In Vitro Binding of $^{67}$Ga to L1210 Cells

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SUMMARY

In vitro methods have been utilized to study $^{67}$Ga binding to L1210 leukemic cells. Uptake of the radioisotope exhibited a striking temperature dependence, approach to saturation, accumulation within the cell, and partial reversibility. Cell fractionation studies indicated distribution of the radioisotope among various cellular components, including soluble macromolecular binders, rather than preferential association with any specific organelle. These results demonstrate the applicability of in vitro methods to studies of the mechanism of uptake of tumor-scanning agents.

INTRODUCTION

Scanning with $^{67}$Ga citrate has been used in the detection and early staging of a broad range of solid tumors and malignant lymphomas (8, 14, 15). A number of in vivo studies with rodents have been directed at elucidating the mechanism of $^{67}$Ga localization. Using autoradiography and cell fractionation techniques, investigators at Oak Ridge (4, 16, 20) have identified "lysosomal-like" granules as the primary sites of $^{67}$Ga localization of AKR/J leukemia, RFT rat fibrosarcoma, mouse lymphosarcoma, and Morris hepatoma cells, as well as a number of normal cells including liver cells, Kupffer cells, parenchymal cells, thymic epithelial reticular cells, and epithelium cells of renal convoluted tubules. Gel filtration of homogenates of the tumors revealed a spectrum of unidentified soluble proteins (16) which appear to bind $^{67}$Ga ionically (13). By contrast, Orii (17) failed to observe any preferential localization of $^{67}$Ga in any subcellular organelle of rat Yoshida sarcoma, liver, or spleen cells and found evidence for at least some nonionic binding of $^{67}$Ga by soluble cytoplasmic proteins obtained from these tissues. Anghileri (2, 3) has drawn attention to the correlation between the intracellular distribution of $^{67}$Ga and $^{4}$Ca in a number of tumors and has demonstrated competitive binding of these isotopes to hydroxypatite, phospholipids, and albumin. The use of cells labeled with $^{67}$Ga to determine sites of metastasis in experimental tumor systems was explored by Hofer and Swarzendruber (13), who concluded that this specific approach was not useful. They did find that L1210 cells incubated with $^{67}$Ga in vivo bound the isotope efficiently.

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performance of isotope binding experiments under clearly defined conditions is a distinct advantage offered by in vitro methods. Dalrymple et al. (6) have reported that transport of $^{67}$Ga across membranes of HeLa cells grown in tissue culture media occurs by a passive mechanism and that a soluble macromolecular receptor for the metal exists in these cells.

The purpose of the present study is to explore in vitro binding of $^{67}$Ga to L1210 leukemic cells harvested as an ascitic tumor from BDF, mice. A dispersed neoplasm was chosen because preparation of single-cell suspensions was convenient and could be accomplished with minimal damage to the cell membrane. Specific objectives of this study were: (a) delineation of the effects on $^{67}$Ga uptake of various experimental parameters such as temperature, dose, and cell concentration; (b) comparison of binding in different media including serum, ascites fluid, tissue culture fluid, 0.9% NaCl solution, and glucose-Locke's solution; (c) determination of the effects of various buffers; and (d) investigation of the intracellular distribution of the isotope in L1210 cells.

MATERIALS AND METHODS

Cell Suspension. L1210 cells were propagated by weekly serial passage at Southern Research Institute (Birmingham, Ala.). Uniformity of cell population was verified by light microscopy after each passage. New cells were obtained from the National Cancer Institute every 4 months. Female BDF, mice (6 to 10 weeks old) were sacrificed by cervical fracture 6 days after i.p. injection of $10^5$ L1210 cells. The peritoneal cavity was flushed twice with 6 ml of iced 0.9% NaCl solution. Multiple perforations of the abdominal cavity were made with a hypodermic needle and the i.p. fluid was dripped into 10 ml of iced 0.9% NaCl solution. The cells were pelleted by centrifugation, washed 3 times in NaCl solution, and resuspended in fresh cold 0.9% NaCl solution. In experiments with glucose-Locke's solution the last wash and subsequent cell dilutions were performed in that medium which was composed of 0.9% NaCl solution, 0.024% CaCl$_2$, 0.042% KC1, 0.02% NaHCO$_3$, and 0.1% dextrose (w/v) (pH 7.1 ± 0.1).

Concentrations of suspensions were determined either by counting the number of cells in a hemocytometer or by determining the PCV$^2$ (1% PCV ≤ 7 x 10$^6$ cells per ml).

Red blood cells obtained from a human donor by

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$^2$ The abbreviation used is: PCV, packed cell volume.
venipuncture were prepared and counted in analogous fashion.

**Cell Irradiation.** A suspension of L1210 cells, which had been washed 3 times in iced 0.9% NaCl solution after harvesting from BDF mice, was exposed to 15,000 R from a $^{60}$Co source. The cells were returned to an ice bath and $^{61}$Ga binding studies were commenced within 2 hr of removal from the host.

$^{61}$Ga **Stock Solution.** $^{61}$Ga citrate (New England Nuclear, North Billirica, Mass.) containing 2 mCi/ml was incubated at 37°, at room temperature, or in an ice bath. At specified time intervals aliquots of iced cell stock suspension were delivered to duplicate tubes, which were vortexed and returned to the incubation bath. The reaction was quenched by transferring the tubes to an ice bath and delivering 2.0 ml iced 0.9% NaCl solution to each tube. The suspensions were washed 3 times with 2.0 ml iced 0.9% NaCl solution and counted before use.

$^{61}$Ga **Binding.** A series of counting tubes containing 0.9% NaCl solution or Locke's solution and aliquots of $^{61}$Ga citrate stock solution in 0.9% NaCl solution were incubated at 37°, at room temperature, or in an ice bath. At specified time intervals aliquots of iced cell stock suspension were delivered to duplicate tubes, which were vortexed and returned to the incubation bath. The reaction was quenched by transferring the tubes to an ice bath and delivering 2.0 ml iced 0.9% NaCl solution to each tube. The suspensions were washed 3 times with 2.0 ml of iced 0.9% NaCl solution. The radioactivity retained by the cells was then determined with a Model 1185 automatic well scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.) calibrated with $^{61}$Ga samples of known activity.

In some experiments it was inconvenient to monitor the entire time course of the reaction. Therefore, the cells were incubated with $^{61}$Ga for 2 hr., quenched, and washed free of unbound radioisotope by the procedure described above.

$^{61}$Ga **Efflux.** Cells preincubated with $^{61}$Ga citrate for 2 hr and washed free of unbound $^{61}$Ga were resuspended in 1.0-ml aliquots of 0.9% NaCl solution or Locke's solution and incubated at 37°. At intervals $^{61}$Ga efflux was estimated by measuring the radioactivity of the supernatant solutions of duplicate tubes.

**Trypan Blue Staining.** Trypan blue (50 µl of a 10% w/v solution) was added to each of a series of counting tubes containing 1.0 ml of 0.9% NaCl solution (or 1.0 ml of glucose-Locke's solution) and 250 µl of a suspension of L1210 cells in 0.9% NaCl solution at 37°. At intervals tubes were removed and the percentage of stained cells was estimated by microscopic examination. Simultaneously, the kinetics of $^{61}$Ga uptake by cells taken from the same stock suspension of L1210 cells was monitored as described above.

**Zonal Fractionation.** L1210 cells isolated from the mouse peritoneal cavity in the above described manner were hemolyzed by brief exposure to 0.2% NaCl solution. Light microscopy revealed no residual red blood cells or any ruptured L1210 cells. The L1210 cells were then incubated for 1 hr at 37° in 20.0 ml NaCl solution containing 5.20 x 10⁻⁷ µmole $^{61}$Ga citrate. The cells were washed 3 times with 0.9% NaCl solution to remove unbound isotope. The cell suspension (20 ml, 4.5% PCV in 0.9% NaCl solution) was homogenized 40 double strokes with a Dounce glass homogenizer.

The zonal fractionation technique has been described in detail by Cline and Ryel (5). A discontinuous sucrose density gradient consisting of 50 ml of 9%, 70 ml of 25%, 85 ml of 35%, 100 ml of 43%, 100 ml of 47%, and 100 ml of 50% sucrose solutions with a 55% sucrose cushion was loaded into a spinning (2000 rpm) B-XIV Spinco zonal rotor (5°) in order of increasing density. The total homogenized sample was layered over the gradient and was followed by a 60-ml overlay of 0.9% NaCl solution. After centrifugation at 30,000 rpm for 35 min, the gradient containing the fractionated components was displaced from the spinning rotor (2,000 rpm) with 57% sucrose. The 280-nm absorption of the effluent was continuously monitored on a Beckman DB-GT spectrophotometer and collected in 20-ml fractions. The sucrose concentration of each fraction was determined on a Bausch and Lomb Abbe 3-L refractometer. The total protein nitrogen was assayed by the Elrod modification (9) of the Lowry technique, and the $^{61}$Ga activity was measured.

**Sephadex G-25 Chromatography.** Washed L1210 cells were incubated at 37° for 2 hr in 7.0 ml of 0.9% NaCl solution containing 1.73 x 10⁻⁶ µmole of $^{61}$Ga citrate per ml. After washing 3 times with iced 0.9% NaCl solution, the cells were homogenized for 15 sec in distilled water in a Potter-Elvehjem homogenizer. The cell suspension was centrifuged for 30 min at 35,000 rpm on a Spinco L2 ultracentrifuge using an SW50L rotor, and an aliquot of the clear supernatant was applied to a 0.9- x 26-cm column of Sephadex G-25 equilibrated and eluted with 0.9% NaCl solution. The eluate was collected in 1.0 ml fractions, for each of which $^{61}$Ga activity and 280-nm absorption were measured. In a separate experiment a sample of $^{61}$Ga citrate in 0.9% NaCl solution was passed through the same column.

**RESULTS AND DISCUSSION**

**Binding Kinetics.** Fig. 1 shows a typical photomicrograph of the suspension of harvested L1210 cells indicating uniformity of blast population, with minimal contamination by red blood cells. A representative kinetic profile of $^{61}$Ga uptake by L1210 cells in 0.9% NaCl solution at 37° appears in Chart 1. The extent of isotope binding to $10^6$ cells (13.6% PCV) was 24.4 and 41.9% of the added dose at 1 and 18 hr, respectively. If it is assumed that the PCV is an upper limit of the volume of intracellular fluid, this represents concentrations against a chemical gradient by factors of 1.8 and 3.1 respectively. It is likely that the PCV is an upper limit of the volume of intracellular fluid, this represents concentrations against a chemical gradient by factors of 1.8 and 3.1 respectively. Since the fractional volume of intracellular fluid is less than the PCV, higher levels of cellular localization are probably attained.

**Radioisotope Dose.** In Chart 2, the extent of 2-hr $^{61}$Ga binding to L1210 cells at various doses of the radioisotope is compared. The curve suggests approach to saturation. Thus, at 1.79 x 10⁻¹⁸ µmole of added $^{61}$Ga about 33% of the radioisotope is bound. At intermediate levels of added $^{61}$Ga the extent of binding is about 11%, while at the highest concentration isotopic binding has decreased to 4.2%.

**Number of Cells.** Varying the number of cells between $10^6$ and $10^8$ had a linear effect on the extent of $^{61}$Ga binding at a

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IO 15 20

TIME (hr)

Chart 1. Kinetics of 67Ga uptake by 10^12 L1210 cells incubated at 37°C in 2.0 ml of 0.9% NaCl solution containing 1.83 x 10^-7 µmole of 67Ga. The initial 5 hr of uptake was monitored in the standard manner. The point at 18 hr was determined by continuing the incubation for 1 sample after all the others had been quenched. To obtain the number of µmoles of 67Ga bound from these charts divide by the indicated power of 10 (e.g., 10^7).

IO'X µMOLES 67Ga BOUND

µMOLES 67Ga ADDED

10^-11 10^-10 10^-9 10^-8 10^-7 10^-6 10^-5

O

Chart 2. The extent of 67Ga binding by 10^12 L1210 cells incubated for 2 hr at 37° in 2.0 ml of 0.9% NaCl solution containing between 10^6 cpm (1.79 x 10^-7 µmole) and 10^7 cpm (1.87 x 10^-6 µmole) of 67Ga citrate in logarithmic intervals of radioisotope concentrations.

dosage level of 1.83 x 10^-7 µmole of the radioisotope (Chart 3). Chart 3 also confirms the report by Dudley et al. (7) that red blood cells have a negligible affinity for 67Ga. Consequently, the minimal contamination of the L1210 suspension by red blood cells had a negligible effect on the extent of 67Ga binding. Chart 3 also shows binding of 67Ga to L1210 cells previously exposed to 15,000 R. Schabel et al. (18) report that after such treatment L1210 cells completely lose their ability to proliferate upon transplantation into a new host. The slope of the binding curve of irradiated cells parallels that of the untreated cells suggesting little change in cellular affinity for 67Ga. 67Ga uptake by L1210 cells therefore appears unrelated to their ability to proliferate upon further transplantation.

Trypan Blue Exclusion. The integrity of L1210 cell membranes in the course of in vitro 67Ga binding studies is demonstrated by the exclusion of the vital dye trypan blue (Table 1). This dye is effectively excluded for at least 4 hr at 37° in either 0.9% NaCl solution or glucose-Locke's solution.

Zinc. 67Zn, the stable decomposition product of 67Ga, was invariably present in 67Ga preparations. Consequently, it was important to ascertain what if any effect zinc had on 67Ga binding. Chart 4 illustrates that over the range of zinc concentrations encountered during the usual 2-week period during which a given sample of 67Ga was used zinc had no

Chart 3. The extent of 2-hr. uptake at 37° of 67Ga as a function of the number of L1210 cells, irradiated L1210 cells, and washed human red blood cells incubated with 1.83 x 10^-7 µmole of 67Ga citrate in 2.0 ml and of 0.9% NaCl solution. Binding of the radioisotope to the counting tube was corrected for by subtracting from each sample a blank reading obtained with a sample lacking cells but otherwise treated in the same manner as the cell suspension. The indicated lines were obtained by least squares analysis of the data.

Table 1

Trypan blue staining of L1210 cells at 37°

<table>
<thead>
<tr>
<th>Incubation in</th>
<th>Time (hr)</th>
<th>10^18 x µmoles 67Ga bound</th>
<th>% fraction stained</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl solution</td>
<td>0.25</td>
<td>4.5</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>6.6</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>11.1</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>18.2</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>31.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Glucose-Locke's solution</td>
<td>0.25</td>
<td>1.8</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>3.3</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>6.8</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>17.5</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>24.5</td>
<td>9.8</td>
</tr>
</tbody>
</table>

* Average of 2 samples. Samples incubated in 1.0 ml of solution containing 1.73 x 10^-4 µmole of 67Ga citrate.
Effect on \(^{67}\)Ga binding. This study also indicates that nitrate ion, the counterion of zinc used in this experiment, did not impede \(^{67}\)Ga uptake.

**Temperature.** Chart 5 summarizes the kinetics of \(^{67}\)Ga binding at 0\(^\circ\), 22\(^\circ\), and 37\(^\circ\) in 0.9% NaCl solution. Two distinct kinetic phases are observed. The first is a rapid uptake of the radioisotope that is largely completed by the time the 1st measurement is made (15 min). Surface adsorption of \(^{67}\)Ga citrate may be involved in this portion of the binding process. Following this rapid phase is a highly temperature-dependent slower phase probably associated with \(^{67}\)Ga transport across the cell membrane. In glucose-Locke’s solution (Chart 6) the initial rapid binding reaction is completely suppressed and only the slower phase of the reaction is observed. The overall level of \(^{67}\)Ga binding is significantly diminished in glucose-Locke’s solution even above the extent associated with elimination of the 1st rapid phase of binding.

**Different Media.** A variety of buffers was investigated in order to study the influence of pH on \(^{67}\)Ga binding to L1210 cells. Table 2 indicates that each of these media decreased the extent of \(^{67}\)Ga binding. Because of this inhibitory effect, it was difficult to systematically monitor the effect of pH variations. However, the pH was essentially stable during the course of a given experiment in 0.9% NaCl solution: starting pH, 6.7 ± 0.3; final pH, 6.9 ± 0.3.

The origin of this inhibition was investigated for glucose-Locke’s solution. Table 2 shows the effects on \(^{67}\)Ga binding of each of the components of this buffer. Both bicarbonate and calcium ions appear to inhibit uptake, the latter effect being consistent with Anghileri’s (3) suggestion of competitive cellular uptake of gallium and calcium. However, only bicarbonate was able to suppress the rapid initial uptake associated with nonzero intercepts in Chart 5. There is also a direct dependence of inhibition of \(^{67}\)Ga binding on the concentration of NaHCO\(_3\) (Table 2). Moreover, Chart 7 illustrates that in the presence of bicarbonate ion elimina-
Table 2
Two-hr in vitro \( ^{67} \text{Ga} \) citrate binding to L1210 cells in various media at pH 6.8 ± 0.4 and 37°

| Medium                          | \( 10^{10} \times \) | \( \mu \text{moles} \) | \( ^{67} \text{Ga} \) bound |
|--------------------------------|----------------------|--------------------------|
| 0.9% NaCl solution              |                      |                          |
| Glucose-Locke's (0.02% NaHCO\(_3\)) |                      |                          |
| Glucose-Locke's (0.03% NaHCO\(_3\)) |                      |                          |
| 0.024% CaCl\(_2\)              |                      |                          |
| 0.042% KCl                     |                      |                          |
| 0.020% NaHCO\(_3\)             |                      |                          |
| 0.100% dextrose                 |                      |                          |
| 0.05 M phosphate                |                      |                          |
| 0.05% Tris                      |                      |                          |
| 0.03% barbital                  |                      |                          |
| 0.1 M Tris-maleic acid          |                      |                          |
| 0.05 M citrate                  |                      |                          |
| Serum                           |                      |                          |
| Hanks' solution*                |                      |                          |
| Ascites fluid                   |                      |                          |

*All media made up in 0.9% NaCl solution except serum, ascites fluid, and Hanks' solution. One ml of 0.8% PCV L1210 cell suspension was incubated with \( 2.60 \times 10^{-10} \) \( \mu \text{mole} \) \( ^{67} \text{Ga} \) citrate for 2 hr. L1210 cells were washed 3 times with 0.9% NaCl solution before and after incubation.

*Contained 5% human serum (v/v).

Chart 7. Kinetics of \( ^{67} \text{Ga} \) uptake at 37° by L1210 cells in normal glucose-Locke's solution (containing \( 1.63 \times 10^{-3} \) \( \mu \text{M} \) CaCl\(_2\)), glucose-Locke's with \( 1.63 \times 10^{-3} \) \( \mu \text{M} \) MgCl\(_2\) replacing CaCl\(_2\), and glucose-Locke's solution lacking either CaCl\(_2\) or MgCl\(_2\). Each sample consisted of 1.0 ml of the indicated solvent to which was added 100 \( \mu \text{l} \) of \( ^{67} \text{Ga} \) citrate stock solution (\( 2.70 \times 10^{-4} \) \( \mu \text{mole} \) in 0.9% NaCl solution) and 250 \( \mu \text{l} \) stock suspension of L1210 cells (4% PCV in 0.9% NaCl solution).

Efflux Kinetics. Chart 8 depicts experiments in which, after monitoring the kinetics of \( ^{67} \text{Ga} \) uptake by L1210 cells for 2 hr, we resuspended the cells in the solvent medium and monitored the efflux kinetics. Only a portion of the bound similar mechanism of inhibition may explain the action of other buffers listed in Table 2.
$^{67}$Ga could be thus removed from the cells. Presumably this fraction is loosely associated with the cell membrane or with the intracellular fluid. The remainder of the $^{67}$Ga appears tightly bound to cellular receptors. Such irreversibility is consistent with the retention of $^{67}$Ga by tumor cells in vivo.

**Zonal Fractionation.** Rate sedimentation zonal centrifugation was used to identify subcellular sites of $^{67}$Ga binding in L1210 cells. Charts 9 and 10 show a representative zonal profile, which contains 7 distinct banding zones numbered in order of increasing density. Table 3 summarizes the percentage of $^{67}$Ga activity, percentage of protein nitrogen, and integrated cytochrome oxidase activity of each of the banding zones. Banding Zone 1 represents the starting zone and is primarily composed of soluble substances originating from cytoplasm, nucleoplasm, and colloidal moieties of subcellular organelles (1). Banding Zone 4 centered at 38.5% sucrose (density, 1.16) had the highest cytochrome oxidase activity (Table 3) indicating localization of mitochondria in this peak. Light microscopy revealed that Banding Zone 7 consisted primarily of whole cells and scattered free nuclei.

The distribution of intracellular $^{67}$Ga in this tumor system is analogous to that in Yoshida sarcoma cells (17). The isotope is not localized in a single subcellular organelle but rather is distributed in various cytoplasmic compartments. This is in contrast to the reports of Brown et al. (4) and Swartzendruber et al. (20) that $^{67}$Ga is localized primarily in "lysosomal-like granules" in the tumors that they have studied.

Chart 11 shows the distribution of $^{67}$Ga after the L1210 cells were disrupted by osmotic shock in distilled water. Under these conditions $^{67}$Ga was concentrated primarily in the soluble phase (Banding Zone 1). There was almost a direct proportionality between protein content and $^{67}$Ga activity in the zonal profile. This indicates that the more vigorous homogenization procedure liberated soluble $^{67}$Ga which may be associated with proteins. This "liberated" $^{67}$Ga was previously either contained within intracellular organelles or solubilized during the homogenization procedure.

**Gel Filtration.** The Sephadex G-25 gel filtration pattern of the soluble fraction obtained from cells disrupted in distilled water is shown in Chart 12. Most of the $^{67}$Ga activity and 280-nm absorption were associated with the void volume, which contained solute of molecular weight in excess of 5,000 daltons. Of the 354,000 cpm ($6.1 \times 10^{-9}$ mole) of $^{67}$Ga applied to the column, 230,000 cpm (65%) were recovered. A similar recovery rate (63.5%) was obtained when 143,000 cpm ($2.5 \times 10^{-9}$ mole) of free $^{67}$Ga citrate were applied to the column, but the elution peak was displaced to higher elution volume (Chart 12, inset). Thus, chromatography on Sephadex G-25 demonstrates the association of $^{67}$Ga with large-molecular-weight species in cellular homogenates. Of the radioactivity recovered from the column, 70% (161,000 cpm) was in the void volume.

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**Table 3**

<table>
<thead>
<tr>
<th>Banding zone</th>
<th>Centered fractions</th>
<th>% sucrose</th>
<th>Density</th>
<th>% of total $^{67}$Ga</th>
<th>% of total protein nitrogen</th>
<th>Integrated cytochrome oxidase activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4, 5</td>
<td>3.0</td>
<td>1.0102</td>
<td>12.76</td>
<td>26.17</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>7, 8</td>
<td>15.6</td>
<td>1.0611</td>
<td>3.72</td>
<td>3.73</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>10, 11</td>
<td>30.0</td>
<td>1.1268</td>
<td>7.89</td>
<td>3.72</td>
<td>0.073</td>
</tr>
<tr>
<td>4</td>
<td>15, 16</td>
<td>38.5</td>
<td>1.1638</td>
<td>11.73</td>
<td>6.11</td>
<td>1.29</td>
</tr>
<tr>
<td>5</td>
<td>20, 21</td>
<td>44.8</td>
<td>1.2012</td>
<td>7.52</td>
<td>5.96</td>
<td>0.186</td>
</tr>
<tr>
<td>6</td>
<td>25, 26</td>
<td>48.0</td>
<td>1.2189</td>
<td>6.99</td>
<td>9.88</td>
<td>0.057</td>
</tr>
<tr>
<td>7</td>
<td>30, 31</td>
<td>52.3</td>
<td>1.2425</td>
<td>37.75</td>
<td>30.65</td>
<td>0.066</td>
</tr>
</tbody>
</table>

* The unit of activity as defined by Smith (19) was the 1st-order rate constant for oxidation of cytochrome c divided by the weight of protein nitrogen (in μg). Integrated values of this quantity for each banding zone are presented here.

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**Figures:**

- Chart 10: The distribution of $^{67}$Ga activity and protein nitrogen in fractionated L1210 cells homogenized in 0.9% NaCl solution.

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Macromolecular $^{67}$Ga binders have also been obtained from homogenates of other tumors (6, 12, 16, 17).

CONCLUSIONS

From the present study the following conclusions can be drawn. $^{67}$Ga bound to L1210 cells is not localized in any specific organelle but is distributed among various subcellular binders. The transport of $^{67}$Ga across the L1210 cell membrane has a number of characteristics comparable with but not conclusive proof of an active process including approach to saturation, a significant temperature dependence, and partial irreversibility. Even though the isotope accumulates within the cell, conclusions about active transport require knowledge of the electrochemical gradient for gallium.

These experiments demonstrate the feasibility of utilizing in vitro methods to explore the mechanism of $^{67}$Ga binding to tumor cells. While L1210 leukemia is a particularly attractive model for such studies, the method can be adapted to studies of other dispersed cell systems including cells grown in tissue culture. Mechanisms of other tumor-scanning agents can be evaluated by a similar procedure. Finally, it may prove feasible to develop in vitro assays of dispersed neoplasms in human body fluids provided that the affinity for $^{67}$Ga (or some other radioisotope) is much greater for tumor cells than for normal cells contained in these fluids.

ACKNOWLEDGMENTS

We extend our sincere thanks to Dr. Frank M. Schabel and Mary W. Trader of the Southern Research Institute (Birmingham, Ala.) for supplying us with BDF, mice with transplanted L1210 ascites tumor cells.

REFERENCES


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Fig. 1. Wright’s stained smear of ascites fluid of BDF, mice 6 days after implantation of \(10^4\) L1210 cells. \(a, \times 520; b, \times 1300.\)
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