TABLE 2. IN VIVO AND EX VIVO QUANTIFICATION OF IN-111 RADIOACTIVITY IN BABOONS (n = 10)

Organ	In Vivo Quantification: Equilibrium % activity	In Vivo Quantification: Final % activity	Ex Vivo Quantification
Whole body	100 ± 0	95.2 ± 3.7*	100 ± 0
Spleen	17.3 ± 3.2	33.5 ± 3.1*	33.7 ± 5†
Liver	15.6 ± 2.0	35.2 ± 6.5*	36.7 ± 7.1†
Bone marrow	—	—	14.4 ± 1.7
Other organs	68.1 ± 4.5	31.4 ± 4	15.5 ± 4.0

* Whole-body In-111 activity did not decrease significantly ($p > 0.05$), but that in the spleen ($p < 0.001$) and liver ($p < 0.0005$) did increase highly significantly with time. (Student's t-test for paired data).
† In vivo and ex vivo quantification of splenic and hepatic In-111 activity did not differ statistically ($p > 0.1$).

Our results confirm the validity of the geometrical mean method for the in vivo quantification of labeled platelets. The results also confirm the findings of Aster (*6*), who in his classical studies showed that the liver and spleen are the major sites of platelet sequestration. The role of the bone marrow has certainly been underestimated in this regard, but it seems to play a relatively subsidiary role to that of the liver and spleen.

ANTHON DUP HEYNS
MATTHEUS G LÖTTER
HARRY F KOTZÉ
HENRY PIETERS
PAULA WESSELS
University of the Orange Free State
Orange Free State
Republic of South Africa

ACKNOWLEDGMENTS

This research was supported by the South African Medical Research Council, Atomic Energy Board and the Research Fund of the University of the Orange Free State.

REFERENCES

1. SCHEFFEL U, TSAN M-F, MITCHEL TG, et al: Human platelets labeled with In-111 8-Hydroxyquinoline: Kinetics, distribution, and estimates of radiation dose. *J Nucl Med* 23: 149-156, 1982
2. KLONIZAKIS I, PETERS AM, FITZPATRICK ML, et al: Radionuclide distribution following injection of ^{111}Indium-labelled platelets. *Br J Haematol* 46:595-602, 1980
3. HEYNS A DUP, 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
4. VAN REENEN O, LÖTTER MG, HEYNS A DUP, et al: Quantification of the distribution of ^{111}In-labelled platelets in organs. *Eur J Nucl Med* 7:80-84, 1982
5. HEYNS A DUP, BADENHORST PN, PIETERS H, et al: Preparation of a viable population of Indium-111-labelled human blood platelets. *Throm Haemostas 42:* 1473-1482, 1980
6. ASTER RH: Studies of the fate of platelets in rats and man. *Blood* 34:117-128, 1969

Reply

We thank Dr. Heyns and his colleagues for their interest in our work with In-111 platelets and their comments on our recent paper in this Journal (*1*).

Since the publication of our paper (*1*), we have (a) validated our in-vivo quantification method based on the modified geometric mean for correction of attenuation, and (b) studied the role of bone marrow in the sequestration of platelets in rabbits (*2*). Our results, details to be published elsewhere, showed that there was good correlation between the values obtained by in vivo quantification and those obtained by postmortem measurements of the radioactivity in the liver and the spleen. In 32 rabbits, $r = 0.854$ for liver and 0.899 for spleen, and the ratios of in-vivo to sacrifice values were 1.05 and 1.10 respectively. Using this method, we also showed that after infusion, the In-111 platelets rapidly accumulated in these two organs, reaching 35% and 12% of the injected dose in the liver and spleen respectively by one day. Thereafter there was little change. On the sixth day, when In-111 platelets had cleared from the circulation, there was 40% in the liver, 14% in the spleen, and 28% in the bone marrow (Table 1). The radioactivity in the bone marrow was derived by assuming that the total bone-marrow mass in rabbits was 2.2% of the body weight (*3,4*). Our earlier estimate of 14% (*1*) was based on measurements we made on three complete rabbit skeletons, freed from adhering muscle and fat after autoclaving the carcass. The radioactivity in bone (approximately 3%) had been subtracted from the total skeleton activity. We suspect that some of the In-111 in the bone marrow may have been lost during autoclaving. Therefore, in our current study, we have used the previously published values (*3,4*) in estimating rabbit bone-marrow mass.

Our results suggest that in addition to liver and spleen, bone marrow plays an important role in sequestering platelets in rabbits. Our results in humans and rabbits (*1,2*) differ from those of Heyns et al. (*5*) in that there was little change of the radioactivity in the liver and spleen after one day. We postulate that the initial localization of In-111 platelets in the liver and spleen was in part due to pooling, while at later days, it was due to sequestration of platelets by the macrophage system in these organs. The role of bone marrow in the sequestration of platelets in man is not clear, since no direct measurement in humans have been carried out. However, in view of the visualization of In-111 radioactivity in areas corresponding to the distribution of bone marrow in *man* (*1,6*), bone marrow may also play an important role in the sequestration of platelets in man. Heyns's recent results in *baboons* (*5*) point to the same conclusion.

URSULA SCHEFFEL
ROBIN HILL-ZOBEL
MIN-FU TSAN
The Johns Hopkins Medical Institutions
Baltimore, Maryland 21205

TABLE 1. BIODISTRIBUTION IN RABBITS AT 6 DAYS AFTER INFUSION

	% INJECTED DOSE*							
	In-111 PLATELETS (n = 19)		Cr-51 PLATELETS (n = 5)		In-111 TRANSFERRIN (n = 4)			
	MEAN	SEM	MEAN	SEM	MEAN	SEM	p†	p‡
Liver	40.00	1.52	22.76	2.08	14.47	—	<.001	<.001
Spleen	14.25	0.80	13.37	2.33	0.46	—	>.5	<.001
Bone marrow	27.53	2.15	26.37	5.13	7.88	—	>.5	<.001
Bone	3.91	0.32	4.30	0.26	5.12	—	>.5	<.05
Muscle	3.82	0.31	4.51	0.64	10.12	—	>.2	>.2
Blood	2.53	0.20	1.71	0.30	1.87	—	>.05	>.1
Kidneys	2.17	0.14	0.96	0.09	4.46	—	<.001	<.001
Lung	0.98	0.61	0.28	0.14	0.55	—	>.5	>.5
Total	95.31	2.71	74.33	3.03	44.92	—	<.005	<.001

* Bone marrow: 2.2% of body weight (BW); bone mass: 10% of BW; muscle mass: 43% of BW; blood volume: 4.25% of BW.

† p value between means of In-111 platelets and Cr-51 platelets.

‡ p value between means of In-111 platelets and In-111 transferrin.

REFERENCES

1. SCHEFFEL U, TSAN M-F, MITCHELL TG, et al: Human platelets labeled with In-111 8-hydroxyquinoline: Kinetics, distribution, and estimation of radiation dose. *J Nucl Med* 23: 149-156, 1982
2. HILL-ZOBEL RL, SCHEFFEL U, MCINTYRE PA, TSAN M-F: ^{111}In-oxine labeled rabbit platelets: In vivo distribution and sites of destruction, submitted for publication.
3. NYE RN: Bone marrow volume in rabbits. *Proc Soc Exp Biol Med* 29:34-37, 1931
4. DIETZ AA: Distribution of bone marrow, bone and bone-ash in rabbits. *Proc Soc Exp Biol Med* 57:60-62, 1944
5. DUP HEYNS, A, LÖTTER MG, KOTZÉ HF, et al: Quantification of in vivo distribution of platelets labeled with indium-111-oxine. *J Nucl Med* 23:943-944, 1982
6. DUP HEYNS, A, LÖTTER MG, BADENHORST PN, et al: Kinetics, distribution and sites of destruction of ^{111}Indium-labeled human platelets. *Br J Haemat* 44:269-280, 1980

Re: A Modified Method for the In Vivo Labeling of Red Blood Cells with Tc-99m

We read with interest the recent paper by Callahan et al. in the Journal (*1*). The authors are to be congratulated for contributing data to further substantiate the modified in vivo erythrocyte labeling technique. We would like to comment briefly on this paper and erythrocyte labeling.

Numerous publications indicate that a wide spectrum of agents can be used for erythrocyte labeling (*2-4*). This is partly the reason for error in the statement: "the lack of a commercially available kit has prevented the (in vitro) method of erythrocyte labeling from gaining widespread acceptance." There are at present a number of commercial kits that both mask stannous ion, binding it weakly to avert hydrolytic precipitation at physiological pH, and have a vehicular function, transporting stannous ion across the erythrocyte cell membrane and locating it within. These agents are stannous-methylene disphosphonate, -glucoheptonate, and -pyrophosphate. It is well known that the erythrocyte is permeable to numerous inorganic and organic anions. All of these kits, along with stannous citrate, migrate electrophoretically an anions. Furthermore, if compounded with Sn-119m as a fraction of the stannous component, they deposit 20-25% of their tin into erythrocytes after 5 min of in vitro incubation, indicating little difference between these agents. The subsequent labeling efficacy of these washed cells with $^{99m}TcO_4^-$ is consistently greater than 98% (Fig. 1).

We further wish to comment on various aspects of labeling that effect image quality in a brief summary of the methods.

IN VITRO

1. This is the most efficient of the methods, has a distinct kinetic advantage, and results in a stannous ion dose less than 1% of that incurred with other methods.
2. It is the least sensitive to quality of the kit, for labeling of stannous ion and pertechnetate occur external to body biochemistry and possible medications.
3. Numerous reagents are available—e.g., stannous-MDP, -PPi, -glucoheptonate—with one quarter of the kit needed for 10 ml of whole blood anticoagulated with heparin or ACD (but not with EDTA).

IN VIVO

1. This method is limited to the Sn-PPi kit (requiring 15 to 20 μg stannous ion/kg) and is extremely sensitive to the quality of the kit.
2. Sn-PPi kits more than 90 days old should not be used, nor should preparations reconstituted more than 30 minutes earlier (sodium ion has an accelerating effect on the hydrolysis of PPi to orthophosphate).
3. Cold Sn-PPi should not be administered through i.v. lines containing dextrose solutions; extravasated cold Sn-PPi results in both high background and urinary bladder activity.

MODIFIED IN VIVO

1. By altering mixing kinetics, this method is less sensitive to the quality of the Sn-PPi kit than is the standard in vivo method.