COMPARISON OF THE METABOLISM OF IRON-LABELED TRANSFERRIN (Fe · TF) AND INDIUM-LABELED TRANSFERRIN (In · TF) BY THE ERYTHROPOIETIC MARROW

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Indium-111-transferrin (TF) has been described as a hematopoietic marrow scanning agent. The purpose of this study was to compare the metabolism of In·TF and Fe·TF by the erythropoietic marrow. There were marked differences observed between the biologic behavior of these two metals bound to transferrin in all parameters studied. These include: (A) slower plasma clearance of In·TF; (B) significantly greater percentage of ^{59}Fe incorporation in the circulating red cells with only minimal labeling of peripheral red cells with indium and rapid disappearance of the indium-labeled cells from the circulation; (C) significantly greater accumulation of ^{59}Fe in the skeleton; (D) irradiated rabbits' legs showed the expected bone and bone marrow decrease in the uptake of ^{59}Fe-transferrin at 48 hr following 500 rads to the right tibia–fibula unit but no change in ^{111}In·TF or ^{99m}Tc-sulfur colloid uptake; (E) in the control nonirradiated legs, 60% of the ^{59}Fe but only 20% of ^{111}In was in the extracted marrow, indicating that the bone concentration of indium was greater than that of ^{59}Fe ($p < 0.001$); (F) a more rapid uptake of ^{59}Fe·TF by reticulocyte-rich blood which at 60 min was nine times that of In·TF. These results suggest that the metabolism of indium is significantly different from that of iron with respect to erythropoiesis. The use of ^{111}In·TF as a bone marrow scanning agent must be approached with caution in view of the high indium-to-iron ratio in normal bone and the unchanged accumulation of indium by bone marrow in which the erythroid marrow has been damaged by irradiation.

In 1967 Stern, et al demonstrated that ionic indium injected intravenously at low pH remained in the circulation for a considerable period of time and accordingly, that the radioisotope form ^{113m}In was a useful blood pool scanning agent (*1*). Because of some chemical similarities to iron, it was postulated that the ionic indium was binding to transferrin in a manner similar to, if not identical to, that of iron. This was subsequently demonstrated to be true (*2*). All of the available radioisotopes of iron suffer from some disadvantage for clinical or experimental use (the extremely low energy of emission of ^{55}Fe; the extremely high gamma energy of ^{59}Fe preventing adequate spatial resolution; the lack of ready availability of the positron-emitting ^{52}Fe). If ^{113m}In bound to transferrin (^{113m}In·TF) behaved in a biologically similar manner as iron bound to transferrin (Fe·TF), it was postulated that it might provide a very useful clinical and research radiopharmaceutical.

As the kinetics of transport of ^{59}Fe·TF in the feto-placental unit of the pregnant rat have been well defined (*3,4*), it was elected to compare the behavior of indium and iron-labeled transferrin in this model. The results of this study have been published elsewhere and showed that there are striking biologic differences in the behavior of these two elements injected bound to transferrin (*5*) (Fig. 1). There was a rapid uptake of ^{59}Fe·TF by the placenta and extremely rapid transfer by the placenta of the ^{59}Fe to the fetus. In contrast there was an equally rapid but progressively increasing uptake of ^{113m}In in the

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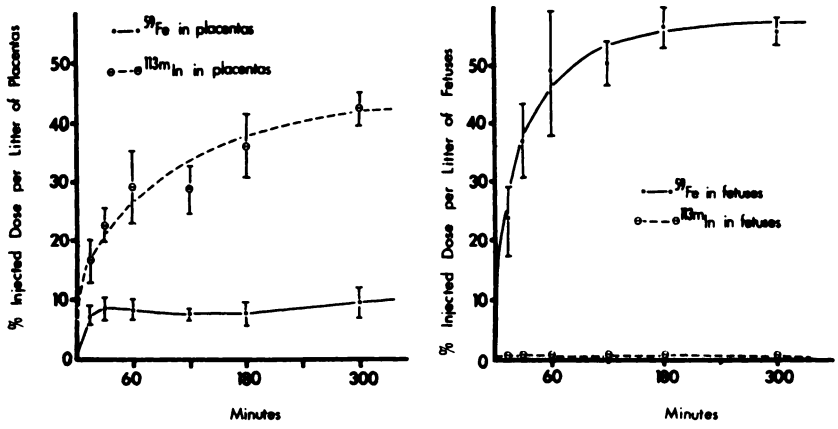

FIG. 1. The kinetics of transport Fe·TF and ^{113m}In·TF to placentas and fetuses were studied after intravenous injection of tracer amounts of these isotopes previously incubated with normal rat plasma into maternal rat. Lefthand graph shows that there was almost immediate appearance of ^{59}Fe in placenta with relatively constant concentration of this isotope throughout period of study. In contrast, there was progressive accumulation of ^{113m}In in placenta. Righthand graph shows there was rapid progressive transport of ^{59}Fe across placentas to the fetuses; in contrast, no significant ^{113m}In activity could be detected in fetuses.

placenta but no transport across the placenta into the fetus. Control studies with iodinated transferrin indicated that the amount of ^{113m}In present in the placenta was far in excess of that amount of transferrin present in the placenta. Concomitantly, pilot studies comparing In·TF to Fe·TF in the erythropoietic system indicated that there were similar gross differences in the behavior of these two compounds (previously unpublished data, detailed below) (*6*).

Recently it has been reported that ^{111}In·TF provides an ideal agent for scanning the hematopoietic marrow of man (*7–10*). This isotope is now readily available in a carrier-free form and if its pattern of distribution did indeed reflect the active erythropoietic marrow, this would provide a readily available and substantially improved agent for bone marrow scanning. The use of ^{52}Fe has been widely described by Van Dyke and coworkers and provides an ideal agent for scanning the erythropoietic marrow (*11,12*) but its availability has been limited because it is cyclotron produced.

Accordingly, this study was undertaken to extend our previous comparisons regarding the biologic behavior of Fe·TF and In·TF by the erythropoietic system.

MATERIALS AND METHODS

It should be emphasized that in all of the studies described here, both the iron and various isotopes of indium used were prelabeled to transferrin prior to injection into the test animal. A serum sample was obtained from the test animal and the unsaturated iron-binding capacity was predetermined, using the Irosorb-59 kit (Irosorb-59, Abbott Labs., North Chicago, Ill.). The amount of serum incubated with the radioactive indium and iron was calculated so that the amount of indium or iron added was less than 50% of the measured values of the unsaturated iron-binding capacity. Iron-59 citrate was used in all of the Fe·TF studies (Mallinckrodt Chemicals, St. Louis, Mo.). Either carrier-free ^{113m}In-chloride (New England Nuclear Sn-113 generator) ($T_{1/2}$ 1.73 hr), carrier-free ^{111}In-chloride ($T_{1/2}$ 2.8 days) (Diagnostic Isotopes, Upper Saddle River, N.J.), or ^{114m}In-chloride ($T_{1/2}$ 50 days) (ORNL specific activity greater than 10 Ci/gm of indium) were used in various experiments depending upon the requirement in terms of the half-life of the isotope of indium.

Technetium-99m-sulfur colloid was prepared using the method of Larson and Nelp (*13*). Samples and the appropriate standards were counted simultaneously in a two-channel automatic gamma sample counter (Packard #5019) with a 3-in. sodium iodide crystal or manually in a 5-in. well counter. The relative counting error was kept to less than 3% according to the criteria of Loevinger and Berman (*14*).

METHODS

Dog studies. For the determination of Fe·TF and In·TF clearance from the plasma and subsequent appearance in the circulating erythrocytes, two 15-kg mongrel dogs were studied. In order to stimulate maximum erythropoiesis, the first dog was phlebotomized prior to the injection of the labeled transferrin. Volumes of 370 ml and 100 ml of blood were removed by arterial puncture (without anesthesia) on 2 consecutive days with simultaneous infusion of equal amounts of normal saline. On the third day, the dog received 27 ml of plasma labeled with 100 μCi of ^{114m}In-chloride and 10 μCi of ^{59}Fe-citrate. The second normal dog received plasma labeled with 4 mCi of ^{111}In-chloride and 10 μCi of ^{59}Fe. Blood samples were obtained at 10, 30, 60, 90 min postinjection and hourly up to 6 hr postinjection; daily for 6 days and 3 times weekly thereafter for the duration of the study. Hematocrit determinations and reticulocyte counts were performed on all samples. The urine was collected daily from

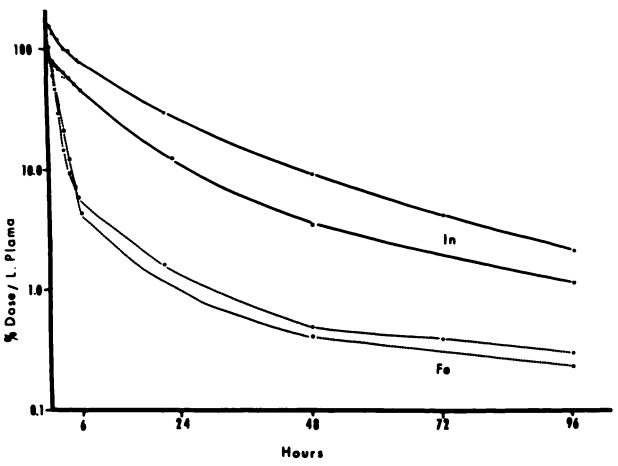

FIG. 2. Rate of plasma clearance of ^{59}Fe·TF and indium·TF after intravenous injection into two mongrel dogs. Note initial rapid exponential clearance of ^{59}Fe from plasma with substantially less than 10% remaining in plasma after 6 hr postinjection; in contrast there was marked delay in clearance of indium from plasma.

the dogs and the amount of ^{59}Fe and ^{114m}In excreted was calculated. In the second dog study, circulating ^{111}In WBC activity was also measured.

Study of uptake of indium and iron by the reticulocyte-rich blood. Reticulocyte-rich rat blood was obtained by injecting Sprague-Dawley rats daily three to five times with a 1% phenylhydrazine–hydrochloride-HCl solution (Fisher Scientific, Fairlawn, N.J.) (2.5 mg/100 gm body wt). Heparinized blood was collected from pentobarbital-anesthetized animals by cardiac puncture and centrifuged at 1400 rpm. The plasma was removed and the red blood cells were washed twice in normal saline and then resuspended in normal saline to approximately the original volume. Rat plasma previously labeled with ^{59}Fe and ^{113m}In or ^{114m}In was added and the erythrocytes and radioactive plasma were incubated together at 37°C in a shaking water bath (Dubinoff Shaker, 50 times per minute). In two experiments, ^{125}I-albumin was added to the incubation mixture as a control. The reaction was stopped at varying intervals by adding 10 ml ice-cold normal saline. Samples were centrifuged at 4°C at 800 rpm and the cells washed four times with normal saline before resuspension and measurement of the radioactivity incorporated into the erythrocytes. The reticulocyte count was obtained on the whole blood prior to the beginning of each experiment.

The separation of the red cells into lysate and stroma was performed as follows: the whole red cells were washed three times in cold normal saline and then lysed either by repeated freeze-thawing or by the addition of distilled water. After centrifugation, the hemolysate-supernatant was removed, the stroma-precipitate washed and then dissolved in 88% formic acid solution prior to measurement of radioactivity.

Tissue distribution in rats. The tissue distribution of ^{59}Fe and ^{111}In·TF was studied in three 280-gm Sprague-Dawley rats. One-milliliter doses of 2 μCi of ^{59}Fe and 15 μCi of ^{111}In bound to plasma were administered intravenously. The animals were sacrificed at 24 hr postinjection and the percent of the dose determined in the whole blood, plasma, washed red cells, liver, spleen, kidney, and skeleton. To obtain the skeleton, the rat carcass was autoclaved at 250°C at 15 psi for 20 min and the adhering tissues removed.

Clearance studies in rabbits. In order to compare the clearance of ^{59}Fe and ^{111}In·TF from the blood of rabbits, nine 2-kg white New Zealand rabbits received 5 μCi of ^{59}Fe·TF and 50 μCi of ^{111}In·TF intravenously. Blood samples were taken by intracardiac puncture at 6 hr from three rabbits and at 18 hr from six rabbits. The whole blood was centrifuged at 2000 rpm for 10 min; the plasma was re-

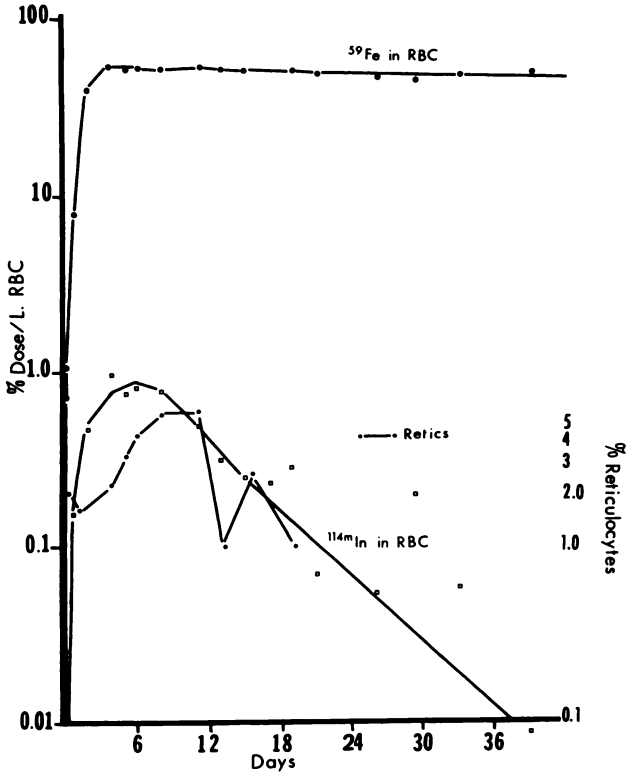

FIG. 3. Appearance of ^{59}Fe and ^{114m}In in circulating red cells after injection of ^{59}Fe·TF and ^{114m}In·TF into previously phlebotomized mongrel dog. There was rapid appearance of significant amounts of the ^{59}Fe in circulating red cells and their ^{59}Fe concentration remained constant throughout duration of the study. In contrast, only 0.8–0.9% of administered dose of ^{114m}In appeared in circulating red cells and there was rapid disappearance of these indium-labeled red cells from circulation. There was rough correlation in amount of indium present in circulating red cells and reticulocyte counts. (Both vertical scales are logarithmic.)

moved and the cellular fraction was resuspended in saline and washed three times prior to measurement of radioactivity.

Radiation of rabbit tibia–fibula. The effect of local bone marrow irradiation on the marrow uptake of ^{59}Fe and ^{111}In transferrin was studied using 3-kg white New Zealand rabbits and the method of Larson and Nelp (*15*), which produces a transient depression of ^{59}Fe uptake but no significant change of marrow colloid uptake. The animals were anesthetized with intravenous pentobarbital (30 mg/kg). The radiation dose of 500 rads was delivered over 3–5 min using 250-keV orthovoltage x-ray to the right tibia–fibula. The dose measurements at skin were made with a standard dose meter at the time of irradiation. It is estimated that the actual marrow dose was somewhat less (on the order of 425–450 rads). The remainder of the rabbit was carefully shielded with lead to assure a radiation dose of less than 2 rads to the control leg.

Forty-eight hr later, the animals were sacrificed. These animals were injected intravenously with 5 μCi of ^{59}Fe·TF and 15 μCi of ^{111}In·TF 6 hr prior to sacrifice; 30 min prior to sacrifice, 250 μCi of ^{99m}Tc-sulfur colloid were injected. A repeat study was performed in an identical fashion except that the ^{111}In-transferrin was injected 48 hr prior to sacrifice to ensure a lower blood level at the time of sacrifice. The right and left tibia–fibula were removed. After freezing the tibia–fibula in liquid nitrogen, as much marrow as possible was removed from the osseous portion and the total radioactivity in the samples was then determined.

RESULTS

Plasma clearance of In·TF in the dog was much slower than that of Fe·TF and did not show the initial rapid exponential clearance as did Fe·TF (Fig. 2). In the first dog, the ^{59}Fe red cell activity reached a peak of 50% of the injected dose per liter at 6 days and remained constant during the 40-day period of study. Maximum ^{114m}In activity was only 0.8 to 0.9% of the administered dose per liter of red cells and the ^{114m}In rapidly disappeared from the circulation (Fig. 3). Whether this was due to accelerated clearance of these In-labeled erythrocytes or rapid elution of the label is not clear. However, there was a rough correlation between the amount of indium present in the circulating red cells and the reticulocyte count (Fig. 3). Ten percent of the injected dose of In and only 0.2% of Fe was excreted in the urine in the first 8 days.

Studies on the second dog using carrier-free ^{111}In showed essentially the same differences in percent incorporation of iron and indium in the circulating

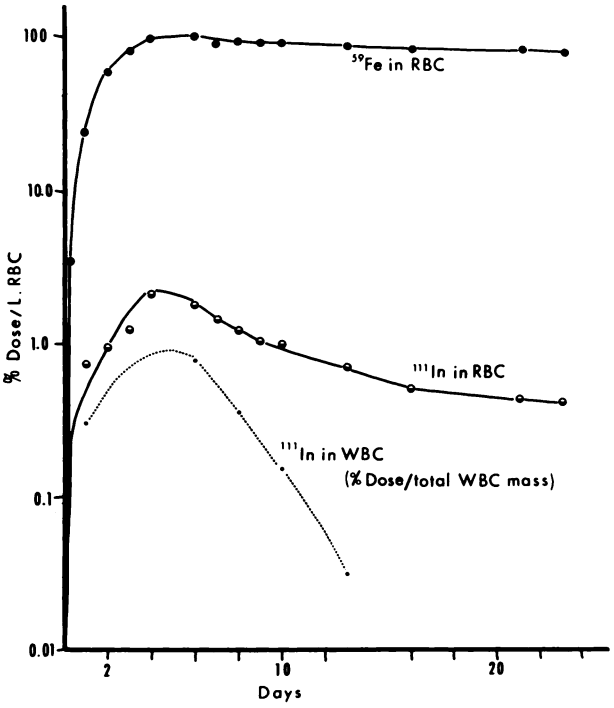

FIG. 4. Studies in second dog injected with ^{59}Fe·TF and ^{111}In·TF showed essentially same differences in percent of incorporation of iron and indium in circulating erythrocytes. There was also transient appearance of small amount of indium present in circulating granulocytes.

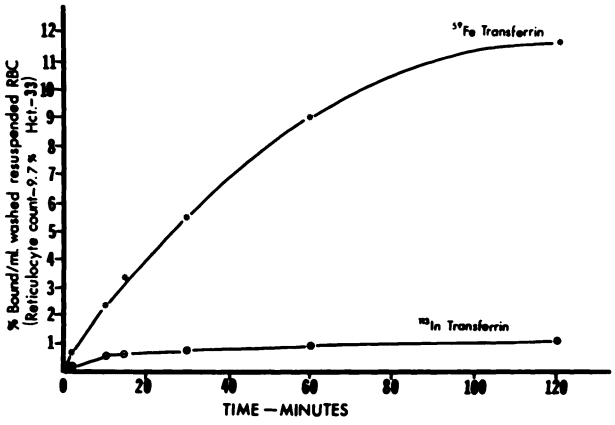

FIG. 5. Reticulocyte-rich erythrocytes were incubated with ^{113m}In·TF and ^{59}Fe·TF at 37°C. There was rapid, progressive accumulation of ^{59}Fe by erythrocytes which was approximately 12 times that of ^{113m}In at 2 hr; concentration of indium associated with erythrocytes remained essentially constant after first 10 min of incubation.

erythrocytes and demonstrated in addition a transient small amount of indium present in the circulating granulocytes (Fig. 4).

Study of relative uptake of reticulocyte-rich blood showed a progressive accumulation of ^{59}Fe transferrin with approximately 12 times as much Fe·TF bound to the erythrocytes at 2 hr as In·TF (Fig. 5). The total iron–indium activity associated with the

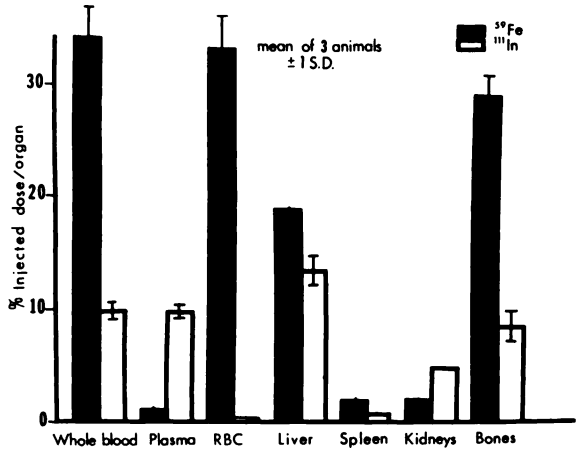

FIG. 6. Tissue distribution of injected ^{59}Fe·TF and ^{111}In·TF in rats 24 hr after injection. Majority of ^{59}Fe present in whole blood at that time was bound to circulating erythrocytes whereas ^{111}In concentration was almost exclusively in plasma. In addition there was far greater accumulation of ^{59}Fe in skeleton than of ^{111}In at this time period.

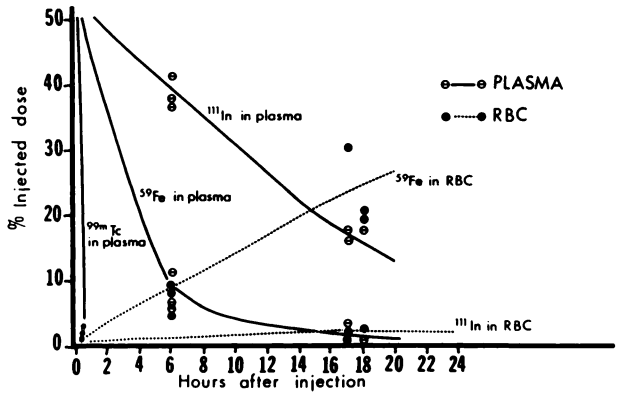

FIG. 7. Similar studies in normal rabbits showed marked delay in clearance of ^{111}In from plasma and rapid appearance of ^{59}Fe in circulating red cells in contrast to very small amounts of ^{111}In appearing in circulating erythrocytes.

erythrocytes after a 60-min incubation at 37°C was determined and the erythrocytes then fractionated into hemoglobin and stroma. These studies showed that after 60 min incubation, the mean uptake of ^{59}Fe per recticulocyte was nine times that of ^{113m}In. At that time more of each isotope was in the red cell lysate but a significant portion remained bound to the stroma. The control studies with added ^{125}I-human serum albumin demonstrated that the washing techniques were adequate (Table 1).

Tissue distribution in rats. Twenty-four hours after injection of indium and iron bound to transferrin striking differences were noted in distribution of these isotopes in the rats. A significant proportion of ^{59}Fe activity was already present in the red cells with very little remaining in the plasma but with significant amounts of indium remaining in the plasma. In addition there was a far greater accumulation of ^{59}Fe in the skeleton than of indium (Fig. 6).

Rabbit studies. There was again demonstrated a marked delay in clearance of indium from the plasma in contrast to that of ^{59}Fe. In addition, there was significant incorporation of ^{59}Fe into the circulating erythrocytes at the termination of the study whereas there was essentially no indium present in the circulating erythrocytes at that time period (Fig. 7).

The irradiated rabbits showed the expected bone marrow decrease of uptake of ^{59}Fe by the irradiated right tibia–fibula, but no change in the In·TF or ^{99m}Tc-sulfur colloid uptake (Fig. 8). Repeat studies in which the ^{111}In·TF was injected 48 hr prior to sacrifice gave identical results. In the control left leg, 60% of the ^{59}Fe but only 20% of the ^{111}In was in the extracted marrow indicating that the bone concentration of indium was greater than that of iron ($p < 0.001$) (Table 2).

TABLE 1. RELATIVE UPTAKE BY RBC AND DISTRIBUTION OF INCORPORATED ISOTOPES BETWEEN RBC LYSATE AND STROMA

	% Ao*/ml of washed RBC											
	Intact washed RBC			Hemoglobin containing lysate		RBC stroma			Ao lysate/ Ao RBC		Ao stroma/ Ao RBC	
Exp. No.	^{59}Fe	^{113m}In	^{125}I-IHSA	^{59}Fe	^{113m}In	^{59}Fe	^{113m}In	Exp. No.	^{59}Fe	^{113m}In	^{59}Fe	^{113m}In
1	14.4	2.70	0.19	7.8	1.70	6.1	0.87	1	0.54	0.63	0.42	0.32
2		0.34	0.01									
3	12.9	1.60		10.6	0.96	2.3	0.61	3	0.82	0.60	0.18	0.38
4	13.3	1.70		9.3	0.99	3.9	0.72	4	0.70	0.58	0.29	0.42
n =	3	4		3	3	3	3	n =	3	3	3	3
$\bar{x}$	13.5	1.58		9.2	1.21	4.1	0.73	$\bar{x}$	0.69	0.60	0.30	0.37
s.d.	0.78	0.97		1.40	0.42	1.01	0.13		0.14	0.03	0.12	0.051
p	<0.001			<0.001		<0.050			<0.500		~0.400	

* Ao—Total radioactivity initially added to suspension.

TABLE 2. % ^{99m}Tc-SULFUR COLLOID, ^{59}Fe, AND ^{111}In ACTIVITY IN MARROW AND TOTAL TIBIA–FIBULA NONIRRADIATED L LEG OF RABBITS

Exp. No.	Extracted marrow			Total tibia and fibula			Ratio of marrow to total bone			p values
	^{99m}Tc	^{111}In	^{59}Fe	^{99m}Tc	^{111}In	^{59}Fe	^{99m}Tc	^{111}In	^{59}Fe	
1	0.076	0.104	0.914	0.190	0.534	1.40	0.400	0.195	0.651	
2	0.096	0.114	1.080	0.207	0.664	1.82	0.463	0.172	0.592	
3	0.173	0.136	1.342	0.400	0.786	2.318	0.434	0.172	0.580	Fe vs In < 0.001
							$\bar{x} = 0.433$	0.180	0.608	Fe vs Tc < 0.005
							s.d. $\pm$ 0.033	0.014	0.039	Fe vs In < 0.001

DISCUSSION

The results of these studies as well as other published data indicate that the metabolism of In·TF and Fe·TF are significantly different with respect to erythropoiesis: (A) the marked delay in plasma clearance of indium bound to transferrin as demonstrated by others in human subjects (*16*) (6.1 hr for ^{113m}In in contrast to 60–120 min for ^{59}Fe), is quite similar to the observations made in this study; (B) the apparent hepatic uptake of indium by the human liver as observed by scanning procedures after the injection of carrier-free ^{111}In bound to transferrin is in striking contrast to the absence of any liver ^{52}Fe activity after erythroid marrow scans with this agent; (C) the marked reduction of In incorporation into reticulocyte-rich red cells in vitro and into circulating red cells in vivo compared with the results obtained with ^{59}Fe·TF; (D) the finding in this study that there is a much higher indium uptake in normal bone as compared with iron and accordingly much lower indium uptake in normal bone marrow as compared with iron; (E) the unchanged accumulation of indium uptake by bone marrow in which the erythroid marrow had been damaged by irradiation. In view of these observations, the use of ^{111}In-transferrin as a bone marrow scanning agent must be approached with caution.

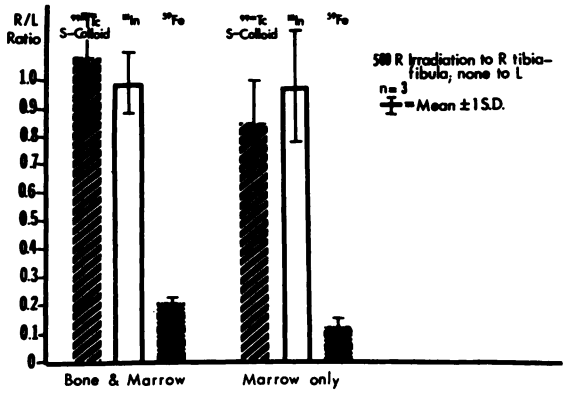

FIG. 8. Forty-eight hours following 500 rads of irradiation to right tibia–fibula unit, there was expected marked decrease in accumulation of ^{59}Fe in irradiated limb compared with control (L) nonirradiated limb; ^{59}Fe uptake was depressed both in total bone and in extractable marrow. In contrast there was no significant change in accumulation of either ^{99m}Tc-sulfur colloid or ^{111}In chloride injected as In·TF in either total tibia–fibula unit or in extractable marrow.

ACKNOWLEDGMENT

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