Ga-67 and Fe-59 Distributions in Mice

Robert G. Sephton, George S. Hodgson, Sunil De Abrew, and Alan W. Harris

Cancer Institute and Walter and Eliza Hall Institute for Medical Research, Melbourne, Australia

Tissue distributions of i.v.-injected Ga-67 citrate and [^{59}Fe] ferric citrate were measured in normal mice and in lymphoid-tumor hosts. The study arose out of previously reported tissue-culture work showing marked transferrin stimulation of Ga-67 and Fe-59 uptakes by cultured cells from mouse lymphoid tumors. In vivo, however, no obvious correlation was found between Ga-67 and Fe-59 tissue distributions; indeed, Ga-67 showed high affinity for tumor tissue and low affinity for hemopoietic tissues, while for Fe-59 the reverse applied. Taken together, these comparisons of kinetics and distributions for Ga-67 and Fe-59 suggest that a tissue's avidity for Ga-67 is strongly influenced by other factors besides the cell population's capacity for transferrin interactions.

J Nucl Med 19: 930–935, 1978

Since its tumor affinity was first reported (*1*), Ga-67 citrate has been widely used in diagnostic imaging of neoplastic and inflammatory lesions (*2*). For certain diseases (e.g., in many cases of malignant lymphoma) the tracer's preferential uptake in diseased tissue can be remarkably high, denoting an active process that is probably of fundamental as well as practical significance. The mechanism by which Ga-67 becomes irreversibly cell-bound has not yet been elucidated.

The in vivo studies described in this paper were stimulated by demonstrations of in vitro analogies between Ga-67 and Fe^{3+}. The phenomenon of Ga-67 transferrin binding has already been reported (*3,4*). Of greater relevance are tissue-culture studies showing that the addition of transferrin to the culture medium promotes large increases in Ga-67 and Fe-59 uptakes by cultured mouse lymphoid-tumor cells (*5,6*). We have been interested, therefore, in exploring possible in vivo correlations between Ga-67 and the classical transferrin label, $^{59}Fe^{3+}$ (*7*). In this paper we present measurements of Ga-67 and Fe-59 tissue distributions in normal mice and in tumor hosts bearing solid tumors from lymphoid lines previously tested in tissue culture.

MATERIALS AND METHODS

Animals. Mice used in this work were of either Balb/c or CBA strains, their ages ranging from 40 to 90 days. The CBA mice were in a specific pathogen-free state, whereas (except in one experiment) the Balb/c mice had been bred in an exposed environment and bore normal bacterial contamination. Except where otherwise indicated, each self-contained experiment was controlled for the animals' sex, age, and environmental history.

Tumors studied were mouse lymphomas (RILQ, WEH17) and mouse myelomas (HPC108, P3K), all being available as cultured cell lines. The former pair originated as radiation-induced thymic lymphomas (*8,9*), the latter as mineral-oil-induced myelomas (*6,10*). With the exception of RILQ (CBA), host animals were Balb/c mice. The hosts were injected subcutaneously with cultured cells (about 5 million cells per mouse) and, after 1–2 wk, developed macroscopic solid tumors.

Tracers. Radiotracers* were Ga-67 citrate (specific activity >30 mCi/μg) and [^{59}Fe] ferric citrate (~10 mCi/mg). Each was diluted to appropriate concentrations in 0.9% NaCl and filtered† immediately before use. Typical administrations were 0.2 μCi Ga-67 and 0.03 μCi Fe-59, as a single mixed injection into the tail vein.

Received Oct. 12, 1977; revision accepted Jan. 18, 1978.

For reprints contact: R. G. Sephton, Cancer Institute, 481 Lt. Lonsdale St., Melbourne, 3000, Australia.

TABLE 1. Ga-67 AND Fe-59 KINETICS IN NORMAL MICE: THE VASCULAR COMPARTMENT

Time (hr)	Plasma		Cells	
	Ga-67	Fe-59	Ga-67	Fe-59
0.05	47.0	54.0	1.0	2.0
0.33	20.0	28.0	0.6	3.7
1.0	21.0	21.5	0.9	3.8
3.0	15.4	7.3	0.7	6.8
7.0	8.7	1.8	0.3	10.6
24.0	1.9	0.36	0.1	27.5
72.0	0.24	0.11	0.01	40.5

Tracer concentrations (as % A_0 per g) in plasma and washed red cells.
Pooled blood from groups of Table 2.

Tissue sampling. At the specified time after injection, the ether-anaesthetized animals were decapitated and blood was immediately collected into preweighed heparinized tubes. Tissues taken included liver, spleen, kidneys, proximal small intestine (about 5 cm length), bone (i.e., both femurs and tibiae), and pieces of macroscopically viable tumor. Intestinal contents were thoroughly expressed and discarded. Bones were scraped clean and, in some instances, marrow was blown out with 0.9% NaCl and kept separately. The samples were immediately weighed, then counted for Ga-67 and Fe-59 radioactivities in a dual-channel well scintillation spectrometer (70–110 keV for Ga-67; 600–1400 keV for Fe-59). Also counted were an aliquot of the injected dose (A_0) and separate Ga-67 and Fe-59 standards to permit "spill-over" corrections where necessary. Tissue concentrations of each tracer were expressed as $\%A_0$ per gram wet weight, and it is in that form that all tissue distributions are presented in the following results.

RESULTS

These measurements referred to distributions of tracer quantities (i.e., $<10^{-5}$ μg Ga, $<10^{-2}$ μg Fe), administered as a mixed injection. Preliminary work had shown that single- or dual-tracer experimental designs gave equivalent results and the latter approach was preferred throughout these studies, not only for convenience but because it permitted direct Ga-67:Fe-59 comparisons for each tissue sample, in each animal.

Tracer distributions in normal mice. Apart from a gradual and progressive clearance from the plasma, the Ga-67 tissue distribution was essentially established within about an hour after injection. The more "active" soft tissues were liver, spleen, kidney, and intestine, which attained Ga-67 concentrations in the region of 3–5% A_0 per gram. For the less active soft tissues (e.g., muscle or lung), Ga-67 concentrations were about an order of magnitude lower, and by 24 hr blood concentrations had also fallen to low levels. Bone concentrations were high (i.e., 10–20%) and marrow concentrations were invariably small. On subdivision of bone samples into joint and shaft sections, the shaft proved generally to have about half the joint's concentration. The Fe-59 distribution patterns were distinguished by high concentrations in spleen, marrow, and blood, reflecting processes of Fe-59 transfer between hemopoietic tissues and the circulation; otherwise, the tracer was distributed fairly diffusely throughout the other soft tissues.

The main points are illustrated by Tables 1 and 2, showing the two tracers' distributions in normal

TABLE 2. Ga-67 AND Fe-59 KINETICS IN NORMAL MICE: TISSUE DISTRIBUTIONS

Tissue	1 hr		3 hr		7 hr		24 hr		72 hr	
	Ga-67	Fe-59	Ga-67	Fe-59	Ga-67	Fe-59	Ga-67	Fe-59	Ga-67	Fe-59
Blood	10.0	12.0	6.7	7.0	3.7	6.1	0.8	13.0	0.15	19.0
Liver	3.5	6.4	4.2	7.8	4.1	7.8	4.5	8.6	3.5	6.6
	±0.35	±0.55	±0.46	±0.48	±0.35	±0.6	±0.1	±0.9	±0.4	±0.7
Spleen	2.8	58.0	2.9	98.0	2.7	117.0	2.3	34.6	1.9	7.9
	±0.45	±23.0	±0.58	±29.0	±0.29	±17.7	±0.35	±6.7	±0.26	±3.4
Kidney	4.3	7.0	4.3	4.5	4.3	3.8	3.6	5.7	2.1	5.6
	±0.28	±1.5	±0.21	±0.7	±0.38	±0.36	±0.5	±0.42	±0.31	±0.58
S. intest.	4.2	6.6	9.5	8.6	4.3	7.4	4.5	8.6	1.2	1.5
	±0.64	±0.66	±0.35	±1.6	±0.89	±1.2	±0.59	±3.0	±0.34	±0.29
Bone (+ marrow)	6.8	14.5	7.1	15.2	8.2	16.8	12.8	10.0	9.0	2.0
	±0.8	±2.0	±1.6	±3.1	±1.5	±3.3	±1.9	±1.7	±1.4	±0.63
Bone (— marrow)	—	—	—	—	7.7	7.3	—	—	—	—

Tissue distributions (% A_0 per g) at specified times after i.v. injection.
Mean values and standard deviations from five mice per group.
Normal mice: male, 60-day-old Balb/c.

TABLE 3. Ga-67 AND Fe-59 TISSUE DISTRIBUTIONS: VARIATIONS WITH AGE AND BACTERIAL LOAD

Tissue	39-d (SPF)		39-d (E)		90-d (E)	
	Ga-67	Fe-59	Ga-67	Fe-59	Ga-67	Fe-59
Blood	0.66 ±0.4	12.8 ±0.2	0.6 ±0.3	15.5 ±1.4	0.8 ±0.2	11.5 ±2.2
Liver	1.7 ±0.6	18.9 ±4.0	3.5 ±0.4	9.5 ±3.6	3.2 ±0.8	7.8 ±3.0
Spleen	0.9 ±0.45	26.0 ±11.0	1.6 ±0.2	35.4 ±9.4	1.4 ±0.3	19.5 ±3.9
Kidney	2.5 ±0.65	11.5 ±1.5	3.0 ±0.3	6.9 ±1.3	2.8 ±0.5	5.0 ±0.9
S. intestine	1.2 ±0.2	6.6 ±0.2	2.3 ±0.6	4.7 ±0.9	2.4 ±0.5	4.1 ±0.9
Bone	24.0 ±2.5	9.3 ±1.2	18.3 ±6.6	8.0 ±2.3	6.6 ±1.7	6.3 ±1.4

Tissue distributions at 24 hr, for 39- and 90-day Balb/c mice (as % A_0 per g tissue).

Mean values and standard deviations from five mice per group.

SPF: specific-pathogen-free (av. wgts. & s.d. (g): body 20, liver 0.98 ± 0.16, spleen 0.08 ± 0.019); E: naturally exposed (av. wgts. & s.d. (g); 39-d: body 21, liver 1.25 ± 0.18, spleen 0.123 ± 0.17); (av. wgts. & s.d. (g); 90-d: body 27, liver 1.6 ± 0.07, spleen 0.12 ± 0.013).

mice, for sampling times extending up to 72 hr. Table 1, presenting measurements of the vascular compartment, shows similar initial volumes of distribution, marked differences in plasma clearance rates, and negligible Ga-67 incorporation into circulating cells. Table 2 shows Ga-67 and Fe-59 distributions for tissues of principal interest. For Ga-67, the typical pattern was quite quickly established and, except for a gradual loss from the intestine, it remained essentially constant for the duration of the study. For Fe-59, the noteworthy feature was high concentrations in spleen and marrow, whose rise and fall marked the conversion from plasma to hemoglobin Fe-59. For other tissues, Fe-59 concentrations were relatively low and static although, as applied for Ga-67, there was a significant long-term decline in intestinal Fe-59 concentrations.

For the present purpose these tables adequately describe the main features of normal Ga-67 and Fe-59 distributions. Table 3 is included, however, to document some of the influences that have been observed in the course of this work—namely, age and bacterial load—and we direct our remarks to the Ga-67 patterns. Bone concentrations are invariably high in younger animals, falling quite markedly with age. Soft-tissue Ga-67 uptakes are significantly decreased in specific pathogen-free, as opposed to naturally exposed mice. In interpreting in vivo re-

TABLE 4. Ga-67 AND Fe-59 TISSUE DISTRIBUTIONS IN TUMOR HOSTS

Tissue	3-hr		6-hr		24-hr	
	Ga-67	Fe-59	Ga-67	Fe-59	Ga-67	Fe-59
Blood	6.0 (5.7–6.4)	6.0 (5.6–6.6)	4.3 (3.9–4.7)	6.5 (4.7–7.6)	0.5 (0.4–0.6)	16.5 (13.6–18.6)
Liver	2.35 (2.3–2.5)	6.2 (5.9–6.5)	3.0 (2.7–3.2)	7.0 (6.8–7.2)	3.2 (2.9–3.5)	7.6 (6.9–8.7)
Spleen	1.3 (1.1–1.4)	53.0 (45–59)	1.6 (1.2–1.8)	46.0 (36–61)	1.7 (1.4–1.8)	24.5 (23.5–26.5)
Kidney	4.0 (3.8–4.3)	3.2 (3.0–3.5)	3.8 (3.0–4.6)	3.0 (2.5–3.5)	2.8 (2.5–2.9)	4.1 (3.8–4.8)
S. intestine	1.2 (1.0–1.5)	5.4 (4.9–6.0)	1.6 (1.2–2.4)	6.8 (5.1–8.7)	0.8 (0.8–0.9)	3.8 (3.3–4.0)
Bone	10.0 (9.7–10.4)	19.3 (18.2–20.1)	11.6 (11–15)	25.3 (24.9–26.4)	11.5 (10.6–12)	12.0 (11.2–12.5)
Tumor	9.2 (8.2–11)	3.2 (2.4–3.4)	10.7 (9.3–15)	4.0 (3.5–4.2)	11.6 (10.4–13.4)	4.0 (3.2–4.8)

RILQ lymphoma (CBA host), average tumor wt 1.5 g.

Mean values (% A_0 per g) and ranges from three mice per time point, three tumor pieces per mouse.

sults—e.g., in terms of tumor Ga-67 avidities—the experimenter must be aware of a range of possible influences on the Ga-67 background—i.e., normal tissue distribution.

Tracer distributions in tumor hosts. Table 4 presents Ga-67 and Fe-59 distributions in CBA mice bearing the RILQ thymic lymphoma as a solid subcutaneous tumor. The measurements refer to sampling times of 3, 6, and 24 hr following the dual-tracer injection. Excluding tumor, the tissue distributions were qualitatively similar to those observed for normal mice. Tumor tissue reached Ga-67 concentrations several times those of the other "active" soft tissues, but there was no evidence of any preferential Fe-59 uptake by tumor. The kinetic features paralleled the previous findings for normal mice: Ga-67 distributions were quickly established, whereas Fe-59 kinetic patterns highlighted that tracer's turnover by the hemopoietic system. A subsequent experiment confirmed that the tumor's strong affinity for Ga-67 becomes manifest very early—even at 30 min after injection (Table 5).

Table 6 compares Ga-67 and Fe-59 tissue distributions for several different tumor models. As suggested by previous results (Tables 2 and 4), sampling at about 7 hr after injection was practiced in order to minimize vascular contributions within tissue-uptake measurements. With some reservations about the P3K myeloma, each tumor type showed an appreciable in vivo affinity for Ga-67, and in no case was there any preferential tumor uptake of Fe-59.

Note that Table 6 refers to experiments performed on different occasions, and there were age and tumor-size variations across the groups represented there. Accordingly we are concerned only with relative tissue uptakes (i.e., tumor/normal tissues; Ga-67:Fe-59), rather than with comparisons of absolute tumor uptakes for the different tumor types. It is perhaps appropriate here to record findings from a properly controlled study of the effect of tumor size (RILQ) on Ga-67 tissue distributions. For small tumors (~0.1–1 g), Ga-67 tumor concentrations and overall tissue distributions were approximately independent of tumor size. With further tumor growth, all tissue concentrations entered into a progressive decline but, even for tumors weighing 4 g, the relativity between Ga-67 tissue concentrations was maintained. Results obtained on the two RILQ groups of Table 6 give an indication of such effects.

TABLE 5. Ga-67 AND Fe-59 DISTRIBUTIONS IN TUMOR HOSTS

Tissue	30 min		24 hr	
	Ga-67	Fe-59	Ga-67	Fe-59
Blood	12.6	33.0	0.5	30.0
Liver	2.3	7.8	1.9	11.0
Spleen	1.9	25.0	1.1	42.0
Kidney	4.3	12.5	2.4	9.0
S. intestine	3.4	8.6	1.8	10.0
Bone	7.7	19.0	16.0	25.0
Tumor	6.3	3.8	12.0	4.6
	(6.0–6.5)	(3.4–4.1)	(10.4–13.2)	(4.3–5.0)

RILQ lymphoma (three mice per time point), showing mean values (% A_0 per g) and including ranges for tumor samples.

DISCUSSION

The principal findings of this study concern the marked differences between Ga-67 and Fe-59 tissue distributions and the rapid kinetics of Ga-67 uptake by both tumor and normal tissues.

In studies of these tumor types as exponentially growing cultured cells, the demonstration of pronounced transferrin stimulation of their Ga-67 and Fe-59 uptakes implied that the action of transferrin is a critical feature of both tumors' cellular uptake mechanisms (*5,6*). Yet in vivo these tumors expressed a high affinity for Ga-67 and a low affinity for Fe-59, while for the major hemopoietic tissues the reverse applied. Operationally we may regard the 7-hr Fe-59 distributions (see Table 6) as a broad reflection of the rate of "complete" transferrin interactions with the various constituent cell populations, "complete" referring to interactions resulting in cellular binding of Fe-59. On that basis, these tumor populations are characterized by low interaction rates, relative even to liver, kidney, or intestinal cell populations.

To explain the high tumor uptakes of Ga-67, one might postulate that those cells have a high frequency for "incomplete" interactions—namely, those resulting in the cellular binding of Ga-67 but not Fe-59. This seems unlikely for, in tissue culture, these tumor cells' Fe-59 uptake responses were even more marked than their Ga-67 responses (*6*; also R. G. Sephton and A. W. Harris, unpublished observations). Alternatively we might suppose that there are marked differences between tumor and normal tissue populations in respect of the *amount* of Ga-67 becoming cell-bound as a result of the transferrin interaction, differences that more than compensate for the tumor cells' relatively low interaction frequency.

The kinetic observations (Tables 2, 4, and 5) are obviously of fundamental interest, though in the context of the preceding discussion their significance is uncertain. A precise quantitative interpretation of

TABLE 6. Ga-67 AND Fe-59 DISTRIBUTIONS IN DIFFERENT TUMOR MODELS

Tissue	RILQ (6)		RILQ (6)		W7 (3)		P3K (2)		HPC108 (4)		MOPC315 (14)	
	Ga-67	Fe-59	Ga-67	Fe-59	Ga-67	Fe-59	Ga-67	Fe-59	Ga-67	Fe-59	Ga-67	Fe-59
Blood	2.0	13.0	3.0	4.6	1.6	7.0	3.6	9.0	4.6	9.0	3.4	8.0
Liver	2.5	4.7	3.0	6.9	2.2	3.5	4.3	6.0	4.7	5.0	3.4	7.0
Spleen	1.7	70.0	2.1	13.0	1.4	42.0	1.6	31.0	2.4	40.0	2.0	63.0
Kidney	2.7	5.0	3.7	6.8	2.1	2.4	8.0	7.0	3.8	1.6	3.5	8.0
Bone	6.5	17.0	10.8	31.0	4.7	9.0	5.5	19.0	5.5	10.0	10.5	16.0
Tumor	12.0	5.0	19.0	5.0	8.5	2.0	9.0	5.0	20.0	2.3	9.8	4.5
	(10.2–15.1)	(4.2–6.3)	(16.5–22.7)	(3.8–6.5)	(7.7–9.4)	(1.8–2.4)	(7.9–10.2)	(4.3–5.7)	(17.1–21.3)	(1.6–2.7)	(8.5–12.8)	(4.0–5.9)
Av. tumor wt. (g)	3.0		0.3		2.5		1.8		0.8		0.5	

Tissue distributions at 7 hr after injection.
Tumor models: lymphomas (RILQ, W7), myelomas (P3K, HPC108, MOPC315).
Mean values (% A_0 per g) from 6, 5, 3 . . . animals per group.

Ga-67 kinetics in tumor hosts is potentially a complex matter—e.g., Ga-67 concentrations in tumor may become progressively diluted during the 6- to 72-hr interval due to progressive tumor growth. It is sufficient to emphasize the relatively high tumor concentrations attained at 30 min and 3 hr, which suggest that the process governing the tissue distribution of Ga-67 is a fast-acting one. It seems unlikely that the direct action of transferrin could commit Ga-67 for cellular uptake at such rates and, by the previous discussion, its action cannot account quantitatively for the distributions attained. Almost certainly processes other than the transferrin effect participate in the in vivo Ga-67 uptake mechanism.

The tissue-culture findings, however, need not be discounted as irrelevant to in vivo questions. Besides showing the decisive role played by transferrin in both Ga-67 and Fe-59 uptake mechanisms, that work also showed significant differences between the two tracers' responses, suggesting that their uptake mechanisms were indeed different in other respects (*6*). Note that the tissue-culture system comprised one cell population (tumor) whose total volume was very small relative to the fluid volume in which tracers—a small amount of "active" transferrin and a 10% fetal calf serum background—were dispersed. Cellular uptake measurements involved separation and washing of cells from radioactive medium, generally following 24-hr exposures (i.e., two cell-doubling times). In the animal, however, tumor and various normal cell populations compete for tracers that are initially distributed in the (serum) circulation, whose volume is comparable with the total cell volume. After "exposures" of a few hours, circulating tracer concentrations become low relative to tissue-bound levels, whose monitoring need involve no washing of the sampled material. It is perhaps not surprising that analogies and differences between Ga-67 and Fe-59 should be expressed quite differently in these two different experimental systems.

Other observations of this study are somewhat secondary but merit some comment. Differences between Ga-67 tissue distributions in exposed and specific pathogen-free mice (Table 3) were modest but significant: we interpret the effect as due to expansion of the reticulo-endothelial component and the enhanced Ga-67 affinity shown by the cells involved. The gradual decline in Ga-67 and Fe-59 intestinal levels may reflect the turnover and loss of labeled cells from that tissue. Regarding Ga-67 uptakes in bone, differences between young and old animals, and between joint and shaft concentrations, are presumably related to differences in the rate of bone formation. It can be shown that Ga-67 adsorbs

to inert bone in vitro and that this "uptake" becomes reduced in the presence of serum in the incubation medium (R. G. Sephton, unpublished observations). Almost certainly, Ga-67 uptake by bone in vivo occurs by passive adsorption to bone-mineral surface in competition with Ga-67 serum binding.

FOOTNOTES

* Radiochemical Centre, Amersham, U.K.
† GSWP: Millipore Corp., Bedford, Mass.

REFERENCES

1. EDWARDS CL, HAYES RL: Tumor scanning with ^{67}Ga citrate. *J Nucl Med* 10: 103–105, 1969

2. JOHNSTON GS, JONES AE, eds: *An Atlas of ^{67}Ga Scintigraphy*, New York-London, Plenum Press, 000

3. GUNASEKERA SW, KING LJ, LAVENDER PJ: The behaviour of tracer Gallium-67 towards serum proteins. *Clin Chim Acta* 39: 401–406, 1972

4. CLAUSEN J, EDELING C-J, FOGH J: ^{67}Ga binding to serum proteins and tumor components. *Cancer Res* 34: 1931–1937, 1974

5. SEPHTON RG, HARRIS AW: Gallium-67 citrate uptake by cultured tumor cells, stimulated by serum transferrin. *J Natl Cancer Inst* 54: 1263–1266, 1975

6. HARRIS AW, SEPHTON RG: Transferrin promotion of ^{67}Ga and ^{59}Fe uptake by cultured mouse myeloma cells. *Cancer Res* 37: 3634–3638, 1977

7. AISEN P: The role of transferrin in iron transport. *Br J Haematol* 26: 159–163, 1974

8. CHEE CA, ILBERY PLT, MOTTA R: Bone marrow transplantation incompatibility modified by F_1 graft inocula. *Rev Europ Etudes Clin et Biol* 17: 53–65, 1972

9. HARRIS AW, BANKHURST AD, MASON S, et al: Differentiated functions expressed by cultured mouse lymphoma cells. II Θ antigen, surface immunoglobulin and a receptor for antibody on cells of a thymoma cell line. *J Immunol* 110: 431–438, 1973

10. HORIBATA K, HARRIS AW: Mouse myelomas and lymphomas in culture. *Exp Cell Res* 60: 61–77, 1970