671. *The Action of Ionizing Radiations and of Radiomimetic Substances on Deoxyribonucleic Acid. Part III. The Molecular Weights of Deoxyribonucleic Acid degraded by X-Rays and by Treatment with a "Nitrogen Mustard."*

By B. E. Conway, L. Gilbert, and J. A. V. Butler.

Molecular weights of deoxyribonucleic acid in aqueous solutions, untreated and degraded by treatment by *X*-rays and di-(2-chloroethyl)methylamine, have been determined by sedimentation and diffusion-constant determinations. The degraded materials have molecular weights which are markedly less than those of the original nucleic acid and exhibit a considerable degree of polydispersity.

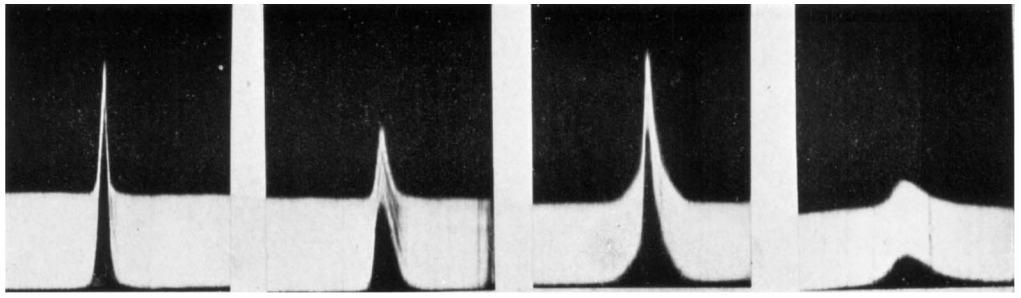
It was shown in Part I (*J.*, 1950, 3411) that the "nitrogen mustard," di-(2-chlorethyl)methylamine, and analogous compounds, when added to deoxyribonucleic acid (calf thymus) solutions, cause a loss of its characteristic viscous behaviour. The effect is similar to that produced by *X*-radiation (Part II, *J.*, 1950, 3418; Taylor, Hollaender, and Greenstein, *Arch. Biochem.*, 1948, **16**, 19). The change of viscosity could be caused by a change in the asymmetry of the molecule or by a disaggregation involving the splitting of the molecule into fragemnts of lower molecular weight. We have therefore determined the sedimentation velocities in the ultra-centrifuge at *ca.* 60,000 r.p.m. (*ca.* 250,000 g.) and the diffusion constants of (1) nucleic acid in the original state, (2) the product of the treatment with di-(2-chloroethyl)methylamine (see Part I, *loc. cit.*), and (3) that of irradiation with *X*-rays (7000 and 40,000 r.).

Fig. 1 shows typical examples of the Schlieren pictures obtained during sedimentation and diffusion. The untreated nucleic acid (Fig. 1*e*) sediments with the characteristic sharp boundary observed by previous workers (Cecil and Ogston, *J.*, 1948, 1382; Kahler, *J. Phys. Coll. Chem.*, 1948, **52**, 676). The diffusion diagrams (Fig. 1*a*) are somewhat asymmetric. The molecular weight, as determined by the methods described below, is of the order of $1{\cdot}5 \times 10^6$. The material treated with the amine shows in the ultra-centrifuge a greater degree of spreading than corresponds to the diffusion picture after the same period of time, indicating heterogeneity with regard to the sedimentation constant (Fig. 1). The diffusion picture itself shows very marked heterogeneity (Fig. 1*b*), which is analysed below. Examples of the sedimentation Schlieren pictures of the *X*-ray-treated material are shown in Figs. 1*g* and *h*, and of the corresponding diffusion pictures in Figs. 1*c* and *d* respectively.

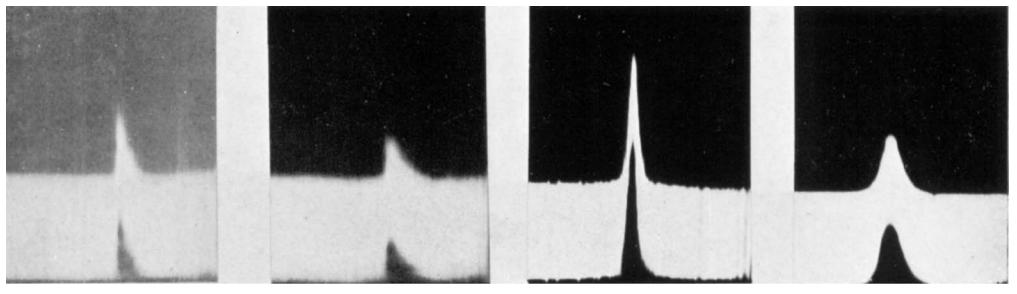
Sedimentation measurements were made in 0·1N-sodium hydrogen carbonate at

FIG. 1.

Schlieren pictures of boundary during diffusion and sedimentation of thymonucleic acid.



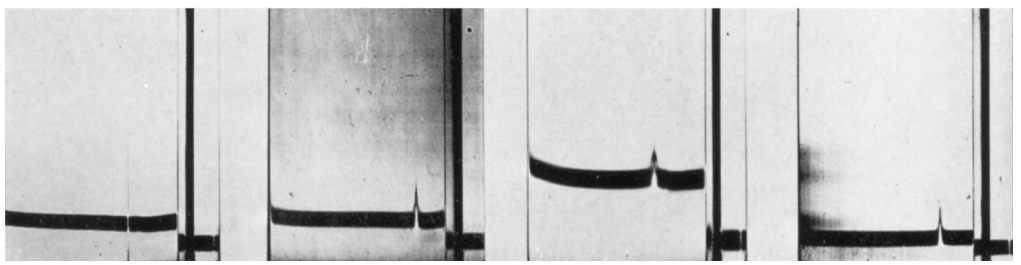
1*a*. 2*a*. 1*b*. 2*b*.



1*c*. 2*c*. 1*d*. 2*d*.

Diffusion photographs :

1*a*. *Untreated* (*A*, 0·2%) *after* 17·2 *hours*. 2*a*. *Same after* 50 *hours*. 1*b*. *Amine-treated* (0·2%) *after* 1·0 *hour*. 2*b*. *Same after* 26 *hours*. 1*c*. *X-Irradiated* (7000 r.) (0·2%) *after* 22 *hours*. 2*c*. *Same after* 45·7 *hours*. 1*d*. *X-Irradiated* (40000 r. *in presence of oxygen*) (0·2%) *after* 4·2 *hours*. 2*d*. *Same after* 25·3 *hours*.



e. *f*. *g*. *h*.

Sedimentation photographs :

e. *Untreated as in* (*a*). *f*. *Treated as in* (*b*). *g*. *Treated as in* (*c*). *h*. *Treated as in* (*d*).

[*To face p*. 3421.

concentrations of 0·2, 0·1, 0·05, and 0·025 wt.%, and diffusion determinations at 0·2, 0·1, and 0·05%. Extrapolation from these results to infinite dilution, which presents considerable difficulties, is discussed below. The mean diffusion and sedimentation constants, corrected to 20°, so obtained are given in the Table, with the extrapolated values for zero concentration and the molecular weights calculated from them. It can be seen that these measurements indicate clearly a significant lowering of the molecular weight in both the amine- and the *X*-ray-treated material.

Thymonucleic acid,	Wt. %.*	$S_{20,}$ $\times 10^{13}$.	$D_{20,}$ $\times 10^{8}$.	$S^{\circ}_{20,}$ $\times 10^{13}$.	$D^{\circ}_{20,}$ $\times 10^{8}$.	$(S/D)^{\circ}$, $\times 10^{5}$.	Mol. wt. from S°_{20} and D°_{20}.	Mol. wt. from $(S/D)^{\circ}$.
Untreated, batch A	0·2	5·7	—	12·0	5·2	2·65	$1·3 \times 10^{6}$	$1·4 \times 10^{6}$
	0·1	6·4	4·05					
	0·05	8·9	4·00					
	0·025	10·3	4·65					
Untreated, batch C	0·2	4·8	—	13·5	5·5	2·74	$1·35 \times 10^{6}$	$1·5 \times 10^{6}$
	0·1	6·0	3·28					
	0·05	8·2	3·70					
	0·025	10·4	4·22					
X-Irradiated (7000 r.)	0·2	6·0	—	12·0	—	—	$<8 \times 10^{5}$	—
	0·1	7·7	8·3					
	0·05	8·8	—					
	0·025	10·6	—					
X-Irradiated (40,000 r.) (O_2 present)	0·2	5·0	—	10·5	15	0·96	$3·9 \times 10^{5}$	$5·3 \times 10^{5}$
	0·1	6·4	7·4					
	0·05	6·9	8·0					
	0·025	8·3	12·1					
Amine-treated, batch C	0·2	5·2	—	10·5	18	0·82	$3·2 \times 10^{5}$	$4·5 \times 10^{5}$
	0·1	6·9	8·75					
	0·05	8·8	9·5					
	0·025	9·6	12·75					
Amine-treated, batch A	0·2	5·8	(0·25) 16·5	10 ± 2	175	—	$3·2 \times 10^{4}$	—
	0·1	8·1	50·0					
	0·05	8·8	104·0					

* The concn. in the diffusion experiments is taken as one-half of that in the lower solution.

Experimental.

The thymonucleic acid was prepared as described in Part I. The amine-treated material was prepared by adding 10 mg. of di-(2-chloroethyl)methylamine to 25 mg. of the nucleic acid. The reaction was allowed to proceed for 5 days, and the product dialysed for 170 hours against frequently changed water. It was then freeze-dried and dissolved as required in 0·1N-sodium hydrogen carbonate.

X-Ray-treated material was prepared as described in Part II, by dissolving the nucleic acid in oxygen-free water in an atmosphere of nitrogen. The solution was irradiated by a 400-kv. *X*-ray machine at the rate of 206 r. per minute to give total dosage of 7000 r. Solid sodium hydrogen carbonate was then added to give a concentration of 0·1N., to prepare the solutions for the diffusion and ultra-centrifugal determinations. Another series of measurements was carried out on the nucleic acid irradiated with 40,000 r. in the presence of air.

Sedimentation velocities were determined in the Spinco ultra-centrifuge at about 250,000 g. (*ca.* 60,000 r.p.m.). The velocity in the case of the degraded samples measured was that of the peak of the refractive-index-gradient curve.

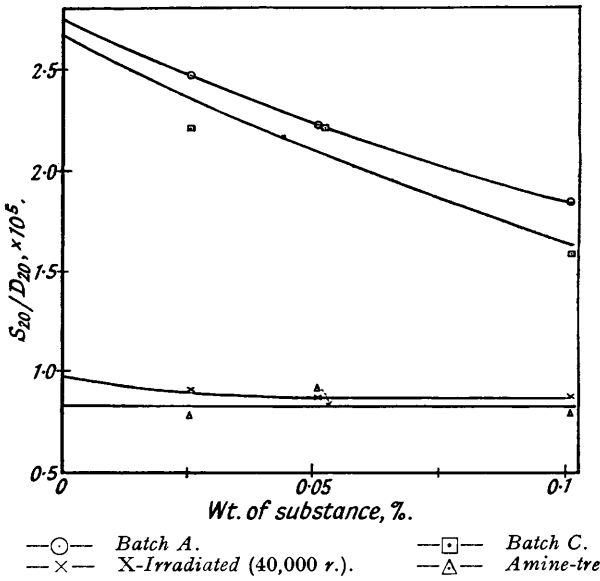
Diffusion constants were determined in the Perkin–Elmer electrophoresis apparatus (Moore and White, *Rev. Sci. Inst.*, 1948, **19**, 700), by Longsworth's scanning method (*Ann. New York Acad. Sci.*, 1941, **41**, 269). The boundary was originally made by sliding two sections of an electrophoresis cell into alignment. Correction of the zero or starting time for disturbance of the boundary while bringing it into view was made by Longsworth's method (*loc. cit.*); see also Stern, Singer, and Davis (*J. Biol. Chem.*, 1947, **167**, 326).

Corrected values (to 20°) of the sedimentation and diffusion constants of the various concentrations are given in the Table. The extrapolation to zero concentration involves much difficulty owing to the marked (and theoretically uncertain) variation with concentration at low values of the latter. The extrapolation of S° agrees with Cecil and Ogston's value although our values of S are somewhat smaller, as the concentration dependence is more marked. There is some indication that in dilute solutions the dependence of S and D on the concentration may be roughly parallel. Singer (*J. Chem. Physics*, 1947, **15**, 341) has discussed the theoretical factors involved in the concentration dependence of S and D and concluded that the same frictional forces are likely to be operative. The ratio S/D, although it may be open to objection on theoretical grounds, is certainly easier to extrapolate (see Fig. 2) and in fact shows little variation with concentration in the degraded samples. Its use is perhaps the best practical

compromise at the present time. The extrapolated values obtained from this ratio and those obtained by separate extrapolations of S and D are given in the Table. The molecular weights are calculated by $M = \frac{RT}{(1 - V\rho)} \cdot \frac{S^\circ}{D^\circ}$, where ρ, the density of the solution, is taken as 1·05, and V the partial specific volume, is taken as 0·55 (Cecil and Ogston, *loc. cit.*).

FIG. 2.

Extrapolation of S_{20}/D_{20} *to zero concentration (thymonucleic acid).*



—○— *Batch A.* —⊡— *Batch C.*
—×— X-*Irradiated* (40,000 *r.*). —△— *Amine-treated.*

DISCUSSION.

Notwithstanding uncertainties arising from the extrapolations and also from the heterogeneity of the substances, there appears to be no doubt that the molecular weight of the nucleic acids is appreciably lowered by treatment with either *X*-rays or the amines. The decrease produced by the chemical treatment is appreciably the greater. One of the samples of amine-treated nucleic acid was apparently appreciably more degraded than the other. This could be due to the formation of a larger quantity of material of comparatively low molecular weight.

Interpretation of these results will necessarily be uncertain until more is known about the nature of the sedimenting unit of mol. wt. $1{\cdot}5 \times 10^6$ of the untreated nucleic acid. There is little evidence to show whether this is a true chemically united molecule or a fairly loose aggregate or micelle made by the association of a number of smaller units. It may perhaps be significant that an entirely different method, *i.e.*, the dielectric dispersion method used by G. and I. Jungner and G. Allgén (*Acta Physiol. Scand.*, 1950, **20**, Suppl. 69; *Nature*, 1949, **163**, 849) gave much lower molecular weights for similar preparations of thymonucleic acid. It must be remembered also that the viscous undegraded acid is inherently unstable since a trace of the enzyme deoxyribonuclease (Kunitz, *J. Gen. Physiol.*, 1950, **33**, 349, 363) causes a rapid loss of the viscous behaviour and a degradation to units which can readily diffuse through cellophane (Carter and Greenstein, *J. Nat. Cancer Inst.*, 1946, **7**, 29) and approach the size of tetranucleotides (Kunitz, *loc. cit.*).

The changes brought about by the amine and by *X*-rays are much less extensive than this and for that reason more difficult to interpret. It does not follow from the results that the changes produced by these agents are necessarily primarily the breakage of the chemical bonds which hold the large molecule together. It is possible that the chemical modification of some of the smaller units may cause a large aggregate to dissociate. These results do not exclude the occurrence of some degree of cross-linking in the treated substances, but they do render it improbable since the overall result is a diminution of molecular weight. The only certain conclusion which is justified at present is that the amine and *X*-rays bring about changes in the physical state of nucleic acid which are on the whole degradative and are empirically similar.

Polydispersity.—Some measure of the inhomogeneity of the untreated and degraded nucleic acids may be obtained from the photographs of the refractive gradient in the diffusing boundary by the method of Gralèn (*Koll.-Z.*, 1941, **95**, [ii], 188) which has been applied by Gralèn and also by Passynsky and Gatovskaja (*Nature*, 1946, **157**, 518) to the polydispersity of rubber.

The method consists in the evaluation of an inhomogeneity coefficient from the difference between the values of D for a given curve calculated by the first and the second moment methods. This coefficient is a measure of the polydispersity and is zero for a homogeneous material.

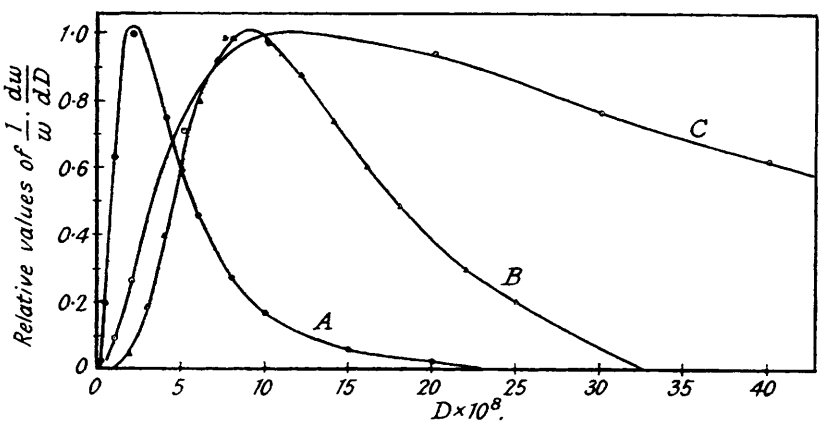
For polydisperse systems showing one maximum (see Kraemer and Lansing, *J. Amer. Chem. Soc.*, 1935, **57**, 1369) on the size-distribution curve, the distribution of diffusion coefficients may be represented by a general logarithmic distribution function of the form

$$dw = \frac{ke^{-Z^2}}{\beta} \cdot dD \text{ where } Z = \frac{1}{\beta} \ln (D/D_0)$$

β is the inhomogeneity coefficient referred to above, D_0 is the value of the diffusion constant corresponding to the maximum of the distribution curve, k is a constant, and dw is the fraction of the material having a diffusion constant between D and $(D + dD)$. Insertion of the parameters D_0 and β, calculated from the first and second moments of the diffusion curve, into the general distribution function, gives the distribution characteristic of the mixture investigated.

FIG. 3.

Polydispersity of original and degraded thymonucleic acid preparations : distributions of diffusion constants.



A. *Untreated, in* 0·1N-$NaHCO_3$.
B. X-*Irradiated, in* 0·1N-$NaHCO_3$ (7000 r.).
C. *Amine-treated, in* 0·1N-$NaHCO_3$.

For a polydisperse system of long-chain molecules non-ideality of the diffusion curve results from the concentration dependence of D in the concentration gradient at the boundary, as well as from polydispersity. However, by computing the polydispersity-distribution function for one boundary at various times, *i.e.*, for various concentration gradients, the extent to which the non-ideality is due to polydispersity and hence the validity of any polydispersity calculation may be estimated.

Calculations of the polydispersity of the untreated thymonucleic acid (the most non-ideal of the solutions investigated) for three different times of diffusion gave good agreement between the three corresponding polydispersity distribution functions.

In Fig. 3 are compared the distributions of diffusion constants for the untreated, the amine-treated, and the X-irradiated nucleic acid samples. It is readily seen that relatively little degradation is brought about by X-irradiation (in absence of oxygen), whereas there is extensive degradation by di-(2-chloroethyl)methylamine to particles having high diffusion constants.

This investigation (Parts I—III) has been assisted by grants to the Royal Cancer Hospital from the British Empire Cancer Campaign, the Jane Coffin Childs Fund for Medical Research, the Anna Fuller Fund, and the Division of Research Grants of the U.S. Public Health Service. We are also indebted to Mr. P. G. Avis for technical assistance and to Miss E. M. Press for analyses. We also thank

Prof. G. A. R. Kon and Dr. W. C. J. Ross for the chloroethyl ethyl sulphide and the similar chloroalcohol used in Part I. We are also indebted to Dr. L. F. Lamerton of the Physics Dept. of the Royal Cancer Hospital, who kindly carried out the irradiations for us.

CHESTER BEATTY RESEARCH INSTITUTE, THE ROYAL CANCER HOSPITAL,
FULHAM ROAD, LONDON, S.W.3. [*Received, July 15th,* 1950.]