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Mechanism of Gallium-67 Accumulation in Tumors

LETTER TO THE EDITOR: We would like to comment on the recent article of Anghileri et al. (*1*), which appeared in the May, 1988 issue of the *Journal*. We believe that none of the data presented supports their conclusion that "Increased permeability of tumor cells is the principal cause of [^{67}Ga]-citrate accumulation by tumors." In fact, there are serious problems with the study as well as their interpretation of the data. We would like to point out the following:

Inhibition of cellular uptake of ^{67}Ga by citrate in vitro has been previously demonstrated (*2,3*). With the current knowledge of nearly 100% binding of ^{67}Ga to serum proteins, notably transferrin, in circulation (*4–6*), it is hard to justify studying ^{67}Ga uptake in the absence of serum and extrapolate the results to the in vivo situation.

To justify their choice of 30 min as the incubation time, the authors indicated that there were 4–5% nonviable cells (Trypan blue positive cells) at 30 min and 6–8% at 1 hr. Furthermore, ^{67}Ga uptake was proportional to the number of nonviable cells, e.g., 8–12% at 30 min and 14–18% at 1 hr (*1*). Preferential uptake of ^{67}Ga by nonviable cells has been previously demonstrated (*7,8*). One wonders whether Anghileri et al. was studying ^{67}Ga uptake by those dead cells. The results presented in Figure 4 showing that ^{67}Ga uptake was higher at 45°C than at 37°C could well be due to the increased number of nonviable cells at 45°C.

The conclusion that [^{14}C]citrate uptake by tumor cells was due to an unmediated physical diffusion was based on a single study which showed that the [^{14}C]citrate uptake as a function of citrate concentrations (0.05 to 2 m*M*) failed to show a saturation phenomenon (Fig. 3). However, the concentrations of citrate used are insufficient to demonstrate that no saturation phenomenon occurs. In fact, the results presented in Figure 3, showed that at citrate concentrations higher than 1 m*M*, there was already a leveling off of citrate uptake. Without doing the experiment with higher concentrations of citrate (e.g., 10–20 m*M*), one can not conclude that citrate uptake does not show a saturation phenomenon.

If Anghileri et al. were correct in concluding that ^{67}Ga uptake follows the physical diffusion of citrate ions into the cells, then one should be able to derive a linear relationship between the fraction of ^{67}Ga taken up by the cells and that of [^{14}C]citrate uptake. Unfortunately, in Figure 3, the uptake of ^{67}Ga was presented as percent of dose, while the citrate uptake was presented as $\mu M \times 10$. Sufficient information was not given to follow recalculation of the data to make such a comparison. Nonetheless, the curves in Figure 3 show that no such correlation exists.

The in vivo study presented in Table 3 could be interpreted differently. Without taking into consideration the multiple factors which may affect the tissue, including tumor, uptake of ^{67}Ga (*9*), one can almost interpret this kind of data anyway he/she wants. It should be pointed out that Hammersley and Zivanovic (*10*) previously have shown that ^{67}Ga uptake in two mouse tumors is independent of citrate concentrations.

Table 1 shows that ~10% (not <10% as stated in the text) of ^{67}Ga taken up by tumor cells could be released by EDTA treatment, while elsewhere in the article the authors indicated that it was 20–30%.

In summary, we believe that the results presented by Anghileri et al. are not new and are not relevant to the mechanisms of ^{67}Ga uptake by tumors.

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REPLY: The inhibition of in vitro cellular uptake of ^{67}Ga citrate is no matter of diagreement; therefore, citrate ion has a defined effect on ^{67}Ga citrate uptake. Concerning the role of plasma proteins (especially transferrin), our work with DS sarcoma tumor cells has shown a dramatic inhibition of in vitro cellular uptake of ^{67}Ga citrate in the presence of blood plasma (*1*). Furthermore, work in progress has also shown, in 20 incubation experiments under the same experimental conditions of the article in question, that in presence of transferrin the ^{67}Ga citrate uptake is equal or as in most cases slightly lower than that corresponding to controls without transferrin. For this reason, we have used an experimental model of cells without plasma proteins in the medium.

As is the consensus in cancer research, the dye inclusion test (Trypan blue in our case) cannot be considered as a viability test, but only as an index of physicochemical changes (affecting the cell permeability) at the cell plasma membrane level (*2*). On the other hand, it is well established that the first effect of hyperthermia is to modify plasma membrane permeability to extracellular molecules and ions (*3*). As a consequence, it is not exact to talk of dead or nonviable cells but of cells with plasma membrane modifications that affect their permeability. For this reason, we have defined an experimental protocol to keep the effects of the number of original dye including cells and incubation time well controlled.

To increase the range of citrate ion concentration was not considered because the highly nonphysiologic concentration of a very complexing molecule as citrate can be expected to drastically change the ionic balance outside and inside the cell, and that the effects on the phospholipid double layer and other permeability regulating systems could become critical. In any case, we stated that under the studied conditions ^{14}C sodium citrate uptake has the characteristics of an unmediated physical diffusion. It is possible, however, that with values over our range of citrate ion concentration the other phenomena above mentioned can play a role, and a saturation could take place. In any case, this saturation will be irrelevant to the mechanisms of uptake under physiologic conditions.

In Figure 3, ^{14}C sodium citrate uptake was presented as μm $\times$ 10, while the uptake of ^{67}Ga citrate was percent of dose. It is not possible to express a *carrier-free* ^{67}Ga citrate uptake on μm concentration basis. For this reason this figure shows only the influence of increasing sodium citrate concentration on carrier-free ^{67}Ga citrate uptake without trying to draw any linear correlation.

The difficulties in interpreting the in vivo results are well explained in our article, with details of the factors affecting them (^{67}Ga dilution, blood plasma citrate, etc.).

There are no discrepancies at all between Table 1, where ~10% of carrier-free ^{67}Ga citrate (in absence of gallium-carrier) is released by EDTA, and the values of 20–30% of carrier-free ^{67}Ga citrate released in the presence of gallium-carrier plus sodium citrate (in the text and corresponding to Figure 5). Simply, these values are not comparable.

To conclude, in scientific research a criticism is valid when sustained by a new interpretation of the experimental facts submitted to the critical appraisal. The fact that our conclusions do not agree with others own hypothesis and conjecture, is not enough to denigrate our work as not new and not relevant to the mechanisms of ^{67}Ga citrate uptake by tumors.

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Per-rectal Portal Scintigraphy: Comparison Between Technetium-99m, Thallium-201, and Iodine-123-HIPDM

TO THE EDITOR: Several works describing the clinical value of per-rectal administration of a radiotracer for the evaluation of portal systemic shunting (PSS) have been reported (*1–5*), and we have read with interest the latest one published in a recent issue of the *Journal of Nuclear Medicine* (*6*). Various tracers have been proposed, but to our knowledge, no comparative study has been performed yet.

In an animal model, we compared technetium (^{99m}Tc) pertechnetate, thallium-201 (^{201}Tl) chloride and iodine-123-(^{123}I)-HIPDM [N,N,N′-trimethyl-N′-(2-hydroxy-3-methyl-5-(123I)iodobenzyl)-1,3-propanediamine] (Medgenix, Belgium) by studying the following parameters: the magnitude of the rectal absorption, the time-variation of the ratio used to evaluate PSS, and the liver uptake of the tracer in the presence of hepatocellular damages.

The rectal absorption was measured in five rats, and the results showed that within 25 min after the per-rectal administration of the radiotracer, the mean absorbed activity amounted to 32% (range 21–41%) for ^{123}I-HIPDM, 29% (range 21–35%) for [^{99m}Tc]pertechnetate, and 17% (range 6–26%) for ^{201}Tl; this last one showing a significant lower absorption ($p < 0.02$).

The variation of the parameter employed to estimate the PSS was evaluated by studying the heart-to-liver ratio for [^{99m}Tc]pertechnetate and ^{201}Tl and the lung-to-liver ratio for ^{123}I-HIPDM vs. time. This ratio remained constant for ^{201}Tl and ^{123}I-HIPDM, but for [^{99m}Tc]pertechnetate, it rapidly in-