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Radionuclide-Induced Evolution of DNA and the Origin of Life

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Summary. Artesian groundwaters of high radionuclide concentration are ubiquitous and may have provided the large, sustained energy sources that were required to drive the multistage process of DNA and primordial cell evolution. The rapid, early development of the genetic code as well as its degeneracy can be attributed to exceptionally high radiation-induced mutation rates in this unique environment. The ability of double-strand DNA to direct enzymatic repair of radiation damage to single strands contributed importantly to its selective evolution. It is postulated that the polymerization of nucleotides took place at elevated temperatures within α-particle tracks of high ion and free-radical density, followed by rapid quenching to ambient conditions. It also is evident that radiation resistance and ploidy were important selection factors in cellular evolution.

Key words: DNA evolution — Genetic code — Groundwater radioactivity — Radiation track properties — Nucleotide polymerization — Radiation repair — Radiation resistance — Chirality

Introduction

The landmark discovery of the double helical structure of DNA (Watson and Crick 1953) has been followed by a revolutionary growth of research in molecular biology and genetics which has improved our knowledge of life processes in remarkable detail. However, it was pointedly noted by Pattee (1969) that theories of chemical evolution do not explain how the hereditary machinery could arise and evolve from reasonable initial environmental conditions. Evidence indicating the extreme improbability that self-replicating systems characteristic of the simplest biologic structures could have arisen by purely random chemical interactions was reviewed and discussed by Walton (1977), who pointed out the need for an energy source and an organizing principle which can provide for the selective development of the self-replicating system. Elsasser (1981) postulated the existence of a principle of creative selection in Nature which gave rise to the origin and stabilization of biological inheritance. What possible agents, mechanisms, and selection processes can account for the rapid and early evolution of the versatile DNA macromolecule with its universal genetic code and, with the help of special proteins, its ability to undergo precise replication and to direct radiation-damage repair? It is the purpose of this paper to present a plausible hypothesis for the evolution of DNA and primordial cells in which natural radionuclides in continental groundwater provided the energy source to produce the essential biomonomers, induce their polymerization in α-particle tracks, transform DNA structures, and provide for the selective evolution of double-strand DNA and primordial cells on the basis of their radiation resistance and life span.

In most discussions of organic synthesis on the primitive Earth, electric discharges, ultraviolet radiation, and thermal sources have been singled out as possible energy sources, with less attention given to ionizing radiation. Calvin (1969) suggested that radioactive ^{40}K was one of the important candidate sources of energy in chemical evolution. Moore and

Sastry (1982) stressed the possible importance of Auger electrons, produced by the electron capture component of ^{40}K decay, as a mutagenic agent in evolution. Dickerson (1978) included emanations from radioactive elements in surface rocks as a possible source. A more concentrated source of radioactivity, hitherto overlooked, is provided by the high concentrations of radioactive emanations and their short-lived radioactive decay products, which are widely observed in artesian aquifers. Concentrations of the radon isotopes ^{222}Rn and ^{220}Rn in wells, mineral springs, and hot springs in Bavaria, Japan, and Arkansas, reported by Kuroda and Yokoyama (1953), ranged from 10^3 to 10^6 pCi l^{-1} (37 to 3.7×10^4 Bq l^{-1}). A large number of groundwater supplies exhibit ^{222}Rn concentrations in the same range (Asikainen and Kahlos 1979; Milvy and Cothern 1989). These high radon concentrations can be attributed to the effective uptake of radon isotopes in subsurface artesian aquifers which percolate through thorium and uranium mineral deposits of high permeability. In addition, observed concentrations of soluble, hexavalent uranium in individual wells ranged up to 10^4 pCi l^{-1} (370 Bq l^{-1}).

During the initial period of cellular evolution, ~4 billion years ago, the specific activities of primordial radionuclides in rocks and minerals exceeded present levels by factors of 1.86, 51, and 1.22 for ^{238}U-, ^{235}U-, and ^{232}Th-series radionuclides, respectively. These factors were calculated using the exponential radioactive decay relationship and half-lives of 4.47×10^9 years for ^{238}U, 7.04×10^8 years for ^{235}U, and 1.41×10^{10} years for ^{23}Th (Lederer and Shirley 1978). The specific activity of ^{40}K in potassium, for a ^{40}K half-life of 1.28×10^9 years, was 8.7 times higher than present levels 4 billion years ago. Due to the ~19.7% abundance of fissionable ^{235}U in natural uranium 4×10^9 years ago, uranium mineral deposits exposed to groundwater sources were subjected to rapid physical and chemical weathering (Kuroda 1956). At that time, the $^{235}U/^{238}U$ activity ratio was ~1.28, indicating that ^{235}U and its radioactive decay products also contributed significantly to the radioactivity of artesian aquifers.

The nature of the mutations required to drive the process of DNA evolution is a key point in the argument presented here. In his critical review of Schrödinger's *What is Life?* Haldane (1945) questions the merits of the Delbrück mutation process (i.e., mutation by small temperature fluctuations) and cites the experimental evidence of Lea and Catchside (1945) which indicates "that many, if not all, lethal mutations produced by irradiating *Drosophila* spermatozoa are due to chromosome breakage followed by restitution." The induction of chromosome aberrations requires substantial energy deposition. Microdosimetric studies (Bauchinger 1983) show that a single short-track electron of ~280 eV (about 14 ionizations) is sufficient to induce a dsb (double-strand break) and a chromosome aberration of the exchange type. A minimum of about 100 eV is required for the induction of a ssb (single-strand break). The effectiveness of densely ionizing α-particle tracks for producing aberrations of chromosomes and chromatids, with an approximately linear dependence of aberration yield on radiation dose, was demonstrated in early experiments by Kotval and Gray (1947). Energy distributions for electron track entities in water and dilute solutions vs primary electron energy are described by Mozumder and Magee (1966). The more energetic electron track entities, blobs (100–500 eV) of high radical concentration and short tracks (500–5,000 eV) of high ion density are effective for inducing ssbs and dsbs in DNA structures. Evans (1977) pointed out that "few mutagens other than ionizing radiations produce single, let alone double, strand breaks in the DNA" and also reminded us "that ionizing radiations produce endonuclease sensitive sites in the DNA, which almost certainly represent base damage, which are converted into single strand breaks by the action of the cells' own endonucleolytic enzymes." In artesian aquifers of high radon isotope concentration the α- and β^--emitting radionuclides would be the primary mutagenic agents responsible for the structuring and restructuring of DNA during the course of primordial cell evolution.

The Prebiotic Setting

Of the thousands of continental groundwater sources of high radionuclide content, some may have been disrupted by comet, asteroid, and large meteorite impacts and others by volcanic activity and seismic events. At least a limited number of these sources should have survived to provide the stable physical and chemical environment required for the long-term evolution of DNA and the primordial cell. It is assumed that the setting for prebiotic organic synthesis was a warm, highly mineralized, artesian spring with ~10^3 Bq ^{222}Rn l^{-1}, a pH between 7.0 and 8.0, and temperatures between 25° and 50°C. A pH near the physiological pH of 7.4 is near optimum for most enzymatic reactions. The inactivation of most enzymes is highly dependent on pH with a zone of maximum stability between pH 5 and 8. Temperatures appreciably exceeding 50°C would be unacceptable due to the high rates of thermal inactivation of enzymes in aqueous solution, usually due to denaturation of the enzyme protein (Dixon and Webb 1979).

Constituents of some artesian aquifers include compounds of the nutrient elements—C, H, O, N, P, and S—other important cellular constituents, including the ions K^+, Na^+, Mg^{2+}, Ca^{2+}, and Cl^-, plus Fe, Zn, Mo, and other trace elements which are essential to life. The primary source of water in such aquifers is rainwater, which will contain soluble gases and a wide range of trace substances present in the primordial atmosphere, including cosmogenic radionuclides as well as biomonomers produced in the atmosphere which may have escaped photochemical destruction. Some groundwater sources also may have included an admixture of highly mineralized juvenile waters containing the reduced gases H_2, CH_4, NH_3, PH_3, and H_2S. Despite the fact that some of the essential nutrient elements supplied by the prebiotic atmosphere, artesian aquifers, and juvenile waters may have been present in reduced chemical forms, their chemistry was significantly altered within the small volumes of α-particle tracks in aqueous solution in which biomonomer production and polymerization took place. α-Particle radiolysis of water produces highly reactive oxygenic free radicals (Magee and Chatterjee 1987) which react with each other and with dissolved constituents. Thus, CH_4, CO, NH_3, and H_2S would be converted to CO_2, HCO_3^-, NO_3^-, SO_4^{2-} and other oxidized chemical species and thence to their reaction products.

Radionuclides as Energy Sources

An equilibrium mixture of ^{222}Rn and its short-lived α- and β^--emitting decay products delivers a total energy of ~20 MeV per ^{222}Rn disintegration, mainly from α-particle interactions. For a concentration of 1,000 Bq l^{-1}, ^{222}Rn and its short-lived decay products contribute 0.10 J l^{-1} yr^{-1}, which gives rise to an absorbed radiation dose of 0.10 Gy (10 rads). Because nearly 96% of this dose is contributed by α activity, the corresponding radiation dose equivalent is about 1.0 Sv (100 rem) yr^{-1}; ^{222}Rn concentrations observed in individual wells range up to 2×10^5 Bq l^{-1}, and activity levels of ^{220}Rn in some artesian waters exceed that of ^{222}Rn by factors of 7 to 15 (Kuroda and Yokoyama 1953); ^{220}Rn, with a half-life of only 56 s, will be much more highly concentrated in the immediate downstream environs of its precursors than is the 3.8-day half-life ^{222}Rn from its ^{226}Ra precursor. Thus, groundwater sources with ~10^4 Bq ^{220}Rn l^{-1} may be almost as common as those with ~10^3 Bq ^{222}Rn l^{-1}. An equilibrium mixture of ^{220}Rn and its decay products delivers ~21.6 MeV per ^{220}Rn disintegration. Thus, 10^4 Bq l^{-1} of ^{220}Rn and its decay products contribute ~1.1 J l^{-1} yr^{-1}. As noted above, 4×10^9 years ago the ^{235}U activity exceeded that of ^{238}U; thus ^{219}Rn and its decay products also contributed as sources of α- and β^--radiation in artesian aquifers. Because the long-lived precursors of ^{222}Rn, ^{219}Rn, and ^{220}Rn—^{238}U, ^{235}U, and ^{232}Th, respectively—have half-lives approaching or exceeding the age of the Earth, the long-term stability of these radionuclide sources is assured, except for those disrupted by catastrophic geological events.

Calvin (1969) and others have stressed the importance of removing the product biomonomers and polymers from the field of the high-energy sources which produced them in order to prevent their degradation. No such removal is required for the ionizing radiation source described here in which most of the radiation dose is delivered in exceedingly small α-particle track volumes and, to a lesser extent, in the secondary electron track entities of β^--particle tracks. Rapid quenching of these minute zones of high ion and free-radical density provides for the effective return of reaction products to ambient conditions without degradation. A radiation dose equivalent of only a few Sv yr^{-1}, delivered mainly in the form of high-LET α-particle radiation, does contribute to a very slow rate of degradation of single-stranded polymers of all types. This provides a unique environment for the selective evolution of double-strand DNA which can direct the enzymatic repair of radiation damage to single strands.

Production of Biomonomers

Biomonomers essential for prebiotic nucleic acid and protein synthesis were variously produced by electric discharges and by UV and ionizing radiation in the atmosphere, as well as by ionizing radiation in groundwater sources of high radionuclide concentration. In a primordial atmosphere with no protective ozone layer, biomonomers were subjected to an uncertain degree of photochemical destruction. The surviving fraction would be deposited in precipitation, concentrated by evaporation, and accumulate in artesian aquifers. Highest biomonomer production rates may have occurred in groundwaters of highest radionuclide concentration. Further concentration of biomonomers may take place on particle surfaces and in aqueous solution at sites where artesian waters percolate through clay mineral deposits.

The effectiveness of ionizing radiation in the production of amino acids and nucleotide bases and sugars has been demonstrated in early experiments by Melvin Calvin and his colleagues and others, reviewed by Ponnamperuma (1967), Calvin (1969), and Lemmon (1970). α-Particle irradiation of aqueous solutions of Fe^{2+} and ^{14}C-labeled CO_2 pro-

duced formic acid and formaldehyde, important intermediates in the synthesis of biomonomers. As Ponnamperuma pointed out, irradiation of formaldehyde in dilute, basic solution produced several sugars including ribose and deoxyribose. Sugars with highest yields were the pentoses and hexoses, a result attributable to the inherent stability of five- and six-membered rings. Electron irradiation of gas mixtures of ^{14}C-labeled CH_4, H_2, NH_3, and H_2O, some with admixtures of phosphorus, produced urea, HCN, glycine, alanine, aspartic acid, and numerous other organic compounds. Urea is an intermediate in the synthesis of the pyrimidine bases. HCN is an intermediate in the formation of adenine, guanine, and other purines by several mechanisms (Ponnamperuma 1967). Electron irradiation of aqueous solutions of CH_4 and NH_3 gave appreciable yields of adenine and dicyandiamide. The relative ease of formation of some of the nucleosides and nucleotides in the presence of phosphate has been demonstrated in a number of experimental studies. As Lemmon (1970) has pointed out, in any watery environment the predominant chemical form of phosphorous is phosphoric acid and its lower oxidation states are unstable. Other aspects of abiogenic syntheses of the biomonomers essential for nucleic acid formation, including the purine and pyrimidine bases, ribose and deoxyribose, nucleosides, and nucleotides, have been widely reviewed and discussed (Orgel 1968; Calvin 1969; Dickerson 1978).

Biomonomers which are essential for prebiotic synthesis of nucleic acids and proteins were, in part, produced in the atmosphere and deposited in precipitation and, in part, produced by ionizing radiation in artesian aquifers of high radionuclide concentration. Due to the high concentrations of oxygenic free radicals generated by the α-particle radiolysis of water, production of biomonomers and nucleotides in this environment actually takes place under oxidizing conditions. Research on the production of nucleic acid components under such conditions is incomplete. However, results of the research on abiogenic synthesis of nucleic acid components by ionizing radiation, discussed by Calvin (1969), indicate that there should be no insurmountable obstacles in the way of such production.

Early Appearance of the Genetic Code

The degeneracy of the genetic code provided the basis for the stochastic model proposed by Sonneborn (1965) who pointed out the importance of deleterious mutations in the early evolution of the code and the selective advantages of its degeneracy in which most synonyms (different codons for the same amino acid) differ in only one nucleotide. Goldberg and Wittes (1966) stressed that the code is designed not only to minimize the adverse phenotypic effects of mutation but also to provide for the increased reliability of protein synthesis. Theoretical studies also indicate that the genetic code evolved under the influence of mutations with synonyms designed to minimize adverse mutation effects (Labouygues and Figureau 1984). The fact that the code is designed to minimize the effects of mutations is consistent with its selective development during exposure to a relatively high frequency of radiation-induced mutations.

The surprisingly early appearance of life on Earth has not been explained by any of the current theories of chemical evolution. In a discussion of evolutionary aspects of degeneracy in the genetic code, Sonneborn (1965) proposed "that the code became frozen by the time that the ancestors of existing organisms had become as complex as bacteria." Rich (1965) described the remarkable universality of the genetic code and pointed out that the protein synthesis mechanism was largely stabilized at an early stage in evolution. Microfossils that resemble bacteria in remarkable detail have been found in sedimentary rocks about 3.4 billion years old (Knoll and Barghoorn 1977). An increased ^{12}C to ^{13}C ratio in sedimentary organic matter nearly four billion years ago is indicative of widespread microbial life at that time (Shidlowski 1988). The unexpectedly early appearance of primordial cells with a highly developed genetic code is less surprising if DNA, RNA, and primordial cells evolved in artesian groundwater of high radionuclide concentration, an environment which provided an exceptionally high frequency of radiation-induced mutations. Thus, if a groundwater source with a radon concentration of 10^4 Bq l^{-1} remained free of serious geological disturbances for a period of one million years, the cumulative incidence of radiation induced mutations would be about equal to that acquired during 10 billion years of Darwinian evolution at spontaneous mutation rates attributable to radionuclides and other mutagens in continental and marine surface waters.

Polymerization in α-Particle Tracks

The questions and problems associated with the prebiotic synthesis of polynucleotides have been widely discussed. The use of thermal and chemical energy sources to drive the polymerization step has been shown to be effective (Fox and Dose 1977) but

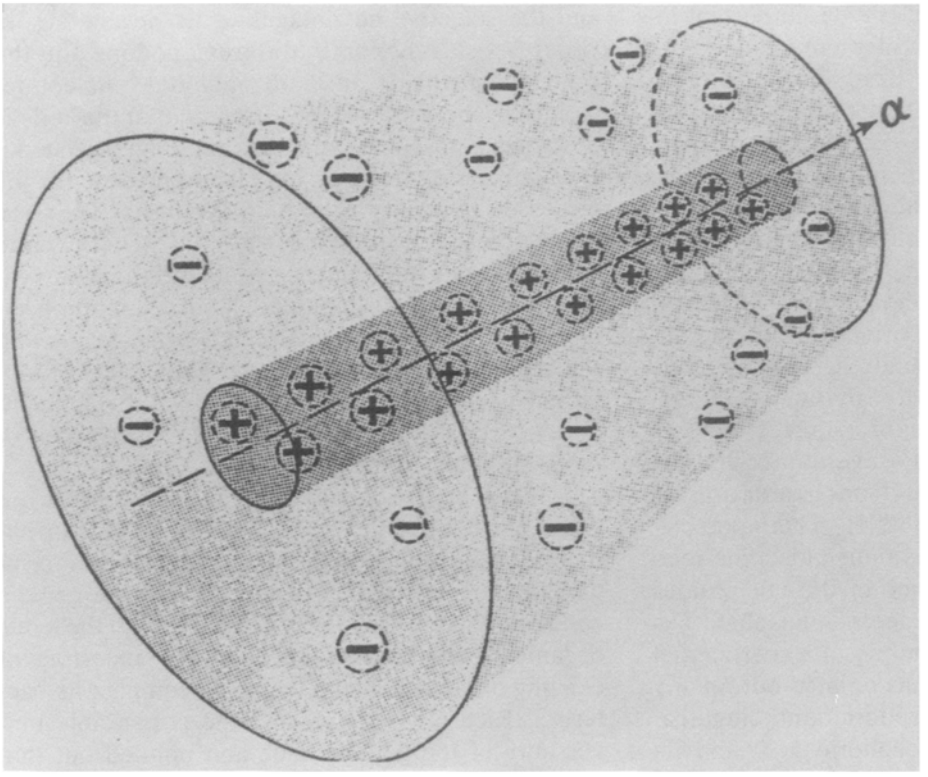

Fig. 1. Idealized schematic of a portion of an α-particle track in water $\sim 10^{-13}$ s after α-particle emission, showing positive ion core and negative ion sheath at ~3 nm radius (after Freeman 1987). The complex, asymmetric α-particle track and its change with time are described in detail by Magee and Chatterjee (1987).

would require several successive environmental changes: concentration of nucleic acid components in surface waters, evaporation to dryness, polymerization at elevated temperatures, and a rapid return to low temperatures. No such sequence of environmental changes is necessary when polymerization takes place at elevated temperatures within α-particle tracks in aqueous solution.

α-Particles are doubly charged helium nuclei of high energy which produce a dense column of ions in short, straight tracks in air, water, and tissue. The complex pattern of energy deposition in α-particle tracks in water, and time scales for thermalization, oxygenic free-radical reactions, and other processes in water radiolysis are described by Magee and Chatterjee (1987). An idealized schematic drawing of a portion of an α-particle track, with a positive ion core and a negative ion sheath, is shown in Fig. 1. This configuration is due to the rapid radial diffusion of free electrons prior to negative-ion formation. This initial separation of charge, the trapping of hydrated electrons, and the subsequent diffusion of ions inhibits the recombination of ions in α-particle tracks to a considerable degree.

Initially, the α-particle track in water produces pressurized water vapor of high temperature and high ion and free-radical density in a volume of only $\sim 2 \times 10^{-3}$ μm^3. Due to rapid radial expansion and diffusion, the track volume returns to ambient temperature within a few microseconds. Upon thermalization, the track of a 6.0 MeV ^{218}Po α-particle, with an energy equivalent of 0.96 pJ (2.3×10^{-13} calories), attains an average temperature about 100°C above ambient. It has been demonstrated that the prebiotic synthesis of polynucleotides can be carried out effectively at elevated temperatures but requires rapid transfer to quenching zones of relatively low temperature to avoid chemical destruction of reactants and products (Fox and Dose 1977). The polymerization of nucleic acid components at elevated temperatures within α-particle tracks, followed by rapid quenching to ambient temperatures, provides a uniquely suitable environment for these processes.

In early studies it was well established that α-particles and other sources of ionizing radiation are very effective in the polymerization of organic compounds in gases (Lind 1961) and in aqueous solution (Haïssinsky 1964). The α-particle irradiation of gaseous C_2H_2 and HCN produced polymers which exhibited long chain structures, with hollow fibers ranging up to 100 nm in length for the C_2H_2 polymer, cuprene. The chain structure of these polymers may be attributable to the linearity and charge distribution of α-particle tracks, illustrated in Fig. 1. Haïssinsky discusses a considerable number of α-particle-induced radiosynthesis and polymerization studies, some with surprisingly high ionic yields which are attributed to chain reactions initi-

ated by high free-radical densities. It is noteworthy that the rate of radiation-induced polymerization of monomers in aqueous solution is accelerated when low concentrations of radiosensitive organic compounds are present.

Important advantages of radiation-induced polymerization compared to its induction by chemical and thermal energy sources are discussed by Charlesby (1987). In particular, ionizing radiation provides for a very wide range of initiation rates, fewer interfering chemical decomposition products, and a minimum of adverse temperature effects. High concentrations of ions and free radicals produced by radiation interactions are very effective in the initiation and propagation of the polymerization process. As Charlesby points out, radiation-induced polymerization is surprisingly effective in dilute aqueous solutions of monomers and polymers due to the indirect action of the radiolysis products of water.

One discussion of radiation-induced polymerization of nucleotides (Fox and Dose 1977, p. 190) took note of the remarkable demonstration of γ-ray-induced polymerization of mononucleotides in aqueous solution (Contreras et al. 1962). In this study the product polymer was continuously removed from the solution by ion exchange in a cyclic process in order to avoid radiation damage to the polymeric material. However, during polymerization of nucleotides in artesian aquifers by α-radiolysis, no such cyclic process is needed because the quenched α-track energy sources conveniently remove themselves from the products.

An important problem in the synthesis of nucleic acid polymers is an effective mechanism for the removal of a molecule of water at each link in the polymer chain in an aqueous environment. Within α-particle tracks, water molecules can be removed from molecules to be polymerized by various mechanisms: evaporation, dissociation, the trapping of free electrons to form hydrated electrons, and the generation of or reaction with oxygenic free radicals. It has been noted (Dickerson 1978) that prebiological coupling reactions could be successfully carried out in aqueous solution if the molecules to be polymerized had been previously coupled to negatively charged ions, such as HPO_4^{2-}. The negative ion sheath of α-particle tracks (Fig. 1) provides a suitable environment for abiogenic synthesis of nucleic acid polymers via this suggested phosphate condensation mechanism. Whatever the dehydration mechanism, the effectiveness of abiogenic synthesis of polynucleotides by α-particle radiolysis can be determined experimentally.

As Orgel (1968), Dickerson (1978), and others have pointed out, it is difficult to explain the 3′-5′ linkage of dinucleotides in organisms because the 2′-5′ linkage and the 5′-5′ linkage appear to be favored chemically for RNA and DNA, respectively. It is suggested here that during synthesis by α-particle radiolysis, induced dipoles within unsaturated purine and pyrimidine rings may lead to a molecular orientation within α-particle tracks which gives rise to a limited yield of dinucleotides with the 3′-5′ linkage, a possibility that can be tested experimentally. Even a very limited yield of dinucleotides with the 3′-5′ linkage may be adequate. The remarkable specificity of ribozymes for selection of 3′-5′-linked substrates in the RNA chain elongation process (and, presumably, in the DNA chain elongation process as well) has been demonstrated by Young and Cech (1989), who showed that the *Tetrahymena* ribozyme selected 3′-5′-linked substrates in the presence of a large excess of dinucleotides with the 2′-5′ linkage. The development of long chains of double-stranded DNA may have proceeded by the Khorana method of gene splinting (Agarwal et al. 1970), in which the sequential addition of short, partially complementary, overlapping single-stranded segments of polynucleotides with the 3′-5′ linkage produced the longer double-stranded complex.

Radiation Resistance as Basis of Selection

In artesian groundwaters of high radionuclide content the selective evolution of double-stranded nucleic acid is to be expected due to enzymatic repair of radiation damage induced in one strand, up to and including most ssbs. The exceptional resistance of double-strand DNA to radiation-induced degradation in aqueous solution under both anaerobic and aerobic conditions has been demonstrated by Schuessler and Hartmann (1985). These authors show that it requires a radiation dose of 30 Gy (3,000 rad) delivered at high X-ray dose rates to induce a limited degree of degradation of DNA. Repair is effected because the base sequence of the undamaged strand serves as a template for the restitution of the damaged strand via the Chargaff base-pairing rules. In this manner, not only double-strand DNA but also double-strand regions of folded, single-strand RNA can undergo radiation damage repair. With the latter exception, single-stranded polymers of all types are subject to a slow rate of irreparable radiation damage by induced strand breakage.

For radon isotopes and their short-lived decay products, ~96% of the total energy deposited by charged particles is contributed by α-particles and only ~4% by β^--particles. However, due to the much greater range of energetic β^--particles, the relative frequency of β^- interactions per μm^3 vol-

ume is more than 15 times that for α-particle interactions. The high frequency of β^--interactions contributed by the decay products of radon is augmented by contributions from δ-rays (secondary electrons in α-particle tracks) and by the β^- decay of ^{210}Bi, ^{40}K, and cosmogenic radionuclides which are also present in artesian aquifers. In general, β^--particles are very effective for induction of ssbs and also induce a limited number of dsbs and chromosome aberrations by several mechanisms. α-Particles and other high-LET radiation induce dsbs in DNA, of which up to 20% are lethal, nonrejoining breaks, and the remainder undergo imperfect restitution resulting in structural aberrations (Ritter et al. 1977). The effects of ionizing radiation on DNA are discussed in detail elsewhere (Hüttermann et al. 1978; Scholes 1983).

The progressive, selective development of double-stranded DNA was driven by a high frequency of α- and β^--radiation interactions. Randomly induced changes in the base sequence provided coding for more efficient and precise replication, for increased effectiveness in radiation damage repair, and for the development of a permeable, protective membrane designed for the selective uptake and retention of essential nutrients, thus contributing progressively to the increased radiation resistance and life span of evolving DNA and primordial cells. The ability of double-strand DNA to repair radiation damage, including most ssbs, provided its unique selective advantage over single-strand polymers of all types. Mitchell (1968) classified organisms in order of increasing radioresistance, as follows: (1) viruses with single-strand DNA (or RNA), (2) viruses with double-strand DNA, (3) haploid bacteria and yeast cells, and (4) diploid cells of yeast and higher organisms. The greater radiation resistance of diploid cells of organisms is reflected in the much higher rates of radiation-induced mutation during meiosis and mitosis when cells are in the haploid state. Human tumors with stem cell lines of higher ploidy are usually more highly resistant to radiation therapy, and cell lines of some aneuploid mammalian tumors are almost immortal in tissue culture (Ohno 1971), further reflecting a radiation resistance, life-span relationship.

Later Stages of Cellular Evolution

Artesian waters emerge into springs, continental surface waters, and marine coastal waters, giving rise to widespread redistribution of primordial cells. Bacteria and other microorganisms are highly concentrated in the surface film of rising bubbles and are injected into the atmosphere as biological aerosols by breaking bubbles at water surfaces (Blanchard 1989). Transported by winds and deposited by rainfall, the early prokaryotes were redistributed in every ecological niche over the Earth's surface.

Prokaryotes deposited by rainfall over continents were, in part, recycled through artesian waters where some of them were again subjected to high rates of radiation-induced mutations for prolonged periods of time. In view of the much greater radiation resistance of eukaryotes than prokaryotes it is possible that important characteristics of the early eukaryotes, including the nuclear envelope, meiosis, and mitosis, also may have evolved in artesian groundwaters. This possibility is independent of the relative influence of symbiosis, which Margulis (1981) so eloquently defends, or of direct-filiation in the evolution of eukaryotes. Margulis suggests that mitosis evolved by a long series of mutations in protists over the better part of a billion years. In some artesian aquifers the same accumulation of mutations would require less than a million years. Cavalier-Smith (1989) classifies the earliest eukaryotes as Archezoa, which includes four phyla that lack mitochondria. *Giardia,* an anaerobe with two identical haploid nuclei and no mitochondria, has been singled out as the possible missing link between prokaryotes and eukaryotes (Kabnick and Peattie 1991). In this connection, it would be of interest to compare the radiation resistance of *Giardia* with that of haploid and diploid cells of closely similar karyotypic sequence.

Chirality of Biopolymers

In a more speculative vein it is of interest to consider another important question—that of the possible influence of α-particle radiolysis of mononucleotides on the chirality of the product biopolymers. The unique chirality of RNA and DNA, exclusively D-ribose and 2-deoxy-D-ribose, respectively, remains unexplained despite extensive research (Bonner 1988). Experimental studies have demonstrated that the oligomerization of homochiral mononucleotides proceeds readily whereas the process is inhibited for racemic mixtures of mononucleotides (Joyce et al. 1984). It is widely recognized that the homochirality of nucleic acids is essential to the replication process. One unanswered question remains. Is the fact that the sugars of DNA and RNA are right-handed rather than left-handed purely a matter of chance or is such right-handedness an unavoidable consequence of the polymerization process? On theoretical grounds Avetisov et al. (1991) propose that prebiotic evolution of homochiral polymers was initiated by a strong, mirror-symmetry breaking process which may have produced a significant excess of one enantiomer,

followed by a process of selective polymeric takeover leading to the formation of oligomers of chiral purity. It is suggested here that the required initial asymmetry may have been the result of chemically induced dynamic electron polarization (CIDEP) of radicals produced by α-particle radiolysis of dilute aqueous solutions of mononucleotides, by mechanisms described by Fessenden (1977), Patterson (1987), and briefly, as follows:

In a review of quantum-mechanical theories of optical rotatory dispersion, Condon (1937) pointed out that an increasing electric field induces corresponding increases in the magnetic moments of polar molecules, accompanied by a complex pattern of charged particle redistribution in which flowing charges may be constrained to move in helical paths. This is an apt description of the early, prethermal events which take place in α-particle tracks in a dilute aqueous system (Magee and Chatterjee 1987). The radioactive disintegration of natural α-emitters in water provides a natural source of ultrafast electron pulse radiolysis, in the subpicosecond domain. The radial redistribution of free electrons in α-tracks, with an initial electron density of $\sim 10^{19}$ cm^{-3}, sets up intense asymmetric electrostatic fields of $\sim 10^5$ V cm^{-1} (Haïssinsky 1964). This natural flash radiolysis process may produce a sufficient degree of spin-polarization of electrons and free radicals to bring about a nonequilibrium distribution of spin-state populations of different chemical reactivities. On this basis, α-particle radiolysis of aqueous solutions of mononucleotides may give rise to a limited yield of dinucleotides of 3′-5′ linkage with an excess of D-sugars. Orientation of the highly polarizable ribose and deoxyribose molecules within the negative ion sheath of α-particle tracks also may influence product yields. The complex structure of α-particle tracks in water exhibits radial energy distributions which vary markedly with distance along the α-track (Chatterjee et al. 1973), seriously complicating any detailed analysis of the physical and chemical processes involved in α-particle radiolysis. Experimental tests look more promising. Demonstration that polymerization of mononucleotides by α-radiolysis produces a limited yield of dinucleotides with 3′-5′ linkage *plus* a significant excess of D-sugars could provide a general solution for the chirality of organisms. This conclusion is dependent on the possibility, pointed out by Yarus (1988), that RNA formed of D-ribose may have a natural selective preference for binding with L-amino acids.

Summary and Conclusions

Natural radionuclides in artesian aquifers may have provided the large, sustained energy sources required for DNA and primordial cell evolution. In particular, radon isotopes and their short-lived radioactive decay products, with groundwater concentrations ranging up to 10^4 Bq l^{-1} and higher, gave rise to a high frequency of radiation interactions capable of contributing effectively to each step in the multistage process of DNA and cellular evolution.

The effectiveness of ionizing radiation in the production of biomonomers essential for the abiotic synthesis of nucleic acids and proteins has been widely demonstrated, notably in experimental studies carried out by Melvin Calvin and his colleagues at Berkeley. Biomonomers produced in the atmosphere by electric discharges and UV radiation are deposited in precipitation and accumulate in artesian aquifers. In groundwaters of high radionuclide concentration the radiolysis of dissolved constituents makes an added large contribution to biomonomer production.

It is well established that α-particles and other sources of ionizing radiation are exceptionally effective in the polymerization of organic compounds in dilute aqueous solution (Haïssinsky 1964; Charlesby 1987). It is proposed here that the abiotic synthesis of DNA was carried out by α-particle radiolysis of aqueous solutions of mononucleotides. The tracks of α-particles provide elevated temperatures and high ion and free-radical densities which drive the polymerization process, followed by rapid quenching to ambient conditions within a few microseconds, thus avoiding the thermal denaturization of reaction products. The polymerization of mononucleotides in aqueous solution by γ-rays was successfully carried out by Contreras et al. (1962). Similar experiments, using mononucleotides and radon isotopes in aqueous solution, should be carried out to test the hypothesis proposed here. Appropriate experimental conditions would include use of a ^{228}Th source to maintain a steady-state concentration of ^{220}Rn of $\sim 10^5$ Bq l^{-1}, a temperature between 40 and 50°C, and a pH near 7.4. Even a limited yield of dinucleotides with the 3′-5′ linkage characteristic of organisms would provide supportive evidence for this hypothesis.

With high rates of radiation-induced mutations as the driving force and with radiation repair and radiation resistance as important factors in the selection process, this hypothesis provides for the selective evolution of double-stranded DNA. The high frequency of radiation-induced mutations in artesian aquifers also can explain the rapid and early development of the genetic code with a degeneracy designed to minimize the adverse effects of deleterious mutations. The relative radiation resistance of various cell types is consistent with their evolutionary sequence and life span. This hypothesis would

explain the origin of life, not as a unique event, but as the unavoidable consequence of the radiolysis of aqueous solutions of compounds of the light elements and other essential nutrients under appropriate conditions over a long period of time.

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