INVESTIGATIVE NUCLEAR MEDICINE

Gallium-67 Uptake by the Liver: Studies using Isolated Rat Hepatocytes and Perfused Livers

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We studied the hepatic uptake of carrier-free Ga-67 using isolated rat hepatocytes and perfused livers. The results indicate that: (a) the liver can take up Ga-67 and secrete it into the bile, even in the absence of transferrin; (b) transferrin inhibits hepatic uptake of Ga-67 and its biliary excretion; and (c) iron deficiency markedly enhances hepatic uptake of Ga-67.

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The liver is a major organ of Ga-67 accumulation, but little is known about the mechanism involved. Several studies (*1–3*) have shown that a reduction of serum unsaturated iron-binding capacity (UIBC), caused by injection of iron or scandium, reduces the hepatic uptake of Ga-67. In contrast, induction of iron-deficiency anemia, which increases the serum UIBC, enhances hepatic uptake of Ga-67 (*3,4*). These in vivo results appear to demonstrate a direct correlation between the serum UIBC and hepatic Ga-67 uptake. Unfortunately, interpretation of these studies is complicated by the fact that the serum UIBC also profoundly affects the plasma level and clearance of Ga-67, as well as its retention by the body (*1–5*). Thus the varying degrees of hepatic uptake at different serum UIBC levels may be a reflection of plasma Ga-67 levels.

To exclude the various possible factors that may affect hepatic Ga-67 uptake in the in vivo models, we have studied Ga-67 uptake by isolated rat hepatocytes and perfused liver preparations. The results suggest that (a) the liver can sequester Ga-67 in the absence of transferrin and excrete it into the bile, (b) transferrin inhibits hepatic uptake of Ga-67 and its biliary excretion, and (c) iron deficiency markedly enhances hepatic uptake of Ga-67.

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METHODS

Preparation of isolated rat-liver cells. Hepatocytes were isolated from male Sprague-Dawley rats by in-situ perfusion with collagenase, as described by Seglen (*6*) and modified by Rubin et al. (*7*). Briefly, after an animal was anesthetized with sodium pentobarbital (50 mg/kg) the liver was preperfused with calcium-free Hepes buffer, then perfused with collagenase (Type CLS II)* in a calcium-containing Hepes buffer. The digested liver was dispersed, and hepatocytes isolated by filtration through a gauze, followed by differential centrifugation, yielding approximately 400 million hepatocytes per rat liver. Final cell suspensions were made in Williams Medium E† supplemented with 10 mmol Hepes and 20 g/l of electrophoretically 100% pure bovine serum albumin. The viability of the isolated hepatocytes, as determined by the trypan blue exclusion test, was 90 ± 4% (mean ± s.d., n = 13) at 30 min, and 85 ± 5% at 4 hr after harvest.

Determination of Ga-67 uptake in hepatocytes. Throughout the experiment, cell suspensions were kept in a shaking water bath of 37° C, in an atmosphere of O_2:CO_2 (95:5%). Aliquots of 2 million hepatocytes in 2 ml medium were placed into plastic tissue-culture tubes and incubated with 0.3–0.5 μCi carrier-free Ga-67 citrate, in the absence or presence of different concentra-

tions of iron-free human transferrin or rat serum. At the end of the incubation period, 4 ml of cold Williams Medium E was added and the cells sedimented by centrifugation, followed by two additional washes with 2 ml Williams Medium E. The percentage of Ga-67 taken up by the cells was determined by counting the radioactivity in the resuspended cells and in the combined washes. Adsorption of Ga-67 to the plastic, as measured in blank tubes, was less than 10% of the radioactivity taken up by the cells.

Perfusion of isolated rat livers. To study Ga-67 uptake by the whole liver, we used a recirculating isolated perfused liver preparation, similar to that described by Miller (*8*). After ACI rats[‡] were anesthetized with sodium pentobarbital (50 mg/kg), the bile duct, portal vein, and thoracic vena cava were cannulated, and the inferior vena cava was tied off. Blood was removed from the liver by initial perfusion with 5 ml heparinized isotonic saline, followed by 50 ml oxygenated perfusion buffer. After separation from its donor, the liver was immediately transferred to the perfusion chamber.[§] The perfusion medium (initial volume = 50 ml) consisted of an oxygenated Krebs-Ringer bicarbonate buffer, supplemented with 40 mg dextrose, 2 g bovine serum albumin (Fraction V) and 500 units heparin. The system was maintained at 37°C, and the pH of the perfusion medium was kept between 7.35 and 7.45 by periodic addition of sodium bicarbonate. The perfusion flow was adjusted to 20 ml/min.

Determination of Ga-67 liver uptake and biliary excretion. The liver was perfused in the presence or absence of transferrin, lactoferrin (~70% pure), or deferoxamine mesylate. After a 1-hr equilibration period; 5 μCi carrier-free Ga-67 citrate were then added to the perfusate. The dose of 5 μCi Ga-67 in 50 ml perfusate was within the range of concentrations to be found in the intra- and extravascular spaces of patients injected with Ga-67 in clinical studies. In some experiments, 5 μCi of either Tc-99m sulfur colloid or Tc-99m DISIDA was also added to the perfusate to determine, respectively, the macrophage or hepatocyte function.

Samples of 0.5 ml perfusate were removed from the reservoir at 5, 10, 20, and 60 min, and at hourly intervals thereafter. Bile was collected hourly. At the end of the perfusion, the original perfusate was disconnected and the liver flushed with 100 ml of fresh medium to remove intravascular Ga-67. The radioactivity in the perfusate samples, bile, wash medium, and liver, which was weighed and divided into five samples, was measured in an automatic gamma counter. Radionuclide crossover contribution was corrected using a computerized stripping program. The results were expressed as percent of the dose originally introduced into the perfusion medium. Glutamic oxaloacetic transaminase (GOT) activity in the perfusate was determined using Sigma assay reagents.

The loss of radioactivity by nonspecific adsorption to the apparatus was monitored in each experiment [% loss = 100% − (% dose in liver + % dose in bile + % dose in perfusate and wash)]. Compared with Tc-99m sulfur colloid (mean percent loss in 2 hr = 3.5 ± 1.0% (s.e.m.), n = 14) and Tc-99m DISIDA (9.0 ± 2.5%; n = 5), Ga-67 citrate showed a strong tendency to adhere to the glass surface of the apparatus (33.7 ± 2.4%, n = 5). The presence of transferrin in the perfusate reduced the amount of Ga-67 loss: 21.5% [12.0–28.4 (n = 3)] at 0.1 mg/ml rat transferrin and 14.9% [12.0–16.8 (n = 3)] at 1.0 mg/ml.

Isolation of rat transferrin. The transferrin was purified from pooled rat serum by salting-out chromatography on Sepharose CL-6B, followed by ion-exchange chromatography on DEAE-Sepharose as described by Sawatzki et al. (*9*). Iron was removed from the purified transferrin by dialysis against 0.4% sodium EDTA (*10*), followed by multiple treatments with 0.1 *M* citrate-acetate buffer at pH 4.5 on CF-25 filters[¶] and subsequent washes with distilled water (*3*). The Ga-67 binding to the final rat transferrin preparation was tested by ultrafiltration, as described by Tsan et al. (*11*); 94–98% of added Ga-67 was bound at a transferrin concentration of 3 mg/ml.

Induction of iron deficiency in rats. Iron deficiency was produced by removing 1.0–1.5 ml blood by cardiac puncture three times weekly for a period of 3 wk, while the animals were kept on a low-iron diet (5958 C).[**] On

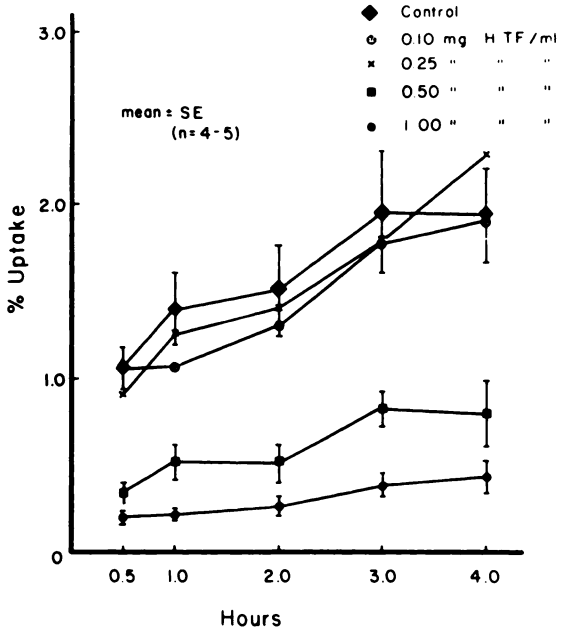

FIG. 1. Ga-67 uptake by hepatocytes in absence and presence of human transferrin (H.TF). Aliquots of 2 million liver cells in 2 ml medium were incubated with Ga-67 citrate for various periods of time, and percentage of added dose taken up by cells was determined. Each point is mean of six experiments. The s.e.m. is given for controls and for 0.5 and 1.0 mg H TF/ml.

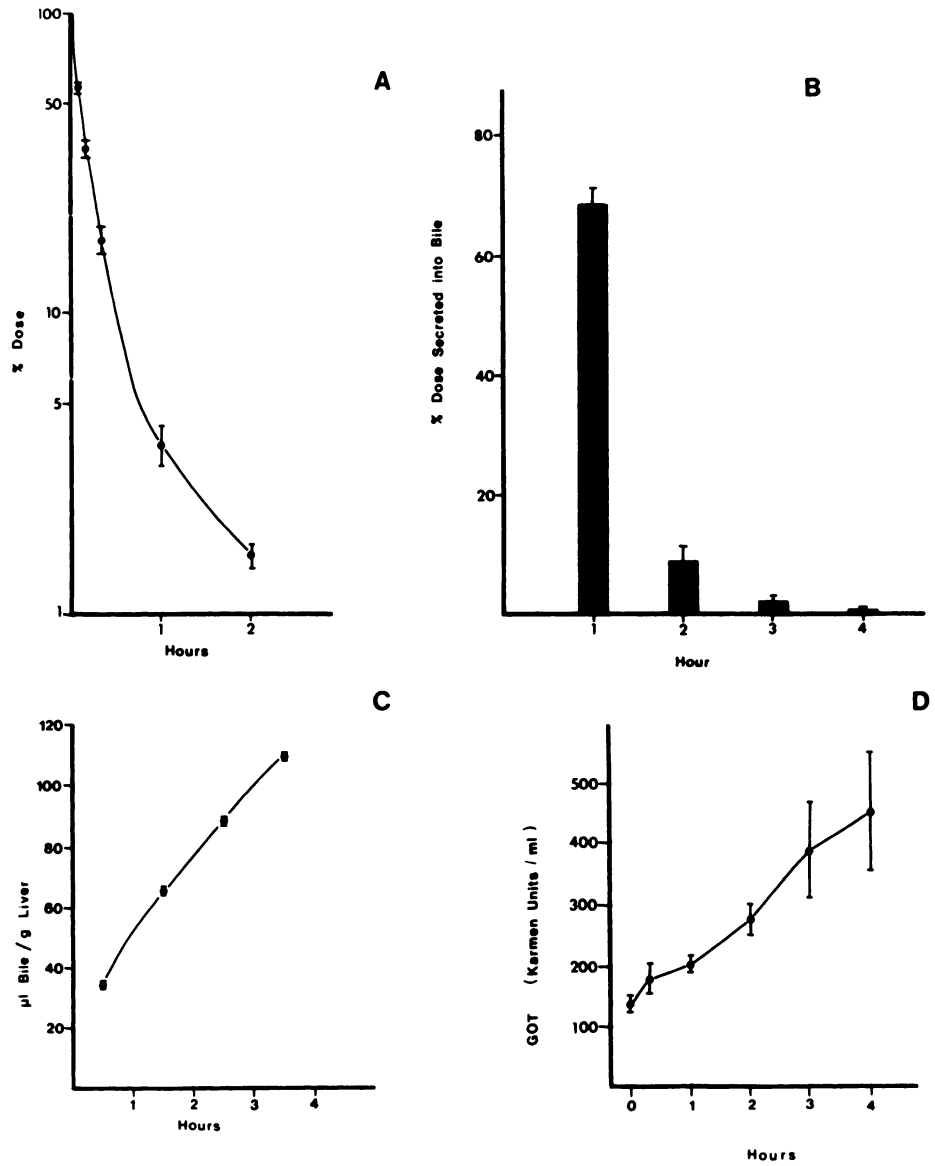

FIG. 2. Assessment of functional integrity of perfused liver. A: Clearance of Tc-99m sulfur colloid from perfusion medium; mean ± s.e.m., n = 14, for each point. B: Biliary secretion of Tc-99m DISIDA; mean ± s.e.m., n = 5. C: Cumulative output of bile during perfusion; mean ± s.e.m. D: Glutamic oxalacetic transaminase (GOT) activity in perfusion medium; mean ± s.e.m.

the day of the experiment, the UIBC and total serum iron-binding capacity (TIBC) were determined using a kit. The serum iron concentration was obtained by subtracting UIBC from TIBC values. Iron concentrations in normal and iron-deficient livers were measured by atomic absorption spectrometry after digestion of the tissue in concentrated nitric acid.

Statistical analysis. Significant differences were determined using Student's t-test for independent means (*12*).

RESULTS

Ga-67 uptake by hepatocytes. Figure 1 shows the time course of Ga-67 uptake by rat hepatocytes. The uptake increased with time, reaching a plateau after 3 hr incubation. Addition of human transferrin at low concentrations (0.1 and 0.25 mg/ml) had no effect on Ga-67 uptake. In contrast, transferrin at high concentrations (0.5 and 1.0 mg/ml) markedly inhibited Ga-67 uptake by hepatocytes (Fig. 1). Similar results were obtained with rat serum: low concentrations (0.005 to 0.125 ml serum/ml medium) had no effect, whereas high concentrations (0.25 to 0.5 ml/ml medium) markedly inhibited Ga-67 uptake by hepatocytes (data not shown).

Functional integrity of perfused livers. The viability of the isolated perfused rat livers was assessed by measuring (a) uptake of Tc-99m sulfur colloid, (b) uptake and clearance of Tc-99m DISIDA, (c) production of bile, and (d) the activity of glutamic oxaloacetic transaminase (GOT) in the perfusion medium. As shown in

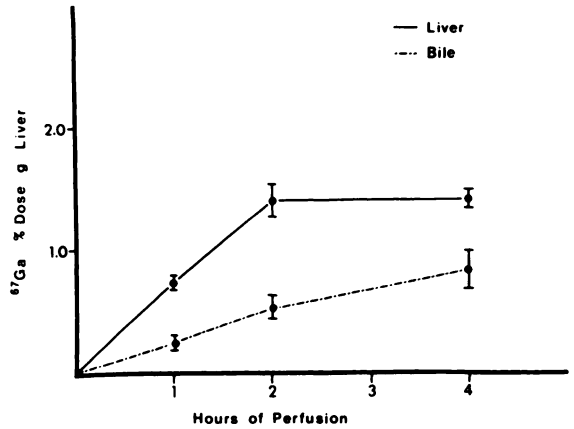

FIG. 3. Time course of hepatic Ga-67 uptake and biliary excretion in perfused rat liver system. Mean ± s.e.m.; n = 5 for each point.

Fig. 2A, the clearance of Tc-99m sulfur colloid was rapid: at 20 min 82% had been taken up by the liver and at 2 hr almost 100% was cleared from the perfusion medium. Tc-99m DISIDA, a biliary agent, was excreted rapidly into the bile; 70% of the dose appeared in the bile at 1 hr (Fig. 2B). There was a progressive production of

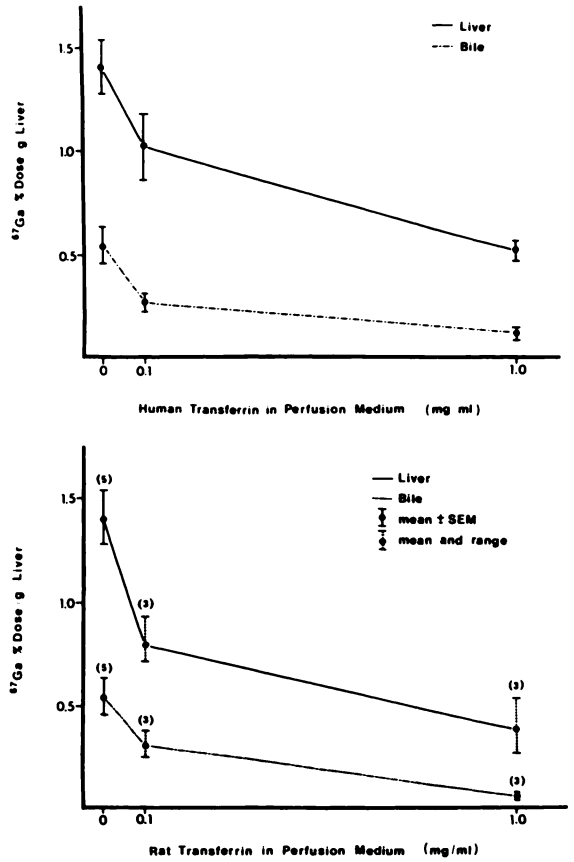

FIG 4. Upper: Effect of human transferrin on hepatic Ga-67 uptake and biliary excretion during 2 hr perfusion period. Mean ± s.e.m.; n = 5 for each point. Lower: Effect of rat transferrin on hepatic Ga-67 uptake and biliary excretion during 2 -hr perfusion period. Number of experiments in parentheses.

TABLE 1. EFFECT OF AMOUNT OF GALLIUM-67 IN PERFUSION MEDIUM (PM) ON LIVER UPTAKE AND BILIARY EXCRETION DURING A 2-hr PERFUSION

	Ga-67 added to PM		p Value
	5 μCi	50 μCi	(n = 5)
	No Transferrin in PM		
	% *⁄g liver (mean ± s.e.m.)	% *⁄g liver (mean ± s.e.m.)	
Liver	1.41 ± 0.13	1.14 ± 0.12	>0.1
Bile	0.55 ± 0.09	0.35 ± 0.05	>0.05
	Human transferrin (1 mg/ml) in PM		
Liver	0.51 ± 0.05	0.51 ± 0.09	>0.5
Bile	0.11 ± 0.03	0.07 ± 0.01	>0.2

* % of Ga-67 added to perfusion medium.

bile by the perfused livers (Fig. 2C). The activity of GOT in the medium rose from 137 Karmen units/ml at the start of the experiment (e.g., after 1-hr equilibration period) to 209 at 1 hr, 276 at 2 hr, and 454 units/ml at 4 hr (Fig. 2D). These GOT values are similar to those reported by Bartosek et al. (*13*). The above results suggest that the functions of our perfused livers were well preserved.

Ga-67 uptake and biliary secretion by perfused liver. Figure 3 shows the uptake and biliary excretion of Ga-67 by perfused livers. Uptake of Ga-67 reached a plateau 2 hr after the addition of Ga-67, indicating an equilibrium between Ga-67 uptake and excretion in the liver at that stage. On the other hand, biliary excretion of Ga-67 increased steadily over the entire 4-hr perfusion period.

The effect of transferrin was then studied. As shown in Fig. 4 (upper), human transferrin at 0.1 mg/ml or 1 mg/ml inhibited hepatic uptake as well as biliary excretion of Ga-67. Since the transferrin receptors of rat hepatocytes may not bind human transferrin, we isolated rat transferrin and then studied its effect on hepatic Ga-67 uptake and biliary excretion by perfused rat livers. As shown in Fig. 4 (lower), similar results were obtained with rat transferrin.

Since varying amounts of Ga-67 were lost during the perfusion, due to nonspecific adherence to the perfusion apparatus, we also studied Ga-67 uptake by perfused livers at two different concentrations of Ga-67 to determine whether changes in the Ga-67 concentration in the perfusion medium would affect the results. As shown in Table 1, whether 5 or 50 μCi of Ga-67 were added to the medium, there was no change in the fraction of Ga-67 taken up by the liver or excreted into the bile. This was the case in the presence or absence of transferrin. These results also showed that the absolute amounts of Ga-67 taken up by the liver or secreted into the bile were

TABLE 2. EFFECT OF LACTOFERRIN AND DEFEROXAMINE ON GALLIUM-67 UPTAKE AND BILIARY EXCRETION DURING 2-hr PERFUSION

	Controls (n = 5)	Lactoferrin (1 mg/ml PM) (n = 3)	Deferoxamine (1 mg/ml PM) (n = 5)
	% *g liver (mean ± s.e.m.)	% *g liver mean (range)	% *g liver mean (range)
Liver	1.41 ± 0.13	0.36 (0.30–0.42)	0.19 (0.12–0.23)
Bile	0.55 ± 0.09	0.014 (0.012–0.016)	0.030 (0.022–0.038)

* % of Ga-67 added to perfusion medium.

TABLE 3. GALLIUM-67 UPTAKE AND BILIARY EXCRETION DURING A 2-hr PERFUSION IN NORMAL AND IRON-DEFICIENT LIVERS

	Control	Iron deficient	p Value
	No transferrin added to perfusion medium		
Liver	1.41 ± 0.13*	2.08 ± 0.18*	<0.025
Bile	0.55 ± 0.09*	0.79 ± 0.20*	>0.2
Liver Fe	167.9 ± 9.4†	25.3 ± 1.4†	<0.001
	Human transferrin (1 mg/ml) added to perfusion medium		
Liver	0.51 ± 0.05*	1.31 ± 0.10*	<0.001
Bile	0.11 ± 0.03*	0.15 ± 0.01*	>0.2
Liver Fe	—	22.0 ± 0.19†	<0.001‡

* % of Ga-67 added to perfusion medium, per g liver; mean ± s.e.m., n = 5.

† μg Fe/g liver.

‡ Difference between controls (no transferrin added) and iron-deficient livers (with transferrin added).

proportional to the amounts of Ga-67 present in the perfusate.

Effect of lactoferrin and deferoxamine. Since transferrin inhibited Ga-67 uptake and its biliary excretion, we next studied the effects of lactoferrin and deferoxamine, both of which bind Ga-67. As shown in Table 2, both markedly inhibited hepatic uptake and biliary excretion of Ga-67.

Effect of iron deficiency. Our results indicate that transferrin inhibits Ga-67 hepatic uptake. This finding is in marked contrast to the in vivo observation that induction of iron deficiency, which increases serum UBIC, enhances Ga-67 accumulation in the liver, whereas a reduction of serum UBIC by the administration of iron or scandium reduces hepatic uptake of Ga-67 (*1–4*). We examined the possibility that enhanced hepatic uptake of Ga-67 was due to hepatic iron deficiency rather than increased serum UBIC. The iron-deficient status in the intact animals was established by measuring the serum iron concentration (66 ± 22 compared with 105 ± 31 μg/dl in normal rats), the serum UIBC (698 ± 28 compared with 349 ± 30 μg/dl in normals) and the hematocrit (26.6 ± 0.5 compared with 46.0 ± 0.5% in normals). After perfusion, hepatic iron content was also measured using atomic absorption spectrometry. As shown in Table 3, iron deficiency markedly enhanced hepatic Ga-67 uptake, but it had no effect on the biliary excretion of Ga-67. This was the case whether transferrin was present in the medium or not.

DISCUSSION

The results presented demonstrate that: (a) the liver is able to take up carrier-free Ga-67 in the absence of transferrin, and excrete it into the bile; (b) transferrin inhibits hepatic uptake of Ga-67 and its biliary excretion; and (c) iron deficiency markedly enhances hepatic uptake of Ga-67. The techniques used in this study obviate the problem inherent in the use of in vivo models, namely, too many variables that complicate the interpretation of the data.

Our finding that iron deficiency increases the accumulation of Ga-67 in the liver is in agreement with a study by Zimelman et al. (*14*), on the uptake of iron in the perfused liver. They showed that iron deficiency significantly enhances hepatic uptake of iron.

In the same study, they also demonstrated that iron (Fe-59 ferrous citrate) is taken up by the liver in the absence of transferrin, and that transferrin inhibits hepatic iron uptake to a significant degree. Since iron tends to be oxidized (and hydrolyzed) at physiological pH, and the accumulation of iron by the perfused liver was extremely rapid (over 40% of the iron was taken up within 1 min), it is likely that the iron in the perfusion medium was converted to a colloidal form and then incorporated by Kupffer cells rather than hepatocytes. In contrast, formation of Ga-67 hydroxide was prevented in our study by use of a sufficiently high concentration ratio of citrate to gallium (10^{17}: 1). The uptake of transferrin-free Ga-67 did not seem to involve phagocytosis, since: (a) Ga-67 uptake in the perfused liver occurred slowly, increasing gradually to ~8% of the dose at 2 hr; and (b) Ga-67 was excreted into the bile. Thus, carrier-free Ga-67 was handled by the liver as are other hepatobiliary agents, and not as a colloid.

In an earlier study we reported that a significant amount of Ga-67 in the gastrointestinal contents originates from the bile (*15*). Our present data confirm the excretion of Ga-67 from the liver through the bile. As can be expected, biliary excretion is dependent on the amount of Ga-67 liver uptake. It is markedly decreased when liver uptake is reduced in the presence of transferrin.

Since there is considerable evidence suggesting that there are transferrin receptors on the plasma membrane

of rat hepatocytes (*16*), one may argue that only trace amounts of transferrin are necessary to promote Ga-67 uptake. It is possible that the bovine serum albumin used in this study had trace contamination of transferrin, and that further addition of transferrin to the system resulted in inhibition of hepatic uptake due to competitive inhibition of receptor binding of the transferrin Ga-67 complex by transferrin. However, using electrophoresis we were unable to detect any contamination of transferrin in the bovine serum albumin used in this study. In addition, we have also studied the effect of low concentrations of human transferrin (2 and 10 μg/ml) on the hepatic uptake and biliary secretion of Ga-67, and there was no effect. Moreover, the serum concentration of transferrin is on the order of 2 mg/ml (*17*). Thus, the liver is normally exposed to a relatively high concentration of transferrin. Our results suggest that when the plasma levels of Ga-67 and intrahepatic iron stores remain constant, the higher the transferrin concentration or UIBC, the lower the hepatic uptake of Ga-67.

Our demonstration that iron deficiency enhances hepatic uptake of Ga-67 is of particular interest. Hayes et al. (*3*), reported that induction of iron-deficiency anemia in rats bearing hepatoma markedly increased hepatic uptake of Ga-67 (a nearly 400% increase), whereas intravenous injection of apo-transferrin increased hepatic uptake by only 60%, although the serum UIBC was similar in both situations (approximately 500 μg/dl, as compared with the normal 200 μg/dl). This discrepancy is most likely due to the iron status of the liver.

We propose that the hepatic uptake of Ga-67 is determined by at least three factors: plasma Ga-67 level, UIBC, and the iron status of the liver. Although these factors may be interrelated, the degree to which they influence hepatic Ga-67 uptake is likely to be quite different. For example, when the serum transferrin binding sites are saturated with iron or scandium, Ga-67 blood levels are greatly reduced due to enhanced urinary excretion, which leads to decreased Ga-67 uptake by the liver. The decreased UIBC and elevated hepatic iron stores may play a secondary role in liver uptake of Ga-67 in this instance. In contrast, in the iron-deficient state, depletion of the hepatic iron stores seems to be the determining factor in liver accumulation of Ga-67. Ga-67 blood levels are actually lower than normal due to increased hepatic and splenic uptake (*3*). According to our studies, the increased UIBC should, in fact, have an inhibitory effect on hepatic Ga-67 uptake; however, this effect is obviously outweighed by the increase in intrahepatic sites available for Ga-67 binding. On injection of transferrin, iron is possibly mobilized from the "labile" iron pool in the hepatocytes, hence, a moderate increase in Ga-67 binding in the liver is observed.

The mechanism of Ga-67 uptake by the liver remains unclear. It is also unclear how iron deficiency enhances hepatic Ga-67 uptake. Further studies are needed to clarify these points.

FOOTNOTES

* Millipore Corporation, Freehold, NJ.
† Gibco Laboratories, Grand Island, NY.
‡ Harlan, Walkersville, MD.
§ MRA Corporation, Clearwater, FL.
¶ Amicon Corporation, Lexington, MA.
** Ralston Purina Company, Richmond, IN.

ACKNOWLEDGMENTS

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