Eur J Cancer Clin Oncol, Vol. 18, No. 7, pp. 661–668, 1982.
Printed in Great Britain.

0277-5379/82/070661-08$03.00/0

Tumor Cell Toxicity of Stable Gallium Nitrate: Enhancement by Transferrin and Protection by Iron*

JANET S. RASEY,† NORMA J. NELSON† and STEVEN M. LARSON‡

Departments of †Radiation Oncology, ‡Radiology, ‡Laboratory Medicine, and ‡Medicine, University of Washington Medical School, and ‡Nuclear Medicine Section, Veterans Administration Hospital, Seattle, Washington, U.S.A.

Abstract—*The cytotoxicity of citrated gallium nitrate (NSC 15200) to EMT-6/UW mouse sarcoma cells growing* in vitro *was assayed as growth inhibition in treated cultures as well as cell survival (colony-forming ability) after acute or chronic exposure to graded doses. Gallium nitrate is both cytostatic and lethal to cells, with some growth inhibition occurring after chronic exposure to low doses* (10 μg/ml) *which kill essentially no cells. Cell kill and growth inhibition were both observed if cells were exposed for* 24 hr *or more to doses greater than* 50 μg/ml. *The growth inhibitory and lethal effects of gallium nitrate were enhanced by the addition of human transferrin to the medium. This enhanced toxicity was consistent with, and proportional to, the increased gallium uptake in the presence of transferrin rather than a direct effect of this iron transport protein. The addition of ferric citrate greatly reduced the toxic effect of the gallium salt. Cells in stationary plateau phase cultures appear to be as sensitive to gallium nitrate as exponentially growing cells.* Ga^{3+} *may mimic* Fe^{3+} *in some aspects of cellular metabolism, and competition between the two metals occurs at the initial uptake step, binding to transferrin, and possibly at other points in cell metabolism.*

INTRODUCTION

GALLIUM nitrate (NSC 15200) and other stable gallium salts have antitumor effects in several rodent tumors and also inhibit the growth of tumor cells *in vitro* [1–3]. The *in vivo* studies employed 10 daily fractionated doses; tumor growth delay, host life extension, and percent cures at 4 months were the endpoints of response. Cells *in vitro* were exposed chronically to gallium nitrate and response was assessed as growth inhibition. Although antitumor activity was verified by these studies, fractional tumor cell survival as a function of gallium dose has not been presented. This can be done to quantify cell kill for cells exposed to radiation and most chemotherapeutic agents if the tumor system used is amenable to cell survival assay by cloning techniques. Response following acute exposure to high doses was not reported in these systems, nor was the effect of cell proliferation on sensitivity measured.

Recently we have utilized the EMT-6/UW sarcoma-like tumor system to study the mechanism of uptake of tracer levels of [^{67}Ga] citrate [4, 5]. This tumor grows *in vitro* as a monolayer culture or *in vivo* as a solid tumor in BALB/c mice. Concentration of [^{67}Ga] citrate by cells in both growth modes has been studied. We have shown that exogenous transferrin is necessary for cells *in vitro* to concentrate [^{67}Ga] above the level in the medium [6], and transferrin has been implicated in the *in vivo* accumulation of this metal ion as well [4]. [^{67}Ga] bound to transferrin has been isolated from lysates prepared from EMT-6/UW tumors after injection of [^{67}Ga] citrate [7].

Uptake and intracellular incorporation of tracer levels of [^{59}Fe] and [^{67}Ga] share several features in common. EMT-6/UW tumor cells concentrate [^{59}Fe] as well as [^{67}Ga] in the presence of transferrin, and the same cell surface transferrin receptor is implicated in uptake of the two metals [5]. Our recent work [8] and reports by Harris and Sephton [9] have

Accepted 8 January 1982.
*These investigations were supported by research grants CA 19899 and CA 24598 from National Cancer Institute DHEW and research funds from the Veterans Administration.

also shown that prior or concurrent exposure of cell cultures to stable iron as ferric citrate inhibits uptake of tracer [^{67}Ga].

We now report the results of our studies on the cytotoxic effect of stable citrated gallium nitrate on EMT-6/UW cells growing *in vitro*, using colony-forming ability (reproductive integrity) of individual cells as well as growth inhibition as endpoints of response. Exogenous transferrin enhances the cytotoxic effect, while stable ferric citrate protects against these salts. These results suggest that better knowledge of iron metabolism in tumor cells would yield insights into the mechanism of the cytotoxicity of gallium compounds.

MATERIALS AND METHODS

EMT-6/UW cells were grown in Waymouth's MB 752/1 medium supplemented with 15% fetal calf serum (Flow Laboratories, Inglewood, CA), 100 units of penicillin and 100 μg streptomycin sulfate/ml. For experiments with exponentially growing cells, cultures were seeded with 10^4 cells/cm^2 in 25 cm^2 Falcon tissue culture flasks or Ambitubes with a flat 5.5 cm^2 growth area (Lux Scientific Corp., Newbury Park, CA). Fed plateau phase cultures were initiated with 5×10^4 cells/cm^2. Medium was changed daily and a stable cell number of $\sim 8 \times 10^5$ cells/cm^2 was attained by day 3 or 4. Depending on the experimental protocol, cultures were supplemented with gallium or iron salts, with or without exogenous human transferrin (Tf) (Type B, greater than 90% iron-free, Sigma Chemical Co., St. Louis, MO). Citrated gallium nitrate (NSC 15200) was provided by the Division of Cancer Treatment, National Cancer Institute, as a solution in distilled water. It was diluted in Waymouth's medium immediately prior to addition to cultures. Iron was added as ferric citrate appropriately diluted in tissue culture medium. The citrated gallium nitrate preparation allows gallium salts to remain in solution at physiological pH. Ga^{3+} citrate is generally favored at low pH (< 4.0), with hydroxides predominating at higher pHs [10]. Because the gallium was presumed to be present as Ga^{3+} citrate, the use of ferric citrate as the competing iron compound was deemed appropriate.

To measure the actual cellular uptake of stable citrated gallium nitrate, it was supplemented with 1.0 μCi/ml carrier-free [^{67}Ga] citrate (Mediphysics, Emeryville, CA) in some experiments. After an incubation of 4–48 hr with stable gallium salt plus [^{67}Ga] citrate, cells were washed, harvested by trypsinization and pelleted by centrifugation, as described by Larson *et al.* [6]. Radioactive medium and the first rinse were saved as standards for counting. Cell pellets and medium samples were counted in a Packard Autogamma well-type scintillation counter. Uptake of radioactive gallium was expressed as % radioactivity incorporated per 10^7 cells, allowing calculation of total gallium uptake as micromoles of gallium salt.

Growth inhibition was determined by performing counts of cell number per flask or tube at various times after addition of cytotoxic and/or protective agents, using a Royco electronic cell counter. Clonogenic cell survival was determined by colony-forming ability of cells trypsinized from treated cultures. Cells were plated at low density in 60-mm Petri dishes containing 5 ml of Waymouth's medium and were allowed to grow undisturbed for 12 days. Colonies were fixed and stained with 0.25% crystal violet in formalin–ethanol fixative. All colonies containing more than 50 cells were counted and cell survival was expressed as (plating efficiency of treated cells)/(plating efficiency of control cells). Plating efficiency of control cultures ranged from 75–90%. The product (total cell number/flask or tube) $\times$ (fractional cell survival) = (number of viable cells/flask) was calculated. This was used to quantitate cell response following chronic exposure to gallium compounds, a useful approach when cells are being born as well as dying during the course of treatment and when the agent being tested is growth-inhibitory as well as lethal. This type of data analysis has been used for cells treated *in vitro* with low dose rate radiation from radium and ^{252}Cf [11].

RESULTS

The growth inhibition of exponentially growing EMT-6/UW cells by graded doses of citrated gallium nitrate is shown in Fig. 1. The ability of exogenous human transferrin to enhance the cytostatic and lethal effect of citrated gallium nitrate is also shown in Fig. 1. The concentration of Tf used, 0.25 mg/ml, was chosen because it was maximally effective in enhancing uptake of tracer levels of [^{67}Ga] citrate [5]. However, a lower Tf concentration, 0.025 mg/ml, was nearly as effective in promoting cytotoxicity of stable gallium nitrate. In studies of *in vitro* uptake of [^{67}Ga] citrate, this lower concentration of Tf also enhanced uptake of the nuclide. Incorporation was typically $\approx$35% of that stimulated by 0.25 mg/ml Tf [5].

(Surviving fraction) $\times$ (total number of cells/culture tube) equals the number of viable cells per culture tube following treatment with citrated gallium nitrate. This is shown in Figs

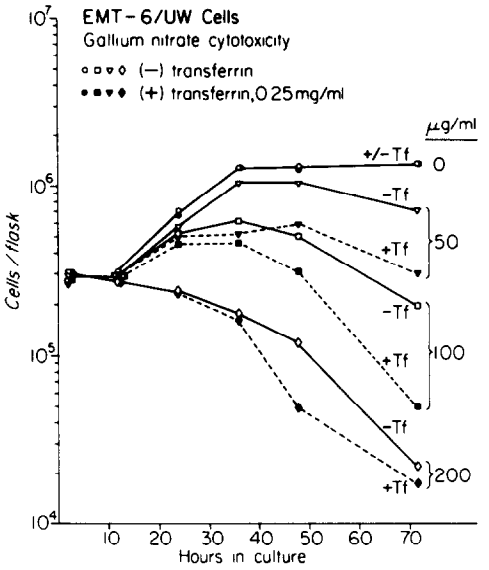

Fig. 1. Growth inhibition of exponentially growing EMT-6/UW cells by various concentrations of citrated $Ga(NO_3)_3$ *(NSC 15200) with or without transferrin* (0.25 mg/ml). *Cultures were established with* 2.75×10^5 *EMT-6/UW cells in* 1.1 ml *Waymouth's medium* + 15% *FCS, containing the specified amounts of* $Ga(NO_3)_3$. *Each point is the average of cell counts done on* 2 *replicate cultures.*

2a–c. Various concentrations of gallium nitrate were added at the time the cultures were established and cells were plated for survival determination 4, 24 or 48 hr later. Acute exposure, even of doses up to 200 μg/ml, produced essentially no cell kill (Fig. 2a). Chronic exposure reduced surviving fraction as well as number of viable cells per tube, but a 48-hr exposure was required before the effect of Tf in enhancing cell kill could be seen (Fig. 2c).

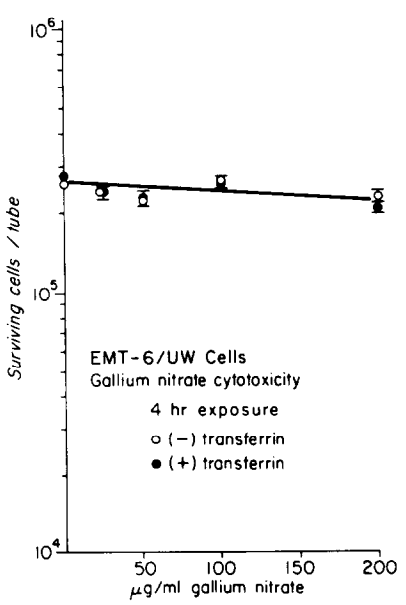

Fig. 2a.

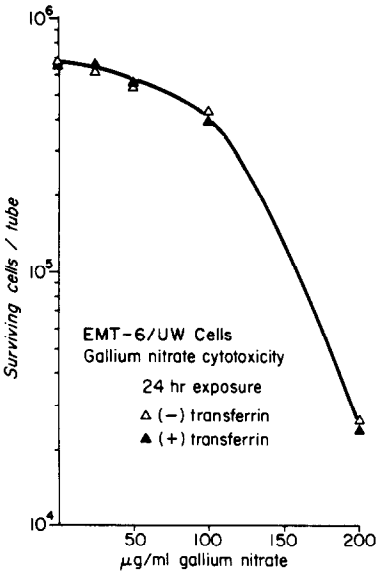

Fig. 2b.

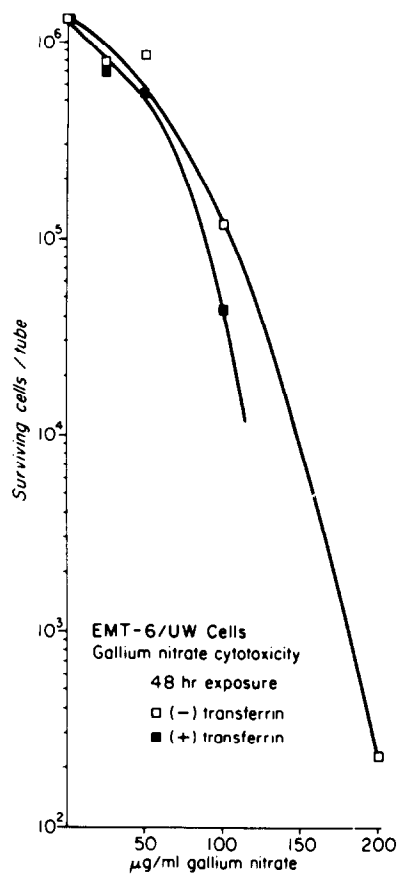

Fig. 2c.

Fig. 2. Cell survival in exponentially growing EMT-6/UW cultures established and allowed to grow for various times in culture medium containing graded doses of gallium nitrate with or without 0.25 mg/ml *transferrin. Surviving cells/culture tube is the product (total cells/tube)* × *(surviving fraction). Surviving fractions were determined by the ability of treated cells to form colonies when plated at low density, as described in Materials and Methods. The control cell plating efficiency was 75–90%. Each point is the mean* ± *standard error for all cell numbers plated at a specified dose. (a) 4-hr exposure; (b) 24-hr exposure; (c) 48-hr exposure.*

Experiments with tracer [^{67}Ga] citrate showed that exogenous transferrin was required for EMT-6/UW cells *in vitro* to concentrate this nuclide above levels in the culture medium [6]. It was therefore assumed that the enhanced cytotoxicity of gallium salts in the presence of added Tf was due to greater uptake, rather than a direct effect of this serum protein. This was tested directly by supplementing citrated gallium nitrate with [^{67}Ga] citrate, either without or with Tf (0.25 mg/ml). Cellular uptake, expressed as μmol $Ga^{+3}/10^7$ cells, is shown in Fig. 3. At each stable gallium concentration there was more uptake in the presence of transferrin and the effect is most pronounced at the long exposure times (48 hr). As noted above, 48 hr was the earliest time at which a difference in surviving fraction and number of viable cells/culture tube was observed between the transferrin-containing and transferrin-free samples. The increased uptake of gallium in the presence of transferrin is consistent with enhanced cytotoxic effect. Table 1 compares gallium uptake (pmol/cell) vs surviving cells/flask expressed as percentage of controls. For the 48-hr exposure time the lowest survival is seen for cells with the highest gallium content.

At 4 hr, gallium content/cell is lower and surviving percentage is slightly higher in cultures containing 25/μg gallium nitrate/ml, with or without Tf, than in cultures exposed at 50 μg/ml. The cell survival data are from a different experiment than the one in which

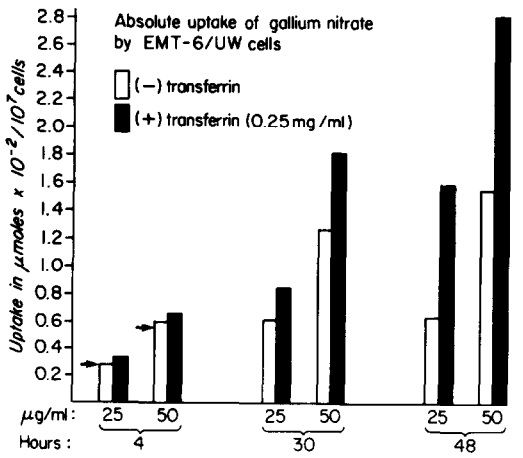

Fig. 3. Absolute uptake of stable citrated gallium nitrate by exponentially growing EMT-6/UW cells in vitro. *Various concentrations of stable gallium salts were supplemented with* [^{67}Ga] *citrate* (1 μCi/ml *medium*), *with or without* 0.25 mg *transferrin*/ml. *Cells were harvested at the indicated times and counted for* [^{67}Ga] *incorporation in a Packard Autogamma well-type scintillation counter. Uptake was then calculated in* μmol *gallium*/10^7 *cells. The horizontal arrows indicate uptake equivalent to equilibration with medium.*

gallium content/cell was measured. Therefore, although the trend is clear, a direct comparison between survival and uptake cannot legitimately be made.

Transferrin is principally an iron transport protein and enhances the uptake of tracer levels of [^{59}Fe] as well as [^{67}Ga] by EMT-6/UW cells [5]. It was therefore assumed there might be other similarities of iron and gallium metabolism by these cells which might relate to

Table 1.

Incubation conditions $GaNO_3$ (μg/ml)	Time	Tf	$Ga(NO_3)_3$ uptake (pmol/cell × 10^4)	Surviving cells/flask (% of control)
0	4	±	0	100%
25	4	+	3.32	85.3 ± 3.8%
25	4	−	2.79	92.9 ± 4.9%
50	4	+	6.45	82.3 ± 4.7%
50	4	−	5.96	87.4 ± 1.3%
0	48	±		100%
25	48	+	15.9	53.8 ± 1.3%
25	48	−	6.3	60.6 ± 2.1%
50	48	+	28.3	42.4 ± 0.4%
50	48	−	15.6	67.5 ± 1.3%

*Surviving cells/flask were determined from the product (total cells/flask) × (surviving fraction). Surviving fraction was determined as the proportion of cells capable of forming a colony when they were plated at low density and allowed to grow undisturbed for 12–13 days, corrected for plating efficiency of untreated control cultures.

the cytotoxicity of stable gallium compounds. Exponentially growing EMT-6/UW cells were exposed to various concentrations of citrated Ga $(NO_3)_3$ alone, ferric citrate alone, or citrated Ga $(NO_3)_3$ plus ferric citrate. All cultures contained 0.25 mg/ml Tf. Ferric citrate alone had little effect on cell growth while citrated gallium nitrate alone was highly toxic, as shown above. Co-incubation with citrated gallium nitrate and ferric citrate markedly decreased the cytotoxicity of the gallium compounds, as measured by inhibition of cell growth (Fig. 4) as well as number of viable (colony forming) cells/culture tube (Table 2).

Because many chemotherapeutic agents are more effective against actively growing cells than stationary (plateau) phase cells, the cytotoxicity of gallium nitrate was tested with fed plateau phase EMT-6/UW cells as well as exponentially growing cultures. The results are shown in Table 3 and Fig. 5 for a 24-hr exposure to gallium nitrate, with treatment beginning at 96 hr of culture age, after a plateau in cell number had been reached. The results in cell number/culture flask as well as number of viable cells/flask (Table 3, Fig. 5) are expressed as percentages of control values. These are compared with results of similar experiments with log phase cells (Table 3). All data are given as percentages because the cell number/control flask is far larger for fed plateau than for exponentially growing cultures.

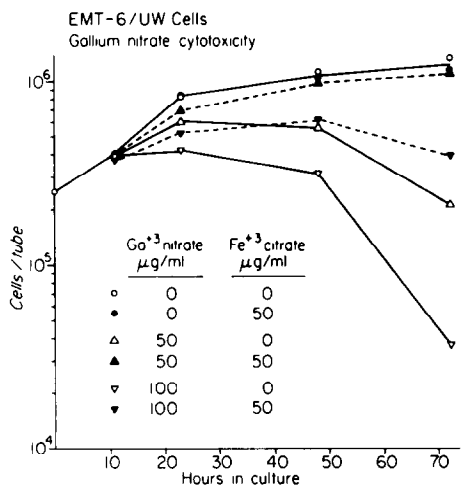

Fig. 4. Effect of iron (Fe^{+3}) on the response of exponentially growing EMT-6/UW cells to citrated gallium nitrate. Cultures were established in medium containing the indicated amounts of citrated gallium nitrate, ferric citrate, or both agents. Each point is the average of cell counts from 2 replicate cultures.

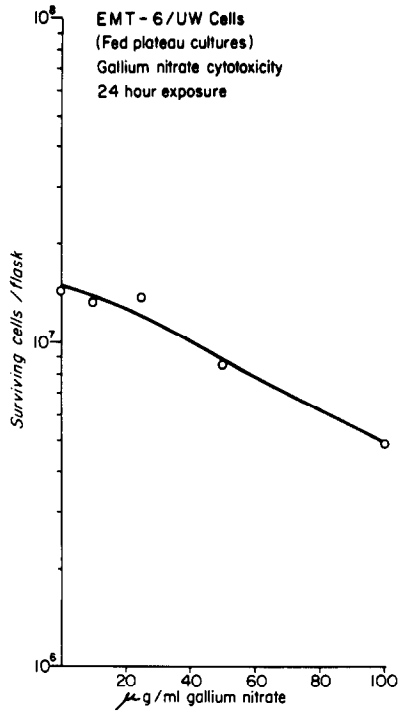

Fig. 5. Cell survival in fed plateau phase EMT-6/UW cultures exposed to graded doses of citrated gallium nitrate for 24 hr *after attainment of a plateau in cell number. All cultures contained* 0.25 mg *transferrin*/ml. *Surviving cells/culture flask is the product (total cells/flask) × (surviving fraction). Surviving fractions were determined by the ability of treated cells to form colonies when plated at low density, as described in Materials and Methods. Control cell plating efficiency was* 83%.

Table 2. Number of surviving cells/culture tube after 48 hr *exposure to citrated* $Ga(NO_3)_3$: *effect of stable* Fe *citrate**

Addition to culture tubes	Number of surviving cells tube	Percentage of control
None (Controls)	1.12×10^6	100
50 μg/ml Fe citrate	1.094×10^6	97.7
50 μg/ml $Ga(NO_3)_3$	8.960×10^4	8.0
50 μg/ml $Ga(NO_3)_3$ + 50 μg/ml Fe citrate	7.494×10^5	66.9
100 μg/ml $Ga(NO_3)_3$	6.266×10^3	0.6
100 μg/ml $Ga(NO_3)_3$ + 50 μg/ml Fe citrate	2.204×10^5	19.7

*All cultures contained 0.25 mg/ml Tf.

Table 3. Total cells/flask and surviving cells/flask as percentages of control values for exponentially growing and fed plateau phase EMT-6/UW cultures: 24 hr exposure to $Ga(NO_3)_3$*

$Ga(NO_3)_3$(μg/ml)	Exponentially growing	Fed plateau phase
	Total cells/flask as % of control	
0 (Control)	$100\left(6.715\times10^5\frac{\text{cells}}{\text{flask}}\right)$	$100\left(1.441\times10^7\frac{\text{cells}}{\text{flask}}\right)$
10	—	95.3
25	88.2	112.8
50	76.5	85.2
100	67.8	78.2
200	33.7	—
	Surviving cells/flask† as % of control	
0 (Control)	$100\left(6.50\times10^5\frac{\text{cells}}{\text{flask}}\right)$	$100\left(1.441\times10^7\frac{\text{cells}}{\text{flask}}\right)$
10	—	92.9 ± 5.3
25	101.7 ± 3.21	96.2 ± 3.56
50	86.4 ± 2.21	59.9 ± 2.87
100	61.0 ± 0.25	34.2 ± 0.41
200	3.67 ± 0.17	—

*Cell numbers attained per culture vessel differ in exponential and fed plateau phase cells. Small culture tubes were used with fed cultures while flasks were used for exponentially growing cells. Therefore results are expressed as percentages of controls (no Ga nitrate added) for comparison. All cultures contained 0.25 mg human Tf/ml in addition to the specified amount of $Ga(NO_3)_3$. Exponentially growing cultures were established in $Ga(NO_3)_3$-containing medium and were harvested at 24 hr culture age. Plateau cultures fed daily reached a plateau in cell number by day 4; at that time they were fed with $Ga(NO_3)_3$ containing medium and harvested 24 hr later.

†Surviving cells/flask was calculated as described in the footnote to Table 1.

The percentage of cells in DNA synthesis is 54.5% in exponential cells and 21.1% in fed plateau phase cells [12]. Stationary cultures are at least as sensitive as log phase cultures using either criterion: cell number/culture vessel or number of viable cells/culture flask or tube. They may, in fact, be more sensitive.

DISCUSSION

Treatment of experimental tumors with gallium nitrate and salts of other Group IIIa metals in the periodic table (aluminium, indium, thallium) indicated some antineoplastic activity for all agents tested [3]. Using tumor growth inhibition as an endpoint of response, gallium nitrate was more effective against a wider range of tumors than were the other compounds tested. Greater than 50% growth inhibition was observed in seven solid subcutaneous rodent tumors treated with gallium nitrate doses at or near the LD_{10} [2]. Gallium nitrate was also effective against some ascites tumors using host life extension as a measure of effectiveness [1], and it inhibited the growth of Walker 256 carcinosarcoma and leukemia L1210 in suspension culture *in vitro* [3]. Exposure to gallium nitrate *in vivo* and *in vitro* was chronic rather than acute. Tissue culture medium was supplemented with gallium salts for a 3-day test growth period [3], and tumor-bearing rats or mice were treated with 10 equal daily injections. These approaches suggested that the gallium salt was toxic due to a gradual accumulation and interference with normal cell metabolism, rather than rapid blockage of a vital cell function. Because gallium nitrate has been studied after chronic exposure only, we chose to examine response to acute as well as continuous exposure of the EMT-6/UW tumor line and to further quantitate tumor cell response in terms of surviving fraction of cells as well as growth inhibition. This would allow separation of cytostatic effects from lethal damage. Furthermore, it seemed appropriate to relate the mechanism of uptake and cyto-

toxicity to our studies of uptake of tracer levels of the tumor-imaging agent, carrier-free [^{67}Ga] citrate, by the EMT-6/UW tumor system. It was assumed that the uptake mechanism would be similar for tracer quantities of the radioactive nuclide and the stable gallium salts.

The investigations of [^{67}Ga] uptake indicate similarities between tracer [^{67}Ga] uptake and iron (Fe^{+3}) metabolism in the cell, which suggested that stable ferric iron compounds might protect cells against the toxic effects of gallium nitrate. Exogenous transferrin is required for EMT-6/UW tumor cells *in vitro* to accumulate [^{67}Ga] citrate above the level in the medium, and transferrin is also implicated in the uptake of [^{67}Ga] by solid EMT-6/UW tumors *in vivo*. The transferrin receptor hypothesis for uptake of carrier-free [^{67}Ga] citrate proposes that [^{67}Ga] is taken up by tumor cells in a step-wise process: (1) [^{67}Ga] binds to transferrin in tissue culture medium *in vitro* or in the blood *in vivo*; (2) the gallium–transferrin complex binds to a cell surface transferrin receptor; (3) the transferrin–metal complex is incorporated into the cell by adsorptive endocytosis; (4) the gallium binds to one or more acceptor molecules within the cell after release from transferrin (5). Because transferrin is principally an iron transport protein, the uptake and intracellular concentration of [^{59}Fe] citrate was also examined in EMT-6/UW cells *in vitro*, either with or without additional transferrin. Tumor cells were found to concentrate large amounts of [^{59}Fe] (up to 50% of the iron in the medium) per 10^7 cells in the presence of small amounts of Tf [5]. These results were correlated with investigations of the mol. wt. profiles of gallium- and iron-binding proteins isolated after labelling of EMT-6/UW tumor cells *in vitro* and *in vivo* [13]. Taken together, they indicate that gallium and iron metabolism in the cell may share several pathways in common.

Iron (either Fe^{2+} or Fe^{3+}) appears to be necessary for the function of the enzyme ribonucleotide reductase [14], and it is also a requirement for proliferation of HeLa cells *in vitro* [15, 16] and compensatory cell profileration in rodent colon after starvation [17]. Most tumors are more rapidly proliferating than many normal tissues, although the dose-limiting normal tissues in chemotherapy are frequently the renewal tissues with rapid cell turnover. Cancer chemotherapy agents directed again ribonucleotide reductase have been given serious consideration [18–20]. Agents which specifically interfere with ribonucleotide reductase (viz. hydroxyurea) are most effective against S phase cells. While this enzyme requires iron, the trace metal certainly has other uses in the cell. Therefore it is not surprising that a substance such as gallium, which interferes with iron metabolism, could kill both proliferating and non-proliferating cells.

This information, taken together with the results of studies reported here, suggests a possible mechanism for gallium cytotoxicity. Stable citrated gallium nitrate is taken up and concentrated in the same manner as tracer [^{67}Ga] or [^{59}Fe]. Gallium may compete with iron for intracellular binding sites and interfere with normal cellular iron metabolism and, consequently, with cell proliferation and survival. This may act through inhibition of ribonucleotide reductase activity, although other mechanisms are possible because iron is a cofactor for other enzymes as well. Studies on the rate of release of [$^{59}Fe^{3+}$] citrate from EMT-6/UW cells, even in the presence of excess stable ferric iron, indicate that release is slow [8]. This suggests that gallium would be cytotoxic only with chronic exposure and would be most damaging under conditions which allow greater accumulation in the cell, i.e. in the presence of exogenous Tf. The slow turnover of iron might allow stable gallium to gradually exert a toxic effect when present in the culture medium for long periods. Our results are consistent with this interpretation.

The ability of stable ferric citrate to protect EMT-6/UW cells from the cytotoxic effects of citrated gallium nitrate certainly relates to the similar mechanisms of uptake of the two metals and may only involve the first step, binding to Tf. Tf has a much higher affinity for iron (binding constant, $K = 9 \times 10^6$ l/mol) than for gallium ($K = 2.5 \times 10^5$ l/mol) in Waymouth's tissue culture medium [5, 21]. Stable ferric citrate greatly reduces uptake of tracer levels of [^{67}Ga] citrate into exponentially growing EMT-6/UW cells *in vitro* when both are added concurrently [8]. A 24-hr pre-incubation with ferric citrate slightly reduces uptake of tracer [^{67}Ga] citrate during a subsequent 24-hr exposure period [8]. It is not yet clear whether the pre-incubation with iron salts saturates gallium-binding macromolecules within the cell.

If ferric iron and gallium do compete for uptake into tumor cells, this may partially explain the low tumor toxicity seen to date with gallium nitrate in cancer chemotherapy [22]. Because iron is a ubiquitous and essential metal in normal human metabolism, a more thorough knowledge of serum iron levels and iron kinetics may be necessary if gallium nitrate is to be administered rationally in patients.

REFERENCES

1. HART MM, SMITH CF, YANCY ST, ADAMSON RH. Toxicity and antitumor activity of gallium nitrate and periodically related metal salts. *J Natl Cancer Inst* 1971, **47**, 1121–1127.
2. ADAMSON RH, CANELLOS GP, SIEBER SM. Studies on the antitumor activity of gallium nitrate (NSC-15200) and other group IIIa salts. *Cancer Chemother Rep* 1975, **59**, 599–610.
3. HART MM, ADAMSON RH. Antitumor activity and toxicity of salts of inorganic group IIIa metals: aluminum, gallium, indium and thallium. *Proc Natl Acad Sci USA* 1971, **68**, 1623–1626.
4. LARSON SM, RASEY JS, ALLEN DR, GRUNBAUM Z. A transferrin mediated uptake of gallium-67 by EMT-6/UW sarcoma II. Studies *in vivo* (BALB/c mice). *J Nucl Med* 1979, **20**, 843–846.
5. LARSON SM, RASEY JS, ALLEN DR *et al.* Common pathway for tumor cell uptake of gallium-67 and iron-59 via a transferrin receptor. *J Natl Cancer Inst* 1980, **64**, 41–53.
6. LARSON SM, RASEY JS, ALLEN DR, NELSON NJ. A transferrin-mediated uptake of gallium-67 by EMT-6/UW sarcoma. I. Studies in tissue culture. *J Nucl Med* 1979, **20**, 837–842.
7. LARSON SM, GRUNBAUM Z, RASEY JS. Demonstration of gallium-67 bound to transferrin in lysates of EMT-6 sarcoma cells harvested from BALB/c mice after prior intravenous injection of ^{67}Ga/citrate. (Abstract) *J Nucl Med* 1980 **21**, P23.
8. RASEY JS, LARSON SM, NELSON NJ. Mechanism of gallium-67 uptake by tumors: similarities of gallium and iron metabolism. *J Nucl Med* 1980, **21**, P23.
9. HARRIS AW, SEPHTON RG. Transferrin promotion of ^{67}Ga and ^{59}Fe uptake by cultured mouse myeloma cells. *Cancer Res* 1977, **37**, 3634–3638.
10. HAYES RL. Factors affecting uptake of radioactive agents by tumor and other tissues. *Tumor Localization with Radioactive Agents.* Vienna, IAEA-MG-50/14, 1976, 29–45.
11. HALL EJ, ROSSI HH, ROIZIN LA. Low dose-rate irradiation of mammalian cells with radium and Californium-252. *Radiology* 1971, **99**, 445–451.
12. RASEY JS, NELSON NJ. Repair of potentially lethal damage following irradiation with X-rays or cyclotron neutrons: response of the EMT-6/UW tumor system treated under various growth conditions *in vitro* and *in vivo*. *Radiat Res* 1981, **85**, 69–84.
13. LARSON SM, RASEY JS, NELSON NJ *et al.* The kinetics of uptake and macromolecular binding of Ga-67 and Fe-59 by the EMT-6/UW sarcoma-like tumor of BALB/c mice. *Proceedings of the 2nd International Radiopharmaceutical Symposium, Seattle,* 19–22 March 1975. Society of Nuclear Medicine, 297–308.
14. MOORE EC, ZCDEK MS, AGRAWAL KC, SARTORELLI AC. Inhibition of ribonucleotide reductase by 1-formyl 180 quinolene thiosemicarbazone and related compounds. *Biochemistry* 1970, **9**, 4492–4498.
15. ROBBINS E, PEDERSON T. Iron: its intracellular localization and possible role in cell division. *Proc Natl Acad Sci USA* 1970, **66**, 1244–1251.
16. ROBBINS E, FANT J, NORTON W. Intracellular iron binding macromolecules in HeLa cells. *Proc Natl Acad Sci USA* 1972, **69**, 3708–3712.
17. STRAGAND JJ, HAGEMANN RF. An iron requirement for the synchronous progression of colonic cells following fasting and refeeding. *Cell Tiss Kinet* 1978, **11**, 513–518.
18. AGRAWAL KC, BOOTH BA, MOORE EC, SARTORELLI AC. Potential antitumor agents 6. Possible irreversible inhibitors of ribonucleotide diphosphate reductase. *J Med Chem* 1972, **15**, 1154–1158.
19. BROCKMAN RW, SIDWELL RW, ARNETT G, SHADDIX S. Heterocyclic thiosemicarbazones: correlation between structure, inhibition of ribonucleotide reductase and inhibition of DNA viruses. *Proc Soc Exp Biol Med* 1970, **133**, 609–614.
20. ELFORD HL, FREESE M, PASSAMANI E, MORRIS HP. Ribonucleotide reductase and cell profiiferation I. Variations of ribonucleotide reductase activity with tumor growth rate in a series of rat hepatomas. *J Biol Chem* 1970, **245**, 5228–5233.
21. LARSON SM, ALLEN DR, RASEY JS, GRUNBAUM Z. Kinetics of binding of carrier free Ga-67 to human transferrin. *J Nucl Med* 1978, **19**, 1245–1249.
22. BEDIKIAN AY, VALDIVIESON M, BODEY GP *et al.* Phase I clinical studies with gallium nitrate. *Cancer Treat Rep* 1978, **62**, 1449–1453.