Note

Estimation of Parameters in Plasma Albumin Turnover by a Modified Double Isotope Method

Ryuhei FUNABIKI,* Nobuhiro HORISAKA** and Atutane OHTA

Department of Agricultural Chemistry,
Faculty of Agriculture,
Tokyo Noko University,
Fuchu-shi, Tokyo 183, Japan

Received November 4, 1983

A pulse labeling technique is suited to single exponential decay kinetics. A deviation from the single exponential is caused by heterogeneity of the system, isotope recycling, non-random breakdown and non-steady states. Isotope recycling could be reduced by using ^{14}C -labeled carbonate, carboxyl-labeled aspartate or carboxyl-labeled glutamate.¹⁾ After pulse labeling with ^{14}C -carbonate, the specific radioactivity of plasma albumin in a rat under steady state decayed by following a single exponential function. When the specific radioactivity was logarithmically plotted against time, a straight line was obtained. The slope of this line gave the degradation rate constant of plasma albumin.²⁾ A long-term uptake experiment, the so called "constant pool method"³⁾ may be also applicable to estimate the fractional turnover rate of plasma albumin using labels minimally reutilized. Cross-checking would be desirable on determining the rate of turnover because the values obtained are likely to be diverse by the possible errors associated with such kinds of experiments. The pulse labeling and uptake experiment are combined to construct a modified double isotope method which permits an estimation of the fractional turnover rate from three sources, that is, a decay rate, an uptake rate and a cross-over point. The same figures should be obtained from the three methods. Data processing by a computer facilitates this approach.

Male Wistar rats weighing 220 ~ 250 g were used. They were housed individually in stainless steel wire-mesh cages and maintained at $22 \pm 1^\circ\text{C}$ in a room with a 12-hour light-dark cycle. The rats were intraperitoneally injected with $52 \mu\text{Ci}$ of DL-glutamic acid- $1\text{-}^{14}\text{C}$ (New England Nuclear Corp.) per 100 g of body weight and fed on a diet containing ^3H -glutamate. Each animal received once a day at 10:00 a.m. the agar gel diet composed of 12 g of dry basal diet, 17 g of 3% agar and $26 \mu\text{Ci}$ of L-glutamic acid- $3\text{-}^3\text{H}$ (New England Nuclear Corp.).

The percentages of components in the dry basal diet were as follows: casein 20; α -cornstarch 32.15; sucrose 32.15; soybean oil 10; salt mixture⁴⁾ 5; vitamin mixture⁴⁾ 0.5 and choline chloride 0.2. No appreciable body weight changes were observed during the experiment. Groups of four or two rats were killed by decapitation at 1, 3, 7, 14 and 21 days after an injection of ^{14}C -glutamate, and blood was taken from each animal; the plasma was obtained by sedimentation of the blood cells. The plasma albumin was prepared according to the procedures of Swick and Ip.²⁾ The plasma albumin obtained showed a single band on disk gel electrophoresis. The method for determining the specific radioactivity (dpm/mg protein) of labeled albumin has been described previously.⁵⁾

The degradation rate constant of the plasma albumin, K_d , was calculated from the decrease of ^{14}C radioactivity by single exponential kinetics, $S = S_0 e^{-k_d t}$ where S is the radioactivity at time t , and S_0 is the initial radioactivity. The synthesis rate constant of the plasma albumin, K_s , was also calculated from the increase of ^3H radioactivity by the relationship $S = S_a (1 - e^{-k_s t})$ where S_a is the ultimate radioactivity. The half-life of the plasma albumin, $t_{1/2}$, was obtained graphically as a first approximation from the cross-over point between the normalized curves (see the footnote for Fig. 1) of the decrease in ^{14}C and the increase in ^3H specific radioactivity. The numerical solution of the equation $e^{-k_d t} = 1 - e^{-k_s t}$ gave the final value of the half-life of the plasma albumin. Data processing was done using the least-squares program with a Sharp MZ 80 microcomputer.

The final values of the fractional turnover rate calculated from the three sources were as follows: (mean $\pm$ standard deviation) 0.209 ± 0.065 per day ($t_{1/2}$ of 3.32 days) from the ^{14}C decrease, 0.159 ± 0.018 per day from the ^3H increase and 0.184 per day from the cross-over point. The smallest turnover rate came from the uptake experiment. Isotope recycling in the uptake experiment is apt to decrease the slope of the line and to increase the ultimate level, which decrease the turnover rate constant. While the ^{14}C in glutamate can be lost as $^{14}\text{CO}_2$ in the Krebs cycle, the ^3H in glutamate has no such way to be excreted, even though a large pool of free glutamate may decrease the effect of isotope recycling. Assuming that no recycling was observed in the decay of ^{14}C -glutamate, while the uptake of ^3H -glutamate had some degree of recycling, we calculated the extent of recycling of ^3H -glutamate compared with ^{14}C -glutamate to be 12% from the equation $K_d - K_s/K_s$. Any delay of increase in the ^3H radioactivity by isotope recycling also sets the cross-over point backwards, which is the reason why the rate constant from the cross-over point has been the mid value among them. However, the difference between values obtained from the three methods was quite minor. The

* To whom reprint requests should be sent.

** Present address: Laboratory of Food and Nutrition, Toita Women's College, Hachioji-shi, Tokyo, Japan.

FIG. 1. Plasma Albumin Turnover.

An increasing curve (—●—) shows the ^{14}C -radioactivity of plasma albumin after an injection of glutamic acid- $1\text{-}^{14}\text{C}$. A decreasing curve (—○—) shows the ^3H -radioactivity of plasma albumin during daily feeding of glutamic acid- ^3H . Normalized values (the ratio of specific radioactivities on each time point to the maximum specific radioactivities, mean $\pm$ standard deviation) were plotted. The curves were obtained by a microcomputer using a non-linear least-squares program.

value of rat albumin turnover, 21% per day ($t_{1/2}$ of 3.3 days) from the pulse labeling technique, was ascertained by the modified double isotope method which provides the parameters from independent tracer kinetics using the same set of animals. The fractional turnover rate of 21% per day obtained in this study is in close agreement with the fractional turnover rate of the rat plasma albumin, 22% per day ($t_{1/2}$ of 3.2 days), found by Swick and Ip.²⁾ They measured the disappearance of labeled albumin after intraperitoneal injection of ^{14}C -labeled bicarbonate. The value obtained in this study is also in agreement with that of Gan and Jeffay,⁶⁾ 20% per day ($t_{1/2}$ of 3.5 days), who infused ^{14}C -lysine to 250 g rats.

The biochemical implication of the turnover of plasma albumin is not known, but there are interesting relationships between the life span of an animal and the turnover rate of its plasma protein.⁷⁾ The albumin turnover rate per unit metabolic mass is constant with increasing body weight,⁸⁾ so that a correlation may exist between albumin turnover and whole body protein turnover which varies as the metabolic mass. Although many methods have been used for measuring and estimating the plasma albumin turnover, the pulse labeling or constant pool method is still useful for studies which compare the protein metabolism of different species. Such an approach could provide helpful clues in attempts to extrapolate from exper-

imental animals to man or large farm animals.

REFERENCES

- 1) J. C. Waterlow, P. J. Garlick and D. J. Millward, "Protein Turnover in Mammalian Tissues and in the Whole Body," North-Holland Pub. Corp., Amsterdam, 1978, p. 383.
- 2) R. W. Swick and M. M. Ip, *J. Biol. Chem.*, **249**, 6836 (1974).
- 3) R. T. Schimke, "Mammalian Protein Metabolism," Vol. IV, ed. by H. N. Munro, Academic Press, New York, 1970, p. 177.
- 4) Q. R. Rogers and A. E. Harper, *J. Nutr.*, **87**, 267 (1965).
- 5) N. Horisaka and R. Funabiki, *Agric. Biol. Chem.*, **42**, 1595 (1978).
- 6) J. C. Gan and H. Jeffay, *Biochim. Biophys. Acta*, **252**, 125 (1971).
- 7) I. M. Spector, *Nature*, **249**, 66 (1974).
- 8) J. C. Waterlow, P. J. Garlick and D. J. Millward, "Protein Turnover in Mammalian Tissues and in the Whole Body," North-Holland Pub. Corp., Amsterdam, 1978, p. 486.