RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Human Platelets Labeled with In-111 8-Hydroxyquinoline: Kinetics, Distribution, and Estimates of Radiation Dose

Ursula Scheffel, Min-Fu Tsan, Thomas G. Mitchell, Edwaldo E. Camargo, Hayden Braine, Michael D. Ezekowitz, Edward L. Nickoloff, Robin Hill-Zobel, Edmond Murphy, and Patricia A. McIntyre

The Johns Hopkins Medical Institutions, Baltimore, Maryland

Platelets from nine normal male subjects were labeled with In-111 8-hydroxyquinoline (In-111 oxine) in the presence of plasma in either "closed" blood transfer packs or in "open" test tubes. The mean labeling efficiencies in these two systems were 27 and 53%, respectively.

Mean survival time of In-111-labeled autologous platelets was 8.76 days, with a standard deviation of 1.05 according to the maximum-likelihood estimate of the gamma-function model. The initial recovery of In-111 platelets in the circulation was 57% with a standard deviation of 11%. The distribution of In-111 platelets in liver and spleen was quantitated by anterior, posterior, and transmission gamma-camera imaging. During the first 30 min, 38% of the injected dose accumulated in the spleen, 13% in the liver. No significant increase in In-111 radioactivity was observed in either of the two organs over a 3–9-day period. The bone marrow was an additional site of In-111 accumulation.

The spleen was the critical organ with respect to radiation dose. The splenic dose was estimated to be 34 rad/mCi In-111 platelets, that of the liver 2.1 rad/mCi.

With the injection of 100–150 μCi of In-111-labeled platelets in normal subjects, giving a splenic radiation of 5 rad, a complete 10-day survival study can be performed and uptake of In-111 in different organs can be measured quantitatively for at least 3–4 days.

J Nucl Med 23: 149–156, 1982

Since the introduction of In-111 8-hydroxyquinoline (In-111 oxine) as a cell label in 1976 (*1*), leukocytes and platelets have been successfully labeled with this agent. In several laboratories In-111 oxine has now replaced the conventionally used sodium chromate(Cr-51) as the platelet label of choice. Indium-111 offers two major advantages: (a) the labeling efficiency of In-111 oxine for platelets is markedly higher than that of Cr-51 and (b) In-111 emits gamma photons with a 184% yield, whereas the photon yield for Cr-51 is only about 10%.

Received July 16, 1981; revision accepted Oct. 5, 1981.

For reprints contact: Ursula Scheffel, 615 North Wolfe St., Baltimore, MD 21205.

The In-111 label has been shown to be stable both in vitro and in vivo (*2,3*). The survival of In-111-labeled platelets was found to agree with that of Cr-51-labeled platelets in animal as well as in human studies (*3,4*). The temporal and spatial distribution of In-111-labeled platelets in normal humans has been studied by Heyns and co-workers (*5*) by determining relative changes in organ radioactivity compared with whole-body radioactivity.

We report here the life span of platelets and their in vivo distribution in nine healthy male volunteers using In-111-labeled autologous platelets. The platelet survival curves were analyzed using three different mathematical models. Quantitative measurements of In-111 radioac-

tivity in liver and spleen were made by anterior, posterior, and transmission gamma-camera imaging. From the data obtained, the radiation doses to single organs and to the whole body were estimated.

METHODS

Preparation of In-111 oxine. For the first five preparations, In-111 oxine was supplied commercially.* For the subsequent four studies In-111 oxine was prepared as previously described (*3*). Briefly, the In-111 oxine complex was formed, then extracted with methylene chloride. In a 5-ml V vial fitted with an adapter, the methylene chloride was evaporated in a stream of sterile nitrogen in a 70 °C water bath. The dry complex was dissolved in 25 μl of ethanol containing approximately 16 μg oxine, followed by addition of 75 μl of isotonic saline. For labeling, 75 μl of the In-111 oxine solution were added to 2 ml of platelet suspension.

Labeling of platelets with In-111 oxine in autologous plasma. Human platelets were labeled with In-111 oxine in the presence of autologous plasma as previously described (*6*). In the first four studies, 500 ml of blood was collected in Fenwal plasmapheresis triple blood packs (closed method). Platelet-rich plasma (PRP) was obtained by 3-min centrifugation at 1400 *g*, and its pH adjusted to 6.5–6.7 with acid-citrate-dextrose (ACD) (NIH-A). The platelets were concentrated into a button by centrifugation at 1400 *g*, the platelet-poor plasma (PPP) was then extracted and the platelets resuspended in 20 to 25 ml of PPP. The yield of platelets by this method was 51% ± 17.2 (s.d.). Approximately 1 mCi of In-111 oxine was then added. The oxine concentration in the labeling suspension was adjusted to 6.25 μg oxine/ml, the concentration previously demonstrated to give optimal labeling efficiency (*6*). After a 90-min incubation, about 100 ml of PPP was added, followed by centrifugation for 15 min at 1400 *g*. After removal of the radioactive supernatant, the platelet button was gently "layered" with an additional 30 ml of PPP, which was then also removed. The labeled platelets were resuspended in the remaining PPP, and, except for an aliquot used to prepare a standard, they were reinfused into the subject.

In the remaining five studies, platelets were harvested and labeled in an aseptic "open" test-tube system, using only 50–100 ml of ACD-blood (*6*). All manipulations were carried out in a laminar-flow hood. PRP was obtained by centrifuging the blood for 10 min at 220 *g*. The yield of platelets by this method was 59.6% ± 18.8 (s.d.). The platelets were sedimented into a button by a second 10-min spin (1000 *g*), then resuspended in 2 ml of PPP and incubated for 50–90 min with 0.6–1.0 mCi In-111 oxine containing ~12.5 μg oxine. Unbound In-111 radioactivity was removed by washing the platelets once with ~10 ml PPP. The labeled platelets were then resuspended in the remaining PPP. Contaminating red blood cells (RBCs) were removed by a final slow spin (5 min at 150 *g*). The supernatant platelet suspension was withdrawn into a syringe; the volume of In-111 platelets infused was determined by weighing the syringe before and after injection.

Survival studies. Nine healthy male volunteers ranging from 20 to 39 yr were studied. All subjects were nonsmokers and did not take any medication (including aspirin) for at least 2 wk before and during the study. Written informed consent was obtained from each individual and the study was approved by the Joint Committee on Clinical Investigation of the Johns Hopkins Medical Institutions. Into the first four volunteers, 240–680 μCi of In-111 platelets were infused. After initial radiation dose calculations, the injected dose was lowered to 77–153 μCi in the succeeding five studies.

Venous blood was obtained at 20 and 90 min after infusion and daily thereafter for 10 days. The hematocrit and platelet count were determined in each sample. Duplicate samples of 5 ml whole blood lysed with saponin, and 2 ml platelet-free plasma (PFP) diluted with 3 ml water, were prepared for measurement of radioactivity. In three of our nine studies (Nos. 2–4) whole-blood radioactivity in each of the blood samples was compared with that of platelets separated from all other cellular elements. For this purpose platelets were harvested from duplicate 3-ml whole-blood samples by diluting the blood with 3 ml normal saline and centrifuging it in plastic test tubes for 6 min at 200 *g*. The supernatant PRP was collected and the remaining cells resuspended in 3 ml saline and centrifuged again (6 min at 200 *g*). The supernatant from this spin was pooled with that from the first. An aliquot of the combined PRP was centrifuged to obtain PFP. The efficiency of platelet harvest in each of the samples was estimated according to the "Recommended methods for radioisotope platelet survival studies" (*7*). Samples of PRP and PFP were prepared for counting. Standards were made by adding 20 μl of the injectate to 5 ml of water. All samples were counted with a less than 3% statistical error in an automated scintillation detector system. The results were expressed as percent of the injected dose in circulating platelets (*3*), assuming a blood volume of 70 ml/kg (*8*). Urinary excretion was measured for 3 days after injection. Samples of 5 ml urine were counted in the same manner as the blood samples.

To estimate mean survival time, the experimental data were subjected to computer analysis. Three mathematical models were used for formal curve fitting: a linear model, an exponential model, and a maximum-likelihood estimate of the integer-ordered gamma function. In addition, a weighted mean of linear and exponential estimates was obtained. These standard methods of curve analysis have been described in detail with appropriate references elsewhere (*3,7*). For each estimate of the

survival time, the residual mean square (RMS) was evaluated as a measure of the precision of the curve fitting. The RMS represents the sum of squares of the deviation of the data values from the fitted line divided by the number of data points minus the number of parameters estimated (three for the gamma model, two for the others). The gamma model has the general form

$$f(t,n) = \frac{a^n}{(n-1)!} t^{n-1} e^{-at} \quad t \geq 0,$$

where t = time from injection, n = number of environmental insults ("hits") to destruction of the platelet, and a = the reciprocal of the mean waiting time between "hits." The mean platelet survival is n/a. The values for n and a are found by iteration on a computer.† The gamma-function model was constructed on the basis of the known platelet physiology and factors influencing platelet survival (*9*). It has been extensively tested (*10*) and is accepted as the standard method of analysis by the International Committee on Standardization in Hematology (*7*). Note that both the exponential and linear models are particular cases of the gamma model. If n equals one, the gamma equation becomes a single exponential; if n becomes infinitely large, this function gives a point distribution, i.e., the linear model.

In vivo quantification of In-111 organ radioactivity. The absolute amount of In-111 radioactivity (A) in liver and spleen at various times after injection was measured with a large-field-of-view gamma camera coupled to a data processing system. A 280-keV medium-sensitivity parallel-hole collimator was used. The camera was peaked for 247 keV with a 20% window. The detector sensitivity in counts/μCi-min (S) was determined with a calibrated standard source of In-111. The standard was prepared by placing a known amount of $^{111}InCl_3$ (150–650 μCi) in a plastic tissue-culture bottle containing an acidified EDTA solution. A 200-K count image of this standard bottle was obtained and stored on magnetic tape. Before injection of the In-111-labeled platelets, the overall effective transmission coefficient (Tr) was determined. For this purpose a 38-cm diameter flood phantom filled with 1 mCi of In-111 was used. A 500-K count image of the flood phantom was made. An additional 500-K count transmission image of the patient's lower chest and abdomen was obtained with the patient supine over the flood source. Liver and spleen areas were outlined with a light pen and the ratio of counts transmitted through the region of interest to unfiltered In-111 counts in the same area was determined.

At various times after infusion of In-111-labeled platelets, the organs of interest were imaged from two diametrically opposed views. The count rates in the regions of interest for both anterior and posterior views (C_A and C_P, respectively) were obtained by using the data processor. The following equation was used to compute the observed activity in the organ of interest:

$$A = \frac{1}{S}\sqrt{\frac{(C_A)\cdot(C_P)}{Tr}}.$$

This in vivo quantification method had been validated in both phantom and animal studies (*11*,*12*).

Radiation dose estimations. The radiation dose was estimated by the "absorbed fraction method" (*13*) and the quantitative data for liver and spleen uptake of In-111 radioactivity obtained in the present study. No human data were available for the calculation of the radiation dose to the red marrow. In normal rabbits 14% of the injected dose was accumulated in the bone marrow at 6 days after injection, at a time when the blood activity in these animals had fallen to less than 3% of the dose (unpublished result, RL Hill-Zobel, et al.). Using these data, a linear uptake of 14% of the injected dose was estimated for the calculation of the radiation dose to the

TABLE 1. LABELING OF HUMAN PLATELETS IN AUTOLOGOUS PLASMA

	Method	No. of platelets per ml suspension	ml of suspension	Incubation time (min)	% labeling	% labeling mean	% labeling s.d.
(1)		2.1×10^9	27.0	90	23.4		
(2)	Closed method	2.4×10^9	31.5	90	35.9		
(3)	In-111 oxine*	3.2×10^9	36.0	90	36.5	27.3	11.1
(4)		1.1×10^9	46.0	90	13.2		
(5)	Open method In-111 oxine*	3.6×10^9	3.5	90	14.0		
(6)		6.0×10^9	2.0	60	39.6		
(7)	Open method	5.3×10^9	2.0	50	59.5		
(8)	In-111 oxine†	6.6×10^9	2.5	60	49.7	53.2	10.8
(9)		4.2×10^9	2.0	60	63.9		

* Commercial preparation.
† Prepared in our laboratory.

TABLE 2. PERCENTAGE RECOVERY AND SURVIVAL (DAYS) OF In-111-LABELED PLATELETS IN NORMAL VOLUNTEERS

	% Recovery*	Mathematical Model Used for Curve Fitting: Linear Days	Linear RMS†	Exponential Days	Exponential RMS†	Gamma Days	Gamma RMS†	n‡	Weighted mean Days
1	46.4	11.59	0.01202	4.30	0.03686	9.92	0.00343	12	9.80
2	61.8	10.10	0.01487	3.58	0.08176	8.76	0.00641	11	9.09
3	60.1	9.93	0.01393	3.17	0.14090	8.69	0.00300	19	9.32
4	54.0	10.67	0.00792	4.14	0.05014	9.52	0.00496	10	9.78
5	40.8	9.93	0.00176	4.01	0.02276	9.43	0.00113	21	9.51
6	80.0	8.98	0.03936	2.88	0.13772	6.35	0.00753	4	7.63
7	57.5	9.69	0.00680	3.67	0.06630	8.87	0.00293	16	9.11
8	59.7	9.38	0.00817	2.80	0.25807	8.96	0.00600	50	9.18
9	53.0	9.22	0.00893	2.92	0.12788	8.17	0.00479	30	8.81

* Estimated by assuming a blood volume of 70 ml/kg body weight (8) and extrapolation of the gamma-function fitted survival curve to time zero.

† RMS = residual mean square.

‡ n = estimated number of hits for the gamma-model curve.

red marrow in man. The radiation doses to gonads and kidneys were estimated taking only the contribution of liver, spleen, and normal blood supply into consideration.

RESULTS

Labeling of human platelets with In-111 oxine in autologous plasma. Platelets from nine volunteers were labeled with In-111 oxine in the presence of autologous plasma. Table 1 presents the efficiencies of labeling, along with the various labeling conditions with regard to platelet concentration and time of incubation. The labeling efficiency markedly improved when platelets were labeled in the "open" test-tube system using "homemade" In-111 oxine (mean percent labeling = 53.2 vs 27.3 with the "closed" method). Since the number of studies with either the "open" or "closed" system was small, and two different sources of In-111 oxine were used, a rigorous comparison of the two methods cannot be made. However, the higher percent labeling in the "open" system was likely because in the test tubes the platelets were more concentrated ($4.4–6.6 \times 10^9$ cells/ml suspension) than in the "closed" blood packs, in which the platelet concentration at labeling ranged between 1.1 and 3.2×10^9 platelets/ml. Differences in the radiochemical purity of the two In-111 oxine sources might also contribute to this observation. In one experiment (No. 5), in which a relatively high number of platelets (3.6×10^9/ml suspension) was incubated with a commercial In-111 oxine preparation, the labeling efficiency was only 14%. The reason for this low labeling yield is not clear.

Initial recovery and survival of In-111-labeled human platelets. Table 2 lists the percentage initial recovery of In-111 platelets and their survivals as estimated by three mathematical models—i.e., the linear, the exponential, and the integer-ordered gamma function model—as well as by the weighted mean of linear and exponential estimation. The mean percent recovery of all nine studies was 57% ± 11 (s.d.) There was no significant difference ($p > 0.5$) in the initial recovery between platelets labeled with In-111 in the "closed" system (studies 1–4) and those labeled in the "open" test tube system (studies 5–9).

With the linear model for estimation, the survival ranged between 9.0 and 11.6 days; with the gamma model, 6.4 to 9.9 days. Very short survival times (2.8–4.3 days) were obtained when the curves were fitted exponentially. The residual mean square value (RMS) is a measure of the precision of the curve fitting. In all nine studies the RMS for the gamma-function model was significantly lower than those of the other two models. Thus, the gamma function fitted our experimental data most accurately.

We also compared two types of blood-sample preparation for the determination of in vivo recovery and platelet survival. In three subjects the In-111 radioactivity of each of the blood samples was measured in platelets separated from all other cellular elements by differential centrifugation, and in whole blood from which the contributing plasma activity was subtracted. Since the platelets in these three studies were harvested and labeled in the closed system, there were some red blood cells contaminating the injectate, ranging between 1 RBC/3000 platelets to 1 RBC/37 platelets. As shown in Table 3, no significant difference in percent recovery or platelet life span was observed between the two methods of sample preparation.

TABLE 3. COMPARISON OF TWO METHODS OF BLOOD-SAMPLE PREPARATION FOR DETERMINATION OF PLATELET SURVIVAL AND INITIAL RECOVERY WITH In-111-LABELED PLATELETS

Subjects	Method of sample preparation	Survival time (days)			% recovery	RBC contamination in injectate. Ratio No. of platelets/RBCs
		Linear	Exponential	Gamma		
2	WB*	10.10	3.58	8.76	61.8	37/1
	SEP†	10.06	3.32	9.25	63.8	
3	WB*	9.93	3.17	8.69	60.1	49/1
	SEP†	9.56	3.32	8.83	61.1	
4	WB*	10.67	4.14	9.52	54.0	3000/1
	SEP†	10.41	3.87	9.65	54.6	

* WB: In-111 radioactivity was determined in whole blood and in platelet-free plasma (PFP). The percent In-111 dose in circulating platelets was calculated by subtracting plasma activity from whole-blood activity.

† SEP: Platelets were separated from whole blood by differential centrifugation, and the efficiency of harvest determined. Indium-111 was measured in PRP and PFP, and PFP activity subtracted from PRP.

In vivo quantification of In-111 organ radioactivity in normal subjects. The In-111 radioactivities in spleen and liver were quantitatively assessed at various times after injection with a computerized gamma-camera system. The results of serial in vivo measurements in eight subjects are given in Table 4. The initial (10–30 min) uptake of In-111 platelets in the spleen ranged from 29 to 52%; that in the liver between 7 and 20%. During the first 24 hr, an average of 37.5% of the In-111 activity was accumulated in the spleen and 13.1% in the liver. In six subjects in which liver and spleen uptake was determined at 3–4 days after injection, there was no significant increase ($p > 0.2$) in either of the two organs over that measured at 30 min or 24 hr. In one volunteer the In-111 distribution was followed for 9 days after infusion. Again, no change in hepatic or splenic uptake of In-111 was observed. Little or no In-111 radioactivity was seen in other organs except in the bone marrow. Especially marked In-111 uptake was observed in the pelvic bones.

Radiation dose. The radiation dose from the injection of 1 mCi of In-111-labeled platelets to selected organs and to whole body was estimated. From the in vivo quantitation, the mean uptakes by liver and spleen at 24 hr were 13.1 and 37.5% of the injected dose, respectively. The rapid uptakes (13% and 38%) in the liver and spleen, respectively, at 10–30 min after injection, and the apparent constancy of percent activity in these organs over the whole 3–9 day observation period, led us to conclude that there was essentially no biologic elimination from

TABLE 4. IN VIVO QUANTIFICATION OF In-111 ORGAN RADIOACTIVITY IN NORMAL SUBJECTS

Patient No. / Time after injection	1	3	4	5	6	7	8	9
	% Dose in spleen							
10–30 min	28.9	33.2	35.6	26.5	30.7	51.9	49.5	48.4
90 min	33.4	35.2	36.1	24.5	30.6	47.1	46.9	40.9
24 hr	27.2	33.3	39.8	26.3	35.7	50.5	42.6	45.7
3 days					43.8	51.3	48.2	53.5
4 days	34.3		41.5					
9 days	32.6							
	% Dose in liver							
10–30 min	20.3	13.5	13.9	16.8	12.1	9.0	10.3	7.2
90 min	17.3	12.8	11.8	20.5	12.7	8.0	10.2	6.9
24 hr	14.1	13.3	14.0	17.7	15.6	9.0	10.1	9.3
3 days					16.6	11.3	15.5	8.0
4 days	16.9		17.0					
9 days	18.8							

TABLE 5. ESTIMATED RADIATION DOSE TO NORMAL SUBJECTS FROM INTRAVENOUS ADMINISTRATION OF In-111 BOUND TO AUTOLOGOUS PLATELETS

	rad/mCi
Total body	0.6
Liver	2.0
Spleen	33.5
Kidneys	1.4
Testes	0.2
Ovaries	0.4
Red marrow*	1.0

* Based on measurement of % dose/g red marrow in normal rabbits (n = 5).

these organs during these times. Therefore, the activity-time integral $\tilde{A}$ was calculated on the basis of the product of administered dose and percent uptake, with effective half-time being considered equal to physical half-life of In-111:

$$\tilde{A} = A_0 \times \% \text{ uptake} \times 67.4 \times \frac{1}{0.693} \quad \mu\text{Ci-hr}$$

Excretion of In-111 was slight: only 1% of the dose was eliminated in the urine during the first 3 days after injection. Fourteen percent of the dose was assumed to be concentrated in the bone marrow over 9 days after injection. The remainder of the radioactivity (35.4%) was assumed to be uniformly distributed throughout the body excluding liver, spleen, and bone marrow. Any decrease in activity was considered due to decay only.

The radiation dose estimates are presented in Table 5. The spleen receives by far the highest radiation dose (33.5 rad/mCi); the doses to liver and kidneys are 2.0 and 1.5 rad/mCi, respectively.

DISCUSSION

It is recommended that labeling of platelets with sodium chromate(Cr-51) be performed in closed blood packs (*7*) using 500 ml of ACD-blood. In our first four studies we have used this "closed" system for tagging human platelets with In-111 oxine. Because of the unwieldiness of the soft plastic bags it is difficult to achieve formation of a platelet button, efficient washing of this button, and adjustment of the platelet suspension to a fixed cell concentration. These difficulties can be avoided by labeling the platelets with In-111 in test tubes using an aseptic technique. The "open" system has two additional advantages: (a) only 50 ml of ACD-blood is needed from normal donors for efficient labeling, and (b) contaminating red blood cells can easily be removed by a final slow centrifugation. Working with the "open" system, however, requires a laminar-flow hood and extreme care in handling the preparation to avoid contamination.

As described previously (*6*), we routinely use autologous plasma as the suspending medium for the labeling of platelets with In-111. Under optimal conditions—i.e., when platelets are concentrated to $> 4 \times 10^9$ cells/ml and the oxine concentration is adjusted to approximately 6-6.5 μg/ml—our labeling efficiency ranges between 40% and 64%. Several methods have advocated labeling of platelets in plasma-free media in order to achieve higher labeling yields (*14-17*). Heyns et al. (*14*) demonstrated that ultrastructural changes of platelet activation do occur when platelets are incubated with In-111 oxine in saline for 30 min at room temperature. However, these changes are largely reversible upon resuspension of the platelets in autologous plasma.

In vivo elution of the In-111 label seems to be slight. Less than 5% of the injected dose was found in the total plasma volume at any time after injection, and excretion of the label in the urine was only 1% during the first 3 days after injection.

The mean survival time of autologous platelets in normal humans was similar to those reported by Heaton et al. (*4*) and Heyns et al. (*5*). For precise assessment of platelet survival time, mathematical analysis rather than visual fitting of the activity-disappearance curves has been shown necessary (*18*). The majority of investigators use a least-squares fit to a linear function. In comparing the accuracy of three different models for curve fitting, including the linear model, we found that the integer-ordered gamma function was superior, but the range of values obtained by gamma-function analysis was wider than with either linear or exponential curve fitting.

Buchholz et al. (*19*) reported a significantly longer survival time for Cr-51 platelets when the radioactivity was measured in the whole blood instead of in platelet buttons, the discrepancy being most likely due to Cr-51 labeling of contaminating RBCs. In our studies with In-111-labeled platelets, similar results were obtained regardless of the type of sample preparation, although RBC contamination was as high as 3%. We have previously shown that RBCs are efficiently labeled with In-111 oxine (*20*). The fact that contaminating In-111-labeled RBCs did not interfere significantly with the platelet life span determinations is compatible with the observation that In-111 elutes rapidly from red cells in vivo (Scheffel et al., unpublished observations). Since the In-111 radioactivity in whole-blood samples was essentially the same as that in separated platelets over the entire 10-day observation period, a transfer of the In-111 label from platelets to other cellular elements in the blood also seems unlikely.

The mean recovery of In-111 platelets, and that of the total In-111 radioactivity in the circulation at time zero, were 57% ± 11 (s.d.) and 61% ± 11, respectively. These

values are lower than the mean In-111 recoveries of 68 to 74% reported by four investigators (*4,5,21,22*), but are similar to the 60% obtained by Vigneron et al. (*23*) as well as similar to the recovery of Cr-51-labeled platelets (*24,25*). Some of the differences in the results of percent recovery reported in the literature are due to the different methods used in estimating the blood volume. Robertson et al. (*22*), for example, estimated a mean recovery of 70% ±21 (s.d.) in five normal male subjects on the basis of 78.6 g blood/kg body weight (B.W.), whereas our recovery data are based on 70 ml blood/kg B.W., a value recommended by the International Committee for Standardization in Hematology (*8*).

Several methods for in vivo quantitation using anterior and posterior gamma-camera imaging have been described (*26–28*), none of which measures overall attenuation directly. In a previous investigation we have shown that transmission scanning can provide an adequate attenuation correction to determine organ activities, with a precision of ~10% (*12*). Using this technique, we found that 38% of the injected dose was taken up by the spleen and 13% by the liver during the first 90 min after infusion. Similar results were obtained by Van Reenen et al. (*29*). These authors determined organ activities by surface gamma-camera imaging and relating the counts in the individual organs to total-body counts measured by whole-body scanning. At later times (3–9 days after injection), our findings differ significantly from those of Van Reenen et al. Whereas an almost threefold increase in liver and a small but significant increase in splenic activity over 9 days were noted by these investigators, we saw no such increase in any of our subjects. Our observation is shared by Peters et al. (*30*), who found activity levels in liver and spleen at 8 days only slightly higher than those at 2 hr after injection. Whether or not this discrepancy is due to the differences in technique of in vivo quantitation is not clear. Calculation of organ dose by simple surface counting may overestimate the activity in the liver by 20%, and underestimate that of the spleen by the same amount (*29*). Surface counting was also used by Robertson et al. (*22*) who, in a recent report, estimated the dose of In-111-labeled platelets from areas of interest in camera images taken over a 75-hr period. Although these authors used only rough approximations in their conversion of surface counts to actual activities in the different organs, their normalized radiation absorbed doses are very similar to ours: doses to spleen, total body, liver, and kidneys were estimated to be 33.5, 0.6, 2.5, and 2.3 rad/mCi In-111, respectively. Our values for the same organs were 33.5, 0.6, 2.0, and 1.4 rad/mCi.

In our study nearly 50% of the injected dose remained unaccounted for. Very little activity was found in the chest region at 9 days after injection, when blood activity was reduced to less than 3%. By that time some In-111 activity was detected in bone-marrow areas, especially in the pelvis. Since total bone-marrow activity cannot be quantitated, in vivo distribution data in animals had to be used. In rabbits, 14% of the In-111 activity was in the bone marrow at 6 days after injection (unpublished results, RL Hill-Zobel, et al.). More detailed studies will be needed to determine more precisely the disposal sites of platelets other than the liver and spleen in man.

The spleen is the critical organ with regard to the radiation dose. Because of the relatively high dose of 34 rad/mCi of infused In-111 platelets, we have restricted the dose of In-111 platelets administered in a single research study to 100–150 μCi. With this dose, a complete 10-day platelet survival study can be performed, and the distribution of In-111 activity in major organs can be followed for at least 3 or 4 days.

The labeling of platelets with In-111 has brought important advances. In vivo visualization of platelet deposition in small clots and areas of vascular damage has been possible for the first time (*31–33*). A relatively simple method for quantitation of In-111 activity in organs of platelet sequestration has become available. With this method, important information on changes in platelet deposition in various disease states should be forthcoming.

In addition, it is hoped that these combined methods will make possible a reassessment of the possible usefulness of radiolabeled platelets in selecting those patients with severe refractory idiopathic (immune) thrombocytopenic purpura (ITP) who will have an immediate favorable response to splenectomy. Chromium-51-labeled platelets have proven unreliable in this regard (*34*), presumably due to the very low photon flux available for external quantification. Despite the limitations of the In-111 platelet labeling method (high initial spleen and liver radioactivity, the limitation of the dose that can be administered to humans, and the relatively short half-life of In-111) it should theoretically be possible with the aseptic open method (a) to harvest and label sufficient autologous platelets and (b) to quantitate, by frequent measurements, the early changes in liver and spleen radioactivity if they parallel the rapid clearance of the labeled platelets from the circulation of such ITP patients.

FOOTNOTES

* Diagnostic Isotopes, Bloomfield, NJ.

† The computer program can be obtained from E. A. Murphy, M.D., 291 Moore Clinic, Johns Hopkins Medical Institutions.

ACKNOWLEDGMENTS

This work was supported by USPHS research grants AM-20812 and GM-10548.

REFERENCES

1. MCAFEE JG, THAKUR ML: Survey of radioactive agents for in vitro labeling of phagocytic leukocytes I. Soluble agents. *J Nucl Med* 17:480-487, 1976
2. THAKUR ML, WELCH MJ, JOIST JH, et al: Indium-111 labeled platelets: Studies on preparation and evaluation of in vitro and in vivo functions. *Thromb Res* 9:345-357, 1976
3. SCHEFFEL U, MCINTYRE PA, EVATT B, et al: Evaluation of indium-111 as a new high photon yield gamma-emitting "physiological" platelet label. *Johns Hopkins Med J* 140: 285-293, 1977
4. HEATON WA, DAVIS HH, WELCH MJ, et al: Indium-111: a new radionuclide label for studying human platelet kinetics. *Br J Haemat* 42:613-622, 1979
5. HEYNS A DU P, LÖTTER MG, BADENHORST PN, et al: Kinetics, distribution and sites of destruction of ^{111}Indium labelled human platelets. *Br J Haematol* 44:269-280, 1980
6. SCHEFFEL U, TSAN MF, MCINTYRE PA: Labeling of human platelets with [^{111}In] 8-hydroxyquinoline. *J Nucl Med* 20:524-531, 1979
7. The Panel on Diagnostic Application of Radioisotopes in Hematology, International Committee for Standardization in Hematology: Recommended methods for radioisotope platelet survival studies. *Blood* 50:1137-1144, 1977
8. The International Committee for Standardization in Haematology (ICSH) The Panel on Diagnostic Applications of Radioisotopes in Haematology: Standard techniques for the measurement of red-cell and plasma volume. *Br J Haematol* 25:801-814, 1973
9. MURPHY EA, FRANCIS ME: The estimation of blood platelet survival II. The multiple hit model. *Thromb Diath Haemorrh* 25:53-80, 1971
10. MURPHY EA: The estimation of platelet survival III. The robustness of the basic models. *Thromb Diath Haemorrh* 26:431-449, 1971
11. NICKOLOFF EL: The physics of left ventricular performance measurements with radioactive tracers. Doctoral thesis, Johns Hopkins Univ., 1977
12. NICKOLOFF EL, SCHEFFEL U, MCINTYRE PA: In-vivo regional and quantification studies. World Federation of Nucl Med and Biol, Washington, D.C., 1978, p 44 (abst)
13. SNYDER WS, FORD MR, WARNER GG, et al: "S", Absorbed dose per unit accumulated activity for selected radionuclides and organs. MIRD, Pamphlet II. New York, Society of Nuclear Medicine, 1975
14. HEYNS A DU P, BADENHORST PN, PIETERS H, et al: Preparation of a viable population of Indium-111-labeled human blood platelets. *Thromb Haemost* 42:1473-1482, 1979
15. WELCH MJ, MATHIAS CJ, DAVIS HH, et al: Modification in the method of labeling platelets with Indium-111. *J Nucl Med* 19:688, 1978 (abst)
16. HAWKER RJ, HAWKER LM, WILKINSON AR: Indium (^{111}In)-labelled human platelets: optimal method. *Clin Sci* 58:243-248, 1980
17. THAKUR ML: Experiences with In-111-oxine. State of the Art Symposium on Radiolabeled Blood Cellular Elements-Current Accomplishments, Immediate Potential, Future Possibilities, New York, 1979. In press.
18. BOLLING DR, MURPHY EA: The estimation of blood platelet survival. VI. Evaluation of the graphical method. *Johns Hopkins Med J* 143:25-31, 1978
19. BUCHHOLZ WM, RODVIEN R, MIELKE CH: Effect of sampling procedure and data analysis on platelet survival studies in man. *Blood* 50 (Suppl) 236, 1977
20. MURRELL M, SCHEFFEL U, WHIPPLE JM, et al: In-111-oxine as a red blood cell label. World Federation of Nucl Med and Biol, Washington, D.C., 1978, 130 (abst)
21. HAWKER RJ: Experience with platelet labelling using Indium-111 oxine. *Br J Radiol* 53:922-923, 1980 (abst)
22. ROBERTSON JS, DEWANJEE MK, BROWN ML, et al: Distribution and dosimetry of ^{111}In labeled platelets. *Radiology* 140:149-176, 1981
23. VIGNERON N, DASSIN E, NAJEAN Y: Double marquage des plaquettes pas ^{51}Cr et ^{111}In. Application a l'étude simultanée de la durée de vie des plaquettes autologues et homologues. *Nouv Presse Med* 9:1835-1837, 1980
24. HARKER LA, FINCH CA: Thrombokinetics in man. *J Clin Invest* 48:963-974, 1969
25. BRANEHÖG I, RIDELL B, WEINFELD A: On the analysis of platelet survival curves and the calculation of platelet production and destruction. *Scand J Haematol* 19:230-241, 1977
26. THOMAS SR, MAXON HR, KEREIAKES JG: In vivo quantitation of lesion radioactivity using external counting methods. *Med Phys* 3:253-255, 1976
27. FERRANT A, CAUWE F: Quantitative organ uptake measurement with a gamma camera. *Eur J Nucl Med* 4:223-229, 1979
28. FLEMING JS: A technique for the absolute measurement of activity using a gamma camera and computer. *Phys Med Biol* 24:176-180, 1979
29. VAN REENEN OR, LÖTTER MG, MINNAAR PC, et al: Radiation dose from human platelets labelled with Indium^{111}. *Br J Radiol* 53:790-795, 1980
30. PETERS AM, KLONIZAKIS Y: Dynamic studies with Indium-111 labelled platelets. *Br J Radiol* 53:923, 1980 (abstr)
31. GOODWIN DA, BUSHBERG JT, DOHERTY PW, et al: In-111-labeled autologous platelets for location of vascular thrombi in humans. *J Nucl Med* 19:626-634, 1978
32. DAVIS HH, SIEGEL BA, JOIST JH, et al: Scintigraphic detection of atherosclerotic lesions and venous thrombi in man by Indium-111-labelled autologous platelets. Lancet 1: 1185-1187, 1978
33. SMITH N, CHANDLER S, HAWKER RJ, et al: Indium-labelled autologous platelets as diagnostic aid after renal transplantation. *Lancet* 2:1241-1242, 1979
34. ASTER RH: Platelet sequestration studies in man. *Br J Haemat* 22:259-263, 1972