PHYSICS AND RADIATION BIOLOGY

Radiotoxicity of Thallium-201 in Mouse Testes: Inadequacy of Conventional Dosimetry

Dandamudi V. Rao, George F. Govelitz, and Kandula S. R. Sastry

University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey, and University of Massachusetts, Amherst, Massachusetts

When Tl-201 is concentrated in mouse testes, the low-energy Auger electrons following its electron-capture decay are found to be much more effective in causing loss of testicular weight and reduction of sperm heads than the energetic beta particles from similarly distributed Tl-204. These results are contrary to expectations based on conventional dosimetry of tissue-incorporated radionuclides, and point to possible underestimation of risks by the currently adopted dosimetric procedures, especially in the case of radionuclides decaying by electron capture and internal conversion.

J Nucl Med 24: 145–153, 1983

Thallium-201 is widely accepted in diagnostic nuclear medicine as the radionuclide of choice for heart function studies. A significant fraction (0.15%) of Tl-201 intravenously injected in humans is found in the gonads, where it has a long biological half-life (*1*) relative to its physical half-life of 73 hr (*2*). Recently, Tl-201 has also been suggested for testicular imaging (*3*). Hossain and Hossain (*4*) indicate that a considerably larger fraction (~0.8%) of the injected dose may be taken up by human testes within the first 24 hr. Like many other radionuclides currently used in nuclear medicine, Tl-201 decays by electron capture (EC) (*2*). The primary inner-atomic-shell vacancies in the daughter Hg atom are filled by a complex series of atomic vacancy cascades involving radiative and nonradiative Auger and Coster-Kronig (CK) transitions (*6*). The resulting emissions are the clinically useful K x rays of Hg and a large number of low-energy electrons with ranges of a few microns or less in biological matter (see Appendix). Since thallous ions behave as analogs of potassium cations (*1*,*5*), Tl-201 in the testes may be expected to be highly concentrated by the cells. Such intracellular concentration of Tl-201 within the testes may result in localized irradiation of cells such as the radiosensitive spermatogonia by the low-energy electrons. In this work we have studied the radiotoxicity of Tl-201, with its EC decay, in comparison with the energetic beta decay of Tl-204 (*7*), distributed similarly in mouse testes. We report that the cytocidal effects of Tl-201 appear to be much more severe than in the companion case of Tl-204, when the biological effects are compared on the basis of the average calculated doses to the testes derived from current dosimetric procedures recommended by the Medical Internal Radiation Dose (MIRD) Committee (*8*) and the International Commission on Radiation Units and Measurements, ICRU (*9*).

Received Aug. 2, 1982; revision accepted Oct. 1, 1982.

For reprints contact: Dr. Dandamudi V. Rao, Dept. of Radiology; NJ Med. School, 100 Bergen St., Newark, NJ 07103.

EXPERIMENTAL MODEL

We have used spermatogenesis in mice as the experimental model to explore the radiobiological effects of the Auger-electron emitter, Tl-201, compared with the beta emitter, Tl-204. While experiments on animals are interesting in themselves, data on radiation effects on spermatogenesis in mice are relevant to man. This is because the events from the division of a stem cell to the production of sperm are essentially the same in mice and man except for the time scale: about 5 wk for mouse compared with about 10 wk for man.

Spermatogenesis is a complex series of events (*10*). The first step is stem cell (A_s) differentiation to produce a pair of cells that, in turn, divide to give type A_1 spermatogonia. These subsequently divide to give rise to a succession of gonial cells, designated as A_2, A_3, A_4, In, and B types. The type B gonia will divide to produce primary spermatocytes, which divide to become secondary spermatocytes. The process of recombination of genes and reduction of chromosome number by half, known as meiosis, occurs during the two spermatocyte divisions. The products of secondary spermatocyte division are spermatids. These proceed, through a succession of stages of differentiation without further division, to become spermatozoa. The spermatogonial cells are known to be very sensitive to radiation in both mice and man (*11,12*). On the other hand, the postgonial cells (spermatocytes, spermatids, spermatozoa) are relatively resistent to radiation injury. This differential radiosensitivity means that the lethal effects will not manifest themselves in reduced testicular weight or sperm-head counts until some time after irradiation. This is the time required by the late-gonial cells surviving irradiation to reach the spermatozoa stage. Thus spermatogenesis may be used as a model to study the radiation effects in vivo (*13*).

The validity of such a model requires that certain criteria be satisfied. First, after a single acute exposure of the testes of the experimental animal (mouse) to an external gamma source, the time necessary for the sperm-head count to reach a minimum has to be experimentally established for the particular strain of the mice used. The decrease in sperm-head count measured at such an experimentally established later time after the initial exposure constitutes acceptable biological effect. Second, in the case of tissue-incorporated radionuclides, the time needed for sperm-head count to reach a minimum, after the initial administration, is the same as for a single exposure to external gamma radiation (*13*) if most of the radiation dose from the radionuclide is delivered to the organ within the first 24 hr. This requires that the effective half-time of the radionuclide in the testes must be quite short.

Male Swiss Webster mice, 6 to 9 wk old and weighing about 30 g each, were used in these studies. The sperm-head counts in several randomly selected animals were determined using procedures described later. No statistically discernible differences in the sperm-head count were observed whether the count was taken 4 wk or 8–10 wk after the mice were originally received. This assures uniform spermatogenesis in the experimental animals. Following single initial exposures of the testes to acute doses (90 rads) of external Cs-137 gamma radiation, the decrease of sperm-head counts, compared with unirradiated controls, was studied over several weeks. The exposed mice were found to have the lowest sperm-head counts on the 29th day following the original radiation insult, in agreement with the results of an earlier study on the same strain of mice (*14*). In spite of the very disparate physical half-lives of Tl-201 and Tl-204 [73 hr vs. 3.8 yr (*2,7*)], these emitters, administered into the testes of these animals, have been found to be cleared from the the organ very quickly, with a 2.5-hr biological half-time for a fast component and a ~9-hr half-time for a relatively longer component (see Fig. 1). These considerations show that the above criteria of the model are fully satisfied.

The central purpose of this work is to investigate the radiobiological effectiveness of low-energy electrons from Tl-201 decay relative to the energetic beta particles from Tl-204. The usual procedure of intravenous injection of radionuclides into the blood stream results in accumulation of only a small fraction of the initial activity in the testes. The i.v. method necessarily results in irradiation of the target (testis) by the penetrating radiations from the bulk of the Tl-201 activity in the rest of the body. Intraperitoneal (i.p.) injection also suffers from a similar disadvantage. In contrast, direct intratesticular (i.t.) injection of Tl-201 effectively eliminates the nontarget-to-testis dose from dosimetric considerations. Since the amount of Tl-201 activity involved in the i.t. mode is not more than a few microcuries (maximum of 25 μCi), the whole-body dose to the animal is small. We have therefore adopted the i.t. route in these experiments.

MATERIALS AND METHODS

Carrier-free Tl-201, and Tl-204 at a specific activity of 3.9 Ci/g were obtained as thallous chlorides. The solutions were heated to dryness and dissolved in normal saline to obtain the required concentrations of radioactivity. The mice were anesthetized under ether and a one-cm long ventral incision was made slightly posterior to where the inguinal canal communicates with the body cavity. The cut continued through the epidermis and dermis into the inguinal canal. The right testis was maneuvered into this opening and partially exteriorized to accept the injection. Using a microsyringe with a 27-gauge needle aligned with long axis, 3 μl of Tl-201 or Tl-204 chloride were injected into the middle of the testis. The organ was then maneuvered back through the canal and the cut was closed with one or two stitches. Mice that were untouched, sham-operated, or injected with stable thallous chloride (up to 3 μg/injection), served as controls. No difference in sperm-head count was observed in the control groups. The animals were killed 28 days after the injection, the time required for late spermatogonial cells to become spermatozoa. The testes were removed, weighed, homogenized in one ml of deionized water, and sonicated for 30 sec. The sperm heads, which are resistant to sonication, were counted by microscope in a hemocytometer to a minimum of 200.

The biological clearance of Tl in mouse testes was studied for the first 48 hr after i.t. injection of 1 to 10 μCi of Tl-201 or Tl-204 chloride, the animals being killed at different times in groups of at least five. The clearance rates were the same whether 1 μCi or 10 μCi of Tl-204 were injected, indicating that any stable thallium present did not affect the elimination of Tl-204.

The detection and assay of the radioactivities of Tl-201 and Tl-204 were performed using the established principles of gamma spectroscopy. A NaI detector, with a well 5 cm deep and 1.27 cm in diameter, was used. In both cases, the Hg K x rays, with average energy of 72 keV, were accepted in the counter with a wide window. While this method was very efficient for Tl-201, it was also fully adequate for Tl-204, which can decay to Hg-204 by EC with a branching ratio of 2.5%, yielding 1.5 K x rays per 100 decays (*7*). The sample-to-detector geometry was the same for all the samples. The detection efficiency for each radionuclide was determined by counting, in the same geometry, Tl-201 and Tl-204 sources of known strength.

Frozen-section autoradiographic studies were performed to identify regions of concentration of thallium in the testes of mice. The animals were killed 18 hr after i.t. injection of 1 μCi Tl-204 chloride. The testes were removed, and immediately embedded in 7% gum tragacanth, and plunged into isopentane at liquid-nitrogen temperature. The frozen testes were then placed in a cryostat and allowed to equilibrate for at least 30 min. To prevent leaching or migration of Tl^+ into liquid emulsion, several slides were dipped in Kodak-NTB3 emulsion and allowed to dry before 10-μm thick sections of the testes were cut and mounted on them. The sectioning was done under a red safe-light. The slides were then stored in a light-tight box. After 1–6 days of exposure, the slides were developed, fixed, and stained using standard procedures. The grains were counted under a microscope in different regions of the seminiferous tubules from randomly selected sections and fields. Compared with the formalin autoradiographic method, the approach adopted here ensures that there is no leaching or movement of thallous ions in the organ after its removal.

RESULTS AND DISCUSSION

Testicular clearance and radiation dose. The data on the testicular clearance studies are graphed in Fig. 1. In spite of the lower specific activity of Tl-204 compared with the carrier-free Tl-201, both radionuclides are effectively eliminated at essentially the same rate. The observed biological half-times are 2.5 hr for the fast component (25%) and 9.0 hr for the longer-lived component (75%) for thallous ions administered by the i.t. route. Such a fast clearance is interesting in view of the relatively avascular nature of the organ. For the longer-lived Tl-204, the effective half-times for the components are 2.5 and 9.0 hr; for Tl-201 the values are 2.4 and 8.0 hr. Within the first 24 hr, about 90% of the injected radioactivity is eliminated, so most of the radiation dose is delivered to the testis in this period.

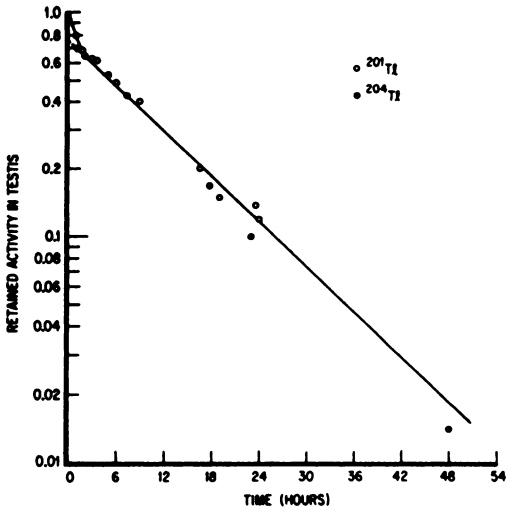

FIG. 1. Biological clearance of intratesticularly injected Tl-201 and Tl-204. Fraction of initial activity retained in testis as a function of time is shown in semi-log plot.

Using the above results, the equilibrium absorbed doses $\overline{D}$ to the testis were calculated following the MIRD and ICRU procedures (*8,9*), which assume homogeneous distribution of the radionuclides as well as the radiation energy in the organ. The average testicular dose $\overline{D}$ in rads is given by (*8,9*)

$$\overline{D} = (A/m)(1/\ln 2)(f_1 T_{1e} + f_2 T_{2e}) \sum_i \Delta_i \phi_i \quad (1)$$

where A = initially administered activity in μCi,
m = average mass of the unirradiated testis (=0.1 g),
f_1, f_2 = relative proportions of the two components (0.25 and 0.75, respectively),
T_{1e}, T_{2e} = the effective half-lives of the respective components in hours,
Δ_i = equilibrium absorbed-dose constant for the i^{th} radiation from the decay, in g-rad/μCi-hr,

and

ϕ_i = absorbed fraction for the i^{th} radiation.

For Tl-204, the beta particles have an average energy of 243 keV, and Δ = 0.507 g-rad/μCi-hr (*7*). The average radius of the nearly spherical testis is about 3 mm, in comparison with the average (95%) range of about 1.4 mm for these beta particles in a water-equivalent medium (*15*). The absorbed fraction ϕ is taken to be unity* for these nonpenetrating radiations, in keeping with the MIRD schema (*8*). The weak (2.5%) EC decay of Tl-

204 (*7*) contributes negligibly to the total dose. The equilibrium absorbed dose to the testis is 54.0 rad per μCi of Tl-204.

In the case of Tl-201, we have used the recent nuclear data (*17*) to be consistent with the current practice of Tl-201 dosimetry. The absorbed fractions ϕ_i are unity for the low-energy conversion electrons and K and L Auger electrons of short ranges, and $\Sigma\Delta_i = 0.08$ g-rad/μCi-hr (*17*) for these electrons. Using $T_{1e} = 2.4$ hr and $T_{2e} = 8.0$ hr, the equilibrium dose to the testis from these nonpenetrating radiations is calculated to be 7.6 rads per μCi of Tl-201. The contribution to the total dose from penetrating x-rays and gamma photons arising in the organ is found to be 0.70 rad per μCi of Tl-201 using Eq. (1) and the following information. The values of Δ_i are taken from Nass (*17*), and those for ϕ_i are calculated using the method of Powsner and Raeside (*18*) for a spherical organ of water-equivalent material. The mass absorption coefficients at various photon energies are obtained by interpolation of the published values (*19*). The quantity $\Sigma\Delta_i\phi_i = 0.0073$ g-rad/μCi-hr. The total dose to the testis from Tl-201 is therefore 8.3 rad per μCi, most of which stems from the Auger and conversion electrons, whereas the dose from Tl-204 (54.0 rad/μCi) is almost entirely due to the relatively high-energy betas.

These dose estimates, based on the assumption of uniform distribution of radiation energy in the organ (*8,9*), suggest that Tl-201 should be much less radiotoxic than Tl-204 on a μCi-to-μCi basis, since about 6 μCi of Tl-201 are needed to deliver as much dose to the testis as 1 μCi of Tl-204. Contrary to this, we have found that Tl-201 decays are almost as effective as Tl-204 decays.

Loss of testicular weight and decrease of sperm-head count. Figure 2 shows the fractional weight loss, and Fig. 3 the reduction in sperm-head population, relative to controls, as a function of the (calculated) dose to the testis from various amounts of Tl-201 and Tl-204 radioactivity injected into the testis. In each case the data show a two-component response, with a sensitive component at lower doses and a relatively less sensitive one at higher doses. Each data point is an average value for

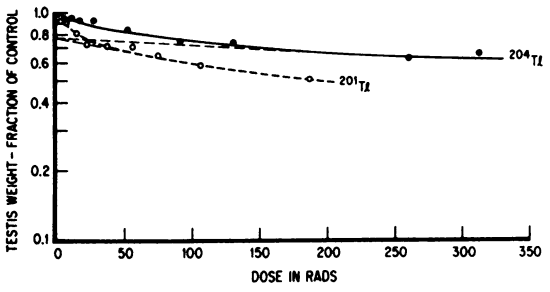

FIG. 2. Fractional weight loss of testes of mice as a function of calculated average dose to the organ from intratesticular injections of Tl-201 and Tl-204 as chlorides.

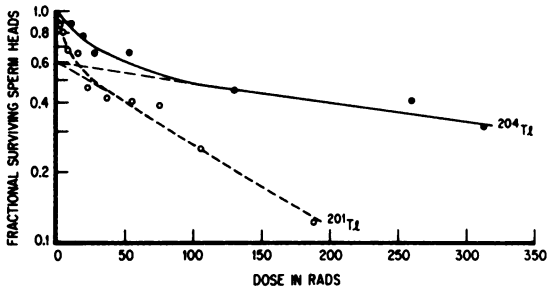

FIG. 3. Sperm-head survival as a function of calculated average dose to mouse testes from intratesticular injections of Tl-201 and Tl-204 as chlorides.

at least five mice, and the standard error of the mean is about 10–15%. Least-squares analysis of the data give

$$W(\text{Tl-201}) = 0.25\, e^{-D/25} + 0.75\, e^{-D/440} \quad (2)$$

$$W(\text{Tl-204}) = 0.25\, e^{-D/57} + 0.75\, e^{-D/1746} \quad (3)$$

for the weight loss W as a function of the dose D in rads to the testis. The decrease in sperm-head count S with dose is given by

$$S(\text{Tl-201}) = 0.39\, e^{-D/7} + 0.61\, e^{-D/127} \quad (4)$$

$$S(\text{Tl-204}) = 0.39\, e^{-D/26} + 0.61\, e^{-D/515} \quad (5)$$

The denominators in the exponents of each term in Eqs. 2 to 5 represent the corresponding values of the dose for 37% survival (D_0) of each component. Values of 'relative biological effectiveness' (RBE) of Tl-201 *relative to Tl-204* were obtained from the ratios of D_0 values for Tl-204 and Tl-201 for each component in Eqs. 2 through 5. These experimental RBE values for the sensitive and insensitive components are 2.3 and 4.0, respectively, for loss of weight, and 3.7 and 4.1 for sperm-head survival. Using the relative proportions of each component as weight factors, the average 'RBE value' is calculated to be 3.8 ± 0.4. This indicates that the radiobiological effects of Tl-201 are two to four times more than expected on the basis of conventional dosimetry.

Our observation of a two-component response in the dose-dependence of the biological effects does not appear to be due to artifacts of the experimental protocol. This response is observed with both Tl-201 and Tl-204. These radionuclides clear from the organ very fast, delivering most of the dose within the first 24 hr. Thus there is no likelihood of either a late effect or chronic irradiation as being responsible for a shifting of attainment of the minimum sperm-head count from the 29th day after initial exposure to a much later period (*20*). In fact, Kohn and Kallman (*21*) demonstrated in 1954 the existence of two components in the weight loss of mouse testis, with very different radiosensitivities for the two. Similar two-component responses have recently been reported (*22,23*) for testicular weight loss following irradiation of some strains of mice by fast neutrons, x rays, and gamma photons. We note that the biological effec-

tiveness of the low-energy electrons compared with energetic beta particles observed in this work is about the same as the RBE of fast neutrons when the effects of neutrons are compared with those of 250 kVp x rays and Co-60 gamma rays. The relatively less radiosensitive component of the dose-response curve may possibly be due to the existence of resting and hence radioresistant spermatogonia. Such a view is plausible according to the model of Dym and Cleremont (*24*) for the stem-cell renewal process. On the other hand, it is possible that the two-component response might be due to differential radiosensitivities within the late spermatogonial cell subpopulations.

The main finding of this work is the high efficacy for biological effect of the radiations from intratesticular Tl-201 compared with those from Tl-204. This is consistent with expectations based on several radiobiological experiments (*25–28*) that indicate that intracellular decays of radionuclides emitting Auger electrons may be much more radiotoxic to mammalian cells in culture than the beta decay of nuclides with similar intracellular distribution. The rest of this discussion is an attempt toward a reasonable understanding of our results.

Auger and Coster-Kronig electrons from EC decay of Tl-201. As a first step, we examined the nuclear decay properties of Tl-201, and evaluated the complete spectrum of radiations to be expected. Our results for conversion electrons, K and L Auger electrons, and x ray and gamma photons agree with the work of Nass (*17*), except for minor differences. The CK and Auger-electron data, not adequately treated so far, are given in the Appendix. The net result is that the average energy released (and absorbed) as nonpenetrating radiation per Tl-201 decay is 43.3 keV rather than 37.5 keV as given by current data. The contribution to the absorbed energy in the testis from the photons is $(10^3/2.13)\Sigma\Delta_i\phi_i = 3.4$ keV per decay of Tl-201. Thus the total average energy deposited in the organ is 46.7 keV per Tl-201 decay compared with the 40.9 keV given by existing data. Therefore, the Tl-201 dose estimate given earlier using the data of Nass (*17*) needs to be enhanced by a factor of 1.14. The average 'RBE value,' corrected accordingly, becomes 3.3 ± 0.3. This implies that the average dose from Tl-201 to the spermatogonial cells is effectively enhanced by this factor. Plausible reasons for such an enhancement are offered below.

Results of autoradiography. A sample autoradiogram of testicular tubular sections is shown in Fig. 4. Over 15,000 grains were counted in numerous tubules. In a separate experiment we have determined that the grain count due to background radiation is ~20% of the total grains counted. The statistical average of the data, after the background correction, gave (48 ± 4)% of the total grains in or close to the 10 μm wide regions containing the gonial stem cells and the differentiating spermatogonia. The remaining grains were found mostly in the rest of the tubule, with a small fraction appearing in intertubular spaces.

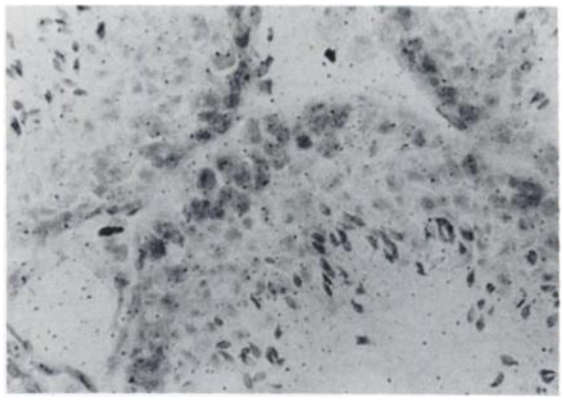

FIG. 4. Sample autoradiogram of seminiferous tubule (x 500) of mouse testis, 18 hr after injection of Tl-204 chloride. Note relatively increased localization of radioactivity in or close to the 10-μm-wide shell containing stem cells and differentiating spermatogonia. On average, total number of grains per tubule is about 500, of which ~100 grains are due to background. Background grains are assigned to different regions assuming that they are, on average, proportional to the respective areas.

In spite of the observed nonuniform distribution, the basic assumption of a uniform equilibrium dose distribution in the organ, inherent in the conventional dosimetric procedures (*8,9*), is expected to be reasonably valid for the energetic Tl-204 betas ($\bar{E}_\beta = 243$ keV), with their low average linear energy transfer of ≤0.2 keV/μm. Since the beta-particle range in water (~1.4 mm) is much larger than the typical cellular diameter (~10 μm), and much less than the size of the testis, the average dose to the spermatogonial cells may not be too different from the average dose to the testis. In sharp contrast to this is the EC decay of Tl-201, with copious emission of low-energy electrons of short range (see Appendix). The enhanced localization of Tl-201 in the shell of tissue containing the spermatogonia in each tubule should result in a considerably higher locally absorbed energy density compared with the average value over the whole testis. This suggests a qualitative explanation for the efficacy of Tl-201 for the biological effects. A more meaningful understanding must be sought at the cellular level.

Concentration effects and enhancement of cellular dose. It is well known that most cells in vivo concentrate potassium cations by a factor of 30–50 relative to K^+ concentration in the extracellular fluid (3–5 m*M*). Since Tl^+ ions behave biologically as analogs of K^+ (*1,5*), it is quite conceivable that the thallous ions may also be highly concentrated by the cells. This may well be the most plausible reason for the observed localization of Tl in the regions containing the spermatogonial cells. Recent in vitro studies (AI Kassis and SJ Adelstein, personal communication) show that V 79 Chinese hamsters lung fibroblasts accumulate $^{201}Tl^+$ to about 130 times the concentration in the external culture medium. If

indeed the spermatogonial cells in vivo accumulate Tl-201 and Tl-204, we can account for the observed dose-enhancement effects.

Let us focus our attention on one of the many cells that may concentrate the radionuclides. For simplicity, we assume that these cells are spheres of 10 μm diameter, and that the intracellular radioactivity is uniformly distributed. The cell's total rate of average absorbed energy $\overline{E}_t$, in keV/sec, stems from two sources: (a) $\overline{E}_c$, from decays within the cell itself, and (b) $\overline{E}_e$, from decays external to the cell. Let V be the volume of the organ containing A μCi of radioactivity at any instant, f_i the fractional volume occupied by those cells that have concentrated the radioactivity, and f_e the fractional volume of the rest of the organ, so that

$$f_i + f_e = 1. \qquad (6.1)$$

We also define the following quantities:

v_c = volume of the cell,
c_e = extracellular concentration of radioactivity in μCi/ml,
c_i = intracellular concentration = nc_e,
n = intracellular concentration factor,
$\overline{E}$ = average energy in keV deposited in the organ per decay, and
$\bar{\epsilon}_c$ = average energy in keV deposited in the cell per decay in the same cell.

The average concentration of radioactivity in the organ is then

$(A/V) = c_e f_e + c_i f_i = c_e (f_e + n f_i)$. Using Eq. 6.1:

$$c_i = nc_e = (A/V)n/[f_i(n - 1) + 1] \qquad (6.2)$$

The quantity $\overline{E}_c = \bar{\epsilon}_c \cdot$ (number of decays per second in the cell) $= (3.7 \times 10^4)\, c_i v_c \bar{\epsilon}_c$. With c_i as given above,

$$\overline{E}_c = (3.7 \times 10^4)\,(A/V)v_c\{n\bar{\epsilon}_c/[f_i(n - 1) + 1]\} \qquad (6.3)$$

To a first approximation, the value of $\overline{E}_e$ is given by the density of the average energy absorbed in the organ per second times the volume of the cell. Thus

$$\overline{E}_e = 3.7 \times 10^4\,(A\overline{E}/V)v_c \qquad (6.4)$$

Since the radioactivity inside the cell is very small relative to the amount present in the entire organ, the total intratesticular activity is used in obtaining $\overline{E}_e$, with the added assumption of uniform distribution of radioactivity and radiation energy in the entire organ (*8,9*). Thus, $\overline{E}_e$ is just the same as that calculated according to the MIRD schema and ICRU procedures. Since the radiobiological effects are governed by the actual dose received by the cell, and current procedures ignore the intracellular contribution $\overline{E}_c$, conventional estimates (*8,9*) of the dose rate are valid only if $\overline{E}_c$ is negligible compared with $\overline{E}_e$. Otherwise the dose rate to the cells may be enhanced relative to the conventional estimate by the factor

TABLE 1. CALCULATED DOSE-ENHANCEMENT FACTORS FOR Tl-201 AND Tl-204 * FOR VARIOUS VALUES OF CONCENTRATION FACTOR, n, AS A FUNCTION OF CELLULAR FRACTION, f_i

f_i \ n	10	50	100	150
0	4.0	16.0	31.0	46.0
	(1.06)	(1.31)	(1.62)	(1.93)
0.05	3.07	5.34	6.0	6.3
	(1.04)	(1.09)	(1.10)	(1.11)
0.10	2.58	3.54	3.73	3.83
	(1.03)	(1.05)	(1.06)	(1.06)
0.15	2.28	2.80	2.87	2.93
	(1.03)	(1.04)	(1.04)	(1.04)
0.3	1.81	1.95	1.97	1.98
	(1.02)	(1.02)	(1.02)	(1.02)
0.5	1.55	1.59	1.60	1.60
	(1.01)	(1.01)	(1.01)	(1.01)
0.7	1.41	1.42	1.43	1.43
	(1.01)	(1.01)	(1.01)	(1.01)

* Values given in parentheses are for Tl-204.

$$N = \overline{E}_t/\overline{E}_e = (\overline{E}_e)/\overline{E}_e = 1 + (\overline{E}_c/\overline{E}_e).$$

Accordingly, using Eqs. 6.3 and 6.4,

$$N = 1 + \{n(\bar{\epsilon}_c/\overline{E})/[f_i(n - 1) + 1]\}. \qquad (7)$$

Inspection of Eq. 7 indicates (a) that N may be significantly larger than unity, even when the intracellular and extracellular concentrations are the same (n = 1), if $\bar{\epsilon}_c$ is comparable with $\overline{E}$; and (b) that situations may exist for which N is considerably larger than 1 depending on n, f_i, and the quality of the radiations.

For the unique once-forbidden beta spectrum of Tl-204 (*7*), $\overline{E}$ = 243 keV, and we estimate $\bar{\epsilon}_c$ = 1.5 keV using the entire beta spectrum of Tl-204 corrected for its deviation from the usual statistical shape of "allowed" beta spectra, and using the calculational procedures given by Kassis et al. (*29*). The value of $\bar{\epsilon}_c$ is not strongly affected by possible deviation from the spherical geometry assumed for the cells. For Tl-201, $\overline{E}$ = 46.7 keV, as noted earlier, and $\bar{\epsilon}_c$ = 14.0 keV when the data on Auger and CK electrons (Appendix), and on conversion electrons (*17*), are used to calculate (*29*) $\bar{\epsilon}_c$.

Listed in Table 1 are dose-enhancement factors N calculated from Eq. 7 for several values of f_i and n. As $f_i \to 0$, N attains large values for Tl-201 with increasing values of the concentration factor. For such a sparsely populated system of cells, N can attain values of the order of 2 even for Tl-204 beta particles, for $n \gtrsim 100$. These considerations may be relevant to in vitro studies. At the other extreme, for a highly cellular organ with closely packed cells ($f_i \sim 0.7$) concentrating the radionuclides, $N \sim 1.4$ for Tl-201, and approaches unity for

Tl-204, even for large values of n. The essential point is that the results in Table 1 clearly show that the dose-enhancement factors of about 3–4, obtained in our experiments for Tl-201, are predicted by Eq. 7 for $n \gtrsim 50$ and an f_i in the range of 0.1–0.15. For Tl-204, the predicted dose enhancement is only $\lesssim 5\%$ in the same range of parameters.

The quantity f_i represents the fractional volume of those cells that have concentrated the thallous ions. It seems reasonable to expect that the spermatogonial cells constitute a major fraction of them. This is consistent with the estimate of Green et al. (*30*) that the entire region of the testis containing spermatogonial cells occupies 17% of the volume of the organ in a close-packed geometry for the tubules. If the cells are distributed randomly in this region, the actual fractional volume occupied by them may be somewhat less. This is consistent with the value of 0.1–0.15 for f_i obtained above. These considerations suggest that the model presented here to account for the observed efficacy of Tl-201, in terms of concentration effects, is on reasonable grounds. Further understanding at the microscopic level should await availability of information on the actual intracellular distribution of Tl-201 in the vicinity of the radiosensitive organelles, such as the DNA.

TABLE 1A. AVERAGE SPECTRUM OF AUGER AND COSTER-KRONIG ELECTRONS IN Tl-201 DECAY

No.	Transition(s)	Yield per 100 decays	Average energy (keV)	Range in unit-density matter, in μm (*39*)
1	$N_{6,7\ A}$	205	0.025	0.0014
2	$O_{1,3\ CK}$,$N_{2\ CK}$,$M_{2\ CK}$, $N_{6,7\ A}$	296	0.038	0.0024
3	$O_{2\ CK}$, $M_{3\ CK}$, $L_{1\ CK}$	46	0.062	0.004
4	$O_{1\ CK}$, $N_{1,2,3\ CK}$, $M_{1\ CK}$, $N_{6,7\ A}$	686	0.090	0.005
5	$N_{2,3,4,5\ CK}$, $M_{2,3\ CK}$, $L_{1\ CK}$	280	0.157	0.0075
6	$N_{1,2,4,5\ CK}$, $M_{1,2\ CK}$, $N_{4,5\ A}$	90	0.260	0.012
7	$N_{2,3,4\ CK}$, $M_{3\ CK}$	50	0.427	0.020
8	$M_{1,2\ CK}$, $L_{1\ CK}$, $N_{1\ A}$	22	0.709	0.038
9	$M_{1,2\ CK}$, $L_{2\ CK}$	5.6	1.388	0.10
10	$L_{1\ CK}$, M_A	212	1.874	0.170
11	L_A	79	8.47*	2.30
12	K_A	3.4	61.07*	65.0

* The corresponding values given in Ref. (*17*) are in error.

SUMMARY AND CONCLUSION

The work reported here provides the first clear evidence for the enhanced radiotoxicity of radionuclides, such as Tl-201, that emit Auger electrons, in the biologically important process of spermatogenesis. When such radionuclides are avidly concentrated by cells, our results emphasize the inadequacy of radiobiological risk estimates based on dose calculations using the simplifying assumptions of the MIRD schema (*8*) and the ICRU procedures (*9*). This is fully consistent with the dosimetric implications (*38*) of Auger electrons at the cellular and subcellular level. Our theoretical model offers a reasonable way to arrive at dose-enhancement factors arising from intracellular concentration effects.

To facilitate clear delineation of the effects of low-energy electrons from Tl-201, it was necessary to adopt the i.t. mode of administration of Tl-201 into the mice. In contrast, Tl-201 is intravenously injected into humans in nuclear medical procedures. The fraction of the initially injected radioactivity reaching the human testis may have a very different residence time and distribution in the organ relative to our experimental system. In view of this, and of the generally recognized uncertainties of extrapolation of the results of controlled laboratory experiments from animals to humans, care should be exercised in projecting our results to applications of Tl-201 in patients. Nevertheless, to the extent that Tl-201 ions entering the human testis behave biologically as analogs of K ions, they should be expected to be concentrated by spermatogonial cells, among others. In that event, the biological effects of the low-energy electrons from Tl-201 in human use may be quite similar to what we find in the case of mice. Accordingly, we hope that the basic research presented here will be of value in the assessment of the biological implications of Tl-201 in human applications.

FOOTNOTE

* For an assumed spherical geometry of the testis, and uniform distribution of Tl-204 in the organ, ϕ is about 0.87 (*16*), but this does not significantly alter the conclusions of this paper.

ACKNOWLEDGMENTS

We thank Mr. H. Oldewurtel, Drs. E. Alger and A. Gona for helpful comments, Dr. C. Haydock for the use of his computer program, and Drs. A. I. Kassis and S. J. Adelstein for personal communication of their results prior to publication.

Supported in part by American Cancer Society Institutional Research Grant to UMDN New Jersey Medical School and PHS Grant No. CA 32877 (DVR) and Biomedical Research Support Grant to University of Massachusetts, Amherst.

APPENDIX

Table 1A gives the average theoretical yields and energies of electrons to be expected from Auger (A) and Coster-Kronig (CK) transitions following the EC decay of Tl-201 to levels in Hg-201,

and finally to the ground state of Hg-201 by internal conversion. In these calculations, we used available nuclear information (*2,31*), theoretical atomic radiative (*32*) and Auger and CK transition rates (*33-36*), and experimental binding energies (*37*). The extremely complex spectrum is simplified by grouping electrons of comparable energies. Estimates for electrons from near-valence shells should be regarded as first approximations because of possible theoretical inadequacies in the use of an independent particle model by McGuire (*34-36*) in the evaluation of transition rates, and also because of possible environmental effects. The nuclear data given by Nass (*17*) should be consulted for conversion-electron spectrum and penetrating radiations.

For each electron group of energy E, its range R (last column, Table 1A) is obtained by interpolation from the experimental data of Cole (Table 3 in Ref. *39*). Note that by virtue of their highly subcellular ranges, electrons in groups 1 through 11, originating from intracellular decays of Tl-201, deposit all their energy in the *same* cell. In calculating, $\bar{\epsilon}_c$, the data of Cole on dE/dR vs. E (Table 3, Ref. *39*) have been used along with the procedures of Ref. (*29*). Our estimate of $\bar{\epsilon}_c$ is not affected even if values of dE/dR vs. E are somewhat different, for the actual case of the spermatogonial cells, from Cole's values for unit-density matter (*39*). This is because the great majority of the electrons have low energies and highly subcellular ranges, while the remaining few are mostly energetic particles (*17*), contributing only minimally to the cellular dose rate, $\bar{\epsilon}_c$, because of their sparsely ionizing nature.

REFERENCES

1. ATKINS HL, BUDINGER TF, LEBOWITZ E, et al: Thallium-201 for medical use. Part 3: Human distribution and physical imaging properties. *J Nucl Med* 18:133-140, 1977
2. SCHMORAK MR: Nuclear data sheets for A = 201. *Nucl Data Sheets* 25:193-234, 1978
3. HOSAIN F, HOSAIN P, SPENCER RP: Testicular imaging with Tl-201 and comparison with other radionuclides. *J Nucl Med* 19:720-721, 1978
4. HOSAIN P, HOSAIN F: Revision of gonadal radiation dose to man from thallium-201. *Third International Radiopharmaceutical Dosimetry Symposium*, HHS Publication FDA 81-8166, Watson EE, Schlafke-Stelson AT, Coffey JL, et al., Eds. U.S. Department of Health and Human Services, 1981, pp 333-345.
5. BARCLAY RK, PEACOCK W, KARNOFSKY DA: Distribution and excretion of radioactive thallium in the chick embryo, rat, and man. *J Pharmacol Exp Ther* 107:178-187, 1953
6. BAMBYNEK B, CRASEMANN B, FINK RW, et al: X-ray fluorescence yields, Auger, and Coster-Kronig transition probabilities. *Rev Mod Phys* 44:716-813, 1972
7. MARTIN MJ, BLICHERT-TOFT PH: Radioactive atoms, Auger-electron, α-, β-, γ-, and X-ray data. *Nucl Data Tables* A8: 1-198, 1970
8. LOEVINGER R, BERMAN M: A revised schema for calculating the absorbed dose from biologically distributed radionuclides. Medical Internal Radiation Dose Committee Pamphlet No. 1, Revised (Society of Nuclear Medicine, New York, 1976)
9. ICRU Report 32: Methods of assessment of absorbed dose in clinical use of radionuclides. International Commission on Radiation Units and Measurements, Washington, D.C., 1979
10. OAKBERG EF: A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal. *Am J Anat* 99:391-413, 1956
11. MEISTRICH ML, HUNTER NR, SUZUKI N, et al: Gradual regeneration of mouse testicular stem cells after exposure to ionizing radiation. *Radiat Res* 74:349-362, 1978
12. ROWLEY MJ, LEACH DR, WARNER GA, et al: Effect of graded doses of ionizing radiation on the human testis. *Radiat Res* 59:665-678, 1974
13. MIAN TA, SUZUKI N, GLENN HJ, et al: Radiation damage to mouse testis cells from [^{99m}Tc] pertechnetate. *J Nucl Med* 18:1116-1122, 1977
14. RAO DV, GOVELITZ GF, SASTRY KSR, et al: Radiotoxicity of ^{125}I *in vivo* and microdosimetry of Auger and Coster-Kronig electrons. *XII International Conference on Medical and Biological Engineering: V International Conference on Medical Physics*, Part IV, 70.6, Jerusalem, Israel, 1979
15. BERGER MJ: Distribution of absorbed dose around point sources of electrons and beta-particles in water and other media. *J Nucl Med* (Suppl. No. 5) MIRD Pamphlet No. 7, 5-23, 1971
16. LOEVINGER R, JAPHA EM, BROWNELL GL: Discrete radioisotope sources. In *Radiation Dosimetry*, HINE GJ, BROWNELL GL, Eds., Academic Press, New York, 1956, pp 693-799
17. NASS HW: New Tl-201 nuclear decay data. *J Nucl Med* 18:1047-1048, 1977
18. POWSNER ER, RAESIDE DE: *Diagnostic nuclear medicine*, Grune and Stratton, Inc., New York and London, 1971, pp 163-164
19. STORM E, ISRAEL HI: Photon cross-sections from 1 keV to 100 MeV for elements Z = 1 to Z = 100. *Nucl Data Tables* A7:565-681, 1970
20. MIAN TA, GLENN HJ, HAYNIE TP, et al: Radiation dose effects on mouse testis from sodium P-32-phosphate: Comparison with sodium Tc-99m-pertechnetate. *Third International Radiopharmaceutical Dosimetry Symposium*, HHS Publication FDA 81-8166, Watson EE, Schlafke-Stelson AT, Coffey JL et al., Eds. U.S. Department of Health and Human Services, 1981, pp 242-249
21. KOHN HI, KALLMAN RF: Testes weight loss as a quantitative measure of X-ray injury in the mouse, hamster and rat. *Br J Radiol* 27:586-591, 1954
22. HORNSEY S, MYERS R, WARREN P: R.b.e. for the two components of weight loss in the mouse testis for fast neutrons relative to X-rays. *Int J Radiat Biol* 32:297-301, 1977
23. MONTOUR JL, WILSON JD: Mouse testis weight loss following high energy neutron or gamma irradiation. *Int J Radiat Biol* 36:185-189, 1979
24. DYM M, CLEREMONT Y: Role of spermatogonia in the repair of the seminiferous epithelium following X-irradiation of the rat testis. *Am J Anat* 128:265-282, 1970
25. HOFER KG, HUGHES WL: Radiotoxicity of intracellular tritium, ^{125}iodine and ^{131}iodine. *Radiat Res* 47:94-109, 1971
26. CHAN PC, LISCO E, LISCO H, ADELSTEIN SJ: The radiotoxicity of iodine-125 in mammalian cells. II. A comparative study on cell survival and cytogenetic responses to ^{125}IUdR, ^{131}IUdR, and ^{3}HTdR. *Radiat Res* 67:332-343, 1976
27. HOFER KG, HARRIS CR, SMITH JM: Radiotoxicity of intracellular ^{67}Ga, ^{125}I and ^{3}H. Nuclear versus cytoplasmic radiation effects in murine L1210 leukaemia. *Int J Radiat Biol* 28:225-241, 1975
28. KASSIS AI, ADELSTEIN SJ, HAYDOCK C, SASTRY KSR, et al: Lethality of Auger electrons from the decay of bromine-77 in the DNA of mammalian cells. *Radiat Res* 90: 362-373, 1982
29. KASSIS AI, ADELSTEIN SJ, HAYDOCK C, SASTRY KSR: Radiotoxicity of ^{75}Se and ^{35}S: Theory and application to a cellular model. *Radiat Res* 84:407-425, 1980

30. GREEN D, HOWELLS GR, HUMPHREYS ER, et al: Localisation of plutonium in mouse testes. *Nature* 255:77, 1975
31. RÖSEL F, FRIES HM, ALDER K, et al: Internal conversion coefficients for all atomic shells, ICC values for Z = 68–104. *Atomic Data and Nucl Data Tables* 21:291–514, 1978
32. SCOFIELD JH: Relativistic Hartree-Slater values for K and L X-ray emission rates. *Atomic Data Nucl Data Tables* 14: 121–137, 1974
33. VENUGOPALA RAO P, CHEN MH, CRASEMANN B: Atomic vacancy distributions produced by inner-shell ionization. *Phys Rev* A5:997–1012, 1972
34. MCGUIRE EJ: L-shell Auger, Coster-Kronig, and radiative matrix elements, and Auger and Coster-Kronig transition rates in j-j coupling. *Research Report* SC-RR-711075, Sandia Laboratories, 1971
35. MCGUIRE EJ: M-shell Auger, Coster-Kronig, and radiative matrix elements, and Auger and Coster-Kronig transition rates in j-j coupling. *Research Report:* SC-RR-710835, Sandia Laboratories, 1972
36. MCGUIRE EJ: N-shell Auger, Coster-Kronig, and radiative matrix elements, and Auger and Coster-Kronig transition rates in j-j coupling. *Report* SAND-75-0443, Sandia Laboratories, 1975
37. BEARDEN JA, BURR AF: Re-evaluation of X-ray atomic energy levels, *Rev Mod Phys* 39:125–142, 1967
38. WRENN ME, PARRY HOWELLS G, HAIRR LM, et al: Auger electron dosimetry. *Health Phys* 24:645–653, 1973
39. COLE A: Absorption of 20-eV to 50,000-eV electron beams in air and plastic. *Radiat Res* 38:7–33, 1969

International Symposium on Single-Photon Ultra-Short-Lived Radionuclides

May 9–10, 1983 **PAHO** **Washington, DC**

The Society of Nuclear Medicine, the American College of Nuclear Physicians, the Department of Energy and Department of Radiological Health are sponsoring the International Symposium on Single-Photon Ultra-Short-Lived Radionuclides on May 9–10, 1983 at PAHO in Washington, DC.

The objective of this symposium is to define the current role and state of the art of development and clinical applications of generator-produced single-photon ultra-short-lived radionuclides (USRN).

This symposium will emphasize the phases of generator production, quality control, dosimetry, instrumentation, and clinical applications of USRN. Principal focus will be on the Ir-191m, Au-185m and Kr-81m radionuclide generators. The sessions are designed to encourage active participation and discussion.

Scientific presentations 12 minutes in length pertinent to objectives of the program are being solicited. Submit 1 page abstracts with supporting data to the ACNP with a registration form. Deadline for abstracts is March 15th. The program committee will arrange with authors for specifics of presentation.

Manuscripts of at least 3 pages long are due on arrival at the symposium; Proceedings will be published by the Department of Energy and DRH.

For further information contact:

Erica Kellison
American College of Nuclear Physicians
Suite 700
1101 Connecticut Avenue
Washington, DC 20036