27. *The Interaction of Schiff's Reagent with Deoxyribonucleic Acids.*

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The intensity of colour produced when a sensitive Schiff's reagent is added to solutions of deoxyribonucleic acids which have been subjected to very mild acid or alkali treatment is found to be greater than before such treatment. The rapidity with which this change occurs and the approximate equality of the effects of acid and alkali suggest an explanation in terms of the hydrolysis of labile $C_{(1)}$–phosphate linkages, the presence of which in deoxyribonucleic acids has already been proposed (Stacey, Li, and Overend, *Nature*, 1949, **163**, 538; Overend, Stacey, and Webb, forthcoming publication).

It has been suggested (Stacey, Li, and Overend, *loc. cit.*; Overend, Stacey, and Webb, *loc. cit.*) that a small proportion of the $C_{(1)}$ atoms of 2-deoxy-D-ribose in deoxyribonucleic acids may be involved in highly labile, polymeric linkages, possibly with phosphoric acid residues, as well as in linkages to the purine and pyrimidine bases. Such phosphate linkages would be very labile to acids, as shown by Friedkin's observation (*J. Biol. Chem.*, 1950, **184**, 449) that 2-deoxy-D-ribose-1 phosphate released 50% of its phosphorus as inorganic phosphate within 10—15 minutes at pH 4 and 23°, free aldehyde being formed at the same time. The phosphates of 2-deoxy-sugars are much more labile towards alkali than are the corresponding phosphates of their normal analogues (Foster, Overend, and Stacey, *J.*, 1951, 987) and substituents are usually much more readily removed from $C_{(1)}$ than from any other position (Cori, Colowick, and Cori, *J. Biol. Chem.*, 1937, **121**, 465; Farrar, *J.*, 1949, 3131). Such compounds would therefore be expected to be hydrolysed rapidly and completely by acid or alkali according to the scheme:

$$\geq C{-}O{-}PO + H_2O \xrightarrow[OH^-]{H^+ \text{ or}} \geq C_{(1)}{-}OH + HO{-}PO$$

(Secondary or primary phosphoryl group, ionised according to pH.)

In this reaction the glycosidic group of the sugar would be revealed and would immediately pass into equilibrium with the straight-chain *aldehydo*-form. Overend (*J.*, 1950, 2769) has shown that many sugars give a red-violet colour with Schiff's reagent (*Annalen*, 1866, **140**, 102) when there is the possibility that they can exist in the *aldehydo*-form (probably owing to the formation of a complex; Wieland and Scheuing, *Ber.*, 1921, **54**, 2527). In particular, 2-deoxy-D-ribose derivatives gave more intense colours than did their normal analogues, especially when substitution prevented the formation of the pyranose form, as in the deoxyribonucleic acids (Brown and Lythgoe, *J.*, 1950, 1990). Ketoses, glycosides, and polyhydric alcohols have been shown to give no colour (Tobie, *Ind. Eng. Chem.*, 1942, **14**, 405; Overend, *loc. cit.*). With this reagent it should therefore be possible to detect the relatively small quantities of free aldehyde groups released from deoxyribonucleic acids by mild acid or alkaline hydrolysis of any labile $C_{(1)}$ linkages of the type suggested above.

The Effect of Acid and Alkali under Mild Conditions.—Aqueous solutions of the sodium salts of deoxyribonucleic acids from herring sperm, calf thymus gland, wheat germ, and mouse sarcoma were treated with acid or alkali, at room temperature. The solutions were neutralised and the pH adjusted to 6·7 by the addition of phosphate buffer. Schiff's reagent was added and the intensity of colour produced was measured on a Spekker absorptiometer at various time intervals after the addition. The reading showed a slow, nearly linear increase with time. From a graph showing this variation (the figure is an example) interpolations were made and the effects of the treatments with acid and alkali ascertained. The experiment was also repeated with herring sperm and thymus nucleic acids which had been dried over phosphoric oxide (110°, 30 minutes, 0·01 mm. Hg) and dialysed, and with a sample of herring sperm nucleic acid which had been irradiated with

ultrasonic waves; the results of these experiments are shown in the Table. Variations in the time of treatment of the nucleic acid with acid or alkali caused no marked difference in the results: for this reason the times of treatment are not included in the Table. The same

Nucleic acid, source and treatment	Extinction coeff. of solution	Normality of acid or alkali	Time of interpolation of Spekker readings (min.)	Increase in Spekker reading after treatment with acid	alkali
Thymus gland, T_1, undried	0·625	0·03	60	0·11	0·09
			110	0·15	0·09
Thymus gland, T_1, dried	0·615	0·03	60	0·07	0·06
			110	0·08	0·06
Thymus gland, T_2, dialysed	0·642	0·025	60	0·14	0·20
			110	0·17	0·20
Thymus gland, isolated by Signer and Schwander	0·661	0·025	60	0·11	0·10
			110	0·11	0·11
Herring roe, H_1, undried	0·639	0·025	60	0·24	0·26
			110	0·23	0·27
Herring roe, H_1, dried	0·639	0·025	60	0·12	0·11
			110	0·12	0·11
Herring roe, irradiated	0·645	0·025	60	0·15	0·17
			110	0·13	0·17
Wheat germ, undried	0·654	0·025	60	0·06	0·08
			110	0·06	0·08
Mouse sarcoma, undried	0·649	0·025	60	0·10	0·16
			110	0·10	0·17
Herring roe, H_2, dialysed	0·648	0·025	60	0·18	0·23
			110	0·18	0·23

qualitative results are obtained whether Spekker readings are interpolated at 60, 110, or 160 minutes in graphs such as those in the figure.

A quantitative comparison between the results for different nucleic acids cannot be made because of variations in the concentrations of the reagents used. However, the following qualitative conclusions may be drawn. In all experiments, treatment of the nucleic acid with acid or alkali increased the intensity of the colour with Schiff's reagent; the effects of acid and alkaline treatment were approximately the same. Drying of the nucleic acids from soft herring roe approximately halved the increase in the intensity of the Schiff's colour effected by treatment with acid or alkali. The decrease in the case of the thymus gland nucleic acid was less than this in one experiment and almost negligible in the other. Drying of the nucleic acids therefore seems to render them more stable to acid and alkali, a conclusion previously derived from titration experiments (Lee and Peacocke, *J.*, in the press). Ultrasonic irradiation of the nucleic acid from herring roe causes a similar reduction (about 60%) in the increase in intensity of Schiff's colour caused by acid or alkali treatment.

The Speed of the Reaction.—Experiments were carried out with thymus and herring sperm nucleic acids in which the time of treatment with acid or alkali was varied between 7 seconds and 30 minutes. The maximum variation of the increase in Spekker reading with time of treatment was only 7—12% of the initial increase caused by 7 seconds' treatment. This initial increase was 0·30 for two samples of nucleic acids from thymus gland (T_1) and 0·25 and 0·17 for two samples from soft herring roe (H_1 and H_3 respectively). Both reactions are apparently complete within 7—12 seconds and only small changes occurred when the nucleic acids were treated for a further period up to 30 minutes.

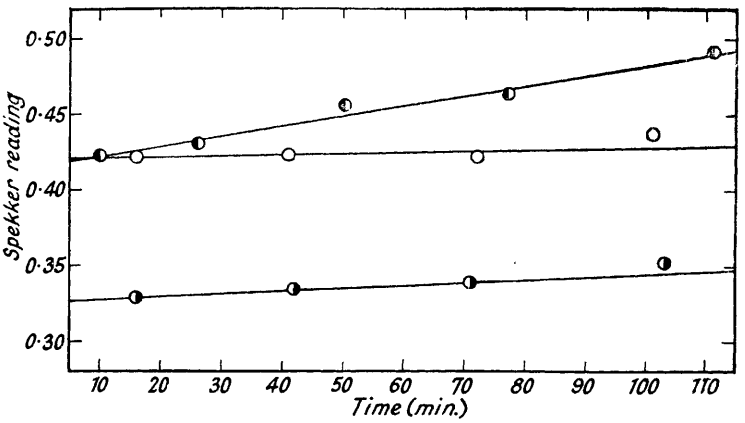
Discussion.—The results show that there is a significant increase in the intensity of the Schiff's colour given by deoxyribonucleic acids after treatment with acid or alkali under very mild conditions. Moreover, this increase is very rapid, and complete within 7—12 seconds. Since the specificity of this reagent towards the aldehyde group has been frequently demonstrated (Wieland and Scheuing, *loc. cit.*), it seems that a rapid hydrolysis of a sugar $C_{(1)}$ linkage has occurred during the treatments.

The purine–sugar and pyrimidine–sugar linkages are known to be very stable to alkali. For example, Levene and his colleagues (Levene and Bass, "The Nucleic Acids," Reinhold Publ. Co., New York, 1931, pp. 180—184) describe methods of isolating pure purine and

pyrimidine deoxyribonucleosides from an enzymic digest of thymus nucleic acid; these involve boiling of the mixture with an excess of aqueous ammonia and then allowing it to stand for 4—5 hours. It therefore seems extremely unlikely that in the very mild conditions of the experiments reported here (approximately 0·03N-alkali, room temperature for 7 seconds to 35 minutes only) hydrolysis of the bonds between the bases and the deoxy-sugar or any breakdown of the sugar ring should have occurred. Moreover, since the reaction caused by alkali is so rapid the possibility of its being due to hydrolysis of this type of linkage can be excluded. It could be explained by the presence in deoxyribonucleic acids of a small quantity of labile sugar $C_{(1)}$ linkages, as already suggested by Stacey *et al.* (*loc. cit.*) for other reasons.

Acid treatment, on the other hand, is known to hydrolyse base–sugar linkages, the bond to the purines being the least stable. Feulgen (*Z. physiol Chem.*, 1917, **101**, 296) found that the $N–C_{(1)}$ bond between the deoxy-sugar and the purine bases was completely broken by 0·15N-sulphuric acid at 80° during 40 minutes, but it has recently been shown that the deoxy-analogue of adenylic acid is converted into adenine at the rate of only

Variation of colour intensity with the time after the mixing of Schiff's reagent and thymus deoxyribonucleic acid (T_1, *undried*).



◑ *Untreated nucleic acid.*
○ *Alkali-treated nucleic acid* (pH 12, 36 *min.*).
◐ *Acid-treated nucleic acid* (pH 2, 36 *min.*).
Final pH *of all solutions* = 6·7.

2% per hour in 0·01N-hydrochloric acid at room temperature (Volkin, Khym, and Cohn, *J. Amer. Chem. Soc.*, 1951, **73**, 1533). Other hydrolyses by Volkin *et al.* indicate that the deoxy-analogue of guanylic acid would be hydrolysed at a similar rate. In our experiments with acid the conditions were the same as in the work of Volkin *et al.*, so there should only be a slow hydrolysis of the purine–sugar linkage, proceeding no further than 1% in the maximum time of acid treatment employed (*ca.* 30 minutes). Since the effect we are describing is rapid and complete well within this 30-minutes period, another explanation of the appearance of these free aldehyde groups seems necessary. There is moreover no possibility that the 2-deoxyribose ring is opening under these mild acidic conditions to give lævulaldehyde derivatives since this requires heating under pressure at 180° with methanolic hydrogen chloride (Deriaz, Stacey, Teece, and Wiggins, *J.*, 1949, 1222). The existence of a small number of labile sugar $C_{(1)}$ linkages would again account for the observations. That the same bonds are being broken by acid as by alkali is further indicated by the observation (see Table) that, within the limits of the experimental variation, the rapid initial release of aldehyde groups from the nucleic acids was approximately the same for the two treatments.

The experiments are seen provisionally to support the hypothesis of Stacey and his co-workers, but this confirmation cannot be regarded as final until the material is available

for model experiments under the same conditions on deoxyribo-nucleosides and -nucleotides and on 2-deoxy-D-ribose-1 phosphate.

Experimental

With the exception of sample H_3, the deoxyribonucleic acids are those described in earlier papers (Lee and Peacocke, *J.*, in the press). Sample H_3 was isolated from herring sperm by similar methods (Mirsky and Pollister, *J. Gen. Physiol.*, 1946, **30**, 101). The ultrasonic-degraded herring sperm nucleic acid was obtained by irradiation of a 0·2% solution of H_1 for 95 minutes, as described previously (Lee and Peacocke, *loc. cit.*). We are indebted to Professor Signer for a sample of thymus nucleic acid isolated in his laboratory (Signer and Schwander, *Helv. Chim. Acta*, 1949, **32**, 853).

In the experiments recorded in the Table, a solution of deoxyribonucleic acid (1 ml.) at the concentration shown in the Table was treated with hydrochloric acid or potassium hydroxide solution; 2 ml. of acid or base (0·05N) were added to the undried and dried thymus nucleic acid solutions, and 1 ml. to the other nucleic acid solutions. After 15—35 minutes the solutions were neutralised and phosphate buffer (3 ml.; 0·05M; pH 7·0) and Schiff's reagent (1 ml.) were added. The resulting solution had a pH of 6·7. The solutions were stoppered to exclude air as much as possible and the intensity of the colour produced was estimated at regular intervals by means of a Spekker absorptiometer with an Ilford 605 filter and a standard solution of a red dye. Readings were not continued beyond 3 hours, owing to the increasing importance of the gradual oxidation of the reagent by air which entered during the removal of the stopper. A control solution in which the nucleic acid was added after the hydrochloric acid, potassium hydroxide, and phosphate buffer was also prepared in order to determine the intensity of colour produced by Schiff's reagent with untreated nucleic acid.

The concentration of a nucleic acid solution was estimated by diluting it 25 times and measuring its extinction at a wave-length of 260 mμ, which is in the region of maximum absorption of the nucleic acids (for the values obtained, see col. 2 of the Table). The atomic extinction coefficients with respect to phosphorus at 260 mμ are 7700, 7500, 7800, and 7300 for calf thymus, herring sperm, mouse sarcoma, and wheat germ nucleic acids, respectively (Dr. S. Laland, private communication).

The increase in Spekker reading after acid or alkali treatment is obtained by subtracting the readings in the appropriate control experiment on untreated nucleic acid from the readings at the same interpolation time in the experiments with treated material. The results were reproducible to within $\pm 0{\cdot}01$ unit with the one batch of reagent.

A sensitive Schiff's reagent was prepared according to Tobie's method (*loc. cit.*) and remained colourless and stable throughout the whole investigation.

For the experiments concerned with the speed of the reactions, the following procedure was adopted. Aliquots (1·5 ml.) of a solution of the nucleic acid (0·67 mg./ml.) were treated with 0·1N-potassium hydroxide (0·5 ml.) for varying times. The solutions were neutralised with 0·01N-hydrochloric acid (0·5 ml.), and the pH was adjusted to approx. 7 by the addition of the phosphate buffer (3 ml.). The time of alkali treatment was reckoned as the time between the addition of the last drop of alkali and the last drop of acid. To each solution Schiff's reagent (1 ml.) was added and the Spekker reading noted at intervals, as above. A similar procedure, with reversal of the order of addition of acid and alkali, was used when the rate of reaction with acid was being examined.

In the latter experiments the point at which interpolations were made in the plot of Spekker reading against time did not significantly affect the results obtained. Spekker readings were again reproducible to within $\pm 0{\cdot}01$ unit.

The authors acknowledge the helpful interest shown by Professor M. Stacey, F.R.S., in this work and a maintenance grant from the Department of Scientific and Industrial Research to one of them (W. A. L.).

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[*Received, August 13th*, 1951.]