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IRON-INDUCED ENHANCEMENT OF ^{67}Ga UPTAKE IN A MODEL HUMAN LEUKOCYTE CULTURE SYSTEM

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The addition of iron, either as Fe dextran or $FeCl_3$, to leukocyte tissue culture medium (containing 20% fetal calf serum) significantly increased the cellular accumulation of subsequently added ^{67}Ga-citrate. This effect of iron was dose-related and decreased, after the initial increase, with increasing concentrations of elemental iron. There was a significant negative correlation ($P < 0.025$) between the concentrations of gallium and iron in the medium, indicating competition between the two elements for the same serum binding sites. Although it is not yet justified to begin clinical trials, these experiments suggest the possibility that prior administration of iron may increase the usefulness of ^{67}Ga scanning for the diagnosis of neoplasms or abscesses. Further testing of this hypothesis in animals with such lesions would seem indicated.

Hayes, et al (*1*) have reported that administration of scandium, simultaneously with ^{67}Ga, to tumor rats and mice increased the excretion of ^{67}Ga and increased the tumor-to-nontumor ratio of ^{67}Ga activity. Since ^{67}Ga scintiscanning is used in the diagnosis of tumors in man (*2–5*), any method which produces similar results could have clinical utility.

Recently Merz, et al (*6*) developed a leukocyte tissue culture technique which is useful for the study of the cellular uptake of ^{67}Ga. These workers have demonstrated a linear relationship between ^{67}Ga concentration and counts associated with the cells. Pressurized washing with normal saline does not significantly reduce the cell-associated counts, while trypsin digestion does. Autoradiographic studies indicate that ^{67}Ga is associated with the cell plasma membrane.

Because gallium seems to bind to the same serum proteins as iron (*7*) and more is known about the potential toxicity and biologic behavior of iron, we examined the influence of iron on the cellular uptake of ^{67}Ga in leukocyte tissue culture.

MATERIALS AND METHODS

Leukocytes were isolated from 20 ml of blood obtained from normal adult volunteers. Heparinized whole blood was centrifuged at 125 g for 15 min. The plasma above the buffy coat was aspirated and discarded. The buffy coat was then removed with an adhering layer of red blood cells. This mixture was treated for 30 sec with 9 cc of distilled water to hemolize the red blood cells. Then a twofold solution of McCoy's 5A medium was added to make the mixture isotonic. Repeat centrifugation at 125 g for 15 min resulted in a button of leukocytes at the bottom of the tube. The supernatant was discarded and the leukocytes were suspended in 7–10 ml of Hank's solution without glucose. The leukocytes were counted with a Coulter counter and further diluted with Hank's salt solution to provide a final concentration of approximately 2×10^6 cells per ml. In all experiments, leukocyte cultures were prepared by adding $4–8 \times 10^5$ cells to 10 ml of McCoy's 5A medium, containing 20% fetal calf serum, in 100-ml glass bottles.

Three sets of experiments were performed. In the first set, sterile aliquots of $FeCl_3$, dissolved in distilled water, were added to the cultures to yield varying concentrations of $FeCl_3$ in the final mixture. Twenty microcuries of carrier-free ^{67}Ga-citrate (Diagnostic Isotopes, Upper Saddle River, NJ) was added immediately after the iron.

The second set of experiments was performed like the first, except that Fe dextran (Imferon) was used

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TABLE 1. EFFECT OF VARYING CONCENTRATIONS OF $FeCl_3$ ON ^{67}Ga ACCUMULATION BY LEUKOCYTES IN CULTURE MEDIUM

[$FeCl_3$]	Mean percent increase of ^{67}Ga on filter*	η
1×10^{-6}	−14.0	1
2×10^{-6}	+20.5 ± 31.5	2
4×10^{-6}	+175.5 ± 24.5	2
5×10^{-6}	73.3 ± 36.3	3
6×10^{-6}	+130.5 ± 13.5	2
1×10^{-5}	+44.7 ± 35.0	3
5×10^{-5}	+79.5 ± 51.5	2
1×10^{-4}	+77.0 ± 52.3	3

* mean ± s.e.

TABLE 2. EFFECT OF VARYING CONCENTRATIONS OF Fe DEXTRAN ON ^{67}Ga ACCUMULATION BY LEUKOCYTES IN CULTURE MEDIUM

Molar concentration of elemental iron in the medium	Mean percent increase of ^{67}Ga on filter*	η	p
1×10^{-6}	−17.0	1	
1×10^{-5}	+13.0	1	
1×10^{-4}	+102.8 ± 29.1	4	<0.05
2×10^{-4}	+146.2 ± 19.1	4	<0.005
3×10^{-4}	+132.3 ± 24.3	3	<0.05
4×10^{-4}	+106.0 ± 10.4	3	<0.01
1×10^{-3}	+9.0	1	
2×10^{-3}	−30.0	1	
1×10^{-2}	−32.0	1	
2×10^{-2}	−66.0	1	

* Mean ± s.e.

TABLE 3. EFFECT OF VARYING CONCENTRATIONS OF $^{59}FeCl_3$ (SPECIFIC ACTIVITY, 1.3–1.5 mCi/μg IRON) ON ^{67}Ga ACCUMULATION BY LEUKOCYTES IN CULTURE MEDIUM

[$FeCl_3$]	Percent dose of ^{67}Ga on filter*	Percent increase of ^{67}Ga on filter*	p
0	0.34 ± 0.02	—	—
0.5×10^{-5}	0.74 ± 0.05	116.0 ± 6.6	<0.001
1×10^{-5}	0.71 ± 0.05	112.6 ± 10.7	<0.005
2×10^{-5}	0.61 ± 0.04	78.8 ± 6.0	<0.001
5×10^{-5}	0.59 ± 0.07	69.4 ± 10.6	<0.005

* Mean ± s.e.

instead of $FeCl_3$. The Fe dextran, with an elemental iron concentration of 0.9 *M*, was diluted with distilled water when final iron concentrations of less than 10^{-4} were required. Otherwise, it was added to the leukocyte culture without prior dilution.

The third set comprised five experiments. Iron-59-$FeCl_3$ (New England Nuclear Corp.) with a specific activity of 43.4 mCi/μg iron was diluted with stable $FeCl_3$ solution to yield a specific activity of 1.3–1.5 mCi/μg iron. Ten microcuries of ^{67}Ga-citrate was added immediately after the ^{59}Fe-containing $FeCl_3$ solution as in the first two sets.

Following the addition of iron and gallium, all leukocyte cultures were incubated at 37° for 2 hr. The medium was then poured into 30-cc syringes, tipped with Swinnex filter holders containing 0.45-μm-pore-size filter membranes. The medium was forced through the membrane with the syringe plunger, resulting in cells being suspended on the membrane. In the third set of experiments, aliquots of the medium, after having passed through the filter, were pipetted into plastic counting tubes. All counting was done in a well counter with a 5-in. sodium iodide crystal. In all experiments, the filters were removed from the filter holders and placed in plastic counting tubes. In the first two sets of experiments ^{67}Ga was counted between 100 and 300 keV. In the third set, ^{67}Ga was counted in a 50–120-keV window and ^{59}Fe in a 900–1,300-keV window. In every case samples were counted in duplicate and averaged. Sufficient counts were obtained to yield a relative standard deviation of less than 1%. Gallium-67 counts were corrected for ^{59}Fe counts entering the ^{67}Ga window by the equation: corrected ^{67}Ga counts $= N_1 - bN_2$, where N_1 is the sample counts in the 50–120-keV window, N_2 is the sample counts in the 900–1,300-keV window, and b is a fraction obtained by dividing the counts of a ^{59}Fe standard in the 50–120-keV window by the counts in the 900–1,300-keV window.

The unsaturated iron-binding capacity of the medium was measured on three separate samples, on different days, using an IROSORB Diagnostic Kit (Abbott Laboratories, Chicago) without modification.

RESULTS

The results of the first set of 18 experiments are shown in Table 1. All concentrations of $FeCl_3$ in the medium above 1×10^{-6} molar resulted in an increase in ^{67}Ga accumulation on the filter. Although, in some cases, the ^{67}Ga on the filter more than doubled, the small number of observations precluded statistic significance.

The results of the second set of experiments, using Fe dextran, are presented in Table 2. The data suggest that the increase in ^{67}Ga on the filter, as a function of iron concentration, has a maximum. At high iron concentrations there appears to be an actual inhibition of ^{67}Ga accumulation on the filter.

Table 3 summarizes the five experiments in Set 3 employing $^{59}FeCl_3$. As shown graphically in Fig. 1, there was a highly significant increase of ^{67}Ga on the filter, with the adhering leukocytes, at each $FeCl_3$ concentration studied. However, this increase de-

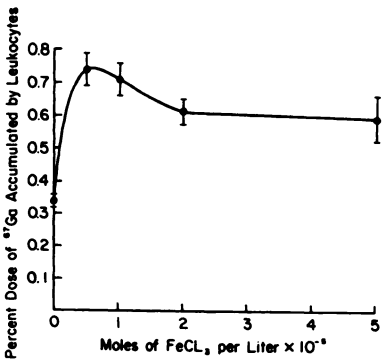

FIG. 1. Percent dose of ^{67}Ga associated with leukocytes in culture medium as function of $FeCl_3$ concentration.

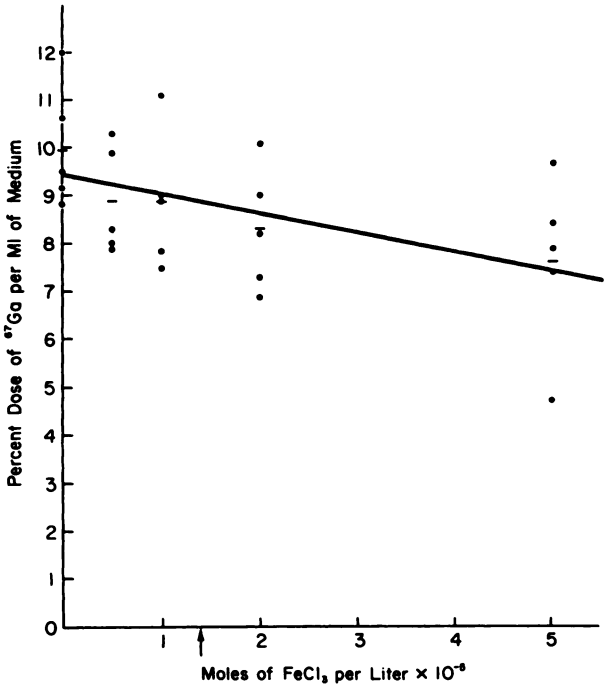

FIG. 2. Correlation between percent of ^{67}Ga per ml of culture medium and iron concentration in culture medium. Each dot represents individual measurement; horizontal dashes are means at each $FeCl_3$ concentration. Arrow on abscissa marks mean unsaturated iron-binding capacity of medium. Medium contains 20% fetal calf serum.

TABLE 4. QUANTITY OF $FeCl_3$ ASSOCIATED WITH LEUKOCYTES IN CULTURE MEDIUM WITH ADDITION OF VARYING CONCENTRATIONS OF $FeCl_3$

[$FeCl_3$]	Percent dose of $FeCl_3$ on filter*	Moles of $FeCl_3$ on filter $\times 10^{-9}$*
0.5×10^{-5}	0.15 ± 0.02	0.77 ± 0.10
1×10^{-5}	0.05 ± 0.008	0.53 ± 0.08
2×10^{-5}	0.03 ± 0.002	0.66 ± 0.03
5×10^{-5}	0.02 ± 0.006	1.29 ± 0.32

* Mean ± s.e.

clined at higher iron concentrations. Analysis of variance shows a significant difference among the means of the percent increases in ^{67}Ga associated with the leukocytes ($P < 0.005$). This provides further evidence that the effect of iron addition has a maximum. Meanwhile, linear regression analysis shows a negative correlation between gallium and iron concentrations in the medium ($R = -0.474$, $P < 0.025$). This is shown in Fig. 2 where each dot represents an individual measurement and the horizontal lines are means of the percent of the original dose per milliliter of ^{67}Ga remaining in the medium at each $FeCl_3$ concentration. The arrow on the abscissa marks the mean unsaturated iron-binding capacity of the medium: $(1.39 \pm 0.03) \times 10^{-5}$ moles per liter.

The quantity of iron on the filter was successfully measured in four of the experiments in the third set and the data are presented in Table 4. The iron going to the filter is relatively constant except at the highest medium $FeCl_3$ concentration studied. However, statistically there is no significant difference with respect to the quantity of iron on the filter between medium $FeCl_3$ concentrations of 0.5×10^{-5} and 5×10^{-5} molar.

DISCUSSION

The presence of iron can cause greatly increased ^{67}Ga accumulation by leukocytes in culture medium. This increased cellular ^{67}Ga uptake is probably not a simple function of iron concentrations but rather one which goes through a maximum and declines. We have not demonstrated any relationship between iron and gallium with respect to the cellular binding of these two elements. It is possible, however, that the effect of iron on cellular accumulation of ^{67}Ga may reflect competition for cell-binding sites at higher iron concentrations. Also, perhaps ^{67}Ga binding to cells results from a metabolic function to which iron is toxic in high concentrations, and the additional ^{67}Ga, displaced from the medium, attaches to glass or other available surfaces comprising the tissue culture system.

Our data support the concept that both gallium and iron bind to the same serum protein(s) (7). In our tissue culture system, increasing concentrations of iron reduced gallium concentration in the medium.

Since one can increase cellular ^{67}Ga uptake by the addition of iron in vitro, the possibility of affecting in vivo ^{67}Ga distribution and excretion needs investigation. Our data would encourage the study of the effects of iron administration on ^{67}Ga metabolism in animals with experimental lesions, provided one is aware that results can be affected by the concentrations of iron employed and the potential toxicity of this element.

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