drazine in aqueous solution from ammonia and chloramine. Our data suggest that a *p*H range of 13.2–13.4 should be maintained if maximum yields of hydrazine are to be obtained.

Acknowledgments.—Our thanks are extended to Mrs. Marlies Zimmer, Mr. R. W. Whitney and Mr. Paul C. Moews for their assistance with the analytical determinations. This investigation was sponsored by the Office of Ordnance Research as one phase of a fundamental study of the synthesis of hydrazine, Contract No. DA-11-022-ORD-828.

URBANA, ILLINOIS

[CONTRIBUTION FROM GIBBS LABORATORY, DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

The Mild Acidic Degradation of Desoxyribose Nucleic Acid

BY CHARLES A. THOMAS, JR.,[1] AND PAUL DOTY

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The molecular weight and size determined by light scattering and the intrinsic viscosity and the rate of production of dialyzable fragments have been studied as a function of time for the sodium salt of desoxyribose nucleic acid (DNA) prepared from calf thymus. The analysis of the data is based upon the statistics of degradation of single chains and double chains. It is found that following the immediate contraction of the DNA molecule upon lowering the *p*H to 2.6 by dialysis a gradual degradation of three types of bonding gets underway. The phosphoester bonds break at the rate of about one bond per weight average DNA molecule per 10 hours. The purines are liberated at about 200 times this rate. Substantial evidence indicates that during the early part of the degradation the two original polynucleotide strands making up the native molecule are held together at a few places by what are thought to be residual hydrogen bonds, clusters of hydrogen bonds or regions of extensive chain entanglement. These, however, also decay with the result that the degradation after about 50 hours is simply that of the random scission of single polynucleotide chains which are constantly losing purine groups.

With the growing acceptance of the Watson–Crick model as representative of the main configurational features of desoxyribose nucleic acid (DNA) and with increasing agreement on the weight, shape and size of this substance in solution, it has become of interest to examine the response of DNA to chemical and physical agents which will disrupt it to a limited extent. In such a study the motive lies in finding out if the two-stranded model can account for the changes induced or whether such evidence demonstrates the presence of other structural features. In addition genetic considerations suggest that the two strands should be separable, a fact which invites the attempt to bring this about and provide, if this is possible, undegraded polynucleotide chains. Finally, there is the possibility that any increase in our knowledge of its molecular behavior may help sharpen the picture of the biological functions and the process of duplication of DNA that is gradually evolving. This present study is concerned with the effects of dilute acid on DNA. The effects of base, heat, denaturing agents and desoxyribonuclease have also been examined in this Laboratory and will be reported shortly.

It is useful to classify what is already known concerning the response of DNA to acid into two parts; the region of mild acidity near *p*H 3 where degradation is not evident and the region of strong acidity where considerable chemical degradation is known to occur. The most remarkable feature of the mildly acid region is the anomaly in the titration curve discovered by Gulland, Jordan and Taylor[2] wherein the first titration from neutral solution to either extreme of the *p*H produces an irreversible change in the titration curve thereafter. The latter titration curve is explicable in terms of the *pK*'s of the same purine and pyrimidine groups that in the Watson–Crick model are involved in hydrogen bonding. The reluctance of native DNA to bind protons, as the initial titration curves show, is properly ascribed to the blocking of the titratable groups due to their involvement in hydrogen bonding.

Coincident with the breakdown of the native, hydrogen-bonded structure by lowering of the *p*H there is a precipitous fall in the specific viscosity.[3] For some time this drop in viscosity was interpreted as a disaggregation or depolymerization. However, by a light scattering investigation[4] it was possible to show that there was no change in molecular weight upon lowering the *p*H to 2.6; there was, however, a marked decrease in the molecular size consistent with the drop in viscosity. Thus a gradual increase in acidity of DNA in saline solution causes no change until near *p*H 3. Then simultaneously there occurs a breakdown of the hydrogen bonded structure, the acceptance of protons by groups that normally titrated at higher *p*H and a contraction of the DNA molecules that corresponds to at least a fifty-fold decrease in the volume they occupy in solution.

Under more strongly acid conditions purines (adenine and guanine) are liberated and internucleotide bonds are broken. Chargaff and co-workers[5] have shown that at *p*H 1.6 and 37° about 24 hours are required for the removal of all the purines. By this time the DNA molecules have been fragmented into several hundred pieces. This degraded product is known as apurinic acid. Exhaustive acidic hydrolysis yields, of course, the pyrimidines, sugars and phosphoric acid.

Thus it is clear that mildly acidic conditions result in changes in macromolecular configuration with no depolymerization (although with perhaps

(1) Atomic Energy Commission Fellow 1952–1954.

(2) J. M. Gulland, D. O. Jordan and H. F. W. Taylor, *J. Chem. Soc.*, 1131 (1947).

(3) J. M. Creeth, J. M. Gulland and D. O. Jordan, *ibid.*, 1141 (1947). Our own measurements of this effect show that when the *p*H is lowered by the addition of buffer having a *p*H of 2.5 the viscosity begins to fall at *p*H 3.1 and reaches 10% of its original value at 2.9.

(4) M. E. Reichmann, B. H. Bunce and P. Doty, *J. Polymer Sci.*, **10**, 109 (1953).

(5) C. Tamm, M. E. Hodes and E. Chargaff, *J. Biol. Chem.*, **195**, 49 (1952).

a very slow loss of purines), while the stronger acidic conditions result in major destruction of the molecule. Our concern lies in exploring the intermediate region where the first stages of degradation can be detected. For this purpose we have employed light scattering measurements to determine molecular weight and shape, intrinsic viscosity and appropriate dialysis experiments. Our reliance on physical methods is necessary because of the insensitivity of chemical methods at this level of degradation.

Experimental Methods

The DNA sample used was that prepared by R. Varin as described in reference 4 and the techniques employed and the values used for the extinction coefficient and the refractive index increment are those described in a recent study of native DNA.[5] That is, the Brice–Phoenix Light Scattering Photometer and multigradient Ubbelohde viscometers with a gradient range of 250 to 70 sec.$^{-1}$ for water have been utilized. In order to change the pH in the light scattering cell continuously while allowing constant observation, an arrangement shown in Fig. 1 was employed. A cellophane membrane was cemented to the open end of the tube by means of Glyptal Cement (General Electric Company) and saline, adjusted to the desired pH, was passed through the unit. Cylindrical light scattering cells were required in this case. Otherwise Erlenmeyer shaped cells requiring no back reflection were employed. The temperature of all experiments was 25°.

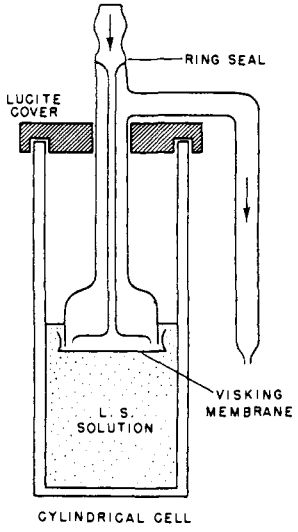

Fig. 1.—Immersion dialysis unit.

Experimental Results

Preliminary Experiments.—After showing that solutions of the DNA sample being used were unchanging in their light scattering characteristics over periods of two weeks in neutral 0.20 M NaCl, the effects of lowering the pH by dialysis were examined. No molecular weight effect could be observed at pH 3 but below this a very slow fall in molecular weight became evident. In one of the first experiments the pH of a DNA solution (63 mg./l.) was lowered to 2.6 by means of the immersion dialysis unit and the reciprocal scattering envelopes[7] were recorded as a function of time as shown in Fig. 2. We note a gradual rise in the intercept corresponding to a decrease in the average molecular weight. The slope, which is proportional to the molecular size (in terms of the radius of gyration) divided by the molecular weight, is seen to remain approximately constant for 120 hours and then begins to increase. This means that the molecular size is decreasing in proportion to the molecular weight.

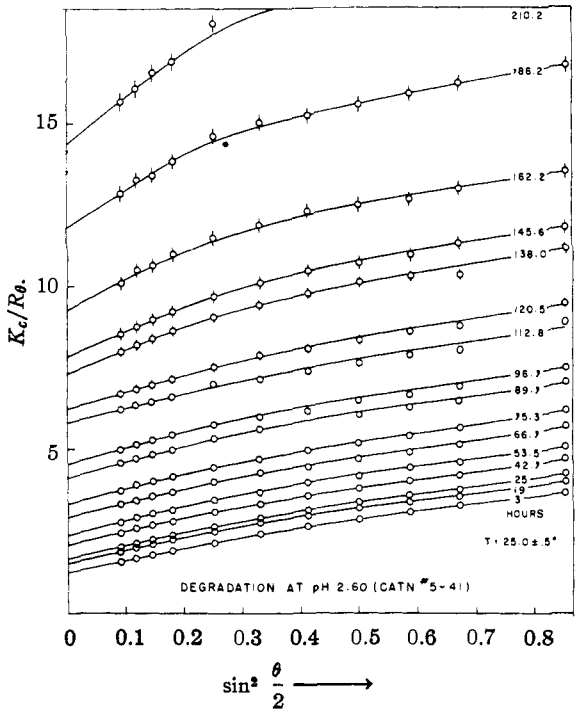

Fig. 2.—Reciprocal scattering envelopes of DNA at pH 2.6 as a function of time.

The rate of degradation was found to be quite pH dependent. The initial rate at 2.7 was about half that at pH 2.6, and it increased about sixfold upon going to pH 2.5. The rate at pH 2.6 was sufficiently slow to account for its not being detected in the brief time of observation employed in the previous study at this pH.[4]

When, in another experiment, the molecular weight had fallen to half its original value the pH was readjusted to 3.85. The molecular weight ceased to fall and remained constant for the 36-hour period over which observations were continued. If reneutralization was carried out after extensive degradation, aggregation occurred.

Light Scattering Observations on Degrading DNA.—A pH of 2.6 was chosen as the highest pH at which a significant molecular weight change could be studied. In each experiment about 25 mg. of DNA was dissolved in 100 ml. of water and this solution was made 0.2 M in NaCl. A rapid dialysis with stirring against pH 2.6 saline was followed by a 30-minute centrifugation at about 100,000 g. This was transferred to the light scattering cell and observations begun less than two hours after the beginning of the dialysis. Concentration was determined by withdrawing a portion from the light scattering cell and determining its optical density. The extinction coefficient, which

(6) M. E. Reichmann, S. Rice, C. A. Thomas and P. Doty, THIS JOURNAL, **76**, 3047 (1954).

(7) This method of plotting the reduced intensity at the angle θ, $R\theta$, conforms with the light scattering equation

$$K\frac{c}{R_\theta} = \frac{1}{MP(\theta)} + 2Bc$$

The concentration must be in units of g./cc. and the constant K equals $2\pi^2 n_0^2 (dn/dc)^2/N\lambda^4$ where n_0 is the solvent refractive index, dn/dc the refractive index increment, N Avogadro's number and λ the wave length of light. The dependence of the reciprocal of the scattering factor $[P(\theta)]^{-1}$ on $\sin^2(\theta/2)$ accounts for the abscissa of the plot. At the concentrations employed the second term in the equation is negligible; consequently Kc/R_θ extrapolated to zero angle may be taken as the reciprocal of the molecular weight.

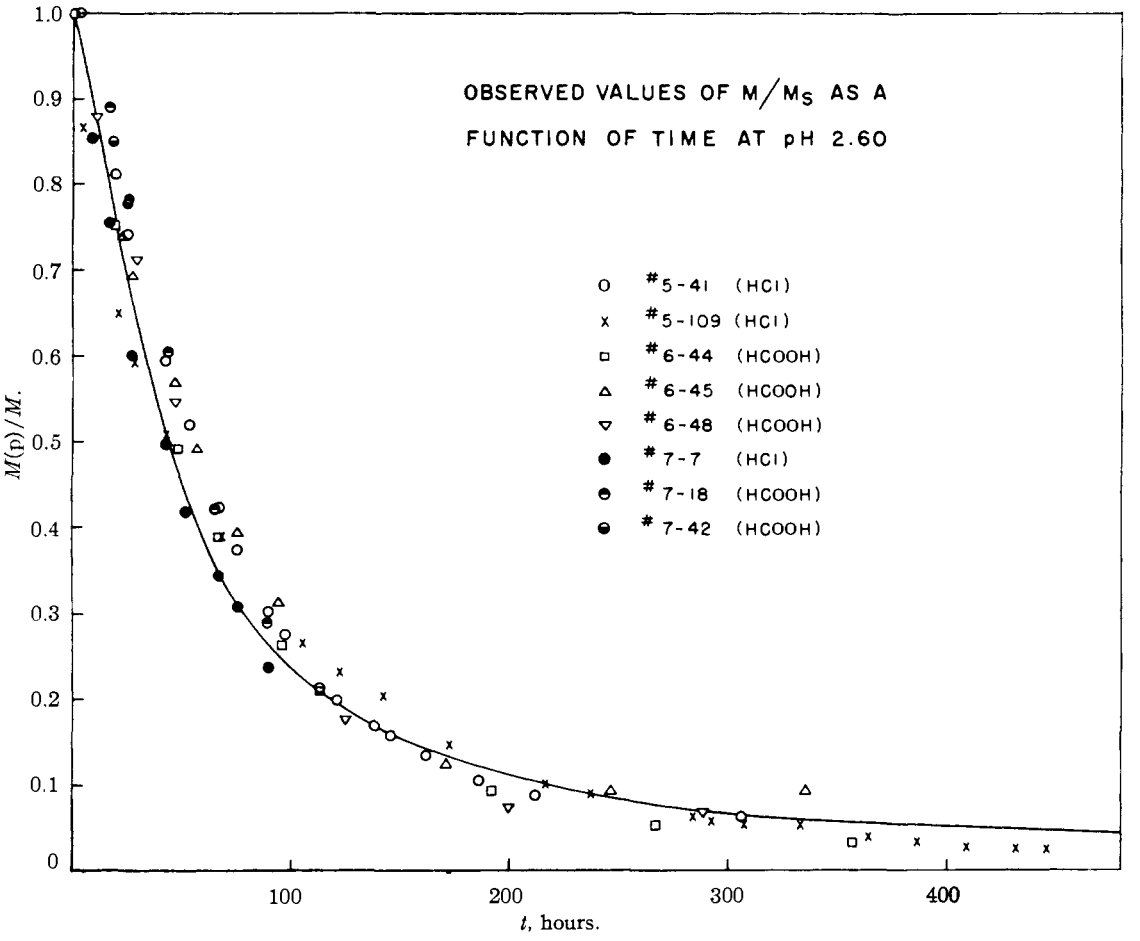

Fig. 3.—Molecular weight decay in DNA solutions at *p*H 2.6 (HCl).

is 213 for neutral DNA solutions, was found to be 273 at this *p*H.

Figure 3 shows the light scattering results of eight degradations at *p*H 2.6. M refers to the starting molecular weight and M(p) to that calculated from Kc/R_0 at the various times of observation. Similar results were obtained at *p*H 2.6 using formic acid instead of hydrochloric acid.

Viscosity Measurements on Degrading DNA.—In order to examine further the variation in shape and size of the degrading DNA viscosity measurements were made in a quartz viscometer that had a flow time of 489 sec. for the solvent (0.2 M NaCl plus HCl to bring *p*H to 2.6). Since DNA at this *p*H does not exhibit a gradient dependent viscosity[4] and since the concentration is so low, the values of the reduced specific viscosity, η_{sp}/c, could be taken as equivalent to the intrinsic viscosity. Measurements of this type showed that η_{sp}/c had a value near 1.0 at the beginning of the degradation and that this value persisted for about 30 hours and then descended slowly reaching about half its value in 160 hours.

Light scattering and viscosity measurements were then carried out on two parts of the same solutions. Typical results are shown in Fig. 4 where in addition to the decay in molecular weight and viscosity, the values of the end-to-end length, R, and the ratio $\overline{R^2}/M$(p) are also plotted. We see here the

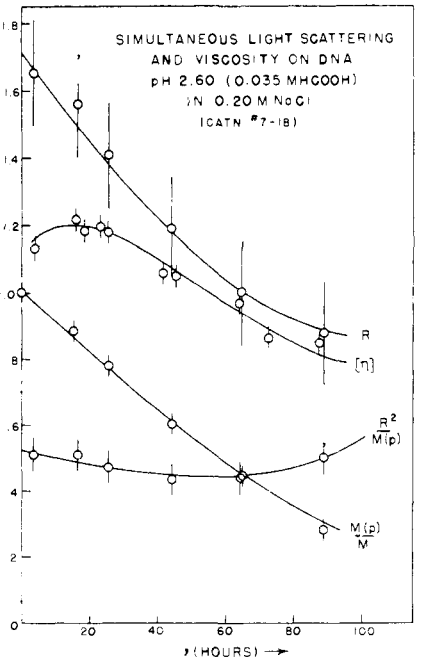

Fig. 4.—The results of simultaneous light scattering and viscosity measurements of DNA at *p*H 2.6. The DNA concentration was 130 mg./l.

characteristic lag in the fall of the intrinsic viscosity and the eventual rise in the R^2/M(p) ratio following an initial decrease.

Dialysis Experiment.—In order to estimate the rate of production of adenine and guanine, which are known to be liberated under these conditions of *p*H and temperature from the work of Chargaff,[5] we have employed his differential spectrophotometric technique to analyze the dialysate during the hydrolysis. The optical densities, D, were measured at 240, 263 and 290 mμ as a function of time, and the concentrations of adenine and guanine in micromoles per liter were calculated from the following expressions

$$C_{\text{adenine}} = 70.3\,(1.54\,\Delta\,263 - \Delta\,240)$$
$$C_{\text{guanine}} = 98.2\,(2.53\,\Delta\,240 - \Delta\,263)$$

where $\Delta\,263 = (D \text{ at } 263) - (D \text{ at } 290)$ and $\Delta\,240 = (D \text{ at } 240) - (D \text{ at } 290)$. Using 6650 for the extinction coefficient with respect to phosphorus it was computed that 28.9 μmoles of phosphorus was present initially in each dialysis bag (total volume 100 cc.). Then from the analytical data of Chargaff and Lipshitz[8] we could compute that 8.38 μmoles of adenine and 6.13 μmoles of guanine should be liberated if all the purines were hydrolyzed. These quantities are entered as horizontal lines in Fig. 5 in which this experiment is summarized.

In Fig. 5 the solid lines represent the calculated adenine and guanine, the dotted lines are calculated on the basis of a first-order rate constant of 0.24%/hour. During the first 200 hours, where most of the other data are taken, the dotted lines reasonably fit the data. This estimated rate of purine liberation is in good agreement with the reported rate of 0.20%/hour at *p*H 3.0 for the splitting of adenine from adenylic acid.[9] At longer times the picture is complicated by the appearance of significant amounts of dialyzable polynucleotide fragments which invalidate the analytical method.

Since the purines constitute only 22% of the mass of the DNA we can calculate from the rate constant that the molecular weight would only be reduced 11% in 300 hours at *p*H 2.6. We have, therefore, neglected any effects of loss of purines in the subsequent considerations.

In order to undertake an interpretation of the data presented in this section we shall digress in the next section to summarize some relations that describe various types of chain degradations.

Chain Degradation Processes.—The principal results of the foregoing section as well as those to be presented subsequently on the enzymatic degradation are expressed in terms of the change in weight average molecular weight as a function of time. Because only a small fraction of the chain bonds are broken it is permissible to assume that the number of bonds broken is directly proportional to the time.[10] The analysis of the experimental results then requires a knowledge of how the weight average molecular weight decays at a constant rate of bond scission for appropriate model systems.

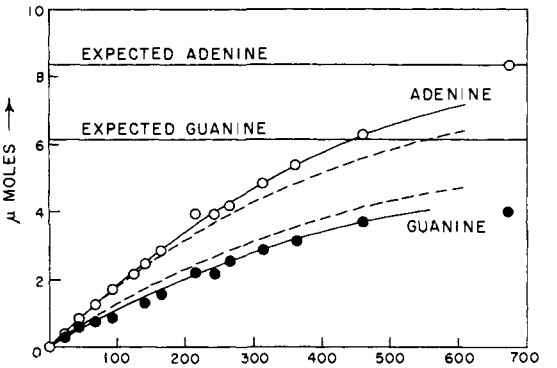

Fig. 5.—Rate of production of adenine and guanine estimated from ultraviolet spectra of dialysate. The dashed lines represent the rates expected for a first-order rate constant of 0.24% per hour. The horizontal lines represent the total adenine and guanine content of the sample.

The development of the statistics of random degradation initiated by Kuhn, carried on by Montroll and Simha[11] and others and brought into particular useful form by Charlesby,[12] covers most of the cases of interest here except that of the degradation of a double chain of Watson–Crick type. The relevant parts of this work and the statistics for the degradation of a double chain are summarized in this section.

Random Degradation.—Using the nomenclature of Charlesby[12] we employ M_n, M_w and M_z for the number, weight and z-average molecular weights and u_1, u_2 and u_3 for the corresponding degrees of polymerization. If w is the weight of a monomer unit, $M_n = wu_1$, $M_w = wu_2$ and $M_z = wu_3$. The symbol p is used to denote the probability of scission of any bond connecting monomeric units and when written in parentheses, (p), following M or u it implies that this refers to the degraded material in contrast to the initial undegraded material. The degree of polymerization of the weight average molecule is given in terms of higher averages by the general relation

$$u_2(p) = u_2 - \frac{1}{3}\,pu_3u_2 + \frac{1}{12}\,p^2u_4u_3u_2 - \ldots \quad (1)$$

The fractional decrease of the weight average molecular weight then becomes, omitting the subscript w

$$\frac{M(p)}{M} = \frac{u_2(p)}{u_2} = 1 - \frac{1}{3}\,pu_3 + \frac{1}{12}\,p^2u_4u_3 - \ldots \quad (2)$$

The reciprocal of this is

$$\frac{M}{M(p)} = 1 + \frac{1}{3}\,pu_3 - \ldots \quad (3)$$

and this suggests that the reciprocal of the fractional decrease in molecular weight be plotted

(8) E. Chargaff and R. Lipshitz, THIS JOURNAL, **75**, 3658 (1953).

(9) E. Volkin, J. X. Khym and W. E. Cohn, *ibid.*, **73**, 1533 (1951).

(10) There appears to be no direct evidence bearing on the validity of this assumption but the alternative possibility must be considered that hydrolysis occurs only after the loss of a purine residue adjacent to a phosphate ester bond [see D. M. Brown and A. R. Todd, *Ann. Rev. Biochem.*, **25**, 327 (1955)]. If this type of activation were a necessary prerequisite for hydrolysis the number of bonds made vulnerable would be proportional to time and the number broken would then be proportional to the square of the time for the early part of the degradation. Since there is no evidence of a continually increasing rate of chain scission in our results the consequences of this mechanism for degradation are not elaborated here. (In the type of plot shown in Figs. 6 and 7 it would have the form of a parabola centered on the ordinate axis).

(11) E. Montroll and R. Simha, *J. Chem. Phys.*, **8**, 721 (1940).

(12) A. Charlesby, *Proc. Roy. Soc.*, **A224**, 120 (1954).

against pu_3 rather than against pu_1 as Charlesby has done.

When the initial distribution is uniform ($u_1 = u_2 = u_3 = u$) the series in equation 1 can be expressed analytically as follows

$$\frac{M(p)}{M} = \frac{2}{(pu_3)^2}\{e^{-pu_3} - 1 + pu_3\} \qquad (4)$$

The reciprocal of this is plotted against pu_3 in Fig. 6. It is interesting to note that at large values of pu_3, this reciprocal plot approaches the asymptote $M/M(\mathrm{p}) = {}^1/_2 + pu_3/2$.

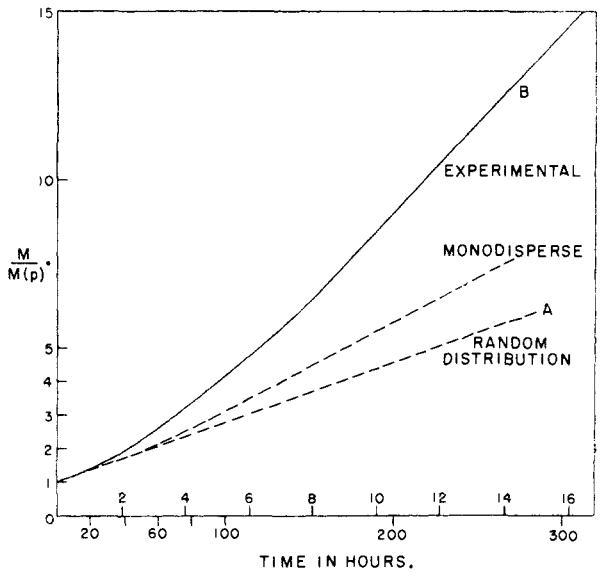

Fig. 6.—Effect of polydisperse starting material on the value of $M/M(\mathrm{p})$ plotted against pu_3, the number of bonds broken per parent molecule of z-average molecular weight (two lower curves). Experimental data plotted against time (upper curve).

When the initial distribution is random or "the most probable one" characterized by $u_2 = 2u_1$, $u_3 = 3u_1$, etc., we have the following

$$\frac{M(p)}{M} = \frac{2u_1/u_2}{1 + pu_1} \qquad (5a)$$

$$\frac{M}{M(p)} = 1 + \frac{1}{3}pu_3 \qquad (5b)$$

The linear plot of this equation is also shown in Fig. 6.

The role of polydispersity in the initial material can now be seen. The degradation of a monodisperse initial material will exhibit upward curvature as shown in Fig. 6. As the polydispersity of the initial material increases to that of the random distribution this curvature disappears and with a further increase in polydispersity the plot would develop downward curvature.[13]

In anticipation of later discussion it is of interest to note that the existence of one or several weak bonds would have the effect of producing initially more rapid molecular weight decay and this would give rise, in the plot of $M/M(\mathrm{p})$ against p, to downward curvature of a kind similar to that produced by excessive polydispersity.

Degradation of a Double Chain.—In so far as the disposition of bonds is concerned, the Watson–Crick structure for DNA can be considered as two parallel chains of the type discussed above and held together by bonds of a different type (hydrogen bonds). Although in the native state these hydrogen bonds exist between all opposite pairs of monomeric units, their number is decreased by the addition of acid or base. Consequently, it appears that an appropriate model at this pH would consist of two chains which are united at regular intervals by inter-chain bonds (cross bonds) which are assumed to be resistant in hydrolysis. If there are f bonds in each single chain between these cross bonds we can calculate the probability of a double chain scission, s, in terms of the probability, p, that any particular chain bond is broken.

The probability of a given bond not being broken in one of the two chains is $(1 - p)$. The probability that f particular bonds located between uniformed distributed cross bonds on one chain are *not* broken is $(1 - p)^f$. Hence the probability that one or more out of the f bonds is broken is $1 - (1 - p)^f$. For the cleavage of the double chain at least one bond must be broken on each chain between the same pair of cross bonds. The probability of this event will be the square of the above quantity.

$$s = [1 - (1 - p)^f]^2 \cong p^2f^2 \qquad (6)$$

The approximation is satisfactory for our range of interest.

Assuming that the chain length distribution in DNA is the random or most probable one, we replace p by s and u_3 by u_3/f. The quantity u_3 now refers to the number of monomeric units in one of the two chains and the ratio u_3/f represents the number of units making up the chain when a unit is thought of as the double chain between cross bonds. These substitutions yield the following expression

$$M/M(p) = 1 + {}^1/_3(p^2fu_3) \qquad (7)$$

The results are plotted in Fig. 7 for several values of f. The expected delay in the molecular weight decay is evident and it becomes increasingly pronounced as the value of f diminishes. In all cases the initial slope is zero in contrast to the single chain degradation shown on the same graph.

This calculation becomes more approximate in character as f becomes large for a number of reasons. The ratio u_3/f is no longer an adequate measure of the number of "effective" bonds. Moreover the asymmetric cleavage of very large rings is not taken into consideration. Finally, with large values of f there is a greater probability that chains of degree of polymerization less than f will be cut from between cross bonds. It can be shown[14] that the contribution of these short chains to the average molecular weight becomes significant if pf exceeds 0.7. For this reason the curves for $f = 500$ and 1000 in Fig. 7 cannot be reliably extended.

(13) Due to a remarkable coincidence this development and the behavior of the curves in Fig. 16 are functionally identical with the analysis of the polydispersity of randomly coiled molecules by light scattering. [Benoit, *J. Polymer Sci.*, **11**, 507 (1953); Benoit, Holtzer and Doty, *J. Phys. Chem.*, **58**, 635 (1954)]. The particle scattering factor $P(\theta)$ plays the role of $M(p)/M$ and the quantity

$$\left[\frac{4\pi}{\lambda'}\sin\frac{\theta}{2}\right]^2\frac{b^2}{6}$$

is the counterpart of p.

(14) C. A. Thomas, Jr., Thesis, Harvard University, 1954.

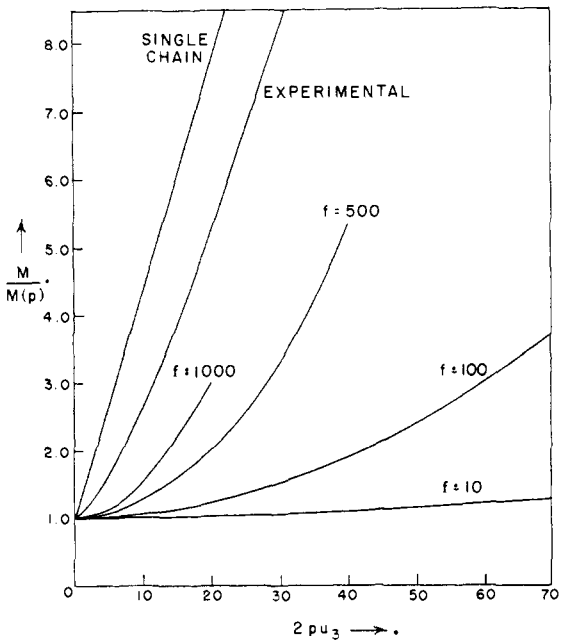

Fig. 7.—Molecular weight decay for random distribution of double chains for various values of f as a function of pu_3, the average number of bonds broken per z-average parent molecule. ($u_1 = 5000$, $u_2 = 2u_1$, $u_3 = 3u_1$).

Interpretation of Results

The Character of the Molecular Weight Decay.—The smooth curve representing the numerous degradations in Fig. 3 is shown in reciprocal form plotted against time in Fig. 6, where it can be compared directly with the theoretical curves. Its most striking feature is the very pronounced upward curvature which is in the opposite sense to that which could be explained by excessive polydispersity and that indeed the early part of the degradation cannot be represented by a random scission process of a single chain. The possibility of the deviation being due to weak bonds or to the two chains (thought to make up the DNA molecule) coming apart is also ruled out since these effects also would give rise to downward rather than upward curvature.

The characteristics of the double chain degradation shown in Fig. 7 seem to provide the clue to the deviations of the degradation from random chain scission. If at pH 2.6 only a few hydrogen bonds remained intact (corresponding to a large f value) but these were also breaking with time (but at a faster intrinsic rate than the chain bonds) we would expect essentially the experimental result shown in the same figure. The initial slope would not be zero because of the finite rate of breaking of the cross bonds, but during the first 100 hours it would appear that they have been largely fractured. Thereafter the decay is essentially that of a single chain.

Since the *initial* molecular weight decrease cannot be interpreted on the basis of random depolymerization of a linear polymer due to the presence of residual hydrogen bonds, we interpret the experimental $M/M(\mathrm{p})$ in terms of p at large values of the time when these hydrogen bonds have been eliminated and the experimental $M/M(\mathrm{p})$ as a function of time approaches a linear relationship. Assuming a random molecular weight distribution and equating the limiting slope of the experimental $M/M(\mathrm{p})$ as a function of time with $^1/_3(\mathrm{d}pu_3/\mathrm{d}t)$ gives 0.159 bonds/hour/z-average molecule or 0.053 bonds/hour/number average molecule. The experimental data have been drawn in Fig. 7 employing this rate constant. Although it is not possible to make an accurate estimate of the number of cross bonds initially present because the actual degradation does not conform in all respects to the simple model described above, it seems likely that there are less than seven effective cross bonds per weight average DNA molecule when the degradation begins.

The Viscosity–Molecular Weight Relation during Degradation.—Results of the simultaneous determination of the intrinsic viscosity and the molecular weight as illustrated in Fig. 4 offer independent support of the conclusions reached in the foregoing section. Data from four such experiments are plotted as log $[\eta]$ against log $M/M(\mathrm{p})$ in Fig. 8. We note that after the molecular weight has been cut by a factor of two, all of the data fall on nearly coinciding straight lines that have a slope of 0.5. This strongly suggests that DNA is in the form of essentially free polynucleotide chains after degradation has lowered the molecular weight to about half its original value. The free polynucleotide chains would appear to obey the familiar viscosity–molecular weight relationship of $[\eta] = KM^{1/2}$ where K is found to be 6×10^{-4}. The exponent of $^1/_2$ indicates that the polynucleotide chains are solvent-entrapping coils as opposed to the extended free-draining character of the native DNA at neutral pH. The nature of the anomalous behavior during the early stages of degradation is apparent when it is noted that the intrinsic viscosity is lower than it would be if the straight lines in Fig. 8 were

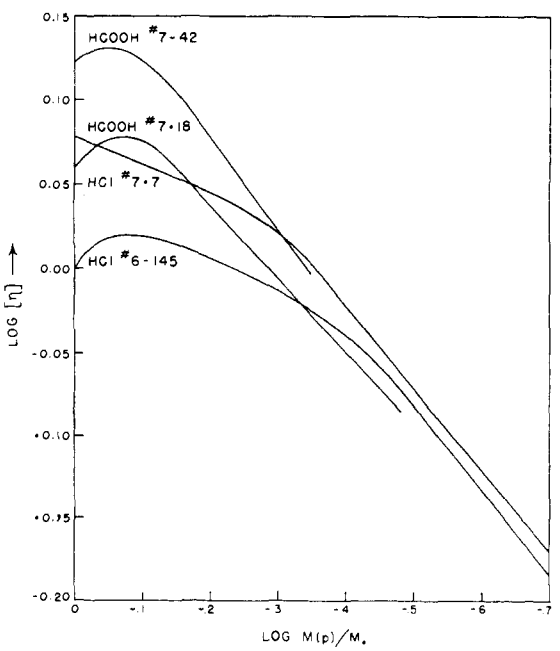

Fig. 8.—Relation between log $[\eta]$ and log $M(\mathrm{p})/M$ during acidic degradation.

extrapolated back to zero time. In other words, during early stages of the degradation the DNA molecules are smaller in size than they would be if their configurations were of the same type as the more degraded species.

This relative contraction of DNA over that expected of single polynucleotide chains would most likely be brought about by the constraining influence of some network points, presumably arising from intact hydrogen bonds or clusters of hydrogen bonds. This diminution in size relative to the randomly coiled single chain frequently has been observed in polymers in which a few network points have been introduced. For example, behavior very much like that shown in Fig. 8 was found for a series of polystyrene fractions having a small amount of crosslinking due to incorporation of divinylbenzene.[15]

Discussion

The general conclusion from this investigation is that upon lowering the *p*H to 2.6 almost all of the hydrogen bonds connecting the two polynucleotide chains dissociate bringing about a drastic contraction of the molecule due to the increased rotational freedom in long sections of the chains. Three types of degradation then appear to get underway. The type on which we have gathered the most information is the hydrolysis of the phosphoester bond: this occurs at a rate of about 0.053 bond per molecule per hour where the molecule is designated as having the number average molecular weight which we have taken as 3.3 million. This corresponds to two nucleotide chains each made of 5000 nucleotides. For the weight average molecule of 6.6 million there would be 0.106 bond broken on the average in the same time.

The second type of degradation is the cleavage of the glycosidic nitrogen bond to free the purine residues. The initial rate of this reaction, calculated from the data in Fig. 5, is about 24 adenine and the same number of guanine molecules liberated per (weight average) DNA molecule per hour.

The third type of degradation is based on inference. From the shape of the reciprocal molecular weight decay plot, Fig. 6, we believe that the remaining hydrogen bonds decay at such a rate that they have nearly disappeared in 50 hours. The viscosity and size measurements support this view since they indicate an opening up of the molecular chains after about 50 hours following which they degrade as single chains.

The rough estimate of the number of hydrogen bonds or clusters of hydrogen bonds remaining intact at the beginning of the degradation is found to be about 7 for a weight average molecule. It is of interest to compare this with the number that would be deduced from titration. The titration curves of Lee and Peacocke[16] carried out at essentially the same ionic strength (0.15 *M*) as used here, show that two of the three equivalents of acid bound per four nucleotides occurs at *p*H 2.8 and that the anomalous titration behavior terminates at this same *p*H. Presumably the two groups that have accepted protons at this point are the 6-amino groups of adenine and cytosine, the same groups that participate in the hydrogen bonds in the native structure. The assignment of *pK*'s to these groups involves some uncertainty. Jordan[17] quotes 3.7 and 4.2 for adenylic and cytidylic acid, respectively; Peacocke[18] assigns values of 4.4 and 4.6 to the corresponding nucleotides. At *p*H 2.6 these two assignments correspond to 3.0 and 0.7% of the nucleotides untitrated and in potentially hydrogen bonding form. These numbers which define, albeit rather vaguely, the upper limit of hydrogen bonds in the DNA molecule adjusted to *p*H 2.6 are larger by a factor of 10 to 40 than the number of cross bonds deduced. This factor could be interpreted as the number of hydrogen bonds in the cluster designated as a cross bond or one could take the view that the few unfilled sites for the protons are not static, but change from one nucleotide to another with the consequence that the formation of hydrogen bonds lags, never being able to keep up with the migration of potential hydrogen bonding sites.

Despite the internal consistency of this view of a few remaining hydrogen bonds which hold the two strands of nucleotides together until the degradation has proceeded for approximately 50 hours, it is important to keep open the remote possibility that these cross bonds are points of extreme chain entanglement arising from the many turns of the two strands about each other in the nature state.

Finally, it is of interest to note that the results presented here and their interpretation do not lend support to the suggestion of Dekker and Schachman[19] that the individual polynucleotide chains are already broken many times in native DNA. The effect of these initial breaks would have been to produce a very rapid decrease in molecular weight early in the degradation whereas the opposite was found to be the case.

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