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Effects of Desferrioxamine on ^{67}Ga Distributions in Tumor-Bearing Mice (40561)

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The radiotracer ^{67}Ga-labeled citrate has been used clinically for tumor detection since 1969 (1), though reasons for its tumor affinity remain uncertain. However studies in several different systems have demonstrated certain analogies between ^{67}Ga and ferric Fe. Tissue culture studies showed that the presence of transferrin in the culture medium enhanced the uptakes of both ^{67}Ga and $^{59}Fe^{3+}$ by cultured mouse tumor cells (2, 3) and cultured human lymphoblasts (4). In contrast, *in vivo* studies have shown few direct analogies between ^{67}Ga and ^{59}Fe distributions in either normal or tumor-bearing mice. In particular, tumor tissues showed high ^{67}Ga and low ^{59}Fe uptakes, while for the hemopoietic tissues the reverse applied (5).

It is established that transferrin is the protein responsible for ^{67}Ga-serum binding (6), and more recently ^{67}Ga-lactoferrin binding has also been noted (7). Our own dialysis studies (unpublished observations) have shown that ^{67}Ga-serum binding becomes markedly reduced in the presence of the iron-chelating agent, desferrioxamine (8). This suggested that desferrioxamine can chelate ^{67}Ga also and that the agent might serve to modify ^{67}Ga distributions *in vivo*.

In this paper we describe further studies of ^{67}Ga distributions in mice, to examine changes due to iv administered desferrioxamine. Their primary purpose was to enlarge our fundamental understanding of the tracer's tumor-seeking properties.

Materials and methods. Detailed descriptions of the mice, tumors, tracers, and basic procedures for these studies have been given previously (4).

All injections were given iv in the tail vein as 0.3 ml vol, with the following materials used in the specified combinations or sequences—carrier-free ^{67}Ga-labeled (0.2 μCi per mouse), [^{59}Fe]ferric citrate (0.03 μCi, 10 ng Fe per mouse), and desferrioxamine (0–5 mg per mouse). The desferrioxamine (Desferal, CIBA–GEIGY, Australia) was prepared as appropriate dilutions in 0.9% NaCl and in some experiments was complexed with ^{59}Fe immediately prior to injection. Used in this form, ^{59}Fe offered a means of monitoring desferrioxamine's tissue distribution and kinetics. Alternative forms for ^{59}Fe were as—^{59}Fe-labeled citrate included with the ^{67}Ga injection, or ^{59}Fe-labeled mouse serum (60-min incubation, 37°) injected minutes before sacrifice for measurements of serum spaces of tissues of interest.

At specified times after injections animals were killed and tissue samples were taken, weighed, and counted for ^{67}Ga and ^{59}Fe activities in a dual-channel scintillation spectrometer (^{67}Ga, 70–110 keV; ^{59}Fe, 600–1500 keV). Aliquots of injected doses (A_0) were also counted and results were expressed as tracer concentrations in the various tissues relative to the injected dose, i.e, as $\%A_0$ per gram wet weight.

Results. Given even 1 hr after administration of ^{59}Fe-labeled citrate, desferrioxamine produced no detectable changes in ^{59}Fe tissue distributions. Throughout this work, we are primarily concerned with the ^{67}Ga changes.

Effects of desferrioxamine, given prior to or simultaneous with ^{67}Ga. In mice receiving 2 mg desferrioxamine together with ^{67}Ga and ^{59}Fe (i.e., as a preincubated mixture), tracer concentrations in all tissues 3 hr later were reduced about 10-fold relative to controls receiving no desferrioxamine. Presumably high proportions of both ^{67}Ga and ^{59}Fe remained desferrioxamine-bound *in vivo* and were thus excreted.

In mice receiving 2 mg desferrioxamine 2 hr before tracer injections, ^{67}Ga distributions were unchanged relative to their untreated controls, i.e., following the chelating agent's excretion, the ^{67}Ga binding properties of serum or tissues remained unaffected.

Desferrioxamine given hours after ^{67}Ga. Normal mice received ^{67}Ga citrate 3 hr prior to the injection of ^{59}Fe-labeled desferrioxamine (1.5 or 5 mg desferrioxamine per mouse), and were sampled at times ranging 2 min to 24 hr postdesferrioxamine. Figure 1

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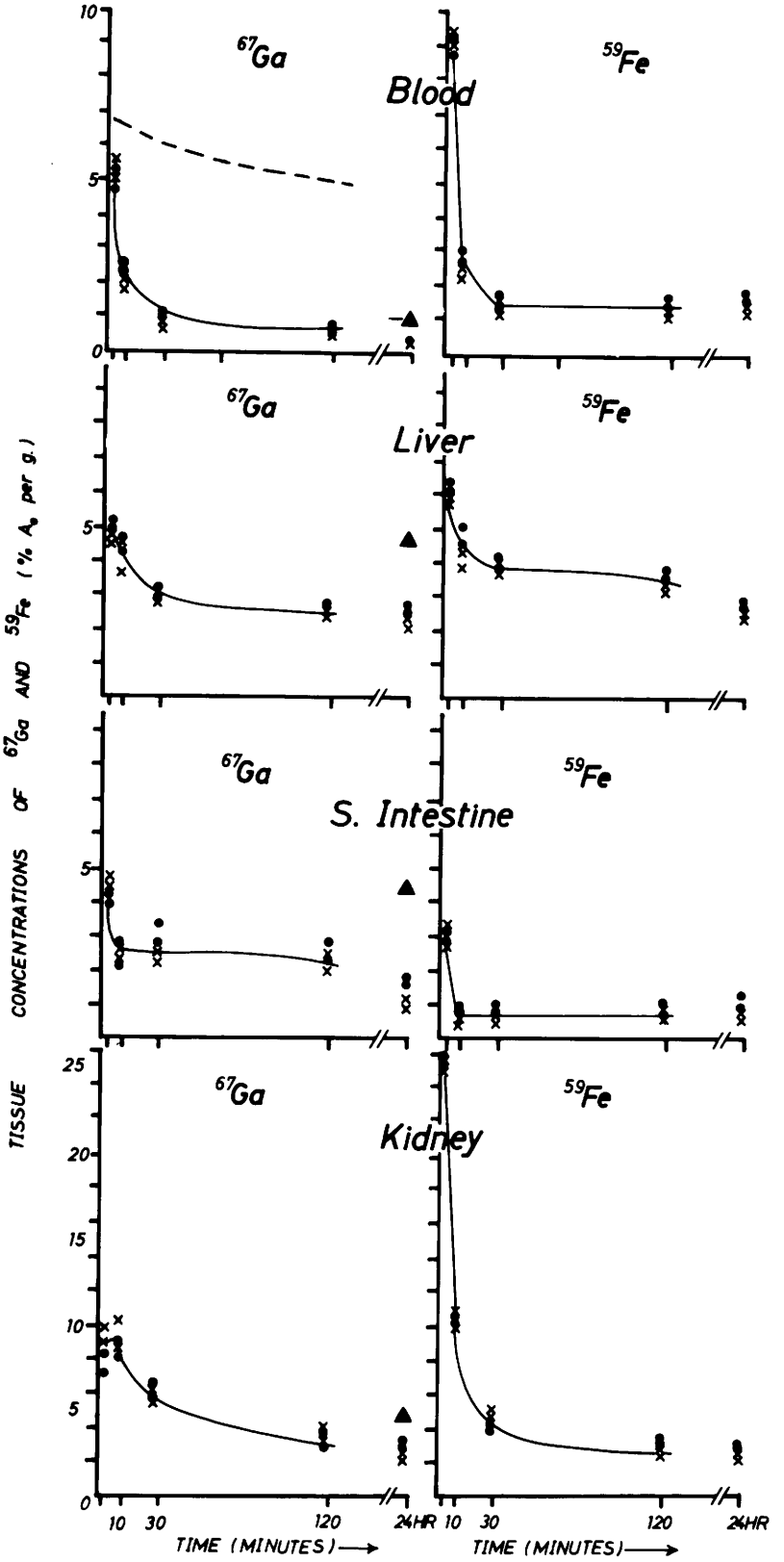

FIG. 1. Kinetics of desferrioxamine-induced changes in ^{67}Ga tissue distributions. Normal Balb/c mice (males, 6 weeks) given [^{59}Fe]desferrioxamine (0.03 μCi, 10 ng Fe) at 3 hr post ^{67}Ga injection. Tissue concentrations (%A_0 per g) measured at 2, 10, 30, and 120 min and finally 24 hr postdesferrioxamine. (●) Individual values, 1.5 mg desferrioxamine per mouse (two mice per time point). (×) Individual values, 5 mg desferrioxamine per mouse. (▲) Mean values for three control animals (24 hr post 0 mg desferrioxamine). (——) Expected blood clearance for control animals (5).

shows the time variation of ^{67}Ga concentrations for tissues of interest, together with ^{59}Fe measurements describing desferrioxamine kinetics. It includes 24 hr ^{67}Ga measurements on untreated mice and, using previously published data (5), it also shows the expected clearance of vascular ^{67}Ga for the untreated case. Following desferioxamine injection, the accelerated clearance of ^{67}Ga from the blood followed quite closely the rapid clearance of ^{59}Fe and the transiently high renal levels of both tracers identify this as the route of excretion of desferrioxamine-chelated ^{67}Ga and ^{59}Fe. For other soft tissues (excepting liver) ^{59}Fe concentrations declined sharply in parallel with blood clearance, while ^{67}Ga concentrations for all tissues approached an intermediate level. The effects of 1.5 and 5 mg doses of desferrioxamine were not significantly different. The effects of desferrioxamine were essentially complete 1–2 hr after administration.

Desferrioxamine effects, given days as opposed to hours after ^{67}Ga. Table 1 shows measurements using CBA mice bearing a solid subcutaneous tumor (RILQ lymphoma), when desferrioxamine was given at either 1 or 48 hr post ^{67}Ga. In accord with previously reported findings, the control measurements showed that ^{67}Ga tissue distributions were essentially established at 1 hr and, apart from the gradual vascular clearance, were relatively unchanged at 48 hr. Nevertheless desferrioxamine's effects on the 1- and the 48-hr distributions were very different. In the former case, ^{67}Ga concentrations in tumor and in other tissues became markedly reduced following 1.5 mg desferrioxamine, and the table also gives some kinetic data on this clearance. In contrast and within the limits of experimental noise, changes in the 48-hr distributions were small, even following 5 mg desferrioxamine. This has been the general experience of other 48-hr experiments, where desferrioxamine's clearance of the vascular component was the only noteworthy feature.

Results of a related experiment (Table II) confirm the pronounced differences between 2 and 48 hr ^{67}Ga distributions with respect to desferrioxamine clearance. In this experiment we also estimated serum spaces of tissues of interest, by sampling ^{59}Fe tissue concentrations at 3 min following injections of ^{59}Fe-labeled mouse serum. Applied to the ^{67}Ga data, these estimations allowed correction for the presence of serum-bound ^{67}Ga in the tissue uptake measurements. The table includes the corrected values for 3-hr cell-bound ^{67}Ga and, while the serum-space corrections were not insignificant for other tissues, tumor uptake was almost totally due to cell-bound ^{67}Ga.

Finally we have found that effects on 3-hr ^{67}Ga distributions could be detected down to 0.1 mg desferrioxamine per mouse, for the CBA mice used in the majority of these experiments. Qualitatively similar desferrioxamine effects were observed using normal or tumor-bearing mice of other strains, but the threshold dose for significant vascular clearance was generally higher, e.g., ~0.5 mg for the Balb/c mice of Fig. 1.

TABLE I. EFFECTS OF DESFERRIOXAMINE ADMINISTRATIONS AT 1 and 48 hr POST ^{67}Ga.[a]

	DFA (1 hr)				DFA (48 hr)		
	0[b]	1.5 mg			0	1.5 mg	5 mg
Tissue	30 min	2 min	15 min	60 min	120 min	120 min	120 min
Blood	10, 11.4	9.5, 7.3	2.8, 2.3	0.8, 0.5	0.18, 0.21	—	—
Liver	2.9, 2.6	2.5, 1.9	1.5, 1.3	1.0, 0.9	3.1, 3.9	2.5, 2.8	2.3, 2.1
Spleen	2.7, 2.9	2.7, 2.0	1.5, 1.0	1.1, 0.9	2.3, 1.8	1.5, 2.1	1.7, 2.3
Kidney	4.2, 4.8	9, 8.5	11.7, 15	2, 4.7	2.3, 2.7	2.8, 2.6	2.8, 2.1
Small intestine	4, 3.4	2.4, 2.3	1.9, 1.6	1.8, 1.3	1.9, 2.4	1.5, 2.3	1.3, 1.8
Bone	7.2, 6.9	5.4, 5.3	3.2, 4.0	3, 3.5	8, 13	8, 12.8	8.2, 11
Tumor	6–8.3	5.5–8.3	3.7–4.5	2–3.3	8–11	6–9	7–10

[a] ^{67}Ga distributions in RILQ/CBA mice (males, 6 weeks). Desferrioxamine (0, 1.5, or 5 mg per mouse) given at 1 or at 48 hr post ^{67}Ga injections, and animals sacrificed at specified times postdesferrioxamine (i.e., 2, 30 ... 120 min). Tissue uptakes expressed as $\%A_0$ per gram, and give individual determinations (two mice per group) and the ranges for tumor tissue (three tumor samples per mouse).

[b] One-hour controls sampled at +30 min, intermediate between 2 and 60 min, and distributions would be relatively constant over that period (4).

TABLE II. CLEARANCE OF CELL-BOUND ^{67}Ga AT 3 AND 48 hr.[a]

	^{67}Ga						DFA-Inaccessible fraction (%)	
	3 hr		48 hr		^{59}Fe-Labeled serum	Cell-bound ^{67}Ga (3 hr, 0)		
	0	DFA	0	DFA			3 hr	48 hr
Whole blood	7.6 ±0.7	0.75 ±0.06	0.15 ±0.06	0.05 ±0.006	49.0 ±3.7			
Serum	13.0	1.25			80.0			
Liver	3.4 ±0.3	1.4 ±0.6	2.5 ±0.3	2.7 ±0.26	8.6 ±0.3	2.0	70	100
Spleen	2.3 ±0.6	0.7 ±0.15	1.1 ±0.3	1.65 ±0.36	4.0 ±1.8	1.65	46	100
Kidney	4.9 ±0.9	3.4 ±0.75	2.2 ±0.35	2.2 ±0.4	7.1 ±0.46	3.75	—	100
Small intestine	3.7 ±0.4	2.8 ±0.25	1.7 ±0.3	1.9 ±0.2	1.2 ±0.4	3.5	80	100
Bone	12.9 ±1.0	7.8 ±0.15	12.2 ±2.0	12.4 ±1.25	3.0 ±0.45	12.4[b]	60	100
Tumor	10.0 ±0.4	4.6 ±0.9	8.2 ±0.7	8.9 ±1.1	1.0 ±0.5	9.85	47	100

[a] Tracer distributions in RILQ:CBA mice (males, 6 weeks). ^{67}Ga: at 3 and 48 hr post ^{67}Ga injections, 1 hr postdesferrioxamine (0 and 1.5 mg per mouse). ^{59}Fe: at 3 min postinjection of ^{59}Fe-labeled mouse serum. For either tracer, tissue concentrations (%A_0 per g) refer to mean values (±1 SD) for four mice per group. Cell-bound ^{67}Ga: ^{67}Ga values (3 hr, 0) corrected for serum-bound ^{67}Ga contributions using tissue/serum ^{59}Fe ratios; e.g., liver = 3.4 − (8.6/80·13) = 2.0. DFA-inaccessible fraction = (+DFA) ^{67}Ga concentrations/cell-bound ^{67}Ga, e.g., (liver, 3 hr: 1.4/2.0 × 100%).

[b] Bone mineral-bound ^{67}Ga.

Discussion. We are concerned chiefly with the significance of desferrioxamine-induced clearance of ^{67}Ga tissue uptakes and the effect's dependence on the time interval between ^{67}Ga and desferrioxamine administrations. Injected 1–3 hr post ^{67}Ga, desferrioxamine caused substantial reductions in ^{67}Ga concentrations of tumor and other tissues; given at 48 hr, however, even relatively large doses had little effect. From previous *in vitro* findings on desferrioxamine's effect on ^{67}Ga-serum binding, the clearance of ^{67}Ga from serum or extracellular spaces is easily understood. However, regarding tumor tissue in particular, previous kinetic findings (5) and the extracellular space measurements of this study (Table II) demonstrated that the tumor's ^{67}Ga content was almost totally cell bound, even at 1-hr post ^{67}Ga injection. Thus it seems that a proportion of cell-bound ^{67}Ga is available for desferrioxamine chelation and removal during the initial phase, and this proportion diminishes progressively with time. Presumably desferrioxamine can distinguish between low- and high-affinity cell binding (i.e., relative to the ^{67}Ga-desferrioxamine affinity) and/or between binding locations which are accessible and inaccessible to desferrioxamine.

From such findings we make this tentative hypothesis. Following iv injection ^{67}Ga moves quite rapidly from the serum to undergo cellular binding which is still reversible by desferrioxamine; more gradually this component becomes irreversibly bound. This "two-phase" hypothesis could plausibly be discussed in terms of membrane and intracellular binding. The former component could readily receive extracellular ^{67}Ga and be equally accessible to desferrioxamine chelation. This membrane-bound ^{67}Ga could then become more gradually internalized and irreversibly bound, e.g., lysosomes (9). The present study does not, however, identify these as the binding locations; nor does it connect directly with tissue culture or subcellular distribution studies where the form and location of reversibly bound ^{67}Ga might not be preserved following washing or other treatments undergone by the sample.

Transferrin's participation in the ^{67}Ga cellular uptake process has been clearly demonstrated in several tissue culture systems (2–4). Assuming this participation applies *in vivo*, transferrin may act either to establish the reversibly bound component, i.e., as a ^{67}Ga-transferrin complex, or to convert reversibly bound ^{67}Ga to the irreversibly bound state.

The present findings do not distinguish between these possibilities, nor do they offer any evidence bearing on the suggested role of lactoferrin (7). Other experimental manipulations causing changes in ^{67}Ga *in vivo* distributions have been reviewed by Hayes (10) and supplemented by Sephton (11). Essentially these relate to effects of competition by other cations (e.g., carrier gallium, scandium, and iron) against ^{67}Ga binding, which are distinct from though complementary to effects of ^{67}Ga chelation.

This study's main contribution has been in the clear demonstration of two levels of cell-bound ^{67}Ga. The readily chelated component is established soon after ^{67}Ga injection and converts more gradually to an irreversibly bound state. The ^{67}Ga avidity of the tumor of this study was expressed at least as much in the former as in the latter component, and this may be an important distinguishing characteristic of ^{67}Ga-avid cells. Desferrioxamine itself promises to be a useful tool in further experimental, possibly even clinical ^{67}Ga studies.

Summary. The iron-chelating agent desferrioxamine caused approximately 10-fold reductions in ^{67}Ga vascular concentrations and substantial ($\sim \times 2$) reductions in ^{67}Ga concentrations of other tissues when injected iv into mice at 2 hr after ^{67}Ga injection. Given 48 hr post ^{67}Ga however, its only noteworthy effect was on the vascular compartment. Tumor was distinguished from other soft tissues by high concentrations of cell-bound ^{67}Ga, of which an appreciable fraction could be chelated and cleared by desferrioxamine given 2 hr after ^{67}Ga. This fraction diminished with time and, at 48 hr, desferrioxamine caused no significant clearance of tumor-bound ^{67}Ga.

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