540. *Deoxypentose Nucleic Acids. Part II.* Evidence for a Labile Polymeric Linkage in Deoxypentose Nucleic Acids.*

By W. G. Overend, M. Stacey, and M. Webb.

Evidence is presented to show that some of the bonds in highly polymeric deoxypentose nucleic acids may involve labile phosphate residues attached at $C_{(1)}$ of the deoxypentofuranose constituents.

It was stated by Stacey, Overend, and Chong-fu Li (*Nature*, 1949, **163**, 538) that some of the highly labile bonds whereby the maximum macromolecular state of native deoxypentose nucleic acid is maintained involve $C_{(1)}$ of the deoxypentose moiety. Further the view was ventured that these might be highly labile glycosidic phosphate linkages. They based their ideas on the fact that highly polymerised deoxypentose nucleic acid isolated from soft herring roes by the well-established and elegant method of Mirsky and Pollister (*J. Gen. Physiol.*, 1946, **30**, 117) could be irreversibly depolymerised to some extent by dialysis for several days, and afforded a product which gave immediately an intense colour with Schiff's reagent (cf. Feulgen, *Z. physiol. Chem.*, 1924, **135**, 203). The dye so formed was completely non-soluble and non-diffusible; the reagent above the dyed nucleic acid remained colourless for more than 30 hours. It was also observed that the N/P ratio of the product was increased, but the purine content was retained. Continuation of studies recently reported (Overend and Webb, *J.*, 1950, 2746) has afforded results which can be interpreted on the basis of the ideas proposed by Stacey *et al.* (*loc. cit.*).

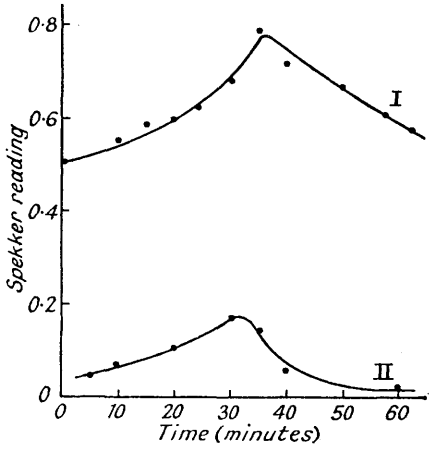
Herring-roe deoxypentose nucleic acid has been dialysed against water and the liquid outside the dialysis sac was collected and evaporated to reduce its volume. In the concentrate it was possible to demonstrate the presence of inorganic phosphate in very small amount. It corresponded to less than 2% of the total amount of phosphorus in the nucleic acid sample used. So far, despite careful search by the chromatographic technique (cf. Vischer and Chargaff, *J. Biol. Chem.*, 1948, **176**, 703, 715), we have been unable to show the presence of basic materials in the concentrate.

Recently (*Research*, 1949, **2**, 99; *J.*, 1950, 2746) we reported a study of the action of ox-pancreas deoxyribonuclease on deoxyribonucleic acid and showed that an initial depolymerisation of the substrate occurs, as evidenced by a rapid fall in viscosity, before cleavage of the nucleic acid into acid-soluble polynucleotides commences. The course of the enzyme reaction has now been followed, by using the supersensitive aldehyde reagent developed by Tobie (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 405) as a modification of Schiff's reagent (*Annal. Chem. Pharm.*, 1866, **140**, 102) (for use of this reagent, see Overend, *J.*, 1950, 2769). The result is shown in the figure. It is clear that, during the initial period when the rapid fall in viscosity occurs, there is an increase in the intensity of colour developed when Schiff's reagent is added. After this period has elapsed the intensity of the colour diminishes again. This point corresponds with the onset of formation of acid-soluble polynucleotides. Control experiments indicated lack of nucleosidase activity in the enzyme preparation used. Reference to the formulæ ($A \longrightarrow E$) indicates a possible explanation of the changes.

It is suggested that in the nucleic acid polymeric chain there occurs infrequently a 2-deoxy-D-ribofuranose unit (*A*) (for evidence that the sugar exists in nucleic acid in the furanose form see Lythgoe and Brown, *J.*, 1950, 1990) which does not carry a glycosidically bound purine or pyrimidine base. Instead the glycosidic linkage (*a*) is a labile phosphate bridge between two nucleic acid chains. In this way it would help to build up the macromolecular structure of the nucleic acid. Recent work with 2-deoxy-pentoses and -hexoses has emphasised the instability of the glycosidic linkage in sugars of this class, especially when they are in the furanose form (see Overend and Stacey, *J. Sci. Food Agr.*, 1950, **1**, 168, for a review of this evidence), and the work of Friedkin and Kalckar (*J. Biol. Chem.*, 1950, **184**, 437, 449) on 2-deoxy-D-ribose-1 phosphate has demonstrated the extreme lability of deoxypentose glycosidic phosphate linkages. When the enzymic depolymerisation commences this bond is hydrolysed and gives (*B*) which can exist in equilibrium with the aldehydo-form (*C*). The latter structure reacts with Schiff's reagent to give a coloured complex (Wieland and Scheuing, *Ber.*, 1921, **54**, 2527; see also Rumpf, *Ann. Chim.*, 1935, **3**, 327). As the depolymerisation proceeds the intensity of colour increases and when the depolymerisation of the nucleic acid is complete, the formation of acid-soluble polynucleotides commences by cleavage of the internucleotides linkages so that a structure

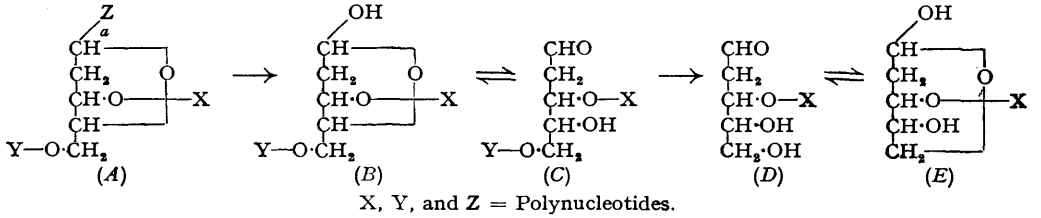
* Part I, *J.*, 1950, 2746.

represented by (*D*) is formed. In a molecule of this type the sugar moiety would co-exist in the aldehyde and pyranose forms, the latter being represented by (*E*). One of us (Overend, *loc. cit.*) has shown that when Schiff's reagent is added to *aldehydo*-2-deoxy-D-ribose co-existing in equilibrium with 2-deoxy-D-ribofuranose [(*B*) and (*C*) are the analogous examples in the nucleic acid molecule] the intensity of colour produced is much greater than when the reagent is added to an equilibrium mixture of the aldehyde and pyranose forms [(*D*) and (*E*) are the analogues in the example being discussed]. Consequently if during the enzymic degradation the Schiff's reagent is added when the reaction has reached the stage represented by (*D*) and (*E*) the intensity of colour developed will be less than if it is added at a stage represented by (*B*) and (*C*).



I. Thymus deoxypentose nucleic acid. *II. Herring-roe deoxypentose nucleic acid.*

Consequently a curve as shown in the figure would be expected. Recently Peacocke and Lee (personal communication; cf. Overend and Peacocke, *Trans. Faraday Soc.*, 1950, **46**, 790) by electrometric titrations have obtained quantitative data which can be interpreted in terms of an irreversible reaction with acid and alkali under very mild conditions (*i.e.*, pH 2 and 12 at 25°). Under the same conditions an increase in intensity of the colour developed with Schiff's reagent was observed. The latter data favour the existence of a labile linkage in the nucleic acid.



X, Y, and Z = Polynucleotides.

Finally we draw attention to an observation we have frequently noted. Several times we have encountered samples of deoxypentose nucleic acids, from both calf thymus gland and soft herring roes, which during deproteinisation cling tenaciously to traces of protein residue which can be detected by the Sakaguchi reaction. In such cases, when fairly vigorous methods are needed to remove these last protein traces, *e.g.*, brief autolysis at pH 3, prolonged dialysis, repeated shaking with chloroform and butanol, etc., the purified nucleic acid frequently reacts rapidly with Schiff's reagent and may have become depolymerised. Consequently the possibility must be considered that these labile glycosidic phosphate linkages may unite the nucleic acid to the protein component of the nucleoprotein at some sites, by linkages other than electrovalent linkages. However, this problem is being studied further. Some of our results appear to indicate that aldehyde groups are liberated when deoxyribonucleohistone is dissociated into deoxyribonucleic acid and histone, by dissolution in 1·0M- or 2·0M-sodium chloride.

Experimental.

The deoxyribonuclease was prepared according to the method described by Overend and Webb (*loc. cit.*), and the nucleic acids used were samples prepared by Mr. Laland, of this department, according to Mirsky and Pollister's method (*loc. cit.*).

A mixture of 1% (w/v) sodium thymonucleate (48 c.c.), veronal buffer (pH 6·92; 24 c.c.), 0·1M-magnesium sulphate (24 c.c.), and distilled water (12 c.c.) was heated at 37°, and then a 0·002% (w/v) solution of deoxyribonuclease (12 c.c.) preheated to 37° was added. At intervals of 5 minutes, aliquots (1 c.c.) of the digest were taken and diluted with distilled water (5 c.c.), and standard neutral supersensitive Schiff's reagent (Tobie, *loc. cit.*) (1 c.c.) was added. The colour was allowed to develop for exactly 15 minutes at 15° and then its intensity was measured in the Spekker photoelectric absorptiometer, Ilford filter No. 605 being used. The experiment was carried out with nucleic acid isolated from both calf thymus gland and soft herring roes. Reults are shown in the figure.

Qualitative Schiff's Test on Fibrous Sodium Deoxyribonucleate isolated from Herring Roe.—(1) *Dialysis.* In a typical experiment, a 1% solution of the sodium nucleate was prepared and shown to give no reaction with the Schiff's reagent while it had a slight positive Sakaguchi test. The solution was treated with a few drops of toluene to manitain sterility and dialysed in a Cellophane bag against running tap water. After 12 hours, a slight flocculent precipitate appeared in the solution, and the Sakaguchi test on the solution was now negative while the Schiff's test was slightly positive. On continuation of dialysis the Schiff's test became intensely positive after 4 days. After evaporation of the solution to small volume, the product was precipitated by the addition of 4 volumes of alcohol at pH 3 and was isolated in the form of a hygroscopic white powder. This was insoluble in water, gave positive tests for purine and pyrimidine bases, and gave immediately an intensely coloured insoluble complex on addition of Schiff's reagent.

In other experiments, traces of phosphate ion were detected in the dialysate.

(2) *Treatment with chloroform–butanol* (4 : 1). The removal of the residual protein was achieved by successively shaking 200 c.c. of a viscid 1% solution of the sodium salt of the nucleic acid with portions of chloroform (20 c.c.) and butanol (5 c.c.) as follows :

Treatment.	Chloroform complex.	Sakaguchi test (on solution).	Schiff's test (on solution).
1	+	+	−
2	+	+	−
3	+	−	−
4	trace	−	−
5	−	−	+ (develops slowly)

The product isolated was fibrous in form.

(3) *Brief acid treatment.* A sample of the fibrous sodium salt of the nucleic acid was precipitated from aqueous solution by the addition of ethanol (3 volumes) containing 1% of hydrochloric acid. The product was washed several times with alcohol. A sample was dissolved in a citrate buffer at pH 3 and heated at 100° for 3 minutes. After isolation the non-fibrous product gave an intensely positive Schiff test on being kept in the reagent for 30 minutes.

From numerous other qualitative observations it appeared that whenever obvious depolymerisation of deoxypentose nucleic acids had occurred the Schiff's test was easy to demonstrate.

The Changes accompanying Dissociation of Deoxyribonucleohistone.—Deoxyribonucleohistone was isolated from calf thymus gland by extraction with 0·002M-sodium arsenate and was precipitated by addition of sodium chloride to 0·14M-concentration (cf. Signer and Schwander, *Helv. Chim. Acta*, 1949, **32**, 853). Hence the nucleoprotein had not been dissociated by dissolution in 1·0M-sodium chloride. A solution of the nucleoprotein (0·8%) was prepared and used for the following experiments :

(i) 0·14M-Sodium chloride solution (4·0 c.c.) was added to the nucleoprotein solution (1 c.c.) and immediately Schiff's reagent (1 c.c.) was also added. A white fibrous precipitate was obtained which slowly developed a very faint pink colour.

(ii) On addition of 2·0M-sodium chloride (4·0 c.c.) to the nucleoprotein solution (1 c.c.), followed immediately by Schiff's reagent (1 c.c.), a bluish-pink fibrous precipitate was formed. The colour slowly became more intense.

(iii) On addition of Schiff's reagent (1 c.c.) to the nucleoprotein solution (1 c.c.) a *white* precipitate of the nucleoprotein was formed. This gradually developed an extremely faint pink colour.

(iv) The magnesium salt of the nucleoprotein was treated with a mixture of 2M-sodium chloride (2 c.c.) and Schiff's reagent (1 c.c.). The solid was instantaneously coloured bright pink.

(v) The magnesium salt of the nucleoprotein was dissolved in 2M-sodium chloride (3·0 c.c.), and Schiff's reagent (1 c.c.) was immediately added. A precipitate formed which rapidly became pink.

(vi) As a control, 2M-sodium chloride (3 c.c.) was added to Schiff's reagent (1 c.c.). No colour developed.

In experiments (iv) and (v) the insoluble magnesium salt of the nucleoprotein was prepared by adding 0·2% magnesium chloride solution (1 c.c.) to the nucleoprotein solution (1 c.c.). The precipitate was collected at the centrifuge (cf. Gilbert, Overend, and Webb, *Exp. Cell. Res.*, in the press). All the experiments were carried out under standard conditions in well-corked tubes.

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

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