plantin, editors. Pergamon Press, Ltd. Oxford. 87.
12. McFarlane, A. S., L. Irons, A. Koj, and E. Regoeczi. 1965. The measurement of synthesis rates of albumin and fibrinogen in rabbits. *Biochem. J.* **95**: 536.
13. Lewallen, C. G., M. Berman, and G. E. Rall. 1959. Studies of iiodoalbumin metabolism. I. A mathematical approach to the kinetics. *J. Clin. Invest.* **38**: 66.