In Vitro Stability and In Vivo Clearance of Fibrinogen or Serum Albumin Labeled with ^{77}Br, ^{131}I, or ^{125}I by Direct or Indirect Synthetic Methods

Linda C. Knight, Sylvia S. L. Harwig, and Michael J. Welch

Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri

Conventional protein iodination involves the addition of an oxidizing agent to the protein solution. Through the use of the acylating agent N-succinimidyl-3(4-hydroxyphenyl)propionate, labeling can be accomplished without subjecting the protein to oxidizing conditions. Fibrinogen and serum albumin labeled with ^{131}I and ^{77}Br by this technique were compared with each other and with ^{125}I-protein prepared by direct iodination using the ICl, chloramine-T, and lactoperoxidase methods. Iodinated proteins have two drawbacks: the high radiation dose accompanying ^{125}I and ^{131}I, and the ease of hydrolysis of the weak carbon–iodine bond. These drawbacks can be overcome by using 56-hr ^{77}Br.

J Nucl Med 18: 282–288, 1977

One of the major concerns in conventional protein iodination is the possibility of altering the properties of the protein by the iodination procedure (*1*). By use of the acylating agent N-succinimidyl-3-(4-hydroxyphenyl)propionate (SHPP), labeling can be performed without subjecting the protein to oxidizing conditions (*2*). The aim of this study is to compare directly iodinated proteins with those iodinated or brominated with SHPP.

As a tracer, 56-hr ^{77}Br has several advantages over currently used isotopes of iodine. Bromine forms a stronger bond to carbon than does iodine: the ≡C–Br bond strengths in aromatic bromine compounds average about 70 kcal/mole (*3*), whereas the ≡C–I bond strengths in aromatic iodine compounds average about 60 kcal/mole (*3*). Thus, brominated compounds should be more stable than their iodinated analogs with respect to hydrolysis, a known problem with radioiodinated proteins (*1*). Radioiodide, which can be released by hydrolysis, is concentrated by the thyroid, delivering a high radiation dose to that gland. Free bromide, on the other hand, is not concentrated by any organ (*4*).

Bromine-77 decays 99% by electron capture and 1% by positron decay (*5*), with major gamma emissions at 242 keV (30%), 300 keV (6%), 520 keV (24%), and 580 keV (7%). The whole-body dose delivered by ^{77}Br in the form of a plasma tracer would be about one-tenth of the dose delivered by either ^{125}I or ^{131}I. The 56-hr half-life of ^{77}Br makes it particularly useful for fibrinogen turnover studies (*6*), which often require several days.

Chloroperoxidase has been found to catalyze the direct labeling of tyrosine and proteins with ^{77}Br at pH 2.8 (*7*). These conditions are too acidic for many proteins, such as fibrinogen, which becomes denatured at low pH. This method offered no advantage as far as hydrolysis rates are concerned, because even acid-stable proteins labeled in this way still hydrolyze quite rapidly (*7*).

An alternative is a two-step method of indirectly labeling proteins. Bromine-77 can be attached to an acylating agent (SHPP) by the chloroperoxidase method. The purified labeled agent can then be at-

Received May 17, 1976; revision accepted Oct. 12, 1976.

For reprints contact: Michael J. Welch, Div. of Radiation Sciences, Mallinckrodt Institute of Radiology, 510 S. Kingshighway, St. Louis, MO 63110.

tached to protein in the absence of acids and oxidizing and reducing agents. This method provides a stable label for fibrinogen without damaging the protein.

MATERIALS AND METHODS

Bromine-77 production. Bromine-77 was produced in the Washington University 52-in. cyclotron by bombarding 2 gm of arsenic pentoxide with 26-MeV alpha particles [$^{75}As(\alpha,2n)^{77}Br$]. The production and separation was carried out according to an adaptation of the method of Helus (*8*). The As_2O_5 powder was pressed onto a grooved aluminum plate and covered with 0.1-mil foil.* After irradiation, the foil was removed and the powder was suspended in 5 ml of deionized distilled water and transferred to a 50-ml pear-shaped flask. Ten milliliters of concentrated sulfuric acid was added, and the flask was then connected to a water-cooled condenser topped with a splash bulb followed by a trap containing 15 ml of water and 20 μl of 0.1 *M* hydroxylamine hydrochloride in aqueous solution. The trap was cooled by an ice bath. Three grams of sodium dichromate was added to the dissolved target material. A stream of nitrogen gas was introduced into the pear-shaped flask containing the reaction mixture. The flask was heated gently until more than 65% of the initial activity had been carried over into the trap. The contents of the trap were made slightly basic by the addition of 1 drop of 1 *N* NaOH. This solution was then evaporated to dryness under vacuum at room temperature. The residue was dissolved in 3–4 drops of 0.25 *M* phosphate buffer, pH 2.8, and the pH was adjusted to exactly 2.8 by the addition of 1 *N* HCl. The solution was used for labeling without further purification. The radiochemical yields obtained were similar to those of other workers (*8,9*).

Materials. Chloroperoxidase was isolated from the growth medium of submerged cultures of *Caldariomyces fumago* by the procedure described by Morris and Hager (*10*). The enzyme concentration of the aqueous solution (in phosphate buffer, pH 2.8) was determined by measuring its spectrophotometric absorbance† against phosphate buffer, pH 2.8, at 280 and 403 nm.

Crystalline SHPP N-succinimidyl-3-(4-hydroxyphenyl)propionate (SHPP) was obtained commercially.‡ Human serum albumin was obtained from Barnes Hospital Pharmacy in a concentration of 250 mg/ml and used without further purification.

The canine fibrinogen used in this study is the Blombäck I-2 (*11*) fraction isolated from pooled frozen dog plasma by glycine precipitation and ethanol fractionation (*12*). The rabbit fibrinogen was isolated from fresh rabbit plasma by ammonium sulfate precipitation (*13*).

Preparation of halogen-SHPP-labeled proteins. The SHPP labeling was carried out in two steps that were similar to the method described by Bolton and Hunter (*2*).

1. At least 2.0 μl (and up to 100 μl in some experiments) of 0.2-mg/ml solution of SHPP in dimethylformamide was placed in the bottom of a 13 $\times$ 150-mm glass culture tube. To this was added 0.1 ml of $Na^{77}Br$ or $Na^{131}I$ in 0.25 *M* phosphate buffer (pH 2.8), 10 μl of 0.3 mg/ml chloroperoxidase, 10 μl of dimethylformamide, and 15 μl of 0.2 *M* H_2O_2. Labeled SHPP was immediately extracted into two 0.25 ml portions of benzene. This extract was evaporated to dryness under vacuum at room temperature.
2. The residue from the benzene extraction was dissolved in 4 drops of borate buffer (0.05 *M* borate, 0.15 *M* NaCl, pH 8.5). Two milligrams of fibrinogen (or serum albumin) was immediately added. The reaction mixture was shaken gently and cooled in an ice bath for 15 min.

Labeled fibrinogen was purified by ammonium sulfate precipitation. One part by volume of saturated ammonium sulfate solution was added to three parts labeled protein solution. After the solution was held for 10 min in an ice bath, the precipitate was centrifuged and washed with a solution containing a 1:3 mixture of saturated ammonium sulfate and normal saline. The precipitate was centrifuged again and dissolved in 0.3 ml of 0.135 *M* NaCl–0.02 *M* Tris hydrochloride buffer, pH 7.4.

Labeled albumin and fibrinogen prepared for in vitro hydrolysis tests were purified by short gel columns packed with Sephadex G-10 and eluted with phosphate buffer (0.13 *M* phosphate, 0.08 *M* NaCl, pH 7.0).

Fibrinogen labeled with ^{131}I was prepared by the SHPP method using the same procedure. Commercial $Na^{131}I$ was used without further purification. For the reaction, the radioiodine solution was diluted with 0.25 *M* phosphate buffer, pH 2.8.

Directly labeled proteins for comparison. For comparison studies, ^{125}I-labeled fibrinogen was prepared by the iodine monochloride method (*13*) and purified by ammonium sulfate precipitation. For hydrolysis studies, ^{77}Br-labeled human serum albumin was prepared by the chloroperoxidase method (*7*), and iodinated human serum albumin was prepared by the lactoperoxidase method (*14*). All labeled proteins were used immediately after preparation, except for ^{125}I-fibrinogen (ICl), which was occasionally stored for up to 2 weeks at $-20°C$ before use.

In vitro studies. In vitro hydrolysis studies were

performed on labeled fibrinogen and albumin by adjusting the pH of the labeled protein solution to 7.0, making the solution 0.08 *M* in NaCl, and maintaining the temperature at 23°C for several days. Aliquots taken at various intervals were analyzed by trichloroacetic acid (TCA) precipitation. Tests of clottability in vitro were performed on labeled fibrinogen using a procedure similar to that described by Regoeczi (*15*). The solution of labeled fibrinogen (in normal saline) was diluted with 0.12 *M* NaCl–0.05 *M* ε-aminocaproic acid solution to a concentration of 0.5 mg/cc. One-tenth milliliter of thrombin (25 units/cc) was added to the tube containing the fibrinogen. After the mixture was cooled in an ice bath for at least 20 min, the clot was removed from the solution on a wire, allowed to synerize, and placed in a clean tube for counting. A solution of 1 *N* NaOH and 5% urea was used to dissolve the clot. The dissolved clot and the supernatant liquid were assayed for radioactivity. For standardization, a TCA precipitation was performed on another sample of labeled fibrinogen.

In vivo studies. Thrombus uptake of labeled fibrinogen was studied in dogs. A thrombus was induced by alteration of the intima of a femoral vein with an electric current (*16*). Using a stainless-steel wire enclosed in a vinyl catheter except at the tip as the electrode, a direct current (5 mA, 2 V) was applied for 1 hr. Labeled fibrinogen was administered 4 hr later. The thrombus and a sample of blood were removed 24 hr after administration of the fibrinogen. The radioactivity and weights of the thrombus and a blood sample were measured. Fibrinogen labeled with ^{125}I by the ICl method was used for comparison with ^{77}Br-SHPP-fibrinogen or ^{131}I-SHPP-fibrinogen.

Clearance studies of labeled fibrinogen were carried out in normal rabbits and dogs, using both halogenated SHPP-fibrinogen and directly iodinated fibrinogen as the thrombus uptake studies. The labeled fibrinogen was administered intravenously. Blood samples were taken over several days. Aliquots of the plasma were tested for clottability and for precipitability of the labeled fibrinogen with trichloroacetic acid. After 2 weeks, a normal dog previously used for a clearance experiment with ^{131}I-SHPP-fibrinogen was reinjected with the agent to test for an antigenic reaction.

TABLE 1. IN VITRO HYDROLYSIS OF LABELED PROTEINS*

A. FIBRINOGEN

	Labeling method	Hydrolysis medium	Percent hydrolyzed: 1 day	Percent hydrolyzed: 3 days
Iodinated	Chloramine-T (*1*)	Saline pH 5.5	19	50
	ICl (*1*)	Saline pH 5.5	9	57
	ICl (*24*)	Saline pH 7	2	8
	ICl (*1*)	Plasma	—	(2.4%/day)
Brominated	Br-SHPP	Saline pH 7	0	0

B. SERUM ALBUMIN

Label	Percent hydrolyzed: 1 day	2 days	4 days	7 days
Br-SHPP	0	0	0	0
Br(CPO)	4	6	14	80
I (lactoperoxidase method)	6	9	13	17
I-SHPP(C-T)	5	6	8	12

* Representative data (results of single trials).

TABLE 2. RELATIVE THROMBUS TO BLOOD RATIOS FOR FIBRINOGEN LABELED BY DIFFERENT TECHNIQUES

	I-SHPP-φ* / I-φ (ICl)	Br-SHPP-φ† / I-φ (ICl)
	0.74	1.38
	1.00	1.17
	1.98	
	1.25	
Average	1.24 ± 0.60	1.27 ± 0.10

* (Radioactivity in thrombus/radioactivity in blood for I-SHPP-φ) / (Radioactivity in thrombus/radioactivity in blood for I-φ (ICl method))

† (Radioactivity in thrombus/radioactivity in blood for Br-SHPP-φ) / (Radioactivity in thrombus/radioactivity in blood for I-φ (ICl method))

RESULTS

The first step of the labeling (attachment of radioactive halogens to SHPP) was carried out with efficiencies as high as 85%. The second step (conjugation to protein) proceeded with efficiencies of about 65% for serum albumin and about 35–50% for fibrinogen. Like Bolton and Hunter (*2*), we find that yields are highly sensitive to slight variations in reaction conditions, especially in the first step.

The yields were not altered appreciably, however, by changing the amount of SHPP in the reaction. With a conjugation-step efficiency of 35%, and assuming no other losses of reactants, the labeling levels were approximately 0.1 molecules of SHPP per protein molecule in the low-level case and about 5 SHPP per molecule in the high-level case, calculated stoichiometrically assuming a molecular weight of 340,000 for fibrinogen. The high-level value may

be inaccurate due to the low solubility of SHPP in aqueous solutions as well as in benzene.

The results of the in vitro hydrolysis experiment are shown in Table 1. In studies on serum albumin (Table 1B) the hydrolysis rates of directly brominated and SHPP-iodinated albumin were not significantly different from those of directly iodinated albumin. The SHPP-brominated albumin, however, remained completely stable toward hydrolysis for over 7 days. In vitro hydrolysis studies on fibrinogen were deemed impractical.

Since fibrinogen is very sensitive to storage conditions, samples of fibrinogen would become cloudy after 2 days at room temperature, indicating that it had denatured. This very obvious alteration in the protein may explain the more rapid hydrolysis after 2 days (Table 1A). The ^{77}Br-SHPP-fibrinogen did not hydrolyze for over 7 days in spite of denaturation. The ^{77}Br-SHPP-fibrinogen and ^{131}I-SHPP-fibrinogen, using fibrinogen from dogs, were found to be more than 90% clottable in vitro, as determined by comparing the total radioactivity found in the clot with the TCA-precipitable radioactivity.

The results of thrombus uptake studies are shown in Table 2. The thrombus-to-blood ratios, defined as

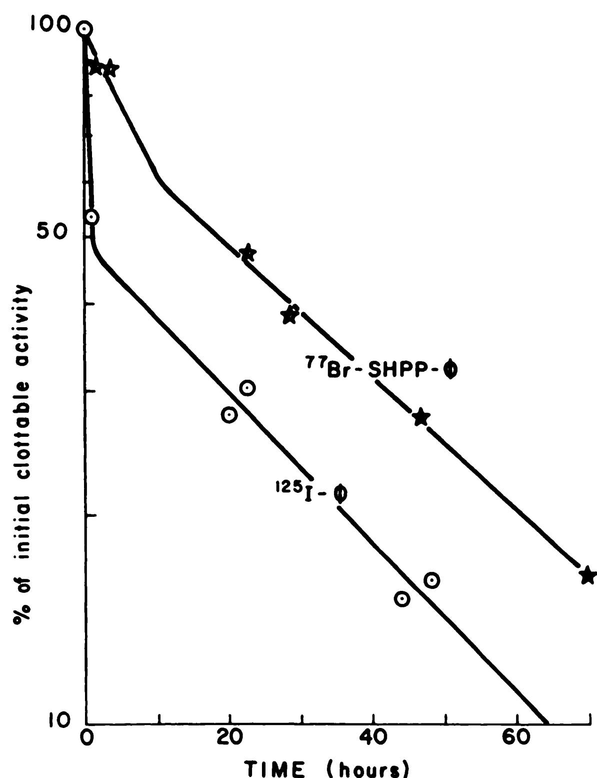

FIG. 1. Plasma clearance of labeled dog fibrinogen: Percent of initial clottable activity present in plasma (log scale) vs. time after injection of labeled fibrinogen. ^{77}Br-SHPP-fibrinogen (stars) is compared with ^{125}I-fibrinogen (circles) (ICl method). Low labeling level (0.1 SHPP/molecule).

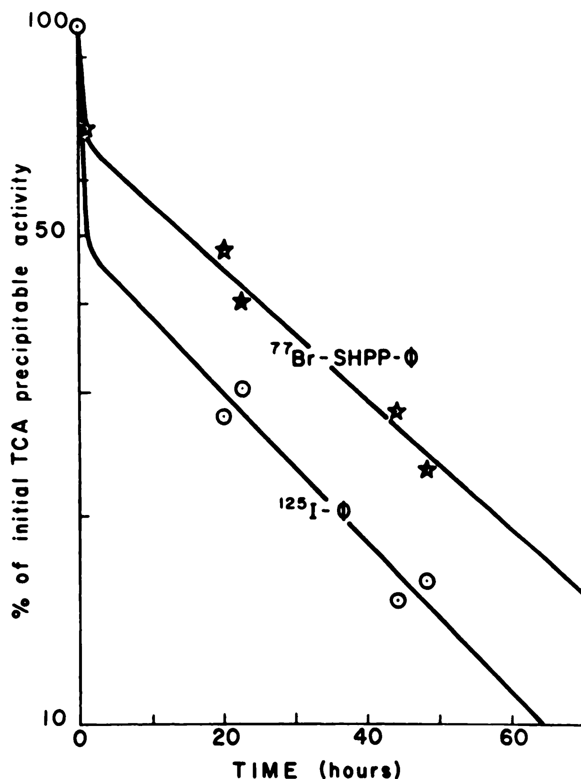

FIG. 2. Plasma clearance of labeled fibrinogen in dog: Percent of initial TCA-precipitable activity present in plasma (log scale) vs. time after injection of labeled fibrinogen. ^{77}Br-SHPP-fibrinogen (stars) is compared with ^{125}I-fibrinogen (circles) (ICl method). Low labeling level (0.1 SHPP/molecule).

$$\frac{\text{radioactivity in thrombus per gram of thrombus}}{\text{radioactivity in blood per gram of blood}},$$

for halogenated-SHPP-fibrinogen are compared with thrombus-to-blood ratios obtained for iodinated fibrinogen (ICl) in the same animals. The means were 1.25 for both ^{131}I-SHPP-fibrinogen and ^{77}Br-SHPP-fibrinogen, i.e., thrombi take up 25% more halogenated-SHPP-fibrinogen than iodinated fibrinogen prepared conventionally.

In five clearance experiments in dogs, ^{77}Br-SHPP-fibrinogen clearance curves had higher Y intercepts than the curves for standard iodinated fibrinogen, but the half-lives were similar (Fig. 1). The curves obtained from TCA data were similar to those drawn from clot data (Fig. 2). Good results were obtained with both high and low levels of SHPP labeling (Fig. 3). When rabbit fibrinogen was labeled by the SHPP method, the fibrinogen frequently became visibly denatured (precipitated) or, if used for an in vivo study, it had a lower Y intercept than the standard (Fig. 4). Figure 5 shows the results obtained when I-SHPP-fibrinogen (rabbit) was labeled at levels of ~0.3 SHPP/molecule and 6 SHPP/molecule and used for clearance experiments. The Y intercepts of the SHPP-labeling curves are lower than those of

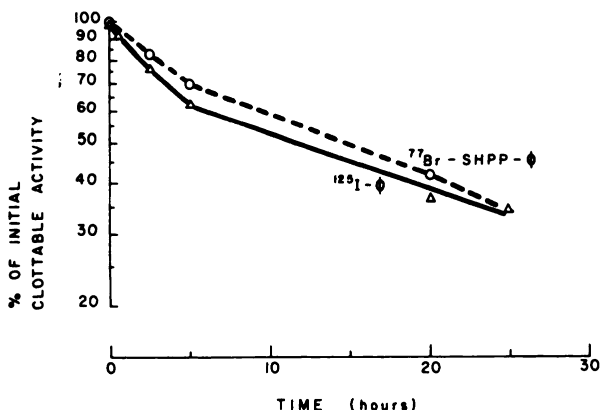

FIG. 3. Plasma clearance of labeled dog fibrinogen: Percent of initial clottable activity present in plasma vs. time after injection of labeled fibrinogen. ^{77}Br-SHPP-fibrinogen (circles) is compared with ^{125}I-fibrinogen (triangles) (ICl method). High labeling level (5 SHPP/molecule).

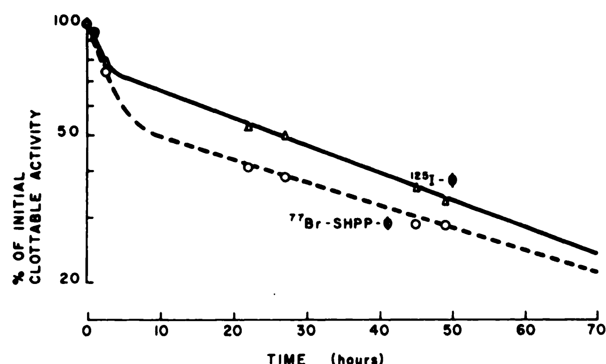

FIG. 4. Plasma clearance of labeled rabbit fibrinogen: Percent of initial clottable activity in plasma vs. time after injection of labeled fibrinogen. ^{77}Br-SHPP-fibrinogen (circles) is compared with ^{125}I-fibrinogen (triangles) (ICl method).

the standard, but the intercepts are the same for both levels of labeling. The clearance half-times, however, are different. The ^{131}I-SHPP-fibrinogen clears at a faster rate than the standard, and the clearance is also faster with more ^{131}I-SHPP groups per molecule. A summary of the clearance characteristics is shown in Table 3.

In the study measuring the clearance rate of a second injection of ^{131}I-SHPP-fibrinogen at 2 weeks after the first clearance study, no change in the clearance curve was noted (Fig. 6).

DISCUSSION

Labeling fibrinogen with halogenated SHPP can lead to a stable biologically active product. Labeling SHPP first, instead of directly labeling amino-acid residues in a protein, permits all the radiohalogen to attach to the stablest possible site, a hydroxyphenyl moiety, and the problem of labeling histidyl and cysteinyl residues is avoided (*14*). Since the SHPP is halogenated before adding the protein, the procedure permits the use of ^{77}Br, which is superior to the commonly used iodine isotopes in terms of radiation dose and stability. Although the radiation emitted from ^{77}Br is not ideal for imaging, the nuclide is suitable for turnover studies (*6*) and also for renal-transplant studies (*17*), especially in patients who have already been studied with radioiodine in another procedure.

The greater stability of the SHPP derivative in dog studies, compared with conventionally labeled proteins, was expected since conventional iodination labels sites in the protein other than those at the most stable tyrosine position (*14*).

The observation that directly iodinated fibrinogen hydrolyzed more rapidly after denaturation may indicate the presence of sulfur–iodine bonds, which are stable in an intact protein and very weak outside the protective protein structure (*18*). This would explain the observation that ^{77}Br-SHPP-fibrinogen did not hydrolyze after denaturation, since all of the halogen is attached to stable hydroxyphenyl

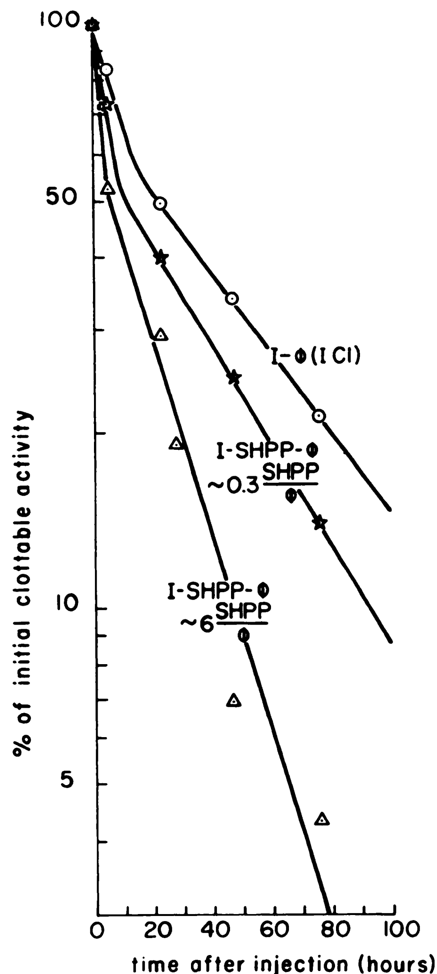

FIG. 5. Plasma clearance of labeled rabbit fibrinogen: Comparison of low (stars) and high (triangles) levels of ^{131}I-SHPP labeling of fibrinogen with ^{125}I-fibrinogen (circles) (ICl method).

TABLE 3. FIBRINOGEN CLEARANCE STUDIES USING HALO-SHPP-ϕ

	Number of trials	Relative amount cleared with $T_{1/2}$* (median)	Range	$T_{1/2}$† / $T_{1/2}$ (ICl) (median)	Range
^{77}Br-SHPP-dog ϕ	5	1.19	0.40	1.20	0.35
^{77}Br-SHPP-rabbit ϕ	2	0.72	0.002	1.07	0.1
^{131}I-SHPP-rabbit ϕ					
0.3 SHPP/molecule	3	0.87	0.01	0.82	0.02
6 SHPP/molecule	3	0.87	0.01	0.40	0.02

* $\frac{\text{Y intercept}}{\text{Y intercept (ICl)}}$

† Half-time of second component of clearance curve for halo-SHPP-ϕ compared with half-time of second component of clearance curve for ^{125}I-ϕ (ICl) in the same animal.

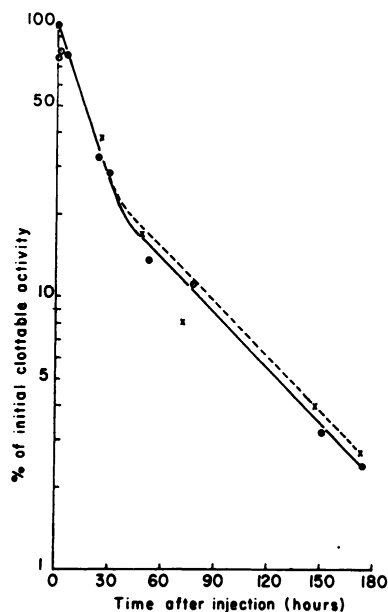

FIG. 6. Plasma clearance of ^{131}I-SHPP-fibrinogen in dog: Percent of initial TCA-precipitable activity present in plasma vs. time after injection of labeled fibrinogen. Two studies done in same animal, 2 weeks apart: First experiment (crosses), second experiment (circles).

groups. An amide linkage between SHPP and protein should be stable as well. The fact that the halogen-SHPP-labeled fibrinogen in rabbits has poorer clearance characteristics than the conventionally iodinated species is consistent with the observation (*19*) that rabbit fibrinogen is more sensitive toward denaturation than dog fibrinogen. This previous work (*19*) would suggest that the SHPP method will be superior with human fibrinogen. When labeling with SHPP, one is adding a larger group to the protein than in the case of direct iodination, so there is a possibility of steric problems as well as a change of overall charge characteristics. This is apparently not a problem with dog fibrinogen. Other species of fibrinogen should be tested with this method before assumptions are made concerning the suitability of the label.

Our experiments with high and low levels of SHPP labeling of proteins indicate that the level of labeling should be kept low. Of course, a halogenated SHPP molecule is quite large; certainly, the introduction of more than one such molecule into a protein could alter the protein's properties. The greater the number of groups that are attached, the greater the chance of attaching one in a position that interferes with the protein's active site.

The high stability of ^{77}Br-SHPP-albumin in the in vitro hydrolysis studies may be explained by bromine's higher bond strength to carbon and the fact that only hydroxyphenyl groups in SHPP are available for labeling. Halogenation of proteins may take place at sites other than tyrosine and some of these other sites are increasingly favored by lower pH (*20*).

In conclusion, SHPP halogenation of dog fibrinogen gives a product whose behavior in clearance and thrombus-uptake studies equals or exceeds that of an ICl-labeled preparation obtained by the preferred method of iodinating fibrinogen (*21*). As ^{125}I-SHPP is now commercially available|| the method affords a simple way of preparing labeled fibrinogen, especially for departments which do not wish to develop their own labeling techniques. The ^{77}Br-labeled fibrinogen has advantages from the radiation dosimetric viewpoint, and ^{77}Br should be easy to produce by spallation reactions in high-energy accelerators (*22*). Bromine-77 can be formed by the decay of ^{77}Kr (*23*), and the pure krypton parent would be formed in high yield by spallation. This should yield a purer form of ^{77}Br than the arsenic pentoxide method can give. As the SHPP method of labeling is applicable to the highly sensitive protein fibrinogen, it should offer a general method of brominating proteins and peptides for in vivo studies.

ACKNOWLEDGMENTS

This work was supported by NIH Grant for Specialized Center of Research in Thrombosis 1 P17 HL 14147. Chloroperoxidase was kindly provided by L. Hager of the University of Illinois.

FOOTNOTES

* Havar foil, Hamilton Watch Co., Lancaster, Pa.

† Hitachi–Perkin–Elmer 139 uv–vis spectrophotometer.

‡ Pierce Chemical Co., Rockford, Ill.

|| Bolton–Hunter ^{125}I reagent is available from New England Nuclear Corp., North Billerica, Mass.

REFERENCES

1. KROHN K, SHERMAN L, WELCH M: Studies of radioiodinated fibrinogen. I. Physicochemical properties of the ICl, Chloramine-T, and electrolytic reaction products. *Biochem Biophys Acta* 285: 404–413, 1972

2. BOLTON AE, HUNTER WM: The labeling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. *Biochem J* 133: 529–539, 1973

3. VEDENEYEV VI, GURVICH LV, KONDRAT'YEV, et al.: *Bond Energies, Ionization Potentials and Electron Affinities.* London, Edward Arnold, 1966, pp 60–63

4. NICHOLSON JP, ZILVA JF: Estimation of extracellular fluid volume using radiobromine. *Clin Sci* 19: 391–398, 1960

5. LEDERER CM, HOLLANDER JM, PERLMAN I: *Table of Isotopes*, 6th ed., New York, Wiley, 1968, pp 212–213

6. COLLEN D, TYTGAT GN, CLAEYS H, et al.: Fibrinogen turnover in physiological conditions in humans. *Br J Haematol* 22: 681–700, 1972

7. KNIGHT LC, KROHN KA, WELCH MJ, et al.: Bromine-77: A new protein label. In *Radiopharmaceuticals,* G. Subramanian, ed. New York, Society of Nuclear Medicine, 1975, pp 149–154

8. HELUS F: Preparation of carrier-free bromine-77 for medical use. *Radiochem Radioanal Lett* 3: 45–50, 1970

9. WATERS SL, NUNN AD, THAKUR ML: Cross-section measurements for the ^{75}As(α,2n)^{77}Br reaction. *J Inorg Nucl Chem* 35: 3413–3416, 1973

10. MORRIS DR, HAGER LP: Chloroperoxidase. Isolation and properties of the crystalline glycoprotein. *J Biol Chem* 241: 1763–1768, 1966

11. BLOMBÄCK B, BLOMBÄCK M: Purification of human and bovine fibrinogen. *Arch Chem* 10: 415–443, 1956

12. MOSESSON MW, SHERRY S: Preparation and properties of human fibrinogen of relatively high solubility. *Biochemistry* 5: 2829–2835, 1966

13. MCFARLANE AS: In vivo behavior of ^{131}I-fibrinogen. *J Clin Invest* 42: 346–361, 1963

14. KROHN KA, WELCH MJ: Studies of radioiodinated fibrinogen. II. Lactoperoxidase iodination of fibrinogen and model compounds. *Int J Appl Radiat Isot* 25: 315–323, 1974

15. REGOECZI E: Iodine-labelled fibrinogen: A review. *Br J Haematol* 20: 649–663, 1971

16. COLEMAN RE, HARWIG SSL, HARWIG JF, et al.: Fibrinogen uptake by thrombi: Effect of thrombus age. *J Nucl Med* 16: 370–373, 1975

17. WINSTON MA, WEISS ER, BLAHD WH, et al.: Use of ^{131}I-fibrinogen in detection of renal transplant rejection. *Invest Urol* 9: 119–123, 1971

18. MEANS GE, FEENEY RE: Electrophilic reagents. In *Chemical Modification of Proteins.* San Francisco, Holden-Day, 1971, pp 175–182

19. HARWIG SSL, HARWIG JF, COLEMAN RE, et al.: Effect of iodination level on the properties of radioiodinated fibrinogen. *Thromb Res* 6: 375–386, 1975

20. KNIGHT LC: *Radiohalogenated Proteins: Preparation and Properties.* Ph.D. Thesis, Washington University, 1976

21. COLEMAN RE, KROHN KR, METZGER JM, et al.: An in vivo evaluation of I-fibrinogen labeled by four different methods. *J Lab Clin Med* 83: 977–982, 1974

22. RICHARDS P, LEBOWITZ E, STANG LG: The Brookhaven linac isotope producer (BLIP). In *Radiopharmaceuticals and Labelled Compounds, IAEA-SM-171138,* vol. 1. Vienna, IAEA, 1173, pp 325–341

23. BLUE JW, BENJAMIN PP: Production of ^{77}Br by the reaction ^{76}Se(α,3n)^{77}Kr. *J Nucl Med* 12: 416–417, 1971

24. ARDAILLOU N, LARRIEU MJ: In vitro studies of radioiodinated fibrinogen. Comparison of ICl and enzymatic methods. *Thromb Res* 5: 327–341, 1974