# Plasma Disappearance of $^{125}$I-Labeled Erythropoietin in Anesthetized Rabbits[1] (36756)

B. L. Roh, L. G. Paulo,[2] J. Thompson, and J. W. Fisher

*Department of Pharmacology, Tulane University, School of Medicine, New Orleans, Louisiana 70112*

Several investigators (1–4) have reported the rate of plasma clearance of erythropoietin (ESF). However, there appears to be considerable variation in the results reported because of the insensitivity of the biological assay methods used for ESF as well as the low specific activity of the crude ESF used. The recent progress in purifying human urinary ESF has made it possible to label ESF with $^{125}$I. The present studies were undertaken to compare the half-time disappearance of $^{125}$I-labeled purified human urinary ESF in plasma of rabbits with that of unlabeled crude human urinary ESF.

*Materials and Methods.* ESF[3] was purified from the urine of patients with severe anemia of hookworm infestation via a technique of benzoic acid precipitation and Sephadex gel filtration (5). The specific activity of the purified ESF was estimated to be 8300 IRP (International Reference Preparation) units/mg of protein using the fasted rat assay for ESF (6). The technique used for labeling ESF with radioiodine was a modification of the method of Greenwood, Hunter and Clover (7). A small amount of ESF (6.25 $\mu$g protein) was reacted with carrier free radio-sodium iodide in the presence of chloramine-T. Sodium metabisulfite was used to stop the reaction. For the evaluation of the efficiency of the iodination reaction, a small portion of the mixture was applied to a chromatographic strip and subjected to a combination of hydrostatic flow and electrophoresis. The labeled undamaged protein was estimated to be 46–54% of the total labeled protein. The undamaged component was quantitatively separated from the labeled damaged component and free $^{125}$I via gel filtration chromatography using a Sephadex G-75 column. The erythropoietic activity of the fractions was determined in exhypoxic polycythemic mice (8). The erythropoietically active fractions were pooled and used in the clearance studies. Five male rabbits weighing 2–2.5 kg were anesthestized with Dial–Urethan and a single dose of labeled ESF was injected into each rabbit via the ear vein. The dosages and specific activities of labeled ESF injected into the 5 rabbits are shown in Table I. Two milliliters of blood were removed at 2, 4, 6, 10, 20, 30, 45, 60, 120, 180, 240, and 300 min and at 12 and 24 hr via a polyethylene tubing which had been previously inserted into the femoral artery of each rabbit. The radioactivity of each plasma sample was counted in a well-type scintillation counter.

In order to characterize the circulating labeled material, plasma samples were fractionated on a Sephadex G-75 column. The results of the analysis revealed that after 2 min the total plasma radioactivity was 88% labeled protein and 12% free $^{125}$I. Further studies of plasma samples showed that the circulating free $^{125}$I was increased to 23% at 10 min and remained at this level thereafter. The labeled protein in the plasma samples was found in the same Sephadex fraction as the labeled ESF before injection. The radioactivity of each plasma sample was corrected for free $^{125}$I and expressed as labeled protein before being plotted on a semilog graph.

The $T_{1/2}$ disappearance studies using labeled ESF were compared with the disap-

[1] Supported by U.S. Public Health Service Grant No. AM-13211-04.

[2] Fellow from Conselho Nacional de Pesquisas, Rio de Janeiro, Brazil.

[3] Supplied by Dr. J. S. Espada, Dept. de Bioquimica, Universidad Nacional del Nordeste, Facultad de Medicina, Corrrientes, Argentina.

TABLE I. Dosages and Specific Activities of $^{125}$I-Labeled Human Urinary ESF Preparations Administered.

| Rabbit no. | Total $^{125}$I injected ($\mu$Ci) | Sp act of $^{125}$I ($\mu$Ci/$\mu$g protein) | Total protein injected ($\mu$g) | Sp act of ESF (units/$\mu$g) | Total ESF injected (units) |
|---|---|---|---|---|---|
| 1 | 59.5 | 27.2 | 2.18 | 2.80 | 6.1 |
| 2 | 69.8 | 26.4 | 2.64 | 2.72 | 7.2 |
| 3 | 14.6 | 21.8 | 0.67 | 3.36 | 2.25 |
| 4 | 14.6 | 21.8 | 0.67 | 3.36 | 2.25 |
| 5 | 111.7 | 84.2 | 1.33 | 2.40 | 3.19 |

pearance of unlabeled partially purified human urinary ESF in 3 anesthetized rabbits. The partially purified unlabeled ESF was taken through the benzoic acid precipitate stage of purification (5) using the urine of a patient with a pure red cell aplasia containing a high titer of ESF. ESF activity was measured in the polycythemic mouse assay and the protein concentration was determined by the method of Lowry *et al.* (9). The specific activity of the ESF was estimated to be 6.8 IRP units/mg protein. The partially purified ESF was dissolved in a phosphate buffer and injected intravenously into each rabbit at dosages of 180, 160 or 135 units. Blood samples were removed at the same time intervals as previously described for the $^{125}$I-labeled ESF studies and plasma ESF titers determined in the exhypoxic polycythemic mouse assay (8). The IRP units of ESF in each plasma sample were determined from an IRP dose–response regression line.

*Results.* Figure 1 shows the plasma disappearance of labeled purified urinary ESF and unlabeled partially purified urinary ESF. The ESF radioactivity disappearance curves show a biphasic pattern, an early rapid phase during the first 60 min and a slower disappearance phase over the next few hours. The regression lines for the rapid and slow phases were constructed according to the method of least squares (10) and the half-time disappearances of both phases were determined. The mean half-time disappearances for the rapid and slow phases for the $^{125}$I-labeled ESF in 5 rabbits were 35.87 $\pm$ 4.8 min and 10.25 $\pm$ 0.48 hr, respectively.

The lines of best-fit for the plasma disappearance of the unlabeled ESF are also shown in the lower portion of Fig. 1. The mean half-time disappearance for the rapid and slow phases in 3 rabbits were found to be 32.39 $\pm$ 8.85 min and 7.98 $\pm$ 0.51 hr, respectively. The differences in the labeled and unlabeled $T_{½}$ plasma disappearances of ESF were not significant.

*Discussion.* The plasma disappearance curves for labeled and unlabeled ESF were found in the present studies to be biphasic. Exponential disappearance curves for sheep plasma ESF in dogs have been previously reported (2). The rapid disappearance phase probably represents primarily distribution. The slow phase is presumed to represent metabolism and excretion. Reissmann *et al.* (3) suggested that the rapid disappearance phase after a single injection of ESF may indicate a larger tissue space for ESF than that of plasma volume.

In the present studies, the half-time disappearances for both rapid and slow phases of $^{125}$I-labeled ESF were approximately in the same range as those of the partially purified unlabeled ESF.

*Summary.* The rate of plasma disappearance of $^{125}$I-labeled highly purified human urinary erythropoietin (ESF) was compared with that of unlabeled partially purified human urinary ESF in anesthetized rabbits. Plasma $T_{½}$ values for both the labeled and unlabeled ESF were essentially biphasic. The mean half-time disappearance of $^{125}$I-labeled ESF was 35.87 min for the rapid phase and 10.25 hr for the slow phase. The half-time disappearances of the unlabeled ESF for the rapid and slow phases were 32.39 min and

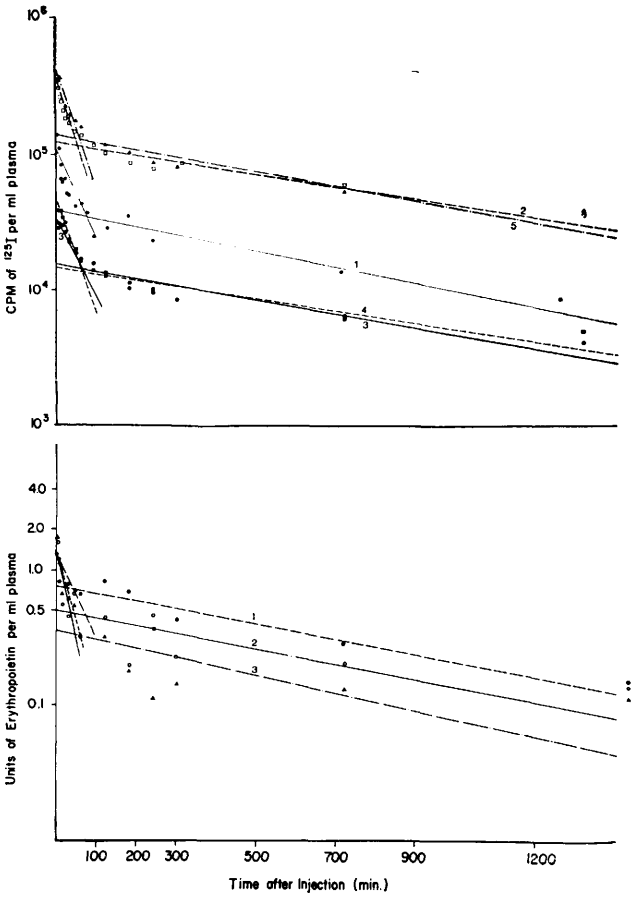


FIG. 1. Plasma disappearance of $^{125}$I-labeled (cpm/ml; upper) and unlabeled (units/ml; lower) erythropoietin following a single intravenous injection in anesthetized rabbits. (Upper) (1, 2, 3, 4 and 5) dosages of 59.5 $\mu$Ci (6.1 units), 69.8 $\mu$Ci (7.2 units), 14.6 $\mu$Ci (2.25 units), 14.6 $\mu$Ci (2.25 units) and 111.7 $\mu$Ci (3.19 units) labeled ESF, respectively. (lower) (1, 2 and 3) dosages of 180, 160 and 135 units (total dose) of unlabeled human urinary ESF.

7.98 hr, respectively. The $T_{1/2}$ values of the $^{125}$I-labeled ESF and the unlabeled ESF are not significantly different.

1. Stohlman, F., Jr., and Howard, D., *in* "Erythropoiesis" (L. O. Jacobson and M. Doyle, eds.), p. 120. Grune and Stratton, New York (1962).

2. Weintraub, A. H., Gordon, A. S., Becker, E. L., Camiscoli, J. F., and Contrera, J. F., Amer. J. Physiol. **207**, 523 (1964).

3. Reissmann, K. R., Diederich, D. A., Ito, K., and Schmaus, J. W., J. Lab. Clin. Med. **65**, 967 (1965).

4. Bozzini, C. E., Nature (London) **209**, 1140 (1966).

5. Espada, J., and Gutnisky, A., Biochem. Med. **3**, 475 (1970).

6. Fried, W., Plzak, L. F., Jacobson, L. O., and Goldwasser, E., Proc. Soc. Exp. Biol. Med. **94**, 237 (1957).

7. Greenwood, F. C., Hunter, W. M., and Clover, J. S., Biochem. J. **89**, 114 (1963).

8. Cotes, P. M., and Bangham, D. R., Nature (London) **191**, 1065 (1961).

9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

10. Dixon, W. J., "Introduction to Statistical Analysis," p. 193. McGraw-Hill, New York (1969).

Received Apr. 19, 1972. P.S.E.B.M., 1972, Vol. 141.