# Studies on Gallium Accumulation in Inflammatory Lesions: II. Uptake by Staphylococcus aureus: Concise Communication

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***Gallium-67 was demonstrated to be taken up in significant amounts by a number of common micro-organisms. The mechanism of gallium-67 uptake was studied in* S. aureus. *It is found to involve two separate processes. One is insensitive to temperature or metabolic inhibitors, and is not inhibited by a high concentration of nonradioactive gallium. This process probably operates through nonspecific binding of Ga-67 to components of* S. aureus. *The second process is not inhibited by metabolic inhibitors either, but it is temperature-sensitive and is inhibited by high concentrations of stable gallium. This component of the Ga-67 uptake is most likely due to a carrier-mediated transport system (facilitated transfer).***

**J Nucl Med 19: 44–47, 1978**

In the preceding paper (*1*), we studied the uptake of gallium-67 by human polymorphonuclear leukocytes (PMN). Since PMN and bacteria are the main components of inflammatory lesions, we studied Ga-67 uptake by a number of common microorganisms. The mechanism of its uptake by *Staphylococcus aureus* was extensively examined and forms the basis of this report.

## MATERIALS AND METHODS

**Materials.** Carrier-free Ga-67 citrate and [$^{14}$C] inulin were obtained commercially. Gallium was present as a complex formed from 9 ng of Ga-67 chloride (containing 2 mCi of Ga-67, specific activity: 4.2 mCi/$10^{-10}$M), 2 mg of sodium citrate, and 6.8 mg NaCl in a final volume of 1 ml. The gallium solution was diluted with 2.5% Na citrate in modified Hanks' solution (without bivalent cation) (*2*). The final solution consisted of the desired concentration of Ga-67 (usually 2 $\mu$Ci/ml) and 0.25% citrate (final pH-7.3). N-ethylmaleimide (NEM), KCN, dinitrophenol (DNP), and sodium iodoacetate were obtained commercially. Gallium chloride ($GaCl_3$) was dissolved in 2.5% sodium citrate.

**Measurement of Ga-67 uptake by microorganisms.** *S. aureus* (ATCC 25923) was obtained from American Type and Culture Collection (Rockville, Maryland), other microorganisms were obtained from the Clinical Microbiology Laboratory of the Johns Hopkins Hospital. They were kept in stock in tryptic soy agar slant and transferred once every month. Before use they were grown overnight in tryptic soy broth at 37° C (the microorganisms were in the stationary phase of their growth curve). After centrifugation at 2,000 g for 10 min at room temperature, the bacteria were washed twice with modified Hanks' solution and diluted to a final concentration of $10^9$/ml according to McFarland Standard (*3*). For the measurement of Ga-67 uptake, 2 ml of bacteria were incubated with 2 $\mu$Ci/ml of Ga-67 in a disposable plastic centrifuge tube for 30 min at 37° C, in a water bath with constant shaking. Previous study (*1*) has demonstrated that Ga-67 does not stick to the plastic centrifuge tubes. At the end of incubation, the bacteria were centrifuged at 2,000 g for 10 min at 4° C and washed twice with 10 ml modified Hanks' solution. Preliminary experiments indicated that further washes did not significantly change the uptake

Received May 17, 1977; revision accepted Aug. 16, 1977.

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**TABLE 1. GALLIUM-67 UPTAKE BY COMMON MICRO-ORGANISMS**

| | Uptake | |
|---|---|---|
| | Mean | Range |
| *S. aureus* | 6,111 (8) | 2,995–19,418 |
| *S. epidermidis* | 419 (2) | 326– 512 |
| *E. coli* | 1,997 (2) | 1,088– 2,907 |
| *S. faecalis* | 1,016 (4) | 400– 1,830 |
| *Salmonella typhimurium* | 2,450 (2) | 2,296– 2,595 |
| *Lactobacillus leichmannii* | 904 (4) | 432– 1,499 |
| *Candida albicans* | 175 (2) | 38– 312 |

Results are expressed as gallium uptake (cpm/100 μg protein) after 30 min. Numbers in parentheses indicate number of experiments.

of Ga-67 by bacteria. Independent experiments—using [$^{14}$C] inulin as an indicator for extracellular fluid contamination—indicated that only two washes were necessary to remove the incubation medium contaminating the initial pellet. The radioactivity was counted in an automatic well counter with a window setting of 80–320 keV. The results were expressed as the amount of Ga-67 uptake per 100 μg of bacterial protein (cpm/100 μg protein). Protein determination was performed by the method of Lowry et al. (*4*) using bovine serum albumin as a standard.

For the study of the effects of metabolic inhibitors on Ga-67 uptake by *S. aureus*, bacteria was preincubated in the presence or absence of 1 mM KCN, Na iodoacetate, NEM or DNP. Ga-67 uptake was then determined as described above. Heat inactivation of *S. aureus* was carried out by heating the organism for 30 min at 80° C in the water bath. For the study of the effects of various cations on Ga-67 uptake, *S. aureus* was incubated with 2 μCi/ml Ga-67 for 30 min at 37° C in the presence or absence of $10^{-6}$M stable $Ga^{3+}$, $Fe^{3+}$ $In^{3+}$, $Al^{3+}$, $Mg^{2+}$, or $Ca^{2+}$. The statistical significance was determined by Student's t test (*5*).

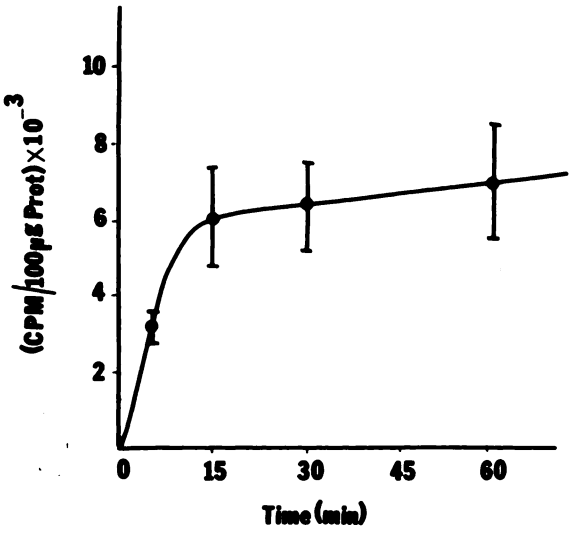


**FIG. 1.** Time course uptake of Ga-67 by *S. aureus*. Results show the mean ± 1 s.e.m. of eight experiments.

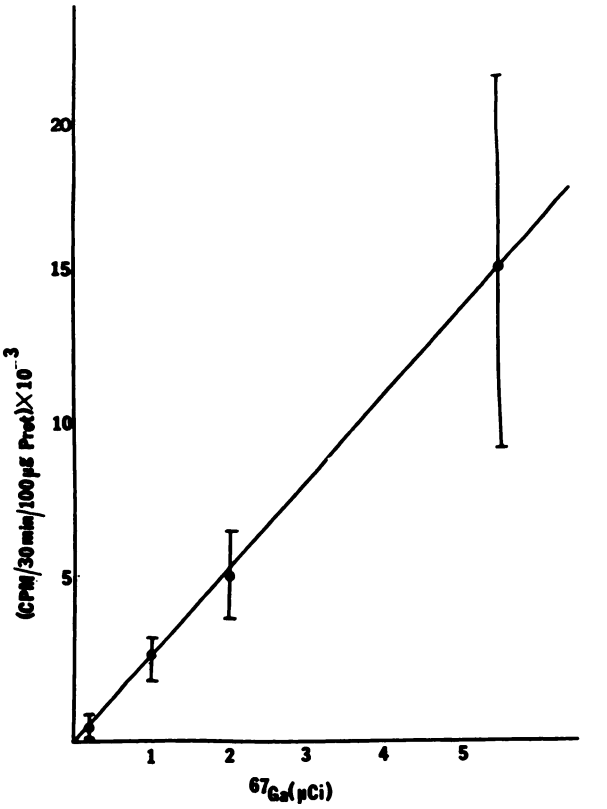


**FIG. 2.** Effect of various concentrations of Ga-67 on its uptake by *S. aureus*. Results show the mean and range of four experiments.

RESULTS

**Gallium-67 utpake by common microorganisms.** Table 1 shows Ga-67 uptake by seven common microorganisms. Although the number of experiments was small in some cases, the results indicate that Ga-67 was taken up by these micro-organisms to a significant degree.

**Gallium-67 uptake by *S. aureus*.** Figure 1 illustrates the time course of Ga-67 uptake by *S. aureus*. The uptake was rapid in the first 15 min of incubation, thereafter there was only a small increase in the uptake for up to 1 hr. Figure 2 demonstrates a linear relationship between the amount of Ga-67 uptake by *S. aureus* and the amount of Ga-67 present in the incubation medium.

In order to determine whether Ga-67 uptake by *S. aureus* is due to simple diffusion or to a carrier-mediated transport system, the effects of tempera-

**TABLE 2. EFFECT OF TEMPERATURE AND METABOLIC INHIBITORS ON Ga-67 UPTAKE BY *S. AUREUS**

| | Ga-67 uptake—30 min incubation (cpm/100 μg protein) | | P value (vs. control) |
|---|---|---|---|
| | Mean | Range | |
| Control | 6,111 | 2,995–19,418 (8) | |
| + KCN | 7,253 | 6,046– 8,564 (3) | >0.3 |
| + Na iodo-acetate | 6,595 | 4,779– 9,596 (3) | >0.8 |
| + NEM | 6,372 | 5,386– 8,272 (3) | >0.8 |
| + DNP | 6,136 | 4,680– 7,146 (3) | >0.9 |
| Heat-killed *S. aureus*† | 2,988 | 1,646– 4,702 (6) | <0.01 |
| Cold (4°)‡ | 4,246 | 3,070– 5,787 (4) | <0.05 |

* Results are expressed as mean and range. *Staphylococcus aureus* was preincubated in the presence or absence of metabolic inhibitors (1 mM) for 30 min at 37°C. Then Ga-67 uptake was measured after another 30 min incubation with 2 μCi of Ga-67 at 37°C. Numbers in parentheses indicate number of experiments.

† Heat inactivation of *S. aureus* was carried out by heating the organism for 30 min at 80°C in water bath.

‡ *S. aureus* suspension was put in an ice bath for 30 min, then 2 μCi Ga-67 was added and Ga-67 uptake determined after 30 min of further incubation in ice.

ture and metabolic inhibitors on Ga-67 uptake were studied. As shown in Table 2, the uptake was markedly inhibited when the experiment was carried out at 4° C. Heat inactivation of *S. aureus* also inhibited Ga-67 uptake. In contrast, various metabolic inhibitors such as KCN, sodium iodoacetate, N-ethylmaleimide, and dinitrophenol (DNP) had no effect. Thus there are at least two components in the uptake of Ga-67 by *S. aureus*. One is temperature-sensitive. This pathway does not require energy, since various metabolic inhibitors have no effect. The second component of the uptake is both temperature-insensitive and resistant to metabolic inhibitors.

**TABLE 3. EFFECT OF VARIOUS CATIONS ON Ga-67 UPTAKE BY *S. AUREUS**

| | Ga-67 uptake—30 min incubation (cpm/100 μg protein) | | P value (vs. control) |
|---|---|---|---|
| | Mean | Range | |
| Control | 6,111 | 2,995–19,418 (8) | |
| + $Ga^{+3}$ | 3,281 | 1,646– 4,702 (5) | <0.05 |
| + $Fe^{3+}$ | 5,899 | 4,011– 9,394 (3) | >0.8 |
| + $In^{3+}$ | 5,906 | 2,861–11,696 (3) | >0.8 |
| + $Al^{3+}$ | 5,521 | 2,770– 5,521 (3) | >0.8 |
| + $Mg^{2+}$ | 5,189 | 3,173– 8,350 (3) | >0.6 |
| + $Ca^{2+}$ | 5,771 | 3,397–10,048 (3) | >0.7 |

* Results are expressed as in Table 1. The cation concentrations were $10^{-6}$M.

If we postulate that the temperature-sensitive component of the *S. aureus* uptake is due to a carrier-mediated transport system, its uptake should be inhibited by stable gallium. As shown in Table 3, gallium at a relatively high concentration ($10^{-6}$M) inhibited Ga-67 uptake by 50%. In contrast, the same molar concentrations of $Fe^{3+}$, $In^{3+}$, $Al^{3+}$, $Mg^{2+}$, or $Ca^{2+}$ had no effect.

Since Ga-67 binds to plasma protein after i.v. injection (*6*), the uptake of Ga-67 by *S. aureus* was also studied in serum. It was found to be essentially the same as that in the modified Hanks' solution: 8579 ± 1685 cpm/100 μg protein (mean ± s.e.m., 7 experiments), compared with 8082 ± 2140.

## DISCUSSION

We have previously demonstrated that Ga-67 is taken up by polymorphonuclear leukocytes (*1*). In the present study we have showed further that Ga-67 was also taken up by seven common microorganisms. In human PMN, the plasma membrane serves as a diffusion barrier and Ga-67 binds only to the plasma membrane (*1*). When this diffusion barrier is disrupted, as in heat-killed PMN, the uptake of Ga-67 increases markedly (*1*). The results shown in the present study suggest that Ga-67 uptake by *S. aureus* is due to a more complicated process with at least two components. One is insensitive to temperature, metabolic inhibitors and a high concentration ($10^{-6}$M) of stable gallium. This is probably due to non-specific binding of Ga-67 to components of *S. aureus*. This fraction constitutes about 50% of the Ga-67 uptake under our experimental conditions, in which 2 μCi of Ga-67 was used. The second component is sensitive to temperature and is inhibited by stable gallium. Thus it has the characteristics of a carrier-mediated transport system. Since metabolic inhibitors have no effect on this component of Ga-67 uptake, it apparently does not require energy and is most consistent with "facilitated transfer," a term used for carrier-mediated transport that does not require energy (*7,8*). In facilitated transfer, the substrate moves across the plasma membrane until the concentrations of the substrate on both sides of the plasma membrane have equilibrated. The linear relationship between the amount of Ga-67 uptake by *S. aureus* and the amount of Ga-67 present in the medium (Fig. 2) is also characteristic of this type of carrier-mediated transport. Whether other microorganisms have the same transport systems for Ga-67 awaits further investigation.

FOOTNOTE

* Dr. Tsan is a recipient of a Research Career Development Award (AI-00194) from National Institute of Allergy and Infectious Diseases.

ACKNOWLEDGMENT

This work was supported by U.S. Public Health Service Research Grants, GM-10548 and AI-13004.

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