Indium and Iron as Tracers for Erythroid Precursors

Marjorie K. Jeffcoat, Barbara J. McNeil, and Michael A. Davis

Harvard Medical School and Peter Bent Brigham Hospital and Children's Hospital Medical Center, Boston, Massachusetts

External visualization and delineation of functional bone marrow is important for diagnostic, prognostic, and therapeutic purposes. Because of difficulties in using the isotopes of iron for bone-marrow imaging, indium-111 chloride has been used extensively for this purpose. In this investigation we attempted to solve the problem of cellular localization of indium chloride by employing a rat model with erythropoietic precursors selectively damaged by lethal intracellular radiation from the Auger electrons of Fe-55.

In the rat, we have shown that the absolute marrow uptakes of indium and iron are different, whereas the absolute uptakes of indium and sulfur colloid are the same. However, in animals whose erythroid activity was partially destroyed with Fe-55, the fractional depressions of iron and indium uptakes were the same and corresponded to the extent of the remaining erythroid activity. In addition, following an in vitro separation of the cellular elements of marrow with iron carbonyl, both iron and indium were found in the erythroid-rich supernatant, whereas sulfur colloid was in the precipitate.

These results indicate that, in the rat, In-111 chloride is an effective in vivo marker for the early phases of iron uptake by the bone marrow.

J Nucl Med 19: 496–500, 1978

The mapping of functional bone marrow is important for diagnostic, prognostic, and therapeutic purposes. Radionuclides of iron are the most logical choice for assessment of erythropoietic activity, but the readily available isotopes Fe-59 and Fe-55 have long half-lives and unfortunate decay schemes. Iron-52, a short-lived, positron-emitting cyclotron product, suffers from high cost and limited availability. Moreover, high-energy radiation emitted during the decay of the Mn-52 daughter degrades the image obtained with annihilation photons (*1*).

The transition element indium is bound to transferrin and is often used instead of iron for the imaging of bone marrow. Indium-111 has a 2.7-day physical half-life, ready availability, a favorable decay scheme, and low cost (*2–4*). However, the validity of indium as a radiotracer for early erythropoiesis has been questioned because of five observations:

1. The absolute uptake of indium by the bone marrow is less than the absolute uptake of iron (*5,6*).

2. The mechanism of the binding of indium to reticulocytes differs from that of iron (*7*), and a larger percentage of the indium bound is elutable by washing.

3. Only a small proportion of (1–4%) injected indium is found in circulating red blood cells (*7,8*).

4. After 500 rads to rat marrow, the proportional decrease in iron uptake is greater than that of indium (*6*).

5. A small number of patients with preleukemia and red-cell aplasia have had normal bone-marrow scans with indium chloride (*9*).

In this study we have attempted to determine whether the initial distribution of indium traces

Received July 8, 1977; revision accepted Dec. 15, 1977.

For reprints contact: Barbara J. McNeil, Dept. of Radiology, Harvard Medical School, 25 Shattuck St., Boston, MA 02115.

erythropoietic or phagocytic bone-marrow function. The erythropoietic elements of rat bone marrow have been selectively destroyed and the relative uptakes of radioiron, radioindium, and technetium sulfur colloid have been determined in the total marrow and separately in the phagocytic and nonphagocytic elements, as a function of remaining erythropoietic activity. A parallel behavior between ionic indium and ionic iron would be expected if the former is a good initial marker for erythropoietic activity, whereas a parallel behavior between indium and technetium colloid would suggest that radioindium traces phagocytic function.

MATERIALS AND METHODS

Iron-55 has been shown to be capable of delivering a lethal intracellular radiation dose to erythropoietic cells in mice with minimal damage to the surrounding reticuloendothelial marrow (*10*). In our study we extended this basic technique in order to allow collection of large marrow samples suitable for cellular fractionation. Hence, we used a larger experimental animal—the rat—and increased the administered dose of Fe-55 about tenfold. In order to avoid the toxicity of free iron, we incubated Fe-55, before injection, with 10 mg of human transferrin for 20 min at 37°C, pH = 2.8.

Twenty-two Sprague-Dawley rats (350 gm) were separated into two groups: 11 control animals were fed a regular diet, and 11 experimental animals were fed an ICN low-iron diet for five days. On the fifth day the experimental animals, under ether anesthesia, were injected with 20 mCi of Fe-55 (1.25 mg carrier iron) through the saphenous vein. The animals continued on the low-iron diet for 3 more days. On the ninth day, under ether anesthesia, all animals were injected with 45 μCi of [^{111}In] indium chloride (no carrier indium), 7 μCi [^{59}Fe] ferric citrate (0.11 mg carrier iron) and 1 mCi [^{99m}Tc] technetium sulfur colloid through the saphenous vein. Five control animals and five experimental animals were studied 5 hr later; the remaining 12 animals were studied 24 hr after injection.

Phagocytic cells were separated from nonphagocytic cells by ingestion of iron carbonyl filings (*11*). The rats were first anesthetized intraperitoneally with 0.3 cc of Nembutal. One femur of each animal was then surgically located and the marrow exposed by scalpel. A small sample of marrow was spread on a coverslip with a camel-hair brush and stained with Wright-Giemsa for determination of the marrow differential and hence of the extent of erythropoiesis (*12*). The remaining marrow was quickly removed from the femur and placed in 2 ml of phosphate-buffered saline at 37°C. Iron carbonyl was added and the cell suspension incubated for 15 min at 37°C to allow the reticuloendothelial cells to ingest the iron carbonyl by phagocytosis. A magnet was then used to draw the carbonyl-laden reticuloendothelial cells to the bottom of the tube. The supernatant was removed with a Pasteur pipette and the entire fractionation procedure was repeated two more times.

The rats were killed with ether while the iron carbonyl separation was in progress. The contralateral femur was exposed and its marrow removed for determination of total marrow radioactivity. The liver and spleen were also removed for determination of radionuclide organ distribution.

The supernatant and cell pellets from the cell fractionation procedure, as well as the samples of whole bone marrow, liver and spleen, were counted for each of the tracers present in a well-type gamma scintillation counter coupled to a dual-channel analyzer. Corrections were made for overlap in the energy spectra.

RESULTS

Bone-marrow uptake and organ distribution. In the control animals at 5 and 24 hr after injection of the radionuclides, the total accumulations of indium and iron in the liver and spleen were similar but less than that of sulfur colloid (Table 1). In the bone marrow there was four to seven times more iron than either indium or technetium. Thus in the bone marrow the absolute uptakes of indium and iron were different, whereas the absolute uptakes of indium and sulfur colloid were the same.

In the experimental animals the fractional depression of the marrow uptakes of In-111 and Fe-59 at both 5 and 24 hr were correlated with each other and varied in accordance with the extent of erythroid activity remaining after Fe-55 injury (Fig. 1a and b). At 5 hr, uptake of these radionuclides was not depressed until 70% of the erythroid activity was destroyed; at 24 hr there was a curvilinear relationship between uptake and erythroid activity. The differences between the iron and indium distribution data at 5 and 24 hr are probably due to the death of several aged precursors and the concomitant rapid maturation time of 34 hr for rat erythroid cells (*13*). At both 5 and 24 hr the distribution of sulfur colloid within the marrow was essentially constant irrespective of the marrow differential counts. Moreover, there was no significant difference between the experimental and control animals in the percentage of the injected sulfur colloid per gram of marrow at either 5 or 24 hr.

Tracer distribution during in vitro separation. At both 5 and 24 hr the relative proportion of iron and indium in the erythroid-rich supernatant fraction was

TABLE 1. SELECTED ORGAN DISTRIBUTION (%) FOR Fe-59 CITRATE, In-111 CHLORIDE AND Tc-99m SULFUR COLLOID IN CONTROL RATS AT 5 AND 24 HR AFTER INJECTION

Tissue	Tracer Uptake*		
	Fe-59	In-111	Tc-99m
Liver			
5 hr	19.3 ± 3.2†	17.1 ± 2.2	76.0 ± 7.0
24 hr	21.0 ± 2.5	19.2 ± 1.2	83.0 ± 4.4
Spleen			
5 hr	0.98 ± 0.2	0.67 ± 0.11	2.9 ± 0.13
24 hr	2.4 ± 0.53	1.6 ± 0.3	2.8 ± 1.5
Bone marrow			
5 hr	4.0 ± 0.13	0.55 ± 0.04	0.71 ± 0.02
24 hr	4.8 ± 0.51	1.30 ± 0.30	1.06 ± 0.34

* Expressed as percentage of injected dose of entire liver and spleen, and as percentage of injected dose per gram of bone marrow.
† Mean ± one s.d.

similar—70% at 5 hr and 75% at 24 hr (Table 2). There was no significant difference between indium and iron. Concurrently only 28% of the sulfur colloid activity remained in the supernatant.

DISCUSSION

Erythropoiesis requires three stages: (a) uptake of iron by the erythroid precursors, (b) transfer of the membrane-bound iron to the cytoplasm, and finally (c) incorporation of iron into the newly synthesized heme and globin moieties. Each of these stages has been studied in detail in both animals and man in attempts to elucidate causes of disturbed erythropoiesis. These same data have also been used to study the suitability of potential radiotracers for erythropoiesis. Although it is clear that any tracer that mimics iron exactly in all three stages is an ideal indicator of active erythropoiesis, a tracer that mimics iron in only the first or second of these stages may still be useful as an indicator for erythroid elements. This appears to be the case for ionic indium.

Iron uptake by the erythroid cell lines has often been studied using reticulocytes because of their ready availability. In rats, rabbits, and man, reticulocyte binding of the transferrin-iron complex takes place in two stages: first, by a relatively weak union that is temperature-independent and is probably secondary to physical adsorption; second, by a stronger union that is dependent upon active metabolism and is inhibited by arsenite and low temperatures (7). Indium mimics iron in the first stage but differs quantitatively in the second. Both metals enter the rat reticulocyte cell—approximately 90% for iron and 20% for indium—but only iron is incorporated into hemoglobin to any extent (7).

In several species the absolute marrow uptake of indium differs from that of iron; in general, at least four times as much administered iron as indium is taken up by the bone marrow (5,6). This difference has led to one of the main objections for using indium as an erythropoietic tracer and was in part responsible for this study. We therefore asked, "Admitting the difference in absolute uptakes, does the relative uptake of indium in compromised erythropoiesis reflect hemopoietic activity in the same way that iron does?" In both our in vitro and in vivo experiments we showed that with selective damage to the erythroid precursors by the Auger electrons

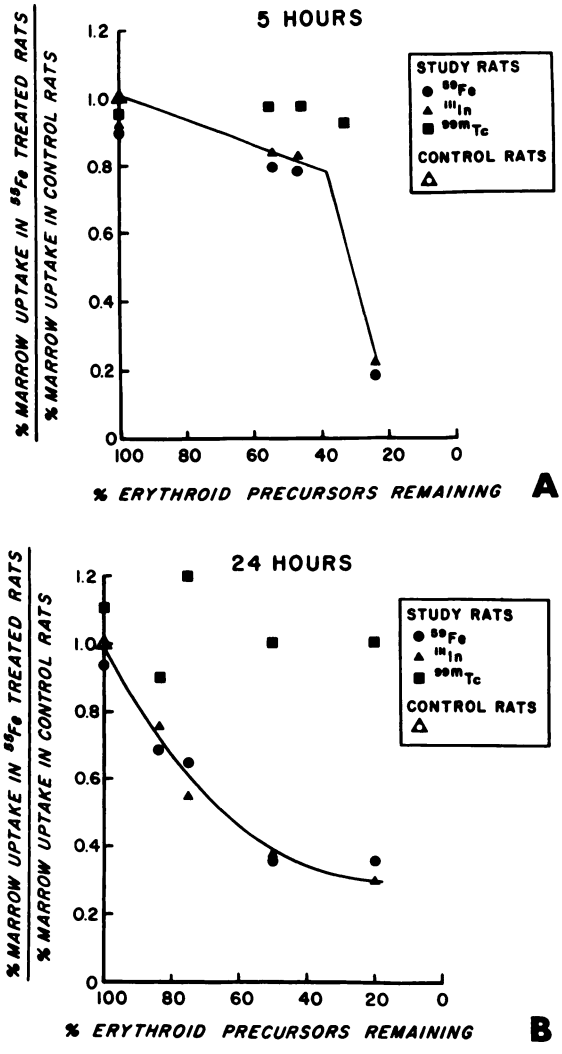

FIG. 1. Effect of Fe-55 ablation on marrow uptake of In-111, Fe-59, and Tc-99m, at 5 hr (a) and 24 hr (b) after injection of these nuclides. On the ordinate is marrow uptake normalized to control animals and on the abscissa the percentage of erythroid precursors remaining. For a given tracer, each point represents a separate animal. At 5 hr there is only a gradual decrease in iron and indium uptake, until nearly 70% of the erythroid elements are killed, whereas at 24 hr there is a continuous and synchronous drop of both iron and indium uptake as more erythroid cells are ablated. At both times, uptake of sulfur colloid is essentially constant.

TABLE 2. IN VITRO BONE-MARROW CELLULAR SEPARATION: % OF BONE-MARROW ACTIVITY IN SUPERNATANT AT 5 AND 24 HR AFTER INJECTION (% IN SUPERNATANT + % IN PRECIPITATE = 100)

	Fe-59	In-111	Tc-99m
5 hr:			
Control (n = 5)	69 ± 2.1	71 ± 1.2	27 ± 2.1
Experimental (n = 5)	66 ± 5.7	71 ± 5.3	30 ± 4.0
24 hr:			
Control (n = 6)	77 ± 4.2	77 ± 2.7	25 ± 3.9
Experimental (n = 6)	74 ± 9.4	77 ± 9.2	25 ± 5.8

* Mean ± one s.d.

of Fe-55, there was a parallel effect on indium and iron but not on sulfur colloid.

Our in vitro data were especially striking because their interpretation cannot be influenced by the fact that some of the carrier iron (particularly that associated with Fe-55) was unbound to transferrin at the time of injection. Phagocytosis of iron carbonyl depends merely on metabolically active phagocytic elements; measurement of tracer activity in the supernatant and precipitate fractions allows nearly complete separation not only of the phagocytic and the nonphagocytic elements but also of their trace element components—in this case, indium and iron.

The iron-binding cells of the marrow have been identified by means of autoradiography in many species (*13,14*), however, autoradiographic data are not yet available for indium. Thus, we must speculate regarding its cellular location in the marrow. One clinical observation may be relevant. Some patients with Diamond-Blackfan disease (congenital red-cell aplasia) have adequate numbers of proerythroblasts in the marrow with virtual absence of later erythroid precursors. A normal thymidine labeling index of the proerythroblasta suggests that there is early death of erythroblast precursors (*15*). Three of our patients in the relapsed form of this disease have had relatively normal indium chloride scans, suggesting that the proerythroblasts of man are capable of binding indium. In other patients with acquired red-cell aplasia and no erythroid precursors of any type, we have observed no uptake of indium by the marrow (*16*).

There are discordances, however, in the reported clinical findings. Merrick and his colleagues studied six patients using both Fe-52 and In-111; they showed good correlation between the two studies in three patients (with myeloproliferative disease, reticulum-cell sarcoma, or myelosclerosis) and poor agreement in three other patients with preleukemia and red-cell aplasia (*9*). We found excellent agreement among indium chloride scans, marrow biopsies, Fe-59 uptake and clinical assessment, in over 40 patients with myelofibrosis, as well as among indium chloride scans, marrow biopsies, and clinical assessment in over 40 patients with aplastic anemia (*17,18*). Similar results in 87 patients with lymphoma were obtained by Gilbert (*19*): 91% of his patients with normal hemopoietic status had normal scans, whereas 94% of his patients with abnormal hemopoietic status had abnormal scans.

A pronounced difference is observed between rat and man in the relative uptake of indium and iron by the externally irradiated bone marrow. Nelp and his colleagues originally showed that external radiation (250–500 rads) transiently affects Fe-59 uptake but not marrow colloid uptake (*20*). McIntyre et al. later showed that 500 rads produce a marked reduction of iron uptake by the rat marrow, as expected, but lead to no change in either indium or sulfur colloid uptake (*6*). In man, a number of clinical studies have shown no such discordance between iron and indium (*3,19,21,23*). Moreover, the observations on the externally irradiated rat marrow are at complete variance with our results on marrow in which erythropoietic cells were selectively irradiated. Selective damage to erythroid precursors in the rat by Fe-55 is supported by unaltered uptakes of sulfur colloid by control and Fe-55 treated marrows at a time when iron uptakes changed dramatically. The reasons for the discrepancies resulting from use of different types (external contrasted with internal) of irradiation in experimental situations are not clear.

We believe that the cumulative data support the following model: most or all erythroid precursors contain transferrin receptors. Both indium-transferrin and iron-transferrin are bound to these receptors; only iron, however, is incorporated to any significant degree into hemoglobin. Admittedly, differences between indium and iron may vary with the species studied, as well as with the hematologic parameter used for comparison. In the rat, our in vitro and in vivo data indicate that the site of early localization of In-111 is not the reticuloendothelial cell, and that in response to a selective depletion of erythroid precursors both indium and iron are depressed proportionally. These data support a large number of human studies that show good agreement between indium uptake and erythroid cellularity.

ACKNOWLEDGMENTS

This work was supported by the Milton Fund, Harvard University.

Dr. McNeil is the recipient of Research Career Development Award K04-GM-00194.

The authors are indebted to Drs. H. Franklin Bunn and Jonathan Glass for helpful discussions in the design of these

experiments and to Lila Tulin, Alice Carmel, and V. King for technical assistance.

This work was presented in part at the 24th Annual Meeting of the Society of Nuclear Medicine, Chicago, June 23, 1977.

REFERENCES

1. ANGER HO, VAN DYKE DC: Human bone marrow distribution shown in vivo by iron-52 and the positron scintillation camera. *Science* 155: 1587–1589, 1964

2. STAUB RT, GASTON E: ^{111}In-chloride distribution and kinetics in hematologic disease. *J Nucl Med* 14: 456–457, 1973 (Abst)

3. LILIEN DL, BERGER HG, ANDERSON DP, et al: ^{111}In-chloride: A new agent for bone marrow imaging. *J Nucl Med* 14: 184–186, 1973

4. HOSAIN F, MCINTYRE PA, POULOSE K, et al: Binding of trace amounts of ionic indium-113m to plasma transferrin. *Clin Chem Acta* 24: 69–75, 1969

5. BEAMISH MR, BROWN EB: The metabolism of transferrin-bound ^{111}In and ^{59}Fe in the rat. *Blood* 43: 693–701, 1974

6. MCINTYRE PA, LARSON SM, EIKMAN EA, et al: Comparison of the metabolism of iron-labeled transferrin (Fe·TF) and indium labeled transferrin (In·TF) by the erythropoietic marrow. *J Nucl Med* 15: 856–862, 1974

7. BEAMISH MR, BROWN EB: A comparison of the behavior of ^{111}In and ^{59}Fe-labeled transferrin on incubation with human and rat reticulocytes. *Blood* 43: 703–711, 1974

8. FARRER PA, SAHA GB, SHIBATA HN: Evaluation of ^{111}In-transferrin as a tumor scanning agent in humans. *J Nucl Med* 13: 429, 1972 (Abst)

9. MERRICK MV, GORDON-SMITH EC, LAVENDER J, et al: A comparison of ^{111}In with ^{52}Fe and ^{99m}Tc-sulfur colloid for bone marrow scanning. *J Nucl Med* 16: 66–68, 1975

10. REINCKE U, BURLINGTON H, CRONKITE EP, et al: Selective damage to erythroblasts by ^{55}Fe. *Blood* 45: 801–810, 1975

11. LEVINE S: Magnetic techniques for in vitro isolation of leucocytes. *Science* 123: 185–186, 1956

12. HARRIS JW, KELLERMEYER RW: *The Red Cell.* Cambridge, Harvard University Press, 1970, pp 309–319

13. PAPAYANNOPOULOU T, FINCH CA: Radioiron measurements of red cell maturation. *Blood Cells* 1: 535–546, 1975

14. LAMERTON LF, BELCHER EH, HARRISS ED: Blood uptake of Fe^{59} in studies of red cell production. In *Kinetics of Cellular Proliferation.* Stohlman F, ed., New York, Grune & Stratton, 1959, pp 301–311

15. HARDISTY RM: Diamond Blackfan anaemia. In *Congenital Disorders of Erythropoiesis.* Stohlman F, ed., New York, Elsevier, 1976, p 98

16. BUNN HF, MCNEIL BJ, ROSENTHAL DS, et al: Bone-marrow imaging in pure red blood cell aplasia. *Arch Int Med* 136: 1169–1172, 1976

17. MCNEIL BJ, HOLMAN BL, BUTTON LN, et al: Use of indium chloride scintigraphy in patients with myelofibrosis. *J Nucl Med* 15: 647–651, 1974

18. MCNEIL BJ, RAPPAPORT JM, NATHAN DG: Indium chloride scintigraphy—an index of severity in patients with aplastic anemia. *Brit J Haematol* 34: 599–604, 1976

19. GILBERT EH, EARLE JD, GORIS ML, et al: The accuracy of $^{111}InCl_3$ as a bone marrow scanning agent. *Radiology* 119: 167–168, 1976

20. NELP WB, GOHIL MN, LARSON SM, et al: Long term effects of local irradiation of the marrow on erythron and red cell functions. *Blood* 36: 617–622, 1970

21. TOUYA JJ, ANSELMI OE, RILEY RF, et al: Metabolism of ^{111}In-transferrin as a measure of bone marrow activity. In *Dynamic Studies with Radioisotopes in Medicine.* Vienna, IAEA, 1975, pp 413–425

SNM POSTCONVENTION SYMPOSIUM AND TRIP TO HAWAII

A postconvention tour to Honolulu and Maui, Hawaii has been set up for July 1–8, following the SNM's 25th Annual Meeting in Anaheim. The package includes round-trip air transportation and hotel accommodations.

The tour has been set up in conjunction with a Postconvention Symposium arranged by the Hawaiian Chapter and the Society. In addition to didactic sessions, a workshop will be conducted on quality assurance in imaging and in vitro procedures.

Application has been made for 20 hours of AMA Category 1 credit, and also for VOICE credits. Reservations for the tour may be made by contacting:

International Professional Meeting Coordinators, Ltd.
49 West 57th St.
New York, NY 10019
(212) 751-4650