PREPARATION AND USE OF ^{123}I-LABELED HIGHLY IODINATED FIBRINOGEN FOR IMAGING DEEP-VEIN THROMBI

John F. Harwig, Michael J. Welch, and R. Edward Coleman

Mallinckrodt Institute of Radiology, St. Louis, Missouri

A method for producing protein-iodination-grade ^{123}I suitable for use with a compact biomedical cyclotron is reported. The preparation of highly iodinated fibrinogen (25 ^{123}I atoms per molecule) is described, and its successful use as a thrombus-imaging agent in experimental animals is reported. This new agent clears from the blood faster than conventional radioiodinated fibrinogen and gives higher thrombus-to-blood activity ratios Thus, the detection of deep-vein thrombi in areas of large blood pool is enhanced, and images can be obtained sooner after administration of the radiopharmaceutical. Induced 4–8-hr-old femoral-vein thrombi in dogs can be well visualized with a scintillation camera as early as 4 hr and as late as 15 hr after administration of 1 mCi of ^{123}I-labeled highly iodinated fibrinogen.

Radioiodinated fibrinogen prepared at moderately high levels of iodination has been investigated as a potential agent for detecting deep-vein thrombosis (*1*). When injected into dogs 4 hr after induction of femoral-vein thrombosis, radioiodinated fibrinogen (containing 25 iodine atoms per molecule) gives thrombus-to-blood activity ratios double those obtained with conventional radioiodinated fibrinogen at 24 hr after injection (*1*). The shorter biologic half-life and lower blood-background activity of the highly iodinated form suggest that it may be more useful for detecting thrombi in regions of large blood pool and that detection may be possible sooner after administration. We have evaluated this new agent for in vivo visualization of thrombi with a scintillation camera.

The good decay characteristics, 13.3-hr half-life, and low radiation dose of ^{123}I make it well suited for nuclear medicine imaging. However, protein-iodination-grade ^{123}I is not available commercially, and most reported methods of producing ^{123}I for radiopharmaceutical preparation use the indirect ^{123}Xe-decay route, which requires large cyclotrons (*2–5*). This communication also describes the production of protein-iodination-grade ^{123}I by a direct method compatible with small medical cyclotrons and an electrolytic method of preparing the ^{123}I-labeled highly iodinated fibrinogen.

MATERIALS AND METHODS

The ^{123}I is produced and isolated by a modification of the method reported by Silvester et al (*6*). Approximately 200 mg of powdered antimony metal of natural isotopic abundance are wrapped in a thin aluminum-foil packet and bombarded with an 8–10-μA beam of 26-MeV alpha particles in the Washington University 54-in. cyclotron. Iodine-123 results from the reaction ^{121}Sb(α,2n)^{123}I.

After the bombardment, the ^{123}I is distilled with a Bantam-Ware apparatus (Kontes Glass Co., Vineland, N.J.), consisting of a 100-ml three-neck round-bottomed flask as the still pot, a 25-ml pear-shaped flask as the receiver, and a distillation head employing a vacuum-jacketed Vigreaux fractionating column and a water-jacketed condenser. The still-pot contents are stirred with a magnetic stirring bar, and the receiver is kept in an ice bath. First, the intact foil packet is placed in the still pot with 10 ml of 9.0 *M* sulfuric acid. Gentle heat is applied with a heating mantel to dissolve the aluminum. Twenty milliliters of concentrated sulfuric acid are then added, and the mixture is heated vigorously to dissolve the antimony. After the still pot is allowed to cool briefly, carrier sodium iodide (50 μg) is added,

Received Sept. 3, 1975; revision accepted Nov. 7, 1975.

For reprints contact: John F. Harwig, Mallinckrodt Institute of Radiology, Washington University School of Medicine, 510 South Kingshighway Blvd., St. Louis, Mo. 63110.

and 20 ml of 0.5 *M* sodium nitrite is slowly added dropwise to the mixture. The still pot is then heated to boiling, and 10 ml of distillate is collected. The distillate is extracted with two 5-ml portions of CCl_4, and the combined CCl_4 extracts are back-extracted with 2 ml of 0.1 *N* NaOH. The NaOH solution is evaporated to dryness at 50°C in vacuo on a rotary evaporator. The residue is reconstituted and neutralized with 200 μl of 1.0 *N* HCl, at which time the ^{123}I is ready for use. To determine radioisotopic purity, samples were examined using a 50-cm^3 Ge(Li) detector coupled to a multichannel analyzer.

Canine fibrinogen is isolated from pooled canine plasma as the Blombäck I-2 fraction (*7*). Highly iodinated fibrinogen (25 iodine atoms per molecule) is prepared by a slight modification of the reported electrolytic technique (*1*). Briefly, the labeling mixture consists of a solution of 4 mg fibrinogen and the purified ^{123}I in 4 ml of 0.02 *M* sodium barbital–0.15 *M* sodium chloride buffer, pH 7.4, giving a 30:1 ratio of carrier iodide to fibrinogen. An anodic potential of +0.4–0.5 V, measured against a saturated calomel electrode, is employed at a current of 10–20 μA. The labeling reaction proceeds at an efficiency of about 60%/hr and is allowed to run until 85–90% of the radioactivity is bound to fibrinogen. The ^{123}I-labeled highly iodinated fibrinogen is separated from the free ^{123}I and other components of the reaction mixture by precipitation with 30% saturated ammonium sulfate and is redissolved in 0.02 *M* tris(hydroxymethyl)aminomethane–0.15 *M* sodium chloride buffer, pH 7.4. Isotopic clottability is determined by Regoeczi's method (*8*), and the rate of blood clearance in dogs is determined by previously reported methods (*1*).

A thrombus was induced in a femoral vein of each of four dogs by the technique of altering the intima with an electric current (*9*). One dog each at 4, 5, 7, and 8 hr after thrombus induction was injected with approximately 1 mCi of ^{123}I-labeled highly iodinated fibrinogen from separate labeling reactions and separate cyclotron runs. Thrombus imaging with a scintillation camera and midenergy (400 keV) collimator (Radicamera, General Electric Co.) was performed 4 hr and 15 hr after injection.

RESULTS

The above method of producing iodination-grade ^{123}I is simple and efficient. Cyclotron yields of about 300 μCi/μA per hour are obtained. Gamma spectroscopy shortly after bombardment indicates the presence of about 2.0% ^{124}I and 0.6% ^{126}I. The total preparation time after bombardment is about 3 hr, with an overall isolation efficiency of about 60%, corrected for decay.

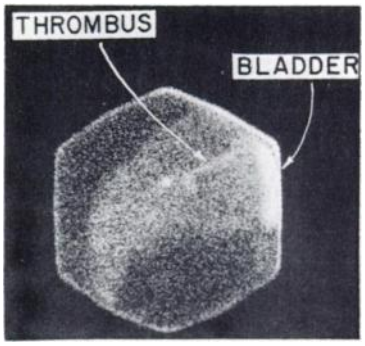

FIG. 1. Scintigram obtained 4 hr after injection of 1 mCi of ^{123}I-labeled highly iodinated fibrinogen in dog shows thrombus induced in right femoral vein 8 hr before injection.

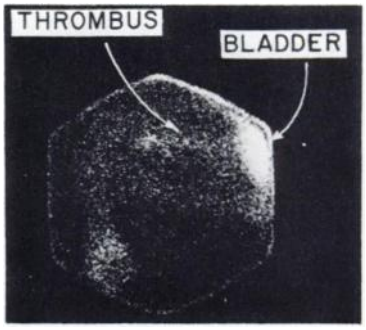

FIG. 2. Scintigram obtained in same dog 15 hr after tracer injection.

The ^{123}I is then suitable for protein iodination. Radiolabeling of fibrinogen with these ^{123}I preparations by the iodine monochloride (*10,11*) and lactoperoxidase (*12*) methods is possible (*13*). However, for the preparation of highly iodinated fibrinogen the electrolytic method is most appropriate (*1*). The modified electrolytic labeling reaction reported here proceeds faster than that previously described (*1*). A labeling efficiency of 85–90%, corresponding to incorporation of about 25 iodine atoms per fibrinogen molecule, is obtained in about 1.5 hr. These preparations have an isotopic clottability of 70–75% and clear from the blood with a $t_{1/2}$ of about 35 hr. An extensive study of the physicochemical properties and in vivo behavior of highly iodinated fibrinogen has been reported previously (*1*).

In the four dogs injected with ^{123}I-labeled highly iodinated fibrinogen, the thrombus was readily visualized each time. None of the induced thrombi failed to accumulate the radiopharmaceutical, and all areas of abnormal accumulation were identified. Figures 1 and 2 show representative images obtained in the dog with the 8-hr-old thrombus. In Fig. 1, taken 4 hr after injection, the thrombus is clearly delineated despite fairly high blood-background activity. In Fig. 2, taken 15 hr after injection, the blood-

background activity has dropped considerably and the thrombus remains readily apparent. The urinary bladder activity in these images does not interfere with visualization of the thrombus.

DISCUSSION

Several methods of ^{123}I production have been reported and compared in recent years (*14–16*). For production of large amounts of ^{123}I the indirect route involving ^{123}Xe decay is probably the preferred method (*17–19*), but this procedure requires a large cyclotron, available at only a few institutions. Most methods reported for preparing ^{123}I-labeled compounds of biologic interest employ the ^{123}Xe-decay technique to effect recoil labeling directly (*2,3*) or through the intermediacy of ICl (*4*) or KIO_3 (*5*). On the other hand, the method described here for ^{123}I production employs the direct $^{121}Sb(\alpha,2n)^{123}I$ reaction and is suitable for the more commonly available compact biomedical cyclotrons. The ^{123}I isolation and purification process reported here yields a material suitable for high-efficiency iodination of fibrinogen and presumably other proteins [previous reports of similar techniques do not mention protein iodination (*6,20*)]. The radioisotopic purity of the present ^{123}I preparations is similar to that reported by other investigators for the direct antimony reaction, where ^{124}I and ^{126}I are the major contaminants (*6,21*), although our purity is not as high as can be obtained by ^{123}Xe decay (*4,19*). Use of isotopically enriched ^{121}Sb for the present preparation would also increase the ^{123}I purity (*14*).

Our previous studies indicated that highly iodinated fibrinogen has advantages over conventional radioiodinated fibrinogen for thrombus detection (*1*). The present work shows that, when labeled with a suitable iodine isotope, highly iodinated fibrinogen can be used to visualize thrombi with a scintillation camera. Although conventional ^{123}I-labeled fibrinogen has been reported to be useful in detecting thrombi by scintiscanning (*22,23*), its slow blood clearance and resulting high background activity may lead to insufficient differentiation between thrombus and blood, at least for several hours after administration. Highly iodinated fibrinogen has been shown to produce high thrombus-to-blood ratios in 4-hr-old thrombi (*1*). The thrombus-to-blood ratios in older thrombi may not be as high, however, if highly iodinated fibrinogen behaves similarly to other thrombus-localizing agents in older thrombi (*9,24*). Nevertheless, visualization of an 8-hr-old thrombus can be achieved with ^{123}I-labeled highly iodinated fibrinogen as early as 4 hr after injection (Figs. 1 and 2). The urinary bladder activity did not interfere with visualization of the thrombus and would be even less of a problem in patients, since clinical imaging of the pelvic area could be performed after the bladder has been emptied. The presence of small amounts of ^{124}I, requiring the use of a midenergy collimator, did not cause substantial image degradation. Highly iodinated fibrinogen labeled with ^{123}I appears to be a promising new imaging agent for actively forming deep-vein thrombi, especially in areas of large blood pool. The efficacy of this agent in older thrombi remains to be determined.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Sylvia Harwig for preparing the fibrinogen, to Robert Feldhaus for assisting with the animal studies, and to John Hood and his staff at the Washington University cyclotron.

This work was supported by an NIH Grant for a Specialized Center of Research, No. 1 P17 HL 14147-04.

REFERENCES

1. HARWIG JF, COLEMAN RE, HARWIG SSL, et al: Highly iodinated fibrinogen: A new thrombus localizing agent. *J Nucl Med* 16: 756–763, 1975

2. WELCH MJ: Labeling with ^{123}I. The reactivity of ^{123}I formed by the decay of ^{123}Xe. *J Am Chem Soc* 92: 408–409, 1970

3. BLUE JW, SODD VJ: ^{123}I production from the β^+ decay of ^{123}Xe. In *The Uses of Cyclotrons in Chemistry, Metallurgy and Biology*, Amphlett CB, ed, London, Butterworth, 1970, p 138

4. LAMBRECHT RM, MANTESCU C, REDVANLY C, et al: Preparation of high-purity carrier-free ^{123}I monochloride as iodination reagent for synthesis of radiopharmaceuticals, IV. *J Nucl Med* 13: 266–273, 1972

5. EL-GARHY M, STÖCKLIN G: A novel method of labeling biomolecules with ^{123}I and ^{125}I. *Radiochem Radioanal Lett* 18: 281–290, 1974

6. SILVESTER DJ, SUGDEN J, WATSON IA: Preparation of iodine-123 by α particle bombardment of natural antimony. *Radiochem Radioanal Lett* 2: 17–20, 1969

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

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

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

10. MCFARLANE AS: Efficient trace-labeling of proteins with iodine. *Nature* 182: 53, 1958

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

12. 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

13. HARWIG JF, HARWIG SSL: Unpublished results.

14. SODD VJ, SCHOLZ KL, BLUE JW, et al: Cyclotron production of I-123: An evaluation of the nuclear reactions which produce this isotope. *BRH/DMRE* 70–4, 1970

15. DEGLUME CH, DEUTSCH JP, FAVART D, et al: Note on the production of ^{123}I for radiodiagnostical purposes. *Int J Appl Radiat Isot* 24: 291–293, 1973

16. SODD VJ: Production and use of ^{123}I. In *Radiopharmaceuticals*, Subramanian G, Rhodes BA, Cooper JF, Sodd VF, eds, New York, Society of Nuclear Medicine, 1975, pp 125–133

17. SODD VJ, BLUE JW: Cyclotron generator of high-purity ^{123}I. *J Nucl Med* 9: 349, 1968

18. LAMBRECHT RM, WOLF AP: The ^{122}Te (^4He, 3n) $^{123}Xe \xrightarrow[2.1\ hr]{B+,\ EC} {}^{123}I$ generator. *Radiat Res* 52: 32–46, 1972

19. FUSCO MA, PEEK NF, JUNGERMAN JA, et al: Production of carrier-free ^{123}I using the $^{127}I(p,5n)^{123}Xe$ reaction. *J Nucl Med* 13: 729–732, 1972

20. THAKUR ML: The preparation of iodine-123 labeled contrast agents. *Int J Appl Radiat Isot* 25: 576–578, 1974

21. NEIRINCKX RD, LAMBRECHT RM, NORTON EF, et al: Quality control of radiopharmaceuticals. XIV. Sodium ^{125}I-iodide. *Int J Appl Radiat Isot* 25: 387–392, 1974

22. DENARDO SJ, DENARDO GL, O'BRIEN T, et al: ^{125}I-fibrinogen imaging of thrombi in dogs. *J Nucl Med* 15: 487, 1974

23. DENARDO SJ, DENARDO GL, CARRETTA RF, et al: Clinical usefulness of I-123-fibrinogen (I-123-F) for detection of thrombophlebitis (TP). *J Nucl Med* 16: 524, 1975

24. COLEMAN RE, HARWIG SSL, HARWIG JF, et al: Radioiodinated soluble canine fibrin: Preparation and potential as a thrombus localizing agent in the dog. *Circ Res* 37: 35–40, 1975

Accepted Articles To Appear In Upcoming Issues

Survey of Radioactive Agents for In Vitro Labeling of Phagocytic Leukocytes. I. Soluble Agents. Accepted 11/11/75.
John G. McAfee and Mathew L. Thakur

A Diagnostic Strategy Using Ventilation–Perfusion Studies in Patients Suspect for Pulmonary Embolism. Accepted 11/11/75.
Barbara J. McNeil

Design and Performance Characteristics of a Whole-Body Positron Transaxial Tomograph. Accepted 11/26/75.
Edward J. Hoffman, Michael E. Phelps, Nizar A. Mullani, Carol S. Higgins, and Michel M. Ter-Pogossian

Intestinal Concentration of ^{99m}Tc-Pertechnetate into Isolated Loops of Rat Bowel. Accepted 12/15/75.
Andrew T. Taylor, Jr., Naomi Alazraki, and James E. Henry

Focal Increased Activity on Scinticisternography: Report of Three Cases. Accepted 12/18/75.
Robert R. McClelland

Double-Labeling of Experimental Acute Myocardial Infarcts with ^{113m}In- and ^{99m}Tc-EDTMP (Preliminary Note). Accepted 12/19/75.
B. Leonard Holman, Alun G. Jones, Michael A. Davis, Joseph Askenazi, and Peter R. Maroko

Mechanism of Localization of ^{99m}Tc-Labeled Pyrophosphate and Tetracycline in Infarcted Myocardium. Accepted 12/29/75.
Mrinal K. Dewanjee and Paul C. Kahn

Techniques for Positron Scintigraphy of the Brain. Accepted 12/29/75.
B. Hoop, D. J. Hnatowich, G. L. Brownell, T. Jones, K. A. McKusick, R. G. Ojemann, J. A. Parker, R. Subramanyam, and J. M. Taveras

Simultaneous Measurement of Percentage Free Thyroxine and Triiodothyronine: Equilibrium Dialysis versus Sephadex Chromatography. Accepted 12/29/75.
Steven M. Snyder, Ralph R. Cavalieri, and Sidney H. Ingbar

Simple Radiometric Techniques for Rapid Detection of Herpes Simplex Virus Type 1 in WI-38 Cell Culture. Accepted 12/29/75.
Nancy D'Antonio, Min-Fu Tsan, Patricia Charache, Steven Larson, and Henry N. Wagner, Jr.

Gallium-67 Uptake by a Malignant Fibrous Histiocytoma (Case Report). Accepted 12/29/75.
David J. Harrowe, Stephen Kessler, Alan A. Jansen, and Steven M. Larson

Radionuclide Angiocardiographic Calculation of the Ratio of Left-to-Right Heart Outputs in a Patient with Unusual Cardiovascular Anatomy (Case Report). Accepted 12/29/75.
J. Anthony Parker, Michael Freed, Donald S. Ahnberg, and S. Treves

Stability of Radiothyroxine Plasma Disappearance Curve despite Catharsis and Unblocked Thyroidal Uptake of Radioiodide. Accepted 12/29/75.
Marguerite T. Hays

Specificity of ^{99m}Tc-Pertechnetate in Scintigraphic Diagnosis of Meckel's Diverticulum: A Review of 100 Cases. Accepted 1/6/76.
Thomas H. Berquist, Nicholas G. Nolan, David H. Stephens, and Harley C. Carlson

Compartmental Models of Cerebral Blood Flow. Analysis Using the 81-keV and 31-keV Photons of ^{133}Xe. Accepted 1/6/76.
W. A. van Duyl, D. Sparreboom, and A. C. W. Volkers

Childhood Pelvic Osteomyelitis Presenting as a "Cold" Lesion on Bone Scan (Case Report). Accepted 1/9/76.
Richard T. Trackler, Kenneth E. Miller, David H. Sutherland, and David L. Chadwick

Unusual Bone Scan Findings in Acute Osteomyelitis (Case Report). Accepted 1/9/76.
Lincoln D. Russin and Edward V. Staab

Field Flood Uniformity Corrections: Benefits or Pitfalls? Accepted 1/9/76.
Thomas N. Padikal, Alan B. Ashare, and James G. Kereiakes

Survey of Radioactive Agents for In Vitro Labeling of Phagocytic Leukocytes. II. Particles. Accepted 1/13/76.
John G. McAfee and Mathew L. Thakur

Subsequent Fertility and Birth Histories of Children and Adolescents Treated with ^{131}I for Thyroid Cancer. Accepted 1/20/76.
Salil D. Sarkar, William H. Beierwaltes, Satinder P. Gill, and Betty J. Cowley

Diagnosis of Cirrhosis and Hepatitis by Quantitative Hepatic and Other Reticuloendothelial Clearance Rates. Accepted 1/21/76.
Sally J. DeNardo, George B. Bell, Gerald L. DeNardo, Robert F. Carretta, Paul O. Scheibe, Thomas J. Imperato, and Peter E. Jackson

Tomographic Images of Blood Pool and Perfusion in Brain and Heart. Accepted 2/17/76.
Michael E. Phelps, Edward J. Hoffman, R. Edward Coleman, Michael J. Welch, Marcus E. Raichle, Edward S. Weiss, Burton E. Sobel, and Michel M. Ter-Pogossian

Determining the Value of Diagnostic and Screening Tests. Accepted 2/26/76.
Barbara J. McNeil and S. James Adelstein