

**RADIOCHEMISTRY  
AND RADIOPHARMACEUTICALS**

**Labeling of Human Platelets with  
[<sup>111</sup>In] 8-Hydroxyquinoline**

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*We have evaluated the factors influencing the labeling of human platelets in the presence of autologous plasma. The labeling efficiency was found to be dependent on a) the time and temperature of incubation, b) the platelet concentration, c) the concentration of citrate ions (in ACD anticoagulant), and d) the concentration of 8-hydroxyquinoline in the suspending medium. Contrary to what was expected, unsaturated transferrin was found not to interfere with the transfer of In-111 from the [<sup>111</sup>In] 8-hydroxyquinoline complex to the platelets. Based on the findings of this study, a protocol was established by which human platelets can be labeled with In-111 in plasma with a labeling efficiency of  $55.5 \pm 9.3$  (mean  $\pm 1$  s.d.) percent.*

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[<sup>111</sup>In]8-hydroxyquinoline (In-111 oxine) has recently been introduced as a gamma-emitting agent for in vitro labeling of platelets with clear advantages over the currently most widely used sodium [<sup>51</sup>Cr] chromate (1). Because of the physical characteristics of In-111, in vivo studies of temporal and spatial distribution and deposition can be performed using In-111-labeled platelets (2-7). The determination of platelet survival with In-111-labeled platelets has been reported (3,8,9).

Several investigators have suggested that the labeling of platelets with In-111 oxine must be performed in a plasma-free medium (1,10) because of possible interference by plasma transferrin. Transferrin is known to have a high affinity for indium cations (11) and indium oxine is a rather weak complex compared with indium transferrin (12,13). On the other hand, it is well established that autologous plasma is the best medium for suspension of pla-

telets during in vitro handling (14). Essential parameters of platelet function and metabolism are changed when platelets are deprived of the plasma-protein environment (15). When labeling platelets with In-111, Goodwin et al. (8) found that the presence of plasma is essential to keep platelets functionally normal as judged by in vivo initial recovery and platelet life span.

In our previous study we have shown that rabbit platelets can be labeled efficiently with In-111 in the presence of plasma (27-67% uptake). When we started to label human platelets using the same method, the labeling efficiency dropped to 3-20%. The present study was therefore designed to evaluate the factors influencing the labeling of human platelets with In-111 oxine in the presence of plasma. Our aim was to establish a protocol by which human platelets, suspended in autologous plasma, could be efficiently and predictably labeled with In-111.

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**MATERIALS AND METHODS**

**Collection of blood and separation of platelets.** We recently described a procedure for the separation

and labeling of rabbit platelets (3). Essentially the same protocol was used for the preparation of human platelets from whole blood. The following minor modifications were introduced. Blood from human volunteers\* was collected into either a) pres-terilized 50-ml round-bottom polycarbonate tubes, or b) in triple blood-pack units. ACD NIH A was used as anticoagulant (7.5 ml ACD + 42.5 ml blood) as well as for acidification of the platelet-rich plasma (PRP) to pH 6.5–6.7 (1 part ACD to 20 parts PRP). With centrifugation speeds and times as described (3), the average platelet yield was 80% when polycarbonate tubes were used and 55% with the triple blood-pack units.

After formation of a platelet button, platelets obtained from 50 ml of blood were resuspended in 2 ml of platelet-poor plasma (PPP), unless stated otherwise. The time for complete resuspension varied from 10 to 60 min.

Platelets were counted by the method of Brecher et al. (16). Clot retraction was used as a measure of platelet function (17,18). The test was carried out on platelet dilutions ranging from 8000 to 2.5 million platelets/cmm.

**Preparation of [In-111] 8-hydroxyquinoline and determination of the 8-hydroxyquinoline (-oxine) concentration.** In-111 oxine was prepared according to the previously described method (3). Briefly, the In-111-oxine complex was formed, followed by an extraction step with methylene chloride. The extraction efficiency did not change significantly between batches (range 79.8–97.4%; mean = 92.1%). On the other hand, spectrophotometric measurements (see below) showed that varying amounts of the originally added 100  $\mu$ g of oxine were extracted into the organic phase (12–44  $\mu$ g, mean  $\pm$  1 s.d. =  $29.4 \pm 11.9$   $\mu$ g). Because of the unpredictable loss of oxine in the extraction procedure, we elected to add routinely an additional 100  $\mu$ g of oxine to each preparation after the evaporation of the methylene chloride. Thus, instead of dissolving the dried In-111 complex in alcohol-saline (1:4), we used a solution of 100  $\mu$ g oxine in 0.1 ml alcohol to dissolve the complex, and diluted thereafter with 0.3 ml of isotonic saline. In experiments testing the effect of the oxine concentration on the percentage of complex formation and on the labeling efficiency, the amount of additional oxine in 0.1 ml alcohol used in dissolving the dried complex was varied from 0 to 400  $\mu$ g.

In all our preparations of In-111-oxine complex, the final 8-HQ concentration was determined spectrophotometrically at 240 nm, using a calibration curve ranging from 0.078 to 5  $\mu$ g oxine/ml.

**Determination of the ratio of free vs. complexed In-111 by "reverse extractability."** Since no suitable

chromatographic system was at hand, the amount of In-111 actually complexed to oxine was determined by a reverse extraction technique. After the In-111 complex was prepared as described above, 10  $\mu$ l of the In-111 complex preparation were introduced into 2 ml of saline. Two milliliters of methylene chloride were added and the solutions thoroughly mixed for 1 min with a Vortex mixer. The two phases were then separated and the radioactivity was counted in both samples. The fraction remaining in saline represented the In-111 not complexed to 8-HQ.

**Labeling of platelets with In-111 and determination of percentage uptake.** In general, incubation of a 2-ml platelet suspension with 25–100  $\mu$ l In-111 oxine was carried out for 30 min at room temperature. The reaction was terminated by addition of 5–10 ml of PPP and immediate centrifugation at 1000g for 15 min. After removal of the supernatant radioactive PPP, the platelet button was washed once with an additional 1–3 ml of PPP.

To determine the labeling yield, the radioactivity of the combined supernatants and the platelets resuspended in a volume of PPP equal to that of the wash solution was counted at 100–500 keV in a 5-in. well scintillation counter. The random counting error was kept below one percent.

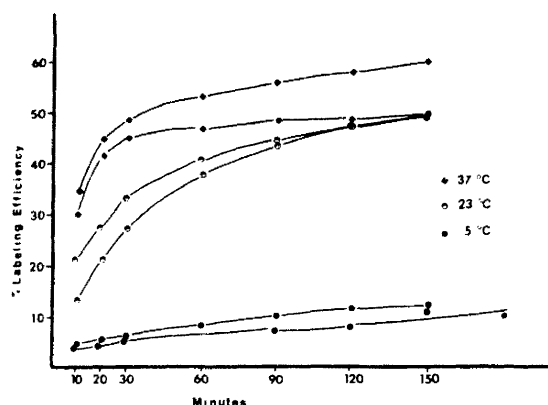
In experiments testing the effect of temperature and incubation time on the labeling, and in those designed to determine whether In-111 eluted from labeled platelets, 0.1-ml aliquots of the platelet suspension were transferred at indicated time intervals to other plastic tubes containing 2.0 ml of ice-cold PPP. The platelets were immediately separated by centrifugation and washed once with 0.4 ml of cold saline. The final platelet button was dissolved in 1 ml of 2 N NaOH.

**Determination of the unsaturated iron-binding capacity (UIBC) of serum.** The UIBC was measured in duplicate serum samples using the Irosorb-59 diagnostic kit† or the Res-O-Mat Fe-59 kit.‡.

## RESULTS

**In-111 uptake by human platelets.** *Effect of time and temperature of incubation on uptake of In-111 by human platelets.* The labeling of human platelets with In-111 as a function of time and temperature is shown in Fig. 1. Whereas at 37°C a plateau in In-111 uptake by the cells was reached after 30 min, at 23°C In-111 incorporation was considerably slower, reaching its maximum at 120 min. At 5°C there was minimal uptake of indium by platelets.

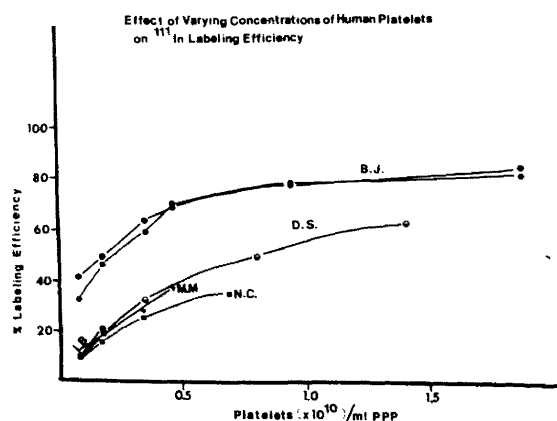
*Effect of Platelet concentration.* Figure 2 shows the dependence of the In-111 uptake on the number of platelets. In these experiments, platelets from several donors were divided into aliquots with in-



**FIG. 1.** Labeling of human platelets with In-111 oxine at various temperatures. Samples of  $9 \times 10^9$  platelets from two normal volunteers were suspended in 2 ml of autologous platelet-poor plasma (PPP) containing ACD, and incubated with In-111 oxine (50  $\mu$ l, containing approximately 12  $\mu$ g of oxine) at either 5°C, 23°C, or 37°C. Aliquots of platelet suspension were removed at different times and platelet-bound radioactivity was determined.

creasing platelet number. As shown in Fig. 2, increase of the number of platelets increased the labeling efficiency. However, when the platelet concentration rose beyond  $10^{10}$ /ml PPP, there was only a slight increase in the labeling efficiency.

**Effect of transferrin and its UIBC.** In order to study the influence of transferrin and its UIBC on the labeling efficiency, platelets from normal donors were labeled a) in saline; b) in transferrin-saline solutions with various amounts of human transferrin, ranging from 3 mg/ml (slightly in excess of the transferrin content in normal serum) down to 0.5 mg/ml; c) in transferrin solutions containing bicarbonate ions; and d) in autologous PPP (Table 1).



**FIG. 2.** Effect of varying concentrations of human platelets on In-111 labeling efficiency. Platelets from five donors were divided into aliquots with increasing platelet numbers. Platelets were incubated with In-111 oxine at room temperature for 30 min; then platelet-bound radioactivity was determined.

The presence of transferrin alone, even at a UIBC level of over 300  $\mu$ g/100 ml, did not lower the percent labeling. Bicarbonate ions, known to be essential for the binding of iron to transferrin (19), were added to the transferrin suspending medium in molar concentrations of twice and ten times the transferrin concentration, but with no observed effect on the labeling efficiency of the platelets. In contrast, PPP containing ACD anticoagulant had a significant lowering effect on the In-111 uptake.

**Effect of anticoagulant.** In order to investigate the role of the ACD anticoagulant, platelets were obtained from a patient with severe iron overload caused by chronic transfusion therapy. They were labeled in saline, in autologous plasma-ACD, and in saline containing the same concentration of ACD as the plasma (Table 2). The determination of the unbound iron-binding capacity in the serum of this patient showed a nearly 100% transferrin saturation. There was a 97% labeling efficiency in saline.

**TABLE 1. EFFECT OF TRANSFERRIN ON LABELING OF HUMAN PLATELETS\* WITH In-111 OXINE**

Suspending medium	pH	UIBC ( $\mu$ g/100 ml)	% label mean (range)	(n)
Saline	5.6	—	97.1 (96.0–98.2)	(3)
TF† 3 mg/ml saline	6.4	337	95.5 (94.5–96.1)	(3)
TF 1.5 mg/ml saline	6.3	172	93.7 (91.3–97.4)	(3)
TF 0.5 mg/ml saline	6.2	62	97.0 (96.2–98.5)	(3)
TF 3 mg/ml $\text{HCO}_3^-$ ‡	7.3	337	96.2 (96.0–96.3)	(2)
TF 3 mg/ml $\text{HCO}_3^-$ **	7.6	337	97.0 (96.9–97.2)	(2)
Plasma (ACD)	6.5	233	25.9 (24.3–27.2)	(3)

\*  $3.5 \times 10^9$  platelets, suspended in 2 ml of the medium of interest.

† Human transferrin, essentially Fe-free, in normal saline.

‡  $\text{NaHCO}_3$ ,  $7.7 \times 10^{-5}$  M in normal saline.

\*\*  $\text{NaHCO}_3$ ,  $3.85 \times 10^{-4}$  M in normal saline.

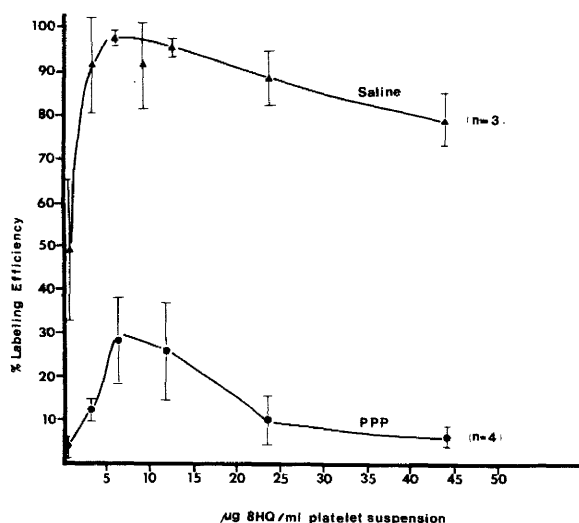
**TABLE 2. EFFECT OF COMPOSITION OF SUSPENDING MEDIUM ON LABELING OF HUMAN PLATELETS\* WITH In-111 OXINE**

Medium	Mean (range)†
Saline-2 ml	96.5 (94.0–98.2)
Autologous ACD-plasma-2 ml‡	64.0 (57.9–70.5)
Saline-ACD (0.6 ml ACD + 1.4 ml saline)	56.3 (44.0–68.6)

\*  $7 \times 10^9$  platelets

† n = 3

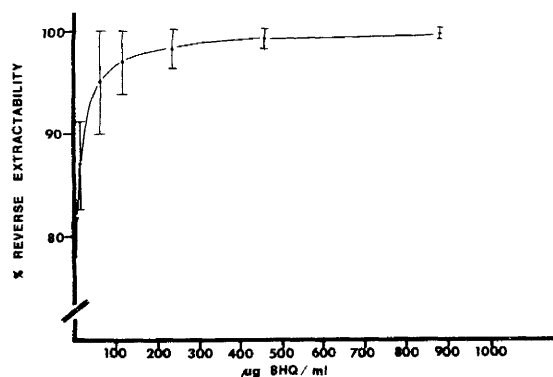
‡ UIBC approximately 40  $\mu$ g/100 ml serum.



**FIG. 3.** Effect of oxine concentration on labeling of human platelets with In-111 oxine. Aliquots of  $5 \times 10^8$  platelets from human donors were suspended in either 2 ml of isotonic saline or in 2 ml of autologous platelet-poor plasma (PPP) containing ACD anticoagulant. This mixture was incubated at room temperature for 30 min with In-111 oxine preparations containing various amounts of oxine.

The labeling efficiency was reduced to 64% in plasma-ACD, and to approximately the same level in the saline-ACD medium. Thus, the citrate ions in the ACD anticoagulant have a significant lowering effect on the In-111 uptake by platelets.

**Effect of oxine concentration.** The effect of oxine concentration on the labeling efficiency was studied using platelets (from normal donors) suspended in either saline or in PPP. Six different preparations of In-111 oxine containing increasing spectrophotometrically determined amounts of the oxine were used. The volume of the In-111-complex added to



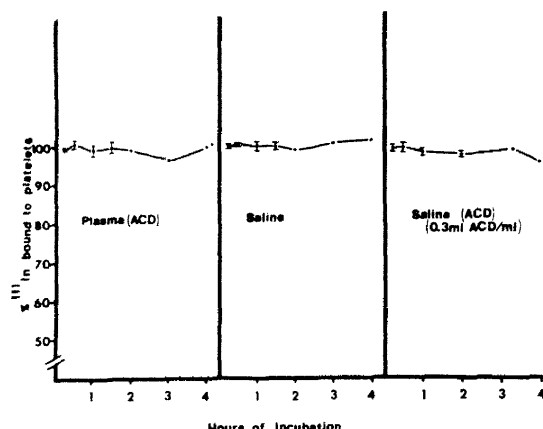
**FIG. 4.** Effect of oxine concentration on reverse extractability of In-111. The term % reverse extractability refers to extraction of In-111-oxine complex from saline back into methylene chloride. This term also reflects amount of In-111 bound in In-111-oxine complex.

the suspended platelets was kept constant. In saline as well as in plasma, the labeling efficiency increased with increasing oxine concentration, up to a maximum at  $6.25 \mu\text{g/ml}$  (Fig. 3). With further addition of oxine, the labeling efficiency in plasma declined rapidly, while that in saline decreased less dramatically.

The increase in In-111 uptake with increasing oxine concentration up to a maximum at  $6.25 \mu\text{g 8-HQ/ml}$  suspension was due most likely to a progressive decrease in dissociation of the In-111-oxine complex. This was shown by determination of the degree of chelation in the In-111 oxine solutions by reverse extraction of the complex from saline into methylene chloride. In Fig. 4 the percent reverse extractability—i.e., the percentage of In-111 extracted into the organic phase representing the chelated In-111 (as opposed to the “free” In-111 in the saline phase)—was plotted against the oxine concentration in the In-111-oxine preparations. There was a considerable amount of nonchelated In-111 in complex preparations at oxine concentrations below  $125 \mu\text{g/ml}$ . At an oxine concentration of  $125 \mu\text{g/ml}$ , however, the extractability, i.e., the amount of chelated In-111, was  $97 \pm 3\%$ . If 0.1 ml of this preparation is added to 2 ml of platelet suspension, diluting the oxine to  $6.25 \mu\text{g/ml}$ , the platelets will be optimally labeled. With further increase in the oxine concentration, the reverse extractability of the In-111-oxine complex remained close to 100%—indicating, as one would expect, that all the In-111 remains bound to the oxine.

As shown in Fig. 3, there was a marked fall-off in the labeling efficiency beyond the optimum oxine concentration. In order to explain this phenomenon, the first question to be answered was whether or not the platelets were damaged by the oxine in concentrations higher than  $6.25 \mu\text{g/ml}$ . No such damage could be detected in either *in vitro* or *in vivo* tests. There was no inhibition of clot retraction even at the highest oxine concentration ( $50 \mu\text{g/ml}$ ). When rabbit platelets were incubated with In-111 oxine at an oxine concentration as high as  $12.5 \mu\text{g}$  per ml of platelet suspension—instead of the 4–8  $\mu\text{g}$  used in the earlier study (3)—the initial recovery and mean survival times were identical to those observed for In-111 platelets in the earlier study (Hill and McIntyre, unpublished observations).

We next investigated whether the decline in labeling efficiency with increasing oxine concentration was due to elution. Figure 5 shows the results obtained by incubating previously labeled and washed platelets for up to 4 hr at  $37^\circ\text{C}$ . The platelets were suspended in three different media: plasma, saline, or saline-ACD. No significant elution occurred in any of the suspending media. In contrast



**FIG. 5.** Elution of In-111 from radiolabeled platelets. In-111-labeled and washed human platelets were resuspended in either plasma-ACD, in saline, or in saline-ACD, and were incubated at 37°C. Aliquots of platelet suspensions were removed at various times and percentage of platelet-bound activity was determined.

to this finding, more than 80% of the previously bound indium was released within 30 min when  $3.45 \times 10^{-7}$  moles/ml (or 50  $\mu\text{g/ml}$ ) of oxine was added to the suspending plasma (Table 3). This finding suggests that an equilibrium exists for indium between the binding sites in the platelet and on the oxine in the medium. In another set of tubes, 8-hydroxy-5-quinoline-sulfonic acid was added to the labeled platelets resuspended in 2 ml PPP in identical molar ratio. The 8-HQ and this sulfonic acid analog bind indium similarly (20), but because of the negative charge of the analog it is unable to penetrate the cell membrane (21). The elution of indium with this analog in the incubation medium was not significantly different from controls to

which neither 8-HQ nor the sulfonic acid analog of 8-HQ had been added.

In order to test whether addition of stable indium to the suspending PPP will lead to release of the radioactive indium from the platelets,  $3.45 \times 10^{-7}$  moles  $\text{InCl}_3/\text{ml}$ —corresponding to approximately ten times the amount of indium ions that can be bound by transferrin (11)—were introduced into the PPP. There was no significant release of In-111 into the PPP medium.

## DISCUSSION

Inherent in any in vitro handling of cellular elements of the blood is the possibility of damage to fragile cells. For platelets, the technical factors of blood drawing, anticoagulant, separation from whole blood, and manipulations during labeling have been studied in detail for many years. The influence of the types of plastic blood containers and of centrifugation times and speeds on the viability of platelets was investigated by Slichter and Harker (23,24). Optimum methods for in vitro preparation of Cr-51-labeled platelets for survival studies have recently been recommended by the Panel on Diagnostic Applications of Radioisotopes in Haematology (14). One prerequisite in these methods is that platelets must be kept in autologous plasma during the entire procedure of harvesting, labeling, and reinfusion.

Because of the known high affinity of indium towards plasma transferrin (11), Thakur et al. (1) proposed the labeling of platelets with In-111 oxine in isotonic saline rather than in a plasma medium. Modifications of the "saline method" have been described lately. They involve either addition of ACD (10), or separation of platelets with a "buttonless saline method" that eliminates platelet-button formation and platelet resuspension after labeling (9). Platelets suspended in saline can be labeled with In-111 with greater than 90% efficiency. As shown in our study, the labeling efficiency is reduced significantly in the presence of ACD-plasma but the loss can be minimized to about 50% if optimum labeling conditions are chosen. This decrease in labeling efficiency can certainly be justified as a trade-off for a more physiologic labeling situation. Our study shows that high labeling efficiency in plasma can be achieved only when platelet and oxine concentrations, as well as the anticoagulant concentration, are carefully balanced. Thakur et al. (25) reported on a low labeling yield (4%) for leukocytes in plasma. Optimum labeling conditions might not have been present in their studies.

Contrary to what we expected, our findings in-

**TABLE 3. PERCENTAGE INDIUM-111 ELUTED FROM LABELED PLATELETS**

Expt.	Control	Addition of 8-HQ <sup>†</sup>	Addition of $\text{SO}_3\text{-8-HQ}^\ddagger$	Addition of $\text{InCl}_3^\S$
I	6.9	82.8	9.5	6.8
II	6.4	82.0	7.0	4.0

<sup>†</sup>  $5 \times 10^{-9}$  In-111-labeled platelets resuspended in 2 ml plasma, incubated for 30 minutes at room temperature with 100  $\mu\text{l}$  ethanol-saline (1:4) added.

<sup>‡</sup>  $6.9 \times 10^{-7}$  moles in 100  $\mu\text{l}$  ethanol-saline (1:4) added to plasma and incubated as in control experiments.  $\text{SO}_3\text{-8-HQ}$  = 8-hydroxy-5-quinoline-sulfonic acid.

<sup>§</sup>  $6.9 \times 10^{-7}$  moles in 100  $\mu\text{l}$  0.05 N HCl added to plasma and incubated as in control experiments.

dicating that, in the absence of citrate ions, transferrin did not significantly affect the labeling of platelets with In-111 oxine. On the other hand, citrate ions in approximately 27-millimolar concentration (as compared with  $2.2 \times 10^{-5} M$  transferrin and indium at around  $2.5 \times 10^{-9} M$ ) strongly interfered with the uptake of In-111 by human platelets. Indium and ferric ions are reportedly very similar in their affinity for transferrin (11). Cavill (26) pointed out that during incubation of normal serum with [ $^{59}\text{Fe}$ ] ferric citrate solutions at high citrate-to-Fe ratio (250:1), significant amounts of the Fe-59 citrate remained unbound for several hours. The kinetics and mechanisms of Fe(III) exchange between citrate and transferrin were studied in detail by Bates et al. (27), who demonstrated that the initial rate of transfer of iron from iron citrate to transferrin decreases with increase of citrate-to-Fe ratio above 20:1. The lack of binding of In-111 to transferrin might therefore be explained by the extremely high ratio of citrate-to-indium in our system ( $10^7$ :1). In addition, the previous study by Thakur et al. (1), showing the formation of In-111-transferrin complex in plasma or in the presence of transferrin, was carried out in the absence of platelets. Our study was performed in the presence of a high concentration of platelets. The affinity of In-111 oxine for platelets has never been investigated previously.

We have shown that the binding of In-111 to platelets is firm but not irreversible. A maximum of 10% of the In-111 elutes from the platelets with incubation at 37°C. This finding compares favorably with the elution under similar conditions of 20–25% of Cr-51 from Cr-51-labeled platelets as reported by Adam et al. (28). However, the presence of oxine outside the In-111-labeled cell leads to elution of the label. This elution takes place only with oxine in the medium but not with the sulfonic acid analog of oxine, which cannot penetrate the membrane.

During the labeling of platelets with In-111 oxine in plasma, an equilibrium is attained between the In-111 bound to the platelet and the In-111 in the oxine complex. The time when this equilibrium is reached depends on the temperature of incubation. At 37°C, 30 min are required for maximum labeling, whereas at 23°C the same degree of labeling can be obtained only if the incubation time is extended to 90–120 min. The low labeling at 5°C is possibly due to solidification of the phospholipid membrane at that temperature.

When human platelets are to be labeled with In-111 oxine in autologous plasma, a balance has to be struck between the guidelines for least-damaging handling of these fragile cells, as established for Cr-51 labeling, and the optimal conditions for labeling

with In-111 as determined in this study. Accordingly, we recommend the following protocol:

1. The platelets should be kept in autologous plasma during the entire procedure of harvesting, labeling, and reinfusion.

2. ACD is the anticoagulant of choice and is also used for acidification of the suspending plasma to pH 6.5 to prevent clumping of platelets.

3. The platelets are concentrated in the ACD-plasma to approximately  $5 \times 10^9$  cells/ml.

4. The platelets are incubated with In-111 oxine for 90 min at room temperature, at an oxine concentration of approximately 6  $\mu\text{g}/\text{ml}$  of suspension. The volume of In-111 oxine added should be less than 50  $\mu\text{l}/\text{ml}$  of suspension.

When all these factors are controlled, human platelets from normal donors can be labeled with In-111 with a labeling efficiency of  $(55.5 \pm 9.3)\%$  ( $\pm 1$  s.d.,  $n = 6$ ). The total time required for platelet separation and labeling will range between 2½ to 3½ hr. Once platelets are labeled with In-111, the label is stable at 37°C incubation for at least 4 hr.

#### APPENDIX A. Platelet Separation from 50 ml Whole Blood, and Labeling Procedure: "OPEN SYSTEM"

1. Draw blood through 18-gauge needle into 50-ml plastic syringe containing 7.5 ml ACD NIH-A solution. Transfer blood into two presterilized 50-ml polycarbonate (PC) tubes (IEC #1650); cap tubes.
2. Spin blood for 15 min at 220 g.
3. Using a 5-ml sterile plastic pipet, transfer supernatant platelet-rich plasma (PRP) into another 50-ml PC tube. Note how many milliliters of PRP are transferred.
4. Add additional ACD to PRP in q.s. to achieve pH = 6.5–6.7. Volume of ACD required is  $\approx 1/20$  of PRP volume.
5. Do platelet count.
6. To sediment the platelets into a button, spin PRP for 15 min at 1000 g.
7. Using a sterile pipet, transfer all platelet-poor plasma (PPP) into another 50-ml PC tube. Leave platelet button undisturbed.
8. Immediately add exactly 2 ml PPP to platelet button and resuspend platelets by gentle repeated suction (do not bubble!) into 5-ml plastic pipet. Wait until platelets are completely unclumped. Patience!
9. Add desired radioactivity of In-111 in 50–100  $\mu\text{l}$  of In-111 oxine solution (alcohol:saline = 1:4). For human platelets, optimum amount of oxine is 12.5  $\mu\text{g}$  8-HQ per 2-ml platelet suspension.
10. Incubate at room temperature for 90 min.
11. Add 5 ml of PPP, mix, and centrifuge 15 min at 1000 g.
12. Transfer radioactive supernatant into another tube.
13. Gently layer platelet button with approximately 1 ml of PPP. Remove PPP and add to first radioactive PPP fraction.
14. Resuspend platelets in PPP as described in Step 8.
15. If there should be considerable contamination with RBCs, they can be removed by a 5-min centrifugation at 150 g.

## APPENDIX B.

"CLOSED SYSTEM" Preparation of In-111-Labeled Platelets  
(This method is an adaptation of the "Recommended Methods for  
Radioisotope Platelet Survival Studies." *Blood* 50: 1137, 1977)

1. Collect 500–600 ml blood into a Fenwal 4R3121 Plasmapheresis Triple Blood Pack using one bag with 75 ml ACD (NIH-A solution) and two empty satellite bags. All subsequent steps are carried out at 20–22°C.
2. Obtain PRP by centrifugation of the blood bag for 3 min at 1400 g (calculated at the bottom of the cup).
3. Extract the PRP from the primary pack into one of the satellite bags. Estimate the volume of PRP by weight (assume wt. = vol.).
4. Through an entrance port inserted into the PRP bag, add ACD (NIH-A solution) in an amount equal to  $1/20$  of the PRP volume.
5. Centrifuge PRP bag (together with the bag containing RBCs and empty bag in one centrifuge cup) for 10 min at 1400 g to form a platelet button on the bottom of the bag.
6. Extract platelet-poor plasma (PPP) into second satellite bag, leaving approximately 20–25 ml PPP with the platelets. Resuspend platelets by gently inverting the bag several times.
7. Estimate volume of platelet suspension by weight.
8. Calculate the amount of oxine necessary for maximum labeling  $[X(\mu\text{g}) = 6.25 \times \text{estimated vol. in ml}]$ . Adjust the oxine concentration accordingly in the vial containing 1 mCi of In-111-oxine, using a solution of nonradioactive oxine (1 mg/ml ethanol). Draw radioactivity into a syringe containing 0.5 ml saline. Through the entrance port, add radioactivity to platelet suspension. Rinse vial, syringe, and port three times with 0.5 ml saline.
9. Incubate for 90 min.
10. Add all but approximately 130 ml PPP from second satellite bag to platelets. Centrifuge platelet suspension (together in one centrifuge cup with second satellite bag and the RBC-bag) for 15 min at 1400 g.
11. Extract radioactive supernatant into RBC bag as completely as possible, without disturbing the packed platelets.
12. Layer platelets with approximately 30 ml PPP (without disturbing platelets), and again remove as much as possible of radioactive PPP by transfer to RBC bag.
13. Resuspend platelets in remaining PPP by gentle inversion of bag. Wait until no clumps are visible. Separate platelet bag from the RBC and the empty bag.
14. Determine the amount of radioactivity associated with the platelets and compare to a standard with known amount of In-111. Usually a dose of less than 200  $\mu\text{Ci}$  of In-111 platelets is given per study. If necessary, use a syringe to remove excess In-111 platelets through entrance port.
15. Infuse labeled platelets through an infusion set without filter.
16. Determine amount of radioactivity remaining in empty bag.

## FOOTNOTES

\* In each instance, written informed consent was obtained on an approved form that meets current National Institutes of Health guidelines.

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‡ Mallinckrodt, St. Louis, MO 63160.

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### 4th ANNUAL WESTERN REGIONAL MEETING THE SOCIETY OF NUCLEAR MEDICINE

**October 18-21, 1979    Monterey Conference Center and Doubletree Inn    Monterey, California**

#### ANNOUNCEMENT AND CALL FOR ABSTRACTS FOR SCIENTIFIC PROGRAM

The Scientific Program Committee welcomes the submission of abstracts of original contributions in nuclear medicine from members and nonmembers of the Society of Nuclear Medicine for the 4th Annual Western Regional Meeting. Physicians, scientists, and technologists—members and nonmembers—are invited to participate. The program will be structured to permit the presentation of papers from all areas of interest in the specialty of nuclear medicine. Abstracts submitted by technologists are encouraged and will be presented at the scientific program. Abstracts for the scientific program will be printed in the program booklet and will be available to all registrants at the meeting.

#### GUIDELINES FOR SUBMITTING ABSTRACTS

The abstracts will be printed from camera-ready copy provided by the authors, therefore, only abstracts prepared on the official abstract form will be considered. These abstract forms will be available from the Western Regional Chapter office (listed below) after March 1, 1978. Abstract forms will be sent to members of the Northern California, Pacific Northwest, Southern California and Hawaii Chapters in a regular mailing in early May, 1979. All other requests will be sent on an individual basis.

All participants will be required to register and pay the appropriate fee.

Please send the original abstract form, supporting data, and six copies to:

**JUSTINE PARKER, ADMINISTRATIVE COORDINATOR**  
**4th Western Regional Meeting**  
**P.O. Box 40279**  
**San Francisco, CA 94140**

**DEADLINE FOR ABSTRACT SUBMISSION: POSTMARK MIDNIGHT, JULY 6, 1979**

THE 4th ANNUAL WESTERN REGIONAL MEETING WILL HAVE COMMERCIAL EXHIBITS AND ALL INTERESTED COMPANIES ARE INVITED. Please contact the Western Regional SNM office (address above). Phone: (415) 647-1668 or 647-0722.