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ACCUMULATION OF RADIOISOTOPES WITH TUMOR AFFINITY II. COMPARISON OF THE TUMOR ACCUMULATION OF ^{67}GA-CITRATE AND ^{201}TL-CHLORIDE *IN VITRO*

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Abstract. The kinetics in tumor cells and various factors affecting the tumor accumulation of ^{67}Ga-citrate and ^{201}Tl-chloride were studied *in vitro*. ^{67}Ga was taken up gradually by tumor cells and its excretion from the cells decreased with time. ^{201}Tl was taken up rapidly by tumor cells. Its excretion was very rapid, indicating that the two nuclides had entirely different kinetics in tumor cells. The uptake of ^{201}Tl by culture cells correlated with that of ^{42}KCl and was inhibited by Ouabain. ^{201}Tl was hardly taken up by nonviable tumor cells. These facts indicate that active transport involving Na-K ATPase is involved in the tumor accumulation of ^{201}Tl. The uptake of ^{67}Ga and ^{201}Tl by tumor cells was not affected by the administration of anticancer agents. The uptake of ^{67}Ga by tumor cells was dependent upon the concentration of transferrin in the medium, which apparently plays a role as one of the pathways of tumor accumulation of ^{67}Ga.

Key wards : ^{67}Ga, ^{201}Tl, tumor accumulation *in vitro*, culture cells.

Imaging with ^{67}Ga-citrate is widely used for the clinical diagnosis of malignant tumors and inflammatory foci of many organs. However, the accumulation mechanism of ^{67}Ga to tumor and inflammatory focus is poorly understood (1). In the author's first report (2), delay in the excretion of ^{67}Ga from tomor cells was shown to be important.

^{201}Tl-chloride was developed as a myocardial scanning agent and its tumor affinity has been demonstrated (3, 4). It is used in the diagnosis of tumors of the thyroid (5); however, studies of tumor accumulation are few and leave much unclear (6).

In the present investigation, the tumor accumulation mechanism of ^{67}Ga-citrate and ^{201}Tl-chloride and the difference in the tumor affinity between the two nuclides was investigated by studying the kinetics in tumor cells *in vitro*.

MATERIALS AND METHODS

Radioisotopes. ^{67}Ga-citrate and ^{201}TlCl were obtained from Daiichi Radioisotope Laboratory (Tokyo, Japan). On the assay date, 1 mCi of carrier-free ^{67}Ga or ^{201}Tl was dissolved in 1 ml of physiological saline, then diluted to the appropriate concentration. ^{42}KCl was a product of the Japan Atomic Energy Research Institute having a specific activity of 7.7-8.0 $\times 10^2$ mCi/g K. [6-^{3}H] thymidine (^{3}H-TdR) was a product of the

Radiochemical Center (Amersham, Holland) with a specific activity of 95 mCi/mg.

Culture cells and conditions. The culture cells were HeLa S3, AS II derived from human ovary embryonic cancer, normal human fibroblasts (normal h.f.), and Yoshida sarcoma (Y.S.). Except for normal h.f., the cells and culture conditions were the same as in my first report (2). All the cells were maintained in Eagle's minimum essential medium (MEM) containing 10% fetal calf serum (FCS).

Measurement of uptake and excretion of tracers in culture cells. The methods of measuring the uptake and excretion of ^{67}Ga in the culture cells were the same as described in the previous report. Briefly, in the experiment using monolayer cells such as HeLa S3, AS II, and normal h.f., the cells were placed in a plastic Petri dish 35 mm in diameter and incubated for 20-24 h at 37°C. Then the medium was exchanged with fresh MEM containing 10% FCS, and ^{67}Ga was added usually in the concentration of 1 μCi/ml-medium and incubated for a further 0.5-24 h. After incubation, the cells were washed 3 times with 2 ml of cold PBS while cooling in ice. In measuring ^{67}Ga uptake, 1 ml of 0.2% trypsin solution was added and the incubation was carried out for 15 min at 37°C, then the cells were peeled from the Petri dishes. The cells were transferred to a test tube, and in order to eliminate the effect of ^{67}Ga attached to Petri dish the cells were washed twice with 7 ml cold PBS by centrifugation at 4°C. The ^{67}Ga activity in the cells was estimated. When measuring ^{67}Ga excretion, the cells were washed 3 times with cold PBS, fresh MEM containing 10% FCS was added, then the cells were incubated for a further 12 h. The residual percent activity of ^{67}Ga in the cells was estimated. In order to promote ^{67}Ga excretion and to prevent resorption of ^{67}Ga, the medium was exchanged at 1, 2, 4, 6, 8 and 10 h.

In the experiment using the cell suspension of Y.S., the cells were poured into a plastic test tube 12 mm in diameter and ^{67}Ga was added as in the case of monolayer cells and incubated. The cells were washed 4 times with 7 ml cold PBS by centrifugation at 4°C, and the ^{67}Ga uptake by the cells was measured. In measuring the excretion of ^{67}Ga, fresh medium was added after washing with PBS and the cells were incubated at 37°C for 12 h without exchanging the medium. The residual percent activity of ^{67}Ga in the cells was estimated.

In measuring the uptake and excretion of ^{201}Tl in the cells, trypsin solution was not used when monolayer cells were peeled from the Petri dish. The reason for this lies in the fact that the excretion of ^{201}Tl from the cell is so rapid that ^{201}Tl taken up by the cells is practically all excreted during the incubation at 37°C for 15 min after the addition of trypsin solution to a Petri dish. Therefore, in measuring the uptake of ^{201}Tl in monolayer cells, after the cells had beed incubated with ^{201}Tl, they were washed 4 times with 2 ml cold PBS while cooling in ice. Then 1 ml of cold distilled water was added and the cells were scraped from Petri dish by a rubber policeman. The distilled water containing the cells was transferred to a test tube and the ^{201}Tl activity was estimated. In the experiment using the cell suspension of Y.S., ^{201}Tl uptake was measured in the same way as ^{67}Ga. The residual percent activity of ^{201}Tl in the cells was estimated one hour after the exchange of medium. The uptake of ^{42}K in cluture cells was also measured by the same method as for ^{201}Tl.

The cell counts when the uptake of ^{67}Ga, ^{201}Tl and ^{42}K were measured were 50-100 × 10^4/dish with HeLa S3 and AS II, 20-50 × 10^4/dish with normal h.f. and 50-100 × 10^4/tube with Y.S.. The diameters of 100 cells suspended in trypsin solution were measured with a micrometer, and the volume of cells was calculated using the average

diameter and assuming that the cells to be spherical.

Preparation of nonviable cells. After placing Y.S. in a plastic test tube, nonviable Y.S. cells were obtained by heating in a water bath for 30 min at 56°C. The viability of these cells was determined by their ability to exclude 0.5% trypan blue.

Effects of anticancer agents. The anticancer agents used were Mitomycin C (MMC), Adriamycin (ADR), 5-Fluorouracil (5-FU), and Neocarzinostatin (NCS). After placing HeLa S3 in a Petri dish and incubating for 20-24 h, anticancer agents of various concentrations were added and the incubation was continued. ^{67}Ga was added 24 h after the addition of an anticancer agent, and its uptake was measured 24 h afterward. The residual percentage of ^{67}Ga in the cells was measured 12 h after the medium was exchanged. ^{201}Tl was administered 48 h after the addition of an anticancer agent, and its uptake was estimated 30 min afterward. Time-lapse changes in cell counts were measured, morphological changes of the cells studied by light microscopy, and the diameter of cells were measured 48 h after the administration of anticancer agents.

The effects of anticancer agents on DNA synthesis were ditermined by measureing the uptake of ^{3}H-TdR by the cells. The cells were incubated with anticancer agents for 48 h at 37°C, then ^{3}H-TdR was added in the concentration of 1 μCi/ml-medium. The cells were incubated for a further 30 min at 37°C, then were washed 3 times with 2 ml cold PBS, and 3 times with cold 5% trichloroacetic acid. Thereafter the cells were lyzed by adding 2 ml of 1N NaOH, further neutralized with HCl, and the activity of ^{3}H-TdR taken up by the cells was measured with a liquid scintillation counter.

The effect of sera on ^{67}Ga and ^{201}Tl uptake. The sera used were normal human serum (HS), rat serum (RS), and FCS. Cells were placed in a Petri dish or a plastic test tube, and after incubation for 20-24 h, washed 3 times with PBS. MEM containing FCS, HS or RS in various concentration was added, then the uptake of ^{67}Ga and ^{201}Tl estimated.

Similarly, the effects of Cohn fraction V (>95% albumin), IV-4 (alpha and beta globulins), II (>95% gamma globulin) of HS, and human apotransferrin (>90% iron free) on the uptake of ^{67}Ga and ^{201}Tl were studied. *In vitro* binding of ^{67}Ga to HS, FCS and human apotransferrin (Tf) was determined by equilibrium dialysis using a cellulose membrane with pore size of 24 Å (excluded more than 12,000 mol. wt.). The membrane was pretreated by boiling for 5 min in 5% sodium carbonate with 50 mM EDTA added. Two ml of MEM containing various concentration of HS, FCS or Tf was incubated with 1 μCi/ml of ^{67}Ga for 24 h at 37°C. After the incubation, 1 ml of the mixture was placed into a cellulose bag, and dialyzed for 24 h at 4°C against 8 ml of MEM (pH 7.2-7.4) with continuous turning. Gallium binding was calculated acording to the following formula:

$$\% \text{ Ga bound} = 100\ (Ci\text{-}C_0)\ Vi/C_0V_0 + CiVi$$

where C_0 = cpm/ml outside membrane; Ci = cpm/ml inside membrane;
V_0 = volume outside membrane; Vi = volume inside menbrane.

RESULTS

Comparison of the kinetics of ^{67}Ga and ^{201}Tl in culture cells. Fig. 1 shows the time-lapse uptake of ^{67}Ga and ^{201}Tl by Y.S.. The abscissa shows the incubation time (contact time) after the addition of ^{67}Ga or ^{201}Tl to the medium. The ordinate indicates the amount of uptake represented in % of ^{67}Ga or ^{201}Tl activity per 10^6 cells against the activity of ^{67}Ga or ^{201}Tl added to the medium. The uptake of ^{67}Ga did not show any marked difference in the contact time between 30 min and

3 h, but from 3 h up to 24 h it increase linearly with contact time, showing a biphasic tendency. In contrast, ^{201}Tl was rapidly taken up by Y.S., and in the contact time of 30 min to 24 h, the uptake of ^{201}Tl was approximately constant. Therefore in the later experiments, the measurement of ^{67}Ga uptake was usually taken at the contact time of 24 h, and ^{201}Tl uptake was measured at 30 min.

The relation between the concentration of ^{67}Ga and ^{201}Tl on the one hand and the uptake of ^{67}Ga and ^{201}Tl by Y.S. on the other is shown in Fig. 2. The up-

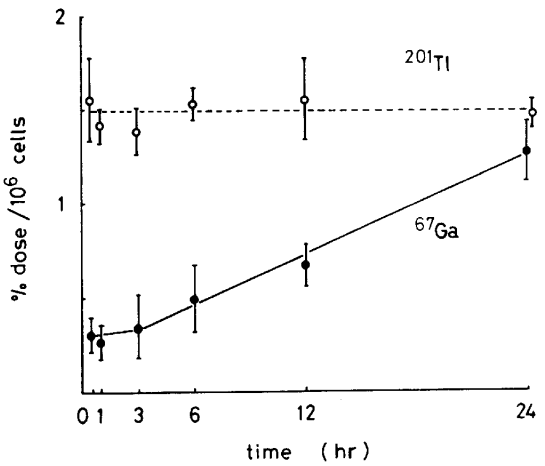

Fig. 1. Time course of ^{67}Ga and ^{201}Tl uptake by Yoshida sarcoma. Cells were incubated with 1 μCi/ml ^{67}Ga or ^{201}Tl for varying intervals at 37°C. Each point represents the mean ± standard deviation for 4 experiments.

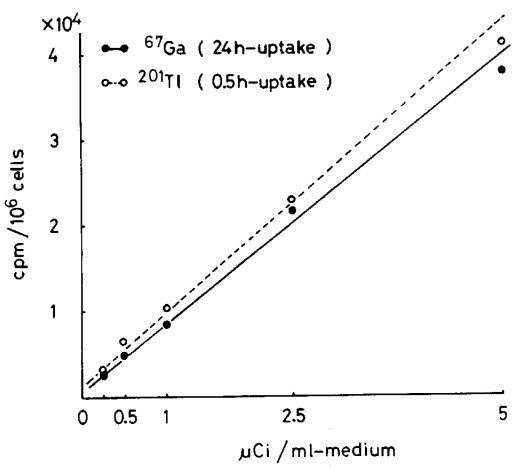

Fig. 2. Effect of various gallium and thallium concentrations on ^{67}Ga and ^{201}Tl uptake by Yoshida sarcoma. Each point represents the mean of two experiments.

take of ^{67}Ga and ^{201}Tl, when the concentrations were changed from 0.25 to 5 μCi/ml-medium, increased in proportion to the concentration and there was no saturation of the uptake.

The effects of contact time on the excretion of ^{67}Ga and ^{201}Tl from Y.S. are shown in Fig. 3. The excretion of ^{67}Ga tended to be prolonged as the contact time grew longer, and in the contact time of 24 h, the residual percentage in the cells at 12 h after the exchange of medium was about 90%. In contrast, the excretion of ^{201}Tl was extremely rapid irrespective of contact time, and the residual percentage in the cells at one hour after the exchange of medium was 4-6%. Moreover, ^{201}Tl excretion from HeLa S3, AS II or normal h.f. was also rapid as for Y.S., and the residual percentage in the cells was about 2% in every cell.

Comparision of ^{201}Tl and ^{42}K uptake. The time-lapse uptake of ^{201}Tl and ^{42}K by Y.S. from 10 min to 120 min after the administration is shown in Fig. 4. ^{201}Tl was taken up rapidly and after 20 min saturation was reached. In contrast, the uptake of ^{42}K increased in proportion to contact time, and the difference in uptake of the two nuclides was probably due to a carrier effect.

A comparison of ^{201}Tl and ^{42}K uptake by various culture cells measured after 30 min is shown in Table. 1. ^{201}Tl uptake per 10^6 cells was greater in normal h.f. than in other tumor cells. However, when ^{201}Tl uptake was represented per 1 mm^3 in consideration of the cell volume, the uptake by tumor cells such as HeLa S3, AS II and Y.S. (although the experimental conditions in Y.S. differed from other cells) was greater than by normal h.f.. This tendency was also true of ^{42}K uptake. ^{201}Tl uptake was greater than ^{42}K uptake in all cells, but the ratio of Tl to K was 2.0-2.8, showing no great difference; hence there was a correlation between ^{201}Tl uptake and ^{42}K uptake.

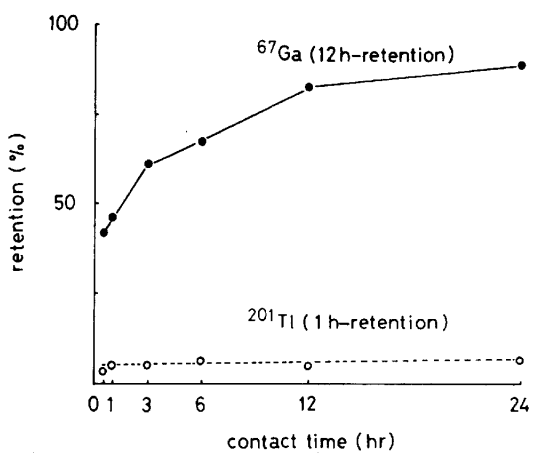

Fig. 3. Effect of contact time on excretion of ^{67}Ga and ^{201}Tl from Yoshida sarcoma. Each point represents the mean of two experiments.

Comparison of ^{67}Ga, ^{201}Tl and ^{42}K uptake by nonviable tumor cells. The uptake of ^{67}Ga, ^{201}Tl and ^{42}K by Y.S. cells rendered nonviable by heating was studied (Table 2). The cell suspension of Y.S. (control) contained 3.7% of nonviable cells but when this cell suspension was incubated at 56°C for 30 min, the percentage of nonviable cells increased to 95.7%. The uptake of ^{201}Tl and ^{42}K by nonviable cells was markedly decreased compared to the control (2-4% of the control). ^{67}Ga uptake by nonviable cells was significantly increased compared to control. This increase was at the contact time of 30 min. It is unclear whether more ^{67}Ga is taken up if the contact time is prolonged.

Effect of Ouabain on the uptake of ^{67}Ga, ^{201}Tl and ^{42}K by tumor cells. It is known that Ouabain inhibits specifically the action of Na-K pump (Na-K ATPase). The

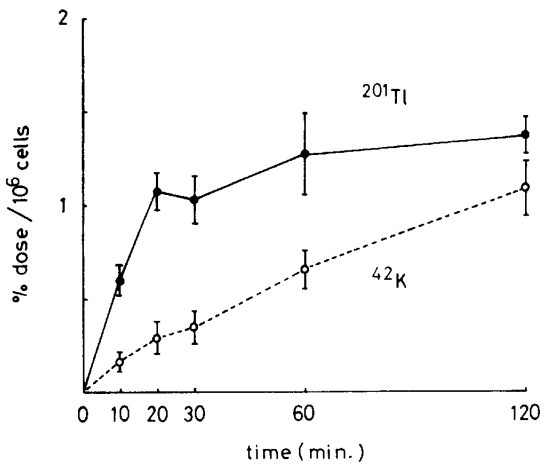

Fig. 4. Time course of ^{201}Tl and ^{42}K uptake by Yoshida sarcoma. Cells were incubated with 1 μCi/ml ^{201}Tl or 1.5 μCi/ml ^{42}K for varying intervals at 37°C. Each point represents the mean ± standard deviation for 4 experiments.

TABLE 1. COMPARISON OF ^{201}Tl AND ^{42}K UPTAKE BY VARIOUS CELLS

	Diameter of cells (μ)	Uptake				
		% dese/10^6 cells[a]		% dose/mm^3 [b]		Ratio
		^{201}Tl	^{42}K	^{201}Tl	^{42}K	Tl/K
HeLa S3	16.8±4.4	3.29±0.37	1.62±0.62	1.31	0.65	2.0
AS II	15.7±2.6	2.50±0.66	1.20±0.14	1.23	0.59	2.1
Normal human fibroblasts	21.8±4.8	3.96±0.88	1.56±0.20	0.73	0.29	2.5
Yoshida sarcoma	13.2±2.4	1.22±0.24	0.43±0.07	1.01	0.36	2.8

a. Cells were incubated with 1 μCi/ml ^{201}Tl or 1.5 μCi/ml ^{42}K for 30 min at 37°C. The values are expressed as the mean ± standard deviation for 4 experiments.

b. Cell volumes were calculated by assuming the cells to be spherical.

effects of Ouabain on the uptake of ^{67}Ga, ^{201}Tl and ^{42}K by Y.S. are shown in Table 3. Ouabain in the concentration of 0-1.0 mM/ml-medium was added to the medium, and ^{67}Ga, ^{201}Tl and ^{42}K uptake was estimated from immediately after the addition to 30 min later. Even if Ouabain was added in the concentration of 0.1-0.5 mM/ml, there was no marked difference in ^{67}Ga uptake from the control without Ouabain. In contrast, ^{201}Tl and ^{42}K uptake tended to be inhibited compared with the control when the concentration of Ouabain was over 0.5 mM. When the concentration of Ouabain was 1 mM, the decrease in ^{201}Tl and ^{42}K uptake was statistically significant. Moreover, the degree of inhibition of ^{201}Tl and ^{42}K uptake by Ouabain was about the same.

Effects of anticancer agents on ^{67}Ga and ^{201}Tl uptake and excretion in tumor cells. Fig. 5 shows the growth response of HeLa S3 in medium without addition of MMC (control) and with MMC added in the concentration of 0.001 μg/ml, 0.1 μg/ml, and 1 μg/ml. With MMC in the concentration of 0.001 μg/ml, the growth curve of HeLa S3 was about the same as of the control, showing no inhibition of proliferation. However, as the concentration of MMC was further increased, the

TABLE 2. ^{67}Ga, ^{201}Tl AND ^{42}K UPTAKE BY NONVIABLE YOSHIDA SARCOMA

	Nonviable cells (%)	0.5h-uptake (% dose/10^6 cells)[a]		
		^{67}Ga	^{201}Tl	^{42}K
Control	3.7	0.44±0.08	1.06±0.14	0.46±0.03
Incubated for 30 min at 56°C	95.7	0.71±0.16**	0.04±0.007*	0.007±0.001*

a. The values are expressed as the mean ± standard deviation for 4 experiments.
* Significantly different from control ($p < 0.01$)
**Significantly different from control ($p < 0.05$)

TABLE 3. EFFECT OF OUABAIN ON ^{67}Ga, ^{201}Tl AND ^{42}K UPTAKE B YOSHIDA SARCOMA

Ouabain (mM/ml-medium)	0.5h-uptake (% dose/10^6 cells)[a]		
	^{67}Ga	^{201}Tl	^{42}K
0 (control)	0.46±0.08 (1.00)	1.26±0.06 (1.00)	0.40±0.09 (1.00)
0.1	0.49±0.05 (1.07)	1.18±0.04 (0.94)	0.41±0.06 (1.10)
0.5	0.45±0.05 (0.98)	0.89±0.03* (0.71)	0.29±0.04 (0.71)
1.0	—	0.89±0.09* (0.71)	0.27±0.01** (0.68)

a. The values are expressed as the mean ± standard deviation for 4 experiments.
The value in the parentheses represents ratio of uptake of Ouabain treatment to control.
* Significantly different from control ($p < 0.01$)
** Significantly different from control ($p < 0.05$)

proliferation of HeLa S3 was inhibited, and with MMC in the concentration of 1 μg/ml, hardly any increase in the cell counts after MMC administration was observed.

Fig. 6 shows the effects of MMC and NCS on the uptake of ^{67}Ga and ^{201}Tl in HeLa S3 and the changes in ^{3}H-TdR uptake and cell diameter. As shown in Fig. 6 (A), with MMC in the concentration of 10^{-3} μg/ml there was no great difference from the control in the uptake of ^{3}H-TdR, but when the concentration of MMC was increased, the uptake of ^{3}H-TdR decreased markedly, indicating inhibition of the DNA synthesis in the cells. The uptake of ^{67}Ga and ^{201}Tl per 10^{6} cells increase markedly with increase in MMC concentration. However, the cells tended to swell with increase in MMC concentration, and representing ^{67}Ga and ^{201}Tl uptake per 1 mm^{3} gave an approximately constant value irrespective of the concentration of MMC.

On the other hand, when NCS was added to HeLa S3, ^{3}H-TdR uptake decreased markedly, together with increase in the concentration of NCS (Fig. 6B). With NCS in the concentration of 0.1 μg/ml, the cells showed marked swelling. Their diameters were about 1.5 times greater than the control. At a concentration of 10 μg/ml, the cells showed atrophy, indicating distinct degeneration. Changes in ^{67}Ga and ^{201}Tl uptake expressed per 10^{6} cells coincided well with this change in cell size, and when the uptake was represented per 1 mm^{3}, an approximatelly constant value was indicated as with MMC.

The effects of various anticancer agents on the uptake of ^{67}Ga, ^{201}Tl, ^{3}H-TdR and ^{67}Ga excretion in HeLa S3 are summarized in Table 4. At all concentrations of anticancer agents shown in this table, the proliferation of cells was markedly inhibited, and ^{3}H-TdR uptake decreased markedly compared with the

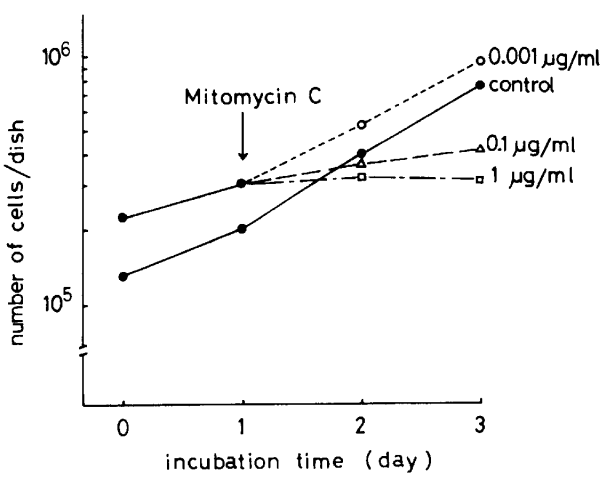

Fig. 5. Growth response of HeLa S3 to various concentrations of Mitomycin C.

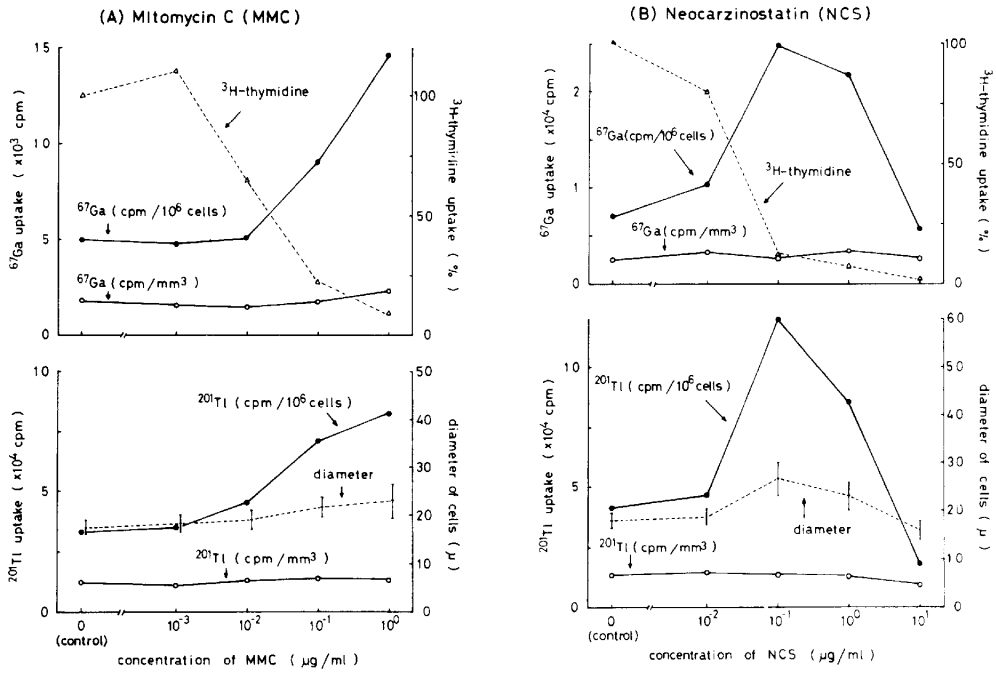

Fig. 6. Effect of various concentrations of Mitomycin C (A) and Neocarzinostatin (B) on ^{67}Ga, ^{201}Tl and ^{3}H-thymidine uptake by HeLa S3. The values shown in table (A) and (B) represent the mean of two experiments. Diameter of cells are expressed as the mean ± standard deviation for 100 cells.

TABLE 4. EFFECTS OF ANTICANCER AGENTS ON UPTAKE OF ^{67}Ga, ^{201}Tl, ^{3}H-TdR AND ON EXCRETION OF ^{67}Ga IN HeLa S3[a]

Anticancer agents[b] (μg/ml-medium)		^{67}Ga		^{201}Tl	^{3}H-TdR
		24h-uptake (% dose/mm^3)	12h-retention (%)	0.5h-uptake (% dose/mm^3)	0.5h-uptake (%)
Control	0	0.23±0.02	88.2±9.2	0.94±0.21	100
MMC	0.1	0.18±0.03	79.4±9.7	0.77±0.33	14
	1	0.22±0.07	70.5±6.7	0.83±0.26	7
ADR	0.1	0.22	71.8	1.26	5
	1	0.33	—	0.75	2
5-FU	10	0.24	73.6	1.07	—
	100	0.32	—	1.08	—
NCS	1	0.28	69.0	0.95	6
	10	0.21	—	0.62	0.6

a. The values of uptake and excretion of ^{67}Ga and ^{201}Tl in control and MMC are expressed as the mean ± standard deviation for 3-4 experiments. The others are the mean of two experiments.

b. MMC: Mitomycin C, ADR: Adriamycin, 5-FU: 5-Fluorouracil, NCS: Neocarzinostatin.

control. However, even with anticancer agents having different action mechanisms, there was no great difference in ^{67}Ga and ^{201}Tl uptake expressed per 1 mm^3. In regard to the effect of anticancer agents on ^{67}Ga excretion, since cell lysis and detachment from the Petri dish occurred with increase in the concentration of the anticancer agent, accurate values could not be obtained; however, the residual percentage of ^{67}Ga in the cells tended to decrease.

Effect of serum on ^{67}Ga and ^{201}Tl uptake in tumor cells. The effects of HS and FCS on ^{67}Ga and ^{201}Tl uptake by HeLa S3 are shown in Fig. 7. When the concentration of HS in the medium was 1% ^{67}Ga uptake became 3-4 times as much as when MEM only was used (Fig. 7A). However, when the concentration of HS was further increased, ^{67}Ga uptake tended to gradually decrease. ^{67}Ga uptake did not show any tendency to increase with FCS and it decreased with increase in the concentration of FCS. ^{201}Tl uptake was markedly inhibited by the addition of HS or FCS to the medium (Fig. 7B). The inhibition was greater with HS. Consequently the effect of serum on the uptake of ^{67}Ga and ^{201}Tl varied with the kind of sera used.

Table 5 shows the effects of various sera on ^{67}Ga uptake in HeLa S3 derived from human tumor and Y.S. derived from rat tumor. In both of these cells with 1% HS and 1% RS, the uptake of ^{67}Ga markedly increased compared to MEM only, but with the addition of FCS this effect was not observed. It was thought that the difference was due to differences in components in the serum itself irrespective of the kind of serum or cell.

The effects of each Cohn fraction of human serum on ^{67}Ga and ^{201}Tl uptake by HeLa S3 are shown in Table 6. Uptake of ^{67}Ga by IV-4 fraction containing transferrin was of the same degree as whole serum. In contrast, all Cohn fractions studied showed no inhibitory effect on the uptake of ^{201}Tl as observable with

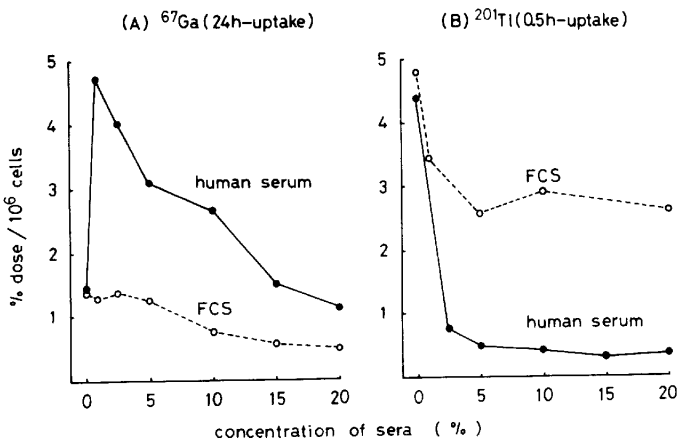

Fig. 7. Effect of various concentrations of human serum and fetal calf serum (FCS) on ^{67}Ga (A) and ^{201}Tl (B) uptake by HeLa S3. Each point represents the mean of two experiments.

whole serum. Next, a study was of the effect of Tf on ^{67}Ga and ^{201}Tl uptake by HeLa S3 (Fig. 8). As a result it was found that ^{201}Tl uptake was hardly affected by the concentration of Tf in the medium; however, ^{67}Ga uptake increased with increase in the concentration of Tf, reaching a peak at the concentration of 50 μg/ml-medium. When the concentration of Tf was further increased, the uptake gradually decreased, showing a tendency similar to HS.

The results for the binding of ^{67}Ga to HS, FCS, and Tf in MEM by equilibrium dialysis using a cellulose membrane are shown in Table 7. In the case of MEM only, ^{67}Ga remaining in the cellulose bag was less than 1%. With

TABLE 5. EFFECT OF VARIOUS KINDS OF BLOOD SERA ON ^{67}Ga UPTAKE BY HeLa S3 AND YOSHIDA SARCOMA

		^{67}Ga uptake (% dose/10^6 cells)[a]	
		HeLa S3	Yoshida sarcoma
MEM only		1.47	1.64
Human serum	1 (%)	5.32	4.51
	10	2.62	2.02
FCS	1	1.47	1.90
	10	0.73	0.98
Rat serum	1	8.30	4.83
	10	2.29	0.94

a. Cells were incubated with 1 μCi/ml ^{67}Ga for 24h at 37°C in MEM containing various kinds of sera. The values are expressed as the mean of two experiments.

TABLE 6. EFFECTS OF COHN FRACTIONS OF HUMAN SERUM ON ^{67}Ga AND ^{201}Tl UPTAKE BY HeLa S3

		Uptake (% dose/10^6 cells)[a]	
		^{67}Ga	^{201}Tl
MEM only		1.80	5.33
Human serum	1 (%)	3.59	—
	5	2.65	0.83
	10	—	0.62
Cohn fraction V	0.5 (mg/ml)	1.81	—
	2.5	1.26	4.74
	5.0	—	5.10
IV-4	0.1	2.54	—
	0.5	3.81	3.47
	1.0	—	3.04
II	0.1	1.25	—
	0.5	1.59	4.63
	1.0	—	4.53

a. Cells were incubated with 1 μCi/ml ^{67}Ga for 24 h or with 1 μCi ml ^{201}Tl for 30 min in MEM containing various concentrations of human serum or Cohn factions. The values are expressed as the mean of two experiments.

the addition of HS or FCS to MEM, the binding of ^{67}Ga to serum markedly increased with the concentration. The binding of ^{67}Ga was greater to HS than FCS. The binding of ^{67}Ga to Tf also increased with increase in the concentration of Tf, and in concentrations of Tf greater than 750 μg/ml, the binding of ^{67}Ga to Tf exceeded 90%.

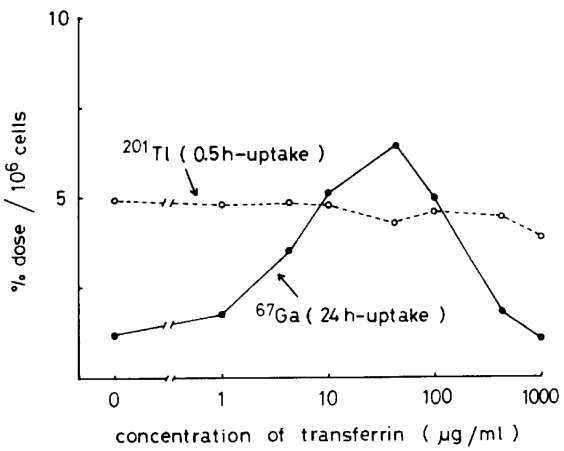

Fig. 8. Effect of various concentrations of human transferrin on ^{67}Ga and ^{201}Tl uptake by HeLa S3. Each point represents the mean of two experiments.

TABLE 7. BINDING OF ^{67}Ga TO VARIOUS CONCENTRATIONS OF SERA AND HUMAN TRANSFERRIN

Concentration of sera (%)		^{67}Ga binding[a] (%)	Concentration of transferrin (μg/ml)	^{67}Ga binding[a] (%)
MEM only	0	0.6	10	1.3
Human serum	1	3.6	50	20.1
	10	57.7	100	45.5
	100	93.6	250	75.3
FCS	1	1.8	500	88.2
	10	19.2	750	92.5
	100	89.1	1000	94.3

a. ^{67}Ga binding to sera or transferrin was measured by equilibrium dialysis with cellulose membrane. The values are expressed as the mean of two expriments.

DISCUSSION

The process by which radioisotopes with tumor affinity accumulate in a tumor may be divided into a) approach to the tumor; b) uptake (adsorption to the cell membrane and passage through); c) localization within the tumor cell and d)

excretion from the tumor cells. In order to develop better tumor scanning agents, it is important to clarify each of these steps as well as to know which of them differ between tumor and normal cells. The excretion of ^{67}Ga was prolonged from tumor cells compared to from normal cells (2). The present study mainly on the second step and compared the kinetics of ^{67}Ga and ^{201}Tl in the *in vitro* experimental system.

^{67}Ga and ^{201}Tl showed entirely different behaviors; namely, ^{67}Ga was first adsorbed to the surface membrane of Yoshida sarcoma then gradually taken up into the cell, from where it was hardly excreted. In contrast, ^{201}Tl was rapidly taken up by Yoshida sarcoma and its excretion was also rapid. This *in vitro* result concurs well with clinical applications using tumor imaging 48-72 h after the administration of ^{67}Ga-citrate. With ^{201}Tl, the tumor imaging was possible about 10 min after its administration.

There have been many reports on the localization of ^{67}Ga in tumor cells (7, 8). The author considers that localization of the cause of the delay of ^{67}Ga excretion is important in the study of the tumor specificity of ^{67}Ga. On the other hand, since excretion of ^{201}Tl is extremely rapid, there are apparently no intracellular organelles or substances that bind firmly and specifically with ^{201}Tl in tumor cells. However, there is a report (9) stating that ^{201}TlCl remains for a relatively long time in malignant tumors of the thyroid gland, stressing the usefulness of so-called delayed scanning. Therefore, further studies on the excretion of ^{201}Tl from tumor cells are required.

Now, ^{201}Tl is the metal that belongs to III A of the periodic table, and the Tl^+ ion has an *in vivo* distribution similar to K and other metals belonging to IA. Regarding tumor accumulation mechanism of ^{201}Tl, Ito *et al* (6), noting a high potassium content in the tumor, found a correlation between the uptake of ^{201}TlCl and that of ^{42}KCl in the tumor of VX-2 cancer-bearing rabbits. In *in vitro* Yoshida sarcoma, ^{201}Tl was taken up more quickly than ^{42}K. This should be due to a carrier effect because ^{201}Tl is carrier-free whereas ^{42}K is not; moreover, a considerable amount of K is contained in MEM. This dose not mean the accumulation mechanisms of ^{201}Tl and ^{42}K differ. Moreover, there is a report (10) that Tl^+ has an affinity about 10 times greater than that of K^+ for the K^+ activating site of (Na^+-K^+)-sensitive ATPase. Therefore, ^{201}Tl can hardly be affected by the presence of K even though it is similar to K in *in vivo*. Comparing the uptake of ^{201}TlCl and ^{42}KCl in various culture cells, the uptake of ^{201}Tl was greater than that of ^{42}K, but there was a correlation in the uptake of the two nuclides which coincided with the *in vivo* results of Ito *et al*. In addition, the uptake per 1 mm^3 of both ^{201}Tl and ^{42}K was 1.7-2.2 times greater by HeLa S3 and AS II cells than by normal human fibroblasts. In relation to the report (11) of the K content in tumor cells being greater, this seems to offer a basis for the tumor affinity of ^{201}TlCl.

Next, in studying the relation between viability of the tumor cells and up-

take, ^{201}Tl like ^{42}K was hardly taken up by nonviable Yoshida sarcoma. Moreover, the administration of Ouabain significantly inhibited ^{201}Tl uptake by Yoshida sarcoma cells. The inhibition of ^{201}Tl uptake was of a similar degree to that of ^{42}K uptake, so active transport with Na-K ATP ase seems to be involved in ^{201}Tl uptake by tumor cells. ^{67}Ga uptake by Yoshida sarcoma was not affected by Ouabain. ^{67}Ga uptake by nonviable Yoshida sarcoma cells at a contact time of 30 min was rather increased compared to that by viable Yoshida sarcoma cells. These findings suggest that ^{67}Ga is independent of active transport at least in the initial stage when ^{67}Ga is adsorbed to the tumor cell surface. In addition, it is known that *in vivo* ^{67}Ga uptake in necrotic regions of tumor is less, but decrease of blood flow to the necrotic region seems to be the cause.

Despite marked inhibition of proliferation of HeLa S3 and the synthesis of DNA by the administration of anticancer agents having various action mechanisms, there was hardly any change in ^{67}Ga and ^{201}Tl uptake per 1 mm^3 of cells. A definite, mutual relation between changes of size such as swelling or atrophy of cells due to the administration of anticancer agent and the uptake of ^{67}Ga or ^{201}Tl expressed per 10^6 cells. This means that uptake is probably correlated to the contact area between the cell and ^{67}Ga or ^{201}Tl. Therefore, the inhibitory action of agents that binding DNA such as Mitomycin C and Adriamycin on DNA synthesis, the antimetabolic action of 5-Fluorouracil, and the action of neocarzinostatin (binds to cell membrane and hardens microtubules) all seem not to affect directly the uptake of ^{67}Ga and ^{201}Tl by tumor cells. In contrast, although cell lysis and detachment from the Petri dish render it difficult to obtain accurate values, the excretion of ^{67}Ga seems to be slightly accelerated. In clinical practice it is known that ^{67}Ga uptake by tumor is decreased after treatment with anticancer agents or irradiation, but there was no decrease of ^{67}Ga uptake by tumor cells after the administration of anticancer agents *in vitro*. Bradly *et al.* (12) reported that, in experiments with cancer-bearing rats, ^{67}Ga uptake by tumor does not change after irradiation given to the tumor region only, but whole-body irradiation induces a decrease in the UIBC and a decrease in ^{67}Ga uptake. Therefore, the decrease of ^{67}Ga tumor uptake is probably explained by indirect factors such as shrinkage of the tumor, decrease in the blood flow to the tumor or its vicinity, and decrease of UIBC rather than the direct action of the anticancer agents or irradiation on tumor cells.

Recently the role of transferrin in tumor accumulation of ^{67}Ga is being emphasized. Larson *et al.* (13-15) reported on ^{67}Ga uptake mediated by transferrin from studies on the kinetics of binding of carrier-free ^{67}Ga to human transferrin and *in vivo* and *in vitro* studies on ^{67}Ga uptake by EMT-6 sarcoma. Transferrin-gallium complex bound to a specific cellular receptor site for transferrin, and later the entire transferrin complex was taken into the cell by adsorptive endocytosis.

In the present study on the effect of human apotransferrin on ^{67}Ga uptake by

tumor cells, marked binding of ^{67}Ga to transferrin was observed by equilibrium dialysis. ^{67}Ga uptake by HeLa S3 was affected by the concentration of transferrin in the medium. ^{67}Ga uptake by HeLa S3 increased with increase in transferrin concentration of the medium, reaching its peak at a concentration of 50 μg/ml; but on further increase of transferrin concentration, the uptake gradually decreased. This result was similar to that of Larson *et al.* (14) using EMT-6 sarcoma, although the concentration of transferrin with which ^{67}Ga uptake reached its peak was different. Therefore, this change of ^{67}Ga uptake due to the concentration of transferrin seems to be explained by the degree of formation of ^{67}Ga-labeled transferrin and fraction of transferrin bound to the cellular receptor, as suggested by Larson *et al.*

The effect of human serum on ^{67}Ga uptake was about the same as that using only transferrin. However, there was no marked increase of ^{67}Ga uptake by HeLa S3 with the administration of FCS. Therefore, the effect of serum on ^{67}Ga uptake differed according to the kind of serum. Moreover, the binding of ^{67}Ga to human serum obtained by equilibrium dialysis was greater than the binding of ^{67}Ga to FCS. In addition, Gams *et al.* (16) reported that many components inhibiting ^{67}Ga uptake exist in the serum. Therefore, the reason for the effects of different sera on ^{67}Ga uptake lies in differences in the binding affinity of ^{67}Ga to the serum (most likely transferrin) as well as to inhibitory components in the serum.

However, there are reports which contradict such a role of transferrin. Vallabhajosula *et al.* (17) noted decrease of pH in the vicinity of tumor tissue accompanied by decreased binding of ^{67}Ga to transferrin, but increased ^{67}Ga accumulation in the tumor. Hayes *et al.* (18) studying the effects of scandium on the tissue distribution of ^{67}Ga in rodents reported that despite decrasse of the binding of ^{67}Ga to transferrin by the administration of scandium, the uptake of ^{67}Ga by the tumor did not decrease. We studied the effect of $FeCl_3$ on ^{67}Ga uptake, and found that ^{67}Ga uptake was markedly increased by $FeCl_3$ in the *in vitro* experimental system in which the ^{67}Ga uptake of tumor cells was affected by the concentration of transferrin (19). Moreover, since ^{67}Ga uptake increased in nonviable tumor cells and ^{67}Ga uptake correlated with the changes of cell size brought about by anticancer agents, the theory of Larson *et al.* that ^{67}Ga tumor accumulation is mediated by transferrin is not comprehensive enough.

On the other hand, the addition of human serum and FCS to the medium markedly decreased ^{201}Tl uptake by HeLa S3. However, the addition of each Cohn fraction of human serum or transferrin caused no remarkable decrease of ^{201}Tl uptake, and the mechanism of inhibition of ^{201}Tl uptake by serum remains unclear. The fact that the uptake of ^{201}Tl by tumor cells *in vitro* was not less than that of ^{67}Ga while *in vivo* ^{201}Tl accumulation in tumor was much less (6), might be related to the inhibitory effect of serum on ^{201}Tl uptake.

Active transport with Na-K ATPase is involved in the tumor accumulation

of ^{201}Tl and the role of transferrin in the tumor accumulation of ^{67}Ga cannot be denied. However, tumor affinity of ^{67}Ga and ^{201}Tl can not be completely explained by these accumulation mechanisms, and further studies are required for the elucidation of tumor specificity of radioisotopes with tumor affinity.

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