Cellular Radiation Doses of Labeled Neutrophils and Platelets

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Radiation doses were calculated for human neutrophils and platelets labeled by phosphorus-32, chromium-51, gallium-67, technetium-99m, indium-111, and mercury-197. The cells were assumed to be spheres with radii of 4.87 μm and 1.07 μm, respectively, with all the radioactivity at either the center or uniformly distributed on the surface. Surprisingly high dose rates were found, due primarily to the small mass and therefore high radioactive concentration and to low-energy electrons, such as Auger electrons. Average total doses to these cells during their effective lifetime in the blood are presented.

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During the past few years, much effort has been expended in incorporating radioactive labels into platelets and leukocytes (*1–6*) for tracer kinetic studies and gamma-camera imaging. The radiation sensitivities of the various types of white blood cells vary widely and radiation damage may affect the function of some. It is important, therefore, to calculate the radiation doses for some typical cells. The nuclides phosphorus-32, chromium-51, gallium-67, technetium-99m, indium-111, and mercury-197 were chosen for these calculations since they currently show the most promise for cell labeling. We have chosen spherical shapes with radii of 4.87 and 1.07 μm to represent neutrophils and platelets, with volumes of 484 and 5.13 μm^3, respectively.

The following emissions contribute to energy deposition: a) gamma photons, b) characteristic x-rays, c) internal-conversion electrons, d) Auger electrons, and e) beta particles. First one must consider the external dose received by the cells primarily during the incubation phase of labeling. Radiation is absorbed by the cells from the surrounding aqueous medium due to radioactivity that has not been incorporated into the cell interior. This incubation usually lasts less than 1 hr. The geometry is usually simple and well defined, such as that of a test tube. The primary contributors to this dose are (a) and (b), x-rays and gamma photons (which coincidentally are those used for imaging), or the high-energy beta rays, (e), of P-32. External doses, which are not provided in this paper, can be easily calculated to ±15% by standard methods (*7*) and are usually of the order of 100 rads or less.

Secondly, one must consider internal dose, the radiation from the radioactivity deposited at the cell membrane or incorporated within the cell. The time periods of biologic interest may be the functional lifetime of the cell within the circulation or the total life within all tissues until cell death.

The photon modes, (a) and (b), are long-range processes in terms of typical cell size; and their energy deposition is dispersed widely about the point of origin. Although (a) and (b) contribute to the external dose during labeling, their contribution

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to a single cell during circulation in vivo is negligible owing to dilution in the relatively large blood volume. The major determinants of the internal dose are the low-energy electrons (many of the type (c) and (e) processes and all of type (d)). They are of short range, depositing essentially all of their energy within the cell, regardless of cell size, and have little effect on the external dose. The high-energy electrons from (c) and (e) have long ranges compared with cell size, leave the cell without depositing much energy, and contribute little to the internal radiation dose. The magnitude of this contribution, however, is a function of cell size. The internal doses are much larger than the external, and only the former have been considered in the following calculations.

Assumptions about geometric factors and radionuclide distribution. Except for P-32, which is discussed separately, we have assumed that the potential path length of an electron is equal to the radius of the cell. This is equivalent to the assumption that the cell is a perfect sphere and all the activity is concentrated at the central point. These cells, however, are not perfect spheres and change in shape. Furthermore, in most cases the activity is more or less uniformly distributed throughout the cytoplasm. For the dose component caused by the higher-energy electrons, the periphery of the cell receives less radiation than the center since the periphery is not irradiated from all sides. However, because the high-energy component is a relatively small contributor to the total dose, this factor does not lead to great uncertainties in the dose calculations. For low-energy electrons, whose range is considerably smaller than the cell diameter, all the energy is deposited within the cell. The average dose, therefore, is independent of cell size and intracellular distribution of activity, and our assumed model leads to accurate estimates. Those electrons with ranges similar to the cell dimensions cause the largest uncertainties, and the dose is then most sensitive to variations in cell size.

The major contributors to the internal radiation dose turn out to be precisely those low-energy electrons (Auger and M-shell conversion) for which the model is relatively good. Therefore our results lead to the correct average doses, but the outer rind has slightly lower doses.

Dose calculations. The absorbed dose rate, $\dot{D}$, in rads/hour is given by

$$D = C \sum_i \Delta_i \phi_i,$$

where

C = the concentration in μCi/g,

Δ_i = the equilibrium dose constant in (g-rad)/μCi-h for the i^{th} emission, and

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

For the calculation of ϕ_i, all of the nuclide is assumed to be at the center of the cell. The percentile distances introduced in MIRD Pamphlet No. 7 (*8*) have been used to calculate the absorbed fractions. The percentile distance, Xp, is defined as the distance an electron must travel to deposit p/100 of its energy. These have been tabulated for water with p's ranging from five to 90 as a function of electron energy as well as the spectrum shape for the beta emitters. Some of these percentile ranges are shown graphically in Fig. 1A.

We have calculated ϕ_i in the following manner. Let r be the radius of the cell scaled by the relative density, which we have taken to be 1.075 for this calculation. For low energy where $X_{90} \ll r$, $\phi = 1$. For intermediate energy defined by $X_5 < r < X_{90}$, $\phi = p$ for that p such that $Xp = r$. For high energies when $r < X_5$, $\phi = (r/X_5)(.05)$.

As discussed above, for the low energy electrons ϕ is very nearly independent of the distribution of activity in the cell. For the intermediate and high energy electrons, the calculated values of ϕ in our

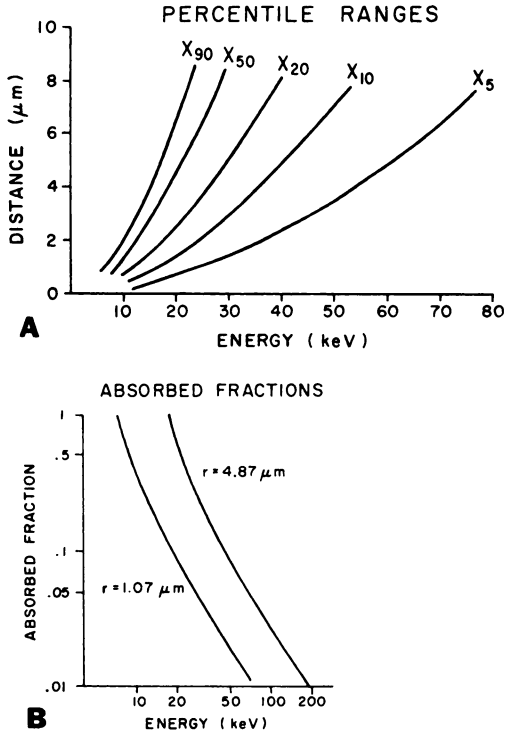

FIG. 1. (A) Percentile ranges in water as a function of energy. (B) energy dependence of absorbed fractions derived from percentile-range data, adjusted for relative density of 1.075.

model probably are overestimates by about 10–20%. However, this leads to inaccuracies of much less than 10–20%, since the high energy component of the dose is relatively small.

Figure 1B gives the dependence of the absorbed fraction so derived on energy for spherical geometries having radii of 4.87 and 1.07 μm and a relative density of 1.075.

The input data and intermediate results for a typical case, Tc-99m labeling a neutrophil, are presented in Table 1. Low-energy electrons, such as the M-shell conversion electrons of the first gamma [MIC (G1), E = 1.6 keV], stop and deposit all their energy within the cell (ϕ = 1) and account for approximately 90% of the dose. High energy electrons, such as the K shell internal conversion electrons [KIC (G2), E = 119.4 keV], deposit only a small fraction of their energy within the cell($\phi \simeq$ 0.02). The average LET for low-energy electrons (1–10 keV) is approximately 10 keV/μm, which is an order of magnitude larger than that for higher energy (approximately 100-keV) electrons. The RBE for such differences may be a significant factor in determining the radiation effects (*9*), but this is not considered in this report.

Table 2, column 3 lists the dose rates at a concentration of 100 mCi/g for P-32, Cr-51, Ga-67, Tc-99m, In-111, and Hg-197 for two cell sizes, 1.07 μm and 4.87 μm.

Phosphorus-32 incorporated in DFP labels the cell membrane and hence is distributed only at the cell surface. Consequently, the calculation of the absorbed fraction is modified slightly. Here r is replaced by 0.64 r because the average potential path through the cell of a beta particle originating on the cell surface is ~32% of the diameter of that cell. It is easily seen that half of the betas travel away from the cell depositing no energy in the cell interior. The percentile range for P-32 beta particles is given in Table 10 of Ref. *8*. The absorbed fraction $\phi = (0.64\ r \div X_5)(0.05)$, where X_5 = 144 μm and r is the density-scaled cell radius. The derived dose rate is listed in Table 2, Column 3.

An example of the use of the data in Column 3 of Table 2 is provided in Table 2 for intracellular radiation dose estimates for the six radionuclides

TABLE 1. INTERNAL DOSE CALCULATION FOR Tc-99m-LABELED NEUTROPHILS (r = 4.87 μm)

Electron emission	Number n_i	Energy E_i (MeV)	Δ (g-rads/μCi-hr)	Φ	Dose rate rads/100 mCi-hr
MIC (G1)	.9860	.0016	.0035	1.0	350
KIC (G2)	.0913	.1194	.0232	.02	46
LIC (G2)	.0118	.1377	.0034	.015	5
MIC (G2)	.0039	.1400	.0011	.015	2
KIC (G3)	.0088	.1215	.0022	.02	4
LIC (G3)	.0035	.1398	.0010	.015	2
MIC (G3)	.0011	.1422	.0003	.015	0
KLL (Auger)	.0152	.0154	.0005	1.0	50
KLX (Auger)	.0055	.0178	.0002	1.0	20
LMM (Auger)	.1093	.0019	.0004	1.0	40
MXY (Auger)	1.2359	.0004	.0011	1.0	110
				Total	~630

TABLE 2. INTERNAL DOSES AND RATES FOR SIX DIFFERENT RADIONUCLIDES USED IN CELL LABELING

Cells	Nuclide	Dose rate for 100 mCi/g (rads/hr)	Dose rate for 1 mCi in cells from 30 ml blood (rads/hr)	Effective $t_{1/2}$ in blood (hr)	Dose while circulating (rads)
Neutrophils	P-32	170	24	5.9	200
(r = 4.87 μm)	Cr-51	800	115	5.95	990
	Ga-67	1700	245	5.57	1970
	Tc-99m	630	91	3	390
	In-111	1300	187	5.5	1480
	Hg-197	3500	503	5.5	3990
Platelets	P-32	35	8.4	82	1000
(r = 1.07 μm)	Cr-51	800	193	93	25900
	Ga-67	1300	313	45	20320
	Tc-99m	520	125	5.7	1030
	In-111	885	213	42	12900
	Hg-197	2800	675	41	39940

used to label neutrophils and platelets, based on certain fixed conditions. It is assumed that all neutrophils or platelets contained in 30 ml of human blood become uniformly labeled with 100% yield after the addition of 1 mCi. The total mass of neutrophils in 30 ml of blood is calculated as 69.7 mg, the product of the number of cells (1.33×10^8), the mean density of 1.08 (*10*), and mean volume of 485 μm^3 (*11*). Likewise, the mass of platelets in 30 ml of blood is calculated to be 41.5 mg: 7.5×10^9 cells times the mean density of 1.07 (*12*) times the mean volume of 5.17 μm^3 (*12*). From these cellular masses, the intracellular concentrations C are calculated as 14.35 mCi/g for neutrophils and 24.1 mCi/g for platelets. Once C values are defined, actual cellular dose rates are calculated (Column 4, Table 2). Assuming the average biologic half-life of neutrophils to be 6 hr and of platelets 4.5 days, the intracellular radiation dose absorbed during their stay in the bloodstream is calculated (Table 2, Column 6).

DISCUSSION

The radiation dose levels listed are surprisingly high, largely because of the relatively small masses of the cells labeled, and especially high for platelets because of their longer biologic half-times and smaller mass. The uncertainty in the dose calculation for neutrophils is particularly great (of the order of 50%) because of uncertainties and variations in the volume of neutrophils (leading to uncertainty in concentration) and variations in their nuclear volume. Dr. John French of the Armed Forces Research Institute estimates the mean neutrophil volume to be only 208 μm^3 (unpublished data).

Furthermore, the neutrophil is not a perfect sphere and changes in shape. These cells have one to several nuclear segments, which can be irregularly shaped and may vary from a few micrometers in diameter down to less than one. The surfaces of these nuclear segments receive almost as much radiation as the average value. However, the central portions of the larger nuclear segments receive less. The values we have calculated are average values, but the actual dose distribution varies due to the cell inhomogeneities. This problem of inhomogeneity is not as great for platelets, since they have no nuclei.

We have specifically excluded the lymphocyte from our considerations. The lymphocyte typically has a diameter of about 5 μm. It contains a large nucleus with a diameter of about 3.5 μm. Most radionuclides used to label lymphocytes are contained in the cytoplasm. The cytoplasm itself would get most of the radiation dose for most of the processes we have discussed. The short-range electrons would deposit energy only in the outer rind of the nucleus, the inner portion receiving energy only from the longer-range electrons. It is known that the extreme radiation sensitivity of the lymphocyte is due primarily to the sensitivity of the nucleus (*7,13*). The dose to a relatively large nucleus would be very inhomogeneous and is not obtained accurately from our simple model. A statistical approach (Monte-Carlo) would be more appropriate in this case (*13*).

In conclusion, we have calculated average doses for neutrophils modeled as spheres with all the nuclide located either at the central point (for Cr-51, Ga-67, Tc-99m, In-111, Hg-197) or the surface (for P-32). Actual doses will differ because of differences in geometry and cell volume and the fact that the nuclide is not uniformly located at the central point but is distributed throughout the cell cytoplasm (or on the cell surface for P-32). Uncertainties also arise from inhomogeneities, including nuclear segments. We estimate that such factors lead to inhomogeneities as large as 50% for neutrophils. Finally, radiation sensitivity may depend not only on the average dose but on other factors such as the LET and the local dose to vital structures within the cell (*13*).

For replicating cells of the marrow with relatively large nuclei (such as lymphocytes and promyelocytes), the nucleus is the site most sensitive to radiation damage. In contrast, platelets, being devoid of nuclei, are highly resistant to irradiation and exhibit normal survival and function after doses as large as 50,000–75,000 rads (*14*). Neutrophils, being fixed postmitotic cells, are generally considered relatively radioresistant, although their radiation sensitivity has not been as thoroughly investigated.

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REFERENCES

1. McAfee JG, Thakur ML: Survey of radioactive agents for in vitro labeling of phagocytic leukocytes. I. Soluble agents. *J Nucl Med* 17: 480–487, 1976
2. Thakur ML, Lavender JP, Arnot RN, et al: Indium-111 labeled autologous leukocytes in man. *J Nucl Med* 18: 1014–1021, 1977
3. Thakur ML, Segal AW, Louis L, et al: Indium-111 labeled cellular blood components: Mechanism of labeling and intracellular location in human neutrophils. *J Nucl Med* 18: 1022–1026, 1977
4. Wistow BW, Grossman ZD, McAfee JG, et al: Labeling of platelets with oxine complexes of Tc-99m and In-111. Part 1. In vitro studies and survival in the rabbit. *J Nucl Med* 19: 483–487, 1978
5. Grossman ZD, Wistow BW, McAfee JG, et al: Platelets labeled with oxine complexes of Tc-99m and In-111. Part 2.

Localization of experimentally induced vascular lesions. *J Nucl Med* 19: 488–491, 1978

6. VAN ROOIJEN N: Labelling of lymphocytes with various radioisotopes for in vivo tracer studies: A review. *J Immun Methods* 15: 267–277, 1977
7. LOEVINGER R, BERMAN, M: A revised schema for calculating the absorbed dose from biologically distributed radionuclides. MIRD Pamphlet No. 1, revised, New York, Society of Nuclear Medicine, 1976
8. BERGER MJ: Distribution of absorbed dose around point sources of electrons and beta particles in water and other media. MIRD Pamphlet No 7, *J Nucl Med* (Suppl No 5) 12: 5–23, 1971
9. ICRU Report 16: *Linear Energy Transfer*, 1970
10. WILLIAMS N, MOORE MAS, SHORTMAN K, et al: Density separation and characterisation of human blood leucocytes, including T & B lymphocytes. *Aust J Exp Biol Med Sc* 52: 491–503, 1974
11. LICHTMAN MA, WEED RI: The monovalent cation content and adenosine triphosphatase activity of human normal and leukemic granulocytes and lymphocytes: Relationship to cell volume and morphologic age. *Blood* 34: 645–660, 1969
12. CORASH L, TAN H, GRALNICK HR: Heterogeneity of human whole blood platelet subpopulations. I. Relationship between buoyant density, cell volume, and ultrastructure. *Blood* 49: 71–87, 1977
13. 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
14. FEINENDEGAN LE, ERTL HH, BOND VP: Biological toxicity associated with the Auger effect. *Proceedings of a Symposium on Biophysical Aspects of Radiation Quality*, IAEA-SM-145/22, Lucas Heights, Australia, 8–12 March 1971; IAEA Vienna, Sept 1971
15. GREENBERG ML, CHANANA AD, CRONKITE EP, et al: Extracorporeal irradiation of blood in man: Radiation resistance of circulating platelets. *Radiat Res* 35: 147–154, 1968