The Incorporation of Ga-67 into the Ferritin Fraction of Rabbit Hepatocytes In Vivo

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Twenty-four hours after the administration of Ga-67 citrate and Fe-59 citrate, rabbits were killed and their livers removed and homogenized. Labile proteins in the filtered liver homogenates were denatured; ferritin was then crystallized from the supernatants by cadmium sulfate. Sephadex G-200 gel filtration of the ferritin fractions was done to determine the distribution of molecular weights in the substances associated with Ga-67 and Fe-59. It was found that Ga-67 was incorporated into the crystallizable ferritin fraction of rabbit hepatocytes with approximately one-sixth the uptake of simultaneously administered Fe-59. Gel-filtration chromatography confirmed that both the Ga-67 and the Fe-59 of the crystallizable ferritin fraction were associated with substances of the appropriate molecular weight for ferritin.

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It is known that the metabolism of gallium in normal tissues parallels that of iron in many important respects. Gallium(III) has an ionic radius of 0.62 Å, whereas that of iron(III) is 0.64 Å (*1*). Gallium, like iron, is bound by the two specific binding sites on transferrin in the serum (*1,2*). Gallium is even incorporated into the hemoglobin fraction of reticulocytes, although to a lesser extent than iron (*3*). On a subcellular level, some investigators report that in tumors gallium is found in association with multiple macromolecular elements, and that it localizes predominantly in the nuclear fraction (*4*). Others report that in normal tissues and in tumors gallium is found largely in association with one or two specific macromolecular elements, and that it localizes predominantly in the lysosomal fraction (*5,6*). In addition, gallium uptake by a ferritin extract in vitro has been reported (*4*). By way of comparison, iron is found in association with many macromolecular elements, including ferritin, and localizes in all the major subcellular fractions, including the lysosomal and nuclear fractions, in proportions that vary with time (*7*). In the present study, the relationship of the metabolism of gallium to that of iron was further defined on the subcellular level by comparing the incorporation of Ga-67 and Fe-59 into the ferritin fraction of rabbit hepatocytes in vivo.

MATERIALS AND METHODS

The experimental animals were New Zealand white rabbits. They were killed 24 hr after the intravenous administration of 10 μCi Fe-59 citrate and 20 μCi Ga-67 citrate. The percent total dose in the liver was determined by reference to suitable counting standards.

The technique of Mazur and Shorr was used (with modifications) to isolate ferritin from other hepatic proteins. The liver was homogenized in a Waring blender, then heated in de-ionized water to 80°C. A coagulum of denatured proteins was filtered out and the solution allowed to cool. The pH was adjusted to 4.6 with 50% acetic acid and the mixture allowed to stand overnight at 4°C. The precipitate that formed was discarded and a 20% solution of cadmium sulfate added to the supernatant, precipitating "crystallizable ferritin." Additional ferritin remaining in the mother liquor was termed "noncrystallizable ferritin" (*8*). At this point, the percent activity of Ga-67 and Fe-59 in each of these fractions was determined with ref-

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erence to the total activity of each nuclide in the liver.

The ferritin fractions obtained were then examined by gel filtration to determine the proportions of Ga-67 and Fe-59 activity actually associated with macromolecules of the appropriate size for ferritin, whose molecular weight is approximately 750,000. Sephadex G-200 was used in a 5- × 100-cm column at 40°C. The upward flow was 15 ml/hr, and 5 ml were collected in each tube. The column was standardized by measuring the elution volumes of dextran blue, horse-spleen ferritin, and bovine transferrin.

RESULTS

The percentages of Ga-67 and Fe-59 found in the various fractions obtained during the ferritin isolation procedure are shown in Table 1. About 8% of the administered activity of both Ga-67 and Fe-59 was found in the liver. For both nuclides, over 80% of the hepatic activity came down in the coagulum of proteins denatured by heat and acid. The uncoagulated activity included both free nuclide and nuclide associated with ferritin. Precipitation of ferritin from this solution by cadium produced crystallizable and noncrystallizable ferritin fractions. The crystallizable fraction contained 1.6% of the liver's Ga-67 and 9.8% of its Fe-59; the noncrystallizable fraction contained 11% of the liver's Ga-67 and 5.5% of its Fe-59.

Gel filtration of crystallizable and noncrystallizable ferritin fractions was done to separate the Ga-67 and Fe-59 found in association with ferritin from those found either free or in association with proteins of lower molecular weight, such as transferrin. It was found that the elution volume of essentially all of the Ga-67 and Fe-59 in the crystallizable fraction was identical to that of the horse-spleen ferritin (Fig. 1). The elution pattern of Ga-67 and Fe-59 in the noncrystallizable fraction however, including multiple peaks, the largest of which corresponded to that of free nuclide. Thus, Ga-67 is found in association with crystallizable rabbit hepatic ferritin in vivo at a concentration approximately one-sixth that of simultaneously administered Fe-59.

TABLE 1. UPTAKE OF Ga-67 AND Fe-59 BY HEPATIC FERRITIN IN RABBITS (24-HR)

Nuclide	Liver uptake (% administered dose*)	Fractional liver uptake (% total liver uptake*)	
		Noncrystalline ferritin fraction	Crystalline ferritin fraction
Ga-67	8.1 ± 0.8 (N = 11)	11.0 ± 1.0 (N = 10)	1.6 ± 0.2 (N = 10)
Fe-59	8.3 ± 0.6 (N = 12)	5.5 ± 2.1 (N = 11)	9.8 ± 2.3 (N = 11)

* Mean ± s.e.

DISCUSSION

Ferritin, along with hemosiderin, is the major storage repository of intracellular iron (*9*). In the present study the 24-hr uptake of Ga-67 into the crystallizable ferritin fraction of rabbit hepatocytes was found to be about one-sixth that of Fe-59. Thus, gallium appears to behave at least somewhat like iron in its interaction with ferritin, as it does in its interaction with transferrin and hemoglobin. Many other metals, however, can interact nonspecifically with transferrin and ferritin, although some do so only in vitro (*2,4*). Similarities between iron and gallium are important in determining the distribution of Ga-67 in normal tissues and the localization of Ga-67 in tumors, although the reported data are somewhat conflicting. Studies of iron metabolism in mouse hepatic tumors have shown that both the Fe-59 uptake and the total ferritin concentration are reduced, especially in rapidly growing tumors (*10*).

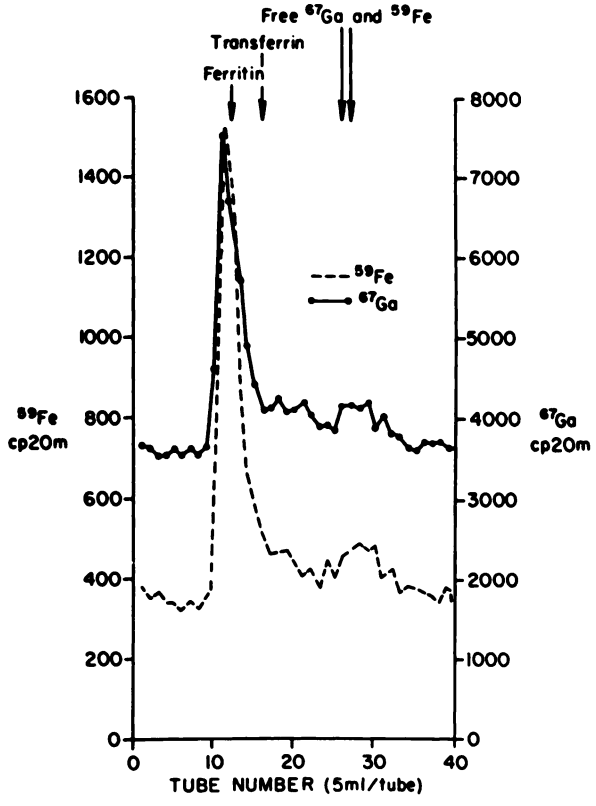

FIG. 1. Sephadex G-200 gel filtration of the crystallizable ferritin fraction obtained from rabbit hepatocytes 24 hr after the administration of Ga-67 citrate and Fe-59 citrate in vivo.

Also, studies of gallium metabolism in various human and animal tumors have shown that Ga-67 localizes predominantly in cell fractions other than the soluble fraction, and that it associates primarily with substances having molecular weights lower than that of ferritin (*4–6*). The role of transferrin in the localization of Ga-67 in tumors is also controversial. Some investigators have reported enhancement of Ga-67 uptake with the addition of transferrin in an in vitro tumor model (*11*), while others have reported inhibition of Ga-67 uptake with the addition of transferrin in another in vitro tumor model (*12,13*).

As a consequence of what is known about the binding of gallium and iron in the blood, some investigators have succeeded in reducing the nontarget activity of Ga-67 in animals by giving parenteral iron dextran, which presumably displaces the Ga-67 from transferrin (*14*). Further clarification of the mechanism of gallium uptake in normal and malignant tissues may lead to other interventions or new radiopharmaceuticals that will improve the quality of tumor scanning.

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