Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 3556–3559, June 1982
Evolution

Intracellular potassium: ^{40}K as a primordial gene irradiator

(electron capture decay/Auger electrons/gene dosimetry/mutations)

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Contributed by Francis D. Moore, March 8, 1982

ABSTRACT **We have been interested in the possibility that the low energy electrons (Auger and Coster–Kronig) emitted after the electron capture decay of ^{40}K may have highly localized radiochemical effects on the genetic material—effects dependent upon the intracellular locus of potassium. We report here that these effects are such that the likelihood of mutagenesis by their impact on DNA is substantial. This suggests that intracellular ^{40}K has played a significant role as a mutagenic agent in evolution.**

Potassium has evolved as the predominant intracellular cation. It is present in most cells—including those of the germ plasm—at high concentrations (≈150 mM) and at a low value (≈3–5 mM) in the extracellular fluid (ECF) (1). The natural potassium mix contains the primordial radionuclide ^{40}K at an isotopic abundance of 0.0118% (2). The biologic implications of the ionizing radiations emitted in the decay of ^{40}K have been of much interest. The average gonadal dose rate has been estimated as 17 mrad/yr, or 50 μrad/day (1 rad = 0.01 gray), from the ^{40}K β-rays of average energy ($\bar{E}_\beta$) of 562 keV and as 2 mrad/yr from the penetrating 1,461-keV γ-rays (3, 4). In comparison, the cosmic-ray dose rate at sea level is about 30 mrad/yr, or 80 μrad/day (3, 5). There is an additional contribution to the radiochemical effects of ^{40}K decay—ignored or hitherto unrecognized—that stems from the orbital electron capture (EC) mode of ^{40}K decay to ^{40}Ar (4). As the inner shell vacancy in the ^{40}Ar daughter atom is filled by a complex cascade of atomic transitions [mostly of Auger and Coster–Kronig (CK) type (6)], electrons of very low energy and short range in biological matter are abundantly emitted. Accordingly, highly localized absorbed energy densities prevail around ^{40}K EC decay sites. Such events occurring in or very closely adjacent to the genetic material should be highly efficient in causing genetic damage and mutations, requiring a reevaluation of their significance in evolution.

^{40}K RADIATION CHARACTERISTICS AND TISSUE DOSIMETRY

Table 1 contains a summary of the radiations emitted, on the average, in ^{40}K decay. The basis for these estimates is presented in the legend. Because potassium is present mostly in soft tissues—which are water equivalent—the ranges of the various electron groups are given for unit density matter, based on Cole's experimental data (12). In developing the necessary dosimetric considerations, we adopt the continuously slowing-down approximation (13) taking into account the experimental range–energy relations and the variation of the rate of energy loss of the electrons as they slow down (12). The average patterns of energy deposition thus obtained agree very well with the expectation values derived from elaborate statistical calculations (14). We assume, for simplicity, that the cells are spherical with a radius of 5 μm. Recent x-ray microanalyses by electron probes reveal that the intranuclear concentration of potassium is at least as high as that in the cytoplasm (15, 16). Accordingly, we adopt a uniform value of 150 mM for the intracellular concentration (c_i) of potassium. The concentration of K in ECF (c_e) is taken to be 5 mM.

The ^{40}K β-rays with $\bar{E}_\beta$ = 562 keV and with a range of about 3 mm in biological matter are sparsely ionizing particles with a rate of energy loss of about 0.1 keV/μm (12). Therefore, they contribute a uniform average dose rate to cells and to the ECF medium, irrespective of whether ^{40}K β-decay occurs in the interior or the exterior of the cell. For a system of cells in a homogeneous tissue large enough to be considered as an infinite medium compared to the β-ray range, the equilibrium β-ray dose rate (D_β) to the tissue is given by:

$$D_\beta \text{ (in rad/day)} = (c_i f_i + c_e f_e) \cdot N_\beta \cdot \bar{E}_\beta \cdot (1.6 \times 10^{-11}), \quad [1]$$

in which f_i and f_e are the fractional volumes occupied by cells and ECF, respectively, and $f_i + f_e = 1$; the β-decay rate (N_β) is 94 disintegrations per day per cm^3 of a solution containing potassium at a concentration of 1.0 mM; $\bar{E}_\beta$ is in keV. With c_i = 150 mM and c_e = 5 mM, we have from Eq. 1:

$$D_\beta \text{ (in rad/day)} = (12.7 f_i + 0.42 f_e)\ 10^{-5}. \quad [2]$$

For an isolated cell or a sparsely populated system of cells, f_i is negligible, and the dose rate from Eq. 2 is thus only about 4 μrad/day originating largely from ^{40}K β-decays that occur in the surrounding ECF medium.

As the cell population density increases, f_i increases, as does the dose rate. Then, D_β can be far in excess of the value for isolated cells. Such a situation obtains when Eq. 2 is applied to large aggregates of single cell organisms or to densely cellular tissues, including vertebrate tissues—such as liver, kidney, endocrine glands, and gonads—both in the embryo and in the adult (17, 18). With K^+ as the predominant intracellular cation at a concentration of about 100 mM in total tissue water, we obtain f_i = 0.66 for densely cellular tissues when c_i = 150 mM (17). The β-ray dose rate to such cells in a dense matrix is about 80 μrad/day, stemming from cross irradiation of cells by β-rays largely of intracellular origin. The β-ray dose rate, enhanced by this "cellular matrix" effect, then approaches the cosmic-ray tissue dose rate (≈80 μrad/day). This effect would be lost were the potassium concentration within cells to be fixed at a low value. These estimates (f_i = 0.66) are based on cellular tissues in which the total tissue substance is comprised ≈66% of cells and ≈33% of extracellular water and solids. This is a conservative mean for densely cellular tissues. Some (e.g., liver and skeletal muscle) exceed this cellular density whereas others are slightly lower (17).

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Abbreviations: EC, electron capture; ECF, extracellular fluid; CK, Coster–Kronig; LET, linear energy transfer.

Over and above the effects of energetic β-rays in a dense cellular matrix, the Auger electrons that accompany the EC decay strongly enhance radiobiological effects traceable to the intracellular locus of potassium. The very short ranges of these low energy electrons, as shown in Table 1, indicate clearly that such emissions would have their major impact on the genetic material only when ^{40}K is present in high concentrations within the nucleus.

By contrast, EC decay events that occur in the cytoplasm or ECF would be of little importance. Considering the cell as a whole—cytoplasm plus nucleus—the average energy deposition in the cell by Auger electrons from an intracellular EC decay event is only 2.17 keV, contributing an average dose of 67 mrad per decay to the cell. The EC decay rate is 11.6 disintegrations per day per cm^3 of a solution containing natural potassium at a concentration of 1.0 mM. For cells of 10-μm diameter, with c_i = 150 mM, the EC decay probability for ^{40}K within each cell is 9.1×10^{-7} per day. Thus, the average total dose rate to the whole cell from the low energy electrons is small, only 6.1×10^{-2} μrad/day—insignificant when compared with the cosmic ray effects ($\approx$80 μrad/day) and those of ^{40}K β-rays ($\approx$80 μrad/day) in a densely cellular matrix. Such considerations have probably been responsible for the neglect of biological effects of EC decay of ^{40}K and other tissue-incorporated radionuclides in the past.

Table 1. Summary of estimated average yields and energies of radiations emitted in ^{40}K decay

Radiation	Average energy, keV	Yield per 100 ^{40}K decays	Range in water, μm
β-rays	562	89	3,200
γ-rays	1,461	11	
K x-rays	2.98	1.0	
K Auger electrons	2.70	7.4	0.32
L_2, L_3 Auger electrons	0.200	16.0	0.008
L_2, L_3 double Auger electrons	0.140	1.5	0.006
	0.025	1.5	0.0011
L_1 Coster–Kronig electrons	0.045	4.4	0.0025
	0.029	1.1	0.0015

The β-ray and γ-ray yields and energies are from Martin and Blichert-Toft (4). In evaluation of the x-ray and Auger electron yields, the primary vacancy distribution in ^{40}Ar after ^{40}K EC decay is calculated theoretically (7). For 100 EC decays, the vacancies in various shells are as follows: K, 76.2; L_1, 9.4; L_2, 0.03; L_3, 11.4; M, 2.9. The K-shell radiation yields are obtained by using a K-shell fluorescence yield of 0.115 and an Auger yield of 0.885 for Ar (6). The distribution of secondary vacancies in the L shell in the radiative and nonradiative K-shell transitions is obtained by an extrapolation of the best fits to experimental data in the range of atomic numbers 20 to 94 and theoretical transition rates (8). There are no radiative transitions in the filling of L-shell vacancies. The L_1 vacancies are almost entirely filled by Coster–Kronig transitions in which the vacancies move to L_2, L_3 subshells with the ejection of very low energy electrons from the M shell. The CK electron spectrum measured by Melhorn for Ar (9) and the estimated total L_1 vacancies (primary and secondary) are used in obtaining L_1 CK electron energies and yields. The average energy of L_2, L_3 Auger electrons is the weighted average of the experimental spectrum reported by Werme *et al.* (10). The yields are based on the final total L_2, L_3 vacancies (146 per 100 EC decays). The double Auger process occurs in the readjustment of the atom following L_2, L_3 vacancies with emission of two electrons from the M shell. According to Carlson and Krause (11), both electrons have continuous energy distributions—their total kinetic energy being 165 eV. The yields and average energies for electrons emitted in this process are based on their experimental data. The β- and EC-decay rates of ^{40}K are calculated by using the branching ratios f_β = 0.89 and f_{EC} = 0.11, $t_{1/2}$ for ^{40}K of 1.28×10^9 yr, and the current isotopic abundance (2, 4). We ignore the small contributions to the dose rate from the penetrating γ-rays of ^{40}K and the K shell x-rays of ^{40}Ar.

RADIOCHEMICAL EFFECTS OF LOW ENERGY ELECTRONS: GENE DOSIMETRY

Recent experiments involving the EC decay of ^{125}I and ^{77}Br (19–23) have shown that Auger electrons of low energy and subcellular ranges cause cytocidal effects fully as drastic as those caused by densely ionizing α-particles of high linear energy transfer (LET) if the decay occurs in the immediate vicinity of DNA or in the nuclear cell water. Using the potassium analogs, ^{201}Tl and ^{204}Tl, we have found that the low energy electrons from the EC decay of ^{201}Tl in mouse testes are much more efficient in reducing the sperm head count (i.e., more damage per decay) than are the energetic β-rays from similarly distributed ^{204}Tl (unpublished data). The remarkable biological impact of EC decay as evidenced by all these experiments is attributed to highly localized energy deposition in the radiosensitive loci of the cell nucleus by the very low energy electrons (19–23). The severe multiple DNA strand breaks (24) caused by the EC decay of ^{125}I, covalently bound to DNA, are also understandable in terms of highly localized absorbed energy densities (unpublished data).

The above experimental findings, only recently available, require a reexamination of the mutagenic efficacy of ^{40}K EC decay in the genetic material. Goodhead *et al.* have shown that low energy photoelectrons ($\approx$280 eV) are more efficient than 250-kV-peak x-rays in producing mutations in V79 Chinese hamster lung cells and in human fibroblasts (25). Their work reveals that the microscopic radiosensitive loci in the nucleus are about 7 nm (or less) in size and that about 300 eV of energy must be deposited in these sites to cause the high LET-type radiobiological effects. In the Auger electron spectrum of ^{40}K EC decay, the 200 eV L-Auger group is the most important one, with a range of about 8 nm (Table 1). Using the data from Table 1 and the range–energy relationships, we estimate the average energy deposited in a sphere of about this size to be 365 eV per EC decay. Thus, ^{40}K decay events meet the mutagenesis criterion established by Goodhead's experiments. If the base damage yields can be assumed to be about the same for the highly localized energy densities of Auger electron doses, it is clear that the EC decay events should be effective in producing base changes.

For consideration of gene dosimetry, we assume that potassium is present in cell water at 150 mM concentration (both in the nucleus and in the chromatin) and we treat the cell as consisting of two phases: the chromosomal matter and the rest of the cell. If, in a cell of 10-μm diameter there are 46 chromosomes—each with an average volume of 0.1 μm^3 (H. I. Kohn, personal communication)—we are concerned with EC decays of ^{40}K in $\approx$1% of the cell volume. An alternative calculation for DNA fraction of cell volume is based on an estimate of 6×10^{-12} g of DNA in mammalian nuclei, of an average (dispersed) density of about 1.0 (H. I. Kohn, personal communication). With cell volume estimated at 5×10^{-10} cm^3, this yields an estimate for chromosomal DNA volume at about 1% of the cell volume. Histones and other proteins closely associated with DNA, to constitute total chromatin, may enhance this value to 2–4% of the cell volume. In either event, the EC decay probability in the chromosome is at least 9.1×10^{-9} per day. When ^{40}K decays by this mode, the gene(s) in its immediate neighborhood are subjected to high densities of absorbed energy (Fig. 1). For each decay, these range from about 50 eV in a sphere of radius r = 1 nm around the site of the event to about 365 eV for a sphere of r = 8 nm and about 2.2 keV for r = 0.32 μm.

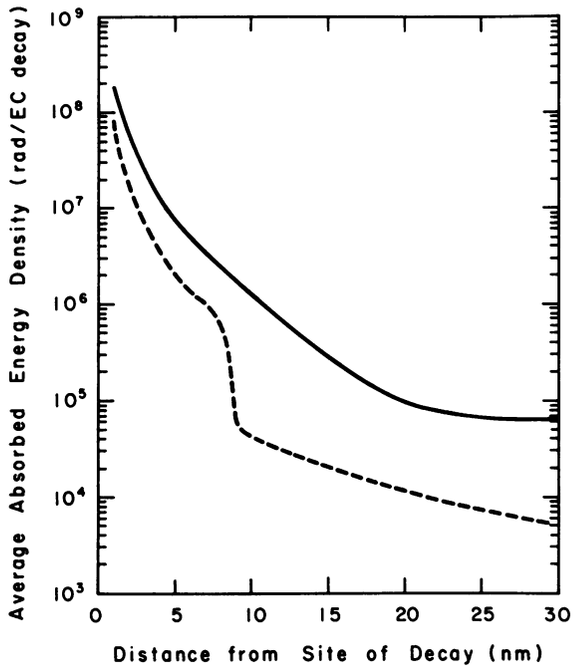

FIG. 1. Spatial distribution of average density of absorbed energy around the site of EC decay of ^{40}K. Auger electron data in Table 1 and the experimental range–energy relations (12) for low energy electrons are used in the calculations. The solid curve represents the primary absorbed energy density within concentric spheres of tissue-equivalent matter as a function of the distance r from the decay site located at the center. The dashed curve indicates the profile of differential distribution of energy density in the annular regions of the concentric spheres as a function of the radius. The width of the annulus here is taken to be 0.5 nm. The sharp decrease between r = 7 nm to r = 8.5 nm occurs at the end of the range of the dominant Auger-electron group of 0.200-keV energy. A sphere of about 8-nm radius is approximately the size of a gene (see text).

These localized energy densities—expressed as the equivalent rad dose per decay—would be 1.9×10^8 rad for a sphere of r = 1 nm, 2.7×10^6 rad for r = 8 nm, and about 260 rad for r = 0.32 μm. The region with r = 8 nm is large enough to contain 3 or 4 nucleosome units of 5-nm average radius (26) with the DNA coiled around them. With 180 DNA base pairs per nucleosome (including the linker DNA), this region contains about 600 base pairs, and it may be viewed as about the size of a gene that can code for a protein with 200 amino acids. Assuming that the molecular weight of the 200 codons is about 4.0×10^5 and using target theory based on the single-hit model of direct action (27), we estimate that a hit delivering 1.4×10^6 rad to the region would have ≈63% efficiency in damaging the structure. The Auger electrons deliver twice this dose to the region and should impact on genes even more effectively. The 365 eV of energy deposited in this region can cause 10–12 ionizations as based on the usually accepted estimate of about 33 eV per ionization (28). The range of radiochemical damage should be considerably larger than 8 nm because of migration of free radicals and indirect action of radiation. Furthermore, the very high local dose of 50 eV or 1.9×10^8 equivalent rad to a region with r = 1 nm from each ^{40}K EC decay site would cause chemical or structural changes in DNA bases or base sequences if—as is to be expected—K^+ is in close proximity to the DNA chain. Because adjacent DNA base pairs are 3.4 Å apart, such a sphere of 1-nm radius contains about 5 or 6 base pairs. With the probability of DNA base damage of 3.3×10^{-10} per rad per base pair for at least one specific type of damage of thymine (29)—and a considerably larger value if we include all possible types of molecular change—the efficacy of EC decay in close proximity to DNA, in producing DNA base changes, may be readily appreciated.

The above considerations show that each ^{40}K decay in the chromosomes should be expected to be efficient in producing mutations. In fact, the total yield of such mutations should be the probability of the decay itself in the chromosomes (9.1×10^{-9} per day), or about 1 event every 100 days in a matrix of 1 million cells. This may be an upper estimate because we have not considered repair processes or the relative genetic significance of the regions hit by Auger electrons. On the other hand, our estimate of cell volume fraction occupied by dispersed chromatin may be low. Current understanding of loci of structural changes required to produce mutations—and their distribution within the gene—is too limited to warrant more detailed estimates.

According to Kohn (30), sparsely ionizing radiations cause mutations at the rate of about 10^{-9} to 10^{-7} per rad per locus per generation. Assuming that human germ cells have about 10^5 loci (P. Rosen, personal communication), cosmic rays and ^{40}K β-rays (each with a dose rate of about 80 μrad/day) produce mutations at a rate in the range of 8×10^{-9} to 8×10^{-7} per day per cell. The mutation yield per cell ($\approx 10^{-8}$ per day) from ^{40}K EC decay is of comparable magnitude. The Auger electron effects are very efficiently produced although the yields themselves are limited by the decay rate in the immediate neighborhood of the genetic material.

Recently available techniques would make possible a laboratory verification of these expectations. Highly enriched ^{40}K/potassium mixtures can now be obtained for media enrichment in which cells could be grown as a mutagenic test system. Alternatively, cellular tissues that contain potassium could be exposed to monochromated synchrotron radiation photons just above the K-shell binding energy of potassium and thus, efficiently excite K-shell vacancies and Auger electron emissions fully analagous to those of ^{40}K. Adopting a suitable mutation-rate-standardized cellular system, such effects could be examined experimentally.

EVOLUTIONARY IMPLICATIONS

The mutagenic role of ^{40}K is thus directly traceable to the high concentration of potassium within cells, the β-ray effects stemming from cross irradiation of cells in a densely cellular matrix, and the Auger effects arising from highly localized irradiation of the gene. If cellular life began some 3.5 billion years ago (31), the ^{40}K activity at that time was about 7 times its current level. The mutagenic effects experienced by species with high intracellular ^{40}K levels [such elevated intracellular concentrations being maintained in higher species by the evolution of an enzyme such as Na^+,K^+-ATPase (32)] would therefore have been severalfold greater in that early epoch of cellular evolution than at present, whereas the cosmic ray effects were presumably constant throughout. The mutation load in germ cells from ^{40}K in primordial times would have been markedly lessened if Na^+—or a mixture of H^+ with Li^+ and Rb^+—rather than K^+ had been selected as the predominant intracellular cations. Considering that some vertebrate cells such as the erythrocytes of certain breeds of dogs and sheep (33, 34) persist with high intracellular sodium (and low intracellular potassium) concentrations, a reversal of the classic roles of sodium and potassium might have served biochemical processes that require a differential cation concentration across the cellular membrane. Natural selection, given a free choice between the two alkali metals, could have favored Na as the major intracellular cation over K, the latter having only 1/20th the cosmic abundance of Na (35,

36). The preference for K over Na by cells, taken together with a high mutation rate due to ^{40}K in early geologic epochs, makes us consider the possibility of species-adaptive advantage from this source—whatever its genesis—and to reemphasize that not all mutations may be adverse. As pointed out by Gould, "The creative process of natural selection works by preserving favorable genetic variants from an extensive pool" (37). Watson states "Too high a rate (of mutations) will so burden the species as to lead to its failure to produce viable progeny, while too low a rate will prevent the emergence of new variants necessary for survival in a changing environment" (38).

These data suggest that through its intracellular site, potassium—as the major cell cation—has participated in the process of evolution by the presence of its primordial radioisotope ^{40}K, increasing species variability through mutation.

The authors thank the following for reading this manuscript during its preparation, helpful criticism, and advice: S. J. Adelstein, A. Hastings, C. Haydock, H. Kohn, W. Haseltine, P. Moore, P. Rosen, and D. Tosteson.

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