

# A REVIEW OF CONDITIONS AFFECTING THE RADIOLYSIS DUE TO <sup>40</sup>K ON NUCLEIC ACID BASES AND THEIR DERIVATIVES ADSORBED ON CLAY MINERALS: IMPLICATIONS IN PREBIOTIC CHEMISTRY

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**Abstract.** This paper describes the possible effects of ionizing radiation arising from long-lived soluble radionuclides within clays, in particular <sup>40</sup>K, at the epoch of the emergence of life on Earth. The free dispersion of soluble radionuclides constitutes an effective *in situ* irradiation mechanism that might have acted upon adsorbed nucleic bases and their derivatives on clays, inducing chemical changes on these organic molecules. Several types of well documented reactions for radiolysis of nucleic acid bases and their derivatives are known, even at low doses (i.e., 0.1 Gy). For example, estimates with a dose rate calculated from <sup>40</sup>K from deep sea clays at 3.8 Ga ago, indicates that over a period of 1000 years the amount of organic material transformed is  $1.8 \times 10^{-7}$  moles/kg-clay.

Although ionizing radiation may also induce synthetic reactions with prebiological interest, all in all these considerations indicate that nucleic acid bases and their derivatives adsorbed on clays were exposed for long periods to degradation conditions. Such situation promotes decomposition of organic molecules rather than protection of them and enhancement of further polymerization, as it has been usually taken for granted.

## 1. Introduction

Adsorption of nucleic bases and their derivatives on clay minerals may have played an important role in primordial organic chemistry. Since its first proposal put forward by Bernal in 1951, it has been frequently argued that in such systems the prebiotic organic molecules may have been adsorbed on such mineral surfaces, concentrated, protected against ultraviolet radiation, and induce an ordered arrangement that probably favored the polymerization of such molecules.

In this article we consider *in situ* radiolytic effects of the long lived radionuclides <sup>40</sup>K, <sup>238</sup>U and <sup>232</sup>Th on adsorbed nucleic acid bases and their derivatives on clays. In particular the effect of <sup>40</sup>K, since this isotope was homogeneously dispersed in oceans and sediments.

Such approach is taken up in turn and applied to nucleic acid bases and their derivatives into several physical conditions: In plain aqueous solutions in a pH range relevant to primordial conditions; in the presence of other molecules; in frozen solutions and crystals.

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TABLE I

Estimated annual dose (Gy/year) at  $3.8 \times 10^9$  years ago at the Earth's crust

Material	$^{40}\text{K}$	$^{238}\text{U}$	$^{232}\text{Th}$
Igneous rock			
Ultrabasic	$2.7 \times 10^{-5}$	$4.73 \times 10^{-7}$	$3.95 \times 10^{-7}$
Basaltic	$5.7 \times 10^{-3}$	$4.73 \times 10^{-4}$	$3.95 \times 10^{-4}$
Granite			
High Ca	$1.73 \times 10^{-2}$	$1.42 \times 10^{-3}$	$8.4 \times 10^{-4}$
Low Ca	$2.89 \times 10^{-2}$	$1.42 \times 10^{-3}$	$1.68 \times 10^{-3}$
Syenites	$3.30 \times 10^{-2}$	$1.42 \times 10^{-3}$	$1.28 \times 10^{-3}$
Sedimentary rock			
Shales	$1.83 \times 10^{-2}$	$1.75 \times 10^{-3}$	$1.18 \times 10^{-3}$
Sandstone	$7.3 \times 10^{-3}$	$2.13 \times 10^{-4}$	$1.68 \times 10^{-4}$
Carbonates	$1.8 \times 10^{-3}$	$1.04 \times 10^{-3}$	$1.68 \times 10^{-4}$
Deep sea sediments			
Carbonates	$2.0 \times 10^{-3}$		
Clay	$1.75 \times 10^{-2}$	$6.1 \times 10^{-4}$	$6.9 \times 10^{-4}$
Seawater*	$2.5 \times 10^{-4}$	$1.42 \times 10^{-6}$	$1.98 \times 10^{-9}$

In the International System of Units, the unit for absorbed dose is called a gray (Gy) and it is defined as an energy deposition of 1 J/kg.

Besides 1 Gy = 1 J/kg = 100 rad.

\* Data from Eisenbud, 1973.

We finally arrive to several conclusions that suggest that prebiotic clay minerals might not be such a safe place that is commonly assumed, due to the radiolytic activity of  $^{40}\text{K}$  within clays.

## 2. Radiation Doses in the Early Earth

In this section we evaluate the radiation doses on the primitive Earth arising from long lived radionuclides as  $^{40}\text{K}$ ,  $^{238}\text{U}$  and  $^{232}\text{Th}$ . These isotopes have a half life as long as  $10^9$  years or more and their present concentration makes it possible to calculate, by backward extrapolation, their concentration and associated radiation doses on the primeval Earth. These calculations will be done on different physical environments of interest to prebiotic chemistry as for example clays, sedimentary rocks, igneous rocks and plain sea water.

Thus, we will first establish the primitive activity of such radionuclides on the basis of the more recent determinations of their concentrations (Klement, 1982). Secondly, the available time for radiation exposure will be estimated by the time interval fixed by the impact constraints on the primitive Earth (Oberbeck and Fogleman, 1989, 1990) and the accepted time for the origin of life at 3.8 Ga (Giga

annum) before present, derived from the isotopic record from carbon in sedimentary rocks (Schidlowski, 1988, 1993).

In order to evaluate the energy deposited by  $^{40}\text{K}$ ,  $^{238}\text{U}$  and  $^{232}\text{Th}$ , we will take into account the energy liberated per one decay. Calculations of the dose rate values were estimated in a similar way by Draganic *et al.* (1991).

In Table I we show annual doses at 3.8 Ga ago from different sources. Further, it has been considered that the geological structure of the Earth and the elemental composition have not change since 3.8 Ga ago (Fox and Dose, 1977).

Potassium is widely distributed throughout the Earth and is likely to be concentrated in the major rock types. It has higher concentrations in acidic igneous rocks and sedimentary aluminosilicates.  $^{40}\text{K}$  has a natural abundance of 0.0117%; a half-life of  $1.2778 \times 10^9$  years, and it decays to  $^{40}\text{Ca}$  by beta particles (89.33%) with 1.325 MeV and to  $^{40}\text{Ar}$  by electron capture (10.67%) emitting a characteristic gamma photon with an energy of 1.46075 MeV (Lederer and Shirley, 1978). Despite its very small isotopic concentration,  $^{40}\text{K}$  plays an essential part in the heat production of the Earth, because it is more abundant than other members with long half-lives. Its natural abundance on the primitive Earth's crust 3.8 Ga ago was around 8 times higher than in the present.

In the actinide series, thorium and uranium are the only naturally occurring elements in the Earth's crust. Acid rocks usually contain more thorium and uranium than mafic rocks. In sedimentary rocks these elements are likely to be more concentrated in argillaceous deposits than in sandstones and limestones (Kabata-Pendias and Pendias, 1986).

Thorium-232 has a half-life of  $1.41 \times 10^{10}$  years, it has a 100% natural abundance, and its decay mode is by alpha particles of 4.016 MeV (77%), 3.957 MeV (23%), and 3.834 MeV (0.2%). Uranium-238 a natural abundance of 99.275%, and half-life  $4.4683 \times 10^9$  years, alpha particles are emitted during its decay with energy of 4.196 MeV (77%) and 4.149 MeV (23%) (Lederer and Shirley, 1978).

It is usually assumed that potassium-40 contributes almost as much energy as the three natural radioactive families toward maintaining the Earth's thermal conditions. Its relative contribution was presumably even more important in the early stages of the Earth's development, some 3 or 4 thousand million years ago.

We calculate that the energy delivered in the prebiotic epoch 3.8 Ga ago from  $^{40}\text{K}$  in clays was  $1.75 \times 10^{-2}$  Gy/year (Gy = 1J/kg). Later on, we will use this figure to evaluate the amount of organic material degraded. We will usually neglect the contribution of the radionuclides thorium and uranium as it is a minor contribution in comparison to that of  $^{40}\text{K}$  (Table I).

### 3. Extent of Adsorption of Nucleic Bases and Their Derivatives on Clay Surfaces

In this section we will mention briefly the more important factors on which depends the extent of adsorption of nucleic bases and some of their derivatives on clay mineral surfaces (specially montmorillonite). Among them: (a) initial equilibrium concentration of the adsorbate in the bulk solution; (b) the nature of the adsorbed molecule; (c) the type of clay mineral and cations associated with it; (d) the pH of the bulk solution; (e) coadsorption phenomena and (f) the degree of polymerization of the organic monomers. We will consider one at a time.

#### 3.1. RELATIVE ADSORPTION VERSUS THE EQUILIBRIUM CONCENTRATION IN THE LANGMUIR EQUATION

Consider the ratio of the substance adsorbed  $a$  and the equilibrium concentration in the bulk solution  $c$  in the Langmuir equation. The application of elementary calculus methods in this function shows that this function is a convex one in  $[0, \infty]$ .

As a result of this analysis we realize that the percentage of binding of a given substance is *variable* and that it depends on the initial equilibrium concentration  $c$  in the bulk solution. The lower is  $c$ , the *higher* would be the percentage binding  $a/c$ . Conversely, the higher is  $c$ , the *lower* would be its percentage binding. An experimental confirmation of this behavior is found in the literature (Ferris *et al.*, 1988).

This situation evidences the relativity of reported data on percentage binding and also makes it difficult to compare experimental results from different laboratories, as they use various initial concentrations in the bulk solutions, ranging from  $\mu\text{M}$  to  $\text{mM}$  (the latter being more common).

#### 3.2. CONCENTRATION FACTOR OF NUCLEIC BASES ON MONTMORILLONITE

One important reason to propose clays as relevant sites for prebiotic chemistry is their capacity to concentrate organic molecules (Bernal, 1951). In this section we will comment on this respect and express the extent of concentration as a *concentration factor*, i.e., as the ratio of calculated concentration of the adsorbate in the interstitial space in the clay to the concentration of the same substance in the bulk solution. We will restrict our calculations to montmorillonite and use a standard specific surface area of  $700 \text{ m}^2/\text{g}$  of clay and a interstitial spacing in the  $c$  axis of  $5 \text{ \AA}$ . Thus, by introducing a factor of  $1/2$  in the calculation of the volume to take into account that in an interstitial space there are *two* internal surfaces, we can calculate the internal concentration in the interlamellar volume of a given quantity of clay. When applied these parameter values to the adsorption of  $3'$ AMP to  $200 \text{ mg}$  of montmorillonite (Ferris *et al.*, 1988) there are about  $18.15 \mu\text{mol}$  of  $3'$ AMP adsorbed in a internal volume of  $0.035 \text{ cm}^3$ . So, there is an internal

concentration of about 0.52 M from an initial bulk solution concentration of 0.025 M, representing a concentration factor of about 20. We notice, however, that the majority of the substance (63%) remains in the bulk solution and does not enter the interstitial space of montmorillonite. Thus, such partition excludes more than half of the substance from the concentration and presumed protection effects suggested by Bernal (1951).

Other examples can be worked out from the literature. Let us calculate particular concentration factors at two extreme values of relative adsorption (at 10% and 95% of adsorption). The former case may correspond, for example, to the adsorption of cytidine on  $\text{Na}^+$ -montmorillonite, at pH 7.5, from a bulk solution concentration of 1 mM (Lailach *et al.*, 1968a). Using the same specific surface area and interlamellar dimension, an analogous calculation leads to a concentration factor about 140. However, notice again that 90% of the organic substance remains outside the interstitial volume of the clay. In the latter case, i.e. 95% adsorption, it may correspond to the adsorption of cytosine on  $\text{Fe}^{3+}$ -montmorillonite, at the same concentrations as before and pH 6 (Lailach *et al.*, 1968b). The calculated concentration factor is about 1300.

Analogous concentration factors may be calculated, ranging from one to three orders of magnitude. However, the most common situation which derives from this review is to have a rather poor adsorption percentage of  $\leq 30\%$  (with 70% of the substance remaining in solution); and very rare situations in which it is observed a high percentage binding of  $\geq 70\%$ .

### 3.3. pH OF BULK SOLUTION AND EXTENT OF ADSORPTION OF NUCLEIC BASES AND THEIR DERIVATIVES ON CLAY SURFACES

Before proceeding we should first clarify aspects related to the pH of the bulk solution used in adsorption experiments, as it is related to expected prebiotic conditions. An obvious environment for prebiotic experiments has been considered to be sea water. The pH of the oceans seems to be in the range  $8.1 \pm 0.2$  and it has not changed very much during the last  $10^8$  years. Its constancy appears to be attributed to aluminosilicate minerals equilibria (Sillén, 1967). However, it has been suggested that the early ocean had a slightly lower pH originated by a higher carbonic acid concentration in the primitive ocean (Walker, 1985). This situation is due to an early Earth without extensive continents. The author speculates on a primitive oceanic pH around 6. However, it is remarked that more precise statements are not possible at the moment, in which it is lacking a better understanding of the processes that control oceanic pH. It is further noticed that the Earth's continents occurred some time after the formation of the Earth, with a period of most rapid growth between about 3 to  $2 \times 10^9$  years ago. So, in this work we will then assume that the pH of the aqueous media about the time of the emergence of life was surely below 8, i.e. close to pH 6.

On the other hand, a review of data on adsorption (mainly in relation to montmorillonite, see Lailach *et al.*, 1968a, 1968b) shows that for the majority of nucleic acid bases the adsorption is largest in the acidic range (i.e. around  $1 \leq \text{pH} \leq 5$ ) and then it *decreases* steadily to a very low adsorption level at  $7 \leq \text{pH} \leq 8$ . In some cases it drops from an adsorption percentage in the range 90–60 (in  $1 \leq \text{pH} \leq 5$ ) to almost null adsorption (in  $7 \leq \text{pH} \leq 8$ ).

Nevertheless, there are several natural situations that attain stronger acidic conditions and thus may improve adsorption binding. For instance the acidity of clay minerals increases as the mineral is dried (Hirschler and Schneider, 1961; Mortland and Raman, 1968; Frenkel, 1974). Further, the influence of the exchangeable cation also plays a role.

These considerations have been applied to fluctuating systems where hydration and dehydration take place in a cyclic manner (Lahav and Chang, 1976). Another possibility is the existence in the prebiotic environment of alternating inputs of acidic fumarolic gases into clay beds, which were exposed also to inputs of organic molecules, and then a subsequent increase of pH by a hydration process (Banin *et al.*, 1985). In the former case it is argued that on the addition of water, desorption takes place and the molecules are redistributed in the system. However, the extent of such redistribution mechanism has not received experimental support.

It should be remarked that a general disadvantage of the preceding models is that they have to resort to very limited and peculiar environments which would develop locally and sparingly. Anyhow, if the local models can be proved to be important to life's origins, they would reinforce the proposal that the polymerization phenomena linked to the emergence of life had to be of an extremely biased character (Mosqueira, 1988).

In this work, then, we will report adsorption data *only* in the 6–8 pH range, which appears to be of prebiotic relevance, because it represents the pH of the more prevalent primitive aqueous environment.

#### 3.4. THE EFFECTS OF INTERSTITIAL CATION AND KIND OF MINERAL SURFACE ON THE EXTENT OF ADSORPTION OF NUCLEIC BASES AND THEIR DERIVATIVES

A review of the literature on these aspects shows indeed a dependence on the extent of adsorption of nucleic bases and their derivatives in respect to the nature of surface mineral and interstitial cation.

An interesting question arises from experiments in which several interstitial cations are used. Some of them are more effective to adsorb organic molecules than others. This situation leads us to examine the relative abundance of these cations in nature. In this perspective, we should expect, *grosso modo*, that the more abundant soluble cation would induce correspondingly the formation of the more abundant interstitial cation montmorillonite. This situation will give us in turn an important clue about the predominance of the cation-montmorillonite couple in the prebiotic environment. A representative abundance to look for is soluble cations in

TABLE II  
 Relative abundances of some soluble cations in contemporary sea water, with respect to Co (and Ni)  
 (adapted from Emsley, 1990)

Soluble cation	Na <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	K <sup>+</sup>	Li <sup>+</sup>	Fe <sup>3+</sup>	Zn <sup>2+</sup>	Cu <sup>2+</sup>	Mn <sup>2+</sup>	Co <sup>2+</sup>	Ni <sup>2+</sup>
Absolute concentration (ppm)	10600	1300	400	380	0.2	0.01	0.01	0.003	0.002	0.0005	0.0005
Relative concentration with respect to Co <sup>2+</sup> (and Ni <sup>2+</sup> )	$2.12 \times 10^7$	$2.6 \times 10^6$	$8 \times 10^5$	$7.6 \times 10^5$	400	20	20	6	4	1	1

seawater (contemporary). It is in this form that it may associate with a given clay surface in the largest combined reservoir of water and clays. In Table II it is shown the relative abundance of some soluble cations in sea water (Emsley, 1990).

It is clear from Table II that the more prevalent types of montmorillonites will be those linked to  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{K}^+$  cations. The other montmorillonite abundances are expected to be totally marginal.

Several studies in the literature are performed with cations that show a fairly good extent of adsorption, but those cations are rather rare in the oceanic medium. For example, notice that according to Table II  $\text{Na}^+$ -montmorillonite is expected to be about  $10^6$  times more abundant than  $\text{Zn}^{2+}$ -montmorillonite (or  $\text{Fe}^{3+}$ -montmorillonite). Other soluble cations, that is  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$ , are still less abundant than  $\text{Zn}^{2+}$  (or  $\text{Fe}^{3+}$ ) (Table II).

However, two factors should be also taken into account that counteract this trend: (a) The greater facility of polyvalent cations to displace monovalent cations, and (b) Particular prebiotic conditions that might have improved the solubility of presently rare cations in the oceanic environment. For example, it has been pointed out that in the early Precambrian the absence of oxygen and the carbon dioxide rich atmosphere, enhanced the solubility of iron, and possibly permitted the formation of iron-rich montmorillonite (Eberl, 1986; Harder, 1988).

Another important remark should be outlined here. It is related with the internal structure of clays. Montmorillonite and the like mineral surfaces may harbor internally interstitial cations due to its particular geometrical arrangement, specially  $\text{K}^+$ , and of most interest its long life radioactive isotope  $^{40}\text{K}$ . However, there are other mineral surfaces susceptible of adsorption with no internal surfaces, as for example gypsum which is an hydrated calcium sulphate ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) (Lazard *et al.*, 1987, 1988), which has been used to adsorb nucleotides. This situation illustrates quite nicely a difference among mineral surfaces in respect to their capability to receive and store (or not) harmful radionuclides within their structure.

### 3.5. COADSORPTION. SYNERGISTIC OR ANTAGONISTIC EFFECTS OF OTHER ORGANIC MOLECULES AND/OR IONIC STRENGTH ON THE EXTENT OF ADSORPTION OF NUCLEIC BASES AND THEIR DERIVATIVES

Up to this point we have referred to adsorption phenomena involving very simple systems constituted by a mineral surface and a single nucleic base or some derivative molecule. We will now make some general remarks concerning coadsorption results.

A review of this aspect in the literature, which is very scarce, shows that indeed the presence of other molecules besides the adsorbate leads both to an increase or decrease in the extent of adsorption, or even do not alter noticeably the degree of binding of certain adsorbates. Further, the ionic strength is another variable which also modifies the extent of binding, and given the same value of ionic strength, there



are cations which show a larger effect on the extent of coadsorption, in particular divalent ions over monovalent ions.

Besides these general remarks, there is one question on which we would like to refer in more detail. It is related to the coadsorption of adenine (A) with thymine (T) and uracil (U). Previous work had demonstrated that T and U are not adsorbed at pH's above 1 (Lailach *et al.*, 1968a, b). In 1969 Lailach and Brindley determined the specific coadsorption of A with T and U in the pH range 1–6 by  $\text{Na}^+$ - and  $\text{Ca}^{2+}$ -montmorillonites. From their data it can be concluded that the coadsorption of T (with A) is null in the pH range 6–8, a fact which has not been generally recognized.

Unfortunately, there are no similar experiments of coadsorption between A and U at pH around 6. However, from another series of experiments between A and T, and A and U at pH 1.2 and 2.0 it is always found that T is adsorbed about 3 times more than U. So we can infer that this behavior would be similar at pH around 6 and expect also a null coadsorption of U (with respect to A) in the pH range 6–8.

In some instances coadsorption phenomena among A and T and/or A and U has not been clearly stated (Lahav and White, 1980; Ponnampertuma *et al.*, 1982). These authors do not state clearly that coadsorption for these molecules is completely over at pH 6 or above. It should be noted that such acidic pH does not correspond to the expected pH of primitive great oceans plus sediment environments.

### 3.6. COADSORPTION INVOLVING POLYNUCLEOTIDES

The degree of polymerization is another variable which strongly alters the extent of adsorption of nucleotide molecules on clays. Large polymer chains usually have strong interaction with surfaces, even though every monomer interacts weakly. This is related to the large number of monomers in each macromolecule (Daoud *et al.*, 1990).

The adsorption isotherm that usually describes the adsorption behavior of large organic molecules and polymers is not the Langmuir equation. According to the initial slope classification of isotherms, the adsorption of polymers results in an H-curve isotherm, which is an extreme version of the Langmuir equation. Its characteristic large initial slope suggest a very high relative affinity of the solid phase for an adsorbing substance. This condition is usually produced either by highly specific interactions between the solid phase and the adsorbing substance or by significant van der Waals interaction contributing to the adsorption processes (Sposito, 1984).

Some results have been produced by Ferris *et al.* (1989) on the adsorption of RNA homopolymers in different conditions of pH and temperature on montmorillonite. They determined that RNA homopolymers exhibit strong adsorption to  $\text{Na}^+$ - and  $\text{Cu}^{2+}$ -montmorillonite and are not readily washed from the clay. It is confirmed that the extent of interaction of polynucleotides is much greater than that observed with mononucleotides which are readily washed from the montmorillonite.

A most interesting result from the same work is the adsorption of mononucleotides onto the clay-homopolymer complex. They determined by the temperature dependence of mononucleotides binding to the clay-homopolymer complex that this binding is by means of Watson-Crick hydrogen bonding of the mononucleotide to the homopolymer adsorbed on the clay surface. In particular Ferris *et al.* (1989) determined that 63% of the uracil of poly (U) have an 5'-AMP associated with them at 2° C. Besides, virtually all of cytosines of poly (C) have an 5'-GMP associated with them at 2° C. Further, no association of 5'-UMP to poly (U) bound to clay was detected.

It is interesting to remark that these results constitute the evidence of a novel 'two story' adsorption which might be of relevance to prebiotic chemistry. This kind of interaction was first proposed by Mosqueira (1979), and applied to a theoretical model describing a cross-autocatalytic prebiotic reaction system, which yielded bistability and hysteresis.

### 3.7. SUMMARY

Adsorption of nucleic bases, their derivatives and homopolymers in a prebiotic system are very complex phenomena, which depends on several environmental constraints. It can be said that the extent of adsorption of these molecules may vary the entire range 0–100%, depending on the conditions. However, we estimated it will prevail a rarity more than 50% degree of adsorption.

## 4. Gamma Radiation Effects on Nucleic Acids Bases and Their Derivatives

The radiation chemistry behavior of purines and pyrimidines and their corresponding nucleic acid derivatives and polymers has prompted continuous and vigorous research, due to its biological importance and its connection with damage in the genetic material by ionizing radiation.

Several types of well documented reactions for the radiolysis of nucleic acid bases and their derivatives are known. We will apply this knowledge to the possible effects of ionizing radiation arising from radionuclides within clays, particularly  $^{40}\text{K}$ , on adsorbed nucleic acid molecules and oligomers on clays in conditions that may be relevant to prebiotic environments.

The effect of irradiation depends on several variables, among them concentration of the organic molecule, physical state, pH of the solution, presence or absence of oxygen, presence of scavengers (compounds that compete for free radicals from water decomposition), radiation dose, radiation rate and type of ionizing radiation. In this survey we focus our attention to the general aspects concerning the radiolysis of nucleic acids bases and their derivatives in different physical conditions.

#### 4.1. RADIOLYSIS OF PURINES AND PYRIMIDINES AND THEIR DERIVATIVES IN AQUEOUS SOLUTIONS

##### 4.1.1. *Pyrimidines and Purines*

In the radiolysis of aqueous solutions, radiation energy is deposited mainly on the water molecules, producing the following radicals:  $\text{H}$ ,  $e^-_{aq}$ ,  $\cdot\text{OH}$ . Hydrogen peroxide and molecular hydrogen are also formed. These radicals attack the solute and form transient species that give rise to the observed products. This effect is known as the indirect action of ionizing radiation. It is the main pathway for the radiolysis of compounds in diluted aqueous solutions (i.e., from very diluted to about 0.5 M). With highly concentrated solutions or pure compounds as crystals, the radiation acts directly on the target molecule. This action is known as the direct effect of radiation. For diluted solutions the decomposition is generally independent of the concentration (Draganic and Draganic, 1973).

There are many works in the literature dealing with the irradiation of aqueous oxygen-free solutions for purines and pyrimidines. Besides, the monomeric organic molecules concentration used in these experiments is in the range  $10^{-4}$ – $10^{-2}$  M. These conditions could be perfectly relevant for chemical evolution studies.

The radiolysis of pyrimidines is well established. In contrast, the radiolysis of purines, is still poorly understood despite the considerable effort that has been made in this area. Some general features of their radiolytic behavior in diluted aqueous solution free of oxygen, at neutral pH have been established. Bases are mainly attacked by the radicals formed from the radiolysis of water. These radicals react by addition to the heterocyclic system.  $\cdot\text{OH}$  addition takes place predominantly at C-5 to give C-5-6yl-Py radical (Jovanovic and Simic, 1986). The  $\cdot\text{OH}$  radical in spite of its high reactivity, exhibits remarkable selectivity and reflects the electrophilicity of  $\cdot\text{OH}$  with heterocyclic rings.

The complexity of such reactions is considerable. To cite one example, upon irradiation of T, there are about fourteen different compounds formed (Infante *et al.*, 1973).

The solvated electron reacts with pyrimidines at practically diffusion-controlled rates by forming the radical anion which is subsequently protonated by  $\text{H}^+$  or a water molecule (von Sonntag and Schuchmann, 1986; Das *et al.*, 1985).

In the case of U, cytosine (C) and T, insoluble dimers of the bases were formed and they had the largest yield in comparison with any other products (Dizdaroglu and Simic, 1984a, 1984b). All these reactions occurred at low dose in the range of 2–50 Gy. Other studies with C have been performed at higher doses in the range of 10–20 kGy (Ponnamperuma *et al.*, 1962).

The reaction of the main water radiolysis species with the purine is a fast process. However the exact sites of the reaction of these various radicals within the purine ring remain mostly speculative (Cadet and Berger, 1985). From quantum chemical calculations it is suggested that radicals attack adenine mainly at N-7 C-8 double bond (Pullman and Pullman, 1961). Subsequent to the attack to C-8 position

with hydroxy radicals, the imidazole ring at the 7,8-bond can be opened. This is the main radiation-induced degradation pathway for A (van Hemmen, 1975). A second pathway after addition of the  $\cdot\text{OH}$  to C-8 is an oxidation reaction. In view of the high reactivity of A, it is surprising that a steady state radiolysis showed low yields of destruction. This suggests that A goes through an overall reconstitution pathway, as was supported by the work of van Hemmen and Bleichrodt (1971). Purine components, especially guanine, have received far less attention (Berger and Cadet, 1985). All these reactions occurred at low dose in the range of 2–50 Gy (Jovanovic and Simic, 1986).

#### 4.1.2. *Nucleosides and Nucleotides*

In the case of nucleosides and nucleotides the experimental observations present a rather complicated picture. In the case of nucleosides, as for example 2'-deoxyguanosine, the radiation-induced degradation products may be classified into several categories according to the nature of the chemical modification on the nucleoside: i) within the purine ring, ii) within the sugar moiety, iii) within both the purine ring and the sugar moiety. From the available data some general features about the mechanism can be drawn: a) Unaltered bases are released on irradiation of nucleosides and deoxynucleosides (Fujita, 1984). This reaction shows a strong dependence of pH for base liberation. Base yields are generally higher at  $\text{pH} > 11$ . b) The opening of the imidazole ring constitutes the main radiation-induced degradation pathway of 2'-deoxyguanosine and its 5'-monophosphate ester in deaerated solution (Berger and Cadet, 1985). Two main decomposition products result from the opening of the imidazole ring and further rearrangement of the sugar moiety. There are other complex pathways which involve the sugar radicals reacting by epimerization, rearrangement, and cyclization reactions giving rise to degradation products. c) In the irradiation of nucleotides, important consequences of attack on the carbohydrate part of the molecule are the immediate liberation of free phosphate and a steady liberation of phosphate in an extended period of several hours after irradiation. The slow liberation speed ups in alkaline solutions. Another consequence of attack on the sugar moiety is the release of the base (Swallow, 1973).

#### 4.1.3. *Nucleic Acids and Oligomers*

Most of the work related to the radiolysis of nucleic acids has been done on poly (U) and DNA. Significant progress has been made in the determination of the mechanism of the radiation-induced decomposition of various DNA model compounds in the solid state as well in aqueous solution. 20–25% of hydroxyl radicals react with the deoxyribose units when solutions of DNA are irradiated. The remaining percentage reacts with various bases on approximately equal basis although T is the most radiosensitive base (Swallow, 1973). Hydrogen atoms appear to react by addition to the bases.

All these chemical changes in nucleic acids give rise to marked changes in the physico-chemical properties of nucleic acids. Even a low dose as 0.1 Gy induces

strand breaks on DNA (Cadet and Berger, 1985). Such low dose is estimated that could be reached in about 10 years of natural radiation in the primitive Earth at 3.8 Ga ago. This estimate stems from the dose rate due to natural radionuclide  $^{40}\text{K}$ ,  $^{238}\text{U}$ ,  $^{232}\text{Th}$  present in deep sea sediments in the primitive Earth 3.8 Ga ago (Table I). The molecular weight decreases when breaks occur sufficiently close to each other in both strands. Also there is a drop of viscosity and lowering of acid-precipitability on both RNA and DNA solutions. This depolymerization is accompanied by the liberation of acid groups and inorganic phosphate (Scholes and Weiss, 1953). Besides this degradation process, there is also evidence that irradiation can produce some crosslinking in such polymers and in pyrimidine oligonucleotides, like TpT; TpTpT; dCpdC in concentrations 0.5 mM (Dizdaroglu and Simic, 1985). Further, radiation-induced crosslinking accounts for about 50% of primary radicals, indicating crosslinking is one of the major processes to be considered in the overall mechanism scheme of radiation-induced DNA damage.

#### 4.1.4. *Radiolysis of Nucleic Acid Bases and Their Derivatives in the Presence of Others Substances. Scavengers, Sensitizers and Protectors*

Radiolysis mechanisms of organic compound in aqueous solutions are complex. When the radiolysis is carried out in the presence of other substances, the reaction pathways can be modified drastically. To get a deeper insight into the reaction mechanism, it is common that some compounds (named scavengers) are deliberately added to a system to react with primary radical species.

One important natural scavenger which has relevance in chemical evolution is the formate ion. (This compound relates to formic acid and formaldehyde, a potential source of sugars according to current views in prebiotic chemistry). The formate ion may react either with  $\cdot\text{OH}$  or  $\cdot\text{H}$  and produce in both cases the formate ion-radical ( $\cdot\text{CO}_2^-$ ), that in turn may react with nucleobases. The reaction of formate radical with pyrimidines is very complex and it follows many pathways. It has been thereby established that the radicals derived from formate increases the degradation of aqueous solutions of C under anoxic conditions (Kamal and Garrison, 1965). Thymidine is also degraded by radicals derived from formate under deaerated environments to produce reduced thymidine compounds and C<sub>5</sub>-linked dimer of no biological interest (Nishimoto *et al.*, 1983).

Further, some substances enhance the decomposition of the compound of interest, this type of substances are call sensitizers. The presence of some transition metals ions like  $\text{Cu}^{2+}$  and  $\text{Cu}^{2+}$  complexes at  $10^{-4}$  M in anoxic conditions act as sensitizers, readily oxidizing the major radical formed by either  $\cdot\text{OH}$  or  $\cdot\text{H}$  adducts of U, and then producing a short-lived carbocation that by deprotonation yields an isopyrimidine (Haysom *et al.*, 1972; Bhattacharyya and Mandal, 1983). Other salts like  $\text{Fe}(\text{CN})_6^-$  have a sensitizing effect towards an aqueous solution of A in anoxic conditions (Cadet and Berger, 1985).

Conversely, some compounds may protect from the decomposition originated by radiolysis, these types of substances are known as radioprotectors. The pres-

ence of KBr 0.1 M in the  $\gamma$ -radiolysis of aqueous solutions of thymidine saturated with either O<sub>2</sub>, N<sub>2</sub>, or N<sub>2</sub>O, respectively, leads to a notable decrease in the number of thymidine decomposition products (Cadet *et al.* 1983). It should be also noted that the presents of KBr in the irradiated thymidine solution prevents any radiation-induced splitting of the N-glycosidic bond of the substrate and subsequent release of free thymine. However, the radicals derived from Br<sup>-</sup> (Br<sub>2</sub>) are active towards the degradation of U and T, and the other purine and pyrimidine 2'-deoxyribonucleosides. Thus, Br<sup>-</sup> plays a protector-sensitizer role, depending of the nature of the target molecule. In the context of chemical evolution, these observations are of particular importance due to the fact that Br<sup>-</sup> is expected to be present in the primitive ocean and it may alter the course of radiolysis. For more details about the radiolysis of water in the primordial ocean consult Draganic *et al.* (1991).

#### 4.2. RADIOLYSIS OF NUCLEIC ACIDS COMPONENTS IN THE SOLID STATE AND FROZEN SOLUTIONS

Among the simplest mechanisms that may have operated on the primitive Earth are evaporation of water, fluctuating environments, and freezing of aqueous solutions. Let us briefly review the radiolysis of nucleic acid bases in such physical states.

##### 4.2.1. *Solid State*

Most of the work related to the irradiation of nucleic acid bases and their derivatives in the solid state have been made in single crystals or glasses. Anions have been observed in irradiated pyrimidines under a wide variety of conditions, but generally at low temperature. Corroborative evidence for anion formation comes from studies on irradiated glasses. Anions formed from thymidine, thymidylic acid, C and 5-methylcytosine have been reported (Sevilla and van Paemel, 1972). Solid nucleic acids give rise to complex ESR spectra on irradiation. One compound which has been identified in DNA is the adduct of hydrogen atom to the T ring.

##### 4.2.2. *Frozen Solutions*

The  $\gamma$ -radiolysis of nucleic acids derivatives in frozen solutions has been carried out, mainly at 77 K. Typically, the radical yield of DNA at this temperature is of the same order of magnitude as in nucleosides and nucleotides.

The radiation induces the formation of primary radical species on the solute and water molecules. However, in contrast to the glassy matrices, presenting a homogeneous spatial distribution of solute molecules, freezing a neutral aqueous solution causes the solute molecule to become excluded from the icy crystal and to gather in dense molecular clusters in the interstices of the solvent crystallites. Their exceedingly high local concentration can thus account for a 'quasi-dry' behavior in respect to ionizing radiation. These arguments do not hold true, completely, in the case of very dilute aqueous solutions (Gregoli *et al.*, 1977). Increasing the temperature causes these species to enter in chemical reactions that will form

different secondary radicals. Although these studies were made with dAMP it is very likely that other nucleic acids components will have similar mechanism (Gregoli *et al.*, 1974).

Irradiation at low doses form many free radicals. The yields of both the primary and secondary species increase with an increase of the concentration of the base (Srinivasan *et al.*, 1970; Gregoli *et al.*, 1977). Thus,  $\gamma$ -irradiation of dTMP, dAMP, and dGMP induces, in each case, the formation of H-addition radicals on the corresponding base moieties (Gregoli *et al.*, 1976).

#### 4.3. IRRADIATION IN THE PRESENCE OF CLAY AND OTHER MINERALS

Ionizing radiation on solids may be viewed as a mechanism that produce defects in the solid itself. The term 'defect' is used in its widest sense, implying any modification of the real crystal lattice from the ideal structure. These defects are responsible for possible chemical reactions of molecules adsorbed in an irradiated solid. There is experimental evidence of formation of defects in solids by radiation at relatively low energy (soft gamma rays, x-rays and  $10^2$ – $10^3$  eV electrons) (Henley and Johnson, 1969).

Energy transfer in clays appears to be an important phenomenon as it may induce chemical reactions (Coyne *et al.*, 1981). In the context of prebiotic evolution the role of clays as transducers of electronic energy through metastable excited states has been outlined (Coyne, 1985). Thus, the energy of radiation may be partially deposited into the clay leading to excitation and ionization. The energy taken up by the solid may be transferred to the adsorbed substance, both the water and adsorbate molecules. Energy transfer to interstitial water produces its chemical decomposition and it raises thus the yield of molecular hydrogen,  $e^-_{aq}$ ,  $\text{H}^\cdot$ , and  $\cdot\text{OH}$  radicals (Garibov *et al.*, 1982). Some of these radicals in the interstitial space may react with adsorbate compounds. The radiolytic products of water can be trapped by a free charged carrier (either an electron or a hole in the clay lattice) and then undergo subsequent chemical transformations. Unfortunately there are not enough studies in this field to draw any clear conclusion.

Another way to produce free radicals from nucleic acid derivatives with relevance to prebiotic chemistry is by cavitation, a phenomenon which takes place when water impinges on water at somewhat low linear velocities. This source of energy gives rise to a free radical chemistry *similar* to the radiolysis phenomena examined in this work (Anbar, 1968; Suslick, 1988).

### 5. Results and General Remarks

Taking into account the previous reviews and considerations, we outline the following remarks and conclusions:

1. In this article we have connected results from different areas. On the one hand we have analyzed the radiolytic behavior of nucleic acid bases and their

TABLE III

Estimates of the transformed material (M) by ionizing radiation present in the different environments in the prebiotic Earth\*

	Interval of time (Ga)		
	3.8 to 3.8025	3.8 to 3.811	3.8 to 3.825
Seawater	$6.46 \times 10^{-6}$	$2.83 \times 10^{-5}$	$6.42 \times 10^{-5}$
Clay	$4.48 \times 10^{-4}$	$1.99 \times 10^{-3}$	$4.47 \times 10^{-3}$
Basaltic	$1.47 \times 10^{-4}$	$6.47 \times 10^{-4}$	$1.46 \times 10^{-3}$

\* Those ranges of time correspond to the years of heavy bombardment of Earth crust according to Oberbeck and Fogleman, 1989.

derivatives. On the other hand, we have reviewed their adsorption characteristics on clays. We remark however, that data on actual radiolysis of these molecules *within clays* is lacking. We expect that the system we have put forward made from these organic molecules adsorbed on clays in the presence of  $^{40}\text{K}$  will result in the formation of radiolytic products. The influence of clays on these prebiotic irradiation processes remains to be actually demonstrated by experiment. However, from what has been said in section 4.3 on heterogeneous catalysis from irradiated clays and the data in this work, a radiolytic degradation of the adsorbed nucleic bases and their derivatives is expected. Besides, the chemical activity of plain non irradiated clays has received ample acceptance since long time ago and it is currently under study. In the context of this study it is particularly important to be also aware of possible chemical outcomes from the interaction of non excited clays in the presence of radiolytic products. It is known for example that free radicals derived from monomers like methyl methacrylate may lose an electron when confined in non irradiated clays, yielding a carbonium ion (Solomon, 1968). Thus, chemical interaction between radiolytic products and irradiated and/or non irradiated clays can be envisaged.

- Let us now evaluate the amount of organic material degraded by the radiolytic effects from  $^{40}\text{K}$  within clays and sea water. To do that we should first establish the time available for radiolysis. We will consider that life originated 3.8 Ga ago. Besides, we assume the impact constraint hypothesis (Oberbeck and Fogleman, 1989, 1990). Under such conditions, the maximum time to originate life was 2.5 to 11 Ma. This period could be extended to 25 Ma if a more conservative estimate is used for the flux of impacting objects before 3.8 Ga for the same range of impactor kinetic energies. In Table III we summarize results for these periods in three different environments. Notice that chemical degradation is higher by two orders of magnitude in mineral surroundings than in plain sea water.



We can calculate that the energy delivered over a period of 2.5 Ma, 11 Ma, and 25 Ma, taking as reference 3.8 Ga ago, from  $^{40}\text{K}$  present in the early ocean ( $1.7 \times 10^{21}$  l), assuming that for each 100 eV the number of molecules affected by the radiation was 0.1 (which is a low value) and an average molecular weight of 100 amu, the amount of material that could be affected by radiation was respectively  $1.1 \times 10^{18}$  g,  $4.8 \times 10^{18}$  g, and  $1.1 \times 10^{19}$  g. (The contributions from  $^{238}\text{U}$  and  $^{232}\text{Th}$  are neglected because they are, respectively, two and five orders of magnitude smaller than that from  $^{40}\text{K}$  (Table I).)

From another perspective, in deep sea clays and using the same assumptions as above, the amount of organic material that could be affected by radiation from  $^{40}\text{K}$  at 3.8 Ga ago is  $1.81 \times 10^{-7}$  moles/kg-clay.

3. It has been shown in section 3.3 that nucleic acid bases and their derivatives are adsorbed in rather low percentage in neutral or slightly alkaline conditions. Thus most of these compounds remain in the bulk solution. Due to this situation it is important to compare radiolysis decomposition with hydrolysis, both in plain sea water. Let us take thymidine for example. Its half-life for hydrolysis is 150 years at  $37^\circ\text{C}$  and pH 7.4 (Shapiro, 1984). On the other hand, the G value ( $G = \text{number of molecules transformed per } 100 \text{ eV}$  delivered into the system) for decomposition of thymidine is 1.98 (Cadet and Teoule, 1972). Taking into account the dose rate of the primitive Earth at 3.8 Ga ago due to  $^{40}\text{K}$ ,  $^{238}\text{U}$  and  $^{232}\text{Th}$  (Table I) the amount of thymidine degraded for the same period of time, that is 150 years, is  $7.69 \times 10^{-9}$  M. So the ratio of degradation due to hydrolysis in comparison with radiolysis is 6–7. Thus, hydrolysis is a degradation process more important than radiolysis in plain sea water. However, the radiolytic degradation *within clays* is 14–16 times larger than the hydrolytic degradation in plain sea water at the same epoch.
4. Inside the clay these compounds are exposed to a higher dose of radiation than in plain sea water (Table I), and the degradation of the organic molecules is greater as we have just seen above. Besides, a highly acidic environment is developed in the interstitial space. We estimate that such conditions adds to the aggressiveness of radiolytic effects of  $^{40}\text{K}$  in the interlamellar space. Thus, one may expect enhanced hydrolytic depolymerization of possible oligomers synthesis within the clay structure. We remark also that studies on the hydrolytic capacities of clays are lacking. In this respect, some known reactions about the catalytic hydrolysis of clays has been studied for disaccharides and esters of carboxylic acids. These examples can give us some indication that hydrolysis inside the clay may be important. McAuliffe and Coleman (1955) made a detailed study for the hydrolysis of ethyl acetate and sucrose. The clays studied were Utah and Wyoming bentonites and hallosyte. They made kinetic studies in the presence of hydrogen-clay and diluted HCl solutions. It was observed that the acid form of both Utah and Wyoming bentonites were more active catalysts than HCl solutions for the ethyl acetate hydrolysis and that H-hallosyte and H-Utah bentonite were slightly more active than HCl solu-

tions for the inversion reaction. Unfortunately, there is a complete lack of experimental data on the radiolysis and hydrolysis effects on nucleic acid components in the presence of clays, therefore, a direct comparison is not possible at the moment. However, we would expect a shorter survival of nucleic acid components within clays in comparison to their survival in plain sea water.

5. Although we have narrowed the pH range of interest to 6–8, there still remain many other variables that affect both the extent of adsorption (of nucleic bases and their derivatives) and their possible radiolytic transformations, as we have just reviewed in this article. This situation introduces a wide variability and uncertainty in outcome, arising from a nonhomogeneous and multicomponent prebiotic environment. Such a situation prevents us to generalize results. More importantly, it introduces to some extent elements of vagueness in theories proposing clays as plausible nucleation sites for the emergence of protobiotic systems.

Alternatively, if clays acted as important nucleation sites, then protobiological synthetic reactions had to proceed much more rapidly than deleterious decomposition reactions to avoid the long term effects of radiolysis and hydrolysis within clays.

6. We referred previously to mineral surfaces with no internal surfaces, as for example gypsum. A general advantage of such surfaces is that it is exposed to radiation doses similar to that of plain sea water.
7. For the above reasons it is proposed a reconsideration of our views concerning the role of clays in prebiotic chemistry; specifically in relation to its dual synthetic-decomposition capabilities. In this article we have outlined plausible prebiotic radiation conditions on nucleic bases adsorbed on clays. We arrive to the conclusion that the environment on the primitive Earth were not ideal for nucleic bases and that background radiation of sufficient dose to destroy these bases and their derivatives existed. Probably clays were not that oasis for organic molecules that Bernal (1951) imagined.

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