In Vivo Effects of Iron-55 and Iron-59 on Mouse Testes: Biophysical Dosimetry of Auger Electrons

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When the Auger-electron emitter, ^{55}Fe, and the beta-emitter, ^{59}Fe, are similarly distributed in the testes of mice, the conventionally calculated average radiation dose to the organ from ^{55}Fe is about 2.6 times more effective in reducing the sperm-head count than the dose from ^{59}Fe. This finding emphasizes the ability of low-energy Auger electrons to damage radiosensitive targets of cells through localized irradiation. The observed efficacy is understandable in terms of dosimetric models based on intracellular distribution studies of the radionuclides.

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Nuclear decays by orbital electron capture (EC) and internal conversion (IC) entail vacancies in the inner electron shells of the residual atoms. The complex series of atomic vacancy cascades that follow are dominated by nonradiative Auger and Coster-Kronig (CK) processes (*1–3*). As a result, numerous electrons are ejected from the daughter atoms. Several of these "Auger" electrons have very low energies and extremely short ranges (a few microns or less) in biological matter (*2,4–6*). When such radionuclides are incorporated into cells, these Auger electrons may locally and selectively irradiate radiosensitive targets in the cells. The biological consequences, of interest to basic radiation biology and to diagnostic and therapeutic nuclear medicine, cannot be predicted *a priori* because of their strong dependence on the intracellular localization and distribution of the radionuclides and on the microscopic patterns of energy deposition by the Auger electrons. In view of this limitation on our current understanding, we have been engaged in an extended program of research on the radiotoxic effects of tissue-incorporated Auger-electron emitters in vivo using spermatogenesis in mice as the experimental model (*5,7–9*).

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The overall objectives of these studies are (a) investigation of the effectiveness of Auger-electron emitters localized in the testes of mice in reducing the number of sperm heads relative to beta emitters distributed similarly in the organ, (b) determination of the intracellular distribution of the radionuclides, and (c) development of biophysically meaningful models for the dosimetry of Auger-electron emitters in vivo.

The present work is a study of the radiobiological effects of the EC decay of iron-55 (^{55}Fe) (*10*) and the beta decay of ^{59}Fe (*10*) following intratesticular (i.t.) administration of the radionuclides. Our results show that the low-energy Auger-electron and x-ray dose to the testis from ^{55}Fe is about 2.6 times more efficient in killing the sperm cells in comparison with the beta and gamma radiation dose from ^{59}Fe, when the average radiation doses to the organ are calculated using the principles of conventional dosimetry (*11–12*). This finding compares well with our earlier report (*5*) that the average testicular dose from the prolific Auger-electron emitter, thallium-201 (^{201}Tl), is about 3.3 times more effective than the dose from the energetic beta rays from ^{204}Tl in causing the same biological effects. As in the case of ^{201}Tl (*5*), the observed efficacy of ^{55}Fe may be explained in terms of an enhancement of the radiation dose rate to the spermatogonial cells over the conventional dose rate estimates (*11–12*) by virtue of the intracellular localization of the Auger-

electron emitter. Such an enhancement of radiation dose rate to the entire cell depends on two parameters, namely, the ratio (n) of the radionuclide concentration within the cells of interest to its concentration in the extracellular medium, and the fraction (f_c) of the total volume of the organ occupied by the cells that have concentrated the radionuclide (*5–6*). The present investigation with ^{55}Fe supports such a parametrization of the cellular dosimetry of Auger-electron emitters. The diffusible nature of $^{201}Tl^+$ radioactive ions precluded determination of its intracellular distribution (*6*). In this work, we have studied the intracellular distribution of the radionuclides of iron. These data have permitted us to extend the dosimetry of Auger electrons to the nucleus of the cell containing the radiosensitive DNA. The observed efficacy of ^{55}Fe is adequately explained in terms of enhanced radiation dose rate to the nucleus of the cell.

EXPERIMENTAL MODEL, MATERIALS, AND METHODS

Experimental model

Spermatogenesis in mice is an experimental model relevant to man (*5,13,14*). The mouse spermatogenic cycle and the radiosensitivities of the different types of cells are discussed in the literature [see, e.g., Fig. 2 in Ref. (*8*)]. The differentiated spermatogonia are the most sensitive to ionizing radiation. The spermatogonial stem cells as well as the postgonial cells are relatively more radioresistant (*8,15*). Because of this differential radiosensitivity, any damage done to the differentiated spermatogonia by radiation can be seen as reduced sperm-head population if counted after the time required by the differentiated spermatogonia to become sperm (elongated spermatids in stages 12–16 (*8,15*) of development). Thus spermatogenesis serves as an effective model to study radiation effects in vivo.

Certain criteria for the validity of the model have been pointed out (*5,7–9*). The biological effects (loss of testicular weight and decrease in sperm-head count) must be determined at a time when the sperm-head count reaches its minimum following the initial exposure to radiation. This elapsed time interval may depend on the strain of the mice as well as on the type of radiation exposure. For an acute exposure to external gamma radiation, and for testes-incorporated radionuclides with very short effective half-lives (a few hours), the sperm-head count reaches the minimum on the 29th day after the initial exposure (*5,7,8*). For radionuclides with long effective half-lives in the testes, the necessary time interval may be quite different (*8,9*), and it must be determined experimentally for each case.

As in our ^{201}Tl work (*5*), the radionuclides are directly introduced into the testes. The advantages of the i.t. mode over other modes of administration of the radionuclides have been pointed out (*5*).

Materials and methods: Experimental animals and radionuclides

Male Swiss Webster mice, 9–10 wk old and weighing about 30 g each, are used in this work. The Auger-electron emitter in this study is ^{55}Fe, which decays to the ground state of ^{55}Mn entirely by the EC mode with a half-life of 2.7 yr (*10*). This radionuclide is of interest for the following reasons.

1. Iron-55 is one of the lightest Auger-electron emitters of biomedical interest in contrast to the heavy ^{201}Tl. The number of Auger electrons following ^{55}Fe decay (see Table 1) is much smaller than in the case of ^{201}Tl (*5,6*). A comparison of the radiobiological effects of the two radionuclides is therefore of basic scientific interest to Auger-electron dosimetry.

2. Iron-55 is a significant by-product of nuclear detonations (*4,16*) and it stays in the biosphere with its long physical half-life. It is also produced by neutron activation in reactors (*16*). Since iron is a biologically essential element, the radioiron distributed in the body pools of stable iron can irradiate blood forming cells (*4*). The effects of ^{55}Fe on spermatogenesis have not been reported.

Another radionuclide of interest to this study is ^{59}Fe, which decays by beta-emission with a half-life of 44.6 days (*10*). Although it is also produced in nuclear detonations and by neutron activation in reactors (*16*), it is not of as much concern environmentally because of its fairly short half-life compared with ^{55}Fe. Nevertheless, ^{59}Fe is widely used in biomedical research and its biological effects on spermatogenesis are of interest. Since both ^{55}Fe and ^{59}Fe should be distributed very similarly in the testis, it is possible to compare directly the biological effects of the low-energy Auger electrons and x-rays from ^{55}Fe decay (Table 1) with those due to the beta and gamma rays (*10*) from ^{59}Fe decay. The radioiron nuclides were obtained* as [^{55}Fe] citrate (specific activity 10.9 mCi/mg or 0.4 GBq/mg) and ^{59}Fe citrate (specific activity 6.6–9.7 mCi/mg or 0.24–0.36 GBq/mg). The radioactive concentrations of the ferrous citrate solutions were adjusted so that a constant volume dose of 3 μl could be maintained

TABLE 1
Theoretical X-Ray, Auger (A), and CK Electron Spectrum Following EC Decay of ^{55}Fe

No.	Group/ transition	Yield per 100 decays	Average energy (keV)	Range (μm) in unit density matter (*19*)
1	K x-rays	26.5	5.95	—
2	K_A	62.0	5.19	0.90
3	L_A	145	0.63	0.033
4	$L_{1\,CK}$	15	0.099	0.0055
5	$L_1, M_{1\,CK}$	21	0.060	0.004
6	$L_{1\,CK}$	7	0.038	0.0025
7	$M_{2,3\,CK}$	172	0.028	0.0017
8	$M_{1,2,3\,CK}$	73	0.017	<0.001
9	$L_1, M_{1\,CK}$	12	0.008	<0.001

for the various amounts of radioactivity injected into the testes of mice.

Using a microliter syringe, the desired activity of ^{55}Fe or ^{59}Fe was injected into the right testes of the mice following a minor surgical procedure (*5*). During the injection, the needle was slowly moved along the long axis of the testis to facilitate a fairly even distribution of the solution in the organ. Mice that were left untouched or injected with normal saline or appropriate amounts of stable ferrous citrate served as controls.

Biological clearance of the radionuclides

The rates of biological elimination of both the radioiron nuclides were determined following i.t. injection 1–3 μCi (0.037 - 0.11 MBq) of ^{55}Fe (specific activity 6.6 mCi/mg or 0.24 GBq/mg) and ^{59}Fe (specific activity 7.6 mCi/mg or 0.29 GBq/mg). Studies were also performed as a function of the amount of stable iron present in the injected radioactive doses. Following i.t. injection of the same amount of radioactivity into several mice, the animals were killed in groups of four to five at various times ranging from 0.25 hr to 34 days postinjection, and the injected testes removed. The remaining ^{59}Fe activity in the testes was assayed by gamma-ray spectroscopy using a NaI detector with a well 5 cm deep and 1.27 cm in diameter. The gamma rays were accepted in a 0.34–2.04 MeV window. All samples were counted under the same geometry. The detector efficiency was determined using standard sources traceable to the National Bureau of Standards. Detection of ^{55}Fe cannot be done easily through standard gamma ray spectroscopy because of the low energy of the x-ray (5.95 keV, see Table 1). Liquid scintillation spectroscopy was therefore used for ^{55}Fe assay. The injected testes were placed in counting vials with 1.0 ml of quaternary ammonium hydroxide and maintained at 50°C for several hours for complete digestion of the testes. The solution was bleached with several drops of 30% hydrogen peroxide and then 15 ml of PCS† was added for counting. The samples were counted using a preset tritium window, which is compatible with the energies of radiations from ^{55}Fe decay (Table 1). Corrections for sample quenching were obtained by the External Standard Ratio technique using counting standards prepared by injecting excised testes with known amounts of ^{55}Fe and preparing them for counting as described above. Experimental samples were also randomly checked for quenching using internal standardization. The fraction of ^{55}Fe or ^{59}Fe radioactivity retained in the testes was determined by comparing the measured activity in each sample against the activity in an injection standard prepared for each group.

Spermatid survival assay

The postinjection time required for the spermatid population to attain the minimum value was determined for ^{55}Fe as well as ^{59}Fe. For this purpose, the mice were initially given i.t. injections with 1.0 μCi (0.037 MBq) of ^{59}Fe or 30 μCi (1.1 MBq) of ^{55}Fe in the standard 3 μl injection volume. These mice were killed in groups of five at various times over a 60 day postinjection period. At each elapsed time interval, the injected testes were removed and weighed. They were then homogenized for 5 sec in 1 ml of deionized water, and sonicated for 30 sec to disrupt all structures except the sperm heads, which are resistant to sonication. The sperm heads were then counted under a light microscope in a hemocytometer to a minimum of 200. These studies showed that the minimum sperm-head count was reached on the 36th day postinjection (see Fig. 2). The biological effects sought were assayed accordingly on the 36th day following i.t. injection of various amounts of ^{55}Fe and ^{59}Fe into the mice.

Radionuclide distribution

The macroscopic distribution of the radionuclides was studied to verify whether the injected dose was spread over a large enough volume so that a sufficient number of the seminiferous tubules were exposed to the radionuclides. A group of mice were given i.t. injections containing 0.8 μCi (0.03 MBq) of ^{59}Fe. The animals were killed 1 day and 4 days later and the injected testes were removed and assayed for ^{59}Fe activity. The testes were then frozen and sliced into 10 to 12 sections each. The sections were weighed and the radioactivity in each was assayed. The data thus obtained on the radioactivity per gram in these slices disclosed that 82% of the retained activity after 1 day was distributed over 67% of the volume of the organ. This initial distribution became more even (81% of activity in 79% of the volume) 4 days later, presumably due to the slow diffusion of the radionuclides over this time scale. In view of this and the long residence time of the radionuclides in the organ (Fig. 1), the observed damage may be regarded as an average effect over the whole organ.

The localization of ^{55}Fe in the organ at the microscopic level was investigated through frozen section autoradiography. These studies were performed on samples prepared 24 hr after i.t. injection at 1.0 μCi (0.037 MBq) of ^{55}Fe using procedures described by Rao et al. (*5*).

As an aid to interpret the results of this work in terms of dosimetric models suitable for Auger-electron emitters, it is necessary to examine whether the radionuclides are concentrated by the cells, and, if so, how the radionuclides are distributed inside the cell. In vitro studies provide qualitative guidance in this regard (*5,6*). The uptakes of ^{55}Fe and ^{59}Fe by Chinese hamster ovary cells (CHOK1) were determined by incubating them for 24 hr at 37°C with 1.0 μCi (0.037 MBq/ml) of ^{55}Fe or 0.5 μCi (0.018 MBq/ml) of ^{59}Fe in the culture medium. The cell nuclei were isolated by a modification of the method of Fraser and Huberman (*16,17*), and the cytoplasmic and nu-

clear fractions of radioactivity determined by liquid scintillation spectroscopy.

The cytoplasmic and nuclear contents of cells in vivo were obtained as follows. One day after i.t. injection of 1.0 μCi (0.037 MBq) of ^{55}Fe, the mouse testes were removed and placed in ice-cold sucrose-TKM buffer with 0.32*M* sucrose, 50 m*M* Tris-HCl (pH 7.5), 25 mM KCl, 5 m*M* $MgCl_2 \cdot 6\ H_2O$, and 1 m*M* phenylmethyl sulfonyl fluoride.‡ The fibrous connective tissue covering the testes was carefully removed. Cells were prepared by gentle squashing of the testes on a brass screen (1 sq mm) and washing the component cells through the screen with TKM buffer. The cells were homogenized in 1 ml of the above buffer in a 7-ml glass homogenizer with a tight fitting Teflon pestle. The homogenate was filtered through four layers of gauze and the filtrate was homogenized with an additional four strokes. Each homogenate was mixed with an equal volume of 20% ficoll (400,000 mol wt)‡ prepared in TKM buffer, then layered over 0.5 ml of 20% ficoll in polyallomer tubes and centrifuged in a Beckman SW60 rotor at 10,000 rpm for 15 min. The supernatant was removed using a Pasteur pipet and the nuclear pellet resuspended in 10 m*M* Tris-HCl pH 7.5. An aliquot of the suspension was removed and the nuclear material solubilized in Ready Solve MP (multipurpose premixed liquid scintillation cocktail for aqueous samples) to estimate the total radioactivity incorporated into the nuclear components. Total proteins in the nuclei were extracted in 2*M* NaCl containing 5*M* urea followed by high speed centifugation to pellet the DNA. Protein and DNA samples were solubilized as stated above and the total radioactivity determined in each of these nuclear components. The cytoplasmic content of radioactivity was obtained from an assay of the supernatant.

RESULTS AND DISCUSSION

Testicular clearance

Figure 1 shows the data on the biological clearance of ^{59}Fe from the testis. The corresponding data for ^{55}Fe are essentially the same and they are not shown. Two components are revealed by these studies. Eighty-five percent of the initial activity was eliminated very quickly from the organ with a 10-min biological half-life, while the rest remained in the testis with a biological half-life of 28.0 days. These clearance rates did not depend on the amount of stable iron present in the injected dose. In contrast with the radioiron nuclides, both ^{201}Tl and ^{204}Tl were eliminated quickly from the testis with a 9-hr half-life for the major component in spite of their very disparate physical half-lives (*5*). The effective half-lives for ^{55}Fe are 6.9 × 10^{-3} days, and 27.2 days for the 85% quickly cleared component, and for the slowly cleared 15% component, respectively. The corresponding values for ^{59}Fe are 6.9 × 10^{-3} days and 17.2 days.

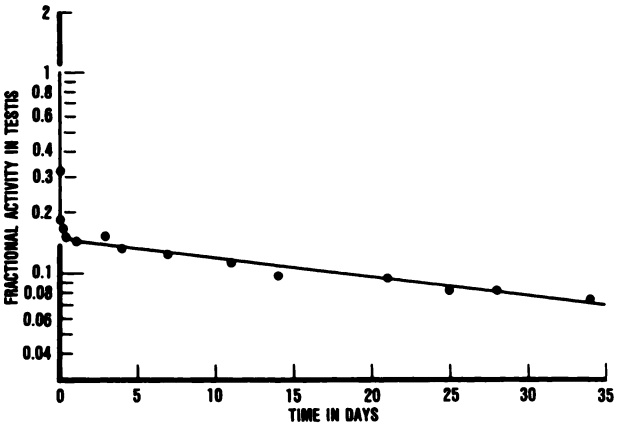

FIGURE 1
Biological clearance of intratesticularly administered ^{59}Fe. Semi-log plot shows fraction of initial activity remaining in testis as function of time

Radiations from ^{55}Fe and ^{59}Fe decay

Available radiation data (*10*) on ^{55}Fe are incomplete in that information on very low-energy CK electrons of interest to local dosimetry of ^{55}Fe is not included. We have theoretically calculated the complete spectrum of radiations following the EC decay of ^{55}Fe. Table 1 is a summary of the complex spectrum. For this calculation, the average vacancy distribution in the various electron shells of the manganese (^{55}Mn) daughter atom per 100 EC decays of ^{55}Fe is obtained using the theoretical expressions for EC probabilities (*10*), the experimental electron binding energies (*20*), and a Q value of 231 keV (*10*) for the EC decay. The primary inner-shell vacancies created are in the ratio.

$$K/L_1/L_2/M_1 = 88.5/9.8/0.04/1.6$$

This information and the calculational approach (including references to data on atomic transitions) recently presented by Kassis et al. (*21*) are utilized in estimating the spectrum in Table 1. Our results on K x-rays, and K and L Auger electrons compare well with available information (*10*). The ranges of the various electron groups given in the last column of Table 1 are obtained by interpolation of Cole's experimental data on ranges of electrons in unit density matter (Ref. (*19*), Table III).

Complete data on the radiations from ^{59}Fe decay are given by Martin and Blichert-Toft (*10*). Two major beta groups occur with maximum energies of 0.457 MeV and 0.274 MeV, the respective yields being 53% and 46%. The corresponding average beta energies are 0.150 MeV and 0.081 MeV, and the average 90% ranges in water are 0.57 and 0.24 mm (*10*). These ranges correspond to radii of spheres of water, centered about a point source, in which 90% of the average beta energy is absorbed (*22*). These estimates (*10*) are based on Berger's approach (*22*).The gamma-photon energies (yields) are 1.099 MeV (56%) and 1.292 MeV (44%), respectively (*10*).

Dosimetric considerations

The mouse testis is nearly spherical with an average radius of about 3 mm and a mass of 0.1 g. The photon energy absorption coefficients (*23*) in water are 0.031 cm^{-1}, 0.029 cm^{-1}, and 23.0 cm^{-1} for the 1.099 MeV and 1.292 MeV ^{59}Fe photons, and the 5.95 keV ^{55}/Mn K x-rays, respectively. The respective absorbed fractions for the testis, assumed to be a sphere of unit density, are 7.2 $\times 10^{-3}$, 6.6 $\times 10^{-3}$, and 0.889. These are calculated for uniform distribution of the radionuclides in the organ using the formulation of Powsner and Raeside (*24*). The average absorbed fraction is 0.97 for the 0.274 MeV beta group, and 0.92 for the more energetic one. These estimates are based on the work of Loevinger et al. (*25*). Since the beta ranges are much larger than typical cell diameters (~ 10 μm) and much less than the size of the testis, ^{59}Fe beta rays emitted in the organ should be expected to irradiate the organ quite uniformly, on the average. In contrast, the Auger and CK electrons of subcellular ranges (Table 1) from ^{55}Fe decay can irradiate intercellular organelles only when ^{55}Fe is localized in the cells. The absorbed fractions for these electrons in the testis are unity.

Since the biological effects are usually parametrized in terms of the average cumulated dose to the organ (*11,12*), the average doses $\bar{D}$ from intratesticular ^{55}Fe and ^{59}Fe are calculated using the relation

$$\bar{D} = 73.8\,(A_O/m)(\Sigma f_i \bar{E}_i \phi_i)\,[\Sigma f_j T_{je}\,(1 - e^{-(\ln 2)t/T_{je}})]. \quad (1)$$

This equation is readily obtained by replacing the instantaneous activity in the conventional expression for the dose rate to the organ [see, e.g., Ref. (*2*); Eq. (1)] with the cumulated activity over the period of time t. The parameters in Eq. (1) for $\bar{D}$ in rad (0.01 Gy) are defined below:

- A_0 = initially injected radioactivity in μCi (0.037 MBq);
- m = mass of the testis=0.1 g;
- $\bar{E}_i$ = average energy of the ith radiation in MeV;
- f_i = average yield of the ith radiation per decay;
- ϕ_i = absorbed fraction for the ith radiation emitted and absorbed in the organ;
- f_j = fraction of the jth component in the clearance curve (j = 1,2);
- T_{je} = effective half-life for the jth fraction for the respective radionuclide in days; and
- t = the time in days for which spermatogonial cells are exposed to the radionuclides from the time of the injection.

Because of the short physical half-life (6 hr) of technetium-99m, and the short effective half-lives (about 9 hr) of the radiothallium isotopes in the testis, the testicular doses were delivered essentially in the first 24 hr (*5,7,8*) in these cases. In view of the long residence time of the 15% component of the radioirons in the testis and the protracted irradiation, the radiation dose to the organ has to be calculated over a time scale consistent with the spermatogenic cycle (*8*). The fact that A_1 spermatogonia take 7 days (*8*) to pass through the stage B suggests calculation of absorbed dose for the first 7-day period. It is desirable, however, to calculate the dose for the first 13-day period. This allows for the exposure of new spermatogonia entering the cycle from the A_{a1} stage preceding the A_1 stage (*8*). Mian et al. (*9*) adopted the same approach in their studies on ^{32}p in mouse testes. Accordingly, the cumulated testicular doses from ^{55}Fe and ^{59}Fe are calculated for the 13-day period postinjection. This dose is 92 rad/μCi (25 Gy/MBq) in the case ^{59}Fe, about 6% of which is from the penetrating gamma rays. The 13-day dose from ^{55}Fe is 4.8 rad/μCi (1.3 Gy/MBq), 25% of which is due to the 5.95 keV x-ray photons.

Biological effects

Sperm-head survival is a more sensitive biological indicator of radiation effects than loss of testicular weight. Accordingly, we present our data on sperm-head reduction only. Data in Fig. 2 show that the minimum sperm-head count is reached on the 36th day following the i.t. injection of ^{55}Fe or ^{59}Fe. The results of sperm-head survival assay performed on this day are shown in Fig. 3 as a function of the average calculated dose to the testis. The surviving fraction S as a function of the dose $\bar{D}$ (in Gy) is fitted by a two-component exponential function:

$$S = a_1 e^{-\bar{D}/D_{01}} + a_2 e^{-\bar{D}/D_{02}}. \quad (2)$$

In Eq. (2), $a_1 = 0.3$ and $a_2 = 0.7$ for both radionuclides;

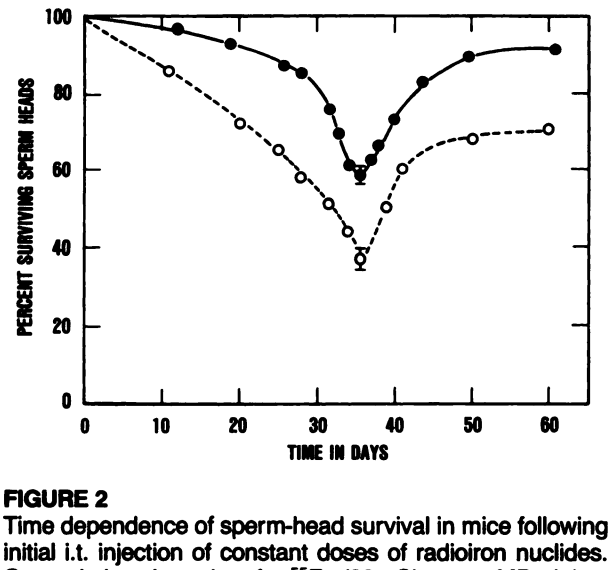

FIGURE 2
Time dependence of sperm-head survival in mice following initial i.t. injection of constant doses of radioiron nuclides. Open circles show data for ^{55}Fe (30 μCi = 1.1 MBq injection dose), and solid circles represent ^{59}Fe data (1 μCi = 0.037 MBq injection dose). Surviving fractions given for each day of assay are obtained in comparison with average sperm-head count in testes of unexposed mice. Minimum sperm-head count is reached 36 days postinjection for both radionuclides. Error bars indicated represent standard deviations of mean values

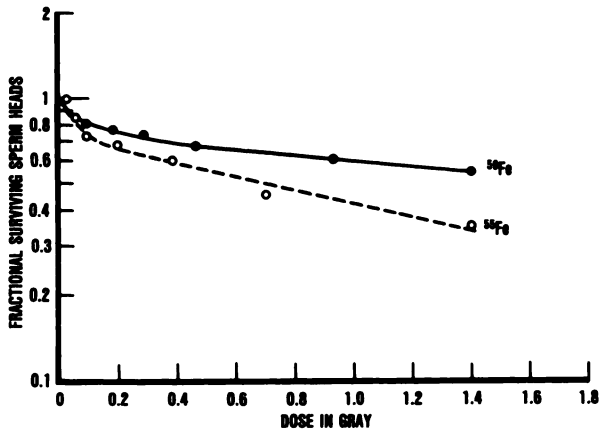

FIGURE 3
Sperm-head survival vs. calculated average radiation dose to testis following i.t. administration of varying amounts of ^{55}Fe or ^{59}Fe radioactivity

D_{01} = 0.09 Gy, and D_{02} = 2.0 Gy for ^{55}Fe; D_{01} = 0.12 Gy with D_{02} = 6.2 Gy for ^{59}Fe. The efficacy of the dose from the low-energy radiations (75% from the Auger and CK electrons) of ^{55}Fe compared to the dose from ^{59}Fe (mostly from the energetic beta rays) may be noted. The "relative biological effectiveness" (RBE) of ^{55}Fe *compared* to ^{59}Fe, calculated from the D_0 values, is 1.3 and 3.1, respectively, for the two components. The weighted average value is 2.6, a_1 and a_2 being the weight factors. It is interesting that the two-component nature of the sperm-head survival curve found by Rao et al. (*5*) in the ^{201}Tl work is also reproduced here. Plausible reasons for this were discussed (*5*) in that paper. Very recent studies in this laboratory show similar response when the testes of the same strain of mice are uniformly exposed to various doses of external x-rays (Rao DV, et al., unpublished data). This indicates that the two-component response observed with the radionuclides may not be the result of artifacts.

Autoradiography

Fig. 4 shows an example of the pattern of localization of ^{55}Fe. Over 20,000 grains were counted under a microscope in numerous tubules in random sections and fields. Statistically, about 75% of the grains in the tubule were found in the basal 10 μm region containing the spermatogonial cells. While these results qualitatively suggest that the spermatogonial cells in vivo might have concentrated the radionuclide, they are not adequate for a quantitative interpretation of the observed efficacy of ^{55}Fe.

Intracellular distribution

At the end of the 24-hr incubation, the CHOK1 cells in vitro concentrated the radioiron nuclides about 20-fold over the radionuclide concentration in the extracellular medium. About 22% of the cellular radioactivity was localized in the nucleus, and the rest in the cytoplasm. Nuclear content of the radioactivity in the testicular cells in vivo was found to be about 15% of the cell content, divided equally between the DNA and the nucleoproteins. This information pertains to all the cells in the organ that might have taken up the radionuclide. Hence it may be considered as an upper limit on the nuclear fraction of the radioactivity in the spermatogonial cells.

RBE value for ^{55}Fe and cellular dosimetry

A plausible explanation for the experimentally obtained RBE value of 2.6 for ^{55}Fe is that the spermatogonial cells and their radiosensitive organelles have effectively received an average radiation dose which is 2.6 times the average dose to the organ, calculated according to the conventional dosimetry of tissue-incorporated radionuclides. We briefly consider below the extent of the validity and limitations of this approach. Improved dosimetric models, which account for such a dose enhancement, are presented.

Conventional dosimetry

This is based on the simplifying assumption that the radionuclides and the radiation energy are uniformly distributed throughout the organ. It is implicitly assumed here that the average dose rate and the cumulated dose to the cell and its nucleus are the same as the average values calculated for the organ as a whole (*11,12*). Let V be the volume of the organ containing A μCi of radioactivity at any time. The average instantaneous rate of energy deposition in the cell (nucleus) per second is given by the product of the volume of the cell (nucleus) and the average density of energy absorbed per second in the organ at that instant. For the spermatogonial cell of volume v_c, this conventional dose rate $(\bar{R}_c)_{CON}$ is given by

$$(\bar{R}_c)_{CON} = (AN_o/V)(\Sigma n_i \bar{E}_i \phi_i)v_c, \qquad (3.1)$$

N_o being the number of disintegrations per μCi. For ^{55}Fe in the testis, the average electron energy $\bar{\epsilon}_e = \Sigma n_i \bar{E}_i$ = 4.22 keV released per decay (Table 1) is also absorbed in the organ (ϕ_i = 1), the average x-ray energy deposited being $\bar{\epsilon}_x = n_x \bar{E}_x \phi_x$ = 1.40 keV with ϕ_x = 0.889. The ratio $\Delta = \bar{\epsilon}_x/\bar{\epsilon}_e$ = 0.332. In terms of these, the conventional dose rate to the cell from ^{55}Fe may be written as

$$(\bar{R}_c)_{CON} = (AN_o/V)\bar{\epsilon}_e (1 + \Delta)v_c. \qquad (3.2)$$

The possibility that the cells may concentrate the radionuclides is ignored in the conventional approach. In spite of this, the average distribution of radiation energy in the organ may be considered to be reasonably uniform when radionuclides, such as ^{59}Fe, emit energetic radiations with ranges or mean free paths much larger than the cell dimensions (*2,5,6*). Conventional dosimetry therefore serves as an adequate approximation in the estimation of radiation dose rates, Eq. (3.1), and cumulated doses, Eq. (1), to the cells and their nuclei when such radionuclides are involved. The basic limitation in the case of Auger-

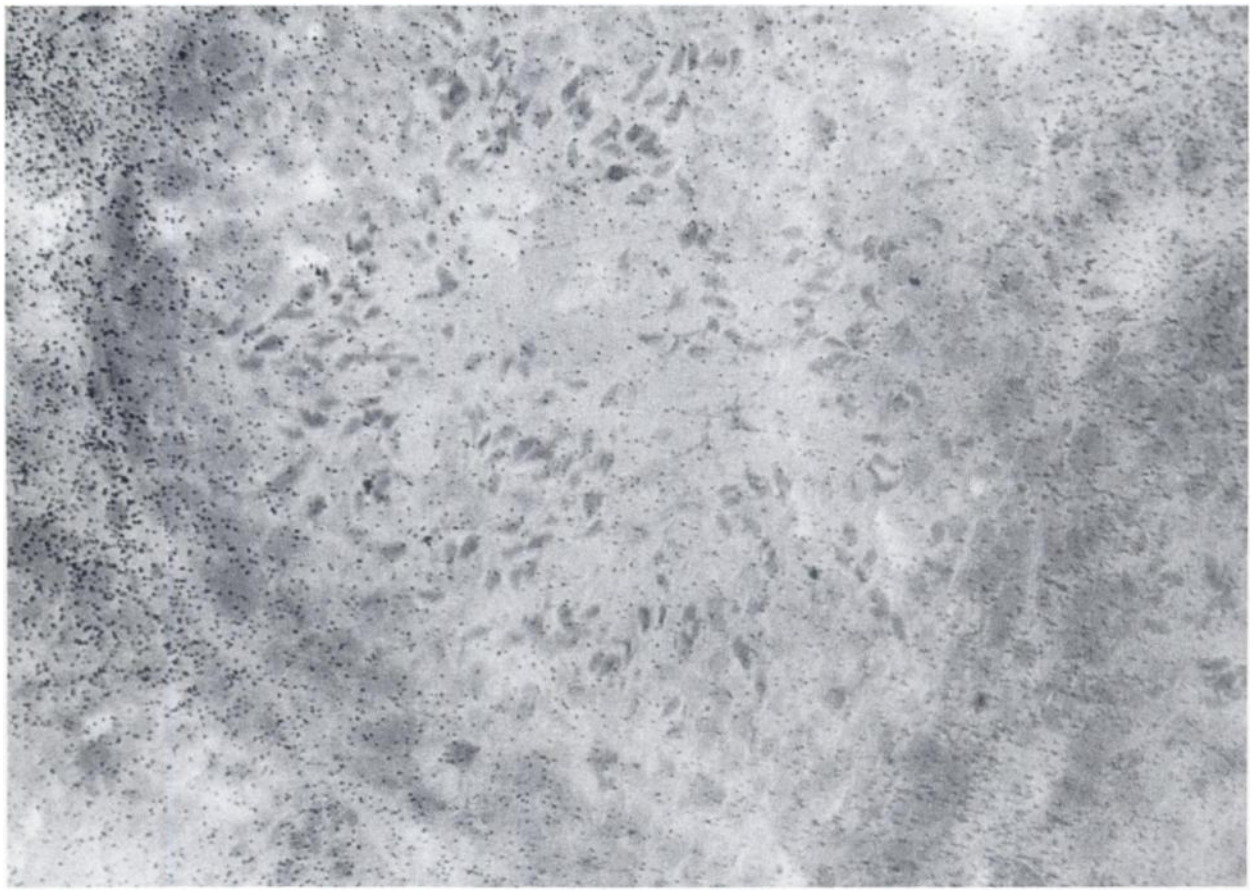

FIGURE 4
Example of autoradiogram of seminiferous tubule (X500) of mouse testis 24 hr after i.t. administration of ^{55}Fe citrate

electron emitters is that this approach does not consider the possibility of localized irradiation of intracellular targets by Auger electrons from decays within the cell. Consequently, conventional dosimetry may underestimate the biological risks of tissue-incorporated Auger-electron emitters. The ^{201}Tl studies in vivo (*5*) and experiments in vitro with ^{201}Tl (*6*) and chromium-51 (^{51}Cr), for example, have emphasized this point. We therefore consider ^{55}Fe dosimetry in vivo taking its intracellular localization into account.

Intracellular concentration: effect on cellular dose rate

The spermatogonial cells and their nuclei are almost spherical and have essentially the same dimensions both in mouse and human testes, with an average cell diameter $d_c = 12\ \mu m$, and a nuclear diameter $d_N = 6\ \mu m$ *(26,27)*. We assume that they are also of unit density. Let C_C be the average concentration (μCi/ml) of ^{55}Fe radioactivity in the spermatogonial cell at any instant, and C_e the average concentration of the radioactivity in the organ external to the spermatogonial cells. Then $C_c = nC_e$, n being the intracellular concentration factor. Let the total volume occupied by these cells be a fraction f_c of the volume of the entire organ. The average instantaneous dose rate $\bar{R}_c$, to the spermatogonial cell consists of two terms: $(\bar{R}_c)_{int}$ from ^{55}Fe decays occurring inside the *same* cell, and $(\bar{R}_c)_{ext}$ from decays taking place external to the cell. Auger electrons from decays inside the cell contribute to $(\bar{R}_c)_{int}$. The x-ray photons from such decays contribute negligibly to $(\bar{R}_c)_{int}$ in spite of the concentration of the radionuclide in the cell since the mean free path (~ 0.04 cm) *(23)* is much larger than the cell diameter (12 μm). Because of their very short ranges (Table 1), Auger electrons from decays external to the cell cannot reach the target cell. Hence, the sole contribution to $(\bar{R}_c)_{ext}$ stems from the penetrating x-ray photons from decays in the whole organ, and it is given by the conventional dosimetry

$$(\bar{R}_c)_{ext} = (AN_o/V)v_c\bar{\epsilon}_x. \qquad (3.3)$$

The quantity $(\bar{R}_c)_{int}$ is the product of the number of ^{55}Fe decays occurring per second within the cell and $\bar{\epsilon}_{ce}$, the average energy deposited in the cell by the Auger electrons emitted per decay in the same cell. This energy $\bar{\epsilon}_{ce} = \phi_{cc}\bar{\epsilon}_e$, ϕ_{cc} being the self-absorbed fraction of the Auger electron energy $\bar{\epsilon}_e$ emitted per decay in the cell. The average number of decays in the cell per second is $N_oC_cv_c$. Using the expression developed by Rao et al. (*5*) for C_c

(see Eq. (6.2), Ref. (5)), we get

$$(\bar{R}_c)_{int} = (AN_o/V)v_c(\phi_{cc}\bar{\epsilon}_e)n/[f_c(n-1) + 1] \quad (3.4)$$

Addition of Eq. (3.3) and (3.4) gives

$$\bar{R}_c = (AN_o/V)(v_c\bar{\epsilon}_e)\{(n\phi_{cc})[f_c(n-1) + 1]^{-1} + \Delta\}. \quad (3.5)$$

The cellular dose rate enhancement factor, $N_c = \bar{R}_c/(\bar{R}_c)_{CON}$, is obtained from Eq. (3.5) and (3.2) as

$$N_c = \{(n\phi_{cc})[f_c(n-1) + 1]^{-1} + \Delta\}(1 + \Delta)^{-1} \quad (3.6)$$

The absorbed fraction ϕ_{cc} readily calculated for uniform distribution of radioactivity in spherical cells using Eq. (3), Ref. (*6*), and the details given therein. For the spermatogonial cells with a diameter $d_c = 12\ \mu m$, we obtain $\phi_{cc} = 0.947$ for the ^{55}Fe electron data in Table 1. For cell diameters 4, 6, 8, and 10 μm, our calculated values of ϕ_{cc} are 0.84, 0.89, 0.92, and 0.94, respectively. Thus ϕ_{cc} is weakly dependent on the cell size. Deviation from the assumption of spherical geometry does not alter the value of this parameter significantly.

The cellular dose rate enhancement factors, N_c, calculated from Eq. (3.6) with the values of ϕ_{cc}, and Δ given above for ^{55}Fe, reveal several interesting features.

1. Assuming a close-packed geometry for the seminiferous tubules in mouse testes, Green et al. (*28*) estimated that the maximum possible volume occupied by the spermatogonial cells constitutes 17% of the volume of the organ. Rao et al. (*5*) have shown that the observed efficacy of i.t. decays of ^{201}Tl for killing the sperm cells is explainable in terms of dose rate enhancement to the spermatogonial cells with f_c in the range of 0.1 to 0.15 for $n \gtrsim 50$. For the same species of mice employed here, it is reasonable to expect that f_c is about the same. For $n \approx 5$–10, Eq. (3.6) predicts dose rate enhancement factors N_c of 2.5 to 3.0 with $f_c \sim 0.1$ to 0.15. Such values of n are consistent with our uptake studies of ^{55}Fe by cells in culture. Thus the observed RBE of 2.6 for ^{55}Fe is understandable in this model.

2. When the cells do not contain ^{55}Fe, $n=0$ and $N_c = 0.25$ for any value of $f_c \neq 1$. The conventional approach overestimates the dose rates to cells if ^{55}Fe decays only occur external to the cells.

3. When $n = 1$, the radioactivity is uniformly distributed throughout the organ. Then $N_c = 0.96$ for all values of f_c. This agrees closely with conventional dosimetry.

4. As $f_c \to 0$, N_c increases with increasing values of n, attaining a value of about 15 for $n = 20$, and 70 for $n = 100$. This observation is particularly relevant to studies with cells in vitro. When the cells are sparsely populated, $f_c \to 0$, and the conventional approach strongly underestimates the actual dose to the cells. This observation is in agreement with the findings of Kassis et al. (*6,21*) in the context of their studies on the toxicity of ^{201}Tl and ^{51}Cr in mammalian cells in culture.

5. Finally, consider a highly cellular organ with cells in a closely packed geometry. If *all* the cells concentrate the radionuclide, $f_c \sim 0.7$ (*5,6*). In this situation, the basic assumptions of conventional dosimetry tend to be valid. Only modest cellular dose rate enhancement should be expected in such cases (*5,6*). For ^{55}Fe, Eq. (3.6) gives $N_c \sim 1.2$ for $n \gtrsim 5$.

Intracelluar distribution: dose rate enhancement to the cell nucleus.

Although the previous model may be adequate, it is appropriate to consider the dose rate to the cell nucleus since it contains the DNA, the target of radiation action on cells. The average instantaneous dose rate to the nucleus, $\bar{R}_N$, may be written as

$$\bar{R}_N = \bar{R}_{NN} + \bar{R}_{NCy} + (\bar{R}_N)_{ext}, \quad (3.7)$$

in which $\bar{R}_{NN}$, the contribution from ^{55}Fe decays in the *same* nucleus, and $\bar{R}_{NCy}$, the contribution to the nuclear dose rate from decays in the cytoplasm of the *same cell*, stem only from the Auger electron. $(\bar{R}_N)_{ext}$ represents the dose rate to the nucleus from decays taking place external to the cell containing the target nucleus of interest. This is simply the dose rate from the x-ray photons:

$$(\bar{R}_N)_{ext} = (AN_0/V)v_N\bar{\epsilon}_x,$$

v_N being the nuclear volume. It is useful to define the following parameters:

f_N = fraction of the volume of the spermatogonial cell occupied by its nucleus;
f_{Cy} = fraction of the volume of the spermatogonial cell occupied by its cytoplasm (with $f_N + f_{Cy} = 1$);
r_N = fraction of the cellular content of radioactivity inside the nucleus;
r_{Cy} = fraction of the cellular content of radioactivity in the cytoplasm (with $r_N + r_{Cy} = 1$);
ϕ_{NN} = self-absorbed fraction of total Auger-electron energy ($\bar{\epsilon}_e$) emitted per decay in the nucleus;
$\phi_{N\leftarrow Cy}$ = fraction of $\bar{\epsilon}_e$ absorbed in the nucleus per decay in the cytoplasm,
$\phi_{Cy\leftarrow N}$ = fraction of $\bar{\epsilon}_e$ absorbed in the cytoplasm per decay in the nucleus, and
dps = disintegration per second.

Then, $\bar{R}_{NN}$ = (dps in the nucleus)$\phi_{NN}\bar{\epsilon}_e = (N_0r_NC_cv_c)\phi_{NN}\bar{\epsilon}_e$. Similarly, $\bar{R}_{NCy} = (N_0r_{Cy}C_cv_c)\phi_{N-Cy}\bar{\epsilon}_e$. Using Eq. (6.2) of Ref. (*5*) for C_c, we can express $\bar{R}_N$ in Eq. (3.7) as

$$\bar{R}_N = (AN_o/V)v_c\bar{\epsilon}_e\{n(r_N\phi_{NN} + r_{Cy}\phi_{N-Cy})[f_c(n-1) + 1]^{-1} + f_N\Delta\} \quad (3.8)$$

The conventional nuclear dose rate $(\bar{R}_N)_{CON}$ is given by replacing v_c by v_N in Eq. (3.2). The nuclear dose rate enhancement factor $N_N = \bar{R}_N/(\bar{R}_N)_{CON}$ is obtained as

$$N_N = \{(n/f_N)(r_N\phi_{NN} + r_{Cy}\phi_{N-Cy}) [f_c(n-1) + 1]^{-1} + \Delta\}(1 + \Delta)^{-1}. \quad (3.9)$$

Using a procedure similar to the calculation of ϕ_{cc}, we obtain $\phi_{NN} = 0.89$ for the spermatogonial cell nucleus with $d_N = 6$ μm. The low-energy electrons (Table 1) emitted in the nucleus cannot go beyond the cell boundary. Hence, $\phi_{NN} + \phi_{Cy-N} = 1$, giving $\phi_{Cy-N} = 0.11$. For uniform distribution of radioactivity in the two source regions, the nucleus and the cytoplasm, we can relate ϕ_{N-Cy} and ϕ_{Cy-N} by the Reciprocal Dose Theorem (see Refs. (*12,25*), e.g.): $\phi_{N-Cy} = (f_N/f_{Cy})\phi_{Cy-N}$. With $d_N = 6$ μm, and $d_c = 12$ μm, $f_N = 0.125$, and $(f_N/f_{Cy}) = 0.143$, for spherical geometry. Hence, $\phi_{N-Cy} = 0.016$. The intracellular distribution studies give $r_N = 0.15$ and $r_{Cy} = 0.85$, if we assume that the nuclear fraction of the radioactivity found in the in vivo studies is indeed representative of the content in the spermatogonial cell nuclei. For these cells, we noted earlier that $f_c = 0.1$ to 0.15. All the parameters in Eq. (3.9) are therefore fixed except for n. Nuclear dose rate enhancement factors of about 2.6 are predicted by Eq. (3.9) for $n = 3.3$ with $f_c = 0.1$, and $n = 3.8$ for $f_c = 0.15$. Thus a modest intracellular concentration factor $n \sim 3$–4 accounts for the observed RBE value of ^{55}Fe in terms of dose rate enhancement to the nuclei of the spermatogonial cells.

RBE values for ^{55}Fe and ^{201}Tl: An intercomparison

The experimental RBE values (3.3 for ^{201}Tl (*5*) and 2.6 for ^{55}Fe) represent the combined effect of the Auger electrons and the penetrating photons emitted concomitantly. Since the photons contribute uniformly to the average cellular and nuclear dose rates irrespective of intracelluar localization of the radionuclides, the efficacy of the Auger-electron component alone should be somewhat higher than the experimental RBE values. The structure of Eq. (3.6) and (3.9) for the cellular and nuclear dose rate enhancement factors shows this effect explicitly. For ^{59}Fe, $\Delta = 0.332$; the corresponding value for ^{201}Tl (*5*) is 0.079. Correcting for this effect, we get an efficacy factor of 3.1 for the Auger-electron component of ^{55}Fe, and 3.5 in the case of ^{201}Tl. These are nearly the same considering experimental uncertainties. There are, however, striking differences between these two radionuclides. The average Auger and CK electron yield is 5.0 for ^{55}Fe decay (Table 1), and about 20 per ^{201}Tl decay (*5,6*). The cells concentrate ^{201}Tl avidly (*6*), while ^{55}Fe is more moderately concentrated. The effective half-lives of the two radionuclides in the testes are also very different. The near equality of the efficacy of the low-energy electrons in both cases, in spite of these differences, is interesting indeed. Whether it is merely a coincidence or indicative of possible significance remains to be determined by further studies.

CONCLUSION

Iron-55 is used in radiobiological studies, though not as a diagnostic imaging agent. Moreoever, it can enter the human blood system through foods such as fish (*4*) by virtue of its extended presence in the biosphere in the event of environmental contamination following atmospheric nuclear detonations or severe nuclear reactor accidents. Perceiving this hazard, Wrenn et al. pointed out the importance of the dosimetry of Auger electrons from ^{55}Fe decay (*4,29*). Reincke et al. (*30*) reported that ^{55}Fe can selectively damage the erythroblasts in the blood of mice. We have presented here the first clear evidence for the efficacy of ^{55}Fe in killing sperm cells when localized in the testes of mice compared to the energetic beta rays from similarly distributed ^{59}Fe.

Several radiobiological experiments (*6,21,31-34*) with mammalian cells in culture have repeatedly drawn attention to the high radiotoxicity of Auger-electron emitters depending on their intracellular localization, and pointed out the need for an understanding of the effects in vivo. Our work with ^{55}Fe in mouse testes is a quantitative contribution in this regard. It confirms the findings of the ^{201}Tl studies (*5,6*) and emphasizes the inadequacy of conventional dosimetry (*11,12*) in assessing the radiobiological risks of tissue-incorporated, Auger-electron emitters that may be concentrated by the cells.

In formulating the dose rate enhancement factors, Eq. (3.6) and (3.9), we have explicitly included the contributions from penetrating radiations, an aspect that was ignored as negligible in the earlier work (*5,6*). The importance of the two parameters, n and f_c, in the cellular dose rate enhancement factor (*2,5,6*) is confirmed in this work, Eq. (3.6). Since the critically important DNA is in the nucleus, the nuclear dose rate enhancement factor (N_N) is biophysically more meaningful. The expression developed for N_N is a new contribution to the dosimetry of Auger-electron emitters in vivo. Inspection of Eq. (3.9) reveals that the parameters n, f_c, r_{Cy}, r_N, and f_N are important in this case. The actual sizes of the cell and the nucleus enter only indirectly in the evaluation of the respective absorbed fractions. The occurrence of f_N, the fraction of the cellular volume occupied by the nucleus, is rather unexpected. Our intracellular distribution studies contributed to an adequate interpretation of the observed RBE value in terms of dose rate enhancement to the nucleus.

Finally, one may ask about the possible role of the low-energy Auger electrons from decays occurring in the proximity of nuclear DNA. Such electrons may indeed cause highly localized irradiation of the DNA (*2*). The present studies cannot answer this question. Experiments are underway to examine these implications.

FOOTNOTES

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