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Studies of the In Vivo Entry of Ga-67 into Normal and Malignant Tissue

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Previous studies of the effect of scandium on the tissue distribution of Ga-67 suggest that Ga-67 makes its initial in vivo entry into normal and malignant tissues by different routes. (Scandium blocking of plasma protein Ga-67 binding increased Ga-67 excretion, decreased its uptake in normal tissues, but had little effect on rodent tumors.) In further studies we have used other methods to alter the plasma binding of Ga-67. Iron saturation of plasma produced effects on Ga-67 tissue distribution similar to those observed with scandium. On the other hand, increasing Ga-67 plasma binding through induction of anemia and administration of apotransferrin produced the reverse of the effects observed with scandium and iron. We conclude that the initial in vivo entry of Ga-67 into tumor tissue involves mainly an unbound or loosely bound form of Ga-67, whereas its uptake by normal soft tissues is strongly promoted by its binding to transferrin.

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Since the initial findings that Ga-67 has a preferential affinity for soft-tissue tumors in humans and rodents (*1*,*2*), numerous suggestions have been advanced as to the basic mechanism involved. These have ranged from hyperpermeability of the plasma membranes of tumor cells (*3*) to the association of Ga-67 with calcium (*4*), transferrin (*5*,*6*), and lactoferrin (*7*).

Based on our observations of the effect of stable gallium and scandium administration on the tissue distribution of Ga-67 in normal and tumor-bearing rodents, we have suggested that the initial entry of Ga-67 into tumor and normal soft tissues occurs by different routes (*3*,*8*). With tumors an unbound or loosely bound form of Ga-67 appears to be involved, whereas with normal soft tissues that route does not seem to be of importance. Figure 1 shows a working scheme of the postulated pathways in the initial biodistribution of Ga-67. The solid lines indicate main pathways. This scheme does not take into account reverse processes and is intended to indicate only the overall movement of Ga-67 in the initial phase of its biodistribution after it enters the vascular compartment. Note that because of the affinity of Ga-67 for plasma proteins (*9*,*10*), under normal circumstances any Ga-67 present in the blood would be contained mainly in plasma Compartment II.

Although our studies with scandium (*8*) support the proposed preferential pathway of Ga-67 from plasma Compartment I into tumor tissue, that evidence relates directly to only one portion of the overall scheme in Fig. 1. We have accordingly attempted to determine the validity of the indicated route from plasma Compartment II into normal tissue by experimentally increasing the Ga-67-binding plasma-protein compartment through both endogenous and exogenous means, using rats bearing Morris 5123C hepatomas. We have also carried out further tests of the indicated route for Ga-67 into tumor from plasma Compartment I by experimentally saturating the iron-binding capacity of plasma using two different iron agents. This communication reports and discusses the findings.

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MATERIALS AND METHODS

Gallium-67 citrate,* scandium oxide† (Sc_2O_3), Im-

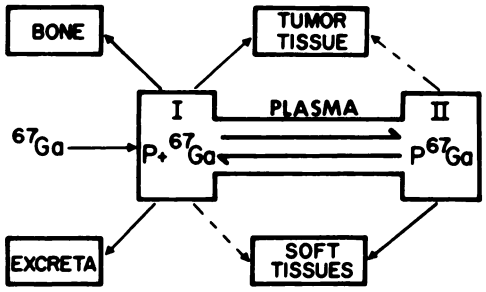

FIG. 1. Proposed scheme of initial entry of Ga-67 into tumor and normal tissues. Solid lines indicate main pathways. This scheme does not take into account reverse processes and is intended to indicate only the overall movement of Ga-67 in the initial phase of its biodistribution after it enters the vascular compartment.

feron,[‡] and rabbit transferrin[‖] were procured commercially. Buffalo rats[§] (five per group unless otherwise indicated) weighed from 250–400 g and were 2.5–4.5 mo of age. In each experiment the animals used were of the same sex and age ±2 wk. Animals were given free access to water and Rodent Laboratory Chow #5001,[¶] except for experiments involving induced anemia in which Low-Iron Diet #5859[¶] was used for both treated and control animals. In each experiment, food was withdrawn after Ga-67 had been administered in order to avoid any effects produced by variations in food intake (*11*).

The transplantable Morris 5123C hepatoma was originally obtained from Dr. Fred Snyder of our Division. It was implanted intramuscularly by trocar into the thigh muscle of the right leg of the Buffalo rats. The animals in each experiment had been implanted with tumor at the same time and from the same tumor. Total tumor weights did not exceed 7% of the rat's body weight. All intravenous injections were made by tail vein.

Rats were administered Ga-67 citrate intravenously at a dose level of 20–50 μCi and were killed by exsanguination following a brief exposure to diethyl ether. Weighed samples of tissue were counted in a well scintillation counter against a standard, and the Ga-67 concentration calculated as percentage of administered dose per gram of tissue, normalized to a body weight of 250 g. Only obviously viable tumor tissue was chosen for radioassay. Whole-body retention and total tumor-compartment uptake of Ga-67 were determined by the geometry-independent technique of Gibbs et al. (*12*). Statistical analyses used Student's t-test.

Scandium citrate was administered intravenously with Ga-67 at a level of 0.5 mg Sc/kg, and the experiment terminated 4 hr later. Scandium citrate solution was prepared from Sc_2O_3 as previously described (*8*).

Ferric citrate was administered intravenously at a level of 140 μg Fe/kg, 10 min before Ga-67, and the experiment terminated 5 hr after Ga-67 administration. The ferric citrate solution was prepared by combining ferric chloride and sodium citrate solutions and adjusting to pH 7 with sodium hydroxide. The citrate-to-iron molar ratio was 3:1. Imferon was injected intramuscularly 4 hr before Ga-67 at a level of 25 mg Fe/kg, and the experiment terminated 24 hr after Ga-67 administration.

Experimental anemia was produced by heart-puncture bleedings twice a week for a period of 3 wk. Ten to 15% of the animal's blood volume was removed at each bleeding; development of anemia was monitored by hematocrit. Control animals received heart punctures without removal of blood. Gallium-67 citrate was administered 3 days after the final bleeding, and the experiment terminated 24 hr later.

Rabbit transferrin was rendered essentially iron-free by multiple treatments with 0.1 *M* citrate-acetate buffer at pH 4.5. PM-30 ultrafiltration membranes[**] were used to concentrate the transferrin solution between

TABLE 1. EFFECT OF SCANDIUM ON THE 4-HR TISSUE DISTRIBUTION OF Ga-67 IN MALE RATS* BEARING 5123C HEPATOMAS

Tissue	Control	Sc (0.5 mg/kg)	Significance
	% Administered Ga-67/g		p
Tumor	3.60 ± 0.47[†]	3.80 ± 0.40	—
Liver	0.68 ± 0.03	0.22 ± 0.01	<0.001
Spleen	1.30 ± 0.13	0.61 ± 0.07	0.001–0.01
Lung	0.82 ± 0.06	0.28 ± 0.02	<0.001
Muscle	0.14 ± 0.02	0.04 ± 0.01	0.001–0.01
Femur	0.54 ± 0.04	0.84 ± 0.02	<0.001
Marrow	1.20 ± 0.11	0.18 ± 0.01	<0.001
Blood	1.40 ± 0.09	0.36 ± 0.02	<0.001
Ga-67 retention (%)	99.0 ± 0.9	75 ± 0.7	<0.001
Tumor wt (g)	16.0 ± 3.3	10.0 ± 1.2	—
Ga-67 in tumor (%)	25.0 ± 3.9	17.0 ± 1.8	—

* N = 5.

† Standard error of the mean.

TABLE 2. EFFECT OF FERRIC CITRATE ON BODY RETENTION OF Ga-67 IN MALE AND FEMALE RATS*

Time (hr)	Male Ga-67 retention (%)			Female Ga-67 Retention (%)		
	Control	Fe cit.†	Diff.	Control	Fe cit.†	Diff.
2	97	92	5	91	78	13
4	96	89	7	88	71	17
12	86	78	8	79	65	14
24	79	72	7	73	64	9

* Four 3-months-old animals per group.
† Ferric citrate administered intravenously, at a level of 140 μg Fe/kg, 10 min before Ga-67 administration.

treatments. Citrate and acetate were then removed by multiple dilutions and concentrations using normal saline. The resulting apotransferrin was administered at a level of 140 mg/kg, 15 min before Ga-67 administration. The experiment was terminated 24 hr after Ga-67 administration.

Serum iron and total iron-binding capacity were determined by the *o*-phenantholine technique, with commercial reagents.††

RESULTS

Table 1 shows the effect produced by scandium on the distribution of Ga-67 in Buffalo rats bearing Morris 5123C hepatomas. The results are similar to those we previously reported for this tumor (*8*). Scandium blocking of plasma-protein Ga-67-binding sites again resulted in dramatic decreases in the uptake of Ga-67 by normal tissues (except for the femur) without producing any effect on the concentration of the radionuclide in tumor. These results are consistent with the pathways indicated in Fig. 1, i.e., scandium administration forced plasma Ga-67 from Compartment II into Compartment I, increasing Ga-67 excretion and bone deposition, which in turn decreased the deposition of Ga-67 in soft tissues. The fact that Ga-67 tumor uptake did not itself increase was apparently the result of the competing excretion and bone deposition processes (see again Table 1).

To further test the effect produced by blocking plasma-protein Ga-67-binding sites, we also saturated plasma-protein iron-binding sites with iron by administering ferric citrate intravenously and a hydrous ferric oxide colloid (Imferon) intramuscularly.

In our experiments with ferric citrate and Imferon, we used female rats rather than males. This choice resulted from a preliminary comparison of the effect of ferric citrate on Ga-67 body retention in male and female rats (Table 2). Statistically significant decreases in the body

TABLE 3. EFFECT OF FERRIC CITRATE ON 5-HR TISSUE DISTRIBUTION OF Ga-67 IN FEMALE RATS* BEARING 5123C HEPATOMAS

Tissue	Control	Fe (140 μg/kg)	Significance
	% Administered Ga-67/g		p
Tumor	3.40 ± 0.28†	3.70 ± 0.46	—
Liver	0.75 ± 0.04	0.56 ± 0.03	0.001–0.01
Spleen	0.77 ± 0.06	0.50 ± 0.06	0.001–0.01
Lung	0.91 ± 0.01	0.68 ± 0.01	0.01–0.02
Muscle	0.14 ± 0.03	0.10 ± 0.01	—
Femur	0.65 ± 0.03	0.77 ± 0.08	—
Marrow	1.10 ± 0.03	0.66 ± 0.12	0.001–0.01
Blood	1.50 ± 0.09	1.30 ± 0.04	—
Ga-67 retention (%)‡	90.0 ± 1.5	84.0 ± 2.2	0.02–0.05
Tumor wt (g)	3.9 ± 0.9	4.6 ± 0.6	—
Ga-67 in tumor (%)	16.0 ± 3.0	21.0 ± 1.9	—
Serum Fe (μg/dl)	150 ± 6	370 ± 10	<0.001
Serum TIBC‖ (μg/dl)	330 ± 4	380 ± 7	—

* N = 5.
† Standard error of the mean.
‡ Body retention at 4 hr.
‖ Serum total iron-binding capacity.

TABLE 4. EFFECT OF IMFERON ON 24-HR TISSUE DISTRIBUTION of Ga-67 IN FEMALE RATS* BEARING 5123C HEPATOMAS

Tissue	Control	Imferon (25 mg Fe/kg)	Significance
	% Administered Ga-67/g		p
Tumor	10.00 ± 0.97†	11.00 ± 1.10	—
Liver	1.40 ± 0.11	0.75 ± 0.05	<0.001
Spleen	0.86 ± 0.07	0.60 ± 0.02	0.001–0.01
Lung	0.33 ± 0.05	0.12 ± 0.00	0.001–0.01
Muscle	0.03 ± 0.01	0.23 ± 0.05‡	
Femur	0.62 ± 0.08	0.58 ± 0.05	—
Marrow	0.91 ± 0.07	0.18 ± 0.01	<0.001
Blood	0.30 ± 0.06	0.12 ± 0.01	0.01–0.02
Ga-67 retention (%)	82.0 ± 1.0	77.0 ± 2.7	0.01–0.02
Serum Fe (μg/dl)	190 ± 16	460 ± 15	<0.001
Serum TIBC‖ (μg/dl)	370 ± 15	400 ± 24	—

* N = 5.
† Standard error of the mean.
‡ See text for explanation of this value.
‖ Serum total iron-binding capacity.

retention of Ga-67 were observed at all time periods for both males and females, but the relative difference between treated and control groups was clearly superior in the females. Accordingly, we chose to use females in both our ferric citrate and Imferon studies. In another preliminary study we found that, following the administration of ferric citrate to female rats at a level of 140 μg Fe/kg (equivalent to 10 mg Fe/70 kg), the plasma-protein iron-binding sites remained saturated through a period of 4 hr. For the ferric citrate study, accordingly, it was decided to determine Ga-67 body retention at 4 hr and then terminate the experiment at 5 hr.

The data in Table 3 indicate that the administration of ferric citrate produced the expected decreases in Ga-67 uptake in normal tissues without altering the uptake in the 5123C hepatoma, although the results were less impressive than those seen with scandium (Table 1). Note that the unsaturated iron-binding capacity of the serum was essentially zero.

The intramuscular administration of Imferon results in a sustained release of iron to the plasma compartment, and this in turn produces a prolonged saturation of plasma iron-binding capacity (*13*). We found that following intramuscular Imferon administration, the 24-hr Ga-67 uptake by normal soft tissue was significantly reduced whereas that in tumor tissue was unaffected (Table 4). Again in the treated animals the serum unsaturated iron-binding capacity was essentially zero, and the whole-body Ga-67 retention was reduced. In a repeat study, the apparent unusual increase in the muscle Ga-67 concentration in the Imferon-treated group shown in Table 4 was found to have occurred because the muscle samples were taken from the thigh used for Imferon injection. The repeat study gave results similar to those shown in Table 4 but with no significant difference being observed for muscle samples taken from the noninjected thighs, whereas Ga-67 uptake in the Imferon-injected thigh was markedly elevated.

Prolonged blood loss decreases serum iron and expands the transferrin pool, which causes a marked elevation in the serum unsaturated iron-binding capacity (*14*). We have utilized this effect to test our proposed pathway for the entry of Ga-67 into normal tissue from plasma Compartment II (Fig. 1).

It is evident from Table 5 that the decrease in serum iron and expansion of the transferrin pool (as indicated by the serum iron and the serum total iron-binding capacity) was coupled with a significant increase in the uptake of Ga-67 by the liver, spleen, and bone marrow. On the other hand, the concentration of Ga-67 in tumor tissue was significantly decreased. These results support our proposed pathway for transport of Ga-67 from plasma Compartment II into normal tissues (Fig. 1), i.e., when the plasma binding sites for Ga-67 are increased, this should result in an increase in the uptake of Ga-67 by normal soft tissues, and at the same time the increased plasma-protein binding of Ga-67 should also interfere with, and consequently reduce, Ga-67 uptake in tumor tissue. Duplicate experiments gave similar results. The reasons for the significant decreases observed in the Ga-67 concentration in muscle and blood are not apparent.

In the study shown in Table 5, a challenge of our scheme was produced endogenously by increasing the binding of Ga-67 to plasma proteins through experimentally inducing anemia. We have also used another

TABLE 5. EFFECT OF ANEMIA ON 24-HR TISSUE DISTRIBUTION OF Ga-67 IN MALE RATS* BEARING 5123C HEPATOMAS

Tissue	Control	Anemic	Significance
	% Administered Ga-67/g		p
Tumor	7.50 ± 0.82†	3.00 ± 0.19	<0.001
Liver	1.30 ± 0.06	6.30 ± 0.27	<0.001
Spleen	2.30 ± 0.26	8.60 ± 0.21	<0.001
Lung	0.51 ± 0.04	0.46 ± 0.11	—
Muscle	0.08 ± 0.01	0.04 ± 0.01	0.01–0.001
Femur	0.96 ± 0.08	0.93 ± 0.06	—
Marrow	2.20 ± 0.19	4.60 ± 0.22	<0.001
Blood	0.35 ± 0.03	0.20 ± 0.01	<0.001
Ga-67 retention (%)	78.0 ± 1.5	83.0 ± 1.5	0.02–0.05
Tumor wt (g)	4.6 ± 0.6	2.2 ± 0.6	0.02–0.05
Ga-67 in tumor (%)	16.0 ± 1.9	4.9 ± 1.3	0.001–0.01
Serum Fe (μg/dl)	180 ± 13	56 ± 2	<0.001
Serum TIBC‡ (μg/dl)	370 ± 5	530 ± 9	<0.001
Hematocrit (%)	44.0 ± 0.3	27.0 ± 0.4	<0.001

* N = 5.
† Standard error of the mean.
‡ Serum total iron-binding capacity.

approach, the exogenous increase of the plasma transferrin level through intravenous administration of rabbit apotransferrin. This was administered at a level that initially increased the circulating transferrin to approximately three times its normal level. Again, as predicted (Fig. 1), the tumor concentration of Ga-67 dropped significantly whereas that in the soft tissues, in general, showed significant increases (Table 6). The high level of serum total iron-binding capacity observed for the transferrin-treated group (770 μg/dl) indicates that excess transferrin was still circulating at 24 hr after apotransferrin administration, although the blood Ga-67 level was not elevated.

DISCUSSION

Substances that are introduced into the vascular pool

TABLE 6. EFFECT OF RABBIT TRANSFERRIN ON 24-HR TISSUE DISTRIBUTION OF Ga-67 IN MALE RATS* BEARING 5123C HEPATOMAS

Tissue	Control	Transferrin (140 mg/kg)	Significance
	% Administered Ga-67/g		p
Tumor	8.00 ± 0.81†	4.60 ± 0.50	0.001–0.01
Liver	1.00 ± 0.08	1.60 ± 0.07	0.001–0.01
Spleen	1.70 ± 0.14	3.60 ± 0.55	0.001–0.01
Lung	0.42 ± 0.05	0.71 ± 0.07	0.01–0.02
Muscle	0.06 ± 0.01	0.10 ± 0.01	—
Femur	0.87 ± 0.07	0.84 ± 0.04	—
Marrow	2.00	3.00‡	
Blood	0.29 ± 0.01	0.30 ± 0.01	—
Ga-67 retention (%)	82.0 ± 1.5	85.0 ± 1.0	—
Tumor wt (g)	8.2 ± 1.3	11.5 ± 1.3	—
Ga-67 in tumor (%)	30.0 ± 3.5	27.0 ± 1.6	—
Serum Fe (μg/dl)	170 ± 9	160 ± 10	—
Serum TIBC‖ (μg/dl)	380 ± 5	770 ± 86	<0.001

* N = 5.
† Standard error of the mean.
‡ Four samples only.
‖ Serum total iron-binding capacity.

TABLE 7. EFFECT OF VARIOUS EXPERIMENTAL PROCEDURES ON TISSUE DISTRIBUTION OF Ga-67 IN RATS BEARING 5123C HEPATOMAS

Treatment	Effect on Ga-67 tissue concentration			
	Predicted for normal	Observed for normal	Predicted for tumor	Observed for tumor
i.v. Scandium citrate*	—	decrease	—	no change
i.v. Iron citrate	decrease	decrease	no change	no change
i.m. Imferon	decrease	decrease	no change	no change
Anemia induction	increase	increase	decrease	decrease
i.v. Apotransferrin	increase	increase	decrease	decrease

* Consult Ref. *8* in addition to Table 1.

are immediately subject to partition between various body compartments. When binding to plasma proteins is involved, this initial interaction can be a major controlling factor in the biodistribution of both natural and foreign substances.

Studies of the binding of gallium by plasma proteins (*9,15*) and our observations on the dramatic effects produced by scandium administration on Ga-67 uptake by normal and malignant tissues (see also Table 1) have suggested the initial biodistribution scheme, as shown in Fig. 1, with one route for Ga-67 uptake into normal soft tissues and a different one for tumors (*8*).

We have now tested these proposed pathways by experimentally altering plasma iron-binding levels (and, as a consequence, Ga-67 binding by plasma protein) in rats bearing Morris 5123C hepatomas. A summary of the results is shown in Table 7.

When Ga-67 binding to plasma transferrin was blocked by iron citrate or Imferon administration, soft-tissue uptake of Ga-67 decreased markedly, whereas that by hepatoma remained the same (Tables 3 and 4). On the other hand, when Ga-67 binding to plasma protein was enhanced by increasing the serum unsaturated iron-binding capacity through multiple bleedings and by apotransferrin administration, the uptake of Ga-67 in soft tissues increased, while that in the hepatoma decreased (Tables 5 and 6).

We interpret these results as strong evidence that different in vivo pathways are indeed involved in the initial entry of Ga-67 into tumor and normal soft tissues. They further suggest that an unbound or loosely bound form of Ga-67 is involved in the entry of Ga-67 into tumors, whereas, conversely, protein binding of Ga-67 promotes its entry into normal soft tissues, particularly those having high reticuloendothelial activities.

Early in our studies of the biodistribution of gallium (unpublished), we observed that the intravenous administration of soluble ferric iron salts decreased the uptake of Ga-67 by soft tissues without appreciably changing tumor uptake. This finding was not pursued at that time because of the known toxicity of iron when given by that route and in that form. In our studies reported here, we have used ferric citrate solely as an experimental agent for the study of the initial entry of Ga-67 into tumor and normal tissues. The dose level used (140 μg Fe/kg), however, corresponds to the amount that has been administered to humans in the past for the in vivo determination of serum total iron-binding capacity (*16*).

Higasi et al. (*17*) and Larson and coworkers (*18*) have observed that administration of hydrous ferric oxide colloid decreased the body retention of Ga-67. In their studies the colloid was administered intravenously, whereas in ours the agent (Imferon) was administered intramuscularly to provide for a continuous release of iron into the vascular compartment. Accordingly the effects seen in our study were more pronounced than those observed by these other workers.

Swartzendruber and Hübner (*19*) and others (*20,21*) have studied the effects of whole-body x-irradiation on the excretion and tissue distribution of Ga-67, and the findings are very similar to those we originally observed with stable gallium (*3*) and later with scandium administration (*8*): increased Ga-67 excretion and bone deposition together with decreased soft-tissue uptake. Bradley and coworkers (*22*) have shown that these irradiation effects were associated with an increase in serum iron. Our experiments with ferric citrate and Imferon (Tables 3 and 4) produced effects similar to those seen with x-irradiation. This strongly suggests that irradiation-induced alterations in the biodistribution of Ga-67 result from an increase in the concentration of Ga-67 in plasma Compartment I (Fig. 1), brought about by the presence of excess iron in plasma Compartment II.

Bradley and coworkers (*23*) have also studied the effect of iron deficiency on the tissue distribution of Ga-67 in normal and tumor-bearing rats. The effects they observed in normal tissue were similar to those we observed (Table 5). However, in that study, as well as in their study of the effects produced by x-irradiation (*22*), they observed entirely different effects on the Ga-67 uptake in the Walker-256 carcinosarcoma than we did with the Morris 5123C hepatoma. That is, with iron-deficiency

anemia they found no change in tumor Ga-67 concentration, whereas with x-irradiation-induced hyperferremia they observed a reduction in tumor Ga-67 concentration, both being the reverse of what we found (see Tables 3, 4, and 5).

Their studies differed basically from ours in that they assayed the entire tumor for Ga-67 content rather than only its viable portion, as we did. Walker-256 tumors tend to be quite necrotic, and the necrotic portion shows much less Ga-67 uptake than does the viable part (*24*). (The fact that they assayed the whole Walker-256 tumor probably accounts for their observation of a considerably lower uptake of Ga-67 in untreated Walker-256 tumors than we did (*2*).) An increase in necrosis in x-irradiated animals, or a primary radiation effect on viable Walker-256 tumor tissue itself, could account for the discrepancy between our results and theirs in the case of hyperferremia. It is difficult, however, to reconcile the discrepancy in the case of iron-deficiency anemia, unless for some reason an increase in Ga-67 concentration was produced in the necrotic portion of Walker-256 tumors, off-setting the decrease that occurs in the viable portion. Or perhaps the extent of the necrosis in this tumor is greatly increased by an anemic state, thus tending to overshadow the effect produced in viable tumor. Our method of producing anemia was different (restricted iron intake plus multiple bleedings) from that used by Bradley et al. (*23*) and, of course, a different tumor type was involved in their study. But the most important point is that when we increased the transferrin level by administering apotransferrin intravenously, the same results were obtained as with anemia. These two studies were thus supportive of each other (see Tables 5 and 6).

The results we have obtained with in vivo administration of apotransferrin (Table 6) are in conflict with many of the in vitro studies that have been reported to date. These reports suggest that the uptake of Ga-67 by tumor tissue is dependent on its binding to transferrin (*5,6,25*).

In vitro studies are obviously extremely useful in isolating biological systems from the multitude of competing factors that exist in vivo; nevertheless, they are still artificial systems. In our own tissue-culture studies, we observed an uptake of Ga-67 by tumor cells far above that expected from simple diffusion, but we could not demonstrate any similarity between the in vitro and in vivo subcellular distributions of Ga-67 (unpublished results). Those results and the ones reported here raise a question as to whether the binding of Ga-67 to transferrin is of importance in the uptake of Ga-67 by tumor tissue. On the other hand, our results do indicate that transferrin is intimately involved in the uptake of Ga-67 by normal tissues.

We feel that the present studies strongly support our earlier contention (*8*) that there is a basic difference in the initial entry of Ga-67 into normal and tumor tissue, i.e., that Ga-67 entry into normal soft tissues is associated with Ga-67 binding by plasma protein whereas Ga-67 entry into tumor tissue involves an unbound or loosely bound form of gallium. No conclusions can be drawn, however, as to the means by which the entries occur, although it is obviously tempting to speculate that entry into soft tissue is through a form of pinocytosis while that into tumor tissue occurs primarily by reversible diffusion followed by an essentially irreversible binding, i.e., by a lysosomotrophic kind of process similar to that identified by de Duve et al. (*26*). The difference between normal-tissue and tumor uptake would therefore have to be attributed mainly to a difference in the plasma-membrane permeability of the two types of tissues. Holley (*27*) and many others have speculated on the likelihood that malignancy is associated with changes in membrane permeability. This would be a much more reasonable explanation than one ascribing special tumor-localizing properties to gallium, since other elements also appear to have this same property, although gallium (and indium) do show a similar unique binding to a particular type of protein in tumors (*28,29*).

FOOTNOTES

* New England Nuclear, North Billerica, MA.
† Alfa Inorganics, Beverly, MA.
‡ Lakeside Laboratories, Inc., Milwaukee, WI.
‖ United States Biochemical Corp., Cleveland, OH.
§ Simonsen Laboratories, Gilroy, CA, and the Mammalian Genetics Animal Production Section, Div. of Cancer Treatment, National Cancer Institute.
¶ Ralston Purina, St. Louis, MO.
** Amicon Corp., Lexington, MA.
†† American Monitor Corp., Indianapolis, IN.

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