
Intracellular Distribution and Radiotoxicity of Chromium-51 in Mammalian Cells: Auger-Electron Dosimetry

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The kinetics of uptake and of radiotoxicity of chromium-51, an Auger-electron emitter, have been studied in V79 lung fibroblasts of the Chinese hamster. Intracellular radioactivity was directly proportional to the incubation period and to the extracellular concentration of the Cr-51. About 14% of the cellular activity was associated with the nucleus, whereas approximately 2% was guanidine-precipitable and therefore bound to DNA. The growth rate of V79 cells was slowed following intracellular incorporation of Cr-51. The cell-survival curve, in terms of colony-forming ability, was of the low-LET type, with a D_{37} of 6.2 pCi/cell. Theoretical dosimetric estimates indicate that, under the given experimental conditions, the mean lethal dose to the cell nucleus was 870 rad. Although this value is somewhat larger than the x-ray D_{37} dose of 580 rad for this cell line, it is more realistic than the gross underestimate obtained by classical MIRD calculations (2–3 rad/cell).

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The discovery (1) that red blood cells of various species could be tagged in vitro with Cr-51 in the anionic hexavalent form of sodium [Cr-51]chromate was followed by the rapid development of techniques for measuring RBC survival time, RBC mass, total blood volume, and spleen-to-liver ratios (2). This led to the widespread use of Cr-51 in cell biology, particularly for the labeling of lymphocytes in immunological toxicity assays (3–6). However, to the best of our knowledge, the radiotoxicity of intracellular Cr-51 has not been investigated. In this paper we report (a) experimental results on the kinetics of uptake and retention of this radionuclide by dividing mammalian cells in culture, (b) its intracellular distribution, and (c) its radiotoxicity as determined by the colony-forming assay (7,8).

Chromium-51 is produced by $^{50}\text{Cr}(n,\gamma)^{51}\text{Cr}$ or

$^{51}\text{V}(d,2n)^{51}\text{Cr}$ reactions. It decays with a 27.7-day half-life (9) to stable V51 entirely by orbital electron capture (EC) and with the emission of highly penetrating 320-keV gamma photons (9.9%) having a mean free path $l_{\gamma} \sim 8.5$ cm (10) in water-equivalent biological matter. The primary EC decay process results in an inner atomic shell vacancy in the V51 daughter atom. Consequently there are emissions of 5.0-keV K x-rays (22%) of vanadium (9) with $l_x \sim 250$ μm (10), and several low-energy Auger electrons (10 eV to 4.38 keV) with subcellular ranges. To understand the observed radiotoxicity, theoretical calculations of the dosimetry of low-energy electrons have been performed using the experimentally determined intracellular content, distribution, and retention of the radionuclide. Our results demonstrate the importance of localized irradiation of radiosensitive targets in the cell nucleus by Auger electrons in causing cytotoxic effects. The present work is in general agreement with published studies on the radiotoxicity of other tissue-incorporated Auger-electron emitters (7,8,11–23).

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MATERIALS AND METHODS

Cell culture

Chinese hamster V79 lung fibroblasts are maintained routinely in our laboratory as monolayers. These cells have a doubling time of about 9 hr when grown at 37°C (95% air-5% CO₂) in minimum essential medium (MEM) supplemented with 15% fetal bovine serum (FBS), 2 mM L-glutamine, 0.1 mM nonessential amino acids, penicillin (5 units/ml), streptomycin (5 µg/ml), and gentamicin sulfate (5 µg/ml). The plating efficiency of these fibroblast-like cells is 60% to 80%.

Radionuclide

Chromium-51 was purchased as sodium chromate* (Na₂⁵¹CrO₄), with its specific activity varying in different lots from 200 mCi/mg to 400 mCi/mg. The desired radioactive concentration was obtained by dilution of the sodium [Cr-51]chromate in Ca²⁺-free MEM. The freshly diluted radioactive solution was sterilized by Millipore filtration (0.22 µm) before use.

Cellular uptake of Cr-51

Logarithmically growing monolayers of V79 cells were trypsinized, suspended in Ca²⁺-free MEM, and counted on a hemocytometer. Cells (4 × 10⁵) were seeded into sterile plastic t-tubes and incubated at 37°C with constant shaking. Four hours later, various concentrations of Cr-51 were added to each tube and the cells reincubated for up to 18 hr. Cell-incorporated Cr-51 activity was determined by layering 100-µl aliquots of cell suspensions over 300 µl of FBS in microfuge tubes, which were then spun for 1 min at 15,000 rpm (22,24). The radioactivity in the pelleted cells was determined in an autogamma scintillation counter.† Counting efficiencies, as determined by blotting precise volumes of a standard Cr-51 solution on filter papers, were 5% to 7%.

Retention of Cr-51

Following an 18-hr incubation with Cr-51, V79 cells were washed free from extracellular radioactivity, resuspended in Ca²⁺-free MEM, and incubated at 37°C on a roller shaker. At varying time periods thereafter (2-50 hr), the cellular Cr-51 content was determined as described above.

Subcellular distribution of Cr-51

Following an incubation (18-hr at 37°C) of 8 million V79 cells at a single radioactive concentration of Cr-51, cells were washed twice in cold, calcium-free salt solution (0.4 mM KH₂PO₄, 0.4 mM Na₂HPO₄·7H₂O, 0.74 mM MgSO₄·7H₂O, 5 mM KCl, 0.12 M NaCl), suspended in cold, hypotonic sucrose buffer (0.25 M, 3 mM CaCl₂, 50 mM Tris, pH 7.0) and kept on ice for 5 min. An equal

volume of the sucrose buffer containing 2% Triton X-100 was added while vortexing, and the nuclei isolated (25). Mitochondria were sedimented by centrifugation of the cytoplasmic fraction at 14,000 rpm for 30 min. Chromium-51 activity associated with various subcellular fractions was determined, and the radioactivity precipitated with trichloroacetic acid and/or guanidine-HCl (6 M) was measured.

Radiotoxicity of Cr-51

Toxicity of Cr-51 was determined by the colony-forming assay (7,8). Briefly, 4 × 10⁵ V79 cells were suspended in Ca²⁺-free MEM and exposed to varying concentrations of Cr-51 for 18 hr, then washed, serially diluted, and sufficient cells seeded in 25-cm² T-flasks to yield 30 to 250 colonies 6 days later. Colonies were fixed in Bouin's fixative, stained with trypan blue, air dried, and counted. The ability of single cells to form visible colonies (≥50 cells) was considered to indicate survival. The survival fractions (S/S₀) were calculated (% growth following exposure to Cr-51 over % growth in the unexposed controls) and plotted against cellular uptake.

EXPERIMENTAL RESULTS

Kinetics of cellular growth

When the V79 cells used in these experiments are grown in monolayers, they have a characteristic doubling time of about 9 hr. In the experiments described in this paper, the cells were suspended in Ca²⁺-free MEM. Under these conditions the cells exhibit a slower growth during the first few hours (Fig. 1) but resume their regular 9-hr doubling time by about 4 hr. When exposed to Cr-51, however, the increase in cell numbers slows, being related to the radioactive concentration in the medium. For example, the incubation of V79 cells at 10 µCi/ml results in the prolongation of the doubling time to about 20 hr (Fig. 1).

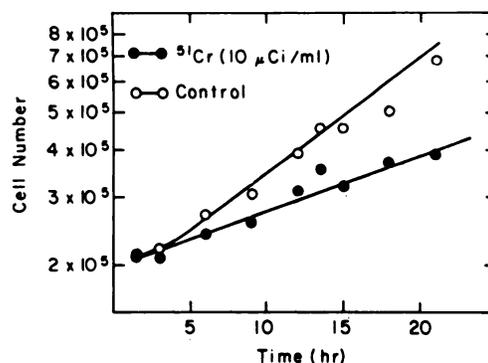


FIGURE 1
Growth of V79 cells suspended in Ca²⁺-free MEM in absence (O) or presence (●) of Cr-51 (10 µCi/ml) as function of incubation time. Each point represents mean of three samples

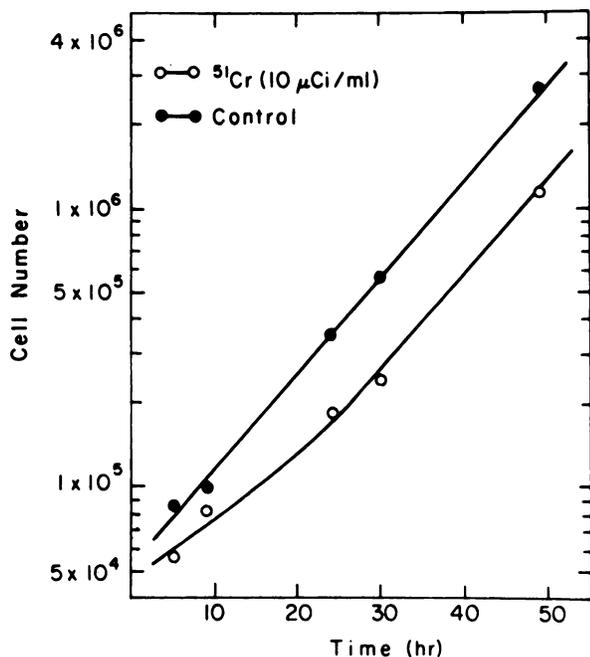


FIGURE 2
Growth of monolayers of V79 cells as function of time following 18-hr preincubation in Ca^{2+} -free MEM in absence (●) or presence (○) of $10 \mu\text{Ci/ml}$ Cr-51. Each point represents mean of three samples

Following the 18-hr incubation with the radionuclide, the cells are washed free of extracellular radioactivity and seeded into T-flasks to determine their survival. We followed cellular growth for up to 50 hr during the T-flask incubation. As expected, and illustrated in Fig. 2, unexposed cells proliferate with a doubling time of 9 hr. Cells pre-exposed to $10 \mu\text{Ci/ml}$ of Cr-51 showed a biphasic growth curve: an early phase with a doubling time of 14 hr lasting for 28-hr postincubation, and a later phase whose slope parallels that of the control, i.e., returns to a doubling time of 9 hr.

Uptake studies

The results of the incubation of V79 cells with a single radioactive concentration of Cr-51 ($20 \mu\text{Ci/ml}$) are illustrated in Fig. 3. Cellular uptake (pCi/cell) is plotted

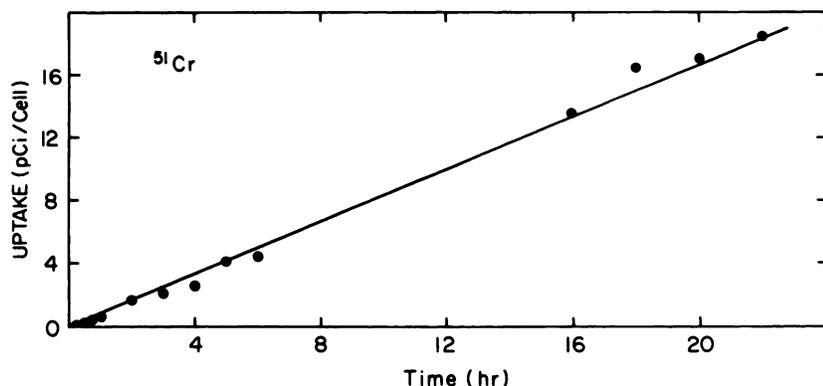


FIGURE 3
Uptake (pCi/cell) of Cr-51 by V79 cells exposed to single radioactive concentration ($20 \mu\text{Ci/ml}$), as function of incubation time (hr). Each point represents average value of three samples

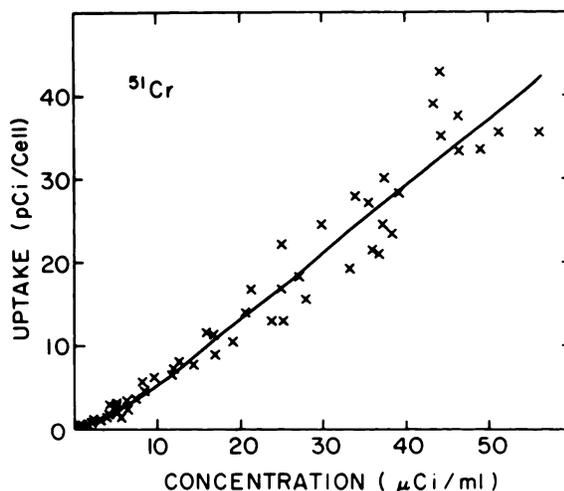


FIGURE 4
Uptake (pCi/cell) of Cr-51 by V79 cells following 18-hr incubation at various radioactive concentrations ($\mu\text{Ci/ml}$). Each point represents average value of three samples

as a function of incubation period (hr). The incorporation of Cr-51 into these cells was dependent on the length of incubation and was linear within the ranges studied.

Figure 4 shows the uptake of Cr-51 by V79 cells following an 18-hr incubation at 37°C . The uptake was dose-dependent and, except for an initial slight toe, the incorporation of Cr-51 was linear when the external radioactive concentration was in the range of 10 to $50 \mu\text{Ci/ml}$.

Retention studies

Figure 5 shows the time-dependence of the cellular radioactivity content following an 18-hr incubation in $10 \mu\text{Ci/ml}$ Cr-51. The biological half-time of this radioactivity, corrected for radioactive decay, was 14 hr during the 28-hr observation period. Since the cells during this time interval also have a doubling time of 14 hr (Fig. 2), the radionuclide must be distributed equally between each daughter cell, remaining bound throughout.

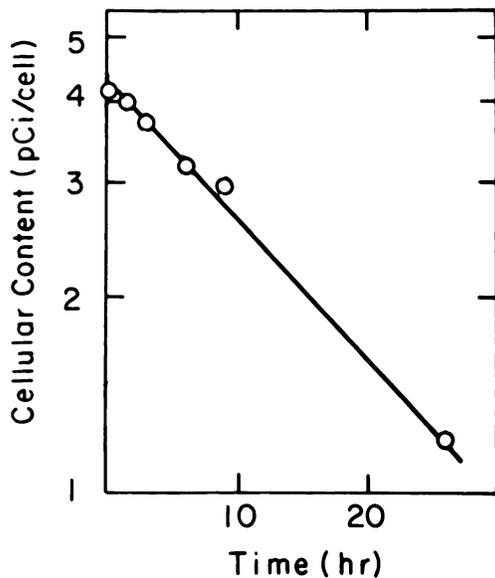


FIGURE 5
Chromium-51 content of V79 cells (18 hr in Cr-51) as function of postexposure time. Each point represents mean of three samples

Intracellular distribution

Chromium-51 content of various subcellular fractions of V79 cells following an 18-hr incubation with the radionuclide is indicated in Fig. 6. Approximately 86% of the intracellular radioactivity was present in the cytoplasm, the bulk of which (~78%) was TCA-soluble. Minimal (<0.2%) Cr-51 activity was associated with the mitochondria. Most of the activity associated with the nuclei (~14% of the total) was TCA-precipitable, but DNA-bound activity, as measured in a fraction precipitable with guanidine-HCl, was only ~14% of the total nuclear activity ($\cong 2\%$ of the whole cell activity).

The subcellular distribution of Cr-51 described above did not change within the next 24 hr. Here too, about 86% of the activity was found in the cytoplasm while the remaining 14% was located in the nucleus.

Clonal survival

The survival fractions (S/S_0) of V79 cells incubated for 18 hr at various concentrations of Cr-51 are plotted in Fig. 7 as a function of cell-incorporated activity (pCi/cell). The survival curve is characterized by a well-defined initial narrow shoulder, followed by an exponential decrease in survival. Using the mean radioactive cellular content at 37% survival as a relative index of toxicity, the D_{37} of Cr-51 in V79 is 6.2 pCi/cell (corresponding to an extracellular radioactive concentration of 10.8 μ Ci/ml). No cytotoxic effects were observed when cells were exposed to equimolar concentrations of stable sodium chromate under similar experimental conditions.

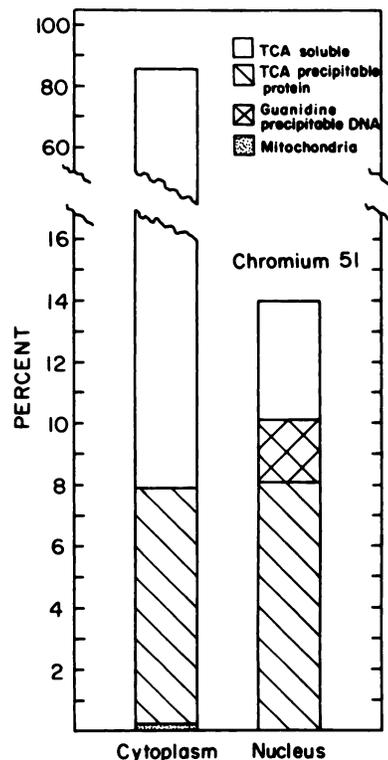


FIGURE 6
Intracellular distribution of Cr-51 in V79 cells following 18-hr incubation in single radioactive concentration

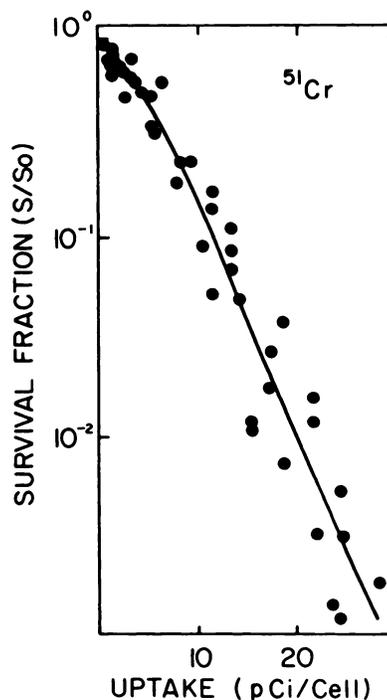


FIGURE 7
Survival of V79 cells following 18-hr incubation at various radioactive concentrations of Cr-51, as function of intracellular content of radionuclide (pCi/cell). Each point represents average value of three flasks

THEORETICAL ESTIMATES

Auger and Coster-Kronig (CK) electronic spectrum following Cr-51 decay

These electrons are the only particulate radiations emitted as a result of the EC decay of Cr-51, and a knowledge of their energies and yields is needed for dosimetric purposes. To obtain this information, the primary vacancies produced in the various atomic shells of the V51 daughter atom by the decay process are calculated using the theoretical expressions (9) for the EC decay probabilities for the various shells, the Q values (9), and the experimental atomic binding energies (26). The calculated initial vacancy distribution per 100 Cr-51 decays on the average is in the ratio

$$K/L_1/L_2/M_1 = 89.3/9.2/0.0/1.5.$$

Using (a) this information, (b) the K- and L-shell radiation transition probabilities of Scofield (27), (c) the K- and L-shell radiationless transition rates of Chen et al. (28), (d) the M-shell radiationless transition rates and energies given by Yin et al., (29), and (e) the work of Larkins (30), in which the effect of multiple electron-defect configurations on electron energies is taken into account, we have evaluated the Auger and CK electron spectrum to be expected, on the average, following the EC decay of Cr-51 in the condensed phase. The experimental electron binding energies (26) and the 'Z/(Z + 1)' rule (31) are used where necessary. Table 1, containing the average values, is a concise presentation of the complicated spectrum. The origin of each electron group is indicated. On the average, a total of about 5.4 Auger and CK electrons is to be expected from Cr-51 EC decay, with extremely short ranges in unit-density matter (32), as indicated in the last column of Table 1. The electron-range information is based on the experimental data of Cole (Table III, in Ref. 32). The K and L Auger energies and yields of Table 1 agree well with the data given by Martin and Blichert-Toft (9) within calcula-

tional uncertainties. The CK-electron data (Table 1) are not available elsewhere.

Radiation dose to the cells: Conventional estimate

The conventional dosimetry (33,34) of tissue-incorporated radionuclides is based on the simplifying assumptions that both the radionuclides and the radiation energy are uniformly distributed throughout the organ or tissue. In this approach, the average absorbed radiation dose to the cells and their nuclei is the same as the dose to the medium. In our system, cells were incubated for 18 hr in 2 ml of the cell culture medium containing varied concentrations of radioactivity in a tube of radius (r) 0.8 cm. In this geometry, the height (h) of the liquid column in the cylinder is 1 cm. Assuming that the cells are uniformly distributed during the course of the incubation, the radiation dose rate is obtained from the relation (33,34)

$$R = (2.13 \times 10^{-3}) C \sum f_i \bar{E}_i \phi_i, \quad (1)$$

where

- R = radiation dose rate (rad/ μ Ci-hr),
- C = concentration of radioactivity (μ Ci/ml),
- f_i = average yield per decay of the i^{th} radiation,
- \bar{E}_i = average energy (keV) of the i^{th} radiation, and
- ϕ_i = absorption fraction for the i^{th} radiation.

The values of ϕ_i depend on the nature of the radiation and on the geometry. For Cr-51, the Auger and CK electrons (Table 1) have very short ranges in biological matter, and $\phi_i = 1$ for the macroscopic tube geometry. The fractions ϕ_γ and ϕ_x for the 320-keV gamma photons and the 5.0-keV x-rays are estimated to be about 0.02 and 0.97, respectively (Table 2). In obtaining these values, we have used the results of Powsner and Raeside (35) and Widman and Powsner (36), who have presented the absorption fractions as a function of the

TABLE 1
Summary of Average Theoretical Auger (A) and CK Electron Spectrum Following Electron-Capture Decay of Chromium-51*

Electron group	Average energy (keV)	Yield per 100 decays	Range (μ m) in unit-density matter (32)
K _A	4.38	67	0.65
L _A	0.449	143	0.023
L ₁ CK	0.091	13	0.005
L ₁ , M ₁ CK	0.046	31	0.003
M _{2,3} CK	0.020	218	0.001
M _{CK}	0.011	53	<0.001
L ₁ , M ₁ CK	0.003	17	<0.0005

* 5.0-keV K x-rays of vanadium are also emitted with average yield of 21.7 per 100 decays.

TABLE 2
Chromium-51: Estimated Average Absorbed Dose
to Nuclei of V79 Cells (18-hr Incubation, 10.8 $\mu\text{Ci/ml}$):
Conventional Dosimetry

Radiation	Absorbed fraction (ϕ)	Dose (rad)
Electrons	1	1.5
X-ray (5 keV)	1	0.45
Gamma-ray (320 keV)	0.02	0.25
Total absorbed dose		2.2

photon energy for spherical and right-circular cylindrical geometries. The linear energy absorption coefficients (μ) in water (10) for the 320-keV and 5.0-keV photons are 0.033 cm^{-1} and 40 cm^{-1} , respectively, the corresponding absorption parameters (μR) being 0.026 and 32. The ϕ_γ and ϕ_x values given above are for a cylindrical shape with $h/r = 1.25$ for the tube geometry. Even for a spherical geometry with $r = 0.8$ cm, the absorption fractions are essentially the same. Accordingly, the precise details of our geometry are not of critical importance for our purpose. Since $\phi_x = 0.97$, we have taken ϕ_x to be unity for the low-energy x-rays in the dose calculation. Using $f_\gamma = 0.099$, $E_\gamma = 320$ keV, and the information on energies and yields for the other radiations from Table 1, we calculate from Eq. (1) a dose rate of 0.0114 rad per hr for 1 $\mu\text{Ci/ml}$ of Cr-51 in the medium containing the cells. Since the total radioactivity concentration in the tube (almost entirely in the medium) at 37% cell survival is 10.8 $\mu\text{Ci/ml}$ and the half-life of Cr-51 is very long (27.7 d) compared with the 18-hr incubation period, we obtain an absorbed dose of 2.2 rad for the cells from exposure to the radionuclide during the incubation at this concentration (Table 2). About 70% of this dose is from the electrons. Since the cells are removed from the radioactive medium and plated immediately after the 18-hr incubation, and since conventional dosimetry (33,34) does not consider radioactivity taken up by the cells during the incubation period, we conclude that the total radiation dose to the cells is only 2.2 rad according to the conventional approach. There are no chemotoxic effects due to stable chromium or to other artifacts. Yet only 37% of the cells have survived the theoretical radiation dose of 2.2 rad! In contrast, the mean lethal dose (D_{37}) of 250-kVp x-rays for the cells used in these experiments is 580 rad (7). Clearly, conventional dose calculations underestimate the actual dose considerably.

The mean radius of V79 cells is 5.1 μm (7) and the cell volume is about 560 μm^3 . With an extracellular concentration of 10.8 $\mu\text{Ci/ml}$, the average cellular uptake of radioactivity is 6.2 pCi/cell at 37% survival (Fig. 7). From these numbers we obtain an intracellular-to-extracellular concentration ratio of about 1000. The excessive intracellular localization of Cr-51 should be expected to irradiate selectively the radiosensitive sites in the cell by the short-range, low-energy Auger electrons

(Table 1). The basic inadequacy of the conventional dosimetric approach is that this aspect is not taken into account, while the Auger-electron energy is spread over macroscopic distances.

Estimation of realistic radiation dose to the cell nucleus

It is widely accepted now that the DNA in the cell nucleus is the primary radiosensitive structure. Accordingly we calculate the total amount of energy \bar{E} deposited in the cell nucleus to obtain the absorbed dose to the nucleus. This energy is given by the product of the total number of disintegrations (N_T) occurring in the cell on the average and the average energy ($\bar{\epsilon}_{\text{CN}}$) deposited in the nucleus per decay in the *same* cell:

$$\bar{E} = N_T \bar{\epsilon}_{\text{CN}} \quad (2)$$

Calculation of the quantity $\bar{\epsilon}_{\text{CN}}$ in Eq. (2) is facilitated by the work of Kassis et al. (7), who have given a generalized energy absorption curve (Fig. 2 in Ref. 7) for V79 cells using a spherical geometry for the nucleus and the cytoplasm. Assuming uniform distribution of hypothetical monoenergetic electron emitters with unit electron yield per decay, they calculated $\bar{\epsilon}_{\text{NN}}$, the average energy deposited in the nucleus per decay in the nucleus, and $\bar{\epsilon}_{\text{NCy}}$, the average energy deposited in the nucleus per decay in the cytoplasm. The details of the calculations are in the appendix of their paper (7). The values of $\bar{\epsilon}_{\text{NN}}$ and $\bar{\epsilon}_{\text{NCy}}$ are given as a function of electron energy by curves C and A, respectively, in Fig. 2 of Ref. 7. Using these results, and the Auger-electron data in Table 1, we obtain $\bar{\epsilon}_{\text{NN}} = 3.43$ keV and $\bar{\epsilon}_{\text{NCy}} = 0.21$ keV for decay of Cr-51 in the nucleus and cytoplasm, respectively (Table 3). Thus nuclear rather than cytoplasmic decay of Cr-51 contributes primarily to the nuclear dose, which is mainly due to the Auger electrons. The intracellular distribution studies presented earlier show that Cr-51 decay per cell occurs with a probability $r_{\text{Cy}} = 0.86$ in the cytoplasm, and $r_{\text{N}} = 0.14$ in the nucleus. With these weight factors, we obtain $\bar{\epsilon}_{\text{CN}} = 0.66$ keV per decay of Cr-51 in the cell.

The total number of disintegrations, N_T , in the cell in Eq. (2) is the sum of the cumulated activities \hat{A}_I and \hat{A}_{PI} during the incubation (I) and postincubation (PI) periods. The area under the uptake curve (Fig. 3) gives \hat{A}_I .

TABLE 3
Average Energy Deposited in Nucleus per Decay in
Source Region

Radiation	Source region	Av energy (keV) deposited
Electrons	Nucleus	3.43
	Cytoplasm	0.21
	Extracellular	Negligible
X, gamma	All source regions	Negligible

TABLE 4
Accumulated Nuclear Dose for D₃₇ Uptake (6.2 pCi/Cell)

Time period	Cumulated intracellular activity (pCi-hr)	No. intracellular decays	Nuclear dose (rad)
0-18 hr (incubation)	55.8	7,430	290
0-28 hr (postincubation)	94.0	12,500	488
29-56 hr (postincubation)	17.6	2,350	92
Total	167.4	22,300	870

Since the uptake per cell at the end of the 18-hr incubation is 6.2 pCi/cell for 37% survival, the contribution of \hat{A}_I to the D₃₇ value of the nuclear dose amounts to 55.8 pCi-hr (Table 4). The postincubation value \hat{A}_{PI} is calculated in two parts, since the effective half-time (T) of Cr-51 in the cells is 14 hr during time t = 0 to 28 hr, and is 9 hr thereafter, as indicated by our experimental data (Figs. 2 and 5). For t = 0 to 28 hr postincubation,

$$(\hat{A}_{PI})_1 = (A_{18})(T_1/\ln 2)[1 - e^{(-\ln 2)(t/T_1)}],$$

where

$$\begin{aligned} A_{18} &= \text{initial postincubation activity in the cell (6.2 pCi/cell),} \\ T_1 &= 14 \text{ hr, and} \\ t &= 28 \text{ hr.} \end{aligned}$$

For t = 29 to 56 hr postincubation,

$$(\hat{A}_{PI})_2 = (A_{28})(T_2/\ln 2)[1 - e^{(-\ln 2)(t/T_2)}],$$

where

$$\begin{aligned} A_{28} &= (A_{18})/4 = 1.55 \text{ pCi/cell,} \\ T_2 &= 9 \text{ hr, and} \\ t &= 27 \text{ hr.} \end{aligned}$$

The contribution from t > 56 hr after incubation is negligible. Hence we obtain $\hat{A}_{PI} = 111.6$ pCi-hr.

The total cumulated activity in the cell is 167.4 pCi-hr from the above estimates (Table 4) and $N_T = 22,300$. The total amount of energy deposited in the cell nucleus is therefore 14.7 MeV. Since the nuclear volume of V79 cells is 270 μm^3 (7), the average cumulated dose (D₃₇) received by the cell nucleus is 870 rad (Table 4). The average dose to the nucleus per decay in the cell is 0.039 rad.

DISCUSSION

This work expands our continuing efforts to understand the biological effects of tissue-incorporated Auger-electron emitters and the various physical and biophysical factors that are important for Auger-electron

dosimetry. We have already investigated the radiotoxicity of the Auger-electron emitters Se-75 (7), Br-77 (8), and Tl-201 (22) in the same V79 cell line. This communication is concerned with the uptake and radiotoxicity of Cr-51. All these studies have repeatedly demonstrated that the intracellular concentration and distribution of the radionuclide—as well as the time-dependent uptake, retention, and elimination—are essential considerations. The physical characteristics of the Auger-electron spectrum and the relative location of the radionuclide in relation to the DNA in the nucleus are additional considerations.

In estimating the D₃₇ dose to the cell nucleus, we have adopted a purely experimental approach in determining the total number of disintegrations occurring in the cell. The intracellular distribution of the radionuclide, which was obtained experimentally, is important for this purpose; that this distribution remained the same in the postincubation period was also verified. From a theoretical point of view, we have used the complete Auger-electron spectrum, the accepted range-energy relations, and energy-loss data in biologically equivalent matter (32). The estimated energy deposition in the nucleus is only weakly dependent on the assumed spherical geometry for the cell (7). As shown in Table 3, the bulk of the radiation dose derives from the short-range Auger electrons emitted from the vanadium daughter deposited within the nucleus.

For the cell line used in this study, the cumulated dose, D₃₇ = 870 rad, is larger than the mean lethal 250-kVp x-ray dose, D₃₇ = 580 rad (7), by a factor of 1.5. This difference may stem from several causes. First, it may reflect the possibility that the nuclear volume (270 μm^3) in the dose calculations might be an underestimate. This is not unreasonable, since cell nuclei become larger as cells grow to divide. Since the volume is proportional to the cube of the radius, effective increases in the nuclear radius of the order of 10-15% could account for the difference. For a given radioactive content, such changes in the nuclear dimensions have negligible effects on the total energy deposited in the nucleus by the electrons, considering their very short ranges (Table 1). In view of

this, the estimated dose to the nucleus (870 rad) may be an upper limit.

Second, the dose rate from the radionuclide is quite protracted as opposed to that from x-rays. Such prolonged exposure allows for the repair of radiation injury, especially for the low-LET components of the damage. The small shoulder on the Cr-51 survival curve suggests that there is a low-LET component to the Cr-51 injury, as previously seen with Se-75 (7) and Tl-201 (22).

Third, the heterogeneity in the intracellular distribution of Cr-51 suggests that decays need not all be equally damaging, since their effectiveness could depend upon the relative distance from the radiosensitive site(s), presumably the DNA. In this regard Cr-51 differs from Tl-201, which is distributed in the water space of the cell including nucleus (22), also from [Se-75]selenomethionine, which is incorporated into cytoplasmic proteins (7), and from Br-77 and I-125 as halodeoxyuridines, which are incorporated into DNA (8,11-21). Our subcellular biodistribution studies show that Cr-51 is heterodispersed in the cell nucleus, with 14% of the nuclear activity bound to DNA (guanidine-precipitable), 57% bound to protein (TCA- but not guanidine-precipitable), and 29% soluble.

The intracellular distribution of Cr-51 has been examined by others. Scaife and Vittorio (37), in studying the kinetics of Cr-51 uptake following x-irradiation of rat thymocytes, have reported that 62% of the radioactivity was localized in the nucleus, as contrasted with the 14% found in this study in V79 cells. Since the nuclear activity is mainly responsible for the radiotoxicity of Cr-51, one wonders whether radiolabeling cells with this radionuclide may produce certain undesirable effects that may interfere with the immunological assay systems in which these cells are being used. On the other hand, Tsang et al. (38) have been unable to detect any measurable toxicity following Cr-51 labeling as measured by vital dye exclusion (trypan blue). While the radioactive content of the leukocytes in their studies (~1 pCi/cell) is probably insufficient per se to exhibit measurable toxicity, the lack of experimental data on the intracellular distribution of Cr-51 does not allow us to draw any conclusions. This is especially important since—as shown in this and earlier studies (5,39,40)—Cr-51 does not leave the cell following intracellular incorporation, and its subcellular distribution remains constant even 24 hr after removal of all extracellular radioactivity.

Considering the interaction of Cr-51 with various subcellular constituents, the question arises as to whether the radiolabeling interferes with the metabolic activity of cells. Whereas the results reported earlier by Szabo et al. (40) did not show any alterations in protein and nucleic acid synthesis in Ehrlich ascites cells following the incorporation of Cr-51, our results with V79 cells produced a definite increase in cell doubling time. These

differences in results may be due to the higher intracellular radioactivity in the present study, the inherent differences in cell type, and/or the different biological endpoints utilized.

Watson (41) has recently raised the question as to whether alternatives should be adopted in lieu of the traditional approaches used to calculate the average radiation dose to an organ from incorporated radionuclides (33,34). In vitro (22) and in vivo (23) studies with Tl-201 following its intracellular concentration in mammalian cells have already pointed out inadequacies in the traditional macroscopic methods. That the conventional dose estimate (2.2 rad) from Cr-51 is essentially negligible compared with the recalculated dose (870 rad) derived from biophysically relevant parameters emphasizes once again the need for taking into account the microscopic distribution of the radionuclide and the energy in the cell. Clearly much work needs to be done in obtaining the necessary information for an improved dosimetry of tissue-incorporated Auger-electron emitters.

FOOTNOTES

- *New England Nuclear Corp.
- † Packard 1145.

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