THE LABORATORY PREPARATION OF INDIUM-LABELED COMPOUNDS

M. H. Adatepe, M. Welch, E. Archer, R. Studer and E. J. Potchen

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

^{113m}Indium, a generator-produced radionuclide derived from ^{113}Sn, has monoenergetic 390-kev gamma radiation and a 1.7-hr physical half-life—features which make it readily applicable to clinical organ scintiscanning. Stern *et al* have developed effective indium-labeled complexes for lung, liver and blood-pool scans (*1,2*). A DPTA chelate of indium has been used for clinical brain scanning (*3*). With these points in mind, the potential broad use of ^{113m}In became obvious, and we undertook investigations to develop a simplified technique for the laboratory preparation of indium-labeled compounds. The methods for the preparation of the indium-labeled complexes described previously (*1,2*) have not resulted in reproducible results in our hands using a 25-mc ^{113}Sn-^{113m}In-generator. Extensive investigations into the factors that influence the reliability of organ localization have led us to significantly modify the proposed techniques and to standardize our laboratory methods. The methods and their applications are reported here.

METHODS

We elute ^{113m}In from a 25-mc ^{113}Sn-^{113m}In generator* daily with 8 ml 0.05 *N* hydrochloric acid (*p*H 1.4). This eluate contains about 5×10^{-9} gm indium (2.6×10^{13} atoms). Tin breakthrough in the eluate is colorometrically determined (hematoxylin) and held to less than 0.002%. The radioactivity is assayed by standard techniques similar to those used for ^{99m}Tc.

The materials used to prepare the indium-labeled colloidal complexes are as follows: the eluate described above; 20% gelatin (USP) solution; sodium phosphate buffer solution (76.1 gm $Na_2HPO_4 \cdot 7H_2O$ 4.6 gm $NaH_2PO_4 \cdot H_2O$ in 500 ml water); ferric chloride solution (485 mg $FeCl_3 \cdot 6H_2O$ in 100 ml water—an excess of Cl^- (dilute HCl) is necessary to maintain stability if this solution is to be stored for any length of time): 0.5 *N* NaOH solution; a *p*H meter and magnetic stirrer. Pyrogen-free, sterile water is used to prepare all the solutions.

The reaction mixture is constantly stirred and *p*H closely monitored throughout the procedures. Sterile techniques are used in addition to autoclaving the final preparation (250°F for 20 min) prior to injection. In addition to assay for radioactivity, each preparation is bioassayed for organ distribution in 100 gm NRL-strain virgin female rats. The particle size of liver, lung and spleen compounds are also checked microscopically on a hemocytometer, and the preparation is not used if there are any particles larger than 100 micron.

Although some overlap of organ labeling occurs in the experimental animal and man, we have observed the greatest specificity (Table 1) with the following preparations:

I. Blood pool. Add 1-ml gelatin solution and 0.5-ml saline to 8 cc of the eluate. The final *p*H of this solution should be kept below 1.7 because when the *p*H approaches 2, larger particles will form that will increase accumulation of the activity in the liver. In this preparation neither phosphate buffer nor NaOH is used. Final *p*H is always below 2.

II. Liver. Add 1 ml-gelatin to the generator eluate and titrate to *p*H 3.5 with sodium phosphate buffer.

III. Spleen. Add 1-ml gelatin to the generator eluate and titrate to *p*H 6 with sodium phosphate buffer.

IV. Lung. To 5 ml of the eluate add 0.15 ml of the ferric chloride solution. Adjust to *p*H 12 with 0.5 *N* NaOH and back-titrate to *p*H 8.5–9 with the gelatin solution.

DISCUSSION

In Table 1, each number represents the mean ($\bar{x}$) $\pm$ the standard error in a group of rats expressed as percent of injected dose per gram of tissue. The "t" test of significance measures the label selectivity with reference to the organ of maximum concentration and the other areas. The preparations I, II, III and IV are as indicated in the text. Note that there is not significant selectivity between the spleen (III) and liver (II) preparations although the spleen preparation (III) significantly increases the spleen activity ($P < 0.001$) in relation to the liver prep-

* Obtained from Neisler/Union-Carbide Co., Tuxedo, New York.

Received Feb. 12, 1968; revision accepted March 27, 1968.

TABLE 1. ORGAN DISTRIBUTION OF ^{113m}I-LABELED COMPOUNDS

Preparation	Whole blood		Liver				Spleen				Lung			
	%i.d./gm	s.e.	%i.d.	s.e.	%i.d./gm	s.e.	%i.d.	s.e.	%i.d./gm	s.e.	%i.d.	s.e.	%i.d./gm	s.e.
I	6	±0.062	7.7	±0.77	1.40	±0.037	0.51	±0.07	0.95	±1.35	2.66	±0.27	2.46	±0.27
			$p < 0.001$				$p < 0.001$				$p < 0.001$			
II	0.53	±0.084	86	±2.51	17	±0.33	1.47	±0.14	3.21	±0.22	1.52	±0.15	1.57	±0.17
	$p < 0.001$						$p < 0.001$				$p < 0.001$			
III	0.17	±0.02	74.27	±4.6	12.1	±1.6	2.88	±0.39	5.59	±0.54	1.77	±0.17	1.78	±0.25
IV	0.29	±0.06	7.34	±2.32	1.66	±0.52	0.68	±0.16	1.57	±0.29	70	±5.82	87.1	±5.90
	$p < 0.001$			$p < 0.001$				$p < 0.001$						

aration (II). The spleen preparation (III) has slightly less affinity for the liver ($P < 0.02$) than does the liver preparation (II). Also note that preparation (IV) (lung preparation) has a lung affinity 12 times greater than the liver uptake.

The final *p*H is controlled by three solutions, namely, sodium hydroxide, gelatin and the sodium phosphate buffer. Gelatin also stabilizes the particles. $FeCl_3$ is added as a carrier in the lung preparations. The *p*H and carrier are the most important factors in determining the size and organ distribution of the indium-labeled compounds. Stern (*4*) recommends that the solution be brought to blood *p*H before injection. In our experience this resulted in an inhomogeneous particle size assessed by Millipore filtration and bioassay. The H^+ ion concentration is 0.33×10^{18} ion/ml (3.16×10^{-7} gm mole/cc) which required only 24 ml whole blood to titer 2 ml of the liver preparation to *p*H 7.4 *in vitro*. Obviously, subsequent *in vivo* experiments failed to define a measurable blood *p*H change in rats or humans. Thus, we have preferred to maintain a constant *p*H prior to injection realizing the blood's buffering capacity.

The liver preparation results in 50 mR/mc to the liver which is somewhat less than the hepatic dose from ^{198}Au liver scans. The amount of indium the patient receives is 10^6 times less than the known toxic dose of this element. The gelatin carrier should have as great a range of safety as the albumin carriers advocated with ^{99m}Tc compounds. Large quantities of gelatin have been infused as a plasma-volume expander without adverse effect (*5,6*). Some have expressed concern over the use of Fe^{+++} as a carrier although the dose of iron injected is less than 80 μg. No adverse effects have been seen in some 500 patients having lung scans.

SUMMARY

These ^{113m}In-labeled compounds are now routinely used in our laboratory for blood pool, liver, spleen and lung scans. The clinical organ specificity of the preparation is remarkably similar to that seen in the bioassay organ distribution studies (Table 1). The clinical scans have improved quality over previous methods primarily due to the greater photon yield. This simplified laboratory preparation and broad application of ^{113m}In-labeled compounds in addition to the relatively low dose and long parent half-life suggest that this may be the system of choice in many clinical situations.

ADDENDUM

It has come to our attention that others have had difficulty obtaining reproducible results in preparing indium colloids using the methods outlined in Ref. *1, 2* and *3*. The size of the generator and variations in generator manufacturing may, in part, be responsible for these problems (*4*). Stern and Scheffel (*4*) have developed modifications (presented at AEC Symposium, Cleveland, September 1967) of the original technique which may give results equivalent to those presented in this paper. There have been no comparative studies between the modified Stern-Scheffel methods and those proposed here.

ACKNOWLEDGMENT

This project was supported in part by the U.S. Atomic Energy Commission Contract No. AT(11-1)-1653 under which this manuscript becomes Document No. CO-1653-35.

REFERENCES

1. Stern, H. S., Goodwin, D. A., Wagner, H. N., Jr. and Kramer, H. H.: Iron (^{113m}In) hydroxide: A new radiopharmaceutical for lung scanning. *Nucleonics* **24**:10, 1966.

2. Goodwin, D. A., Scheffel, U., Wagner, H. N., Jr. and Kramer, H. H.: Indium-113m colloid: A new radiopharmaceutical for liver scanning. *Nucleonics* **24**:65, 1966.

3. Stern, H. S., Goodwin, D. A., Scheffel, U., Wagner, H. N., Jr. and Kramer, H. H.: In-113m for blood-pool and brain scanning. *Nucleonics* **25**:62, 1967.

4. Stern, H. S.: Personal communication.

5. Gray, I. and Pulaski, E. J.: Metabolism of plasma substitutes, II oxypolygelatin. *Proc. Soc. Exp. Biol. Med.* **78**:421, 1951.

6. Jacopsen, S. D. and Smyth, C. J.: Gelatin as a substitute for plasma. *Arch. Internal Med.* **74**:254, 1944.