Localization of Gallium-67 During Embryogenesis (36965)

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(Introduced by T. T. Odell, Jr.)

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That isoenzyme and protein patterns of leukemic tissues of adult mice resemble those of the corresponding fetal tissues but differ from those of normal adult tissues has been previously noted (1). In addition, more recent studies have demonstrated that sera from leukemic mice show specific protein alterations that are identical to those seen in sera of pregnant mice (2). Previous studies have shown that gallium-67 (^{67}Ga) preferentially localizes in the thymus and lymph nodes of leukemic AKR mice and in the spleen and liver of BALB/c mice with Rauscher leukemia (3). Gallium localization in bone and neoplastic tissues was first reported by Dudley and associates (4, 5), and the radionuclide ^{67}Ga was later shown to localize in many nonosseous human tumors (6–10) and in several experimental animal tumors (11). The mechanisms involved in the localization of ^{67}Ga have not been clearly defined, although there is good evidence that the isotope concentrates in lysosomal bodies (12). Because of the isoenzymatic similarity of leukemic and fetal tissues and similarities in serum protein alterations in mice bearing such tissues, and since ^{67}Ga concentrates in murine leukemic tissues, the present study was undertaken to determine the degree of ^{67}Ga concentration in embryonic and associated tissues in the mouse. Localization of ^{67}Ga in such tissues would extend previous similarities between embryogenesis and leukemogenesis in the mouse and could provide a possible model system for studying the mechanism of ^{67}Ga uptake *per se*.

Materials and Methods. Mice. Specific-pathogen-free BALB/c female mice were raised and maintained at the animal facilities of the Biology Division, Oak Ridge National Laboratory. The day on which vaginal plugs were observed after mating the previous evening was considered Day 0 of the gestation period.

Isotopes. ^{67}Ga-Citrate, in essentially carrier-free form, was obtained from the Isotopes Division, Oak Ridge National Laboratory. It was prepared for injection as previously described by Hayes *et al.* (11). Sterile aqueous [^{3}H]TdR (sp act 6 Ci/mmole) was obtained from Schwarz BioResearch Inc., Orangeburg, NY. To determine isotope uptake in various tissues and organs, virgin or pregnant mice were injected intravenously (iv) with either 1 μCi of ^{67}Ga or 1 mCi [^{3}H]TdR and were sampled 24 hr later. Spleen, thymus, and liver tissues of the adult mice and the embryos and decidua were removed from the treated mice, weighed, and fixed in Bouin's fixative, and their radioactivity was determined by counting in a deep-well scintillation counter. The ^{67}Ga radioactivity was expressed as cpm localized per gram of tissue divided by 1% of the counts remaining in the inoculum 24 hr after injection. The amount of [^{3}H]TdR in embryos and associated decidua was determined by precipitating aliquots of sonicated tissue extracts with trichloroacetic acid (TCA) onto scintillation counting discs and counting in a liquid scintillation counter. The radioactivity was expressed as cpm per microgram of protein following protein determinations of the extracts.

Autoradiography. For the preparation of autoradiograms, pregnant mice were injected iv with 1 mCi of either ^{67}Ga or [^{3}H]TdR. Tissues were removed 24 hr after injection of either isotope, and the autoradiograms were

[1] Operated by Union Carbide Corporation under contract with the U. S. Atomic Energy Commission.

TABLE I. Concentrations of ^{67}Ga in Whole Embryos and Associated Maternal Tissue.

Age of embryo (days)	No./time point	Activity[a] (mean ± SE)
5	13	7.48 ± 1.03
6	13	7.15 ± 0.57
8	18	10.45 ± 0.16
9	9	13.59 ± 0.90
10	14	11.45 ± 0.44
12	4	5.38 ± 0.16
13	8	5.86.± 0.30
14	4	6.31 ± 0.19
15	6	6.07 ± 0.35
16	8	5.77 ± 0.28
17	4	3.22 ± 0.11
18	4	4.35 ± 0.38

[a] Expressed as cpm localized per gram of tissue divided by 1% of the counts remaining in the inoculum 24 hr after injection.

prepared as previously described (3). Briefly, this involved the fixation of tissues in either 2.5% glutaraldehyde (pH 7.3) or Bouin's fixative. Tissues fixed in glutaraldehyde were subsequently washed in 0.06 *M* *s*-collidine (pH 7.4). Paraffin sections about 5 μm thick were dipped in Kodak NTB-2 nuclear track emulsion. The sections were exposed and developed in Kodak D-19 and stained with hematoxylin and eosin.

Results. Uptake of ^{67}Ga in whole embryos. As shown in Table I, the total incorporation of ^{67}Ga in whole embryos was related to the time of gestation. Embryonic uptake of ^{67}Ga was ~7% at 5 days and reached a maximum of 13.5% at Day 9 of gestation. Beginning with Day 12, the total ^{67}Ga counts per embryo declined.

Uptake of ^{67}Ga in adult tissue. The concentration of ^{67}Ga in the spleen and liver of pregnant mice did not differ significantly from that in virgin mice (Table II). In contrast, thymus tissue of pregnant mice showed a progressive decline in ^{67}Ga localization throughout the gestation period (Table II).

Uptake of [^{3}H]TdR in whole embryos. The incorporation of [^{3}H]TdR per microgram of protein progressively declined in embryonic tissue with increasing gestation time (Table III).

Autoradiography of embryonic and adult tissues. Autoradiograms of embryonic and deciduate tissues labeled with ^{67}Ga were prepared on Days 6–14 of gestation. Embryos at Day 6 showed scattered labeling, but on Days 8–13 labeling was localized in the decidua, fetal membranes, and limb buds. By Day 14, most of the ^{67}Ga was localized in the skeleton. The uterine mucosa of virgin mice did not localize ^{67}Ga. In contrast to the intense labeling of embryonic and deciduate tissues with ^{67}Ga, crypt cells of the intestinal tract of normal or pregnant mice did not localize ^{67}Ga. Autoradiograms of fetal and deciduate tissues labeled with [^{3}H]TdR revealed scattered labeling with no specific concentration in fetal membranes or decidua, although at 6 days the decidua and embryo did contain labeled cells. Conversely, the crypt cells of the intestinal tract from adult mice were intensely labeled with [^{3}H]TdR.

Discussion. Between Days 8 and 10 of the gestation period, ^{67}Ga was localized in embryonic and associated decidual tissues at levels comparable to those previously observed in leukemic tissues of AKR mice with spontaneous leukemia and BALB/c mice with Rauscher leukemia (3). The reason for the marked localization of ^{67}Ga during this time period is unknown, although Days 9–

TABLE II. Concentrations of ^{67}Ga in the Liver, Spleen and Thymus of Virgin and Pregnant Mice.

Days of gestation	No./time point	Activity[a] (mean ± SE)		
		Liver	Spleen	Thymus
Virgin	6	5.85 ± 0.23	5.67 ± 0.39	4.06 ± 0.61
5–7	11	5.21 ± 0.31	4.28 ± 0.39	3.29 ± 0.33
8–11	11	4.48 ± 0.38	4.55 ± 0.63	2.35 ± 0.28
12–16	13	4.26 ± 0.28	5.68 ± 0.35	1.15 ± 0.14
17–19	7	4.43 ± 0.28	4.42 ± 0.35	0.43 ± 0.037

[a] Expressed as cpm localized per gram of tissue divided by 1% of the counts remaining in the inoculum 24 hr after injection.

TABLE III. Concentrations of [^{3}H] Thymidine in Whole Embryos and Associated Maternal Tissue.

Age of embryo (days)	No./time point	cpm/μg protein (mean $\pm$ SE)
6	5	283.75 $\pm$ 40.87
7–8	6	74.12 $\pm$ 16.11
11–12	10	31.29 $\pm$ 5.55
13–15	13	30.45 $\pm$ 3.13
16	5	44.60 $\pm$ 3.67
17–18	15	3.62 $\pm$ 0.66

11 in murine embryogenesis are characterized by rapid organogenesis (13). The uptake of ^{67}Ga in the embryonic tissues at other time periods ranged from 4 to 8%, which is comparable to levels in normal adult mouse tissues (3). Autoradiograms of embryonic and decidual tissues from mice injected with ^{67}Ga revealed a marked concentration of the isotope in the limb buds, cartilaginous precursors of skeletal structures, the decidua, and especially in the fetal membranes at Days 9–10 of gestation. The localization in the cartilaginous precursors was not surprising, as it is well documented that gallium is a bone-seeking element. That isotope incorporation into embryonic bone is not responsible for the peak concentration of ^{67}Ga at Day 9 of gestation is indicated by the drop in total uptake by Day 12 in spite of continual uptake into bone, as revealed by autoradiographic examination. Intestinal crypt cells showed no ^{67}Ga localization, indicating that the ^{67}Ga concentration in fetal membranes and in decidual tissue is not a reflection of rapid cell turnover but more likely is associated with some characteristics of embryonic development and growth. The uptake of [^{3}H]TdR in embryonic or decidual tissue at 6 days was marked, but declined progressively during the gestation period. However, the intestinal crypt cells showed a marked concentration of [^{3}H]TdR, which again indicates that uptake of ^{67}Ga as opposed to [^{3}H]TdR is not a reflection of cellular division. It is interesting to note in this regard that a recent study by Fogh (14) indicated rather marked localization of ^{67}Ga in the mammary tissue of pregnant women.

The only obvious alteration in the localization of ^{67}Ga in the tissues of pregnant mice compared to tissues from virgin mice was the progressive decline in uptake by the thymus throughout the gestation period. The decline appears to be somewhat selective, since neither the weight nor the histologic appearance of the thymus during various times of gestation indicated any obvious differences between pregnant and virgin mice. Previous studies of normal and leukemic thymus tissue of AKR mice (12) have indicated that ^{67}Ga is concentrated in phagocytic cells. Whether the pattern of ^{67}Ga uptake in the thymus of pregnant mice indicates loss of phagocytic cells remains to be determined.

Thus, just as leukemic and pregnant mice respond with identical alterations in serum protein profiles, they also selectively concentrate the isotope ^{67}Ga into leukemic and embryonic tissues, respectively. This observation extends the previously reported similarities between embryogenesis and leukemogenesis. In addition, the present results suggest, as do the results of Fogh (14), that the study of ^{67}Ga localization during the course of embryogenesis may prove to be useful for determining the mechanism and significance of ^{67}Ga localization in other systems.

Summary. Isotope uptake in embryos and tissues of pregnant mice was determined 24 hr after intravenous inoculation of pregnant mice with either ^{67}Ga or [^{3}H]TdR. ^{67}Ga concentration in embryos and associated maternal tissue at 6–8 days was comparable to values reported previously in normal adult mouse tissues, whereas in embryos at 9–11 days, it equaled or surpassed concentrations reported in leukemic tissues of adult AKR and BALB/c mice. By Day 13, ^{67}Ga counts were diminished in the embryos. The ^{67}Ga concentration in the thymus of pregnant mice decreased progressively with increasing gestation time, as did [^{3}H]TdR uptake in embryonic tissue. Autoradiograms revealed that the ^{67}Ga localized in fetal membranes, limb buds, and vertebrae of the embryos and in the decidua. Conversely, embryos and decidua labeled with [^{3}H]TdR indicated no such selectivity. ^{67}Ga did not localize in crypt cells of the intestinal tract, whereas [^{3}H]TdR did. Therefore, incorporation of ^{67}Ga in the fetal tissues and decidua did not appear to be related to DNA synthesis. In addition,

^{67}Ga localization during embryogenesis may prove to be a useful model system for determining the basis and general significance of ^{67}Ga localization in nonosseous tissues.

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