64. *Deoxypentose Nucleic Acids. Part III.* Some Effects of Ultrasonic Waves on Deoxypentose Nucleic Acids.*

By S. G. Laland, W. G. Overend, and M. Stacey.

It has been demonstrated that ultrasonic waves disaggregate deoxypentose nucleic acids in dilute aqueous solutions. Changes accompanying this degradation have been investigated and the products have been examined. Results indicate that the products are of relatively high molecular weight. The decrease in viscosity of deoxypentose nucleic acids induced by sonic irradiation has been correlated with changes in the pattern of the titration curves, which can be explained, at least in part, by a decrease in hydrogen bonding. That this is not the entire explanation of the process, however, is indicated by the observed increase in the proportion of secondary phosphate groups. The mechanism of the degradation is discussed. Comment is made on the value of ultrasonic irradiation for rupturing cells during the preparation of nucleic acids from bacterial sources.

Recently we have been interested in isolating, from bacterial sources, various macromolecules and in particular deoxyribonucleic acid (Stacey, *J.*, 1947, 853; Overend, Stacey, Ungar, and Webb, *J. Gen. Microbiol.*, 1951, **5**, 268). In order to disrupt bacterial cells without using chemical reagents some workers (Flosdorf, Kimball, and Chambers, *Proc. Soc. Exp. Biol. N.Y.*, 1939, **41**, 122; Flosdorf and Kimball, *J. Immunol.*, 1940, **39**, 287) have subjected them to ultrasonic vibrations (cf. Zill and van Wagtendonk, *Biochem. Biophys. Acta*, 1951, **6**, 524, for the influence of ultrasonic vibrations on the activity of paramecium). Although this treatment is often effective, we have noted that the deoxypentose nucleic acid obtained from cells disrupted by this procedure gave aqueous solutions of a much lower viscosity than those isolated from similar cells disrupted in a mechanical shaker or a bacterial mill. Consequently it was considered of interest to examine the effect of ultrasonic waves on the chemical and physical properties of deoxypentose nucleic acids.

A further reason for this investigation was to study the nature of hydrogen bonding in deoxypentose nucleic acids. This formed part of an examination of the mode of aggregation of deoxypentose nucleic acids, using the electrometric titration technique (Overend and Peacocke, *Trans. Faraday Soc.*, 1950, **46**, 790; Lee and Peacocke, *J.*, 1951, 3361). Gulland *et al.* (see *Soc. Exp. Biol. Symp.*, 1947, **1**, 1) have proposed that the state of aggregation of deoxypentose nucleic acid is maintained essentially by hydrogen bonds. Evidence for this was drawn from the relation between the marked drop in viscosity of the nucleic acid with its loss of structural character, when acted upon by acid and alkali under very mild conditions, and the effect of these agents in releasing groups for titration in their usual pH ranges. The titration curves of the acid- and the alkali-treated deoxypentose nucleic acid were apparently the same and to account for this it was suggested that the hydrogen bonds linked amino- and enolic hydroxyl groups on the thymine and guanine residues. On this hypothesis, the discrepancy between forward- and back-titration curves of acid- and alkali-treated material should be some criterion of the state of hydrogen bonding of a particular deoxypentose nucleic acid. High-frequency sound waves are known to lower the viscosity and progressively degrade some natural (Flosdorf and Chambers, *J. Amer. Chem. Soc.*, 1933, **55**, 3051; Szent-Gyorgyi, *Nature*, 1933, **131**, 278; Szalay, *Z. physikal. Chem.*, 1933, *A*, **164**, 234; Stacey, *Research*, 1951, **4**, 48; Lockwood, James, and Pautard, *ibid.*, p. 46) and synthetic (Melville and Murray, *Trans. Faraday Soc.*, 1950, **46**, 996) polymers, so that it was thought that electrometric titration of nucleic acids irradiated for varying periods of time with ultrasonic waves would yield valuable information on their state of aggregation and particularly on their mode of hydrogen bonding. A brief preliminary account of the effect of ultrasonic waves on deoxypentose nucleic acids has already been published (Laland, Overend, and Stacey,

* Part II, *J.*, 1951, 2450.

Research, 1950, **3**, 386) and this work has now been amplified and extended (cf. Stern and Goldstein, *J. Polymer Sci.*, 1950, **5**, 687).

The ultrasonic waves (frequency, 213 kc. per sec.) were produced by the well-known method of driving a quartz crystal with the output from a valve oscillator (cf. Melville and Murray, *loc. cit.*). The deoxypentose nucleic acids examined were prepared from calf thymus gland, herring soft roes, and wheat germ. Extensive analytical data concerning these samples will form the subject of a separate communication.

On irradiation, with ultrasonic waves, of 0·2% aqueous solutions of the nucleic acids the viscosity decreased rapidly (see Fig. 1). After irradiation the nucleic acid solutions always showed a pH value less than that of the original solution, contrary to report by

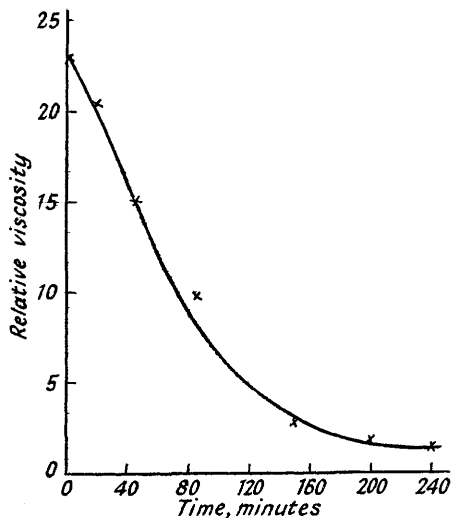
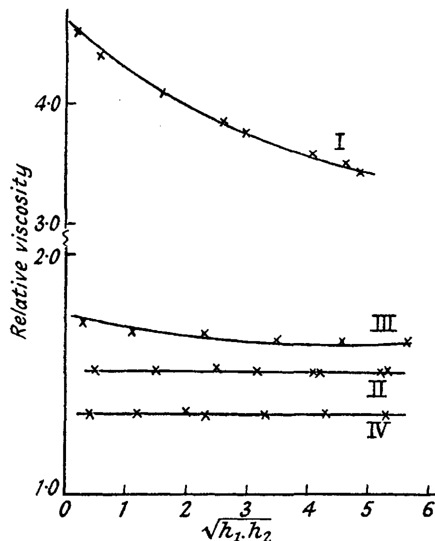


FIG. 1. *Change in viscosity of calf thymus deoxypentose nucleic acid on ultrasonic irradiation.*

FIG. 2. *Anomalous viscosity of nucleic acids.*

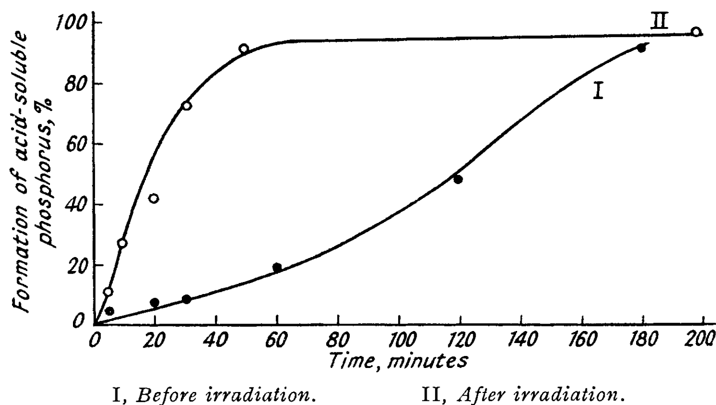


I, *Herring roe nucleic acid.*
II, *Herring roe nucleic acid after ultrasonic irradiation for* 95 *minutes.*
III, *Wheat germ nucleic acid.*
IV, *Wheat germ nucleic acid after ultrasonic irradiation for* 95 *minutes.*

Stern and Goldstein (*loc. cit.*) (however, our time of irradiation was more prolonged). The change in pH of our irradiated samples was shown by electrometric titration (Lee and Peacocke, *loc. cit.*) to be correlated with an increase in the number of free primary and secondary phosphoryl groups in the samples. The full significance of this finding will be discussed elsewhere (Overend, Peacocke, and Stacey, forthcoming publication). It is interesting to note that Greenstein, Taylor, and Hollaender (*Cold Spring Harbor Symp.*, 1947, **12**, 237) found no change in titratable acid groups on *X*-irradiation of thymus deoxyribonucleic acid. Furthermore in their work there was no change in acid precipitability; although the ability to be precipitated out of 95% ethanol in the presence of sodium chloride was completely lost in the *X*-irradiated material, ultrasonic-irradiated nucleic acids retained this property. Negligible amounts of ammonia could be detected (method of King, Haslewood, and Delory, *Lancet*, 1937, **1**, 866) in ultrasonic-irradiated solutions of deoxypentose nucleic acid from wheat germ or herring soft roes, but extended irradiation of solutions of calf thymus deoxypentose nucleic acid resulted in some loss, as ammonia, of the nitrogen present (cf. Greenstein *et al.*, *loc. cit.*; Scholes, Stein, and Weiss, *Nature*, 1949, **164**, 711). (Extended irradiation of calf thymus nucleic acid was necessary since it gave more highly viscous solutions than did the other nucleic acids used, and consequently required longer for degradation.) Negligible amounts of inorganic

phosphate were formed and the products from the irradiation were still relatively insoluble in acids. The refractive indices of the native and corresponding irradiated samples in solution were identical, as also were their ultra-violet absorption spectra, thus showing that no groups exhibiting strong absorption were liberated during the irradiation. It is interesting to compare the identity of the absorption spectra of native and sonic-irradiated nucleic acids with the result obtained during the enzymic degradation of deoxypentose nucleic acid. When pancreatic deoxyribonuclease acts on a deoxpentose nucleic acid substrate a fall in viscosity of the substrate occurs, and we find that simultaneously there is a large increase in the intensity of the ultra-violet absorption spectrum (cf. Kunitz, *J. Gen. Physol.*, 1950, **33**, 349). Hence it would seem that the changes in viscosity induced in deoxypentose nucleic acids enzymically and by ultrasonic irradiation are different in character. Solutions of the native and the irradiated materials were dialysed in

FIG. 3. *Action of deoxyribonuclease on herring roe deoxypentose nucleic acid.*



I, *Before irradiation.* II, *After irradiation.*

Cellophane bags at 37° against distilled water. Negligible amounts of material of low molecular weight passed through the membrane, and no free purine or pyrimidine bases were detected. Comparative measurements on diffusion rates with the simple apparatus of Gage (*Trans. Faraday Soc.*, 1948, **44**, 253) on the native and the irradiated samples showed no significant difference, confirming the absence in the irradiated material of polynucleotides of low molecular weight. Hence it seems that although some disaggregation of the deoxypentose nucleic acids is induced by ultrasonic irradiation, the products are still of relatively high molecular weight. Further proof of the disaggregated nature of the irradiation products was provided by an examination of some of their physical properties. Unlike the native deoxypentose nucleic acids which showed structural (anomalous or non-Newtonian) viscosity in the Frampton viscometer (*J. Biol. Chem.*, 1939, **129**, 233), the irradiated products exhibited no anomalous behaviour (see Fig. 2). Since structural viscosity is a reflection of molecular asymmetry and in this case also probably an index of the degree of polymerisation, this result indicates that disaggregation has occurred. Moreover, in the simple apparatus of Edsall and Mehl (*J. Biol. Chem.*, 1940, **133**, 409) the irradiated products did not show the birefringence of flow characteristic of the native material (cf. Greenstein, *Adv. Protein Chem.*, 1944, **1**, 209, and Sparrow and Rosenfeld, *Science*, 1946, **104**, 245, concerning the non-Newtonian viscosity and streaming birefringence of flow of dilute aqueous solutions of deoxypentose nucleic acids). Stern and Goldstein (*loc. cit*) state that the deoxyribose content, as determined colorimetrically by the Dische reaction (*Mikrochem.*, 1930, **8**, 4), was unchanged before and after ultrasonic irradiation of deoxypentose nucleic acids and we have confirmed this. However, after irradiation the nucleic acids gave a somewhat more intense colour when a supersensitive Schiff's reagent (Tobie, *Ind. Eng. Chem. Anal.*, 1942, **14**, 405) was added. This result correlates with earlier evidence (Overend, Stacey, and Webb, *J.*, 1951, 2450) for the existence, in nucleic acids of the deoxypentose type, of a labile polymeric bond which is possibly a glycosidic phosphate linkage (cf. Overend and Stacey, *Nature*, 1949, **163**, 583).

Electrometric titration results supported the view that ultrasonic vibrations disaggregate deoxypentose nucleic acids and indicated that some of the hydrogen bonds are broken (Lee and Peacocke, *loc. cit.*). Enzyme studies on the irradiated material confirmed this. It has been demonstrated that the action of ox-pancreas deoxyribonuclease on deoxypentose nucleic acid results in an initial decrease in viscosity without the formation of acid-soluble polynucleotides (Overend and Webb, *Research*, 1949, **2**, 99; *J.*, 1950, 2746). When the fall in viscosity is complete, onset of formation of acid-soluble polynucleotides commences. When the irradiated samples of deoxypentose nucleic acid were incubated, under identical conditions at pH 7·0, with this enzyme, little further decrease in viscosity occurred and the formation of acid-soluble material commenced immediately (see Fig. 3). It would appear that ultrasonic irradiation disaggregates the nucleic acids to less complex polynucleotides which are susceptible to the nucleotidase action of the enzyme.

The evidence presented shows, that when nucleic acids are isolated from micro-organisms, disruption of the cells by ultrasonic vibrations should be avoided if undegraded samples are required.

It is possible to make some observations on the mechanism of the depolymerisation of deoxypentose nucleic acids by sonic irradiation and to compare this with the *X*-irradiation of these macromolecules. Greenstein *et al.* (*loc. cit.*) showed that, on *X*-irradiation of thymus nucleic acid, the drop in viscosity initiated by the *X*-rays continued after cessation of the irradiation, for a period of about eight hours, whereafter the viscosity levelled off and approached the value consequent on the spontaneous drop of the control specimens. Scholes and Weiss (*Nature*, 1950, **166**, 640) have shown that free radicals, particularly the hydroxyl radical formed by the action of the *X*-radiation on the water of the solutions, was responsible for this effect. Both Butler and his co-workers (*Nature*, 1950, **165**, 714; *J.*, 1950, 3411, 3418, 3421) and Limperos and Mosher (*Amer. J. Roentgenol. Radium Therapy*, 1950, **63**, 681) have also proposed radical mechanisms for the breakdown of deoxypentose nucleic acids in aqueous solutions by *X*-rays and it seems that under suitable conditions hydroxyl radicals and hydrogen atoms are both effective (cf. Lea, "Actions of radiations on living cells," Macmillan Co., New York). Although after the ultrasonic irradiation the viscosity of the solutions of nucleic acid continued to fall very slightly, the change was in no way comparable with the effect observed after *X*-irradiation of similar solutions. Hence it is clear that a radical mechanism is not responsible for the sonic degradation of nucleic acids. Results reported by Melville and Murray (*loc. cit.*) indicated that the degradation by ultrasonic vibrations of dilute solutions of poly(methyl methacrylate) and polystyrene leads to molecules rather than to radicals.

Other possible mechanisms whereby the degradation is induced are (*a*) oxidative fission and (*b*) cavitation. It is also possible that the effect could be thixotropic. At various times evidence for these effects have been examined. Freundlich and Gillings (*Trans. Faraday Soc.*, 1938, **34**, 649), investigating the action of ultrasonic waves on aqueous solutions of gelatin, and toluene solutions of rubber, found that the lowering in viscosity was not permanent. Therefore they concluded that the viscosity change was merely a thixotropic effect and was not due to molecular degradation. These experiments left some doubts as to the results of previous workers who had relied on viscosity measurements alone to prove degradation. The possibility of the viscosity fall being due to a thixotropic effect has been disposed of, however, by Brohult's experiments on the degradation of hæmocyanin (*Nature*, 1937, **140**, 408) and more fully by Schmid and Rommel (*Z. physikal. Chem.*, 1940, *A*, **185**, 98) who worked with chemically better defined synthetic polymers. The latter workers were also able to exclude oxidative fission as the cause of the viscosity fall, a conclusion confirmed by Melville and Murray (*loc. cit.*).

Melville and Murray have shown that degradation can be accomplished without cavitation, but that the breakdown is not so marked as when it is allowed to take place. We have observed that on storage irradiated solutions of nucleic acids do not regain their original viscosities and hence that the effect is not thixotropic, and that irradiation in an atmosphere of nitrogen freed from oxygen causes only a very small difference in the rates of decrease of the viscosity, compared with an irradiation in air. This indicates that oxidative fission can play only a very minor part in the change. It would seem that,

concerning the mechanism of the depolymerisation of nucleic acids by sonic vibrations, Oster's experiments (*J. Gen. Physiol.*, 1947, **31**, 89) on the effect of this treatment on tobacco mosaic virus are of special interest. Electron micrographs lead to the view that the individual virus particles are broken first into halves and then into quarters through the cavitation effect resulting from the sonic vibrations. It is our opinion that sonically irradiated deoxypentose nucleic acids are broken down in similar manner. First, the hydrogen bonds are broken, and then the main polynucleotide chain is ruptured. This leads to the formation of more primary and secondary phosphoryl groups than are found in the native nucleic acid. Stern and Goldstein (*loc. cit.*) have provided sedimentation data which would support this viewpoint. That main valency bonds may be broken through the application of purely mechanical forces to fibre structures is demonstrated by recent experiments by Lundgren and Stein (Abs. of Paper Presented at 114th Meeting of the Amer. Chem. Soc., Portland, Oregon, Sept., 1948).

Experimental

The apparatus used for the generation of the ultrasonic waves consisted of a valve oscillator with two Osram MT 16 valves in parallel, coupled to an X-cut quartz crystal. The applied voltage was about 10 kv and the anode current about 70 milliamp. At maximum output the total energy dissipated by the crystal was *ca.* 100 w, of which about 5% was converted into sound energy. Details of construction of the apparatus and crystal mounting are as described by Melville and Murray (*Trans. Faraday Soc.*, 1950, **46**, 996).

In order to obtain maximum sound energy from the crystal, it is necessary to supply the alternating voltage to the crystal at the same frequency as the fundamental frequency of the crystal. This fundamental frequency is fixed by its dimensions and consequently the oscillator must be tuned to the frequency of the crystal. When the crystal is vibrating under oil, a fountain of oil is thrown up from the surface above the crystal face, and this fountain forms a convenient tuning indicator. Consequently the oscillator was tuned by adjusting the variable condensers in the anode and coupling tuned circuits, until a maximum fountain was obtained.

Samples of Deoxypentose Nucleic Acids Used.—The nucleic acids used were isolated from calf thymus gland, herring soft roes, and wheat germ. The nucleic acid from calf thymus gland was isolated essentially by Mirsky and Pollister's method (*J. Gen. Physiol.*, 1946, **30**, 117). Protein was removed by the Sevag procedure (Sevag, Lackman, and Smolens, *J. Biol. Chem.*, 1938, **124**, 425). The nucleic acid from herring soft roes was isolated in a similar manner, except that it was freed from protein by saturation with sodium chloride (cf. Hammarsten, *Biochem. Z.*, 1924, **144**, 383). Deoxypentose nucleic acid was prepared from wheat germ by the method of Laland, Overend, and Webb (*Acta Chem. Scand.*, 1950, **4**, 885).

Irradiation Procedure.—The solution being irradiated was contained in a glass vessel (capacity, 100 c.c.) which was placed on the surface, and directly above, the fountain of oil. Throughout the irradiation the temperature was kept at 20—22°, by a water cooling system. In each experiment a 0·2% solution (50 c.c.) of the deoxypentose nucleic acid was used.

Viscosity Changes of the Nucleic Acids During Sonic Irradiation.—Viscosity measurements were carried out in an Ostwald viscometer at 25°, on aliquots (4 c.c.) of the nucleic acid solution being irradiated. Initial measurements were usually made 10 minutes after sonic irradiation had commenced. When no appreciable fall in viscosity was noted, the product was isolated by evaporation to dryness in the frozen state. Typical viscosity changes observed for calf thymus deoxypentose nucleic acid, for example, are shown in Fig. 1.

pH *Changes induced by Sonic Irradiation* (cf. Goldstein and Stern, *J. Polymer. Sci.*, 1950, **5**, 687).—Measurements were carried out with a Cambridge Instrument Co. pH meter. Results were:

Source of nucleic acid	Time of irradiation (min.)	pH of solution, initial	final	Change in pH after irradiation
Calf thymus gland	240	5·62	5·00	−0·62
Herring soft roe	95	6·06	5·50	−0·56
Wheat germ	95	5·90	5·46	−0·44

Examination of the Irradiated Samples.—A comparison was made of the properties of the nucleic acids before and after ultrasonic irradiation.

(a) *Precipitation tests* (cf. Greenstein *et al.*, *Cold Spring Harbor Symp.*, 1947, **12**, 237).

Precipitation tests were carried out by adding absolute ethanol (2 vols.) to solutions (0·5%) of the nucleic acids in 1M-sodium chloride. Results were as follows :

Source of nucleic acid	Nature of the precipitate
(i) Thymus gland :	
before irradiation	Immediate formation of an extremely fibrous ppt.
after irradiation	Slow formation of a flocculent ppt.
(ii) Herring roe : before and after irradiation	Immediate formation of a fibrous ppt.
(iii) Wheat germ : before and after irradiation	Immediate formation of a fibrous ppt.

(b) *Refractive index.* Measurements were carried out with a Hilger refractometer on 0·3% aqueous solutions at 20° :

	Thymus gland	Herring roe	Wheat germ
Before irradiation	1·3359	1·3357	1·3355
After irradiation	1·3359	1·3355	1·3358

(c) *Dialysis.* In a typical procedure an accurately weighed amount of the sample (dried in the frozen state) was dissolved in freshly distilled water (6 c.c.). The solution was placed in a Cellophane dialysis sac which was then suspended in a tube containing water (20 c.c.). The amount of material which had dialysed in 4 hours at 37° was determined from observations on the intensity of the absorption exhibited at 260 mμ by the liquid surrounding the dialysis sac. The following results were obtained :

Sample of nucleic acid	Time of irradiation (min.)	Initial concn. (mg./c.c.)	Dialysis (%)
(1) Thymus gland : before irradiation	0	2·3	0·55
after irradiation	240	2·4	0·90
(2) Herring roe : before irradiation	0	2·3	0·51
after irradiation	95	2·4	0·86
(3) Wheat germ : before irradiation	0	2·4	0·34
after irradiation	95	2·2	1·09

(d) *Diffusion.* The experiments were carried out in the apparatus described by Gage (*Trans. Faraday Soc.*, 1948, **44**, 253) (cf. Overend and Webb, *J.*, 1950, 2746) with filter paper (Whatman, No. 54) as the diffusion membrane. An aqueous solution of the sample (0·0056 g./l.) was introduced into the cell after being preheated at 37° for 30 minutes, and diffusion allowed to proceed for 3 hours at 37° against distilled water (1 l.). The amount of diffusion was determined by measuring the intensity of the ultra-violet absorption of the liquid in the cell. Decreases in the intensity of absorption before and after diffusion were calculated. Only with the nucleic acid isolated from calf thymus gland was there any observable decrease in the intensity of absorption, and this was very small.

(e) *Viscosimetric behaviour.* The viscometer described by Frampton (*J. Biol. Chem.*, 1939, **129**, 233) was used for this investigation. All measurements were carried out at 25° ± 0·05° on 0·1% solutions. The results obtained ($\sqrt{h_1 . h_2}$ being the geometric mean of the hydrostatic pressure in centimetres) are shown in Fig. 2.

(f) *Streaming birefringence of flow.* The streaming birefringence of flow of solutions of the native and irradiated deoxypentosenucleic acids was determined in the apparatus of Edsall and Mehl (*J. Biol. Chem.*, 1940, **133**, 409) (this only gives a rough estimate of the degree of streaming birefringence of flow of a solution). Results obtained were as follows :

Source of nucleic acid	Time of irradiation (min.)	Concn. (%)	Degree of streaming birefringence
Herring roe	0	0·3	+, +
	95	0·3	—
Wheat germ	0	0·3, 0·6, 1·2	–, –, –
	95	0·3	—
Thymus gland	0	0·3	+, +, +
	240	0·3	—

(g) *Ultra-violet absorption spectra.* Measurements of the intensity of the ultra-violet absorption of the various samples in aqueous solution were made with a Unicam spectrophotometer, before and after irradiation, with the following results :

	Nucleic acid from herring roe, before	herring roe, after	thymus gland, before	thymus gland, after	wheat germ, before	wheat germ, after
$P(\varepsilon)_{260\ m\mu}$	7500	7620	7700	7920	7300	7300

($P(\varepsilon)_{260\ m\mu}$ is the molecular extinction coefficient at 260 mμ calculated by using the phosphorus content as the standard of reference. This term was first used by Chargaff and Zamenhof, *J. Biol. Chem.*, 1948, **173**, 327.)

(h) *Behaviour towards Schiff's reagent.* The sample of nucleic acid (*ca.* 2 mg.) was dissolved in freshly distilled water (8 c.c.) contained in a thoroughly cleaned boiling-tube. Supersensitive Schiff's reagent (Tobie, *Ind. Eng. Chem. Anal.*, 1942, **14**, 405) (1 c.c.) was added and then the tubes were securely sealed. After 90 and 210 minutes the intensity of the colour developed was measured with the Hilger (Spekker) photoelectric absorptiometer and Ilford filter No. 605 (λ = 5500 Å). All measurements were made under controlled conditions of temperature. Results, before and after irradiation, were as follows :

	Nucleic acid from					
	thymus gland,		herring roe,		wheat germ,	
	before	after 240 min.	before	after 95 min.	before	after 95 min.
ε_p at 550 mμ after 90 min. ...	110	142	96	130	108	140
ε_p at 550 mμ after 210 min. ...	275	280	250	275	275	300

($\varepsilon_p = RA/cd$ when A = atomic weight of phosphorus, R = instrument reading, c = g. of phosphorus per l. of nucleic acid solution, and d is the thickness of the cell in cm.)

(i) *Action of deoxyribonuclease.* (1) Viscometric changes. The viscometric changes were observed which resulted from the action of a 0·0018% aqueous solution of deoxyribonuclease (prepared according to McCarty's method, *J. Gen. Physiol.*, 1946, **29**, 123) on a digest consisting of a solution of the deoxyribonucleic acid (before and after irradiation) (0·13%) which was 0·18M. with respect to both magnesium sulphate and veronal buffer (pH 6·92). An Ostwald viscometer was used and all the observations were made at 37° on aliquots of 4 c.c. Typical changes in viscosity observed are shown in Fig. 3.

(2) Formation of acid-soluble polynucleotides. The substrate and enzyme preparations used were identical with those already described, except for the experiments with wheat germ nucleic acid. In this case the concentrations of the nucleic acid and enzyme were 0·11% and 0·0004% respectively and the digest was 0·022M. with respect to magnesium sulphate and the veronal buffer. At intervals, aliquots (2 c.c.) were withdrawn and acid-insoluble material was precipitated by addition of 5N-hydrochloric acid (0·3 c.c.). The precipitate was removed at the centrifuge, and the phosphorus content of the supernatant liquid was determined by Allen's colorimetric method (*Biochem. J.*, 1940, **34**, 858). The following results were obtained :

Herring roe nucleic acid									
Before irradiation :									
Time (min.)	5	20	30	40	60	120	180	225	
Formation of acid-sol. P (% of total P)	5·5	7·9	7·9	18·8	18·8	48·0	92·0	100·0	
After irradiation :									
Time (min.)	5	10	20	30	50	200	260		
Formation of acid-sol. P (% of total P)	10·6	27·0	41·5	73·0	91·0	96·0	100·0		
Thymus gland nucleic acid									
Before irradiation :									
Time (min.)	20	40	60	80	116	146	176	305	380
Formation of acid-sol. P (% of total P)	5·5	9·5	10·0	9·5	15·2	24·0	29·5	71·0	96·0
After irradiation :									
Time (min.)	10	20	40	60	90	120	150		
Formation of acid-sol. P (% of total P)	9·0	9·3	20·5	30·0	41·5	64·5	83·0		
Wheat germ nucleic acid									
Before irradiation :									
Time (min.)	5	15	30	50	70	95			
Formation of acid-sol. P (% of total P)	45	61·5	82	87	91	96			
After irradiation :									
Time (min.)	5	15	30	50					
Formation of acid-sol. P (% of total P)	58	70	85	98					

Estimation of the Inorganic and Acid-soluble Phosphorus formed during Irradiation.—A solution of the irradiated nucleic acid sample in distilled water (5 c.c.; 2 mg. of nucleic acid per c.c.) was placed in a centrifuge tube and precipitated by the addition of 5N-hydrochloric acid (0·5 c.c.). The precipitate was removed at the centrifuge and analyses were carried out on aliquots (2 c.c.) of the supernatant liquid for inorganic phosphate and organic-bound acid-soluble phosphorus compounds. In addition the total phosphorus content of the original nucleic acid solution was determined.

Source of irradiated nucleic acid	Phosphorus present as inorg. phosphate (% of total phosphorus)	Acid-soluble phosphorus (% of total phosphorus)
Calf thymus gland	1·0	2·0
Herring soft roes	1·0	2·0
Wheat germ	1·0	4·0

Changes occurring during and after irradiation.—(a) *Liberation of ammonia* (cf. King, Haslewood, and Delory, *Lancet*, 1937, **1**, 866). A solution of nucleic acid (2 c.c.), irradiated as described, was diluted with distilled water (5 c.c.) and standard Nessler's reagent (1 c.c.) was added. The intensity of the colour developed after 5 minutes was measured in the Spekker photoelectric absorptiometer with Ilford filter No. 602. Calibration of the intrument with a standard solution of ammonia gave a means of measuring the ammonia content of the nucleic acid solution. Results were as follows:

Source of nucleic acid	Time of irradiation (min.)	Nitrogen present as ammonia (% of total N content of the nucleic acid solution)
Herring soft roes	90	$<0·6$
Wheat germ	110	$<0·6$
Calf thymus gland	240	1·25

It was shown that by use of this method it was possible to detect 0·0025 mg. of ammonia.

In control experiments it was possible to demonstrate that any ammonia formed during irradiation was not evolved: a 0·2% solution of herring roe deoxypentose nucleic acid containing added ammonia (0·0144 mg. of nitrogen per c.c.) was submitted to ultrasonic irradiation at 20° and pH 5·5 for 120 minutes; the ammonia in the solution was estimated by the above method before and after irradiation; there was no appreciable change.

(b) *Change in viscosity after irradiation.* A 0·2% solution of herring roe deoxypentose nucleic acid was submitted to ultrasonic irradiation for 20 minutes. The viscosity of the solution was determined 3 minutes after the cessation of the irradiation, and thereafter the solution was maintained at 25° and its viscosity measured at intervals. A similar experiment was carried out with calf thymus gland nucleic acid, except that the initial irradiation treatment was maintained for 60 minutes. Results were as follows:

Herring roe nucleic acid

Time (min.)	3	9	90	255	960
$\eta_{rel.\ H_2O}$	2·14	2·11	2·08	2·08	2·07

Calf thymus nucleic acid

Time (min.)	5	960
$\eta_{rel.\ H_2O}$	13·6	12·1

(c) *Ultrasonic irradiation in an atmosphere of nitrogen.* A solution of calf thymus deoxypentose nucleic acid (0·07%; $\eta_{rel.}$ 3·65) (20 c.c.) was placed in a closed glass vessel, and nitrogen (freed from oxygen by passage over a heated copper spiral) was bubbled through the solution for 1 hour. The vessel was then sealed and the solution irradiated with ultrasonic vibrations for 35 minutes. After this period the relative viscosity of the solution was 1·62, whereas the control solution irradiated in the presence of oxygen had a relative viscosity of 1·47 (cf. Melville and Murray, *loc. cit.*, for an account of the slight variations of viscosity which occur in a series of experiments under apparently identical conditions).

The authors are indebted to Professor H. W. Melville, F.R.S., and his colleagues for advice and for use of their sonic oscillator. This work was supported by grants from the British Empire Cancer Campaign (Birmingham Branch) and from Imperial Chemical Industries Limited.

THE CHEMISTRY DEPARTMENT, THE UNIVERSITY,
EDGBASTON, BIRMINGHAM, 15.

[*Received, July 28th,* 1951.]