Lawrence Berkeley National Laboratory
Recent Work

Title
THE RADIATION CHEMISTRY OF NUCLEIC ACID CONSTITUENTS

Permalink
https://escholarship.org/uc/item/2jm3j6pk

Author
Ponnamperuma, Cyril Andrew.

Publication Date
1962-06-01
DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.
UNIVERSITY OF CALIFORNIA
Lawrence Radiation Laboratory
Berkeley, California
Contract No. W-7405-eng-48

THE RADIATION CHEMISTRY OF NUCLEIC ACID CONSTITUENTS

Cyril Andrew Ponnamperuma
(Thesis)
June 1962
THE RADIATION CHEMISTRY OF NUCLEIC ACID CONSTITUENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>The chemical basis of heredity</td>
<td>2</td>
</tr>
<tr>
<td>II.</td>
<td>The nucleic acid constituents</td>
<td>10</td>
</tr>
<tr>
<td>III.</td>
<td>Survey of previous work</td>
<td>25</td>
</tr>
<tr>
<td>IV.</td>
<td>Experimental techniques</td>
<td>32</td>
</tr>
<tr>
<td>V.</td>
<td>The radiation decomposition of adenine</td>
<td>50</td>
</tr>
<tr>
<td>VI.</td>
<td>The radiation decomposition of cytosine</td>
<td>74</td>
</tr>
<tr>
<td>VII.</td>
<td>The radiation decomposition of guanine</td>
<td>81</td>
</tr>
<tr>
<td>VIII.</td>
<td>The radiation decomposition of nucleosides and nucleotides</td>
<td>91</td>
</tr>
<tr>
<td>IX.</td>
<td>Conclusion</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>Acknowledgments</td>
<td>122</td>
</tr>
</tbody>
</table>
ABSTRACT
THE RADIATION CHEMISTRY OF NUCLEIC ACID CONSTITUENTS

The chemical effect of ionizing radiation on nucleic acid constituents was studied with the intention of finding those effects which may be of significance in understanding the origin of a radiation mutation.

C$^{14}$-labeled purine and pyrimidine bases, nucleosides, and nucleotides were irradiated in aqueous solution in the absence of oxygen. A Co$^{60}$ $\gamma$-source was used for the radiation. The radiation decomposition products were analyzed by paper chromatography, uv absorption spectrophotometry, and liquid scintillation counting. Co-chromatography with known amounts of inactive carrier was used to identify the formation of trace quantities of decomposition products.

Several reactions mediated by the free radicals produced by the radiolysis of water were investigated. Among these may be listed:

1. Destruction of purine and pyrimidine bases
2. Destruction of nucleosides
3. Destruction of nucleotides
4. Release of sugar from nucleosides and nucleotides
5. Release of sugar phosphate from nucleotides
6. Release of phosphate from nucleotides
7. Deamination of amino bases to hydroxy bases in the free bases, nucleosides, and nucleotides
8. Opening of the imidazole ring of purine bases
9. Formation of $8$-hydroxypurines
The G values for bases, nucleosides, and nucleotides destroyed were approximately of the same order. Guanine, however, was anomalous in its behavior. As the free base, guanine appeared to be far more resistant to radiation than guanosine or guanylic acid.

Of special significance is the deamination of amino bases to hydroxy bases. The conversions observed were:

- Adenine to hypoxanthine
- Adenosine to inosine
- Adenylic acid to inosinic acid
- Cytosine to uracil
- Cytidine to uridine
- Cytidylic acid to uridylic acid
- Guanine to xanthine

The G values for these reactions were small (0.1 or less). But since chemical deamination is a recognized agent in the production of artificial mutants, the occurrence of such a reaction in the radiolysis of nucleic and constituents might suggest a possible explanation for the origin of a radiation mutation.
I. THE CHEMICAL BASIS OF HEREDITY

The principal interest in the radiation chemistry of nucleic acid constituents stems from the important role the nucleic acids play in hereditary processes. Cytological, genetic, and chemical experiments performed during the last two decades have established almost beyond question the function of the nucleic acids in heredity.\(^1,2\)

Two types of nucleic acids

There are two known kinds of nucleic acids - deoxyribose nucleic acid (DNA), and ribonucleic acid (RNA). Both are polymers of nucleotide units which, in turn, consist of a purine or pyrimidine base, a sugar residue, and a phosphate group. In DNA the sugar is deoxyribose; in RNA it is ribose. Both types of nucleic acids are present in plant and animal cells. While RNA is found mainly in the cytoplasm, DNA occurs almost exclusively in the nucleus of the cell. Animal viruses contain DNA, or RNA, or both. Plant viruses, however, contain only RNA.\(^3\) The RNA content ranges from 6% in the tobacco mosaic virus to 35% in the turnip yellow mosaic virus.

Cytology

Cytochemical experiments have shown DNA to be located in the chromosomes, the cellular structures that carry the genes.\(^2,4,5\) The amount of DNA is essentially constant in the diploid somatic nuclei of various tissues within a species, while it is present only in half quantities in the
haploid sperm cells. Moreover, the various proteins of the nucleus do not have the same distribution. The classic work of Morgan and his co-workers established the parallelism between the organized chromosome movements and the movements of the genetic determinants deduced from Mendelian genetics. This led to the acceptance of the chromosome as a gene carrier. Cytochemical data has located the DNA in the chromosomes, while cytogenetic data has shown that the chromosomes must carry the genes.

The phenomenon of transformation

From the field of genetic chemistry comes direct evidence that a chemical substance is involved in genetic processes and that this substance is DNA. The discovery of the biological phenomenon of transformation and the nature of the transforming principles thus afforded the first possibility of a chemical approach to the problem of heredity.

In 1928, Griffiths reported that from mice injected with living non-encapsulated rough colony type (R) pneumococci mixed with heat-killed encapsulated smooth (S) variants, it was possible to recover living (S) pneumococci. The new feature, once established, was retained and reproduced in subsequent generations as if a new gene had been added to the genetical make-up of the receptor cells. Although this experiment was repeated by other workers, it still appeared possible that the seemingly heat-killed preparation could have contained some heat-resistant bacterial forms.

In 1944, however, Avery and his co-workers prepared
purified extracts of the transforming principle and showed that this had all the properties of a highly polymerized DNA. Chemical analysis and biochemical studies have established that the transforming principle is DNA. No evidence has yet been produced to the contrary.

Several workers have studied the infection of Escherichia Coli with bacteriophages of the type T. These coliphages are composed of 60% protein and 40% DNA. The DNA appears to be mechanically confined by a protein membrane. Hershey and Chase have demonstrated that the DNA plays a major role in this process. In a study of the T2 and T6 infecting phages, it was shown that the DNA fraction of the phage enters the cell while the protein coat remains outside. These investigators grew bacteriophage in bacteria in the presence of radioactive sulfur and radioactive phosphorus. As sulfur is present in certain amino acids, the viral proteins became labeled with radioactivity. Radioactive phosphorus, on the other hand, was incorporated into the nucleic acids. When bacteria were infected with these doubly-labeled phages, only radioactive phosphorus was detected indicating that the nucleic acid of the phage, and not the protein, had entered the host cell.

The action of ultra-violet radiation

Further evidence for the genetic function of DNA is furnished by the action of ultra-violet radiation. The action spectrum of uv light in producing mutations in corn
and fungi is equivalent to the uv absorption curve of the nucleic acids.\textsuperscript{12, 13} The dimerization of pyrimidines by uv radiation, recently reported, probably accounts for changes in the structure of the nucleic acid molecule which may give rise to mutations.\textsuperscript{14, 15}

**Chemical mutagens**

Evidence for the chemical nature of the gene can also be found in the effects of certain mutagenic agents like the nitrogen and sulfur mustards to which the nucleic acids are highly susceptible. Sulfur mustard was one of the first chemical substances which was clearly shown to produce a mutation. The methods used to demonstrate the reaction of these substances with DNA do not resemble the milder conditions of biological experiments. However, mustards in high concentrations react readily with phosphate and amino groups of nucleic acids producing alkylated derivatives. With less vigorous treatment, loss of viscosity and depolymerization takes place.\textsuperscript{16, 17} Mustard derivatives having only a single reactive halogen group have been shown to produce both mutations\textsuperscript{18} and depolymerization.\textsuperscript{17} It has also been found that mustard treatment blocks the synthesis of DNA in the amphibian embryo and in E. Coli.\textsuperscript{18, 19} Nitrous acid, which deaminates purine and pyrimidine bases, has been widely used in the production of mutants for experimental study.\textsuperscript{20, 21}

There is also a group of mutagens which is structurally related to some of the nucleic acid constituents. The
methylated purines, like caffeine and theophylline, are effective mutagens for E. Coli.\textsuperscript{22} It is possible that these bases interfere with the normal incorporation of purines into the nucleic acid molecule.\textsuperscript{23}

There is evidence to show that, like DNA, RNA can also function as a carrier of genetic information. RNA isolated from TMV has an infectivity which exists in the absence of virus protein.\textsuperscript{24,25,26} A similar finding has been reported with the mengo encephalitis virus; RNA isolated from the cells of this virus is also able to produce an infection in the absence of protein.\textsuperscript{27} There is some indication, too, that in the turnip yellow mosaic virus it is the RNA which controls virus multiplication.\textsuperscript{28,29}

In recent years, a large number of biochemical experiments has directed attention to the role which microsomal particles play in protein synthesis. These particles are composed of 50\% RNA and 50\% protein. The protein synthesis is strictly dependent on the integrity of the RNA. In liver cells, all the protein synthetic activity has been shown to be carried out by the microsomal particles.\textsuperscript{30} It has also been claimed that cytoplasmic RNA, or its precursor, is synthesized in the nucleus and transmitted to the cytoplasm.\textsuperscript{31} The common belief is that the RNA molecule carries in the sequence of its bases some, or perhaps all, of the information necessary to achieve the synthesis of individual protein molecules.\textsuperscript{32}
**Ionizing radiation**

The classic work of Muller\(^3\)\(^\text{3}^\) established the mutagenic action of x-rays. The frequency of x-ray mutations, as studied in the fruitfly Drosophila, increased linearly with the dose in rads.\(^3\)\(^4\) There appears to be no lower threshold to the mutagenic action of ionizing radiation. Assuming that human genes resembled those of a mouse more than those of a fruitfly, Muller estimated that 1 roentgen produces about $6 \times 10^{-4}$ mutations in one human reproductive cell, and that 80 roentgens would double the frequency of naturally occurring mutations.

**The origin of a radiation mutation**

There is an uncontested axiom that a chemical or physical process is the basis of any manifestation in life. Since the role of the nucleic acids in hereditary processes has been established almost unequivocally, the origin of a radiation mutation must be linked to a change in one of the units of the nucleic acid molecule. Recent findings in radiation chemistry have made it impossible to apply the target theory of radiation to mutagenesis. Much of the action of ionizing radiation on organic molecules is mediated by the activated water molecules. The study of the radiation decomposition of the nucleic acid constituents might thus give us a clue to the origin of a radiation mutation.
REFERENCES


II. THE NUCLEIC ACID CONSTITUENTS

The composition of the nucleic acids has been deduced from the results of chemical and enzymatic hydrolys...
Sugars

As early as 1909, it was recognized that the nucleic acid prepared from yeast contained a pentose sugar and that this was ribose. This was subsequently confirmed by Gulland. The nature of the sugar in DNA was clarified when Levene and Mori isolated deoxyribose from the guanine nucleoside of DNA. The sugar in the DNA's of several mammalian tissues was chromatographically identical with the deoxyribose found in thymus DNA.

Nucleosides

A purine or pyrimidine base condenses with a pentose or deoxypentose sugar to form a nucleoside. Adenine combines with ribose to give adenosine, and with deoxyribose to give deoxyadenosine. The nucleosides are N-glycosides. Union between base and sugar occurs at position 9 of the purine bases and position 3 of the pyrimidines. The linkage has a β-configuration, and the sugar is in the furanose form.

Nucleotides

The nucleotides are phosphoric esters of the nucleosides. The ribose nucleosides have 3 free hydroxyl groups on the sugar ring, and can form 3 possible nucleoside monophosphates. Adenosine thus gives rise to adenosine-5′-phosphate, adenosine-3′-phosphate and adenine-2′-phosphate. The deoxyribose nucleoside can give rise only to the 3′ and 5′ monophosphates.
Structure of DNA

The DNA molecule consists of two polynucleotide chains wound around each other in a helical manner (Figure 4). The pitch of the helix is 34 Å and there are 10 nucleotides per turn of each strand. The nucleotides are arranged in such a manner that the bases are hydrogen bonded to each other, and the chains are held together by these hydrogen bonding pairs (Figure 4). This structure was first proposed by Watson and Crick in 1953,\(^{17}\) and has since been corroborated by experimental evidence.\(^{18}\)

A striking feature of this structure is that specific base pairing is postulated. An examination of the models shows that only the adenine-thymine and guanine-cytosine pairs can bond together and at the same time fit into the two-strand helix (Figure 5). This conclusion is in complete agreement with the analytical data indicating that in DNA the molar ratios of adenine to thymine and guanine to cytosine are unity,\(^{19}\) and that the sum of the purine nucleotides equals the sum of the pyrimidine nucleotides.\(^{20,21}\)

Replication of DNA

The hereditary information probably resides in the nucleotide sequence of DNA.\(^{22}\) The molecule is very long, so that the permutations of arrangements of nucleotide bases could have enough specificity to completely define and differentiate one organism from another. As some method of molecular replication is necessary for the transmission
of hereditary characteristics, it has been suggested that the structure itself contains an indication of how this replicating process may be carried out. Each strand in the DNA molecule may serve as a template for replication. The evidence that chromosomal material also replicates like a duplex structure supports this theory.

**Structure of RNA**

In comparison with DNA, less is known of the structure of RNA. A 3'-5'-internucleotide chain has been postulated for the main RNA chain (Figure 6). Base composition analysis has shown that the amount of 6-keto (guanine and uracil) groups equals the amount of 6-amino (adenine and cytosine) groups.

The macromolecular structure of RNA has not yet been completely elucidated. RNA is unstable chemically compared to DNA, and most extraction procedures probably disrupt the molecular configuration. The same x-ray diffraction pattern is produced by oriented fibers of RNA prepared from a variety of sources. Although this pattern is not well resolved, it is sufficiently characteristic to enable one to assert that RNA, isolated from plant, animal, bacterial, and viral sources, has a common underlying configuration. The planar purine and pyrimidine rings in the molecule are oriented at right angles to the fiber axis.

The preparation, however, of synthetic polynucleotides having all the characteristics of RNA has greatly augmented
the structural studies of RNA.\textsuperscript{28} The fiber diffraction pattern produced by synthetic polyribonucleotides is identical with that of native RNA.\textsuperscript{29} (Figure 7). These studies have given rise to the suggestion that RNA may undergo molecular replication in a manner very similar to that of DNA. In addition, the formation of three-stranded polynucleotide molecules (Figure 8) by enzymic synthesis has led to the belief that such mechanisms may be important in the formation of RNA from a two-stranded DNA molecule.

The present study deals with the effect of ionizing radiation on some of the nucleic acid constituents. The compounds examined were:

1. **Bases** - adenine, guanine, and cytosine.
2. **Nucleosides** - adenosine, guanosine, and cytidine.
3. **Nucleotides** - adenylic acid, guanylic acid, uridylic acid, and cytidylic acid.
4. **Nucleic acid** - RNA.
Fig. 1. Purines, pyrimidines, nucleosides and nucleotides.
Fig. 2. Ring structure of pyrimidine and purine.
Fig. 3. Structures of some trace purines and pyrimidines from nucleic acids.
Fig. 4. Single chain of DNA and double helix.

(A) Chemical formula of a single chain of deoxyribonucleic acid.

(B) The figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis.

[J. D. Watson and F. H. C. Crick, Nature, 171, 964 (1953)]
Fig. 5. Base-pairing in DNA
Fig. 6. The chemical structure of a section of an RNA molecule.
Fig. 7. Schematic representation of polyadenylic acid. Each band represents the ribose-phosphate backbone, while the bars stand for pairs of adenine bases.29.
Fig. 8. The hydrogen bonding between three hypoxanthine bases in three-stranded polyinosinic acid.30.
REFERENCES


30. Ibid., p. 67.
III. SURVEY OF PREVIOUS WORK

There is considerable literature on the radiation chemistry of nucleic acids and related compounds. In vivo or in vitro studies of the polymer have been primarily concerned with the physico-chemical changes produced. The main purpose of the investigations on the chemical effect of ionizing radiation on the nucleic acid constituents, however, has been to discover those chemical changes which may be of biological significance, and to identify the products which may reflect interference with the genetic function of the nucleic acids.

Effect of radiation on the polymer

Alexander and Stacey\(^1\) irradiated dry herring sperm DNA with Co\(^{60}\) rays and with 1.2 MeV electrons. Equal doses of $\gamma$-rays and 1.2 MeV electrons produced the same amount of degradation. Radiation produced "hidden" breaks of the backbone which were revealed as molecular weight changes only after dissociation (Figure 1). For a dose of $1.8 \times 10^5$ rads, a decrease of only 20\% was observed in salt solution, whereas in urea a 75\% change was observed. The true number of breaks in the polymer appear to be revealed when the hydrogen bonds were disrupted by urea.

The energy required to break a bond was found to lie between 10 and 20 eV although the anticipated value from viscosity measurements was 100 eV per break. This may be due to the fact that the energy absorbed by the molecule
was not confined to the initial ionization, or excitation, but may be transferred along the molecule to a more labile bond before a chemical reaction occurs.

Cox and co-workers\(^2\) studied the effect of $\gamma$-irradiation on aqueous solutions of sodium deoxyribonucleate from herring sperm. Titration studies of the irradiated DNA showed that the majority of the cross-linking hydrogen bonds in DNA remained intact and that the DNA was still largely in the double helical form. The irreversible forward-titration curve of DNA from pH 7 down to pH 2.5 differs from the reversible back-titration curve. This difference depends on the number of hydrogen bonds present which link the acid titrable amino groups of cytosine and adenine to the $-\text{NHCO}$ systems of guanine and thymine. Changes in the relative positions of these curves can be used to estimate the effect of any process on the hydrogen bonds of DNA.\(^3\)

On irradiation, the hydrogen bonds gradually disappeared but, even when the viscosity had become very low, about 60% of the hydrogen bonds still remained intact. The rupture of bonds, however, began at much lower doses than those causing an appreciable change in viscosity. These changes in hydrogen bonding show that even low doses of such radiation might impair replication of the DNA molecule with consequent genetic effects.

Scholes and Weiss\(^4,5\) found that, in aqueous solutions, both DNA and RNA were degraded to polynucleotides. According
to them, free radicals formed by the radiolysis of water were responsible for this process \( H_2O \rightarrow H^+ + \cdot \text{OH} \).\(^6\) Evidence for this indirect nature of the breakdown came not only from the observed depolymerization of DNA with chemically induced free radicals,\(^7\) but also from the inability of x-rays to depolymerize sodium nucleate in the dry state or in frozen aqueous solution.\(^8\)

When 0.2% aqueous solutions of DNA and RNA were irradiated at a dose level of \( 10^6 \) rads in the presence of oxygen, the following effects were observed:\(^9\) breakage of the ester linkages leading to the liberation of inorganic phosphate, and fission of the glycosidic linkages with the liberation of free bases and ammonia.

On the basis of their results, Scholes and Weiss concluded that the loss of structural viscosity on irradiation of DNA solutions can be attributed to a loss of hydrogen bonding as a result of an elimination of some of the amino groups.

**Effect of radiation on nucleic acid components**

The degradation of the nucleotides has been interpreted in terms of two reactions: attack on the base followed by hydrolytic release of ribose, and attack on the ribose moiety followed by release of free base.\(^10,11\) The base attack was considered to be the major pathway. Ammonia was formed when nucleotides, nucleosides, or free bases, were irradiated. The yield of ammonia depended on the pH of the solution.
Small but definite yields of ammonia were obtained from uracil, which contains no primary NH₂ group, indicating that the nitrogen of the pyrimidine ring also contributes to the formation of ammonia. Scholes and Weiss concluded, however, that deamination of the enzymic type, e.g., the conversion of adenine to hypoxanthine, did not take place under these conditions.

In further studies¹² on the chemical effect of ionizing radiation on purine and pyrimidine ribonucleotides in aqueous solution, Scholes et al reported the formation of intermediate labile phosphate esters which lead to a post-irradiation release of inorganic phosphate. They suggested that the radiation-induced lability of the nucleotides was due to the formation of activating carbonyl groups in the sugar components.

**Formation of hydroperoxides**

The same group of workers has reported the formation of hydroperoxides when RNA and DNA were irradiated in aqueous solution.¹³ Investigation of a number of ribonucleotides, and corresponding free bases, has shown that the formation of the hydroperoxides is associated with the pyrimidine bases.

The thymine hydroxy-hydroperoxide was rather stable and could be isolated from the irradiated solution.¹⁴ Cytosine hydroperoxide was found to be unstable, under neutral and alkaline conditions.¹⁵ The yields of hydroperoxide from neutral uracil solutions were comparable to
those obtained from thymine. Dimethyl uracil also yielded a hydroperoxide in somewhat similar amounts.\textsuperscript{16} A pyrimidine glycol has also been isolated from a solution of cytosine irradiated in the absence of oxygen.

**Opening of the imidazole ring**

When guanosine and guanylic acid in aqueous solutions were irradiated, a compound similar to that produced by the alkylation of guanylic acid was formed.\textsuperscript{18} Spectral and chromatographic data suggested that this compound was 2,4-diamino-5-formamido-6-hydroxypyrimidine. The radiation degradation of guanylic acid and guanosine was thought to be strikingly different from that of the corresponding adenine derivatives. Further work,\textsuperscript{19} however, has indicated that the opening of the imidazole ring is a general result of the irradiation of purine nucleosides and nucleotides.

**Effect of radiation on a diphosphate**

Nems\textsuperscript{20} studied the effect of irradiation on a dilute solution of adenosine diphosphate. He found that the absorbency at 260 m\textmu{} decreased linearly with the x-ray dose up to $3 \times 10^5$ rads. The principal uv absorbing product was adenine. The monophosphate was formed only in trace quantities. Several irradiation products were present, but were not identified.
Fig. 1. A diagrammatic representation of the effect of urea on irradiated DNA which shows how breaks hidden in the dimeric structure (b) become apparent in urea solution when all the hydrogen bonds are broken.
REFERENCES

IV. EXPERIMENTAL PROCEDURE

Irradiation

Most of the irradiations described in this work were done with a high intensity Co\textsuperscript{60} source.\textsuperscript{1} A few irradiations of nucleotides in the solid state were performed with the linear electron accelerator of the Radiation Laboratory.\textsuperscript{2}

The method of operation of the Co\textsuperscript{60} source is illustrated in Figure 1. The cobalt is held in a center unit and movable shields and sample holders are placed above and below it. The lower shield and sample holder introduces a sample within a ring of vertically held Co\textsuperscript{60} containing tubes. The upper shield and sample holder unit permit the introduction of a sample in the annular space between the outer center shield and the Co\textsuperscript{60} tubes. For the experiments described here, only the lower shield and sample holder were used.

For irradiations of solutions, 250 µl of a solution of each compound were sealed in glass tubes under vacuum. To eliminate dissolved oxygen, a steady stream of nitrogen was bubbled through the solution for about ten minutes. The nitrogen above the solution in the glass tube was then pumped out while the solution was frozen in liquid nitrogen. The tubes were sealed when the pressure had dropped below 20 microns. The tubes used were 14 mm in diameter and 30 mm long. As the geometry of the Co\textsuperscript{60} source could be adjusted to accommodate a holder from 1" to 6" in diameter, several samples could be irradiated at the same time.

For the irradiation of solids, the samples were
vacuum-sealed in capillary tubes of 3 mm diameter. The capillaries were then placed in holders in which they could be exposed to the Co\textsuperscript{60} Y-rays or to the electron beam of the linear accelerator.

**Dosimetry**

Although many methods are available for the determination of high dose rates of ionizing radiation, the Fricke ferrous sulfate dosimeter\textsuperscript{3,4} appears to be the easiest to use. The results obtained are highly reproducible, the extent of the reaction is easily detected, accurately measured, and proportional to the dose.\textsuperscript{5}

A solution of Fe\textsuperscript{++} saturated with oxygen was exposed to radiation for various lengths of time. The amount of Fe\textsuperscript{+++} formed:  

\[
\text{Fe}^{++} + \frac{1}{2} \text{O}_2 + 2\text{H}^{+} \rightarrow \text{Fe}^{+++} + \text{H}_2\text{O}
\]

was found by direct reading of the absorbency of the solution at a wavelength of 305 m\textmu, with a Beckmann DU spectrophotometer. Assuming that the extinction coefficient for Fe\textsuperscript{+++} is 2130 and the G value for the reaction 15.5, the curves shown in Figure 2 were obtained.

To find the dose level at which a sample was irradiated, a solution of FeSO\textsubscript{4} was exposed to the Y-rays at the same time. The intensity of radiation, corresponding to the optical density of the FeSO\textsubscript{4} solution, was obtained from the graph. At a 1" setting of the Co\textsuperscript{60} slugs, the intensity was 5 x 10\textsuperscript{6} rads per hour.

**Paper chromatography**

The radiation products were analyzed by descending
paper chromatography. Whatman No.4 filter paper, washed with oxalic acid, was used.

More than eighty solvent systems have been described for the separation of nucleic acid components by paper chromatography. Several of those which showed distinct advantages over the others were investigated. The solvent systems examined were:

1. Isopropanol-HCl
2. Isobutyric acid-NH₃
3. Isobutyric acid-NH₃-EDTA
4. Methanol-NH₃
5. Pyridine-H₂O
6. Butanol-urea
7. Butanol-propionic acid-water
8. Phenol-water
9. n-Butanol
10. Water (pH 10)
11. Propanol-NH₃-water
12. Butanol-acetic acid-water
13. Amyl alcohol-H₂O

In the initial experiments, the radiation decomposition products of C¹⁴-labeled nucleotides were analyzed by using isobutyric acid-ammonia-EDTA in one dimension and butanol-propionic acid in the other. In later work, however, propanol-ammonia-water was used in the first dimension instead of isobutyric acid-ammonia-EDTA. The propanol-ammonia-water system had several advantages over the other.
Well-defined spots were obtained for all purine and pyrimidine bases, nucleosides, and nucleotides. The rate of flow of solvent was much faster. The unpleasantness of working with the evil-smelling isobutyric acid, the odor of which is evident from chromatographic papers even after the papers are dry, was eliminated.

The $R_f$ values for the series of compounds tested with the two systems, propanol-ammonia-water and butanol-propionic acid-water, are listed in Table I. The relative positions of the compounds in a two-dimensional chromatogram are shown in Figures 3, 4, and 5.

The RNA bases, nucleosides, and nucleotides were obtained from the National Biochemicals Corp., Cleveland, Ohio. The deoxy compounds were supplied by Schwarz Bio-Research, Inc., Mount Vernon, New York. The formamidopyrimidines were a gift of Dr. G.B. Brown of the Sloan-Kettering Institute for Cancer Research, New York.

The butanol-propionic acid solvent was prepared by mixing equal parts of butanol saturated with water and 60% propionic acid. The mixture was prepared immediately before use. The propanol-ammonia solvent consisted of $n$-propanol mixed with a solution containing 75% of 0.380 $\text{M}$ ammonium hydroxide. Here, again, the two liquids were mixed just prior to use.

Aqueous solutions of the nucleic acid components contained 1 $\mu\text{g}/\mu\text{l}$. Xanthine and guanine, which were poorly
soluble in water, were dissolved in 1 N HCl. Twenty-five micrograms were spotted on paper for chromatography.

The chromatograms were run for six hours at an average temperature of 27°C. In both systems, the solvent front had run approximately 40 cm from the origin.

In all the radiation studies described, C\textsuperscript{14}-labeled compounds were used. By placing an x-ray film in contact with a chromatogram, an autoradiograph was obtained. Each spot on the autoradiograph represented a different compound.

Preparation of Shadowgrams

The uv absorbing characteristic of purines and pyrimidines enables them to be easily detected. A quartz screen fluoresces when placed over a uv source. If a chromatogram containing spots of uv absorbing material is intercepted between the quartz screen and the uv source, darkened areas may be noticed on the screen corresponding to the spots.

A permanent record of the distribution of uv absorbing material on the chromatogram can be made with the aid of a shadowgram.\textsuperscript{15} These can be prepared by laying the chromatogram over Kodagraph photographic paper (14" x 17") and shining a uv source (G.E. Hanovia lamp - 115 watts) over it for 10 seconds. The uv absorbing areas appear as well-defined white spots on a dark background. Figure 6 is a shadowgram of a unidimensional chromatogram of adenine-2-C\textsuperscript{14} and cytosine-2-C\textsuperscript{14}. The corresponding autoradiograph is shown alongside.
**Liquid scintillation counting**

The radioactivity of the breakdown products was assayed by liquid scintillation counting. The spots were eluted from chromatograms with 0.1% formic acid solution. A convenient method of elution was to suspend the wedge of paper containing a spot from the needle of a hypodermic syringe bent upwards in the form of a hook (Figure 7). A battery of these syringes was mounted inside an airtight perspex box. The volume of eluant was easily regulated in this way. The spots were eluted directly into 2 ml volumetric flasks, and the volume made up to mark by the addition of water.

The liquid scintillator solution was made up of:

- toluene 2000 ml
- dioxane 2000 ml
- ethanol 1200 ml
- napthalene 260 gm
- PPO 26 gm
- POPP 0.5 gm

An internal standard of 100 µl of toluene-$\text{C}^{14}$, equivalent to 12,3000 dpm, was used.$^{21}$

The counting was done at room temperature in the detector unit built in this laboratory.$^{22}$ This apparatus is illustrated in Figure 8. The cell compartment is embedded in lead brick shielding. The coincidence operating phototubes are arranged face to face. The whole apparatus is mounted on an open bench without further shielding from light.

The use of this equipment at room temperature eliminated
difficulties that arise from diminished solubilities at low temperatures, from phase separation in the scintillator, and from condensation of atmospheric moisture in the solution. The counting efficiency was about 65%.
## Table I

$R_f$ Values of Purine and Pyrimidine Bases, Nucleosides and Nucleotides in Two Solvent Systems

<table>
<thead>
<tr>
<th>Compound</th>
<th>Propanol-Ammonia-Water</th>
<th>Butanol-Propionic Acid-Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>0.64</td>
<td>0.79</td>
</tr>
<tr>
<td>Cytosine</td>
<td>0.62</td>
<td>0.60</td>
</tr>
<tr>
<td>Guanine</td>
<td>0.37</td>
<td>0.55</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>0.55</td>
<td>0.58</td>
</tr>
<tr>
<td>Thymine</td>
<td>0.75</td>
<td>0.74</td>
</tr>
<tr>
<td>Uracil</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Xanthine</td>
<td>0.41</td>
<td>0.50</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.65</td>
<td>0.63</td>
</tr>
<tr>
<td>Cytidine</td>
<td>0.60</td>
<td>0.46</td>
</tr>
<tr>
<td>Guanosine</td>
<td>0.36</td>
<td>0.37</td>
</tr>
<tr>
<td>Inosine</td>
<td>0.52</td>
<td>0.40</td>
</tr>
<tr>
<td>Uridine</td>
<td>0.54</td>
<td>0.47</td>
</tr>
<tr>
<td>Xanthosine</td>
<td>0.43</td>
<td>0.32</td>
</tr>
<tr>
<td>Deoxyadenosine</td>
<td>0.78</td>
<td>0.70</td>
</tr>
<tr>
<td>Deoxyctydine</td>
<td>0.75</td>
<td>0.60</td>
</tr>
<tr>
<td>Deoxyguanosine</td>
<td>0.59</td>
<td>0.50</td>
</tr>
<tr>
<td>Deoxyinosine</td>
<td>0.70</td>
<td>0.49</td>
</tr>
<tr>
<td>Deoxyuridine</td>
<td>0.73</td>
<td>0.58</td>
</tr>
<tr>
<td>Thymidine</td>
<td>0.76</td>
<td>0.73</td>
</tr>
<tr>
<td>Adenylic acid 2'</td>
<td>0.34</td>
<td>0.24</td>
</tr>
<tr>
<td>Adenylic acid 3'</td>
<td>0.35</td>
<td>0.25</td>
</tr>
<tr>
<td>Adenylic acid 5'</td>
<td>0.29</td>
<td>0.19</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>0.22</td>
<td>0.19</td>
</tr>
<tr>
<td>Guanylic acid</td>
<td>0.11</td>
<td>0.16</td>
</tr>
<tr>
<td>Inosinic acid</td>
<td>0.24</td>
<td>0.10</td>
</tr>
<tr>
<td>Uridylic acid</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>Deoxyadenylic acid</td>
<td>0.39</td>
<td>0.26</td>
</tr>
<tr>
<td>Deoxyctidylic acid</td>
<td>0.35</td>
<td>0.24</td>
</tr>
<tr>
<td>Deoxyguanylic acid</td>
<td>0.22</td>
<td>0.17</td>
</tr>
<tr>
<td>Deoxyuridylic acid</td>
<td>0.35</td>
<td>0.17</td>
</tr>
<tr>
<td>4-Amino-5-imidazole carboxamide</td>
<td>0.64</td>
<td>0.66</td>
</tr>
<tr>
<td>4-Amino-5-formamido-6-hydroxypyrimidine</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>4,6-Diamino-5-formamido-pyrimidine</td>
<td>0.49</td>
<td>0.53</td>
</tr>
<tr>
<td>2,4-Diamino-5-formamido-6-hydroxypyrimidine</td>
<td>0.21</td>
<td>0.30</td>
</tr>
<tr>
<td>2,4,5-Triamino-5-formamido-pyrimidine</td>
<td>0.22</td>
<td>0.37</td>
</tr>
<tr>
<td>4,5,6-Triaminopyrimidine sulfate</td>
<td>0.57</td>
<td>0.67</td>
</tr>
</tbody>
</table>
Fig. 1. Co$^{60}$ $\gamma$-ray source
A. Co$^{60}$ containing tubes. B. Lower shield and sample holder. C. Upper shield and sample holder. D. Center shield.
Fig. 2. Ferrous sulfate dosimetry. Optical density at 305 m\(\mu\) and yield of Fe\(^{+++}\) vs dose.
Fig. 3. Chromatography map of purines and pyrimidines.
Fig. 4. Chromatography map of ribose nucleosides and nucleotides.
Fig. 5. Chromatography map of deoxyribose nucleosides and nucleotides.
Fig. 6. Shadowgram and autoradiograph of a unidimensional chromatogram of adenine-2-$\text{C}^{14}$ and cytosine-2-$\text{C}^{14}$.
Fig. 7. Method of eluting material from spots on paper chromatograms.
Fig. 8. Apparatus for liquid scintillation counting.
1. Preamplifier. 2. Multiplier phototube housing.
REFERENCES


V. THE RADIATION DECOMPOSITION OF ADENINE

The action of ionizing radiation on a solution of adenine gives rise to two types of effects. In one case, the purine ring is preserved while, in the other, the ring structure is destroyed. Deamination, or the conversion of adenine to hypoxanthine, and the formation of 8-hydroxy-adenine are examples of the first type. The second type of radiation effect is characterized by severe damage to the molecule. In the latter case, the principal results observed were the formation of water-insoluble volatile products, the decrease in the optical density at 262 μ, and the opening of the imidazole ring (Figure 1).

Deamination of adenine

Deamination takes place quite readily when an amino-purine or aminopyrimidine is hydrolyzed with acid. Ten percent HCl converts cytosine into uracil. Nitrous acid deaminates adenosine to inosine and guanosine to xanthosine. A similar conversion takes place enzymatically. Intestinal deaminases, for example, convert adenine to hypoxanthine, adenosine to inosine, and adenylic acid to inosinic acid. The uv irradiation of adenine solutions has been reported to give trace quantities of hypoxanthine.

Previous workers had concluded that deamination of this type did not take place under the influence of ionizing radiation. With the aid of radioactive tracer techniques, however, it has been found in this study that adenine is converted into hypoxanthine. The yield is small, but
Adenine-2-Cl\textsuperscript{14} of specific activity 1.3 mc/mmole was obtained from Isotope Specialties, Inc., Burbank, California. Two hundred and fifty microliters of solution were irradiated at different dose levels, from \(10^6\) to \(2 \times 10^7\) rads.

The irradiation products were analyzed by paper chromatography, using Whatman No.4 paper and propanol-ammonia-water and butanol-propionic acid as solvents. Twenty-five \(\mu l\) of the irradiated solutions of adenine were spotted on paper together with 25 \(\mu g\) of inactive hypoxanthine as carrier. The ultraviolet-absorbing areas were detected with the aid of shadowgrams. Autoradiography with x-ray films showed darkening of the film corresponding to the hypoxanthine spot on the shadowgram. The radioactivity could have come only from the adenine, showing the conversion of adenine to hypoxanthine. The chromatography of the control was done at the same time as that of the irradiated samples. Thus, bacterial deamination could have been, at most, a minor effect.

In one experiment, the hypoxanthine spot was eluted with 0.1% formic acid and re-chromatographed with fresh carrier hypoxanthine using butanol-water\textsuperscript{9} and isobutyric acid-ammonia-EDTA\textsuperscript{10} as solvents. The radioactivity area exactly coincided with the hypoxanthine uv absorption, thus confirming the formation of radioactive hypoxanthine.

The hypoxanthine spots were eluted with 0.1% formic acid and counted with a liquid scintillation counter having
an internal standard of C$^{14}$-labeled toluene. The same technique was used for the estimation of residual adenine. The results of the experiments are shown in Table I.

The biological importance of a deamination of this type may be very great. If it would be legitimate to extrapolate from a high dose level to a low dose level, we may have a possible clue to the origin of a radiation mutation. When the ribonucleic acid of tobacco mosaic virus was heated with HNO$_2$, the amino bases were converted to the hydroxy bases without splitting of the nucleotide chain. Deamination of a large number of nucleotides resulted in the inactivation of the molecule.$^{11}$ More gentle treatment by controlled action of HNO$_2$ gave a maximum number of mutations when an average of 1 deamination resulted per 6000 nucleotides.$^{12}$

The Watson and Crick structure for DNA postulates a hydrogen bond between adenine and thymine in the double-strand structure.$^{13}$ Because hypoxanthine, xanthine, and uracil normally exist in the keto form,$^{14}$ the deamination of adenine to hypoxanthine will result in the disruption of this hydrogen bond. From theoretical considerations, Lavalle$^{15}$ pointed out that deamination of adenine would result in the replacement of an adenine-thymine base pair by a guanine-cytosine pair, altering the molecular code of heredity.

**Formamidopyrimidines**

Scholes and Weiss$^{16,17}$ have shown that in the x-irradiation of aqueous solutions of purine nucleotides and
nucleosides, the purine ring system is the most sensitive to radiation damage. Hems$^{18,19}$ examined the attack on the base in greater detail, and found that when aqueous solutions of guanosine and guanylic acid were irradiated in the absence of oxygen, the imidazole ring of the purine system opens to form the corresponding 4-amino-5-formamidopyrimidine riboside. This product was found to be similar in uv spectral characteristics to the products obtained by the alkylation of guanylic acid.$^{20}$

In the present study of the radiation products of aqueous solutions of adenine, the 4-amino-5-formamido-6-hydroxypyrimidine and the 4,6-diamino-5-formamidopyrimidine have been identified (Figure 2).

Twenty-five microliters of an 0.1% solution of adenine irradiated at $2 \times 10^6$ rads were chromatographed with 25 µg of each of the inactive 4,6-diamino-5-formamidopyrimidine and 4-amino-5-formamido-6-hydroxypyrimidine.$^{21}$ Two-dimensional chromatograms were run with propanol-ammonia-water and butanol-propionic acid-water as solvents. The positions of the inactive formamido compounds were found with the aid of shadowgrams. X-ray film autoradiographs of the same chromatograms showed darkened areas corresponding to the spots on the shadowgram. These spots were eluted with water and re-chromatographed in two dimensions, using butanol-water$^9$ and isobutyric acid-ammonia-EDTA.$^{10}$ In each case, there was complete coincidence between the darkening of the x-ray film and the uv absorbing spots as shown by the
shadowgram.

The identity of the 4,6-diamino-5-formamidopyrimidine produced by the radiolysis of adenine was further confirmed spectrophotometrically and chemically. The spot of paper cut out from the chromatogram was washed with ether to remove any impurities that might interfere with the uv spectrum. This was then eluted with water, and its spectrum observed with a Cary uv spectrophotometer (model II). At pH 7 the spectrum (Figure 3) had a maximum at 262 μm and was identical with that of an authentic sample of 4,6-diamino-5-formamidopyrimidine. When hydrolyzed with N HCl, the compound eluted from the paper was converted completely to 4,5,6-triaminopyrimidine. Heat cyclization for two hours at 200° C converted over 95% of the compound into adenine, leaving about 1% unchanged.

The yields of 4,6-diamino-5-formamidopyrimidine produced when solutions of adenine are irradiated at different dose levels are shown in Table II.

The maximum yield of the 4-amino-5-formamido-6-hydroxy pyrimidine obtained was only 0.5%. The amount eluted was not sufficient for chemical tests. This compound probably arose from the breakdown of the hypoxanthine produced during the irradiation.

The formation of these formamido compounds may be of some biological importance, especially in the light of the work of Scholes and Weiss, 16,17 and Hems, 18,19 with the nucleosides and nucleotides. The imidazole ring opens before
the loss of the sugar moiety. It is possible that, under the influence of radiation, a ring opening of this type could take place while the nucleotide is still attached to the double helical DNA. This type of ring opening has been observed with the mutagenic nitrogen mustards when they react with the nucleic acids. The opening of the imidazole ring while the monomeric nucleotide is still part of the long chain acid will interfere with the orderly process of replication of the DNA molecule.

8-hydroxyadenine

A second compound formed by the radiolysis of adenine without destruction of the purine ring was identified as 8-hydroxyadenine (Figure 4). The steps by which the identity of this compound (hereafter referred to as compound B) was established will now be described.

As the chromatographic characteristics of compound B were very similar to those of the 4,6-diamino-5-formamido compound already identified, it was thought to be the isomer of the 4,6-diamino-5-formamidopyrimidine. The latter is presumably formed by the fission of the C-8 to N-9 bond of adenine. If the ring opening took place by the fission of the N-7 to C-8 bond, the 5,6-diamino-4-formamido compound might also be formed.

Although several methods are available for the preparation of the 4,6-diamino-5-formamidopyrimidine, the 5,6-diamino-4-formamido compound has not been reported in the literature. Any attempt at formylation has resulted
in the production of the 5-formamido compound.

Compound B was unaffected by hydrolysis with 1 N HCl for 1 hour. In addition, heat cyclization at 200°C produced no adenine from it, whereas a formamidopyrimidine would have been expected to give a quantitative yield of adenine. The possibility of compound B being the 5,6-diamino-4-formamido compound was, therefore, ruled out.

When the radiolysis was repeated with adenine-8-C\textsuperscript{14}, the resulting chromatogram gave an autoradiograph identical with that obtained from irradiated adenine-2-C\textsuperscript{14} solution. No radioactivity was lost when compound B from adenine-8-C\textsuperscript{14} was hydrolyzed with 1 N HCl. This clearly indicated that both carbon atoms 2 and 8 of the purine system were present in compound B. It was therefore considered highly probable that the purine ring was intact in this product.

A shadowgram of adenine-2-C\textsuperscript{14} solution irradiated at 2 x 10\textsuperscript{6} rads and chromatographed on Whatman No.4 paper in two dimensions indicated that compound B was uv absorbing. The spectrum of the material was masked by the heavy background produced by the oxidation products of the solvents. As adenine derivatives are almost insoluble in ether,\textsuperscript{26} the interfering substances were removed by washing the paper containing compound B with ether before eluting it with water. Fig. 5 shows the uv spectrum of compound B at pH 2, 7, and 10.

Since compound B was readily produced, giving a yield of 5.5% at 2 x 10\textsuperscript{6} rads, it was thought probable that it was
the result of a simple process of hydroxylation. 6-N-hydroxylaminopurine, isoguanine, adenine 1-N-oxide, and 8-hydroxyadenine are possible hydroxylation products of adenine (Figure 4).

Adenine 1-N-oxide is formed by the action of $\text{H}_2\text{O}_2$ on a solution of adenine. $^{27,28}$ As radiolysis products of organic solutions are very similar to the reaction products of $\text{H}_2\text{O}_2$, $^{29}$ adenine 1-N-oxide could be produced by the irradiation of adenine. It has also been reported that adenine 1-N-oxide is formed by the radiolysis of adenine. $^{30}$ However, adenine 1-N-oxide has a characteristic uv absorption spectrum with maxima at 231 m$\mu$ and 262.5 m$\mu$. $^{29}$ The molar absorbancy at 231 m$\mu$ is $41.5 \times 10^3$ while at 262.5 it is only $8.1 \times 10^3$. Although adenine 1-N-oxide has $R_f$ values very similar to compound B in 4 different solvent systems; propanol-ammonia-water; butanol-propionic acid-water; isobutyric acid-ammonia-EDTA; and n-butanol-water, complete coincidence between adenine 1-N-oxide and compound B was not obtained when the chromatograms were examined with the aid of shadowgrams and autoradiographs. This established that compound B was not adenine 1-N-oxide.

It could be assumed that compound B was isoguanine which is found in the radiation products of adenine 1-N-oxide. $^{31}$ But, the uv absorption spectrum of isoguanine at pH 7 had two maxima at 238 m$\mu$ and 286 m$\mu$, $^{32}$ indicating that compound B was not isoguanine.

Nor could compound B be identical with 6-hydroxylamino-
purine. Although the latter has very similar UV spectral characteristics to those of compound B at pH 2 and pH 7, it is unstable in basic solution whereas compound B is stable. Besides, the 6-hydroxylaminopurine reduced alkaline phosphomolybdate reagent, as well as silver nitrate, and produced a deep blue color with ferric chloride. None of these reagents was affected by compound B.

Its spectral characteristics and stability to heat and acid treatment seemed to indicate that compound B was 8-hydroxyadenine. The latter was prepared by passing phosgene into a solution of 4,5,6-triaminopyrimidine sulfate. One gram of 4,5,6-triaminopyrimidine sulfate was dissolved in 40 c.c. of 10% sodium hydroxide and phosgene was passed in for 2 hours. 5 c.c. of 2 N sulfuric acid was added to the reaction mixture. On cooling, the 8-hydroxyadenine sulfate separated. The product was re-crystallized from 2 N H$_2$SO$_4$. Analytical data were as follows:

For (C$_5$H$_5$ON$_5$)$_2$H$_2$SO$_4$

Calculated  C,30.03%  H,3.02%
Found  C,30.05%  H,3.10%

Twenty-five micrograms of 8-hydroxyadenine were co-chromatographed with 25 of adenine-2-C$^{14}$ solution irradiated at 2 x $10^6$ rads. The radioactivity of compound B corresponded to the UV absorption of the 8-hydroxyadenine. This coincidence was obtained in 4 different solvent systems establishing that compound B was 8-hydroxyadenine. This was further confirmed by the UV absorption spectra of compound B.
which had the same characteristics as that of 8-hydroxyadenine at pH 2, 7, and 10.32 (Figure 6).

**Breakdown of the ring system**

To estimate the amount of radioactivity lost when a solution of adenine-2-\(^{14}\)C is irradiated from $10^6$ to $2 \times 10^7$ rads, 25 microliters of each irradiated solution were counted with a liquid scintillation counter, using an internal standard. The loss of activity may be due to several causes - the release of \(^{14}\)C-labeled volatile material, the adsorption of \(^{14}\)C-labeled compounds on the glass container, or the chelation of radioactive products by trace impurities. However, in every case, the loss of activity is a measure of the breakdown of the molecule.

In a second series of experiments, a solution of adenine-8-\(^{14}\)C was irradiated and the loss of radioactivity was determined. The adenine-8-\(^{14}\)C was obtained from Nichem Inc., Bethesda, Md., and had a specific activity of $10 \text{ mc/m mole}$. These results indicated the part of the purine system - the pyrimidine or the imidazole - which was more sensitive to radiation (Figure 7). The loss of radioactivity increases linearly with the dose. The greater decomposition of adenine-8-\(^{14}\)C solutions shows that the imidazole ring is more labile to radiation than the pyrimidine ring. In view of the resonance stability of the pyrimidine ring, it is hardly surprising that the imidazole ring is attacked more easily than the pyrimidine ring.
Residual adenine

In the series of irradiations of adenine-2-$^{14}$C, the amount of adenine destroyed was measured. The residual adenine was separated from the decomposition products of radiation by two-dimensional paper chromatography. The adenine spots were detected with the aid of shadowgrams. The spots were then eluted with 2 ml of 0.1% formic acid. The eluant was made up to 5 ml and 200 $\mu l$ of each counted with a liquid scintillation counter. In Figure 8, the percentage of adenine destroyed is plotted against radiation dosage. From 0 to $2 \times 10^6$ rads, there is a steep rise in the curve. From $2 \times 10^6$ to $20 \times 10^6$ rads, the slope is less and the destruction of adenine is a linear function of the dose.

The effect of irradiation on the uv absorption spectra of an 0,1% solution of adenine at pH 7 is shown in Figure 9. All measurements were made with a Cary recording spectrophotometer (model 14). The optical density decreases with increasing radiation. At $20 \times 10^6$ rads, there is no peak visible, confirming the findings by paper chromatography that the adenine is entirely destroyed.
TABLE I

Formation of hypoxanthine from adenine by ionizing radiation. In all experiments, the initial total activity was $4.1 \times 10^5$ disintegrations per minute (dpm).

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Dose (10^6 rads)</th>
<th>Residual adenine (%)</th>
<th>Hypoxanthine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Act. (dpm)</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>87.7</td>
<td>3602</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>75.4</td>
<td>3807</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>60.1</td>
<td>7840</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>44.4</td>
<td>3839</td>
</tr>
</tbody>
</table>
TABLE II

Formation of 4,6-diamino-5-formamidopyrimidine and 8-hydroxyadenine from 0.1% solution of adenine-2-C14

<table>
<thead>
<tr>
<th>Dose in $10^6$ rads</th>
<th>4,6-diamino-5-formamidopyrimidine</th>
<th>8-hydroxyadenine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>3.8</td>
<td>5.5</td>
</tr>
<tr>
<td>5</td>
<td>3.5</td>
<td>10.5</td>
</tr>
<tr>
<td>10</td>
<td>1.7</td>
<td>9.9</td>
</tr>
<tr>
<td>20</td>
<td>0.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Fig. 1. Chromatogram of an aqueous solution of adenine-2-\( ^{14} \text{C} \) irradiated at \( 2 \times 10^6 \) rads.
Fig. 2. Formamidopyrimidines from adenine and hypoxanthine.
Fig. 3. UV spectrum of 4,6-diamino-5-formamidopyrimidine formed by the radiation decomposition of adenine.
Fig. 5. UV spectrum of Compound B.
A. At pH 7.  B. At pH 2.  C. At pH 10.
Fig. 6. UV spectrum of 8-hydroxyadenine.
A. At pH 7.  B. At pH 2.  C. At pH 10.
Fig. 7. Loss of volatile radioactivity on the irradiation of adenine-2-C\textsuperscript{14} and adenine-8-C\textsuperscript{14}. 
Fig. 8. The radiation decomposition of adenine.
Fig. 9. UV spectrum of irradiated adenine at pH 7.
A. Unirradiated B. $2 \times 10^6$ rads. C. $5 \times 10^6$ rads.
D. $10 \times 10^6$ rads. E. $20 \times 10^6$ rads.
REFERENCES

21. These compounds were kindly supplied by Dr. George B. Brown of the Sloan-Kettering Cancer Research Institute, New York.


VI. THE RADIATION DECOMPOSITION OF CYTOSINE

In the nucleic acids, there are three pyrimidine bases — cytosine, uracil, and thymine. Uracil is found only in RNA, while thymine occurs only in DNA. Cytosine, however, is a constituent of both DNA and RNA. The chemical effect of ionizing radiation on this pyrimidine is, therefore, of special interest in a study of the radiation chemistry of nucleic acid constituents.

Several workers have described the formation of hydroxy-hydroperoxides from pyrimidines under the influence of radiation. Sinsheimer and Hastings\(^1\) showed that the action of uv light on uracil in aqueous solutions produces a well-defined photo-product, 4-dihydro-5-hydroxy-dimethyluracil. Scholes and Weiss\(^2,3,4\) have described the formation of hydroxy-hydroperoxides when aqueous solutions of uracil and dimethyluracil were irradiated with Co\(^{60}\) \(\gamma\)-rays or 200 kv x-rays in the presence of air. In the absence of oxygen, however, dimethyluracil gave 4-dihydro-5-hydroxy-dimethyluracil and the corresponding 4,5-glycol. Ekert and Monier\(^5\) recently demonstrated the formation of a pyrimidine glycol when a solution of cytosine was irradiated in the absence of oxygen.

The principal chemical effects of ionizing radiation on the purines adenine and guanine were the breakdown of the ring system and deamination.\(^6,7\) These two effects have now been observed in the case of the pyrimidine cytosine.
The breakdown of the pyrimidine ring

Cytosine-2-C\(^{14}\) of specific activity 45.6 \(\mu\)C/mg was supplied by Schwarz BioResearch Inc., Mount Vernon, New York. Two hundred and fifty microliters of an 0.1% solution of cytosine in water were sealed under vacuum after dissolved oxygen was expelled by bubbling nitrogen through the solution. The solutions were irradiated at different dose levels from \(10^6\) to \(20 \times 10^6\) rads.

To estimate the amount of radioactivity lost on irradiation, probably volatile water-insoluble C\(^{14}\) compounds, 25 \(\mu\)l of each of the irradiated solutions were diluted to 5 ml. Aliquots (100 microliters) of this solution were measured by liquid scintillation counting, using an internal standard.

The residual cytosine was separated from the radiation decomposition products by 2-dimensional paper chromatography, using Whatman No. 4 paper washed with oxalic acid. Propanol-ammonia-water was used as a solvent in one direction, and butanol-propionic acid-water in the other. Twenty-five microliters of the irradiated solutions were spotted on each chromatogram. The distribution of radioactivity on the chromatogram was recorded by autoradiography with x-ray film. The amount of residual cytosine was estimated by eluting the cytosine spots with 0.1% formic acid and counting aliquots of the elute using a liquid scintillation counter with an internal standard of C\(^{14}\)-labeled toluene. The results are shown in Table I.
The effect of irradiation on the UV absorption of an 0.1% solution of cytosine is shown in Figure 1. The measurements were made with a Cary recording spectrophotometer (Model 11) at a pH of 7. The optical density decreases with increasing radiation. This, however, is only an approximate indication of the breakdown of the pyrimidine ring as some of the radiation products may absorb at this wavelength.

The results shown in Table I indicate that cytosine is less sensitive to ionizing radiation than the purines adenine and guanine. At a dose level of 20 x $10^6$ rads, adenine was completely destroyed, and the loss of radioactivity as volatile material amounted to 46.8%. With cytosine, however, 13.5% of the original material was left intact and only 8.5% was lost as volatile compounds.

**Deamination of cytosine**

The deamination of adenine and guanine by ionizing radiation has already been described. The conversion of cytosine to uracil has now been observed.

The irradiation products of cytosine-2-Cl were co-chromatographed with non-radioactive uracil as carrier. The uracil was detected in the chromatogram with the aid of shadowgrams. There was darkening of the x-ray film corresponding to the UV absorbing area of the shadowgram. This coincidence was obtained with 4 different solvent systems - butanol-propionic acid-water; propanol-ammonia-water; butanol-formic acid-water; and n-butanol.
The radioactivity could have come only from the cytosine, establishing the conversion of cytosine to uracil. The maximum yield of uracil (Table I) is 3.7% at $2 \times 10^6$ rads. This is significantly larger than the maximum of 1.9% at $5 \times 10^6$ rads observed in the deamination of adenine.\textsuperscript{6}

As cytosine occurs both in DNA and RNA, the conversion of cytosine to uracil may have more biological implications than the deamination of adenine or guanine. This change would give rise to an unnatural base in DNA and, if replication occurs, a cytosine-guanine base pair would ultimately be replaced by an adenine-thymine pair. With RNA, it is conceivable that the altered nucleic acid undergoes identical replication.\textsuperscript{12} In either case, an altered base would presumably lead to a change in protein composition.\textsuperscript{13,14,15}
TABLE I
Radiation decomposition of cytosine-2-C\textsuperscript{14}

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Dose in rads</th>
<th>Loss of volatile radioactivity</th>
<th>% cytosine destroyed</th>
<th>% uracil found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.01</td>
</tr>
<tr>
<td>1</td>
<td>$10^6$</td>
<td>0.0</td>
<td>9.8</td>
<td>2.7</td>
</tr>
<tr>
<td>2</td>
<td>$2 \times 10^6$</td>
<td>0.0</td>
<td>10.2</td>
<td>3.7</td>
</tr>
<tr>
<td>3</td>
<td>$5 \times 10^6$</td>
<td>0.0</td>
<td>39.0</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>$10 \times 10^6$</td>
<td>0.0</td>
<td>53.5</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>$20 \times 10^6$</td>
<td>8.5</td>
<td>36.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Fig. 1. UV spectrum of irradiated cytosine at pH 7.
A. Unirradiated. B. $1 \times 10^6$ rads. C. $2 \times 10^6$ rads.
D. $5 \times 10^6$ rads. E. $20 \times 10^6$ rads.
REFERENCES

11. R.D. Hotchkiss, J. Biol. Chem. 175, 315 (1948).
VII. THE RADIATION DECOMPOSITION OF GUANINE

Since guanine has the same ring structure as adenine, the radiation decomposition of guanine would be expected to be very similar to that of adenine. However, observations on the effect of radiomimetic alkylating agents on nucleic acids have shown that the imidazole ring of deoxyguanylic acid is very sensitive to the action of these chemicals.\(^1,2\) The radiation degradation of guanylic acid and guanosine has also been reported to be different from that of the corresponding adenine derivatives, especially with regard to the opening of the imidazole ring.\(^3\) It was, therefore, considered to be of some importance to examine the effect of ionizing radiation on a solution of guanine to see whether the pattern of decomposition of guanine differed markedly from that of adenine.

The solubility of guanine in water is extremely low.\(^4\) The free base has a solubility of 0.004% at 40\(^\circ\). A detailed study of the radiation effects on a solution was thus made very difficult. A broad picture, however, of the radiation decomposition has been obtained.

Scholes and Weiss\(^5\) found that when an 0.1% solution of guanine at a pH of 1.28 was irradiated in the presence of air with 200 kv x-rays at a dose of 4 x 10\(^6\) rads, ammonia, oxalic acid, and guanidine were formed. They also noted the presence of small amounts of hydroxylamine.\(^6\)

In this study, the irradiations were done in aqueous solution in the absence of air. As in the case of adenine,
the effects observed were the deamination of guanine to xanthine, the conversion of guanine into 8-hydroxyguanine, the opening of the imidazole ring, the formation of water-insoluble volatile products, and a marked change in the uv spectrum of the solution.

Opening of the imidazole ring

The imidazole ring of adenine opened to give the 4,6-diamino-5-formamidopyrimidine. Guanine would, by analogy, be expected to give the 2,4-diamino-5-formamido-6-hydroxy-pyrimidine. Co-chromatography with an authentic sample of the latter compound indicated a yield of only 0.9%. This result is surprising as the opening of the imidazole ring to give the corresponding formamidopyrimidine riboside has been reported to take place readily when solutions of guanosine and guanylic acid were irradiated.

Deamination of guanine

Guanine-8-C\textsuperscript{14} sulfate \((\text{C}_5\text{H}_5\text{ON}_\textsubscript{5})_2\text{H}_2\text{SO}_4\) of specific activity 2.18 mc/mmmole was obtained from the Oak Ridge National Laboratory. An 0.004% solution was used for these experiments.

The method of irradiation and chromatographic analysis was the same as that described for the radiation decomposition of adenine. Two hundred and fifty microliters of a solution of inactive xanthine in 1 N HCl containing 0.1 mg xanthine per ml were chromatographed together with 500 \(\mu\)l of guanine sulfate solution irradiated at 2 \(\times\) 10\textsuperscript{6} rads. An autoradiograph with x-ray film showed darkening of the film,
corresponding to the inactive xanthine as detected by a shadowgram. The same result was obtained in 4 different solvent systems: propanol-ammonia-water, butanol-propionic acid-water, n-butanol-water, and ammonia-isobutyric acid-EDTA. As no radioactive xanthine was detected in the blank experiment in which guanine-8-\textsuperscript{14}C sulfate was chromatographed with inactive xanthine, the radioactivity of the xanthine spot in the irradiated material must have come from the guanine, showing the conversion of guanine to xanthine. At 2 x 10\textsuperscript{6} rads, the yield of xanthine was 0.63\%.

Formation of 8-hydroxyguanine

As 8-hydroxyadenine was formed when an aqueous solution of adenine was irradiated, it was expected that guanine would give 8-hydroxyguanine.

The 8-hydroxy compound of guanine, or 2-amino-6,8-dihydroxy purine, was prepared according to the method of L.F. Cavalieri and A. Bendich. One gram of 2,4,5-triamino-6-hydroxypyrimidine sulfate and 2.5 gm of urea were intimately mixed and fused at 180° for 1 hour. The melt was extracted with 20 c.c. of 12 N NaOH. Suspended insoluble material was removed by filtration. The filtrate was acidified with glacial acetic acid. The yield was 20\%.

Two hundred and fifty microliters of a solution of guanine-2-\textsuperscript{14}C sulfate irradiated at 2 x 10\textsuperscript{6} rads were co-chromatographed with 25 \( \mu g \) of inactive 8-hydroxyguanine. An autoradiograph with x-ray film showed darkening of the film corresponding to the inactive 8-hydroxypurine as detected by
a shadowgram. The same result was obtained in the 4 different solvent systems described above in the case of xanthine. The yield of the 8-hydroxy compound was 0.9%.

Breakdown of the purine ring

The amount of radioactivity lost when solutions of guanine-8-C\textsuperscript{14} sulfate and guanine-2-C\textsuperscript{14} sulfate were irradiated from $10^6$ to $2 \times 10^7$ rads was determined by counting aliquots of the irradiated solutions with a liquid scintillation counter. Guanine-2-C\textsuperscript{14} sulfate of specific activity 9.2 $\mu$C/mg for this experiment was obtained from the Southern Research Institute, Birmingham, Alabama.

As described in the study on adenine, the loss of radioactivity may be due to the release of C\textsuperscript{14}-labeled volatile material, adsorption on the glass surface, or chelation by trace impurities. However, the loss of radioactivity was considered to be a measure of the radiation damage.

The results are shown in Figure 1. As expected, the guanine-8-C\textsuperscript{14} sulfate with the radioactive carbon atom in the imidazole ring has lost more activity than the molecule with the label in the pyrimidine ring.

UV absorption spectrum of irradiated guanine

The uv spectra of a solution of guanine sulfate irradiated at 3 different dose levels is shown in Figure 2. At $5 \times 10^6$ rads, the absorption peak has disappeared completely. This is in marked contrast to that of adenine where, even at $10^7$ rads, the absorption peak can still be
observed.

Radiation products of guanine

A chromatogram of a solution of guanine-2-C\textsuperscript{14} irradiated at 2 x 10\textsuperscript{6} rads is shown in Figure 3. Several spots can be seen. In contrast to this picture, the autoradiograph of a chromatogram of adenine-2-C\textsuperscript{14} irradiated at 2 x 10\textsuperscript{6} rads shows fewer radiation decomposition products. The guanine destroyed on increasing the radiation dose from 10\textsuperscript{6} to 2 x 10\textsuperscript{7} rads is shown in Table I.
TABLE I

Guanine destroyed on irradiation

<table>
<thead>
<tr>
<th>Dose in rads</th>
<th>% guanine destroyed</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^6$</td>
<td>34</td>
</tr>
<tr>
<td>$2 \times 10^6$</td>
<td>61</td>
</tr>
<tr>
<td>$5 \times 10^6$</td>
<td>98</td>
</tr>
</tbody>
</table>
Fig. 1. Radiation decomposition of guanine-2-C^{14} sulfate and guanine-8-C^{14} sulfate.
Fig. 2. UV spectrum of irradiated guanine-2-C\textsuperscript{14} sulfate at pH 7.
A. Unirradiated. B. $2 \times 10^6$ rads. C. $5 \times 10^6$ rads.
Fig. 3. Chromatogram of guanine-2-C$^{14}$ sulfate irradiated at $2 \times 10^6$ rads.
REFERENCES

7. These compounds were kindly supplied by Dr. George B. Brown of the Sloan-Kettering Cancer Research Institute, New York.
11. R.D. Hotchkiss, J. Biol. Chem. 175, 315 (1948).
VIII. THE RADIATION DECOMPOSITION OF NUCLEOSIDES AND NUCLEOTIDES

Some of the chemical changes which occur when nucleosides and nucleotides are irradiated have been reported by Scholes and Weiss.\textsuperscript{1,2} Among the results described were the formation of free ribose from the nucleoside, and the dephosphorylation of the nucleotide.\textsuperscript{3} Hydroperoxides have also been shown to be formed from the nucleotides and nucleosides of the pyrimidines.\textsuperscript{4}

In this investigation, \textsuperscript{14}C-labeled nucleotides and nucleosides were irradiated in aqueous solutions to determine whether the changes observed in the free base also took place in the nucleoside and the nucleotide. Of special interest was the question of deamination.

Nucleosides

\textsuperscript{14}C-labeled adenosine, guanosine, and cytidine were obtained from Schwarz BioResearch, Mount Vernon, New York. The following were the specific activities of each:

- Adenosine-8-\textsuperscript{14}C: 5 \(\mu\)c/mg
- Cytidine-2-\textsuperscript{14}C: 4 \(\mu\)c/mg
- Guanosine-2-\textsuperscript{14}C: 3.1 \(\mu\)c/mg

Two hundred and fifty microliters of 0.1% aqueous solutions of these nucleosides were irradiated in the absence of air at a dose of 2 \(\times\) 10\(^6\) rads. The radiation products were chromatographed in two dimensions, using butanol-propionic acid-water\textsuperscript{5} and propanol-ammonia-water\textsuperscript{6} as solvents. In every case, there was a release of free
base, adenine from adenosine, guanine from guanosine, and cytosine from cytidine.

Co-chromatography with inactive carrier, combined with autoradiography and uv shadowgrams, demonstrated the deamination of these bases. Hypoxanthine was detected in the radiation products of adenosine, xanthine in guanosine, and uracil in cytidine. The yield of free bases and hydroxy bases formed from the nucleosides is shown in Table I.

In adenosine and cytidine some deamination took place before the release of the ribose to give inosine and uridine. However, if any xanthosine was formed from guanosine it was too small to be detected.

In addition to hypoxanthine, 4,6-diamino-5-formamidopyrimidine and 8-hydroxyadenine (formed in the radiolysis of adenine) were also detected in the radiation decomposition products of adenosine. The formation of the 4,6-diamino-5-formamidopyrimidine (3%) and 8-hydroxyadenine (4%) seems to indicate that the fission of the glycosidic linkage takes place before the attack on the base.

The uv spectra of 0.1% solutions of adenosine, guanosine and cytidine, irradiated at 2 x 10^6 rads, are shown in Figures 1, 2, and 3. In the case of adenosine, there is a decrease of about 40% in the optical density of the solution at 260 mµ. In guanosine, the peak at 252 mµ has disappeared and a new peak has appeared at 225 mµ, where the unirradiated solution showed a minimum. In cytidine, the absorption has disappeared almost completely, possibly due to the destruction of the chromophore in the pyrimidine ring on the formation
of a pyrimidine glycol.\textsuperscript{7}

**Nucleotides**

The following nucleotides, uniformly labeled with $^{14}C$, were obtained from Schwarz BioResearch, Mount Vernon, New York:

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3'$ adenylic acid</td>
<td>0.5 $\mu$C/mg</td>
</tr>
<tr>
<td>cytidylic acid (mixed isomers)</td>
<td>1.0 $\mu$C/mg</td>
</tr>
<tr>
<td>$3'$ guanylic acid</td>
<td>0.1 $\mu$C/mg</td>
</tr>
<tr>
<td>uridylic acid (mixed isomers)</td>
<td>1.0 $\mu$C/mg</td>
</tr>
</tbody>
</table>

In all these nucleotides, except the uridylic acid, the radiochemical impurities as detected by scanning a unidimensional paper chromatogram added up to more than 0.1%. The presence of the impurities may have been due to self-irradiation.\textsuperscript{8} The cytidylic, guanylic, and adenylic acid were recrystallized from water. The resulting product gave only one radioactive spot on a unidimensional chromatogram run in a solution of isobutyric acid-ammonia-EDTA.\textsuperscript{9}

In one experiment, 0.1 mg of solid uridylic acid was vacuum-sealed in a capillary tube 3 mm in diameter and exposed to the electron beam of a linear accelerator.\textsuperscript{10} At $1.2 \times 10^8$ rads, no evidence of decomposition was obtained. At $1.4 \times 10^8$ rads, about 5% decomposition was obtained. Uridine and uracil were both detected.

In subsequent experiments, 0.1% solutions of the nucleotides were irradiated in vacuo at dose levels ranging from $10^6$ to $10 \times 10^6$ rads. The uv absorption spectra of these nucleotides are shown in Figures 4 to 7. In the case of cytidylic acid, there is a marked drop in the optical
density between A and B. This may be explained in terms of the formation of the pyrimidine glycol by the attack of OH radicals on carbon atoms 4 and 5 of the pyrimidine ring.

Since the purine or pyrimidine base of nucleotides is easily released by radiation, it was thought highly probable that ribose or ribose phosphate would be liberated. To test this possibility, twenty-five microliters of each of these solutions were chromatographed in two dimensions, together with twenty-five micrograms each of ribose and ribose phosphate. The solvents used were phenol-water and butanol-propionic acid-water. Autoradiographs were made after a week's exposure of the chromatograms to x-ray film. The chromatograms were then sprayed with an aniline hydrogen pthalate spray, prepared by dissolving 0.93 gm of aniline and 1.66 gm of pthalic acid in 100 ml of water-saturated butanol. One microgram of ribose could be detected easily by means of the color reaction given by this reagent. The sprayed chromatograms were heated for 5 minutes at 105°C. Superimposing the autoradiographs on the sprayed chromatograms showed no darkening of film corresponding to the areas where free carrier sugars gave color reactions with the aniline hydrogen pthalate. It must, therefore, be concluded that when nucleotides are irradiated at $2 \times 10^6$ rads in the absence of air ribose, or ribose phosphate, is not formed.

The amount of free base and nucleoside released when each of the nucleotides is irradiated at $2 \times 10^6$ rads is shown in Table II. In the case of adenylc acid, about
1.0% hypoxanthine was also formed. 4,6-diamino-5-formamidopyrimidine and 8-hydroxyadenine were both detected. The yield of each was about 0.5%.

RNA

Several workers have described the physico-chemical changes produced when nucleic acids were irradiated in solution.\textsuperscript{12,13} Many of these changes have been explained in terms of the fission of sugar phosphate bonds in the backbone of the molecule, and the rupture of hydrogen bonds.\textsuperscript{14}

It is of considerable importance to know whether the changes which the base - nucleoside or nucleotide - undergoes when irradiated occur in the case of the polymer.

An exploratory experiment was performed with RNA to find out whether adenylic acid could be deaminated\textsuperscript{15} to inosinic acid when a solution of RNA was irradiated at 2 $\times$ 10$^6$ rads.

RNA randomly labeled with C$^{14}$ of specific activity 30 $\mu$C/mg, was obtained from Schwarz BioResearch, Mount Vernon, New York. Two hundred and fifty microliters of an 0.1% solution of RNA were irradiated at 2 $\times$ 10$^6$ rads.

Several methods have been described for the hydrolysis of nucleic acids to the constituent nucleotides, nucleosides, and free bases.\textsuperscript{16} Many of these methods give rise to deamination. Thus, 1 N alkali at 37° C has caused 10% to 33% deamination in cytidylic acid.\textsuperscript{17} Cytidylic acid was deaminated to the extent of 2% N/100 NaOH and 12% in N/10 NaOH, but no deamination was detected at pH 11.\textsuperscript{18} This latter method was, therefore, used for the hydrolysis of RNA
in this experiment.

One hundred microliters each of RNA irradiated at $2 \times 10^6$ rads, and unirradiated RNA, were treated with 100 of N/500 NaOH at 37° C for 24 hours. Twenty-five microliters of each solution were then chromatographed two-dimensionally, using inactive inosinic acid as carrier. As the radioactive spots on the autoradiograph were not well-defined, the spots corresponding to the inosinic acid were cut out and re-chromatographed in isobutyric acid-ammonia-EDTA. A trace of radioactivity (0.01%) was detected in the inosinic spot corresponding to the blank, and 0.5% in that from the irradiated material.

In a further experiment, the irradiated RNA solution was subjected to paper ionophoresis$^{19}$ at 600 volts for 6 hours in propionate buffer at pH 3.4. About 0.5% radioactivity was detected in the spot corresponding to the inactive carrier inosinic acid. It appears, therefore, that deamination takes place at every level - in the free base, the nucleoside, nucleotide, and the polymer.
TABLE I

Formation of bases and hydroxy bases from nucleosides irradiated at $2 \times 10^6$ rads

<table>
<thead>
<tr>
<th>Residual Nucleoside</th>
<th>Base</th>
<th>Hydroxy Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine 18%</td>
<td>Adenine</td>
<td>Hypoxanthine 2%</td>
</tr>
<tr>
<td>Cytidine 20%</td>
<td>Cytosine  9%</td>
<td>Uracil 0.5%</td>
</tr>
<tr>
<td>Guanosine 25%</td>
<td>Guanine</td>
<td>Xanthine 0.2%</td>
</tr>
</tbody>
</table>
### TABLE II

Formation of bases and hydroxy bases from nucleotides irradiated at $2 \times 10^6$ rads

<table>
<thead>
<tr>
<th>Residual Nucleotide</th>
<th>Base</th>
<th>Hydroxy Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylic acid 26%</td>
<td>Adenine 40%</td>
<td>Hypoxanthine 1.0%</td>
</tr>
<tr>
<td>Cytidylic acid 18%</td>
<td>Cytosine 7%</td>
<td>Uracil 1.8%</td>
</tr>
<tr>
<td>Guanylic acid 10%</td>
<td>Guanine 8%</td>
<td>Xanthine 1.5%</td>
</tr>
</tbody>
</table>
Fig. 1. UV spectrum of irradiated adenosine at pH 7.  
A. Unirradiated.  B. $2 \times 10^6$ rads.
Fig. 2. UV spectrum of irradiated guanosine at pH 7.
A. Unirradiated. B. $2 \times 10^6$ rads.
Fig. 3. UV spectrum of irradiated cytidine at pH 7. 
A. Unirradiated. B. $2 \times 10^6$ rads.
Fig. 4. UV spectrum of irradiated adenylic acid at pH 7.
A. Unirradiated. B. $1.2 \times 10^6$ rads. C. $6 \times 10^6$ rads.
D. $12 \times 10^6$ rads. E. $18 \times 10^6$ rads.
Fig. 5. UV spectrum of irradiated cytidylic acid at pH 7.
A. Unirradiated. B. 10^6 rads. C. 5 \times 10^6 rads.
D. 10 \times 10^6 rads.
Fig. 6. UV spectrum of irradiated guanylic acid at pH 7.
A. Unirradiated. B. $10^6$ rads. C. $2 \times 10^6$ rads.
D. $5 \times 10^6$ rads. E. $10 \times 10^6$ rads.
Fig. 7. UV spectrum of irradiated uridylic acid at pH 7.
A. Unirradiated. B. $6 \times 10^6$ rads. C. $1.2 \times 10^6$ rads.
D. $12 \times 10^6$ rads.
REFERENCES

IX. CONCLUSION

The chemical effect of ionizing radiation on nucleic acid constituents was studied with the intention of finding those effects which may be of some significance in understanding the origin of a radiation mutation. The reactions which appeared to be of biological importance were therefore examined. Among these may be listed the following:

1. Destruction of bases
2. Destruction of nucleosides
3. Destruction of nucleotides
4. Release of sugar from nucleosides and nucleotides
5. Release of sugar phosphate from nucleotides
6. Release of phosphate from nucleotides
7. Deamination of amino bases to hydroxy bases in the free bases, nucleosides, and nucleotides
8. Opening of the imidazole ring of purine bases
9. Formation of 8-hydroxypurines

These radiation effects were studied in aqueous solution, and must be considered to have arisen through the mediation of the free radicals produced by the radiolysis of water,

\[ \text{H}_2\text{O} \rightarrow \text{H}^+ + \cdot \text{OH} \]

The G values, or number of molecules destroyed, transformed, or produced per 100 electron volts, are presented in Tables I - IX. As all the radiations done in this study were in the absence of oxygen, some values culled from the literature have been included for purposes of comparison.

The G values for destruction of bases (Table I) show that there is no oxygen effect when the free bases are irradiated in aqueous solution. The purines are clearly more
stable than the pyrimidines. This sensitivity of the pyrimidines arises from the ease with which the pyrimidines give rise to hydroperoxides when irradiated in water.\textsuperscript{1,2} Guanine appears to be less sensitive to radiation than adenine. The concentration effect for adenine is not significant. For the pyrimidine thymine, however, there is a 50% increase in the G value for every tenfold increase in concentration.

All the nucleosides (Table II) were irradiated at approximately the same concentration. The G values indicate that they are equally sensitive to radiation. Again, there is no apparent oxygen effect.

The sensitivity of the purine and pyrimidine nucleotides to radiation is about the same (Table III). The G values for nucleotide destruction are not markedly different from those of the free bases and the nucleosides. Guanine is the only anomaly in that the free base is much more stable to radiation than the nucleoside or the nucleotide. Here, too, there seems to be no apparent oxygen effect.

While sugar is released (Table IV) when nucleosides are irradiated in the presence or absence of O\textsubscript{2}, no sugar or sugar phosphate (Table V) appears to be formed when nucleotides are irradiated. The presence of the phosphate group must enhance the destruction of the sugar moiety. Scholes and Weiss\textsuperscript{3} have also reported that they were unable to detect ribose or ribose phosphate from irradiated solutions of adenylic acid.
The release of phosphate (Table VI) is the same for both purine and pyrimidine nucleotides. This is significantly lower than the value for nucleotides destroyed. The attack on the sugar and the free base thus appears to be a major pathway of attack on the nucleotide molecule by free radicals generated in the radiation of the aqueous solutions.3

Table VII gives the G values for deamination or the conversion of the amino bases into the hydroxy bases. Adenine is converted into hypoxanthine, guanine into xanthine, and cytosine into uracil. At the nucleoside level, adenosine yields inosine, and uridine is formed from cytidine. No xanthosine, however, was detected from guanosine. Inosinic acid was formed from adenylic acid, and uridylic acid from cytidylic acid. The formation of xanthyllic acid from guanylic acid was not investigated. The G value decreases from the base through the nucleoside to the nucleotide. Of the free bases, the pyrimidine cytosine appears most sensitive to this reaction.

In Tables VIII and IX are given the G values for two reactions of the purines - the opening of the imidazole ring and the formation of the 8-hydroxypurines. The yield of 4,6-diamino-5-formamidopyrimidine from adenine is considerably higher than the corresponding 2,4-diamino-5-formamido-6-hydroxypyrimidine from guanine. Similarly, the yield of the 8-hydroxyadenine is considerably greater than that of the 8-hydroxyguanine. These results are consistent with the low G value for the destruction of guanine when the free base
is irradiated.

All the reactions described here were studied at very high dose levels in order to obtain sufficient material for the identification of the products. However, if it would be reasonable to extrapolate these results to low levels of radiation, they would probably have great significance in radiobiology, especially with reference to the origin of a radiation mutation.

Those reactions which would do gross damage to the replicating molecule as base destruction, release of sugar, etc., would, no doubt, be lethal. But a change which still allows replication to take place would inevitably result in a mutation.

Deamination by chemical agents like nitrous acid is known to give rise to mutations. This method, indeed, has been used for the production of artificial mutants in the tobacco mosaic virus. That deamination of the same type takes place when nucleic acid constituents were exposed to ionizing radiation is, perhaps, of great significance in understanding the origin of a radiation mutation. The deamination took place at every level, in the free base, the nucleoside, the nucleotide, and the polymer. The conversions observed were:

1. Adenine to hypoxanthine
2. Adenosine to inosine
3. Adenylic acid to inosinic acid
4. Guanine to xanthine
5. Cytosine to uracil
6. Cytidine to uridine
7. Cytidylic acid to uridylic acid
A glance at Figure 5 of Chapter II shows that, when adenine is changed to hypoxanthine, the hypoxanthine would pair with cytosine instead of with thymine at the next replication. The cytosine would then seek out its normal partner, guanine. An adenine-thymine pair would thus be replaced by a cytosine-guanine pair. Similarly, the change of cytosine into uracil would convert a cytosine-guanine pair into an adenine-thymine pair. The conversion of cytosine into uracil would, moreover, give rise to an unnatural base in the DNA molecule. In the light of recent evidence for the existence of the genetic code, an altered base would change the molecular code of heredity.
TABLE I

Bases destroyed

1. In the absence of $O_2$

<table>
<thead>
<tr>
<th>Base</th>
<th>Conc. in moles per liter</th>
<th>$G$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>$7.5 \times 10^{-3}$</td>
<td>0.9</td>
</tr>
<tr>
<td>Guanine</td>
<td>$0.3 \times 10^{-3}$</td>
<td>0.1</td>
</tr>
<tr>
<td>Cytosine</td>
<td>$9.0 \times 10^{-3}$</td>
<td>1.5</td>
</tr>
<tr>
<td>Uracil</td>
<td>$8.9 \times 10^{-3}$</td>
<td>1.2</td>
</tr>
<tr>
<td>Thymine</td>
<td>$7.9 \times 10^{-3}$</td>
<td>2.0</td>
</tr>
</tbody>
</table>

2. In the presence of $O_2$ (G. Scholes, J. Ward, and J. Weiss, *J. Mol. Biol.* 2, 379 (1960))

<table>
<thead>
<tr>
<th>Base</th>
<th>Conc. in moles per liter</th>
<th>$G$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>$2 \times 10^{-4}$</td>
<td>1.09</td>
</tr>
<tr>
<td>Guanine</td>
<td>$2 \times 10^{-4}$</td>
<td>1.09</td>
</tr>
<tr>
<td>Cytosine</td>
<td>$2 \times 10^{-4}$</td>
<td>2.05</td>
</tr>
<tr>
<td>Uracil</td>
<td>$2 \times 10^{-4}$</td>
<td>1.93</td>
</tr>
<tr>
<td>Thymine</td>
<td>$2 \times 10^{-4}$</td>
<td>1.89</td>
</tr>
</tbody>
</table>

3. Concentration dependence of $G$ values - in the presence of $O_2$ (G. Scholes et al., *ibid.*)

<table>
<thead>
<tr>
<th>Base</th>
<th>Conc. in moles per liter</th>
<th>$G$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymine</td>
<td>$2 \times 10^{-3}$</td>
<td>2.76</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^{-4}$</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^{-5}$</td>
<td>1.23</td>
</tr>
<tr>
<td>Adenine</td>
<td>$2 \times 10^{-3}$</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^{-4}$</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^{-5}$</td>
<td>0.65</td>
</tr>
</tbody>
</table>
TABLE II

Nucleosides destroyed

1. In the absence of $O_2$

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Conc. in moles per liter</th>
<th>$G$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>$3.8 \times 10^{-3}$</td>
<td>1.5</td>
</tr>
<tr>
<td>Guanosine</td>
<td>$3.8 \times 10^{-3}$</td>
<td>1.3</td>
</tr>
<tr>
<td>Cytidine</td>
<td>$4.1 \times 10^{-3}$</td>
<td>1.7</td>
</tr>
<tr>
<td>Uridine</td>
<td>$4.1 \times 10^{-3}$</td>
<td>1.8</td>
</tr>
<tr>
<td>Thymidine</td>
<td>$4.1 \times 10^{-3}$</td>
<td>1.7</td>
</tr>
</tbody>
</table>

2. In the presence of $O_2$ (G. Scholes, J. Ward, and J. Weiss

\[ J. \text{Mol. Biol.} 2, 379 (1960). \]

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Conc. in moles per liter</th>
<th>$G$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>$2 \times 10^{-4}$</td>
<td>0.92</td>
</tr>
<tr>
<td>Uridine</td>
<td>$2 \times 10^{-4}$</td>
<td>1.72</td>
</tr>
<tr>
<td>Thymidine</td>
<td>$2 \times 10^{-4}$</td>
<td>1.75</td>
</tr>
</tbody>
</table>
## TABLE III

Nucleotides destroyed

1. In the absence of $O_2$

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Conc. in moles per liter</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylic acid</td>
<td>$2.9 \times 10^{-3}$</td>
<td>1.1</td>
</tr>
<tr>
<td>Guanylic acid</td>
<td>$2.7 \times 10^{-3}$</td>
<td>1.2</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>$3.1 \times 10^{-3}$</td>
<td>1.3</td>
</tr>
<tr>
<td>Uridylic acid</td>
<td>$3.1 \times 10^{-3}$</td>
<td>1.0</td>
</tr>
</tbody>
</table>

2. In the presence of $O_2$ (G. Scholes, J. Ward, and J. Weiss *J. Mol. Biol.* 2, 379 (1960))

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Conc. in moles per liter</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylic</td>
<td>$2 \times 10^{-4}$</td>
<td>0.75</td>
</tr>
<tr>
<td>Uridylic</td>
<td>$2 \times 10^{-4}$</td>
<td>1.65</td>
</tr>
</tbody>
</table>
TABLE IV

Sugar released from nucleosides and nucleotides

1. Nucleosides

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Conc. in moles per liter</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>$3.8 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
<tr>
<td>Guanosine</td>
<td>$3.8 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
<tr>
<td>Cytidine</td>
<td>$4.1 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
<tr>
<td>Uridine</td>
<td>$4.1 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
<tr>
<td>Thymidine</td>
<td>$4.1 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
</tbody>
</table>

2. Nucleotides

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Conc. in moles per liter</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylic acid</td>
<td>$2.9 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
<tr>
<td>Guanylic acid</td>
<td>$2.7 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>$3.1 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
<tr>
<td>Uridylic acid</td>
<td>$3.1 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
</tbody>
</table>
**TABLE V**

Sugar phosphate released from nucleotides

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Conc. in moles per liter</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylic acid</td>
<td>$2.9 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
<tr>
<td>Guanylic acid</td>
<td>$2.7 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>$3.1 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
<tr>
<td>Uridylic acid</td>
<td>$3.1 \times 10^{-3}$</td>
<td>0.0</td>
</tr>
</tbody>
</table>
TABLE VI
Phosphate released from nucleotides

1. In the absence of $O_2$ (M. Daniels, G. Scholes, and J. Weiss J. Chem. Soc. 3771 (1956)).

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Conc. in moles per liter</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine - 3' phosphate</td>
<td>$2.9 \times 10^{-3}$</td>
<td>0.18</td>
</tr>
<tr>
<td>Adenosine - 5' phosphate</td>
<td>$2.9 \times 10^{-3}$</td>
<td>0.25</td>
</tr>
<tr>
<td>Cytidine - 3' phosphate</td>
<td>$3.1 \times 10^{-3}$</td>
<td>0.13</td>
</tr>
</tbody>
</table>

2. In the presence of $O_2$ (M. Daniels et al, ibid.)

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Conc. in moles per liter</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine - 3' phosphate</td>
<td>$2.9 \times 10^{-3}$</td>
<td>0.34</td>
</tr>
<tr>
<td>Adenosine - 5' phosphate</td>
<td>$2.9 \times 10^{-3}$</td>
<td>0.23</td>
</tr>
<tr>
<td>Cytidine - 3' phosphate</td>
<td>$3.1 \times 10^{-3}$</td>
<td>0.36</td>
</tr>
<tr>
<td>Base</td>
<td>Conc. in moles per liter</td>
<td>Product</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Adenine</td>
<td>$7.5 \times 10^{-3}$</td>
<td>Hypoxanthine</td>
</tr>
<tr>
<td>Guanine</td>
<td>$0.3 \times 10^{-3}$</td>
<td>Xanthine</td>
</tr>
<tr>
<td>Cytosine</td>
<td>$9.0 \times 10^{-3}$</td>
<td>Uracil</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Conc. in moles per liter</th>
<th>Product</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>$3.8 \times 10^{-3}$</td>
<td>Inosine</td>
<td>0.03</td>
</tr>
<tr>
<td>Guanosine</td>
<td>$3.8 \times 10^{-3}$</td>
<td>Xanthosine</td>
<td>0.00</td>
</tr>
<tr>
<td>Cytidine</td>
<td>$4.1 \times 10^{-3}$</td>
<td>Uridine</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Conc. in moles per liter</th>
<th>Product</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylic acid</td>
<td>$2.9 \times 10^{-3}$</td>
<td>Inosinic acid</td>
<td>0.01</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>$3.1 \times 10^{-3}$</td>
<td>Uridylic acid</td>
<td>0.02</td>
</tr>
</tbody>
</table>
# TABLE VIII

Opening of the imidazole ring of purine bases

<table>
<thead>
<tr>
<th>Base</th>
<th>Conc. in moles per liter</th>
<th>Product</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>$7.5 \times 10^{-3}$</td>
<td>4,6-diamino-5-formamidopyrimidine</td>
<td>0.4</td>
</tr>
<tr>
<td>Guanine</td>
<td>$0.3 \times 10^{-3}$</td>
<td>2,4-diamino-5-formamido-6-hydroxypyrimidine</td>
<td>0.002</td>
</tr>
<tr>
<td>Base</td>
<td>Conc. in moles per liter</td>
<td>Product</td>
<td>G</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------------</td>
<td>---------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Adenine</td>
<td>$7.5 \times 10^{-3}$</td>
<td>8-hydroxy-adenine</td>
<td>0.28</td>
</tr>
<tr>
<td>Guanine</td>
<td>$0.3 \times 10^{-3}$</td>
<td>8-hydroxy-guanine</td>
<td>0.001</td>
</tr>
</tbody>
</table>
REFERENCES


ACKNOWLEDGMENTS

I wish to thank Professor Melvin Calvin for his inspiring direction. I am especially grateful to him for having stimulated my interest in a wide range of scientific research - from the spin of an electron to life in outer space.

I am also deeply indebted to Dr. Richard M. Lemmon for his continued help, counsel, and guidance during the course of this research.

This work was done under the auspices of the U.S. Atomic Energy Commission.
This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.