in $C_1^{(R,\omega}$. The equation is balanced in the chemical sense if (regard + as a product symbol) both sides have the same prime factorization. It is easily seen that this is equivalent to saying that $x - y$ satisfies the definition of balance given in § 2.

THEOREM 4. *Let x and y be 1-chains in a subcomplex of a chemical complex; if x and y are homologically equivalent, then $x \rightleftharpoons y$ is balanced. The converse holds in the nonproper subcomplex.*

When the chemical complex is applied to a problem, the product symbols will have particular properties, determining a special complex. In the detailed paper we consider the theory of a few special complexes which appear to be important chemically.

The only law of chemistry which goes into the construction of our complexes is conservation of mass. Therefore they are by no means applicable only to chemistry. It is easy to conceive of a wide class of combinatorial problems which could be put into the language of algebraic complexes, as has been done here for chemistry.

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*ESR STUDY OF γ-IRRADIATED POLYNUCLEOTIDES**

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Electron spin resonance investigations of radiation-induced free radicals in DNA and RNA, their constituent bases, and their component nucleosides and nucleotides have previously been made.[1–13] This study is concerned with homopolymers and copolymers of certain of the nucleotides. Some results on three dry polynucleotides at room temperature have previously been reported by Muller.[9]

Perhaps the most important knowledge gained from these previous ESR studies of the nucleic acids and their constituents is that H atoms released from bound water, perhaps also from the sugar group, can add to certain of the base rings (apparently to all except cytosine) to produce H-addition radicals. (*See* Note added in proof.) This H-addition was proved for thymidine by analysis of the single crystal;[13] for DNA, by deuterium substitution[12] and by irradiation of DNA under H_2 pressure.[11] For guanine, adenine, and uracil, as well as for thymine, it was strikingly proved by subjection of the powdered samples to H

and D bombardment.[14] We have also shown that O-H radicals from a column of gaseous OH will add to the uracil ring in a similar manner.[15] By similar bombardment we have more recently shown that H-addition occurs both on the uracil ring and on at least one of the purine rings in RNA. Despite the evidence for H-addition in the various nucleic acid bases, H-addition has been definitely proved only on the thymine ring of DNA. However, a triplet found[6] in trout sperm DNA containing 20 per cent H_2O and $^1/_{10}$ per cent protein has been interpreted[11] as possibly arising from an H-addition on a purine ring.

Homopolynucleotides and copolymer nucleotides are synthetic compounds having structure, and in many cases behavior, similar to that of the natural polynucleotides DNA and RNA. They have only one or two kinds of bases in the whole macromolecule, whereas natural polynucleotides have four. A study of radiation damage to polynucleotides shows what might happen to DNA and RNA under the same conditions. The advantage of studying the synthetic compounds lies in the greater simplicity of their structure.

The polynucleotides included in this study are ammonium salt of polyuridylic acid (Poly U), potassium salt of polycytidylic acid (Poly C), polyadenylic acid (Poly A), polyinosinic acid (Poly I), and the copolymers Poly AU and Poly CU, which have each of the specified nucleotides in equal quantity spaced randomly along the polymer chain. Although inosine base compounds are not present in DNA and RNA, Poly I is pertinent to this study because its structure resembles closely that of polyguanylic acid, which was not available.

Experimental Procedure.—All the polynucleotide samples studied were spongelike compounds (obtained from Miles Chemical Co.). They were cut into small pieces with a scalpel, evacuated in a sample tube for at least 10 hr, and sealed. Some of the samples before being sealed were moisturized by either H_2O or D_2O vapor. In these cases, pure water vapor was allowed to enter the whole vacuum system after evacuation for 10 hr. The samples were kept for 20–60 min under 100 per cent relative humidity and were then sealed. Both dry and moist samples were irradiated by γ rays from Co^{60} at 195°K (dry-ice temperature) except when otherwise specified. Samples were exposed to a dose rate of about 3 mr/hr for 2–6 hr. The second derivative curves of the actual absorption lines were first recorded at 77°K. As the samples were slowly warmed in a dewar, spectra were recorded every 5 min. The observations were made at a frequency of 9000 Mc/sec. Spectroscopic splitting factors g for all the observed resonances are near that of the free electron spin, 2.0023.

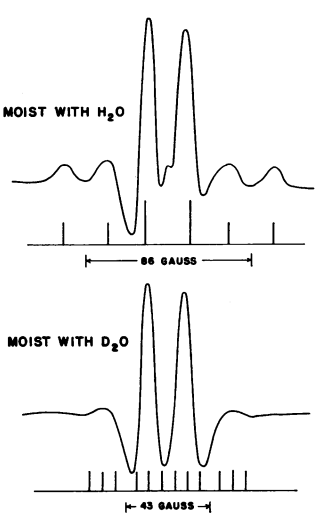
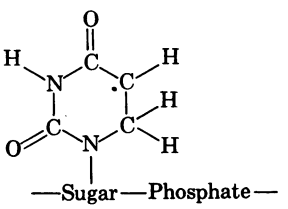


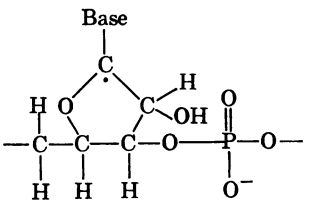
FIG. 1.—Second derivative ESR curves for γ-irradiated polyuridylic acid moisturized with H_2O and D_2O (irradiated at 195°K, observed at 77°K). The bars at the base represent the theoretical pattern expected for a radical formed by H-addition (*upper*) and D-addition (*lower*) on $C_{(6)}$ of the uracil ring.

Poly U and Poly C.—By exposure of the previously evacuated samples to H_2O vapor before γ irradiation, we were able to produce ESR signals from H-addition radicals in Poly U (but not in Poly

C) similar to those produced by H bombardment of uracil.[14] Evidence for this is shown in Figure 1, where the bars give the theoretical pattern expected for the H-addition radical (I).



(I)



(II)

Proof that the H atoms added to the Poly U ring come from the absorbed H_2O is obtained when samples exposed to D_2O vapor are similarly irradiated and observed. The latter samples show evidences for D-addition radicals, none for H-addition radicals (see bottom curve of Fig. 1).

The large difference in the $C_\beta H_2$ proton coupling for the H-addition radicals in uracil[14] (33 gauss) and in Poly U (43 gauss) indicates that the H-addition occurs on different carbons of the rings for the two. The smaller coupling observed for uracil is in agreement with the H-addition on $C_{(5)}$, as theoretically predicted by Pullman and Mantione.[16] The larger couplings of Poly U can be accounted for if the H-addition occurs on $C_{(6)}$ in the polymer and if a spin density of the order of 0.1 is on $N_{(1)}$. The $C_\alpha H$ coupling of 20 gauss indicates that the spin density on C_α or $C_{(5)}$ of radical (I) is 0.76.

In dry, evacuated samples of γ-irradiated Poly U and Poly C, no evidence was found for H-addition radicals. The resonances obtained after irradiation of the samples at 195°K and observation at 300°K and lower temperature appear to consist of a doublet of spacing about 20 gauss with a sharp singlet between the doublet components (see Fig. 2). The sharp singlet and the doublet clearly arise from different radicals since they decay at different rates. At lower temperature, especially in moist samples, the doublet components as well as the singlet are relatively sharp and are better resolved than in Figure 2 (compare with Fig. 1). As the temperature of the irradiated samples is raised above room temperature, both the doublet and the sharp singlet decay, leaving in both samples a somewhat broader singlet.

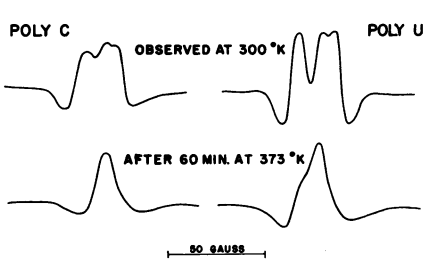


FIG. 2.—Second derivative ESR curves for dry samples of γ-irradiated polycytidylic acid and polyuridylic acid (in vacuum at 300°K).

The 20-gauss doublets of Poly U and Poly C, together with the sharp singlets, are found in the other polynucleotides of this study. These resonances must therefore arise from the common constituent, the ribose or the phosphate group. If the doublet splitting is due to a proton, the coupling mechanism must be that of hyperconjugation since the sharpness of the components indicates that the coupling is isotropic. It might arise from a radical of type (II), in which coupling to a single proton through the mechanism of hyperconjugation is possible. This radical would be formed by the loss of an H atom from the ribose component.

TABLE 1

CHARACTERISTICS OF PRINCIPAL RESONANCE SIGNALS OBSERVED

Probable radicals	Compound	Proton Coupling (gauss) $C_\alpha H$	$C_\beta H$	Characteristics of resonance
H-addition radicals of base rings	Poly U	$a_1 = 20$	$a_2 = a_3 = 43$	Appears or increases with presence of water; presence of D_2O causes different pattern; in Poly U less stable, in purine polynucleotides more stable than the doublet resonance
	Poly A		$a_1 = a_2 = 37$	
	Poly I		$a_1 = a_2 = 39$	
H-abstraction radicals of ribose group	Poly U		18	Sharp doublet; sensitive to presence of water; insensitive to difference between H_2O and D_2O; in Poly U more stable, in purine polynucleotides less stable than H-addition radicals
	Poly C		20	
	Poly A		21	
	Poly I		19	
	Poly CU		20	
	Poly AU		20	

An interesting feature of the ESR for the Poly U and Poly C samples exposed to either H_2O or D_2O vapor is the enhancement of the sharp 20-gauss doublet. This suggests that besides the more direct mechanism of production of radical (II), the H or OH from the dissociated H_2O might also abstract H from the ribose component to form H_2 or H_2O and a radical such as (II) which gives the doublet. In moist samples neither radical (I) nor (II) is stable at room temperature. The resonance of both radicals decays within minutes after the samples are brought to room temperature. We were unable to produce signals of H-addition radicals in Poly C even in moisturized samples. This is in agreement with the negative results on H-bombarded cytosine.[14] If H-addition radicals are formed in Poly C, they are evidently too short-lived or unstable for detection in these experiments.

Proton couplings and other information about the principal resonances are summarized in Table 1. The proton couplings for the H-addition radicals are similar to, but understandably somewhat different from, those of the corresponding free bases.[14]

Poly A and Poly I.—When samples of dry, evacuated Poly A or Poly I were irradiated at 195°K and observed at 77°K, no pronounced signals from H-addition radicals could be observed, although such signals did appear after the irradiated samples were warmed to 300°K. When the samples were exposed to water vapor

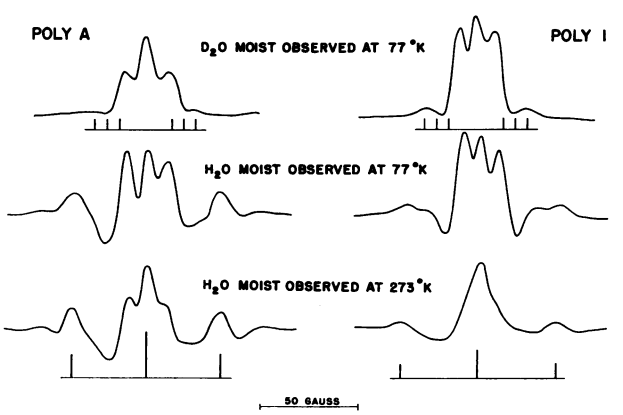
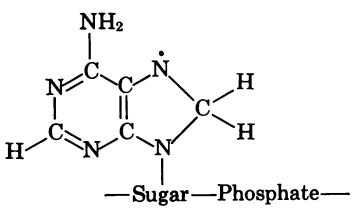
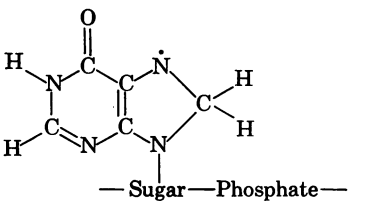


FIG. 3.—Second derivative ESR curves of γ-irradiated polyadenylic acid and of polyinosinic acid moisturized with H_2O and D_2O (irradiated at 195°K). The bars represent the theoretical pattern expected for radicals formed by D-addition (*upper*) and H-addition (*lower two*) on $C_{(8)}$ of the purine rings.

before irradiation at 195°K, strong signals corresponding to H-addition radicals in the purine bases[14] were obtained for both Poly A and Poly I. These persisted after the samples were warmed to 273°K (see Fig. 3). The triplet component in the resonance indicated by the bars is believed to result from the radicals (III) or (IV), in which H-addition occurs on $C_{(8)}$.



(III)



(IV)

The observed proton couplings are listed in Table 1. Proof that the added H comes from absorbed water was given by observation of γ-irradiated samples moist with D_2O. These gave the resonance expected for radicals formed by D-addition on $C_{(8)}$. The outer shoulders on the Poly A curve are due to anisotropic N^{14} coupling, as was earlier explained.[14] Interestingly, no such strong signals were obtained when the moisturized samples were irradiated at 77°K and observed without warming.

It is of interest that a doublet of about 20 gauss splitting and a sharp singlet like those found for Poly U and Poly C are also observed for Poly A and Poly I at below room temperature. The doublet is enhanced by treatment with water vapor and decays as the temperature is raised to 300°K or higher. As mentioned earlier, this doublet, which is observed in all the polynucleotides studied, may arise from radicals like (II), formed in the ribose sugar group. The sharp singlet may come from radicals formed on the phosphate group.

Figure 4 shows resonances for dry Poly A and Poly I irradiated and observed at room temperature. A component of the triplet arising from the H-addition radicals (III) and (IV) is noticeable, although this component is not as strong as it is for the moist samples. Unlike those for the moist samples, these H-addition radicals are relatively stable in dry samples at room temperature, but they disappear when the samples are warmed to 100°C for an hour.

Poly CU and Poly AU.—The copolymers offer the possibility of observing transfer of radiation damage from one nucleotide to another, or possible protection of one unit by the other.

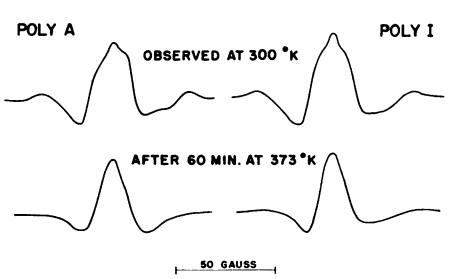


FIG. 4.—Second derivative ESR curves of γ-irradiated dry samples of polyadenylic acid and of polyinosinic acid (irradiated in vacuum at 300°K).

Figure 5 shows a comparison of the ESR signals of irradiated Poly CU and Poly AU with those for the corresponding monopolymers. All samples were dry. After thorough evacuation, they were given the same γ-ray dosage of $\sim 10^7$ r at 195°K and were otherwise treated in the same manner. The curve for Poly CU resembles that for Poly C much more than that for Poly U; that for Poly AU resembles that for Poly U more than that for Poly A. From this,

we might conclude that the relative ease of radical formation in the different units of the dry samples is in the order C > U > A. The doublet component, which is thought to arise from the common ribose unit, is present in the ESR for all the species.

At the lowered temperature the doublet of Poly CU and of Poly AU is enhanced by moisture just as it is for the homopolymers; it is likewise unstable at the elevated temperatures.

So far we have not been able to produce signals from H-addition radicals in the moistened copolymers, but this may be due to the small quantities of the moistened samples used. We are now obtaining larger quantities of these copolymers for a more thorough study of H_2O effects. We are also investigating other copolymers of the nucleotides and will report the results in a later communication.

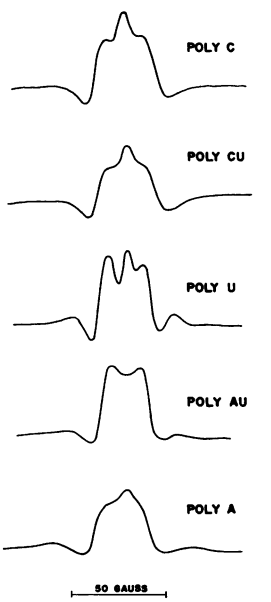


FIG. 5.—Comparison of the ESR spectra of γ-irradiated Poly C and Poly U with their copolymers Poly CU and Poly AU. (Dry samples in vacuum irradiated at 195°K, observed at 77°K.)

Relevance of Results for the Nucleic Acids.—From these results it is evident that the radiation products of bound water are a source of considerable damage to the nucleic acids. Hydrogen atoms released from the H_2O readily bond to the purine and pyrimidine rings of all the homopolymers examined, except possibly in Poly C. We could not obtain samples for the homopolymer of thymine, but H-addition has already been proved for the thymine ring in DNA itself. Further evidence is obtained that H-abstraction from the ribose sugar group either by H or O-H produced from dissociation of bound water is probably a potent source of indirect damage to RNA. Probably such H-abstraction occurs also for the deoxyribose group of DNA, but we have not yet been able to obtain samples for similar studies of the deoxyhomopolynucleotides. ESR evidence to be described in a later report[17] shows that H atoms from an atomic spray will add directly to the uracil ring and also to one of the purine rings in RNA.

All the dry polynucleotides when warmed above room temperature for short times or when allowed to stand for a long time at room temperature after irradiation give slightly asymmetric singlet resonances very similar to those originally reported[2] for DNA and RNA at room temperature. This singlet ESR, which is generally the only pattern observed for dry, evacuated samples of DNA or RNA irradiated and observed at room temperature, might result from any or all of the nucleotide components.

The disappearance of the signals from the H-addition reactions as the temperature of the sample is raised may result from a loss of the added H to restore the original ringed structure, thereby healing the damage to that group. On the other hand, it could be caused by a second addition reaction which would permanently alter the ringed structure.

From the preliminary study of Poly CU and Poly AU, the probability of radiation damage to these units in dry nucleic acids seems to be in the order C > U > A. Although synthetic polymers containing thymine were not available for the present

study, it appears from the thymine-like resonance already observed in DNA that the thymine ring would have a higher probability of damage from H-addition reactions than would any of these units. Failure to observe H-addition radicals for other rings in DNA or RNA may often be due to their instability or short lifetimes at the observation temperature rather than to their failure to be formed. Thus the relative probability of radiation damage to different nucleotides is not necessarily measured by the relative strengths of their observed ESR signals.

There is considerable evidence that water in the cell can increase the biological damage[18–20] of radiation, although small amounts of moisture have been found to exert a protective effect on certain seed cells[21, 22] and on artemia cysts.[23] Butler[24] found that viscosity changes occur in DNA as a result of radiation products of H_2O. Latarjet *et al.*[25] have shown that the indirect damage to DNA by the irradiation products of water persists in frozen samples; they suggest that these effects depend on the ability of radiation products of water, H and OH radicals, to migrate in the frozen samples. This interpretation agrees with results obtained here and with the earlier ESR results of Patten and Gordy[26] which show no evidence of H-addition or H-abstraction radicals when samples of DNA, RNA, or their basic constituents containing various amounts of H_2O are irradiated and observed at 4.2°K.

Note added in proof: We have now been able to detect free radicals formed by H-addition on the cytosine ring at lowered temperatures. The radicals are unstable at room temperature.

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