The Tissue Distribution of Gallium Radionuclides

Hoffer, Huberty, and Khayam-Bashi, in their paper entitled "The Association of Gallium-67 with Lactoferrin" (pp. 713–717, this issue), have added an interesting observation on the interaction of gallium (at the carrier-free level) with yet another constituent of the biological complex in mammalian tissue. They may well have uncovered an important factor involved in the overall in vivo behavior of this element. Their findings are, again, one more example of the quite fascinating interactions that this simple substance has with the fabric of many biological processes. In spite of the fact that gallium has not as yet been shown to have any essential trace element function, perhaps it does have one, but operative only at concentrations far below those normally considered to be of importance! Deficiencies would thus be quite unlikely to be observed because of the natural abundance of gallium.

Before commenting further on the observations of Hoffer and his co-workers, it seems logical that I first include in my remarks a brief review of the present knowledge concerning factors involved in the tissue distribution of gallium radionuclides.

The radionuclides of gallium hold an unusual place in the development of nuclear medicine. Attention was initially called to them during the late 1940s by Dudley and his co-workers (*1*), who suggested that the bone-seeking characteristics of this element might be the basis for the use of its radionuclides (initially, Ga-72) in the diagnosis and possible treatment of malignancies of bone. Diagnostic attempts made at that time were premature, mainly because good instrumentation was lacking, and it was not until the early 1970s that the real potential of gallium radionuclides in the diagnosis of cancer and inflammatory processes was recognized (*2*). Why an element closely akin to aluminum should show such an unusual affinity for malignancy and inflammatory lesions is indeed intriguing.

Factors that affect the gross tissue distribution of Ga-67 in animals and man include: presence of tumor, inflammation, age, sex hormone status, lactation, pregnancy, exposure to ionizing radiation and the amount of the element present in the dose administered (*3, 4*). It is quite remarkable that such a simple substance is involved in so many physiological processes. Indeed, one might conjecture that gallium radionuclides may ultimately find their greatest use as tracers for biological processes rather than as agents for the detection of cancer and inflammatory lesions.

When Ga-67 is administered intravenously in a simple chelate form, i.e., citrate, it is bound almost immediately to plasma proteins, probably mainly to transferrin. The subsequent entry of Ga-67 into cells and its association with subcellular components is controlled to some extent by this binding to plasma proteins (*3*). By 24 hr the tissue distribution of Ga-67 is essentially complete. Uptake of Ga-67 in transplanted animal tumors is mainly associated with viable rather than necrotic tissue (*2*). It is not now clear how Ga-67 makes its entry into various cell types. Entry could occur by endocytosis (of protein-bound Ga-67) (*5*), diffusion (hyperpermeability of tumor cell plasma membranes) (*3, 6*), or exchange of transferrin-bound Ga-67 with lactoferrin, as suggested by Hoffer and his co-workers in their paper.

Swartzendruber et al. (*7*) first showed by electron microscopic autoradiography that in both cancerous and normal murine tissues, Ga-67 was associated with lysosomes. Brown et al. (*8*) and Haubold and Aulbert (*9*) confirmed this observation using subcellular tissue fractionation techniques. Other workers (*6, 10*) have on the other hand reported finding large amounts of Ga-67 associated with the soluble portion of tumor tissue homogenates rather than with isolated cell organelles.

In work in our laboratory with various transplanted animal tumors, we have found that the technique used in homogenation and subsequent handling of such preparations can be of importance (*11*). The organelles of some tumors are quite friable, and even the gentlest homogenation technique can lead to disruption of organelles and loss of contained Ga-67 to the supernatant fraction of the homogenate. This occurrence is attested to by the presence of large amounts of acid phosphatase, a

lysosomal enzyme, in the soluble fraction along with increased amounts of Ga-67 activity under such conditions (*3*). The presence of a mucilagenous material in most tumors also produces difficulty in subcellular fractionations. This material tends to act as a trapping agent unless it is removed and, when present, results in loss of small Ga-67-binding organelles to the nuclear pellet in the first stage of subcellular fractionation. This phenomenon may account for the unusual amount of Ga-67 found by others in the nuclear fraction (*6*).

Brown et al. (*11*) have also identified a microsomal fraction having a high affinity for Ga-67. Interestingly enough, in hepatomas these small particles appear to have a much greater avidity for Ga-67 than they do in liver (*11*).

When rat and mouse tumors containing Ga-67 are homogenized in distilled water and the soluble fraction subjected to G-200 gel filtration, approximately 50% of the Ga-67 present in the extract is found to be associated with a protein fraction having a molecular weight of $4\text{–}5 \times 10^4$ daltons (*12*). Similar gel filtration studies with normal liver indicate the presence of much lower levels of this particular Ga-67-binding macromolecular material (*12*). Some other normal tissues show a rather high association of Ga-67 with this fraction, e.g., the thymus (*13*), but, when the absolute concentrations of Ga-67 present in normal and tumor tissue are compared, it is apparent that tumor tissue contains by far the highest level of this Ga-67-binding material.

This particular Ga-67-binding protein has now been purified in our laboratory. We find it is a glycoprotein with a molecular weight of 4.5×10^5 daltons containing approximately 50% protein based on amino acid analysis; it is sensitive to heat and pH and is readily saturated in vivo by quite small amounts of stable gallium (*12*).

Hoffer and co-workers suggest in their paper that Ga-67 bound to lactoferrin might be a basic intracellular agent involved in the localization of Ga-67 in some normal tissues and also in inflammatory lesions, since lactoferrin is known to be present in high concentration in granulocytes and a number of normal tissues that have high uptakes of Ga-67 (spleen, bone marrow, etc.). They propose, on the basis of their work reported here, a possible transfer of Ga-67 from plasma transferrin to lactoferrin in mammary tissue during periods of lactation. This may well occur, but it should be pointed out that, during gestation, lactation, and involution in animals, very dramatic increases in lysosomal enzymes occur in the mammary glands, indicating that lysosomes may well be involved in the increased uptake of Ga-67 seen in breast tissue and in milk (*14*).

Although Hoffer et al. indicate there is little information on the level of lactoferrin in tumors, it would not be out of order to speculate that at least a portion of the Ga-67 uptake by tumors may occur as the result of inflammation. Lactoferrin, however, has a stated molecular weight of 85,000 to 90,000 in contrast to the 45,000 molecular weight macromolecule we have found binding most of the Ga-67 in tumor tissue. In view of these in vivo findings, it appears unlikely to us that lactoferin is involved in a major way in the uptake of Ga-67 by tumor tissue.

In studies reported to date on the interactions of gallium radionuclides with biological systems, we have extensive information of gallium's organ and tissue distribution, but information on the subcellular distribution and the possible mechanism(s) involved in its cellular uptake is far from satisfactory. The results obtained by some workers are in agreement, while those of others are contradictory. Even so, one senses that we have with the subcellular distribution of gallium the possible existence of a set of processes of major biological importance. This belief is supported by the fact that In-111, the rare earth radionuclides, and even the actinides show considerable similarities to Ga-67 in their cellular and subcellular distributions, suggesting similar pathways for entry of these materials into cells (*4*).

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