38. *Chemical Action of Ionising Radiations in Solution. Part XVII.* Degradation of Deoxyribonucleic Acid in Aqueous Solution by Irradiation with X-Rays* (200 kv).

By M. Daniels, G. Scholes, J. Weiss, and C. M. Wheeler.

The viscosity changes in aqueous solutions of deoxyribonucleic acid irradiated with *X*-rays (200 kv) have been studied, with particular reference to the effects of molecular oxygen. The radiation-induced loss in viscosity can be ascribed to scission of the internucleotide phosphate bonds, after attack by the free radicals produced primarily by the action of the radiation on the solvent, water. The slow post-irradiation viscosity loss ("after-effect"), which occurs in solutions irradiated in the presence of oxygen, is accounted for on the basis of the labilisation of the carbon–phosphate linkages, *i.e.*, intermediate formation of labile phosphate esters which can undergo slow hydrolysis. Hydroperoxides have been detected in nucleic acid solutions irradiated in the presence of oxygen, but appear to be associated only with the pyrimidine bases. The Lipmann–Tuttle test for acyl phosphate was positive, but inconclusive.

The loss of viscosity and of streaming birefringence which occurs on irradiation of aqueous solutions of deoxyribonucleic acid (DNA) with ionising radiations has been studied by several workers.[1-3] It was concluded from sedimentation data and from electron-micrographs that the effect of these radiations (*e.g.*, *X*-rays, γ-rays) was to break the long asymmetric DNA particles into fragments of variable dimensions. An "after-effect" has also been observed, namely, slow post-irradiation decrease of viscosity at room temperature, the significance of which has been emphasised by Butler and Conway [4,5] who concluded that it occurred only if molecular oxygen was present during irradiation.

In our earlier publications [6,7] it was shown that definite chemical changes took place in the nucleic acids on irradiation of their dilute aqueous solutions with *X*-rays, as a consequence of chemical reactions with the free radicals (OH and H) produced primarily from the water.[8]

It has been suggested [7,9] that intermediate formation of labile phosphate esters, which can undergo subsequent slow hydrolysis, may be largely responsible for the post-irradiation viscosity loss.

We now report a more detailed investigation of the viscosity changes in DNA solutions on irradiation with *X*-rays (200 kv), with special reference to the effects of oxygen.

Fig. 1 gives some *typical* results obtained from (equilibrated) 0·1% (w/v) solutions irradiated with a total dose of 7100 rep ("rœntgen equivalent, physical").

When atmospheric oxygen is present, an initial loss of viscosity takes place during the irradiation and a slow post-irradiation loss of approximately equal magnitude. That this is typical is apparent from the results with several samples shown in Table 1. The "after-effect" in solutions containing oxygen persists when the viscosity is measured in the presence of sodium chloride.

Under oxygen-free conditions in aqueous solutions (Fig. 1*a*), there is a post-irradiation

* Part XVI, *J.*, 1956, 3771.

[1] Wegmuller, Ph.D. Thesis, Berne, 1942; Sparrow and Rosenfeld, *Science*, 1946, **104**, 245; Taylor, Greenstein, and Hollaender, *Arch. Biochem.*, 1948, **16**, 19.
[2] Butler, *Canad. J. Res.*, 1949, *B*, **27**, 972.
[3] Limperos and Moscher, *Amer. J. Roentgenol.*, 1950, **63**, 681, 691.
[4] Butler and Conway, *J.*, 1950, 3418.
[5] Conway and Butler, *J.*, 1952, 834.
[6] Scholes, Stein, and Weiss, *Nature*, 1949, **164**, 709; Scholes and Weiss, *ibid.*, 1950, **166**, 640; *idem*, *Biochem. J.*, 1953, **53**, 567.
[7] *Idem*, *ibid.*, 1954, **56**, 65.
[8] Weiss, *Nature*, 1944, **153**, 748.
[9] Scholes and Weiss, *ibid.*, 1953, **171**, 920.

viscosity loss which is about one-third of that obtained in the presence of oxygen.[10] However, when the viscosity of these solutions is measured in the presence of sodium

TABLE 1. *Total loss of relative viscosity and extent of " after-effect " in the irradiation of* DNA *solutions* (0·1% *w*/*v*) *with* X-*rays* (200 kv) *in the presence of atmospheric oxygen. Total dose* = 7100 *rep. Temp.* 25°.

DNA Sample	Viscosity before irradn.	Total viscosity loss	" After-effect "	After-effect / Total loss	10^3k (from eq. 1)
Signer	8·42	4·02	2·50	0·62	1·26
Signer (measured in NaCl)	1·66	0·23	0·15	0·65	28·2
Schwander	4·92	1·94	1·30	0·67	20·8
Bütler	5·61	2·20	1·43	0·65	2·02
Butler	6·34	2·19	2·15	0·98	1·21
Herring-sperm nucleic acid	3·06	0·65	0·44	0·68	5·08

chloride there is no further drop in the viscosity (Fig. 1*b*). A similar observation has also been reported by Conway.[11] On the basis of current views on the effects of neutral salts on polyelectrolytes,[12] it might be assumed that the post-irradiation viscosity loss in oxygen-

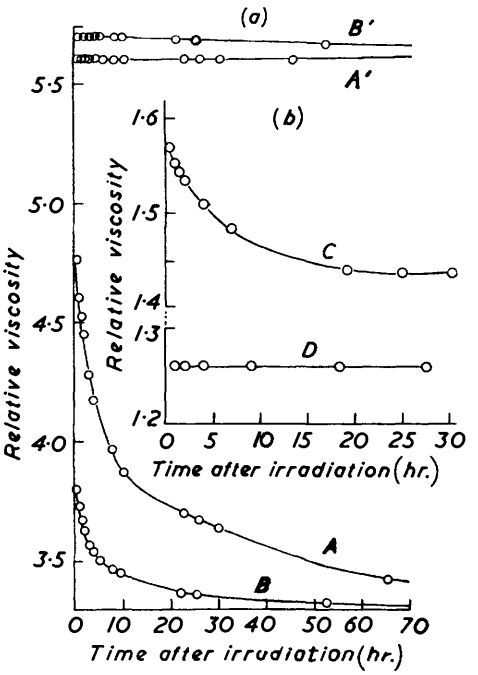


FIG. 1. *Change of relative viscosity of* 0·1% (*w*/*v*) *aqueous solutions of* DNA *after irradiation with* X-*rays* (200 kv). *Total dose* 7100 *rep.* (*a*) *Curve A : irradiation in the presence of atmospheric oxygen. Curve B : irradiation* in vacuo. *A' and B' refer to the corresponding unirradiated controls.* (*Bütler* DNA). (*b*) *Solutions containing* 0·1M-*sodium chloride, added after the irradiation. Curve C : irradiation in the presence of atmospheric oxygen. Curve D : irradiation* in vacuo. *The unirradiated controls were constant at relative viscosities of* 1·62 *and* 1·40 *respectively* (*Signer* DNA).

free solutions, in the absence of salt, is due to slow re-equilibration of the fractured DNA particles consequent upon irradiation; this may be attributed to an association occurring when insufficient electrolyte is present to complete the counter-ion atmosphere of the DNA particles.[11,13]

From Fig. 1 it is apparent that definition of the *initial* viscosity loss involves some considerable latitude in the extrapolation over the first half-hour after irradiation. However, on further examination it was found that the rate of loss of viscosity during the major part of the after-effect (both in the presence and absence of sodium chloride) can be satisfactorily represented by the relation :

$$1/(\eta_t - \eta_\infty) - 1/(\eta_0 - \eta_\infty) = kt \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

[10] Cf. Daniels, Scholes, and Weiss, *Experientia*, 1955, **11**, 219.
[11] Conway, *Brit. J. Radiol.*, 1954, **27**, 49; *Nature*, 1954, **173**, 579.
[12] Cf. Doty and Ehrlich, *Ann. Rev. Phys. Chem.*, 1952, **3**, 81; *J. Polymer Sci.*, 1954, **12**, 159.
[13] Wall, Huizenga, and Grieger, *J. Amer. Chem. Soc.*, 1950, **72**, 2636, 9228.

where η_0, η_t, and η_∞ are, respectively, the relative viscosities immediately after irradiation, at time t, and at infinity. Consequently, a plot of $1/(\eta_t - \eta_\infty)$ against t gives values for the relative viscosity immediately after irradiation and for the experimental rate constant k governing the decay. Fig. 2 is a typical reciprocal plot and shows that the initial loss is a well-defined quantity. The constants (k), obtained in this way, are given in the last column of Table 1.

In the salt-free solutions the rate of re-equilibration is about ten times faster than that

FIG. 2. *Reciprocal plot of the difference between relative viscosities at time t (η_t) and at infinity (η_∞) after irradiation with X-rays* (200 kv) *in the presence of atmospheric oxygen* [0·1% DNA, *Butler*].

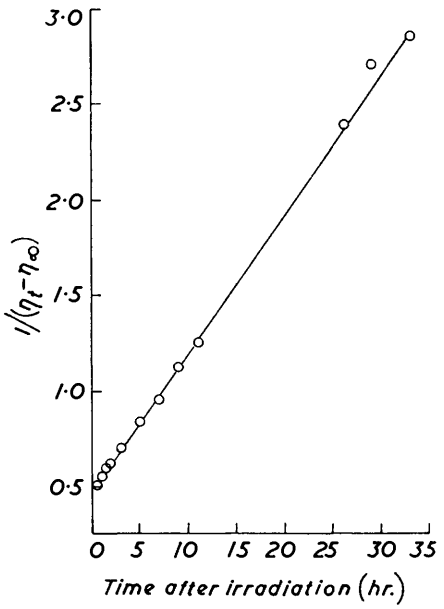
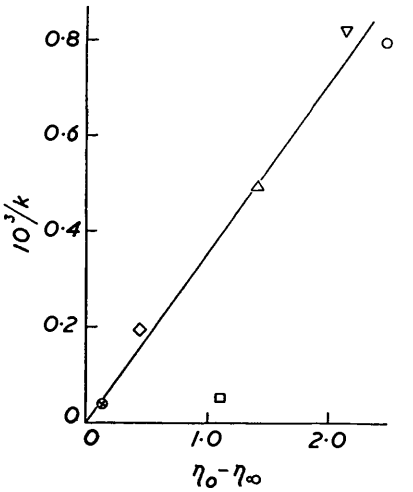


FIG. 3. *Variation of* $(1/k)$ [*from eq.* 1] *with* $(\eta_0 - \eta_\infty)$ *for solutions of different samples of nucleic acid irradiated with* X-*rays* (200 kv) *in the presence of atmospheric oxygen at constant dose* (7100 rep.) *and at constant concentration* (0·1% *w/v*).



○ *Signer acid.* ⊗ *Signer acid* (*measured in* 0·1M-NaCl). △ *Bütler acid.* □ *Schwander acid.* ▽ *Butler acid.* ◇ *Herring-sperm acid.*

of the after-effect; the latter, therefore, is the rate-determining process for the observed viscosity losses.

Equation (1) can be rearranged to:

$$(\eta_0 - \eta_t)/(\eta_t - \eta_\infty) = k(\eta_0 - \eta_\infty)t = k't \quad . \quad . \quad . \quad . \quad . \quad (2)$$

which is similar to a relation noted by Conway and Butler.[5] In contrast to k, the value of k' is nearly constant for all the samples of DNA investigated. This is shown in Fig. 3 where $(\eta_0 - \eta_\infty)$ has been plotted against $1/k$: a straight line passing through the origin is obtained, leading to an average value of $k' \simeq 2{\cdot}75 \times 10^{-3}$ min.$^{-1}$.

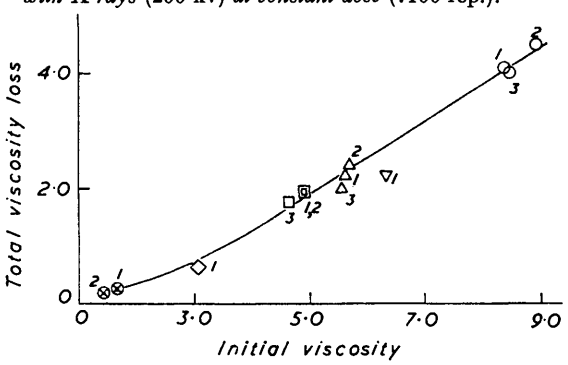
It has been found that the initial viscosity loss is greater in the solutions irradiated *in vacuo*, to such an extent that the *total* loss of viscosity is practically independent of the presence of oxygen. Where DNA solutions were saturated with hydrogen (1 atm.) the viscosity changes were practically indistinguishable from those observed *in vacuo*: Table 2 shows that, except in one instance (Bütler DNA, 0·1%), the total loss of viscosity in the presence of hydrogen is only a few per cent less than that *in vacuo*, and this difference, in fact, is largely cancelled, when account is taken of the different initial viscosities of the DNA solutions (see below). It appears, therefore, that the overall breakdown of the nucleic acid in terms of viscosity loss is the same whether irradiation is carried out in the presence

of oxygen, *in vacuo*, or in the presence of hydrogen. This was found to be the case for 0·05% as well as for 0·1% nucleic acid solutions.

For constant dose and constant weight concentration, there is a linear relation between total viscosity loss and initial viscosity, shown in Fig. 4 where are plotted the experimental points obtained from 0·1% solutions of the various samples of nucleic acid irradiated under various conditions. A similar relation also holds with 0·05% solutions, the plot lying above that for the 0·1% solutions. Butler,[2] using 0·5% DNA solutions and radium γ-rays, also observed a proportionality between the viscosity change and the initial viscosity.

It might be thought that the observed after-effects were due in whole, or in part, to reaction of DNA or of radiation-damaged DNA with the hydrogen peroxide produced during irradiation in the presence of oxygen. The concentration of hydrogen peroxide in these solutions is rather low (of the order of 10^{-5}M) and since it has been shown that the viscosities of pure samples of DNA (*e.g.*, the Signer preparations) are unaffected by hydrogen peroxide up to a concentration of 10^{-3}M it is most unlikely that the after-effects can be

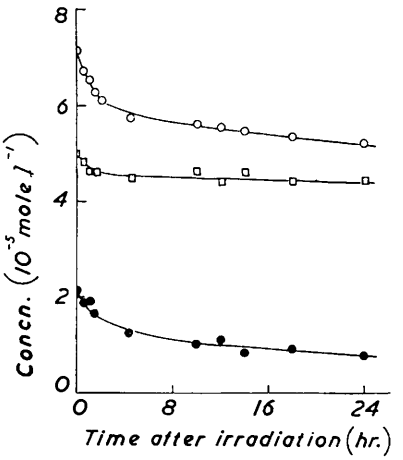
FIG. 4. *Total viscosity loss as a function of initial viscosity. 0·1% w/v solutions of different* DNA *samples irradiated with* X-*rays* (200 kv) *at constant dose* (7100 rep.).



○ *Signer acid.* ⊗ *Signer acid (measured in* 0·1M-NaCl). △ *Bütler acid.* □ *Schwander acid.* ▽ *Butler acid.* ◇ *Herring-sperm acid.*

(1) *In the presence of atmospheric oxygen.* (2) In vacuo. (3) *In the presence of hydrogen* (1 *atm.*).

FIG. 5. *Post-irradiation decay of hydrogen peroxide and of the hydroperoxides in* DNA *solutions* (0·1% *w/v Signer*) *irradiated with* X-*rays* (200 kv) *in the presence of oxygen* (1 *atm.*). *Total dose* $3·2 \times 10^4$ rep.



○ *"Total peroxides" (by iodide reagent).* □ *Hydrogen peroxide (by titanium reagent).* ● *Hydroperoxides.*

TABLE 2. *Total loss of relative viscosity on irradiation of* DNA *solutions with* X-*rays* (200 kv) *in the presence of atmospheric oxygen,* in vacuo, *and in the presence of hydrogen* (1 *atm.*). *Total dose* = 7100 *rep. Temp.* 25°.

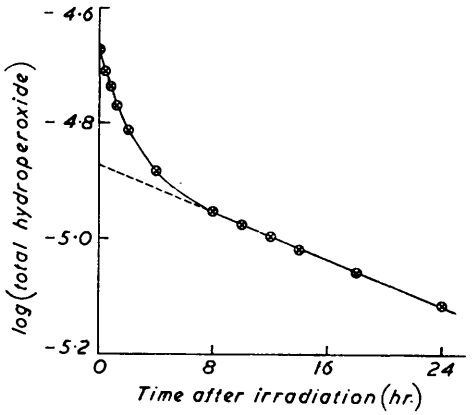
DNA solution		η_i	η_∞	$(\eta_i - \eta_\infty)/\eta_i$	DNA solution		η_i	η_∞	$(\eta_i - \eta_\infty)/\eta_i$
Signer (0·1%)	In O_2	8·42	4·40	0·474	Bütler (0·05%)	In O_2	3·46	1·62	0·532
	In vac.	8·94	4·60	0·486		*In vac.*	3·38	1·63	0·518
	In H_2	8·44	4·44	0·474		In H_2	3·26	1·66	0·491
Schwander (0·1%)	In O_2	4·92	2·98	0·394	Signer (0·1%)	In O_2	1·66	1·43	0·139
	In vac.	4·92	2·98	0·394	(measured in	*In vac.*	1·44	1·26	0·125
	In H_2	4·64	2·86	0·384	NaCl)				
Bütler (0·1%)	In O_2	5·61	3·41	0·392					
	In vac.	5·70	3·36	0·411					
	In H_2	5·59	3·59	0·358					

η_i = relative viscosity before irradiation.
η_∞ = relative viscosity at time = ∞ (after irradiation).

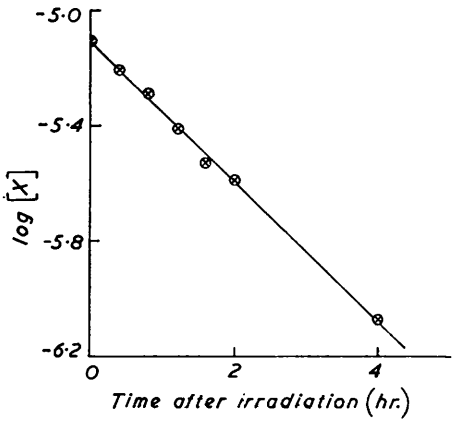
associated with a hydrogen peroxide–DNA reaction. On the other hand, it remains possible that reaction between hydrogen peroxide and radiation-damaged DNA may result in some viscosity loss. To test this the DNA solutions were freeze-dried immediately after irradiation—it was found that this process removed practically all the hydrogen peroxide. Sufficient 0·1M-sodium chloride was added to the residue to reconstitute a 0·1% DNA solution and viscosity measurements were carried out as soon as possible. In this way it was shown that the total viscosity loss was independent of the presence of hydrogen peroxide produced during irradiation. This confirms similar observations by Conway and Butler [5] which, however, were made in the absence of salt.

The irradiated DNA solutions were tested for the presence of hydroperoxides by comparing the yields of total estimatable peroxide by means of (*a*) titanium sulphate and (*b*) potassium iodide. (In general, hydroperoxides can oxidise iodide but do not give the coloured complex with the titanium reagent, except when they are decomposed to hydrogen

FIG. 6. *Rates of post-irradiation decay of the hydroperoxides in* DNA *solutions* (0·1% *w/v Signer*), *irradiated with* X-*rays* (200 kv) *in the presence of oxygen* (1 *atm.*). *Total dose* $3{\cdot}2 \times 10^4$ *rep.*



(*a*) *Logarithm of the total hydroperoxide concentration plotted against time after irradiation.*



(*b*) *Logarithm of the concentration of the more rapidly decomposing hydroperoxide* (X) *plotted against time* (*obtained from the extrapolation in Fig.* 6*a*).

peroxide under the conditions in question.) In solutions irradiated in the presence of oxygen, greater " peroxide " yields were obtained with the iodide reagent, suggesting that hydroperoxides were produced under these conditions. For example, in DNA solutions (0·1% Signer) irradiated with a total dose of $\sim 3{\cdot}0 \times 10^4$ rep. the yield of hydroperoxides corresponded to $G \sim 1{\cdot}0$ (molecules/100 ev) and the yield of hydrogen peroxide to $G \sim 1{\cdot}5$. Post-irradiation decay of both the hydrogen peroxide and the hydroperoxides was observed (see Fig. 5). The decay curve for the hydroperoxides can be accounted for on the basis of the occurrence of two concurrent first-order processes, the slower of which accounts for about two-thirds of the total hydroperoxides. A plot of the logarithm of the hydroperoxide concentration against time gave the graph shown in Fig. 6*a*; the slope of the linear portion corresponds to a first-order rate constant of $\sim 4 \times 10^{-4}$ min.$^{-1}$. The concentration of the " slow component " could then be estimated, and subtraction of this from the total hydroperoxide yield over the same period gave figures which on logarithmic plotting were also found to be a linear function of time (Fig. 6*b*). Decomposition of the faster component, therefore, also conforms to a first-order process, with a rate constant of $\sim 1 \times 10^{-2}$ min.$^{-1}$. (A hydroperoxide reaction was also given by ribonucleic acid solutions irradiated in the presence of oxygen.)

However, preliminary experiments with several monoribonucleotides [14] (the corresponding deoxyribonucleotides were not available) indicated that hydroperoxide formation is apparently associated with the oxidation of certain pyrimidine bases: *e.g.*, a hydroperoxide was formed on irradiation of solutions of cytidine-3′ but not of adenosine-3′ or adenosine-5′ phosphate. It is, therefore, possible that the two hydroperoxides found in the irradiated DNA solutions are derived from the two pyrimidine components; further work in this direction is under way. The possible rôle of hydroperoxides in the viscosity after-effect is discussed below.

If it is assumed that a " true " after-effect occurs only if oxygen is present during the irradiation, there is a certain parallelism with the formation of acyl phosphates by irradiation,[15] as for this oxygen is also required. Acyl compounds could be formed by attack at position 5′ of the sugar component in DNA and slow hydrolysis at room temperature could then break the chain and lead to a loss of viscosity. To test this some experiments were carried out with the Lipmann–Tuttle colour reaction for acyl phosphate.[16] A positive reaction was observed in the irradiated DNA solutions but was also found in the irradiated solutions of several purine and pyrimidine derivatives where, in some cases, no acyl compound could feasibly be formed (see Table 3). These experiments do not eliminate the posibility of the presence of acyl compounds in the irradiated nucleate solutions but are clearly inconclusive owing to the relative non-specificity of the Lipmann–Tuttle reaction.

TABLE 3. *Lipmann–Tuttle reaction in aqueous solutions of the nucleic acids and related compounds irradiated with* X-*rays* (200 kv) *at* pH 6·5—7·0. *Total dose* $1{\cdot}3 \times 10^5$ *rep.*

Solution irradiated	Spekker readings [a]	
	Room temp.	After 30 min. at 100°
In oxygen		
DNA (Signer) 0·1%)	0·043	0·065
	0·052	0·061
DNA (herring sperm) 0·05%	0·020	0·040
Ribonucleic acid (BDH) 0·1%	0·020	0·035
Adenosine-5′ phosphate 0·1%	0·033	0·027
Yeast adenylic acid 0·1%	0·033	0·020
Yeast cytidylic acid 0·1%	0·022	0·016
Adenine 0·04% [b]	0·010	—
Ribose-5′ phosphate (Ba salt) 0·06% [b]	0·021	—
In vacuo		
DNA (herring sperm) 0·05%	0·004	0·007
Adenosine-5′ phosphate 0·01%	0·002	—

[a] Spekker reading of 0·1 corresponds to $G \simeq 1$ (in terms of acetyl phosphate). [b] Total dose $6{\cdot}5 \times 10^4$ rep.

DISCUSSION

The chemical changes which take place on *X*-irradiation of aqueous nucleic acids (DNA or RNA) include an increase in the number of titratable acid groups and the formation of inorganic phosphate, of small amounts of free purine bases, and of ammonia.[6,7] Glycosidic ester, and internucleotide linkages are thus broken, indicating that the radicals (H, OH) attack both the base and the sugar components. The decrease in viscosity in irradiated DNA solutions must, therefore, be attributed, in the first instance, to these radical processes.

According to the recent views of Crick and Watson [17] the structure of DNA can be represented by two intertwined polynucleotide chains, helically arranged about a common axis and held together by hydrogen bonding between paired purine and pyrimidine bases.

[14] Daniels, Scholes, and Weiss, *J.*, 1956, 3771; Scholes, Weiss, and Wheeler, *Nature*, 1956, **178**, 157.
[15] Scholes and Weiss, *Nature*, 1954, **173**, 267.
[16] Lipmann and Tuttle, *J. Biol. Chem.*, 1943, **159**, 21.
[17] Crick and Watson, *Proc. Roy. Soc.*, 1954, *A*, **223**, 80

On this basis, it seems unlikely that attack on a base with the consequent destruction of its hydrogen bond can lead to any appreciable change in the size or shape of the DNA molecule and hence to any loss in viscosity. Attack on the deoxypentose components, on the other hand, can lead to the scission of the internucleotide links ($C_{(3')}$ or $C_{(5')}$) and this must be regarded as the main cause of viscosity loss.

As regards the mechanism of the chemical degradation, the fact that the *total* loss of viscosity is independent of the presence of oxygen or hydrogen seems to indicate that the total primary radical attack is the same under all these conditions, although the detailed mechanism may be different.

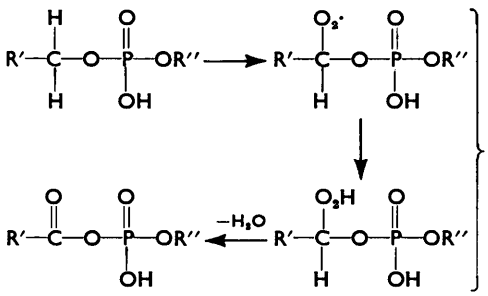
Immediate fracture of the DNA strands may result from an attack by OH radicals only or by both OH and H formed by the action of the radiation on the water, viz., RH $\longrightarrow$ R· $\longrightarrow$ fission, where RH represents nucleic acid and R· a nucleate radical. In the presence of molecular oxygen, when the H atoms are effectively transformed into HO_2, reactions of the type, RH + HO_2 $\longrightarrow$ R· + H_2O_2, appear unlikely, since they would give fairly high yields of hydrogen peroxide whereas the observed value of $G \simeq 1{\cdot}5$ is even less than the maximum ($G \simeq 2{\cdot}3$) to be expected if the hydroperoxy-radicals simply dismutate ($2HO_2 \longrightarrow H_2O_2 + O_2$).

Introduction of molecular hydrogen into the system displaces the H : OH ratio to higher values owing to the reaction,[18] $H_2 + OH \longrightarrow H + H_2O$, whose occurrence at least to some extent, is indicated by the reduced yield of ammonia and increased yield of inorganic phosphate on irradiation in the presence of hydrogen;[7] the latter observation indicates that reductive processes are involved in the degradation of the sugar phosphate part of the DNA molecule. At this stage, however, it is difficult to decide the chemical rôle of hydrogen atoms in the viscosity loss. The yields of hydrogen gas in irradiations carried out *in vacuo* are fairly low ($G \sim 1$), which seems to eliminate dehydrogenation reactions of the type, RH + H $\longrightarrow$ R· + H_2. On the other hand, reductive dephosphorylation[19] appears possible and might lead to chain scission. In the presence of molecular oxygen radicals R· formed primarily might, by a reaction R· + O_2 $\longrightarrow$ RO_2·, lead to peroxy-radicals which may subsequently form the labile product. Hydroperoxides could be formed by reduction of the RO_2· radical, *e.g.*:

$$RO_2 + O_2^- \longrightarrow RO_2^- + O_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad (a)$$

$$RO_2^- + H^+ \rightleftharpoons RO_2H \quad . \quad . \quad . \quad . \quad . \quad . \quad (b)$$

where O_2^- arises from the equilibrium $HO_2 \rightleftharpoons H^+ + O_2^-$. The intermediate formation of organic peroxy-radicals could also lead to an acyl phosphate, possibly also *via* a hydroperoxide; this, on the basis of reactions (*a*, *b*) can be represented as follows, where R′ and R″ are remainders of the polynucleotide chain:



(*c*)

The viscosity " after-effect " could be associated with labilisation of the carbon–phosphate linkages according to the reactions (*a*, *b*, *c*). Originally, we suggested that the after-effect

[18] Cf. Stein and Weiss, *J.*, 1949, 3245.
[19] Atherton, Openshaw, and Todd, *J.*, 1945, 382.

might be due to the production of a 4'-carbonyl group in the deoxypentose, but this could not be so if one accepts the view that only the viscosity measurements carried out in the presence of salt are significant. Under this proviso, a " true " after-effect would occur only in solutions irradiated in the presence of molecular oxygen, whereas the formation of a 4'-carbonyl group should occur on irradiation in the presence as well as in the absence of oxygen, as has been found in the irradiation of several monoribonucleotides.[14] With regard to reactions (*a*, *b*), if the hydroperoxides shown to be formed in DNA solutions irradiated in the presence of oxygen were produced in the sugar component, the internucleotide bonds would probably be labilised: however, since the hydroperoxides are probably associated only with the pyrimidine bases (see above), their decomposition could hardly be responsible for the after-effects. Further, the observed rates of the hydroperoxide decay (Figs. 5 and 6) appear to be incompatible with the rate of loss of post-irradiation viscosity even though one cannot expect here a simple relation between chemical change and viscosity loss.

If we ascribe the viscosity after-effect to slow hydrolysis of labile phosphate esters, the decay should follow first-order kinetics. Equation (1) bears a resemblance to the well-known equation for a second-order reaction, but this is purely formal. It is known that random degradation of high polymers by first-order bond fission can be expressed by a somewhat similar relation involving the degree of polymerisation.[20] For polyelectrolytes, such as nucleic acid, however, the theoretical relation between viscosity and degree of polymerisation is uncertain and this prevents rationalisation of equation (1) on a kinetic basis; neither k' nor k would represent the actual rate constants of the chemical reaction since they are empirical quantities and may include many other factors.

Moreover, in view of the phosphodiester nature of the internucleotide linkage, chain breaking such as envisaged for the after-effect will not, in general, liberate inorganic phosphate, but should afford mainly phosphomonoester groups. Free phosphate can only arise from end-group oxidation; the absence, at room temperature, of any significant post-irradiation release of phosphate from the irradiated DNA solutions is not surprising in view of the high degree of polymerisation of the material and the fact, that, at the dose level employed, on the average, a maximum of only one in about 150 nucleotide molecules is attacked.

Experimental

Irradiations.—The source of *X*-rays was a Victor Maximar therapy tube, without filters and operating at 200 kv and 15 ma. Irradiations were carried out in Pyrex glass vessels of the type previously described.[21] The dose rate (determined by the ferrous dosimeter,[22] $G_{Fe^{3+}} = 15{\cdot}5$) was 1185 rep./min. in the viscosity experiments and 6000 rep./min. in those concerned with the detection of hydroperoxide and acyl phosphate.

Evacuation was effected with a two-stage oil-pump backed by a mercury diffusion pump, the pressure of oxygen in equilibrium with the solution not exceeding 10^{-5} mm. Irradiation in the presence of hydrogen was carried out by saturating an evacuated solution with hydrogen, which was purified by passage over palladised asbestos and copper gauze at 400°, and then through an activated charcoal trap, immersed in liquid nitrogen.

Triply distilled water, used for all solutions, was prepared by distilling ordinary distilled water from alkaline permanganate and then from phosphoric acid in a " Baraglass " still of the type described by Smith.[23] The DNA solutions were equilibrated at 25° for *ca.* 10 hr. before irradiation.

Viscosity Determinations.—These were carried out in an Ostwald viscometer, in a thermostat at 25° ± 0·01°. As soon as possible after irradiation (duration ~6 min.) the solution was transferred to the viscometer and allowed to reach the equilibrium temperature. The first

[20] Ekenstam, *Ber.*, 1936, **69**, 549, 533; Kuhn, *Ber.*, 1930, **63**, 1503.
[21] Farmer, Stein, and Weiss, *J.*, 1949, 3241.
[22] Farmer, Rigg, and Weiss, *J.*, 1955, 582.
[23] Smith, *Chem. and Ind.*, 1938, **57**, 936.

measurement was carried out about 30 min. after irradiation and subsequently at intervals until viscosity became constant. For measurements in sodium chloride solution, the procedure was as above, except that after irradiation sufficient concentrated sodium chloride solution was added to bring the solution to the required molarity (the volume change was negligible).

Reproducibility was very good. Differences (±5%) in the viscosity of control solutions could be generally ascribed to variations in the weighing of small amounts of the fibrous DNA and to variations in water content of " air-dried " samples. The viscosity change on irradiation was reproducible within ±2%.

Freeze-drying.—The apparatus used was based on a model described by Holtzman.[24] The solution (100 ml.) was frozen in thin layers in four 250 ml. flasks, which were then attached to a central flask immersed in solid carbon dioxide–methanol. The whole apparatus was continuously evacuated by an oil-pump until sublimation into the central vessel was complete. A water-reservoir was included in the apparatus, so that at the end of the freeze-drying operation, a little water vapour could be condensed on to the powder remaining; this prevented loss on opening of the apparatus to the air.

Hydrogen Peroxide and Hydroperoxide.—Hydrogen peroxide was determined by use of the titanium sulphate reagent according to Eisenberg's method.[25] After addition of the reagent, precipitated nucleic acid was removed by centrifugation and the optical density then measured in a " Unicam " spectrophotometer (S.P. 500) at 405 mμ. Total peroxide was determined by iodide according to Hochenadel's procedure,[26] the measurements being made in the " Unicam " instrument at 353 mμ.

Lipmann–Tuttle Reaction.[16]—This was adapted as follows: To 20 ml. of the irradiated solution were added 10 ml. of acetate buffer (pH 5·4) and 5 ml. of a mixture of equal volumes of a 28% w/v hydroxylamine hydrochloride and 14% (w/v) sodium hydroxide solution. After 10 min. at room temperature (or 30 min. at 100°), 2·5 ml. of concentrated hydrochloric acid and 5 ml. of ferric chloride solution [5% (w/v) in 0·1N-hydrochloric acid] were added. The colour (brown-red) was then measured on a " Spekker " colorimeter with a blue-green filter (Kodak 549). With irradiated nucleate solutions, precipitation occurred on addition of the acid and ferric chloride; after 5 min. the precipitate was removed by centrifugation, before the measurement.

For the nucleic acids (Table 3), greater values were obtained after *heating* in the presence of the hydroxylamine reagent, probably because heating reduces, to some extent, the subsequent amounts of nucleic acid precipitated on addition of acid, and so increases the amount of soluble ferric complex. With the nucleotides, heating results in a loss of colour and is presumably due to decomposition. Probably, therefore, the observed values in the irradiated nucleate solutions give only a lower limit.

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[24] Holtzmann, *Science*, 1950, **111**, 550.
[25] Eisenberg, *Ind. Eng. Chem. Anal.*, 1943, **15**, 327.
[26] Hochenadel, *J. Phys. Chem.*, 1952, **56**, 587.