## RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

# Indium-111 Tropolone, A New High-Affinity Platelet Label: Preparation and Evaluation of Labeling Parameters

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**Platelets were labeled with a new neutral, lipid-soluble metal complex of indium-111 and tropolone. Unlike oxine, which must be dissolved in ethyl alcohol, tropolone is soluble in isotonic saline. Platelet labeling with In-111 tropolone can be performed in both acid-citrate-dextrose (ACD)-plasma and ACD-saline media within two hours' time. Labeling efficiency has been 80–90% in ACD-saline and 60–70% in the ACD-plasma medium.**

**Optimum concentrations for the labeling of platelets with In-111 tropolone were 5 μg/ml in ACD-saline and 10 μg/ml in ACD-plasma, using a 15-min incubation at room temperature. A kit formulation for convenient routine preparation of In-111-labeled platelets has been developed. Seven parameters of platelet labeling were studied: concentration of tropolone, citrate, plasma proteins, and calcium ions; also platelet density, temperature, and pH of incubation medium. Their effects on the mechanism of platelet labeling with lipid-soluble tracers are discussed.**

**J Nucl Med 22: 981–987, 1981**

Indium-111 oxine, a chelate of In-111 with 8-hydroxyquinoline, was introduced in 1976 by Thakur and associates as a label for granulocytes (*1*) and platelets (*2*). Since then we and others have optimized the various platelet-labeling parameters, performed comparative survival studies with Cr-51-labeled platelets, and used labeled platelets in thrombosis research (*3–8*). However, platelet labeling in plasma with In-111 oxine, as recommended by a panel on diagnostic application of radioisotopes in hematology (*9*), resulted in inconsistent and low labeling efficiency. Also, because of the poor solubility of oxine in aqueous solution, a small amount of ethyl alcohol is needed as a solvent for In-111 oxine, but the probability of injurious effects of alcohol on platelet functions was demonstrated by Haut and Cowan (*10*).

We have developed a new water-soluble, high-affinity platelet label that has demonstrated a higher stability constant and a higher (olive oil/citrate buffer) partition coefficient than In-111 oxine. The chelating ability of tropolone was used by Dyrssen (*11*) in the separation of metal ions by solvent extraction and by Pitt and Gupta (*12*) for iron removal in iron-storage disease. Indium-111-labeled tropolone permits efficient, consistent, and convenient platelet labeling in either an acid-citrate-dextrose (ACD)-saline or an ACD-plasma medium. We have also developed a kit for platelet labeling, which may lead to more widespread use of In-111-labeled platelets in investigations of arterial thrombosis and thrombocytopenia.

Received Apr. 29, 1981; revision accepted July 20, 1981.

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## MATERIAL AND METHODS

**Preparation of sterile ACD anticoagulant kit.** Four grams of anhydrous citric acid, 11.2 g of anhydrous trisodium citrate (or 12.78 g of trisodium citrate dihydrate), and 6.0 g of dextrose were dissolved in 500 ml of distilled water. The solution was membrane filtered (0.22 μm), and 8-ml and 25-ml aliquots were stored in sterile 10-ml or 50-ml vials. The former are used for blood collection, the latter for preparation of the ACD-saline

kit. The pH of the ACD solution was ~5.0 and osmolarity was ~300 mOsm.

**Sterile ACD-saline kit for platelet labeling with In-111 tropolone.** Thirty-six milliliters of ACD solution were mixed with 250 ml of sterile isotonic saline, and pH was adjusted to 6.5, with 1-ml aliquots transferred to a disposable polystyrene tube; 4.0 ml of sterile 1 *N* NaOH was used to titrate. The solution was membrane filtered (0.22 $\mu$m) and aliquots of 10–30 ml were transferred to sterile vials. This stock solution contained 3.02 mg of citrate ion per milliliter. We find stainless steel needles more suitable than aluminum needles in the transfer of 1 *N* NaOH solution. The batches were tested for osmolarity (~300 mOsm), sterility (culture for 7 days with brain-heart infusion broth with 5% beef extract, and 5 days with blood-agar medium), and apyrogenicity (*Limulus* amebocyte lysate test*).

**Preparation of In-111 tropolone.** Eight to ten mg of tropolone † (weighed by microbalance) was dissolved by vortexing in sterile isotonic saline in a sterile polystyrene tube. The volume was adjusted to a concentration of 1 $\mu$g/$\mu$l of saline. The pH was adjusted to 7.4 with 0.1 *N* NaOH and the solution was sterilized by membrane filtration (0.22 $\mu$m). When it was capped at room temperature, we found that this stock solution yielded constant labeling efficiency for almost 3 mo.

Twenty microliters of tropolone solution was transferred to a 12-ml polypropylene tube with a sterile micropipette, and 300–1,200 $\mu$Ci of In-111 chloride was added dropwise. The solution was mixed for 2 min, 3.5 ml of ACD-saline was added, and the pH of the solution was adjusted to 6.5 with sterile 0.1 *N* NaOH, using a precalibrated pH meter. We found this solution of In-111 tropolone in ACD-saline suitable up to 1 wk after preparation. We also found that the 1-ml syringe with a polypropylene holder (No. 7022D) for the needle (27 gauge, ½ in. long) introduced minimal aluminum contamination in the preparation of ACD-saline. Ten micrograms of tropolone were used in the In-111 tropolone preparation for platelet labeling in plasma, and the pH of the final In-111 tropolone solution in a small volume was adjusted to 7.0 with 0.1 *N* sterile NaOH. Sterile stock solutions of 0.1 *N* NaOH and 0.1 *N* HCl for pH adjustment were kept in sterile polystyrene tubes in the refrigerator.

For the determination of optimum conditions of platelet labeling with In-111 tropolone, we studied seven parameters: plasma proteins, citrate, tropolone, calcium ion concentration, platelet density, temperature, and pH of incubation medium. Platelets were obtained from five healthy mongrel dogs and counted with a commercial counter.‡ The details of each of these experiments are described below.

**Tropolone concentration and platelet-labeling efficiency.** Fifty microcuries of $^{111}InCl_3$ was added to aliquots of stock solution of tropolone in saline; the pH was adjusted to 6.5. Four-milliliter aliquots of ACD-saline stock (pH = 6.5) were added and $2.2 \times 10^9$ plasma-free platelets were added and incubated for 30 min at room temperature. They were then washed with ACD-plasma. Labeling efficiency was determined after removal of the small fraction of platelet aggregates (less than 5% of total radioactivity). For plasma labeling, the platelet pellet was resuspended in 0.5 ml of plasma and transferred to In-111 tropolone in a small volume at pH 7. Labeling efficiency in percent was then determined by dividing the radioactivity of In-111 in the platelet pellet by the total radioactivity in the platelet pellet and washings, and multiplying by 100. The radioactivity was measured in an ionization chamber.

In most of these labeling experiments, dog blood was collected in ACD-anticoagulant (86 ml of blood/16 ml of ACD) with a 19-gauge needle. Platelet-rich plasma was obtained by spinning the whole blood in a 40-ml sterile centrifuge tube (round-bottom polycarbonate, screw cap) at 200 *g* for 10 min. Four to eight milliliters of platelet-rich plasma was transferred to a 12-ml polypropylene centrifuge tube with screw cap. (These caps maintain sterility during labeling and also prevent loss of carbon dioxide and the consequent increase in plasma pH, which can adversely affect platelet function.) The platelet-rich plasma was then spun at 1600 *g* to obtain the platelet pellet. For plasma labeling, these platelets were suspended in 0.5 ml ACD-plasma with a sterile polyethylene pipette. After incubation for 15 min with In-111 tropolone at room temperature, 2.5 ml of ACD-plasma was added and the platelets were washed by spinning at 1600 *g* for 10 min, then resuspended and washed again with 3 ml of ACD-plasma. Finally, any residual platelet aggregates were removed by spinning at 100 *g* for 5 min. Residual red blood cells were lysed by incubation with 5 ml of 0.2% saline for 30 min. Hemoglobin-associated radioactivity was removed by centrifugation at 1600 *g* for 10 min. The platelet-labeling efficiency was determined by measuring radioactivity with an ionization chamber in the platelet-pellet washings and platelet aggregates. The only difference in labeling procedure between In-111 oxine and In-111 tropolone was that with the latter no washing of the platelet

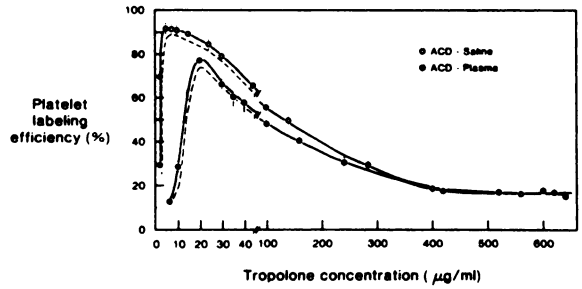


**FIG. 1.** Effect of tropolone concentration on platelet labeling in plasma and ACD-saline. Dashed line is platelet In-111 after erythrocyte lysis.

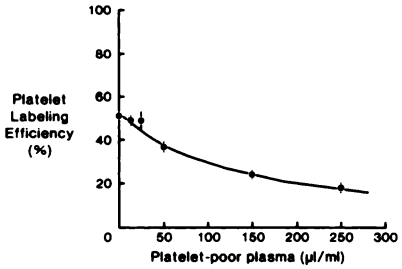


**FIG. 2.** Effect of plasma proteins on platelet-labeling efficiency.

pellet with ACD-saline was necessary, thus eliminating one step.

**Plasma protein concentration.** The effect of plasma on platelet-labeling efficiency was determined by adding increasing amounts of plasma to $2.2 \times 10^9$ platelets obtained from 4 ml of platelet-rich plasma. Variable amounts of platelet-poor plasma were added directly to 50 $\mu$Ci of In-111 tropolone containing 50 $\mu$g of tropolone in 4 ml of ACD-saline. The platelets were labeled and washed as before, and platelet-labeling efficiency determined.

**Incubation temperature and time.** This was studied by adding $2.2 \times 10^9$ platelets to 50 $\mu$Ci of In-111 tropolone (20 $\mu$g tropolone) in 4 ml ACD-saline. The platelet labeling was determined after incubation at 4°C, room temperature (~25°C), and 37°C for periods of 5–120 min.

**Number of platelets.** Platelets obtained from 1, 2, 3, 5, 8, and 12 ml of platelet-rich plasma were incubated with In-111 tropolone in 0.5 ml of plasma or 4 ml of ACD-saline at room temperature for 30 min, and labeling efficiency was determined.

**Concentration of citrate ion in ACD-saline.** Citrate chelates calcium ion and a host of trace-metal impurities in the incubation medium, and chelation of free $Ca^{2+}$ prevents activation of the coagulation cascade. Since citrate ion is an essential anticoagulant for platelet harvesting and labeling, we studied the effect of increasing amounts of citrate ion on platelet-labeling efficiency. Platelets, $2.2 \times 10^9$, from 4 ml of platelet-rich plasma were incubated for 30 min at pH 6.5 with 50 $\mu$Ci of In-111 tropolone containing 50 $\mu$g of tropolone. The citrate ion concentration varied from 1.5 to 177 mg/ml, and platelet-labeling efficiency was determined.

**Concentration of calcium ion in ACD-saline.** Platelets, $1.2 \times 10^9$, from 4 ml of platelet-rich plasma were centrifuged to form a platelet pellet. Variable amounts of $Ca^{2+}$ ion were mixed with 50 $\mu$Ci of In-111 tropolone in 4 ml of ACD-saline. These mixtures were added to the platelet pellet and incubated for 30 min at room temperature, and platelet-labeling efficiency was determined.

**Hydrogen ion concentration in ACD-saline.** The pH of ACD-saline was adjusted to 5, 6, 6.5, 7, 8, 9, 10, and 11 with 0.5 *N* HCl or 0.5 *N* NaOH. An aliquot of 50 $\mu$Ci of In-111 tropolone solution (50 $\mu$g of tropolone) was then added and final pH adjustments were made to the above-mentioned values. Platelets, $2.2 \times 10^9$, from 4 ml of platelet-rich plasma were incubated in triplicate for 30 min at room temperature, and platelet-labeling efficiency was determined.

**Plasma incubation on release of In-111 label.** Indium-111-labeled platelets, tagged in ACD-saline or plasma medium, were resuspended in ACD-plasma and checked for In-111 release during 6 hr at room temperature. Aliquots of platelets were centrifuged at 1, 2, 4, and 6 hr, and radioactivity in plasma and platelets was determined with the ionization chamber. More than 95% of the In-111 was bound to platelets at 6 hr after labeling in either ACD-saline or ACD-plasma.

**Aggregation of control and In-111-labeled platelets.** Blood of conscious, healthy mongrel dogs that had not received any drugs or participated in any experiments in the previous month was collected from a jugular vein into ACD solution. Platelet counts‡ of all tested suspensions were adjusted to 300,000/mm$^3$ with autologous platelet-poor plasma. Platelet aggregation (*13*) was then examined at 37°C with a single-channel platelet aggregometer.‖ The ADP used was from equine muscle. To control any effect of aging on aggregation, platelet-rich plasma and suspensions prepared from it were tested alternately and within 4 hr of the time of venepuncture.

## RESULTS

Increasing tropolone concentration decreased platelet-labeling efficiency, with a peak value of about 5–6 $\mu$g/ml in ACD-saline and 10 $\mu$g/ml in ACD-plasma (Fig. 1). It is also evident from Fig. 1 that labeling in plasma rather than ACD-saline led to lower platelet-labeling efficiency. Increasing the plasma proteins decreased platelet-labeling efficiency (Fig. 2). Slight loss of labeling efficiency resulted after red-cell lysis for platelets labeled in either ACD-saline or ACD-plasma, but the general shape and peak of tropolone concentration did not change.

The platelet-labeling efficiency increased with both increasing incubation time and increasing temperature (Fig. 3). The rate of increase in labeling efficiency with

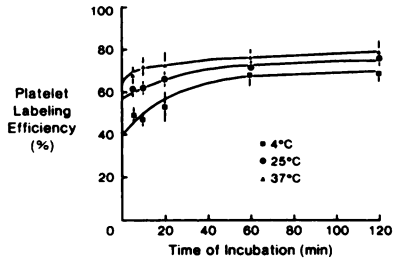


**FIG. 3.** Effect of temperature and time of incubation on platelet-labeling efficiency.

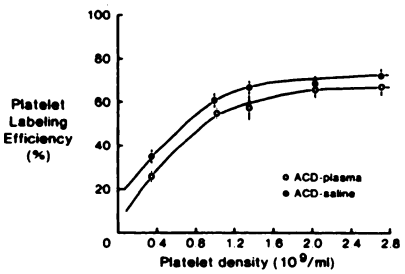


**FIG. 4.** Effect of platelet density on platelet-labeling efficiency.

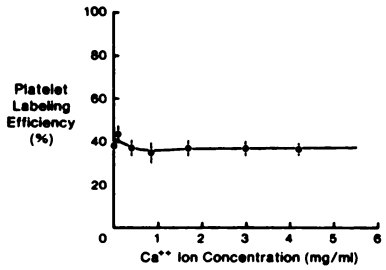


**FIG. 6.** Effect of $Ca^{2+}$ ion concentration on platelet-labeling efficiency.

incubation time was higher at 4°C and lower at 37°C. No further gain in labeling efficiency was observed after 60 min of incubation.

With either ACD-saline or ACD-plasma as incubation medium, the labeling efficiency increased and reached almost a plateau value at $2.8 \times 10^9$ platelets. ACD-saline labeling led consistently to higher labeling efficiency (Fig. 4).

Increasing concentration of citrate ion decreased platelet-labeling efficiency (Fig. 5). If this curve is extrapolated to zero citrate concentration, the platelet labeling increases to its maximum value of about 87%. When unlabeled In-111 tropolone was kept for a week at room temperature, dissolved in ACD-saline with 3 mg/ml added citrate, there was no loss of platelet-labeling potential.

Calcium ions at various concentrations did not affect platelet-labeling efficiency when platelets were labeled with In-111 tropolone in ACD-saline (Fig. 6). There is always excess citrate ion available to chelate excess calcium ions. The platelet pellets were also easily dispersed in the ACD-plasma solution, although excess $Ca^{2+}$ was available for promoting aggregation.

The highest platelet-labeling efficiency was obtained at pH 9 (Fig. 7) when platelets were labeled with In-111 tropolone in ACD-saline. At this pH the platelet membrane may be most permeable because of reversible loss of phospholipid and cholesterol. At higher or lower pH values, the platelet-labeling efficiency decreased.

Similar effects of these parameters on platelet-labeling efficiency have been observed by several investigators (*2–6*) when platelets were labeled with In-111 oxine in Tyrode buffer, ACD-saline, or ACD-plasma.

Aggregation of platelets to which tropolone was added, in the presence of ACD-plasma or ACD-saline, was diminished in comparison with the parent platelet-rich plasma (Fig. 8). The reduction was comparable whether the In-111 tropolone was added in the presence of ACD-plasma or of ACD-saline. Platelet aggregation was also comparable when tropolone was used in place of In-111 tropolone.

## DISCUSSION

The structures of the three chelating agents, oxine, acetylacetone, and tropolone, are shown in Fig. 9. Both oxine and tropolone molecules form five-membered octahedral and neutral complexes with In-111 and a variety of other divalent and trivalent cations (*11*) and are used for the analytic separation of metal ions by solvent extraction. Acetylacetone forms a six-membered ring with metal ions. Most of these complexes are efficiently extracted at low pH in chloroform and other lipid-solubilizing organic solvents. The structure of the 1:3 complex of In-111(tropolone)$_3$ is shown in Fig. 10; indium ion loses its ionic characteristics as it is buried in the organic envelope of tropolone. It may then diffuse through a lipid membrane like a neutral ionophore. Our preliminary studies indicate that In-111 tropolone has a higher lipid solubility than In-111 oxine and In-111 acetylacetone. The partition coefficient for olive oil/ACD-saline was found to be $3.54 \pm 0.28$ for In-111 oxine, $7.93 \pm 1.04$ for In-111 acetylacetone, and $18.18 \pm 1.79$ for the In-111 tropolone complexes. This five-fold increase in lipid solubility permits more efficient cellular extraction of In-111 tropolone from both ACD-saline and ACD-plasma. Tropolone has been evaluated for the sequestration of ferric ion in iron-storage disease (*12*).

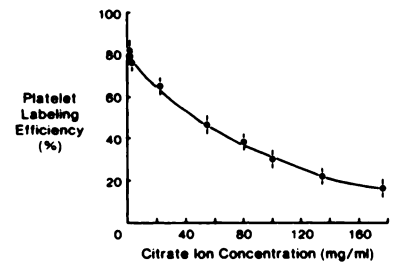


**FIG. 5.** Effect of citrate ion concentration on platelet-labeling efficiency.

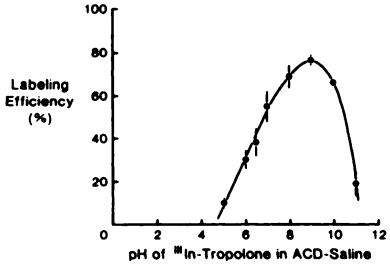


**FIG. 7.** Effect of pH on platelet labeling with In-111 tropolone in ACD-saline solution.

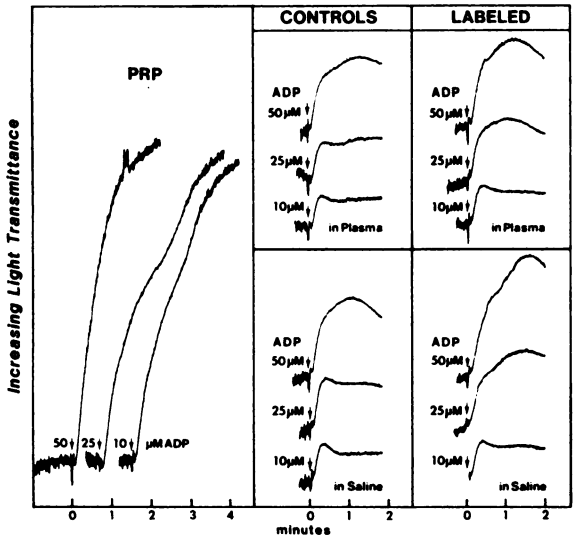


**FIG. 8.** Typical aggregation tracing of control and labeled canine platelets with ADP. As platelets aggregate, light transmittance increases. *Left:* normal platelet-rich plasma (PRP). *Center* (controls): platelet suspensions containing tropolone. *Right* (labeled): platelet suspensions containing In-111 tropolone. *Top:* tropolone added in presence of plasma. *Bottom:* tropolone added in presence of ACD-saline. Time and concentration of ADP additions are indicated.

Its toxicity is minimal in the microgram amounts used for platelet labeling. The $LD_{50}$ of tropolone in mice by intraperitoneal administration was in the range 15–200 mg/kg, and that of oxine by subcutaneous and intraperitoneal administration 30 mg/kg and 88.8 mg/kg, respectively (*14*). This toxicity is of no significance, because most of the tropolone carrier, like oxine, is probably released as a result of ligand exchange inside the platelets and is thus removed from them during washing with ACD-plasma solution.

The major advantage of In-111 tropolone is its solubility in isotonic saline; hence, no solvent that could adversely affect platelet function—such as ethyl alcohol in the case of oxine and HEPES or Tris buffer in the case of acetylacetone—is necessary for cell labeling.

The decrease in labeling efficiency at low temperatures (Fig. 3) may be due to freezing of cholesterol and reduction of permeability at lower temperature. When platelets are suspended in ACD-saline, there is a rapid loss of cholesterol and phospholipid from the platelet membrane (*15–17*). The enhancement of membrane permeability increases incorporation of In-111 tropolone, and therefore labeling efficiency, when platelets are incubated in ACD-saline rather than plasma. Similar observations were made by Scheffel and associates (*3*) when human platelets were incubated with In-111 oxine in plasma. For an investigator with minimal experience in cell labeling, the ACD-saline medium might be more suitable than ACD-plasma, because the former could provide higher labeling efficiency and viability and could tolerate trace metals better than the plasma medium.

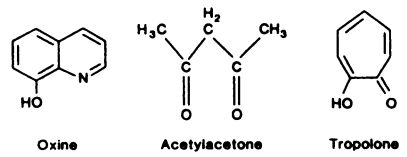


**FIG. 9.** Structures of three chelating agents that form neutral lipid-soluble complexes with In-111 cation.

The results of platelet labeling with excess citrate ion (Fig. 5) indicate that when platelets are labeled in ACD-saline, the labeling efficiency will not be affected by the metal-ion contaminants—for example, traces of $Cd^{2+}$ ion from $^{111}InCl_3$; $Al^{3+}$ ion from the aluminum needle; or $Ca^{2+}$, $Mg^{2+}$, or $Fe^{3+}$ from the saline solution or the glass vial or residual plasma. The kinetics of reverse exchange leading to the formation of In-111 citrate may be slow, but excess citrate might increase intracellular citrate ion. This might lead to the formation of In-111 citrate, which could diffuse out again during washing (*11*).

Although several investigators (*1–3*) have suggested chloroform extraction of In-111 oxine, with removal of chloroform by evaporation and redissolution in ethyl alcohol before platelet labeling, we found this step unnecessary. Because In-111 ion is carrier-free, the formation of In-111 oxine in the presence of excess oxine is assured. Although 75–95% of the In-111 complex is extracted with chloroform, it is not known how much of the oxine is extracted along with the corresponding In-111 complexes, for we also found that most of these lipid-soluble tracers were absorbed to glass and polymer surfaces. Solvent extraction of In-111 oxine or In-111 tropolone and other manipulations thus introduce an unknown parameter about the level of oxine or tropolone, and these levels are of critical importance in platelet labeling (Fig. 1).

Aggregation of platelets by ADP was reduced by the handling procedures required to label the platelets with In-111 tropolone. The presence or absence of plasma during the labeling procedure did not affect the degree of reduction of platelet aggregation. Neither the In-111 radioactivity nor the tropolone appeared to affect platelet function, inasmuch as the reductions of platelet aggre-

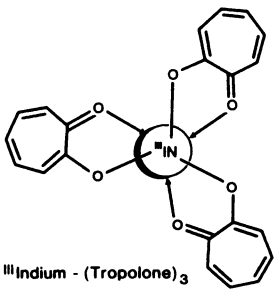


**FIG. 10.** Structure of In-111 tropolone. Three molecules of monovalent tropolone anion combine with one central trivalent In-111 ion to form an octahedral complex of In-111(tropolone)$_3$.

gation of control and labeled platelets were comparable. Thus the reduction in platelet aggregation appears to be totally explicable by physical handling (for example, centrifugation, resuspension) and not by the labeling itself. During labeling manipulations—especially centrifugation, pelleting, and dispersion—platelets might lose some ADP and require more exogenous ADP for their aggregation. This loss of ADP from platelet organelles is a reversible process. Reimers and associates (*15*) pointed out that a partial degranulation process does not affect platelet survival. These observations are in agreement with our preliminary platelet-survival studies.

The intracellular ligand exchange between In-111 oxine and cytoplasmic protein was studied by Hwang (*16*) and by Pandian and associates (*17*). The time-integrated perturbation factors can be related to the rotational correlation time of the environment of the In-111 nucleus. Indium-111 platelets showed rotational correlation times of half to a third of that for In-111 oxine. This suggests that, inside the platelet, the environment of the In-111 nucleus changes from that of oxine to that of a large molecule. Our work on In-111 oxine and In-111 tropolone with red blood cells suggested that red-cell membrane retained 50–60% of the total In-111 inside the red cell (*18*). This result suggests that an equilibrium is established inside the cell whereby the phospholipid and proteins of membranes of cell and organelles share In-111 along with cytoplasmic proteins. Perturbed angular correlation studies (*16,17*) of different In-111 oxine and In-111-labeled platelets also support this hypothesis. It is only after lysis of the cell that these In-111-bound protein and In-111-labeled organelle fragments are released into the circulation and sequestered mainly by the reticuloendothelial system.

Our preliminary work suggests that both lipoproteins and transferrin sequester the neutral In-111 complexes. As we increase the amount of plasma proteins, more In-111 complex is incorporated into lipoproteins and transferrin, and less is available for cellular uptake. Also, the variability of cell labeling in plasma within the same species of animals could easily be accounted for by variations in the amount of lipoproteins, transferrin, and metal ions in plasma.

Our preliminary studies with Sephadex gel chromatography indicate that the cytoplasmic protein carrier of In-111 has a molecular weight of 50,000–55,000 daltons. A similar molecular weight for In-111-bound platelet protein was reported by Hudson and associates (*19*) for platelets labeled with In-111 oxine. Variations of intraplatelet distribution of In-111 were observed by Eakins and associates (*20*) when platelets were labeled in ACD-saline and ACD-plasma. As long as In-111 is bound to platelet organelles or cytoplasmic platelet proteins, and the membrane integrity is intact, platelet recovery, survival, and imaging may not be affected whether the platelets are labeled in an ACD-saline or an ACD-plasma medium. The present knowledge of simplified platelet labeling with In-111, and the acceptable radiation dose (*21*) for platelet-survival and imaging studies, should lead to more widespread use of In-111-labeled platelets in the future.

## FOOTNOTES

* Microbiological Associates.
† Aldrich Chemical Co.
‡ Coulter ZBI or S-Plus counter.
‖ Model 330, Chrono-Log Corp.

## ACKNOWLEDGMENTS

The authors greatly appreciate the encouragement of Heinz W. Wahner, M.D., and the expert technical assistance of Mr. Sushital Chowdhury, Mr. James A. Rosemark, and Mr. John Q. Stropp.

This investigation was supported in part by Grants HL-24602 and HV-92915 from the National Institutes of Health, Public Health Service.

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## RADIOPHARMACEUTICAL SCIENCE COUNCIL WORKSHOP ON

### The Choice of the Appropriate Animal Model in Radiotracer Design

**January 28, 1982** **Phoenix Hilton** **Phoenix, Arizona**

The Radiopharmaceutical Science Council will hold a one-day workshop held in conjunction with the midwinter meeting of the Society of Nuclear Medicine. The program for the workshop will include the following invited papers as well as contributed papers.

Edward A. Carr, M.D. "Animal Models for Study of Radiopharmaceuticals that are Substrates for (Catecholamine) Uptake$_1$ and Uptake$_2$"
Brian M. Gallagher, Ph.D. "Monoclonal Antibodies: The Design of the Appropriate Carrier and Evaluation System"
Adrian Nunn, Ph.D. "Species Differences and the Need for Multiple Animal Models for Hepatobiliary Agents"
Michael J. Welch, Ph.D. "Labeled Cells"
Leonard I. Wiebe, Ph.D. "Oncological Models for Screening Potential Diagnostic Radiopharmaceuticals"

Participants are encouraged to make hotel reservations at the meeting site by contacting the Registrar, SNM, 475 Park Ave. So., New York, NY 10016. A registration fee of $35.00 will be collected at the entrance to the meeting room.

For further information contact the Co-Organizers: William Eckelman, President, RPSC, or Richard M. Lambrecht, President-Elect, RPSC, c/o the National Office.

Abstracts should be sent to Richard M. Lambrecht, Ph.D., Dept. of Chemistry, Brookhaven National Laboratory, Upton, NY 11973.

**Deadline for the receipt of abstracts is January 2, 1982.**

## SNM REFERRAL SERVICE

The SNM Referral Service is accepting applications from employers and job applicants. The Service lists positions wanted and positions available in the following nuclear medicine fields: Physician, Technologist, Scientist, Commercial, and Other.

The fee for job applicants is $5.00 for SNM members and $50.00 for nonmembers. For employers, the fee is $50.00 for each position listed.

To obtain more information and an application form, please write to:
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