67Ga Binding to Human Serum Proteins and Tumor Components

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SUMMARY

67Ga is known to concentrate in a variety of malignant tumors. Immunoelectrophoresis combined with autoradiography of human serum incubated with 67Ga showed that the radionuclide is associated with transferrin, apart from a weak binding to β-lipoprotein. The thermodynamic constant for gallium was estimated and indicated that gallium citrate in the presence of serum proteins occurred in a colloid form bound to transferrin.

Ultracentrifugation of tumor homogenates obtained from patients who had received i.v. injections of 67Ga citrate for scintigraphy demonstrated that 67Ga is preferentially bound to proteins in the nuclei fraction. Acrylamide gel electrophoresis of extracts of cancer tissue indicated that intracellular 67Ga is associated mainly with a fast-migrating substance, perhaps a protein, with a molecular weight lower than that of ferritin.

INTRODUCTION

A number of authors, Higasi et al. (12), Winchell et al. (26), Vaidya et al. (24), and Fogh and Edeling (8), have confirmed the observation by Edwards and Hayes (7) that carrier-free 67Ga has a high and rather specific affinity for neoplastic tissue. Most malignant lesions (and some benign) can be observed by 67Ga scintigraphy. At present nothing is known about the biological behavior of gallium. The purpose of the present work was to study 67Ga transport in the blood and the mechanism of 67Ga accumulation in malignant tissues.

MATERIALS AND METHODS

Carrier-free 67Ga used as a citrate was provided by Philips-Duphar, Wees Petten, Netherlands (DRN 3103). Blood was obtained either from healthy human adults among the laboratory personnel or from patients who had received i.v. injections of 2.0 to 2.5 mCi 67Ga citrate for scintigraphy at the Department of Nuclear Medicine, University Hospital, Copenhagen, Denmark (8).

Neoplasm tissue samples were obtained from patients who had been examined by 67Ga scintigraphy shortly before operation. All of the tumors studied were subjected to histological examination. The various tumor types are shown in Tables 1 and 2.

CHEMICALS

When not otherwise stated all chemicals were of highest obtainable purity from British Drug Houses, Poole, Dorset, England. However, the agar used was Difco Special Noble Agar from Difco Laboratories, Detroit, Mich., and the Freund adjuvant [containing heat-killed tubercle bacilli (Mycobacterium tuberculosis, H 37 Rv)] was also from Difco. The Sephadex G-200 (Medium) was from Pharmacia, Stockholm, Sweden.

Qualitative Assay of the Binding of 67Ga to Serum Proteins

The gallium binding to proteins of normal human serum was evaluated by combining immunoelectrophoresis with autoradiography as previously described (5). Normal serum was incubated with 67Ga (50 µCi/ml) at 37° in a water bath for 1 hr. Immunoelectrophoresis using 1.5 µl serum was performed as described by Clausen (3). The procedure was carried out as a micromethod on microscopic object slides (7.5 x 1 cm) in buffered agar gel (1% w/v Difco Special Noble Agar dissolved by heating to 95° in a 0.05 M sodium barbital buffer, pH 8.6) at 180 V (7 V/cm for 1.5 hr in an LKB apparatus; LKB, Stockholm, Sweden). After electrophoretic separation, 60 µl polyvalent antiserum produced in goats were used. The immunization schedule used for production of antiserum against normal human serum in goats was as described by Clausen (3), whereby goats were given injections of 0.5 ml Freund's adjuvant (Difco). The immunization was repeated every 3rd week for 5 months. After the last immunization, the antiserum was isolated by blood centrifugation after the animals were bled. Specific antisera against individual serum proteins [against human albumin, human serum transferrin, γ-globulins (immunoglobulins IgG, IgA, and IgM), α-2-lipoprotein, and α-2-macroglobulin] were obtained from the Dutch Red Cross, Central Laboratories, Amsterdam, Holland. After immunodiffusion for 16 hr, the slides were washed in 0.9% (w/v) NaCl solution for 2 days and finally for 1 hr in distilled water. Thereafter, the slides were dried at 45° and autoradiography was carried out (3).

For autoradiography ordinary X-ray films (Kodak RP/L54) were used. After autoradiography for 1 to 3
Table 1

"Ga concentration (cpm) in tumor, surrounding normal tissue, and blood from 13 patients

<table>
<thead>
<tr>
<th>Patient and Ga No.</th>
<th>Tumor/Blood</th>
<th>Tumor/surrounding tissue</th>
<th>Tumor type</th>
<th>Location of tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. C. 61/70</td>
<td>88</td>
<td>70</td>
<td>Hodgkin’s disease</td>
<td>Mediastinum</td>
</tr>
<tr>
<td>I. P. 26/71</td>
<td>53</td>
<td>70</td>
<td>Hodgkin’s disease</td>
<td>Mediastinum</td>
</tr>
<tr>
<td>F. N. 70/70</td>
<td>316</td>
<td>338</td>
<td>Reticulum cell sarcoma</td>
<td>Mediastinum</td>
</tr>
<tr>
<td>L. K. 128/71</td>
<td>2.1</td>
<td>2.1</td>
<td>Thymoma</td>
<td>Mediastinum</td>
</tr>
<tr>
<td>N. H. 121/71</td>
<td>25</td>
<td>14</td>
<td>Anaplastic carcinoma</td>
<td>Thyroid gland</td>
</tr>
<tr>
<td>J. P. 177/71</td>
<td>8</td>
<td>9</td>
<td>Anaplastic carcinoma</td>
<td>Thyroid gland</td>
</tr>
<tr>
<td>E. D. 129/70</td>
<td>8</td>
<td>9</td>
<td>Adenocarcinoma</td>
<td>Breast</td>
</tr>
<tr>
<td>I. S. 22/71</td>
<td>12</td>
<td>12</td>
<td>Adenocarcinoma</td>
<td>Breast</td>
</tr>
<tr>
<td>R. C. 47/71</td>
<td>1.5</td>
<td>1.5</td>
<td>Adenocarcinoma</td>
<td>Breast</td>
</tr>
<tr>
<td>J. L. 13/71</td>
<td>8</td>
<td>15</td>
<td>Anaplastic carcinoma</td>
<td>Breast</td>
</tr>
<tr>
<td>J. S. 31/71</td>
<td>30</td>
<td>30</td>
<td>Meningioma</td>
<td>Brain</td>
</tr>
<tr>
<td>J. J. 20/71</td>
<td>2.5</td>
<td>3.6</td>
<td>Oligodendroglioma</td>
<td>Brain</td>
</tr>
<tr>
<td>E. K. 103/71</td>
<td>1.2</td>
<td>0.3</td>
<td>Malignant melanoma</td>
<td>Eye</td>
</tr>
</tbody>
</table>

* Ratio = "Ga concentration in tumor (cpm/g wet tissue) and in surrounding normal tissue or blood (cpm/ml).

Table 2

"Ga concentration in subcellular fractions of 3 "Ga-containing tumors

<table>
<thead>
<tr>
<th>Fraction content</th>
<th>Fraction</th>
<th>Center</th>
<th>Solid part</th>
<th>Periphery</th>
<th>Center</th>
<th>Solid part</th>
<th>Periphery</th>
<th>Squamous cell carcinoma, CJ Ga-52/70 [cpm/g, wet wt], solid tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei, 800 x g, 10 min</td>
<td>L*</td>
<td>142</td>
<td>35</td>
<td></td>
<td>27,881</td>
<td>64,905</td>
<td>37,412</td>
<td>339,953</td>
</tr>
<tr>
<td>SP</td>
<td>12,637</td>
<td>8,273</td>
<td>34</td>
<td>410</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondrial, 12,000 x g, 20 min</td>
<td>L</td>
<td>0</td>
<td>41</td>
<td>33</td>
<td>12,033</td>
<td>25,578</td>
<td></td>
<td>346,198</td>
</tr>
<tr>
<td>IP</td>
<td>313</td>
<td>1,639</td>
<td>793</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>42</td>
<td>93</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microosomal, 100,000 x g, 60 min</td>
<td>L</td>
<td>9</td>
<td>17</td>
<td>0</td>
<td>4,431</td>
<td></td>
<td></td>
<td>303,459</td>
</tr>
<tr>
<td>IP</td>
<td>185</td>
<td>7,450</td>
<td>294</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>46</td>
<td>89</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant from microsomal fraction</td>
<td>L</td>
<td>12</td>
<td>19</td>
<td>6</td>
<td>13,618</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP</td>
<td>113</td>
<td>200</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>105</td>
<td>126</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* L, lipid; IP, interphase; SP, supernatant protein.

weeks, the immuno-electrophoretic slides were stained with Amido black, destained in 5% v/v glacial acetic acid, and dried at 45°. By comparison of the results of the immuno-electrophoretic tracing with those of the autoradiography, the binding proteins for ⁶⁷Ga could be localized:

**Separation of Protein by Agar Gel Electrophoresis.** Separation of serum proteins preincubated with ⁶⁷Ga in vivo or in vitro was effected in a 1% (w/v) Difco Special Noble Agar dissolved by heating (95°) in 0.05 M sodium-barbital buffer, pH 8.6. The separation was performed below light petrol ether (25) in a transverse trough in the gels cast on microscopic slides (7 x 1 cm). After separation at 7 V/cm for 28 min, the slides were either submitted to immunodiffusion, as described for immuno-electrophoresis, or dried under filter paper and assayed autoradiographically (cf. the immuno-electrophoretic procedure). After autoradiography, the slides were stained with Amido black for detection of proteins and destained in 5% glacial acetic acid. Some slides were, however, stained for observation of lipids with 1% (w/v) Sudan Black dissolved in 60% (v/v) aqueous ethanol and destained in 50% (v/v) aqueous ethanol.

**Separation of Proteins by Acrylamide Electrophoresis.** Acrylamide electrophoresis of soluble tumor proteins was carried out after an in vivo uptake of ⁶⁷Ga citrate and also on horse ferritin (2% w/v) (Pentex, Inc., and Miles Laborato ries, Kankakee, Ill.) incubated in vitro with 40 μCi ⁶⁷Ga citrate and/or 4 μCi ⁵⁹Fe citrate per 100 μl ferritin. The electrophoresis was performed in 5% (w/v) acrylamide gels in a vertical apparatus (Shandon Ltd., London, England), using a gel cast of 70 x 6.5 mm. The electrophoretic separation was made in a discontinuous buffer system with 0.4 M Tris-HCl buffer (pH 8.9) in the gel and 0.04 M Tris-glycine buffer (pH 8.3) in the anode and cathode compart-
Quantitative Assay of the Protein Binding of $^{67}$Ga to Human Serum Transferrin

This was made by the Sephadex equilibration method (2). The basic principle of this method involves a loading of the Sephadex columns (Sephadex G-100; Pharmacia, Uppsala, Sweden) swelled in an appropriate buffer containing the $^{67}$Ga as a citrate in variable amounts and at different temperatures. By application of human serum or purified human serum transferrin (Behringwerke, Marburg/Lahn, Germany) to which $^{67}$Ga (citrate derivative) was added, the proportion of the distribution of $^{67}$Ga between the protein peak and the peak localized to the elution volume in which the free gallium was located, the binding constants could be estimated as described by Bull (1).

On the basis of the data obtained (temperature, 22°C), using the equation for binding of drugs to serum proteins described by Klotz (14) and Klotz et al. (15, 17), it was possible to estimate the intrinsic binding constant, $K_0$, and the total number of binding sites, $n$, on every protein molecule. By accepting the multivalency of transferrin with $n$ binding sites, each of which may bind gallium, and by assuming that the free energy of the combination of a protein site with a binding molecule is the same for all such sites and is not affected by the number of gallium molecules already bound to the protein molecule, it is possible to use the law of mass action for the number of bound molecules per transferrin molecule ($r$) as a function of the concentration of free gallium (A) (19).

$$ r = An/(K + A) \quad (A) $$

This equation can be transformed into:

$$ 1/r = (K/n) \times (1/A) + (1/n) \quad (B) $$

From the data obtained by Sephadex filtration, the value of $r$, i.e., the amount of gallium bound per mole of transferrin, could be evaluated as a function of the amount of free, nonbound gallium ($A$). Statistically, Equations A and B are based upon the assumption of an independent binding of each gallium molecule, which in turn is dependent on: (a) the number of available sites; and (b) freedom from the influence of the bound sites. This assumption is valid when $n$ is high, because then the statistical factor in brackets in the equation:

$$ RT \log_e (k_i - 1/k_1) = RT \log_e (n - (i - 2)/(n - (i - 1)) - i \over i - 1 $$

is determined only by the last factor in brackets (16). As shown below in the experimental system used in the present work, $n$ is equal to 14 sites/molecule. In this investigation, the affinity constant $K_0$ for the binding of gallium nitrate was assayed by Scatchard's plots (Bull (1)) in which $r/A$ to $r$ is indicated by the formula

$$ r/A = K_0(n - r) $$

where $K_0$ is the average equilibrium constant.

From the intrinsic binding constant, the individual binding constants, $k_1$, $k_2$ ... $k_i$, corresponding to every single reaction of every site, may be estimated. In this paper, the $k_1$ value was estimated from the relationship:

$$ k_1 = ([n - (i - 1)]/i)k $$

The free energy provided by the gallium binding to the 1st site was estimated from the equation $\Delta F_0 = RT \log_e k_1$, where $\Delta F_0$ refers to the Gibbs standard free energy (temperature 25°C and pressure 1 atmosphere, both being constant).

Also, since

$$ \Delta H_0 /RT^2 = d \log_e K_0/dT $$

the change in standard enthalpy at constant pressure ($\Delta H_0$) as a function of temperature change was evaluated by using the same system as described above at different temperatures ($t = 30, 35, and 45°$). Finally, $\Delta S_0$ was estimated (temperature constant) by the equation $\Delta F_0 = \Delta H_0 - T\Delta S_0$.

The temperature of the Sephadex column was regulated by fitting the whole column with a water jacket made of a plastic mantle, in which the inlet and outlet pipes of the water jacket were connected to a thermostat equipped with a circulation pump (Tecam Thermonstat). The temperature of the column was regulated with an accuracy of ± 0.1°. The elution fluid used for filtration was also regulated by means of water bath equipped with a thermostat.

For a viable quantitative assay for the binding of gallium to serum transferrin, 500 µl of 1% (w/v) serum transferrin were mixed with a 500-µl solution of gallium nitrate dissolved in physiological isoosmotic sodium citrate of nitrate. The gallium concentration was increased gradually from 0.005 to 10 mg/mg transferrin in physiological sodium citrate (0.005, 0.018, 0.043, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 mg/mg transferrin). After incubation for 1.5 hr. 3.5 µCi carrier-free $^{67}$Ga were added to every sample, and the mixture was applied on a Sephadex G-200 column (45 x 1 cm), previously equilibrated with the above-mentioned concentrations of gallium dissolved in physiological sodium citrate. The columns were eluted with physiological sodium citrate. Fifty drops were collected per glass in an LKB fraction collector equipped with a drop counter and an automatic fraction collector (LKB Ltd., Stockholm, Sweden). On every fraction collected, the total protein was assayed by the Folin reaction (20), and the radioactivity was
measured by means of a well-type scintillation counter (Packard 3003). By plotting \( r/(A) \) against the concentration of gallium nitrate in the assay mixture, the binding constant and the change in free energy for the binding of gallium to transferrin could be assayed, as indicated above.

By assay of the binding constant at different temperatures as noted, the changes in the thermodynamic constants could be assayed following the formula outlined above.

**Studies on the Distribution of Gallium in Tissue (see Tables 1 and 2)**

For examination of subcellular fractions of \(^{67}\)Ga-containing tumors, the \(^{67}\)Ga concentrations (cpm/g of tissue) in tumors, surrounding normal tissue, or blood (cpm/ml) were determined by means of a well-type scintillation counter (Packard 3003). Samples of tumor tissue (2 g) were disintegrated in a Potter-Elvehjem disintegrator with 5 volumes of 0.9% (w/v) NaCl solution and subjected to ultracentrifugation in a Sorvall Superspeed centrifuge (Sorvall Ltd., England). The crude nuclei fraction was isolated by centrifugation (at 800 \( \times \) g for 10 min, 4\(^{\circ}\)). The supernatant of the 800 \( \times \) g fractions was subjected to centrifugation at 12,000 \( \times \) g\(_{\text{max}}\) for 20 min at 4\(^{\circ}\), (Rotor SS 34) for isolation of mitochondria. The supernatant from the crude mitochondrial fraction was centrifuged in Rotor 25.2 in a Spinco L-2 ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.) at 100,000 \( \times \) g\(_{\text{max}}\) for 1 hr at 4\(^{\circ}\). Each fraction was suspended in 5 volumes of 0.28 M sucrose and recentrifuged at its respective g value. No effort was made to isolate lysosomes and the Golgi apparatus, but such studies are being prepared. The particular fraction and the supernatant of the microsomal fractions were extracted with 20 volumes of chloroform : methanol (2:1) while being agitated in a shaking apparatus for 20 hr. The chloroform phase, as well as the interphases, were reextracted in chloroform : methanol. The combined chloroform phases of every fraction were agitated with the theoretical upper phase (9). Finally, the lipid phase, interphase, and aqueous phase were evaporated and counted for radioactivity (see above).

In other experiments, the tissue homogenate was centrifuged at 3000 \( \times \) g, and the supernatant was studied for \(^{67}\)Ga-binding proteins by electrophoresis.

**RESULTS**

**\(^{67}\)Ga Transport in Blood.** \(^{67}\)Ga is eliminated rather slowly from the blood, and there is no significant difference in its elimination in cancer patients as opposed to normal controls. Forty-eight and 72 hr after injection, only 5 and 2\%, respectively, of the dose remained in the blood. The elimination curve for \(^{67}\)Ga in the blood of a normal person is shown in Chart 1. The curve is complex, but it may be broken down on a semilogarithmic graph into 3 rectilinear components with half-lives of 5 to 10 min, 2 hr, and 48 hr, respectively.

Determination of hematocrit percentage and measurements of radioactivity in whole blood and plasma from patients who had received i.v. injections of \(^{67}\)Ga for scintigraphy showed that the radionuclide is found in the plasma fraction exclusively.

**Binding of \(^{67}\)Ga to Serum Proteins.** Fig. 1 shows electrophoresis combined with autoradiography of human serum incubated in vitro with \(^{67}\)Ga citrate. Apart from a weak binding of \(^{67}\)Ga to the \(\beta\)-lipoprotein, virtually all of the bound \(^{67}\)Ga was found to be associated with transferrin. The electrophoresis did not reveal binding to any other serum proteins when antiserum against pooled human serum and monospecific antisera were used.

**Thermodynamic Binding Data for the Binding of \(^{67}\)Ga to Human Serum Transferrin.** Chart 2 (upper) demonstrates the relationship between the proportion of gallium, expressed as moles of the nitrate derivative used to bind transferrin as a function of the amount of free gallium nitrate in the assay system containing gallium nitrate and transferrin. From the plot, the equilibrium constant for the protein binding could be calculated by the formula \( K = 1.772 \) liters/mole.

From the last-mentioned chart, the change in free energy is calculated as \( \Delta F^{\circ} = -4.78 \) kcal/mole. The plot shows...
Ga Binding

Chart 2. Upper, the Scatchard plot of \( r/A \) as function of \( r \). A linear relationship is obtained at \( r \) values below 0.04 mg/mg. Ordinate, \( r/A \) (ml/mg); abscissa, \( r \) (mg/mg). Lower, the relationship between \( \log K \) (expressed in arbitrary units) as a function of 1000/\( T \). A linear relationship is obtained only at temperatures above 37°. \( \log K \) (\( K \) is the average equilibrium constant expressed in arbitrary units). Ordinate, 1000/\( T \).

Affinity of \(^{67}\)Ga to Different Types of Human Neoplasms.

Table 1 shows the ratio between the \(^{67}\)Ga concentrations in the lesion (cpm/g of tissue) and in the surrounding normal tissue or blood (cpm/ml). Hodgkin’s granuloma and reticulum cell sarcoma generally show significantly higher uptake of \(^{67}\)Ga than most other tumors. Otherwise, the affinity of \(^{67}\)Ga for malignant tumors does not seem to be significantly different for the various kinds of tumors.

Previously published studies on carcinomas of the breast showed no correlation between \(^{67}\)Ga uptake and the degree of differentiation of the tumor (8). In the present study, the target/nontarget ratio was less than 2 in one case of adenocarcinoma of the breast (Patient R. C., Ga-47/71) and in a case of malignant melanoma of the eye (Patient E. K., Ga-103/73). Neither of the 2 tumors was detected by scintigraphy. In the above mentioned report, Fogh and Edeling (8) observed that 4 of 19 malignant breast tumors could not be observed by scintigraphy. Histological examination showed no significant difference between these 4 tumors and the 15 in which \(^{67}\)Ga was accumulated.

\(^{67}\)Ga Location in Malignant Tumors. In order to determine whether some \(^{67}\)Ga is bound to lipids of the tumors, 1 part of tumor tissue containing \(^{67}\)Ga was mixed with 4 parts of 1-butanol and 4 parts of 0.9% (w/v) aqueous NaCl in a Potter-Elvehjem disintegrator at 600 rpm for 2 min. After separation of the phases, only 21.6% of the \(^{67}\)Ga activity of the tumor was present in the butanol phase. This indicates that \(^{67}\)Ga is bound preferentially to proteins. This finding is consistent with the results of ultracentrifugation, which will be discussed.

Studies on the subcellular distribution of \(^{67}\)Ga by ultracentrifugation demonstrate that the major part of \(^{67}\)Ga is located in the crude nuclei fraction in Hodgkin’s granuloma and in an anaplastic carcinoma. In a squamous cell carcinoma, the activity in the 3 fractions was nearly the same. Following phase separation of each of the fractions in Hodgkin’s granuloma into a lipid phase, a water-soluble phase, and an interphase, most of the radioactivity was observed in the interphase, which consists mainly of membranous and/or denatured proteins.

Acrylamide gel electrophoresis of ferritin incubated with \(^{67}\)Ga citrate and \(^{59}\)Fe citrate (Chart 3) showed that the major part of \(^{67}\)Ga in the gel was located in the ferritin area. A high concentration of \(^{67}\)Ga was also found in a band close to the leading edge, but in this location little \(^{59}\)Fe was present. Acrylamide gel electrophoresis of crude extract of lung cancer tissue (Chart 4, Patient C. J., Ga-52/70 (compare with Chart 3)) revealed a minimal amount of \(^{67}\)Ga concentrated in the area with mobility as ferritin. On the other hand, a fraction with mobility close to the leading edge contained a high concentration of \(^{67}\)Ga. Planimetry of the curve depicting the radioactivity counts in the gel as a

![Graph showing relationship between r/A and r](image)

![Graph showing relationship between \( \log K \) and 1000/\( T \)](image)

![Graph showing distribution of radioactivity along the gel](image)
Chart 4. Upper, distribution of radioactivity along the gel (cpm/mg gel). Lower, polyacrylamide gel electrophoresis of extract of lung cancer tissue containing 67Ga. The leading edge is to the right. The dark bands indicate location of proteins.

function of the length of the gel revealed that 33% of the total radioactivity was found in the areas with mobility as ferritin (cf. Chart 3). A similar distribution of radioactivity was found following acrylamide gel electrophoresis of tissue from a patient with primary liver carcinoma (Patient S. H., Ga-29/70).

DISCUSSION

The present data indicate that 67Ga, when present in the blood, is at least partly related to transferrin. Chemical textbooks do not clearly define the nature of gallium nitrate or citrate, especially as to whether the compound is of an ionic, freely dissociable nature or a complex colloid when added to serum. It has been suggested that gallium compounds, like the corresponding aluminum derivatives, easily form hydroxides and alums at neutral pH (13). The thermodynamic constant, therefore, cannot be taken as an exact value, but only as an indicator of the binding phenomena occurring. However, the thermodynamic data of the present paper, revealing a standard free energy change of -4.78 kcal/mole, an enthalpy change of 1.02 kcal/mole, and a positive change in entropy, support the idea that the gallium citrate occurs in the presence of proteins in a colloid form, that is bound to transferrin mainly by van der Wals' forces and by hydrophobic bonds similar to the bindings of dyes and sulfonamides to albumin (3, 15, 17).

The results of our studies on the binding of 67Ga to serum proteins are in accordance with the results obtained by Hartman and Hayes (11) and Gunasekera et al. (10), who demonstrated that 67Ga is bound to certain proteins in serum, especially that of transferrin.

Our ultracentrifugation work, showing that 67Ga in tumor tissue is located in the nuclei fraction, is not comparable to the results of Schwartzendruber et al. (23), who, by means of zonal ultracentrifugation and electron microscopic autoradiographs, found that 67Ga is associated with lysosomes or related particles in tumor cells of animals. A detailed subcellular fractioning, or an attempt to isolate the Golgi apparatus and the lysosomes, was not undertaken in the present study.

Our current observation that serum transferrin may be an acceptor not only for serum iron, but also for serum 67Ga, raised the possibility that gallium, when accumulated in tumor tissue, might be associated with ferritin. Bearing on this were the findings of Richter (21, 22), who found that abnormal ferritins occur in some human carcinomas. In addition the data of Linder et al. (18) demonstrated that rapidly proliferating tissue has a higher affinity to iron than other tissues. On the other hand, these same authors found low ferritin content in hepatomas, which, according to our experience, accumulates 67Ga sufficiently to be observed by scintigraphy.

The results of the present study on 67Ga distribution in malignant tumors, however, indicate that 67Ga in the acrylamide gel is bound to several proteins, among which are areas with mobility, such as ferritin. The acrylamide gel electrophoretic studies may also indicate that 67Ga is associated with a rapidly migrating substance, perhaps a protein, which has a molecular weight lower than that of ferritin [M.W. 747,000 (6)]. However, of the total 67Ga radioactivity present in the protein zones of the acrylamide gels, about 33% was localized to zones with mobility, such as ferritin.

In the acrylamide gel electrophoretic studies, horse ferritin was used, but previous studies have shown that human serum proteins have antigenic groups in common with animal serum proteins (4). Our results show that 67Ga in the blood is firmly bound to transferrin and that the transferrin molecule has 14 binding sites for gallium when this is in a colloid state, as it is in serum. The studies on the distribution in tumor tissue indicate that 67Ga may be associated with a protein of relatively low molecular weight. It is, however, not proven that 67Ga is bound to a specific protein or proteins. Further studies are necessary to elucidate the nature of this 67Ga binding substance.

ACKNOWLEDGMENTS

The authors express their gratitude to Anna Fosdal for assistance with the English rendering and the preparation of the manuscript.

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**\( \text{Ga Binding} \)**

Fig. 1. Immunoelectrophoretic tracing of human serum separated by agar gel electrophoresis below light petrol ether followed by immunodiffusion. The application troughs are in the middle of the slide. The antibody trough is located between the 2 application troughs. The anode is to the left. A, immunoelectrophoretic tracing of 10 µl human serum incubated with 0.01 \( \mu \text{Ci} \) \( \text{Ga} \); B, immunoelectrophoretic tracing of 15 µl human serum incubated with 0.015 \( \mu \text{Ci} \) \( \text{Ga} \). A and B are developed by immunodiffusion for 16 hr with 100 µl horse antiserum against human serum, washed with 0.9% (w/v) NaCl solution for 1 day, and deionized in distilled water for 1 hr. After the slide was dried, the pattern was stained with Amido black for protein and with Oil Red for lipids. C and D are autoradiographic prints of A and B. It is seen that transferrin (to the right of the application trough) and a trace of \( \beta \)-lipoprotein (to the left) contain radioactivity. For experimental details, see text.


67Ga Binding to Human Serum Proteins and Tumor Components

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