Compounds Which Mediate Gallium-67 Transfer from Lactoferrin to Ferritin

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The influence of various low molecular weight compounds on the transfer of ^{67}Ga from human lactoferrin (LF) to horse spleen ferritin (HoFE) has been examined in vitro. When LF*^{67}Ga complex was placed in competition with HoFE using a dialysis system the initial transfer rate (TR) of ^{67}Ga to HoFE was slow and continuous. In the presence of 1 m*M* pyrophosphate (PP_i) ascorbate and adenosine triphosphate (ATP), the TR was dramatically enhanced. This effect was concentration sensitive since reduction of the ATP to 0.1 m*M* eliminated the enhancement. Other intracellular compounds did not significantly influence the TR. Although PP_i and ascorbate ions yielded larger TR's, ATP was more effective in the promotion of ^{67}Ga transfer to HoFE. When the LF/HoFE concentration ratio was decreased, in the presence of ATP, the transfer of ^{67}Ga was significantly increased. These results suggest that ferritin present intracellularly could remove and retain ^{67}Ga entering the cell in the form of a LF*^{67}Ga complex. Moreover, increased synthesis of ferritin and cytosolic phosphate compounds would appear to enhance this process.

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Although the exact mechanism of gallium-67 (^{67}Ga) localization in tumors and abscesses is still unclear, evidence has accumulated that iron binding molecules play a role. In an abscess, it is likely that transferrin (TF), the iron transport protein, is involved in delivery to the site (*1*). We have postulated that ^{67}Ga could then be transferred to lactoferrin (LF) and finally deposited in ferritin (FE) by a receptor mediated process (*2*).

There is strong evidence supporting this hypothesis. Increased concentrations of LF, which has both bacteriocidal and bacteriostatic properties, has been detected in fluids obtained from patients with a variety of inflammatory diseases (*3–5*). This increase has been correlated with the presence of polymorphonuclear leukocytes (PMNs) at the inflammatory site. In vitro evidence has shown that 85% of the LF present in the secondary granules of PMNs is exocytosed upon phagocytic challenge (*6*). Thus, LF, deliberately deposited to aid PMN function, provides the abscess exudate with a high concentration of ^{67}Ga avid molecules. Under these conditions, ^{67}Ga can be transferred from TF to LF (*7*). Lastly, Van Snick et al. (*8*) have demonstrated that iron borne by LF was bound to murine macrophages and subsequently transferred to FE present in the cells in a receptor mediated process. A large number of low affinity LF receptors have also been detected on the surface of human monocytes and macrophages (M/M) (*9,10*).

The object of this study was to examine in vitro this final process, the translocation of ^{67}Ga from LF to horse spleen ferritin (HoFE). Although it is not known if LF and FE interact directly, it is possible that these proteins may interact through a membrane. For example, LF may be enveloped in an endocytic vesicle of M/M and the ^{67}Ga transferred through this membrane to FE. To explore this possibility, we have investigated, initially, the ability of various compounds to stimulate translocation using a dialysis membrane to separate these two proteins.

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MATERIALS AND METHODS

Materials

The LF* was used without further purification. However, the LF*^{67}Ga (specific complex of ^{67}Ga bound to LF) eluted as a single peak on a Sephacryl-300 column and was greater than 90% pure as measured by SDS electrophoresis. HoFE was used without further purification and contained about 15% aggregates as measured on an S-300 column. The HoFE was purchased from two sources† and the results for each preparation were qualitatively the same.

Dialysis experiments and mediators

These were performed as previously described (*7,11*). The LF*^{67}Ga was prepared similarly to the TF*^{67}Ga (specific complex of ^{67}Ga bound to TF). The buffer used was 50 m*M* *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES) containing 100 m*M* NaCl and 0.4 m*M* $NaHCO_3$, pH 7.4. A 500 μl aliquot of a stock mediator solution was added to 5 ml of the LF*^{67}Ga solution to achieve the appropriate final mediator concentration. Mediators were chosen because (a) these compounds stimulate transfer of iron from TF to HoFE; (b) they are potential intracellular chelates; or (c) are elevated in some tumor cells. One milliliter of the LF sample containing ~2–4 μCi ^{67}Ga/ml (1 μCi/ml = 25 p*M*) was then placed in the right and buffer plus mediator in the left chamber of the dialysis cells. After an hour, 100 μl of HoFE solution or buffer plus mediator was placed in the left chamber of the dialyzer cell. Analysis of the LF and HoFE solutions (*12*) indicated an initial iron saturation of 26 and 2%, respectively. A relative amount of nuclide bound to LF was computed from the equation:

$$\text{Difference \% Bound (D\%B)} = (R - L)/(R + L) \times 100, \quad (1)$$

where R = radioactivity in the chamber which contained LF*^{67}Ga, potentially free ^{67}Ga and the appropriate mediator, and L = radioactivity in the chamber which contained either buffer or HoFE*^{67}Ga (specific complex of ^{67}Ga and HoFE), free ^{67}Ga and the appropriate mediator. If the concentrations of these species are substituted in Eq. 1, then:

$$\text{D\%B} = \frac{(\text{LF*}^{67}\text{Ga}) - (\text{HoFE*}^{67}\text{Ga})}{(\text{LF*}^{67}\text{Ga}) + (\text{HoFE*}^{67}\text{Ga}) + 2\,(^{67}\text{Ga})_f} \times 100, \quad (2)$$

where (LF*^{67}Ga), (HoFE*^{67}Ga) and (^{67}Ga)$_f$ are the concentrations of the LF*^{67}Ga, HoFE*^{67}Ga and nonprotein bound ^{67}Ga, respectively. As noted previously (*11*) it can be seen from Eq. 2 that D%B is a measure of the shift of nuclide from LF to HoFE. However, D%B is only a relative value since the presence of proteins on both sides of the dialysis membrane precluded direct assay of the protein-bound ^{67}Ga. To distinguish between the protein and nonbound activity, an additional experiment was required at the termination of the dialysis protocol (see below).

Twenty microliters were removed from each chamber at appropriate times and the activity present measured. Data points represent the means of at least duplicate samples of the D%B values. Experiments were repeated at least twice except where noted. The equilibrium time was estimated from the plateau of the D%B versus time plots.

Determination of ^{67}Ga transferred from LF to HoFE

After the dialysis experiments were completed all the samples in the right chambers were combined and chromatographed as described by Weiner et al. (*11*) to determine the percentage of nuclide bound to LF, and the free fraction. The procedure was repeated with the left chamber samples to obtain the fraction of ^{67}Ga transferred to HoFE. A different procedure was used to estimate the LF-, HoFE-, and nonprotein-bound activity at times other than at the completion of the experiment. The D%B at the particular time and an estimate of (^{67}Ga)$_f$ from the values obtained at completion were inserted into Eq. 2. This equation was then solved for (LF*^{67}Ga) and (HoFE*^{67}Ga). Lastly, an additional equation:

$$(^{67}\text{Ga})_T = (^{67}\text{Ga})_f + (\text{LF*}^{67}\text{Ga}) + (\text{HoFE*}^{67}\text{Ga}), \quad (3)$$

where (^{67}Ga)$_T$ is the concentration of ^{67}Ga added initially, was used to determine separately (LF*^{67}Ga) and (HoFE*^{67}Ga). Means values from at least duplicate experiments with most relevant mediator compounds are presented.

Determination of transfer rates

The transfer rate (TR) was determined as the linear least squares of the plot, D%B versus time, using values from 0–25 hr depending on the attainment of equilibrium. The R value was always >0.93.

HoFE concentration dependence

These dialysis experiments were performed as described above, except that a large stock solution of LF was prepared. This was done to maintain a constant 17 μM LF concentration in each experiment. The ATP concentration was also kept constant at 1 m*M*. The final HoFE concentration was varied from 0.3 to 30 μ*M* by adjusting the volume of the stock HoFE solution added to the left chamber.

Statistical analysis

The analysis variance and Newman-Keuls multiple range test were used for data analysis (*13*).

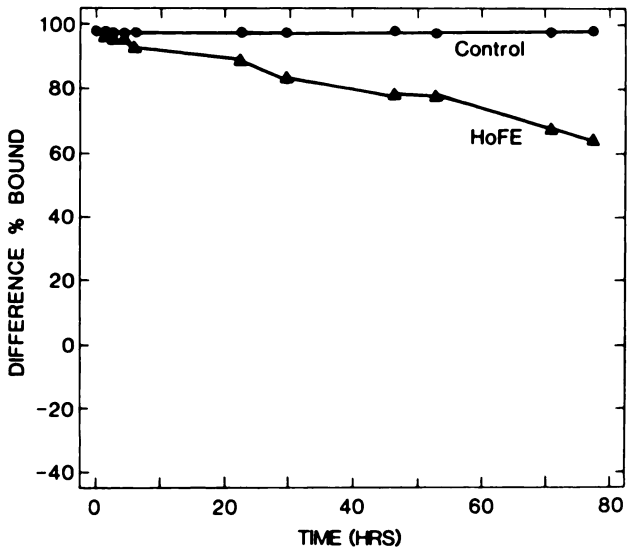

FIGURE 1
Equilibrium dialysis of preformed LF*^{67}Ga (17.5 μM) in competition with HoFE (3 μM) without mediators. LF* ^{67}Ga solution was placed on right side and HoFE (▲) or buffer solution (●) was added to left side dialysis cell chambers. Aliquots were removed from chambers and D%B was computed from (R − L)/(R + L) × 100 where R and L are radioactivity in right and left chambers, respectively. ^{67}Ga concentration = 3.1 μCi/ml. Data points represent means (N ≥ 2) and this is one of seven experiments. Incubation temperature was 37°C and buffer was 50 mM HEPES buffer containing 100 mM NaCl, 0.4 mM $NaHCO_3$, pH = 7.4 and 0.2% (w/v) NaN_3

RESULTS

Dialysis experiments

When the preformed LF*^{67}Ga was challenged by HoFE in the absence of mediators, there was a small, slow, shift of radionuclide from LF to HoFE (Fig. 1). For example, at 80 hr, the D%B was 60% for the LF*^{67}Ga in competition with HoFE. When either pyrophosphate (PP_i) or ascorbate ions were added to the incubation mixture, there was a dramatic large and rapid decrease in D%B in the presence of HoFE which slowed considerably after 20 hr (Fig. 2). This suggested that ^{67}Ga was translocated to HoFE, there was an increase in free ^{67}Ga or both. Some increase in nonprotein bound nuclide was likely, since without a HoFE challenge, PPi ions reduced the D%B compared to the D%B completed in the absence of mediators (Figs. 1 and 2). Abscorbate ions also caused a small decrease in the control D%B. The very low values of the challenged D%B indicated that at least a portion of ^{67}Ga was transferred to HoFE.

Since adenosine-triphosphate (ATP) was the most effective transfer agent in previous experiments with TF (*11*), its influence on LF was investigated. The addition of ATP at 1 mM concentration resulted in a large reduction in the D%B when LF was challenged by HoFE (Fig. 3). This translocation appeared similar to the diminution in D%B caused by PP_i or ascorbate, however, the decrease was greater (−65% compared with −20%) and it required longer to plateau. Moreover, ATP in the absence of HoFE did not increase the presence of free ^{67}Ga (Fig. 3). In an attempt to mimic the cytoplasmic millieu we examined the interaction of ATP with another physiological chelator, citrate. The addition of citrate ions (30 μM) did not change the appearance of D%B compared with time curve compared to ATP alone (Fig. 3). The presence of 30 μM citrate alone had little effect up to 30 hr but did enhance the translocation after 30 hr.

Effect of mediators on transfer rates

To obtain a more quantitative estimate of the ability of these compounds to promote D%B reduction, the TR's, a measure of the initial movement of ^{67}Ga to HoFE, were calculated and are shown in Table 1. In the presence of ascorbate, PP_i, ATP and ATP plus citrate, the TR's were significantly increased while citrate and the components of ATP had little influence. This effect of ATP was concentration dependent. A reduction in the ATP concentration to 0.1 mM decreased the TR almost two orders of magnitude, although this was not significantly different than the mediator-free case. Ascorbate produced the highest TR which was significant compared to the other high TR's ($p < 0.01$). While the differences between PP_i and the ATP-citrate combination were not significant, both TR's were different than the TR for ATP alone ($p < 0.001$). Since the TR was, in part, an indication of the ability of these compounds to stimulate the dissolution of the LF*^{67}Ga, the addition of citrate had an additive effect.

The effect of mediators on the amount of ^{67}Ga transferred

A more important aspect was the actual ability of these compounds to transfer ^{67}Ga to HoFE. The high TR compounds reduced the LF-bound nuclide, compared with the mediator-free condition ($p < 0.05$) as might be expected (Table 2). However, the presence of ascorbate did not appear to significantly enhance nuclide bound to HoFE. While ATP and ATP plus citrate did significantly promote the greatest transfer compared to all other compounds ($p < 0.05$), little ^{67}Ga was translocated when the ATP concentration was reduced which was consistent with the observed TR. PP_i did not appear to enhance transfer of nuclide compared to the mediator-free case and clearly increased the presence of nonprotein activity alluded to earlier. The components of ATP actually appeared to retard formation of HoFE*^{67}Ga.

From Table 2 it appeared that ascorbate and the other promotors were modest compared to the media-

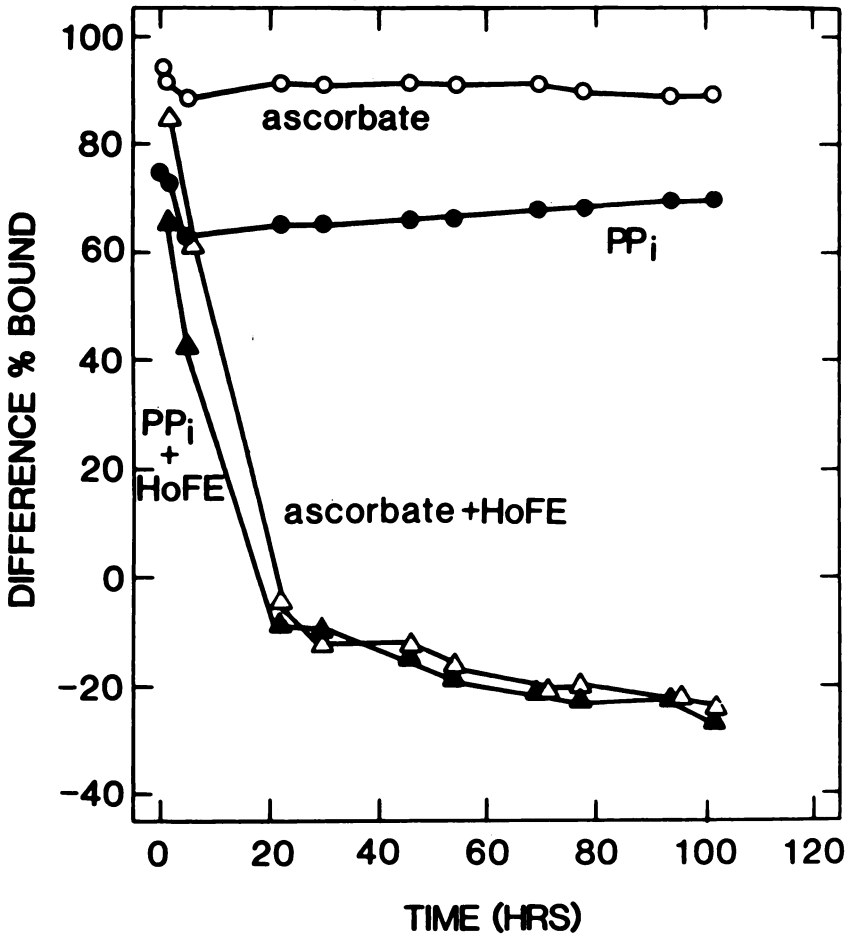

FIGURE 2
Equilibrium dialysis of preformed LF*^{67}Ga in competition with HoFE solutions containing 1 m*M* ascorbate (O, △) and PP_i (●, ▲) ions. LF*^{67}Ga solution containing ascorbate or PP_i was placed on right side and HoFE (△, ▲) or buffer solution (O, ●) was added to left side of dialysis chambers. LF and HoFE concentrations were 25.2 μ*M* and 2.5 μ*M*, respectively. ^{67}Ga concentration = 2.5 μCi/ml for ascorbate and PP_i. Data points represent means (N ≥ 2) and each experiment repeated twice. Other conditions and procedures described in Fig. 1 legend

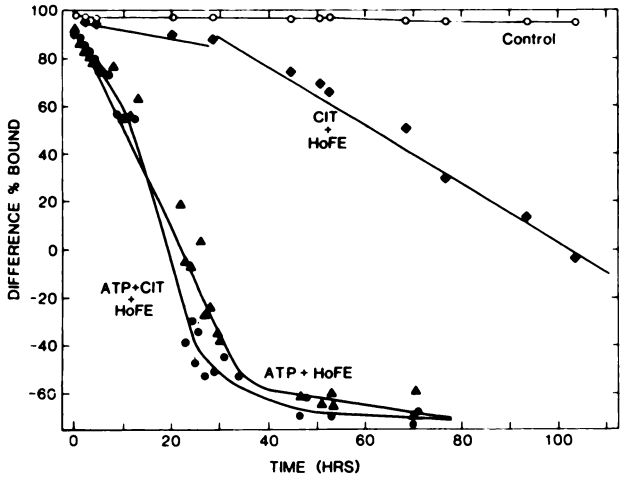

FIGURE 3
Equilibrium dialysis of preformed LF*^{67}Ga in competition with HoFE solutions containing: 1 m*M* ATP (▲, O), 1 m*M* ATP plus 30 μ*M* citrate (●, O) and 30 μ*M* citrate ions (♦, O). LF*^{67}Ga solution plus mediator was placed on right side while HoFE (●, ▲, ♦) or buffer (O) plus mediator solution was added to left side. LF and HoFE concentrations were 20 μ*M* and 3 μ*M*, respectively. ^{67}Ga concentration = 3.4, 3.2, and 1.9 μCi/ml for experiments containing ATP, ATP + citrate, and citrate, respectively. Data points represent means (N ≥ 2) and each experiment repeated at least twice. Other conditions and procedures described in Fig. 1 legend

tor-free experiments, at most a two-fold increase in the activity transferred to HoFE. However, the mediator-free experiment was carried out substantially longer than the equilibrium time required for either experiment which contained ATP or ascorbate. While the reaction with ATP came to equilibrium in about 37 hr and little nuclide was transferred afterward, in the absence of mediators there was a slow continual shift of ^{67}Ga. The longer time interval essentially allowed equalization of the effects between the slower and faster promoters. Therefore, to attempt to examine the mediators on a more equal footing, the percentage of activity present in both LF and HoFE at 20 and 70 hr has been calculated (Table 3). For the LF and HoFE values, the difference between the mediator-free condition and adenosine monophosphate (AMP), adenosine, orthophosphate (P_i), and 0.1 m*M* ATP as mediators was not significant (values not shown). The small negative value for the percent bound to HoFE at 20 hr for the mediator-free case indicates that our assumption of a constant nonprotein activity is not quite valid. However, the 70-hr value is more reasonable; and this implies that time was required to achieve this percentage of nonprotein-bound activity. Thus, at 20 and 70 hr little activity had yet been transferred to HoFE in the absence of mediators. In contrast, at 20 hr, considerable

TABLE 1
Influence of Mediator Compounds on the Transfer Rate of ^{67}Ga from Lactoferrin to Horse Spleen Ferritin

Mediator*	Transfer rate† %/hr	Range	Significance‡
No mediators	−0.57§	(−.30 − −.78)	—
Ascorbate	−4.40	(−4.3 − −4.5)	p <0.001
PP_i	−3.60	(−3.4 − −3.7)	p <0.001
ATP	−2.12	(−1.9 − −2.3)	p <0.001
ATP + 30 μM citrate	−3.40	(−3.1 − −3.6)	p <0.001
0.1 mM ATP	−0.03		NS
30 μM citrate	−0.32		NS
AMP	−0.27		NS
Adenosine	−0.58		NS
P_i	−0.26		NS

* Concentration of each 1 mM except as indicated, pH 7.4 and containing 0.2% NaN_3 or 0.4% benzyl alcohol.

† Estimated from initial slope (0–25 hr) of D%B versus time curve obtained from dialysis experiments.

‡ Compared with no mediators and analyzed using Newman-Kuels Multiple Range Test.

§ Means calculated from at least duplicate experiments except for experiments containing 0.1 mM ATP, citrate, AMP, adenosine and P_i which were performed once.

nuclide had been shifted to HoFE in the presence of all mediators except citrate. Compared on the same time frame, these mediators caused at least a 30-fold increase in ^{67}Ga translocated to HoFE. Hence, the differences not easily apparent in Table 2 can clearly be seen in Table 3. PP_i removed the most activity from LF compared to the others ($p < 0.05$) while the ATP-citrate combination was better than ATP alone ($p < 0.05$) at 20 hr. At 70 hr, the effect of ATP equals the ATP plus citrate result and now both have transferred significantly more than the others ($p < 0.05$). Citrate ions which looked like a good promoter in Table 2 were not effective at 20 hr. However, at 70 hr the effect of these ions was no longer significantly different than the effect of either ascorbate or PP_i.

Effect or HoFE concentration of transfer

The effect of HoFE concentration on ^{67}Ga transfer in the presence of 1 mM ATP was investigated with a constant LF concentration. An increase in HoFE concentration increased the amount of ^{67}Ga transferred and produced a subsequent reduction in LF-bound ^{67}Ga (Fig. 4). Both processes plateaued at high HoFE concentration. At 3 μM HoFE, most of the ^{67}Ga bound to LF has already been transferred and a further increase in HoFE concentration had little effect; hence, the plateau. In contrast, there was no leveling off in the TR. At the lower HoFE concentrations the rate of increase was slight; however, once over ~0.8 μM, the TR was dramatically increased.

DISCUSSION

What is the mechanism of promotion?

How is it possible that these low molecule weight compounds stimulate the translocation given that LF has a higher affinity for ^{67}Ga than TF (*7*)? Although the translocation is thermodynamically favored (HoFE has a greater affinity for ^{67}Ga than LF), there exists a kinetic barrier which is only overcome in the presence of these compounds. These data and previous work implies that the two most important factors of this mediation effect are (a) the ability of these compounds to stimulate gallium exchange from the metal binding site on LF, and (b) the formation of a stable, soluble, high affinity hydrolysis product, $Ga(OH)_4^-$, for inter-protein transport (*14*). ATP does not appear to stimulate the uptake of ^{67}Ga binding by HoFE (*15*) which would shift the equilibrium toward HoFE. Nor does ATP form a high affinity complex which, like EDTA, would remove the metal ion from LF (*16,17*). Moreover no decrease in the D%B for LF*^{67}Ga was observed in the presence of 1 mM ATP and without HoFE. Thus it is likely that ATP interacts with LF and causes destabilization of the metal ligand bonds. It is well known that phosphate containing compounds interact with TF mediating iron release (*18,19*). This ATP-protein interaction yields an increase in the exchange rate and the formation of the gallate anion. Gallium falls into a class of metal ions

TABLE 2
Effect of Various Mediators on ^{67}Ga Transfer from Lactoferrin to Horse Spleen Ferritin

	^{67}Ga distribution at end of equilibrium dialysis experiments*				
Mediator†	Lactoferrin %	Ferritin %	Nonprotein %	Incubation time (hr)	Equilibrium time‡ (hr)
None	47§	37	15	142	—
Ascorbate	19	57	19	165	23
PP_i	3	37	42	165	23
ATP	3	76	11	71	37
ATP + 30 μM citrate	3	78	10	71	33
0.1 mM ATP	74	4	5	93	—
Lactate	16	67	10	189	—
30 μM citrate	17	63	20	140	—
AMP	62	17	10	331	—
Adenosine	64	11	8	162	—
P_i	61	13	7	162	—

* Samples removed from dialysis chambers and chromatographed to determine distribution of ^{67}Ga. For other details see Methods.

† Concentration of each, 1 mM, except as indicated; also contained 0.2% NaN_3, pH = 7.4.

‡ Estimated from the plateau of D%B versus time plot.

§ Means obtained from at least duplicate experiments except for experiments containing, 0.1 mM ATP, lactate, AMP, adenosine and P_i which were performed once.

TABLE 3
Percentage of ^{67}Ga Bound to Lactoferrin and Horse Spleen Ferritin, as Function of Time, Estimated from D%B*

Incubation time	20 hr			70 hr		
Mediator†	LF (%)	HoFE (%)	Sig‡	LF (%)	HoFE (%)	Sig‡
None	91§	−7	—	83	2	—
ATP	58	31	$p < 0.005$	10	80	$p < 0.005$
ATP + 30 μM citrate	42	50	$p < 0.001$	7	84	$p < 0.005$
Ascorbate	45	39	$p < 0.005$	29	54	$p < 0.05$
PP_i	24	34	$p < 0.005$	13	46	$p < 0.05$
30 μM citrate	83	−3	NS	41	39	$p < 0.05$

* Percentage bound values estimated from D%B values using Eq. 2 and Eq. 3. For details, see Methods.

† Concentration of mediator 1 mM except where noted.

‡ Significance. Values for both LF and HoFE at each time compared with values obtained from mediator-free experiments and upper p values reported. Newman-Keuls Test used.

§ Mean values calculated from at least duplicate experiments.

which generally form labile complexes (*20*). This lability is simply enhanced in the LF*ATP complex (ATP specifically bound to LF). The other possibility is that ATP, or more likely ascorbate, could increase the flow of iron from HoFE to LF. When TF and HoFE are incubated together, iron is exchanged in both directions with the net flow toward TF‡ (*12*). Then the iron and ^{67}Ga would exchange (*21*), or iron binding to LF would reduce the free ligand (LF) concentration. In either case, the gallate ion would be produced to bind FE.

Gallium, under these conditions, forms a high affinity soluble species and not the insoluble gallium hydroxide. Harris and Pecararo (*14*) have calculated the percentages of the gallium hydrolysis species in solution using the best available overall formation constants. The distribution is primarily $Ga(OH)_4^-$ (98.4%), some $Ga(OH)_3$ (1.6%) and only traces of the remaining species. Even though the gallate ion is relatively soluble, its presence is limited by the solubility of $Ga(OH)_3$ (solubility = 40 nM, (*22*)). However, in our study of these experiments, the total concentration of gallium is at most 0.1 nM. In addition, these investigators have shown that the gallate ion has formation constant which is comparable to the TF*Ga complex (within an order of magnitude depending on conditions). Therefore, it is likely that $Ga(OH)_4^-$ is the predominant species involved in interprotein transport.

Comparison of LF and TF experiments

The similarities we have observed in these and previous experiments with TF and HoFE (*11*) are most likely related to the similar metal binding site on both proteins (*23*), while the differences are related to the different affinities of these two proteins for metal ions. An anion binding site on both proteins would explain the effect of the mediators. Compounds which were effective for LF were also effective for TF while the obverse was true. A mediator which had little stimulative effect for LF was also ineffective for TF. There were some quantitative differences, particularly in regard to the kinetics of ^{67}Ga movement and the presence of nonprotein bound activity. Generally, the time required for the mediators to work was shorter for LF (*11*). In addition, the phosphate compounds caused more free ^{67}Ga with TF than LF. This suggests that the rate-limiting step in the transfer process is the dissolution of $Ga(OH)^-$ before gallium can bind to HoFE. TF which has a lower affinity for gallium is less resistant to the formation of the gallate. Any increase in $Ga(OH)^-$ concentration reduces the thermodynamic pressure for gallium binding to HoFE, which in turn increases equilibrium times.

The role of LF and HoFE in ^{67}Ga localization

Our results are consistent with the suggestion that a LF*^{67}Ga complex present at an abscess or tumor site could transfer its gallium to FE located intracellularly. The LF*^{67}Ga complex would form, bind to the receptor on M/M, and either transfer the ^{67}Ga directly to surface FE or be incorporated into an endocytic vesicle, and then transfer the nuclide to FE. The reasons for the increased presence of LF at an abscess site have been

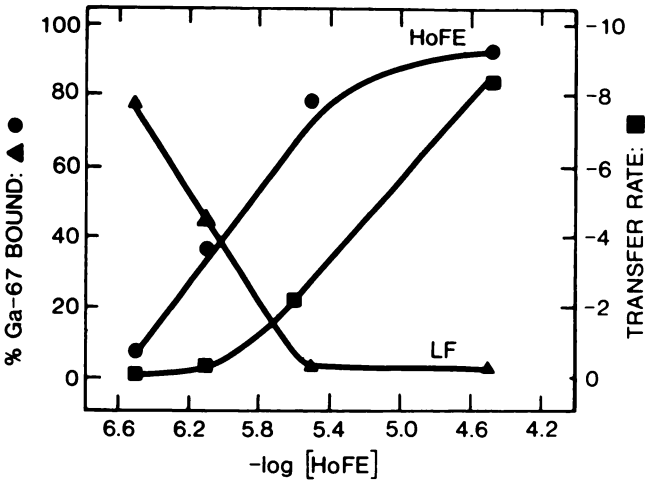

FIGURE 4
Influence of HoFE concentration on transfer of ^{67}Ga from LF to HoFE in presence of 1 mM ATP. Equilibrium dialysis experiments were performed with 17 μM LF*^{67}Ga in right and various HoFE concentrations (0.3 to 30 μM) containing ATP, in left chambers. ^{67}Ga concentration = 2.8–4.3 μCi/ml. Transfer rates (■) were computed from initial slope of D%B versus time curves. After 112 hr incubation for each experiment, samples were chromatographed to determine ^{67}Ga activity bound to HoFE (●) and LF (▲). Results from single set of experiments except for 3 μM HoFE which was repeated twice. For other conditions and procedures see Fig. 1 legend

TABLE 4
Calculation of Putative Intracellular Lactoferrin: Ferritin (LF:FE) Ratios in Normal and Various Disease States

Item	LF concentration* μg/ml	LF molecules/cell† ×10⁵	LF:FE ratio‡ Monocytes	LF:FE ratio‡ Macrophages
Control	0.44	4	6:1	.4 - .5:1
Pseudogout	22	180	250:1	17 - 21:1
Sepsis	127	750	1040:1	70 - 90:1
Gout	223	1050	1400:1	100 -121:1

* LF concentration was measured in synovial fluids from normals and patients with various diseases (*5*).

† Number of LF molecules bound to adherent monocytes computed using 2×10^8 receptors per cell with an affinity constant = 4×10^5 L/M(*9*).

‡ Computed assuming all LF molecules incorporated interact with total FE pool = 72×10^3 molecules/monocyte (*33*). FE concentration increased 12–15-fold (Skikne, Cook, unpublished results) in macrophages.

well documented (*2*) and more recent evidence has added substantiation (*24–27*). Data suggest that PMNs are attracted early to the site in high concentrations while macrophages appear later in lower concentrations (*26,28*). Results using an animal model imply that as the lesion progresses, many PMNs are destroyed while macrophage remained viable (*28*). Animal model experiments have suggested that M/M are an important cell type in ^{67}Ga localization (*29,30*). The above experimental results are consistent with preliminary clinical observations that imply indium-111 white blood cells (predominately PMNs) are more useful for acute abscess detection whereas ^{67}Ga scintigraphy has a higher sensitivity in chronic or subacute infections (*31*). Thus, even though PMNs contain a high concentration of LF, it is possible that time may be required for the cell destruction or degranulation to yield a sufficient LF concentration in the exudate. Lastly, our results imply that increased ferritin concentration enhances ^{67}Ga deposition. In experimental animal models for inflammation, liver ferritin synthesis is increased and serum ferritin levels are elevated in patients with inflammatory disease (*32*).

While the above is a qualitative description of the process, some numerical estimates of this process can be provided. The only caveat is that we have used human LF and horse spleen FE in our experiments. However, preliminary experiments using human liver FE (*16*) suggest qualitative agreement with the data presented here. Bennett and Skosey (*5*) have quantitated the LF present in synovial fluids from patients with a variety of diseases. Using these data and a value for monocyte ferritin (*33*), we can show (Table 4) that in the non-inflammatory case little LF is bound to the monocytes and little LF*^{67}Ga would enter the cell. However, in inflammations, due to pseudogout, the number of LF molecules bound, increases almost two orders of magnitude, and the FE concentration in macrophages increases 12–15-fold (Skikne, Cook, unpublished results). This reduces the LF:FE (endocytosed LF and intracellular FE) ratio from 250:1 in monocytes to 17-21:1 in macrophages. In light of our data, this ratio would be much more conducive to nuclide translocation. These rough calculations suggest that our in vitro data is consistent with an in vivo transfer in an abscess.

Elevated synthesis of both of these proteins in tumors could yield a scenario similar to that described above. LF and FE concentrations are increased in a variety of ^{67}Ga positive human cancers (*34,35*) and other human cancers (*36,37*). Experiments with animal models have shown that a FE fraction from rabbit liver contains significant amounts of ^{67}Ga (*38*). In contrast, little ^{67}Ga was detected in FE fractions from a variety of rat tissues including tumor (*39*). Moreover, the cause and reason for any increase is unclear, although deSousa, Broxmeyer, and others have provided in vitro evidence that both LF and FE are inhibitors of granulopoesis (*40*). Thus, derangements in white cell synthesis, either primary or secondary to the disease state, could yield elevated protein levels. Further investigation will be required to establish the reasons for these elevated levels in tumors and their precise relationship to ^{67}Ga localization.

How would mediators influence localization?

With this understanding of the promotion mechanism, we can surmise how these compounds could effect ^{67}Ga deposition. Variations in diphosphate compounds or ascorbate could modulate transfer. Alterations in cytoplasmic ascorbate concentration have been observed in various pathological states (*41,42*). Intracellular nucleoside triphosphates (NTPs) concentrations are about 2–15 m*M* (*43*) and are well above the threshold level we have observed (See Table 2). However, preliminary results (*16*) suggest that increases in ATP concentration up to 5 m*M* further stimulate transfer. Moreover, other phosphate compounds, e.g., 2,3 phosphodiglyceric acid, inositol hexaphosphate and other NTPs (*18,19*), would likely be effective. Our results also suggest that other chelate molecules could act at least additively. Although the role of only one compound, citrate, was examined, other physiological chelators, e.g., lactate, polyamines or sulfate compounds, would probably act as an adjunct to the influence of phosphate containing compounds.

FOOTNOTES

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† Miles Laboratories, Elkart, Indiana and Sigma Chemical Co., St. Louis, MO.

‡ Please note that in our previous article (*J Nucl Med* 24:608–614, 1983) the net flow was erroneously stated to be towards ferritin.

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