

THE EFFECTS OF POTASSIUM 40 ON THE
MUTATION RATE IN ESCHERICHIA COLI

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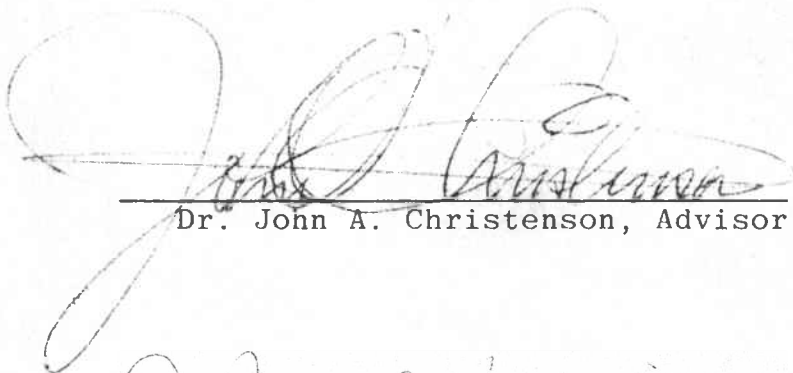
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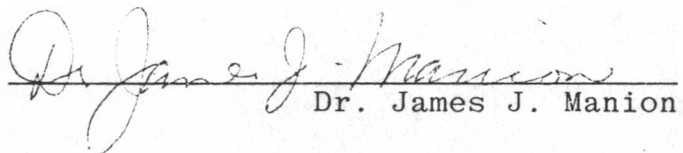
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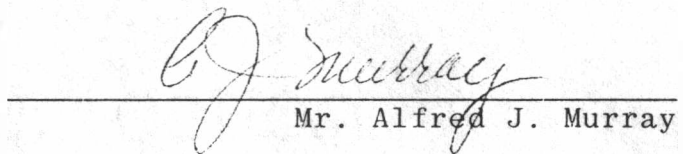
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ABSTRACT

Escherichia coli was grown in synthetic medium containing either 50 uM potassium 39 or 50 uM natural potassium. The 39K represented more than a 100 fold decrease in the radionuclide potassium 40. The cultures were then allowed to grow in a subambient radiation incubator. The mutation rates were then measured by back-mutation to valine resistance and compared.

The E. coli grown in the medium containing the natural potassium showed a substantially higher mutation rate than those grown in the potassium 39 media. This shows that the 40K in the natural potassium does play a significant role in the total mutation rate of these cells.

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INTRODUCTION

Investigators have recently become interested in the biological consequences of the decay of the primary radionuclide potassium 40 (40K).

Investigations into the decay of 40K by Auger and Coster-Kronig electrons have implicated that they may play a major role in the natural mutation rate of cells. It has been suggested that the close proximity of this radionuclide to the genetic material of all cells may have contributed greatly to the evolution of life (15).

The purpose of this investigation was to determine whether or not there actually was a significant contribution to the mutation rate by 40K. Escherichia coli was chosen as a test species as it is highly radiosensitive, it can readily be tested for mutations, and it has a low potassium requirement. The last reason was important due to the cost and scarcity of purified isotopes of potassium.

LITERATURE REVIEW

Potassium

Potassium is the seventh most abundant element in the earth's crust with an abundance of 2.5% by weight (2). As it and most of its compounds are highly water soluble, it is found mainly in aqueous environments such as sea water and brine wells. Small amounts of potassium form insoluble clays with silicon, oxygen, and aluminum (19).

Naturally occurring potassium is composed of four isotopes. The isotopes and percent abundance are listed below (16, 17).

<u>ISOTOPE</u>	<u>% ABUNDANCE</u>
POTASSIUM 39	93.08+0.04
POTASSIUM 41	6.91+0.04
POTASSIUM 40	0.0119+0.001
POTASSIUM 42	TRACE

Potassium is easily detected by mass spectroscopy and is identified by its strong line spectra at 766.5nm and 769.9nm (19).

Potassium and Escherichia coli

Potassium has evolved as the primary intracellular cation of almost all living cells including E. coli.

Its intracellular concentration may be from 20 to 50 times that in extracellular fluid (8). In E. coli, the potassium concentration is approximately 330 mM for normally growing cells (5).

Potassium is maintained at its high intracellular concentration by the action of active transport systems located in the cell membrane (8,17). In E. coli, there are at least three such systems that are independently acting. These systems are designated as TrkA, TrkD, and Kdp. The TrkA is the major transport system with regards to capacity. It has a K_m value of 1.5 mM, and its gene is located near the streptomycin resistance gene. The second system, TrkD, has a capacity around one-fifth that of the first system. Its K_m value is 0.5 mM. Its gene is located near the isoleucine-valine biosynthesis operon. The last system, the Kdp system, is a high-affinity system with a K_m value of less than 10^{-3} mM and has a capacity approximately equal to that of the second system. This transport system is normally repressed by the concentration of potassium and is only observed in potassium-starved cells. Its gene is located near the galactose operon (7).

Potassium has many functions in E. coli. Two major functions of potassium that are closely related are osmo-regulation and acid-base (or electroneutrality) regulation. Since potassium is found within the cell entirely in its ionized state (K^+), and because of its

low molecular weight, it can greatly affect the osmolality of the cell. In E. coli. it has been shown that potassium is the major controlling factor for cellular osmolality (4). Acid-base regulation is aided by the transport of potassium into the cell. The uptake of potassium into E. coli. cells is accompanied by an excretion of H⁺ ions into the surrounding medium. This process helps maintain the correct internal pH of the cells (4).

Potassium is also necessary for many enzymatic processes with E. coli. Many of these enzymes require potassium to become active. Most are kinases involved with the phosphorylation of various molecules. One such process is the transfer of a phosphate group from ATP to pyruvic acid by pyruvate kinase during glycolysis. Potassium is also required for the synthesis of proteins by ribosomes (11,17).

Potassium 40 Decay

Potassium 40 undergoes radioactive decay to form either calcium 40 (B-decay) or argon 40 (electron capture). The decay of potassium 40 arises from the disintegration of a neutron to form a proton thus yielding the 40Ca. This reaction follows the equation ${}_{19}^{40}\text{K} \rightarrow {}_{-1}^0\text{B} + {}_{20}^{40}\text{Ca}$. The electron capture of potassium 40 occurs when an electron in one of the inner shells is absorbed into the nucleus. Electron capture of potassium 40 follows the equation ${}_{19}^{40}\text{K} + {}_{-1}^0\text{e} \rightarrow {}_{18}^{40}\text{Ar} + \text{energy}$. The energy

released following electron capture can be in the form of γ -rays, x-rays, Auger electrons, or Coster-Kronig electrons.

B- particles are high energy electrons emitted from the nucleus during the transition of a neutron to a proton (3). Auger electrons are low energy photoelectrons emitted as an alternative to x-rays after the creation of a vacancy in one of the subshells by electron capture (2). Coster-Kronig electrons differ from Auger electrons in that these electrons are not emitted from the atom following electron capture. They are instead transferred between the subshells of an atomic shell. This is possible only when the subshells involved have the same principle quantum number. This results in the transition of a vacancy of an electron from one subshell to a higher one (1).

Potassium 40 is a long lived radioisotope with a half-life of 1.26×10^9 years. This yields an average of 105 disintegrations per day per cm^3 in a solution containing 0.1 mM natural potassium. Of these disintegrations, 94 result in B-decay and 11 result in electron capture (14,15). Table 1.

Table 1. Estimated average yields and energies of 40K decays. Estimations based on research done by Moore and Sastry (1982). Linear energy transfers calculated by dividing average energy by distance traveled.

Radiation	Average energy (KeV)	Yield/100 40 K decays	Range in water (um)	Linear Energy transfer (Kev/um)
B - rays	562.0	89.0	3,200.0	1.76
γ - rays	1,461.0	11.0	--	0.00
K x - rays	2.98	1.0	--	0.00
K Auger electrons	2.70	7.4	0.32	8.44
L1, L2 Auger electrons	0.200	16.0	0.008	25.00
L2, L3 double Auger electrons	0.140 0.025	1.5 1.5	0.006 0.0011	25.30 22.70
L1 Coster-Kronig electrons	0.045 0.029	4.4 1.1	0.0025 0.0015	18.00 19.30

Mutational Effects of Ionizing Radiation

Mutations are changes in the DNA of a cell by insertion, deletion, or replacement of one or more bases in a chromosome. These changes usually result from the misreading of the base sequence of the chromosome by DNA polymerase during replication (12). Radiation causes mutations either directly by ionizing one of the bases or indirectly by reacting with intermediate molecules in the cytoplasm. This reacting with other molecules leads to excitation of the molecules, formation of ions, or formation of free radicals. These intermediates can then react with the DNA causing mutations.

Mutations resulting from direct ionization of the DNA bases usually arise from one of two ways. The first is a rearrangement in the structure of the base causing it to resemble a different base. This will then be read as the other base during replication. The other way direct ionization can cause mutations is by breaking the sugar-phosphate backbone of the polynucleotide strands (12,18). Recent experiments have shown that these radio-sensitive loci of the chromosomes are about 7 nm long and that approximately 300 eV of energy must be deposited in this space to cause a mutation (6).

In the indirect method of excitation, the intermediate molecule absorbs the energy of the radiation and gives it up in the form of UV light. This UV light

can then react with pyrimidine bases in the chromosomes forming dimers. These dimers can then result in either a T-A to C-G transition or a G-C to A-T transition in the replicated DNA (12,18).

The formation of ions by radiation is the result of a deposition of energy equal to or greater than the binding energy of the electron shell. This increase in energy causes the ejection of an electron forming an ion pair. An ion pair consists of the atom which ejected the electron (positive ion) and the atom which received the electron (negative ion). The average energy needed to form an ion pair in air is 33.7 eV and is approximately the same as that needed in biological systems. These ions can then react with DNA in much the same manner as that of direct ionization.

The last method of indirect mutation is by the forming of free radicals in the cytoplasm. Free radicals are molecules which have an unpaired, nonbinding electron which is available for quickly forming a bond (19). These molecules are highly active and react readily with DNA to form mutations. Since water is the most common constituent in cytoplasm, most free radicals are those formed by water. These include hydrogen peroxide (H_2O_2), hydride radical (H^*), hydroxide radical (OH^*), and the superoxide radical (O_2^*). These are important in that they can travel through the cytoplasm and thus delocalize the area of effect of radiation (3,18).

Effect of Potassium 40 on Mutation Rate

The average total gonadal dose of radiation in man is approximately 136 urad/day at sea level. This dose consists of approximately 80 urad/day from cosmic rays, 50 urad/day from B-decay of 40K, about 5.5 urad/day from γ -decay of 40K, and trace amounts from other natural radionuclides. This means that approximately 40% of all natural radiation arises from the decay of potassium 40 (9,15).

Moore and Sastry (1982) have suggested that the B-decay of 40K in densely cellular tissues may contribute the equivalent of 80 urad/day. This arises from the cross irradiation of nuclei by the same B-particle. In sparsely cellular media (such as E. coli. in suspension culture), the dosage would remain relatively constant at 50 urad/day.

Moore and Sastry (1982) have also suggested that the electron capture and resulting Auger electrons may be more important than previously thought. The Auger electrons of most importance are the L1, L2 electrons. These electrons can deposit 365 eV of energy in a sphere the size required to cause mutations. This exceeds the experimental energy requirement of 300 eV needed for mutations to occur. This shows that if potassium 40 is in close proximity of the DNA when one of these electrons is emitted it could cause a mutation. The

mutation rate caused by this form of electron capture decay has been estimated to be approximately 10^{-8} mutations/day/cell. This is comparable to the rate caused by cosmic and 40K B-radiations (15).

MATERIALS AND METHODS

Reagents and Bacteria

Nicotinic acid, biotin, and L-leucine were purchased from Nutritional Biochemicals Co. Bacto-Dextrose and Bacto-Agar were obtained from Difco Labs. Sodium-phosphate monobasic and potassium chloride were purchased from Fisher Scientific Co. Citric acid and sodium-ammonium-phosphate were obtained from Mallinkrodt, Inc. Magnesium sulfate was obtained from American Drug and Chemical Co., ferrous sulfate from E.K. Industries, Inc., thiamine from Sigma Chemical Co., L-arginine from Vega, and calcium chloride from Matheson, Coleman, and Bell Manufacturing. Purified potassium 39 was obtained from Oak Ridge National Laboratory. All water used was doubly distilled and then deionized through a Millipore deionizer.

Escherichia coli of the strain K-12 343/113 valine sensitive was kindly provided by Ursula K. Smith of Argonne National Laboratory.

Media

The medium used was a Vogel-Bonner medium modified to be potassium free (VB K+ free). See Table 2 for

stock solutions.

The components of solution II of the trace salts solution were dissolved in 80 ml of water and then brought to a total volume of 90 ml. The two solutions were then combined to form 100 ml of solution. The VB (50X) K⁺ free and the 50% Bacto-Dextrose solutions were heated to dissolve the constituents. All stock solutions were then autoclaved for 15 min at 121° C. They were then stored at 4° C until used. The stock solutions were then combined to form the complete media and sterile potassium chloride was added. Valine agar was autoclaved for 15 min at 121° C. See Table 3 for complete media and valine agar.

The complete medium without the added potassium was analyzed by optical emission spectroscopy for potassium contamination. The medium contained approximately 4.245 ug K⁺/liter. This is the equivalent of 0.108 uM K⁺ background contamination. The final concentration of potassium in the complete medium was 50 mM. This reduced the background contamination to a negligible amount.

Procedures

Media were prepared and petri plates were poured with 10 ml of the valine agar/plate. The 39K medium was then placed in either culture tubes containing 5 ml each or steril 50-ml Erlenmeyer flasks containing

45 ml each. The nK complete medium was placed in sterile 50-ml Erlenmeyer flasks in like amounts. All containers were then aseptically sealed.

One culture tube containing 5 ml of the 39K complete medium was then inoculated from a stock slant of the valine sensitive E. coli. This was then incubated at 35° C for 24 hrs. One ml of this culture was then placed into each of two 50-ml Erlenmeyer flasks containing 45 ml 39K complete medium and nK complete medium respectively. These flasks were shaken and 5 ml of these solutions were pipeted into two sets of eight culture tubes each. One set of the 39K complete medium and one set of the nK complete medium.

Two sets were then immediately placed in an incubator in a "low radiation" room. This was done to minimize interference from background radiations. The tubes were incubated for 48 hr at 36° C. The tubes were then removed, and three valine agar plates were inoculated from each tube with 1 ml. These plates were labeled and placed in an incubator for 24 hrs at 35° C. The plates were then removed and the number of colonies on each was recorded.

The remaining cultures from each of the tubes were immediately checked for total colony forming units (CFU). The cultures were diluted 1:1 with distilled, deionized water and their absorbance of 500 nm light were then measured on a Beckman 1500 spectrophotometer. All read-

ings were measured against a solution of uninoculated nK complete media diluted 1:1 with distilled, deionized water. These readings were recorded and then converted to total CFU by the conversion factor of 3.58×10^8 CFU per absorbance unit.

This entire procedure was then repeated for five trials.

Table 2. Composition of stock solutions used in Vogel-Bonner growth medium.

VOGEL-BONNER SOLUTION (50X)

10.0 g MgSO₄ 7 H₂O
109.4 g Citric acid monohydrate
500.0 g K₂HPO₄
175.0 g Na(NH₄)HPO₄
H₂O to 1 l

VOGEL-BONNER SOLUTION (50X): MODIFIED BY HOYT AND LUCKEY - K⁺ FREE

10.0 g MgSO₄ 7 H₂O
109.4 g Citric acid monohydrate
407.5 g Na₂HPO₄
175.0 g Na(NH₄)HPO₄
H₂O to 1 l

TRACE SALTS SOLUTION

Solution I.
100.0 mg CaCl₂ 2 H₂O
H₂O to 10 ml

Solution II.
10.00 mg MgSO₄ 7 H₂O
0.025 mg FeSO₄ 7 H₂O
H₂O to 90 ml

3% L-ARGININE SOLUTION (W/V)

3.0 g L-arginine
H₂O to 100 ml.

1% NICOTINIC ACID SOLUTION (W/V)

1.0 g nicotinic acid
H₂O to 100 ml

50% BACTO-DEXTROSE SOLUTION (W/V)

50.0 g. Bacto-Dextrose
H₂O to 100 ml

Table 2. (cont.)0.1% BIOTIN SOLUTION (W/V)

0.1 g d-biotin
H₂O to 100 ml

3% L-LEUCINE SOLUTION (W/V)

3.0 g L-leucine
H₂O to 100 ml

1% L-VALINE SOLUTION (W/V)

1.0 g L-valine
H₂O to 100 ml

Table 3. Composition of complete media and valine agar.

nK COMPLETE MEDIUM (NATURAL POTASSIUM)

20 ml V-B (50X) K+ free solution
1 ml trace salts solution
10 ml 50% Bacto-Dextrose solution
1 ml 3% L-arginine solution
1 ml 1% nicotinic acid solution
1 ml 0.1% d-biotin solution
1 ml 3% L-leucine solution
3.7267 g nKCl
H₂O to 1 l

39K COMPLETE MEDIUM (POTASSIUM 39)

Same as nK complete medium except that 3.7276 g of 39KCl is substituted for the nKCl.

VALINE AGAR

15 g melted Bacto-Agar were added to 1 l of nK complete medium. To this solution was added 1 ml 1% L-valine solution.

RESULTS

Data were obtained only from the first three trials since the last two trials became contaminated. The overall mean mutation rates were 3.883×10^{-8} valine mutants/bacterium for the 39K cultures and 2.089×10^{-8} valine mutants/bacterium for the nK cultures. Standard deviations for the two cultures were 5.994×10^{-8} valine mutants/bacterium and 4.480×10^{-8} valine mutants/bacterium respectively.

Considering the 39K cultures as the expected and the nK cultures as the observed, the following statistical analysis was performed (10).

$$\begin{array}{ll} m_n = 3.883 \times 10^{-8} & m_{39} = 2.089 \times 10^{-8} \\ \sigma_n = 5.994 \times 10^{-8} & \sigma_{39} = 4.480 \times 10^{-8} \\ n_n = 96 & n_{39} = 96 \end{array}$$

STUDENT'S "T" METHOD:

$$t = \frac{\sqrt{n} |m(\text{expected}) - m(\text{observed})|}{\sigma(\text{expected})}$$

$$t = \frac{\sqrt{96} |2.089 \times 10^{-8} - 3.883 \times 10^{-8}|}{4.480 \times 10^{-8}}$$

$$t = 3.924$$

$$t(0.2\%) = 3.19$$

$$t > t(0.2\%)$$

Using this method of comparing means, it is less than 0.2% probable that these differences in mutation rate happened by chance.

DISCUSSION AND CONCLUSIONS

The 34% increase in mutation rate demonstrates that the potassium 40 in natural potassium does have an effect. This is expected since it is estimated to contribute 40% of the total natural radiation to a cell from B-particles and γ -rays (12,18).

The increase in mutation rate of the nK cultures over the 39K cultures is small since most of the cosmic radiation had been eliminated by the "low radiation" room shielding. Therefore, it is unlikely that the electron capture and resulting Auger electrons play a significant role in the overall mutation rate as had been suggested by Moore and Sastry (1982).

If cellular life emerged 3.5 million years ago (13), the potassium activity was about seven times its current level. The mutagenic effects of potassium at that time would have favored cells which maintained it in high intracellular levels. The use of potassium as an intracellular cation would have given such cells a species-adaptive advantage as they could mutate quicker than cells which did not maintain an intracellular, radioactive cation (20).

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