The Metabolism of Transferrin-bound ^{111}In and ^{59}Fe in the Rat

By Michael R. Beamish and Elmer B. Brown

The metabolism of transferrin-bound indium and iron were compared by in vivo studies in the rat. Rats were injected with serum transferrin labeled with ^{111}In and ^{59}Fe, and, at intervals ranging from 20 min to 5 days after injection, they were killed. They were perfused through the portal vein with 200 ml of 0.9% saline, and the residual radioactivity, expressed as a percentage of the injected dose, was measured in liver, spleen, kidney, muscle, washed red cells, red marrow, and femur. At 5 days, 76% of the injected ^{59}Fe was recovered in the red cell mass; only 1%–2% of ^{111}In could then be recovered. Uptake and release of the ^{111}In label by the femur was markedly less than that of the ^{59}Fe. Whereas 85% of the injected ^{59}Fe could be recovered from the circulating red cells, liver, and spleen, only about 15% of the injected ^{111}In could be so recovered. Approximately 35% of the injected ^{111}In was excreted, and 43% was recoverable from the carcass. The subcellular distribution of the two isotopes in the liver at timed intervals following intravenous injection was studied. While 35% of the ^{59}Fe activity in the homogenate was associated with ferritin, only 4% of the ^{111}In could be so identified. The results indicate a significant difference between the metabolism of ^{111}In and ^{59}Fe in the rat and make it unlikely that the metabolism of ^{111}In in man bears much similarity to that of iron.

REPORTS OF THE uptake of transferrin-bound indium by reticulocytes,[1,2] its incorporation into heme by red blood cells,[3] and preliminary clinical studies with ^{111}In as a marrow-imaging agent that suggest uptake by the red bone marrow[1,2,4] have led to the suggestion that indium behaves in a manner similar to iron.

We have previously compared the uptake of ^{111}In and ^{59}Fe bound to serum transferrin by both rat and human reticulocytes in vitro and have shown that while ^{111}In binds to specific reticulocyte iron receptor sites, there is little intracellular transfer or incorporation of the metal into hemoglobin or heme.[5]

Although ^{111}In is being used increasingly as a marrow-imaging agent in clinical evaluation of sites of erythropoiesis, there have been few reports of its metabolism in vivo. The purpose of this study is to compare certain aspects of the metabolism of indium with that of iron.

MATERIALS AND METHODS

Indium was purchased as carrier-free $^{111}InCl_3$ dissolved in 0.2 *N* HCl (specific activity 1.0 mCi/ml) from Diagnostic Isotopes Inc., and iron as $^{59}FeCl_3$ dissolved in 0.2 *N* HCl

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(specific activity 15.0 mCi/mg) from International Chemical and Nuclear Corp. Fresh serum, clotted from blood in iron-free glass tubes, was obtained from normal adult Sprague-Dawley rats. ^{111}In and ^{59}Fe were bound to transferrin by incubation in serum for 30 min at 37°C. Chromatography with Sephadex G-150 equilibrated in 0.1 *N* NaCl, 0.01 *M* $NaHCO_3$ was used to confirm complete binding to transferrin.

The labeled serum was injected into the dorsal penile vein of 15 male Sprague-Dawley rats (200-250 g). The rats were divided into five groups and were killed at timed intervals after injection. Five to six milliliters of heparinized blood were collected from the orbital sinus of each animal before death, the hematocrit was measured, and aliquots of plasma and thrice-washed red cells were obtained for measurement of radioactivity.

The portal vein was cannulated and perfused with 200 ml of 0.9% saline, during which time the hepatic vein was cut to allow drainage of the perfusate and intermittently clamped in order to raise the perfusion pressure. This technique permitted most of the vessels and tissues below the diaphragm to be perfused free of visible blood. Tissue samples of liver, spleen, kidney, and thigh muscle were taken for measurement of radioactivity. Both femurs were isolated and scraped clean of soft tissue. Bone marrow was removed from the shaft of one femur by splitting with bone forceps. The remaining diaphyseal bone was washed free of marrow with saline. The whole femur, washed bone, and red marrow were measured for radioactivity using a dual channel Packard gamma scintillation spectrometer. Hematocrit and hemoglobin estimations were performed by standard techniques.[6]

The organ and tissue radioactivities were expressed as a percentage of the injected radioactivity. The percentage uptake by the red cell mass was calculated from the product of the radioactivity in washed red cells obtained from 1 ml of heparinized blood and the blood volume. The blood volume was calculated as 6 ml per 100 g body weight.[9]

Estimation of Whole Body, Fecal, and Urinary ^{111}In Losses

Fresh normal rat serum labeled with ^{111}In was injected into three rats as described above. The rats were placed in metabolic cages and fed a standard laboratory diet. Urine and feces from each of the three animals were collected separately. Whole body, fecal, and urine radioactivity was measured at timed intervals during a 130-hr period with a multiprobe whole body gamma scintillation counter designed for small animals. Results were corrected for physical decay by reference to a standard. The activities were expressed as a percentage of the whole body activity initially present 10 min after injection.

Subcellular Fractionation of Liver

Serum transferrin doubly labeled with ^{59}Fe and ^{111}In was injected into four male adult rats. Their livers were perfused with 150-200 ml of 0.9% saline at 90-min, 9-, 24-, and 64-hr intervals after injection. The organs were removed, blotted, weighed, minced with scissors, and mixed with 3 volumes of cold 0.25 *M* sucrose, 0.001 *M* EDTA solution. Each liver was then homogenized with a single pass in a Potter Elvejhem homogenizer with a motor driven Teflon pestle. Subcellular fractionation was carried out as described by de Duve et al.,[7] and nuclear (N), mitochondrial (M), lysosomal (S), microsomal (P), and supernatant (S) fractions were obtained. Acid phosphatase was assayed by the method of Gianetto and de Duve.[8] The radioactivity in the supernatant fraction present as ferritin was measured by using an antiferritin serum produced in rabbits against purified rat liver ferritin prepared by the method of Drysdale and Munro.[16] The purity of the ferritin was confirmed by polyacrylamide gel disk electrophoresis and by total iron and protein estimations.

RESULTS

The distribution of the ^{59}Fe and ^{111}In radioactivity in the circulating red cells and organs at timed intervals after intravenous injection is shown in Table 1. There was a progressive increase in the percentage uptake of the ^{59}Fe in the red cell mass with respect to time; by the fifth day after injection, 76% of the injected ^{59}Fe label was recovered there. In contrast, there was minimal uptake of

Table 1. Organ Radioactivity

Time		Red Cells	Liver	Spleen	Kidney	Muscle (per g)	Whole Femur	Femur Marrow	Femur Bone
20 min	^{59}Fe	1.67 ± 0.56	0.68 ± 0.17	0.41 ± 0.07	0.50 ± 0.09	0.014 ± 0.008	0.26 ± 0.12	0.21 ± 0.042	0.04 ± 0.007
	^{111}In	0.26 ± 0.10	0.73 ± 0.13	0.27 ± 0.02	0.54 ± 0.09	0.017 ± 0.001	0.12 ± 0.05	0.09 ± 0.013	0.03 ± 0.006
8 hr	^{59}Fe	7.60 ± 1.20	7.05 ± 0.97	3.30 ± 1.17	0.37 ± 0.07	0.18 ± 0.130	1.95 ± 0.09	1.02 ± 0.240	0.43 ± 0.010
	^{111}In	0.24 ± 0.06	7.20 ± 0.48	0.83 ± 0.17	1.71 ± 0.05	0.16 ± 0.047	0.24 ± 0.01	0.10 ± 0.027	0.04 ± 0.006
24 hr	^{59}Fe	52.50 ± 2.70	7.80 ± 0.71	1.84 ± 0.09	0.35 ± 0.01	0.04 ± 0.003	1.45 ± 0.05	0.55 ± 0.062	0.21 ± 0.113
	^{111}In	0.90 ± 0.05	11.50 ± 0.92	1.00 ± 0.11	2.02 ± 0.04	0.09 ± 0.008	0.45 ± 0.02	0.12 ± 0.008	0.12 ± 0.046
3 days	^{59}Fe	67.60 ± 2.10	8.50 ± 0.97	1.04 ± 0.23	0.39 ± 0.04	0.03 ± 0.002	0.43 ± 0.06	0.26 ± 0.028	0.03 ± 0.002
	^{111}In	1.10 ± 0.02	11.60 ± 1.00	1.29 ± 0.14	2.28 ± 0.11	0.09 ± 0.009	0.36 ± 0.06	0.16 ± 0.018	0.07 ± 0.007
5 days	^{59}Fe	76.20 ± 1.76	8.37 ± 0.50	1.05 ± 0.17	0.36 ± 0.04	0.03 ± 0.003	0.15 ± 0.03	0.09 ± 0.023	0.02 ± 0.004
	^{111}In	1.23 ± 0.04	11.30 ± 1.90	1.43 ± 0.18	1.84 ± 0.06	0.12 ± 0.007	0.32 ± 0.02	0.14 ± 0.087	0.07 ± 0.006

Organ distribution of ^{59}Fe and ^{111}In over a 5-day period after intravenous injection. Each result represents the mean ± SE from three animals and is expressed as a percentage of the injected radioactivity.

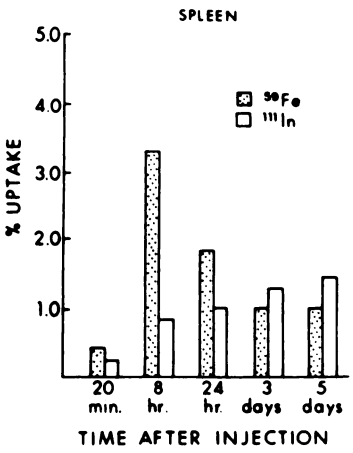

Fig. 1. The uptake of ^{111}In and ^{59}Fe into the spleen at timed intervals following intravenous injection of ^{111}In- and ^{59}Fe-labeled serum.

^{111}In label by the red cells, so that only 1.2% of the label was recovered in the red cell mass 5 days after injection. The ^{59}Fe activity in the spleen rose to a maximum of 3.3%, 8 hr after injection, following which there was a fall in radioactivity probably indicative of splenic erythropoiesis (Fig. 1). In contrast, the ^{111}In activity increased with time, reaching a maximum of 1.4%, 5 days after injection, suggesting a major uptake by nonerythroid cells.

The expected uptake and release of ^{59}Fe was seen in the whole femur and femoral red marrow (and minimally in washed bone), reaching maximal values 8 hr after injection of 1.95%, 1.02%, and 0.43%, respectively (Fig. 2). A similar, but significantly lower, uptake was observed with ^{111}In; maximal values 24 hr after injection were 0.45%, 0.12%, and 0.12%, respectively. The release of ^{111}In was less marked than that for ^{59}Fe. The ratio of the maximal whole femur

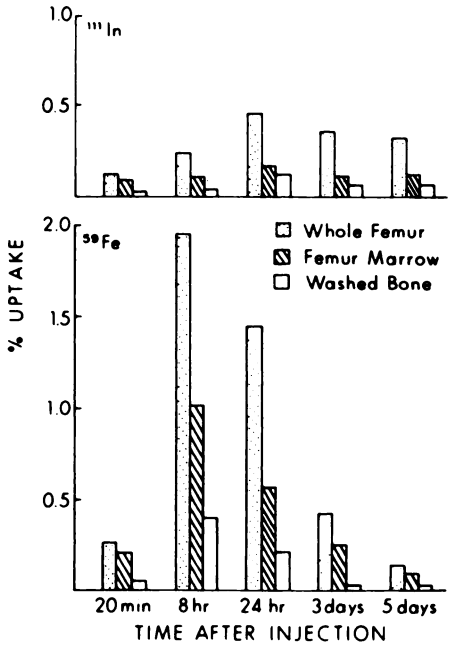

Fig. 2. The uptake of ^{111}In and ^{59}Fe into whole femur, femur marrow, and washed bone obtained from the femoral shaft after removal of the marrow, at timed intervals following intravenous injection of ^{111}In- and ^{59}Fe-labeled serum. The uptake is expressed as a percentage of the injected activity.

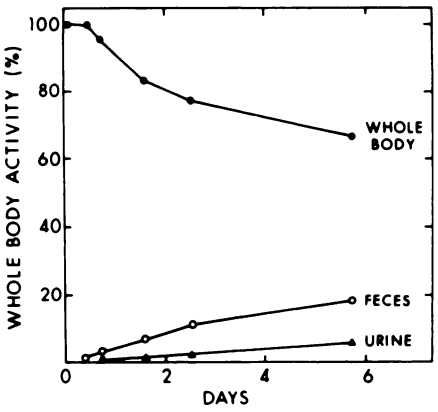

Fig. 3. Whole body, fecal, and urine loss of ^{111}In from three rats following intravenous injection of ^{111}In-labeled serum. Mean activity, corrected for decay, is expressed as the percentage of the whole body activity present immediately following injection. Fecal activity (0—0) and urine activity (▲—▲) represent the cumulative sum of the combined samples.

activity divided by the activity remaining at 5 days was 13.4:1 for ^{59}Fe and 1.4:1 for ^{111}In, indicating a 93% and 29% release, respectively, from the whole femur. These data indicate that the majority of the ^{111}In taken up by whole bone remains in that organ, suggesting limited uptake and release by erythroid marrow.

The uptake of the ^{111}In radioactivity in kidney and muscle was significantly greater than that for ^{59}Fe, with ^{111}In:^{59}Fe ratios of 4:1 in muscle and 5:1 in kidney 5 days after injection.

At 5 days, approximately 85% of the ^{59}Fe label was recovered from the blood and viscera shown in Table 1, while only about 15% of the ^{111}In label could be similarly recovered, suggesting a more generalized tissue distribution or rapid excretion of ^{111}In. The extent of ^{111}In excretion was studied by measuring whole body, fecal, and urine radioactivities in three rats at timed intervals after intravenous injection (Fig. 3). Whole body loss was insignificant during the initial 12-hr period; subsequently, there was a 34% loss during the 120-hr period of study. There was a corresponding appearance of radioactivity in both feces and urine, so that the cumulative losses were 18% and 6%, respectively, during this period. While these results offer a partial explanation for the low recovery of the ^{111}In label mentioned above, they also indicate that the unaccounted for activity must remain in the carcass. This postulate was investigated by examining the carcass of a rat after the removal of the thoracic and abdominal viscera 5 days following an intravenous injection with ^{111}In-labeled serum. The carcass was found to contain 43% of the injected label. Muscle, skin, and bone were separated by dissection and found to contain 14%, 16.5%, and 6.0%, respectively, of the injected label.

Liver Fractionation Studies

The distribution of ^{59}Fe and ^{111}In in subcellular fractions of liver from rats perfused at timed intervals following intravenous injection of doubly labeled serum was studied in order to compare the intracellular metabolism of the two isotopes (Fig. 4). Initially, most of the ^{59}Fe activity was in the soluble (S) fraction. During the next 24 hr, there was a progressive rise in the microsomal (P) fraction. At all times, over 70% of the activity was located in either P or S fractions. In contrast, less than 25% of the ^{111}In activity was recoverable in either

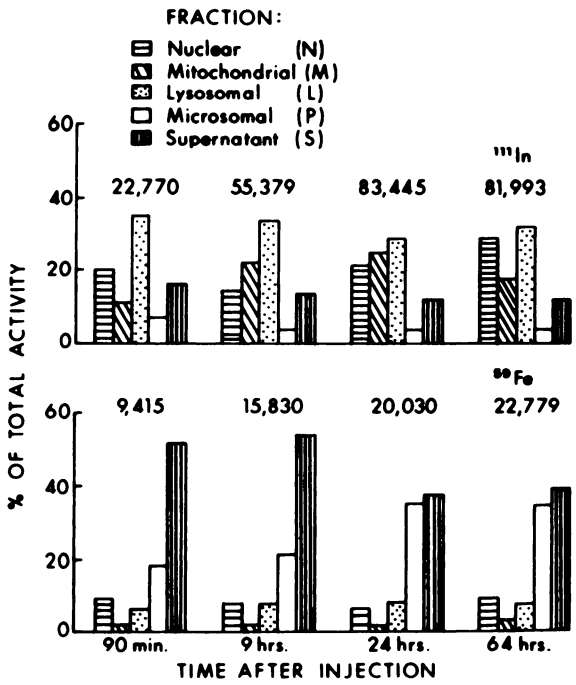

Fig. 4. Hepatic uptake and subcellular distribution of ^{111}In and ^{59}Fe after intravenous injection of ^{111}In- and ^{59}Fe-labeled serum into four rats. The subcellular fractions were prepared as described in the text. The numbers above the columns represent the total radioactivity per gram of liver. The mean activities of the fractions are expressed as a percentage of the activity in the whole homogenate.

P or S fractions. Initially, 35% of the radioactivity was localized in the lysosomal (L) fraction, and this percentage remained fairly constant throughout the period of study. Between 14% and 24% was found in the nuclear (N) fraction. Although there is significant cross contamination of particulate fractions, particularly microsomal (M) and L fractions, acid phosphatase assays on these fractions demonstrated reasonable purity and maximal activity of the L fraction.

The ^{59}Fe in the S fraction was shown to be ferritin associated by the following criteria: (1) The ^{59}Fe remained in the supernatant solution after heating at 75°C for 10 min and centrifugation at 10,000 *g*. (2) Virtually 100% of the activity in the S fraction was precipitable by an antiferritin antiserum prepared in the rabbit against purified rat liver ferritin. In contrast only 32% of the ^{111}In present in the S fraction could be precipitated by antiferritin antiserum. Thus, 24 hr after injection, 35% of the ^{59}Fe radioactivity and 4% of the ^{111}In activity could be identified as ferritin associated. The association of ^{59}Fe activity with the P fraction probably indicates the incorporation of ^{59}Fe into ferritin, as apoferritin synthesis has been demonstrated in both membrane-bound and free polyribosomes.[11]

DISCUSSION

Although ^{111}In is used in clinical medicine as a scanning and tumor-localizing agent,[1,2,4] there have been few complete in vivo studies of its metabolism following intravenous injection. Recently, several authors have drawn attention to its similarity to iron on the basis of its capacity to be bound selectively by transferrin,[1,2] its uptake by reticulocytes after in vitro incubation in the trans-

ferrin-bound state,[1,2] and its apparent incorporation into the heme molecule.[3] Preliminary clinical studies with $^{111}InCl_3$ as a bone marrow scanning agent have demonstrated a distribution similar to that of red marrow,[1,2] and it has been suggested that the in vivo metabolism of indium is similar, if not identical, to that of iron.[1]

Hosain et al.[12] have demonstrated in vivo complete binding of ^{113m}In to transferrin 5 min after an intravenous injection to patients and have confirmed these results with in vitro starch gel electrophoretic studies on plasma doubly labeled with ^{113m}In and ^{59}Fe. Reports on the uptake of indium-bound transferrin by reticulocytes have been conflicting. Lilien et al., in a preliminary communication, have reported remarkably similar rates of uptake of transferrin-bound ^{111}In and ^{59}Fe on incubation with human reticulocytes.[1] However, other workers performing in vitro incubations with rabbit and rat reticulocytes have reported ^{111}In uptakes consistently only 10% that of ^{59}Fe.[2,13]

Previous studies in this laboratory have shown that the uptake of transferrin-bound ^{111}In by human and rat reticulocytes was usually between 20% and 30% and 5% and 12% that of the ^{59}Fe controls, respectively.[5] In contrast to ^{59}Fe, where the isotope is rapidly transferred into the cell and incorporated into heme, over 90% of the ^{111}In of reticulocytes appears to remain on the surface membrane, as judged by its reflux following reincubation with unlabeled serum. Furthermore, we have been unable to demonstrate binding of the remaining cell-associated ^{111}In to either hemoglobin or heme.

The data presented in this paper, comparing the metabolism of ^{111}In and ^{59}Fe in the rat, indicate marked differences in the behavior of these two isotopes. Five days after intravenous injection of serum labeled with ^{59}Fe and ^{111}In, 76% of the ^{59}Fe and only 1.2% of ^{111}In was recovered from the circulating red blood cells (Table 1). While the uptake and release of ^{111}In by the marrow was significantly decreased in comparison with ^{59}Fe, there was a progressive increase of ^{111}In in muscle, kidney, and liver over the period of study (Table 1). Further studies on a rat carcass 5 days after injection with ^{111}In demonstrated 14.0% and 16.5% of the injected activity in muscle and skin, respectively. The localization of ^{111}In-transferrin in these tissues may be the result of extravascular pooling or the attachment of ^{111}In activity from the perfusate following portal vein perfusion (see Materials and Methods) and the virtual absence of significant plasma activity 24 hr after injection make cell-associated activity more likely than extravascular pooling.

While there is usually about a 10% loss of whole body ^{59}Fe activity over a 7-day period after intravenous injection in the rat,[14] the presented data show a 35% loss of ^{111}In from the whole animal during the 5½-day period following intravenous injection of the isotope. The majority of the activity was recovered in the feces (18%) and urine (6%). Marked differences between the ^{111}In and ^{59}Fe isotopes were also observed when liver subcellular fractions obtained by differential centrifugation were examined at timed intervals after intravenous injection of the two isotopes. Over 70% of the ^{59}Fe present in the whole homogenate could be recovered in either the microsomal or the supernatant fractions during the period of study, and 35% of the total homogenate activity 24 hr after injections could be identified as associated with ferritin. In contrast,

less than 25% of the ^{111}In could be recovered in either microsomal or supernatant fractions, and only 4% of the total activity in the homogenate could be identified as ferritin associated. A significantly high percentage (35%) was constantly associated with the lysosomal fraction.

Other workers have demonstrated significant differences between the metabolism of indium and iron with respect to erythropoiesis in rats, dogs, and rabbits[13] and with respect to placental and fetal uptake in rats.[15]

There is little published data on the metabolism of indium in humans. While clinical studies with ^{111}In have demonstrated a distribution similar to that of the anticipated red marrow distribution in patients,[1,2,4] only 4% of the injected label was recovered in circulating red cells 12 days after injection.[2] Excretion studies have been reported for one patient: a 15% loss occurred into feces and urine over the 10-day period of study.[10]

The data presented in this paper indicate that there is limited uptake and release of ^{111}In in the bone marrow following intravenous injection, and most of the isotope remains bound to tissues not primarily concerned with iron metabolism. Furthermore, the absence of significant activity in the circulating red cells and absence of a significant association with ferritin in the liver indicate that, in the rat, ^{111}In does not follow the normal metabolic pathways of iron metabolism.

The sum of presently available data makes us believe that the metabolism of indium in human subjects bears little similarity to that of iron. Until further information is obtained with regards to the cellular distribution of indium in the bone marrow, the results of bone marrow scanning obtained with isotopes of indium will remain difficult to interpret.

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