13. *Nucleotides. Part X.* Some Observations on the Structure and Chemical Behaviour of the Nucleic Acids.*

By D. M. Brown and A. R. Todd.

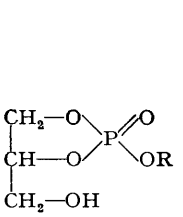
The chemistry of the isomeric mononucleotides obtained on hydrolysis of ribonucleic acids is reviewed and their behaviour is shown to parallel that of the glycerol monophosphates. This leads directly to an understanding of the hydrolytic behaviour of nucleic acids. The bearing of this and of other evidence on the problem of the general structure of the ribonucleic acids is discussed.

Since 1949 knowledge of the degradation products of the ribonucleic acids has undergone considerable change. Until that year it was believed that only four mononucleotides could be obtained by either chemical or enzymic hydrolysis of ribonucleic acids. These mononucleotides had been formulated as the 3'-phosphates of adenosine, guanosine, uridine, and cytidine in the light of degradative studies by Levene and Harris (*J. Biol. Chem.*, 1933, **101**, 419) on yeast adenylic and guanylic acids, the two pyrimidine nucleotides being tacitly assumed to have an analogous structure. The structures of the basic nucleosides had been completely established (see Lythgoe and Todd, *Nucleic Acid Symp. Soc. Exp. Biol.*, 1947, **1**, 15, Camb. Univ. Press, and numerous papers on nucleoside synthesis by Todd *et al.*, *J.*, 1943—1949). The fact that only four nucleotides could be isolated was a source of considerable difficulty when attempts were made to formulate the ribonucleic acids as polynucleotides in which individual nucleosides were joined together by phosphodiester linkages, and it led to various assumptions regarding the mode of linkage and reactivity towards hydrolytic agents which were difficult to accept or even to test experimentally (see Gulland, *J.*, 1938, 1722; 1944, 208; Tipson, *Adv. Carbohydrate Chem.*, 1945, **1**, 193; Schlenk, *Adv. Enzymology*, 1949, **9**, 455). Knowledge which has accrued since 1949 in other laboratories, taken in conjunction with our own investigations in the nucleotide field, has led to fresh considerations which appear to us to throw some light on the problems of the structure and chemical reactivity of the nucleic acids.

The separation of two isomeric adenylic acids from alkaline hydrolysates of yeast ribonucleic acid by Carter and Cohn (*Fed. Proc.*, 1949, **8**, 190, and subsequent papers) marked an important step forward in nucleic acid chemistry, and it was soon followed by the separation of the other three nucleotides into pairs of isomers (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 1471, 2811; Loring, Luthy, Bortner, and Levy, *ibid.*, p. 2811). With respect to the isomeric adenylic acids, termed adenylic acids *a* and *b*, Carter (*ibid.*, p. 1466) suggested that these were adenosine-2' and -3' phosphate, the *b* isomer representing the long-known yeast adenylic acid, although he also considered α β-isomerism at the "*N*-glycosidic" linkage (position 1') as a possible alternative explanation. That either of the isomers was adenosine-5' phosphate was excluded on chemical and enzymatic grounds. Direct comparison of adenylic acid *a* with synthetic adenosine-2' phosphate was impossible because synthetic compounds hitherto regarded as nucleoside-2' phosphates were shown not to have this structure, the benzylidene nucleosides used in their preparation being 2' : 3'-benzylidene derivatives and not 3' : 5'- as had been earlier believed (Brown, Haynes, and Todd, *J.*, 1950, 2299). Moreover, since benzylidene adenosine had been used as an intermediate in the synthesis of yeast adenylic acid (Michelson and Todd, *J.*, 1949, 2476) the identification of adenylic acid *b* with this material did not prove that the phosphate residue was attached to $C_{(3')}$; the synthetic method was ambiguous in the sense that the phosphate group in the final product could be either at $C_{(2')}$ or $C_{(3')}$. We have already (Brown and Todd, preceding paper) presented our evidence for the view that adenylic acids *a* and *b* are the 2'- and the 3'-phosphates of adenosine, although not necessarily respectively, and that they are identical with the two isomeric nucleotides obtained by phosphorylating 5'-trityl adenosine.

* Part IX, preceding paper.

The adenylic acids *a* and *b* are readily converted into a mixture of each under acid conditions (Brown, Haynes, and Todd, *loc. cit.*), and the same observation has been made on the isomeric pyrimidine nucleotides (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 2811). On the other hand, the isomers are stable, without interconversion, under alkaline conditions (Brown and Todd, *loc. cit.*). These experiences find their counterpart in the chemistry of the glycerol phosphates. Thus, glycerol α-phosphate, which is unaffected by alkali, is rearranged in warm dilute acid solution to an equilibrium mixture of glycerol α- and β-phosphate (Bailly, *Compt. rend.*, 1938, **206**, 1902; 1939, **208**, 443, 1820; Verkade, Stoppelenburg, and Cohen, *Rec. Trav. chim.*, 1940, **59**, 886). This rearrangement has been shown to be intramolecular (Chargaff, *J. Biol. Chem.*, 1942, **145**, 455) and it has also been observed with the phosphatidic acids (Baer and Kates, *J. Biol. Chem.*, 1950, **185**, 615). Thus far the isomeric nucleotides and the glycerol phosphates appear to be analogous. In the latter series, it was postulated (Verkade *et al.*, *loc. cit.*; Chargaff, *loc. cit.*) that the phosphoryl migration occurred by way of an intermediate cyclic phosphate ester (I; R = H), although the substance itself was not isolated. We assumed that the isomerisation of the adenylic acids involved the cyclic intermediate (II). Work in progress in these laboratories (with Mr. D. Magrath) has shown that this and other cyclic nucleoside-2′ : 3′ phosphates are accessible substances and that they break down readily on hydrolysis to give a mixture of the 2′- and the 3′-phosphates.



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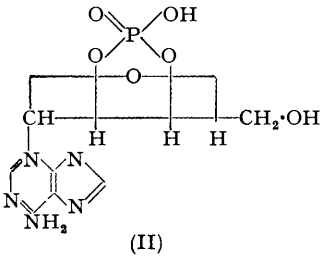

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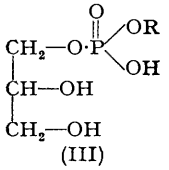

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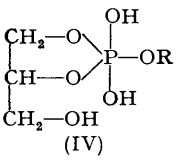

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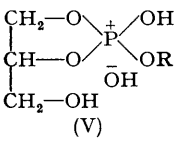


(V)

Some further points in the chemistry of the glycerol phosphates deserve mention. Bailly and Gaumé (*Bull. Soc. chim.*, 1935, **2**, 354) showed that whereas glycerol α-phosphate is stable to alkali, glycerol α-(methyl hydrogen phosphate) (III; R = Me) is readily hydrolysed under alkaline conditions to methanol and a mixture of glycerol α- and β-phosphate; no methyl phosphate is produced. Baer and Kates have described a similar hydrolysis of the choline phosphate (III; R = choline residue); choline was liberated and a mixture of glycerol α- and β-phosphate resulted (*J. Biol. Chem.*, 1948, **175**, 79). Alkaline degradation of the lecithins results in phosphoryl migration (Baer and Kates, *ibid.*, 1950, **185**, 615). Presumably the same factor operates in the case of 2-hydroxyethyl dimethyl phosphate which, under the action of dilute alkali, forms 2-hydroxyethyl phosphate with loss of methanol (Bailly and Gaumé, *Bull. Soc. chim.*, 1936, **3**, 1396). It is noteworthy that 2-methoxyethyl dimethyl phosphate is hydrolysed by dilute alkali to 2-methoxyethyl methyl hydrogen phosphate which is stable towards alkali, a result which is in accord with the generally recognised stability of dialkyl esters of phosphoric acid toward alkaline reagents (cf. Kosolapoff, "Organo-Phosphorus Compounds," Wiley, New York, 1950, p. 232).

It thus appears that the prerequisite for alkali lability in dialkyl phosphates is the presence of a hydroxyl function in proximity to the phosphoryl group. That interaction between neighbouring hydroxyl and phosphoryl groups takes place has been stressed by Kumler and Eiler (*J. Amer. Chem. Soc.*, 1943, **65**, 2355) who have shown that the polyol

and sugar phosphates are abnormally strong acids in comparison with the monoalkyl phosphates.

It is well known that the triesters of phosphoric acid are sensitive both to alkali and to acid (Kosolapoff, *op. cit.*). We envisage a mechanism for the hydrolysis of the hydroxylated dialkyl phosphates in which a neutral cyclic phospho-triester is involved. Thus, in the alkaline hydrolysis of glycerol α-(methyl hydrogen phosphate), (I; R = Me) is produced and immediately hydrolysed to methanol and the cyclic phosphate (I; R = H) which then gives rise to glycerol α- and β-phosphate. No other major products are to be expected, since it is evident that, although the three ester linkages in the cyclic triester must be of comparable reactivity, yet only by fission of the bond retaining the singly-linked substituent can degradation of the molecule occur. Baer and Kates (*J. Biol. Chem.*, 1948, **175**, 79) had earlier proposed a cyclic orthophosphate intermediate (IV; R = choline residue) in the alkaline fission of the choline glycerol α-phosphate. The phosphonium structure (V) may also be considered.

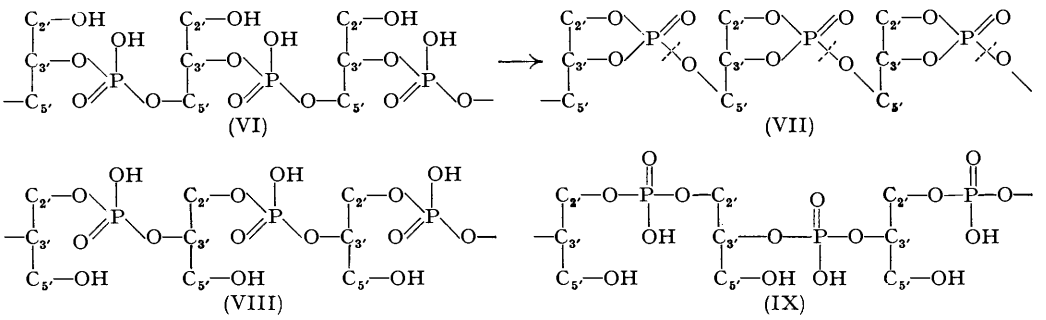
Fonó (*Arkiv Kemi, Min., Geol.*, 1947, **24**, *A*, No. 33, pp. 14, 15) has already pointed out the importance of the neighbouring hydroxyl groups in causing lability of diesters of phosphoric acid containing a glycerol or ethylene glycol residue and has also postulated the existence of a cyclic triester intermediate in their hydrolysis. In this important though little quoted paper he also advanced the view that the alkali-lability of ribonucleic acid, in contrast to the alkali-stability of deoxyribonucleic acid, may be attributed to the extra hydroxy-group present at $C_{(2)}$ in the sugar residues of the former, *i.e.*, that ribonucleic acids are analogous to glycerol alkyl phosphates in their hydrolytic behaviour. On the evidence available to him at the time of his paper, however, he was unable to develop the theme further, nor did he appear to appreciate fully its implications.

During our synthetic experiments we have had occasion to hydrolyse 5′-trityl adenosine-2′ (and -3′) dibenzyl phosphate with 80% acetic acid (Bredereck, Berger, and Ehrenberg, *Ber.*, 1940, **73**, 269) to effect the removal of the trityl and one benzyl group. It is known that monodebenzylation takes place smoothly under comparable conditions in derivatives of adenosine-5′ dibenzyl phosphate (Baddiley and Todd, *J.*, 1947, 648; Brown, Haynes, and Todd, *loc. cit.*). We found that a considerable proportion of nucleotide (adenylic acids *a* and *b*) was produced, involving the loss of both benzyl groups. Furthermore, when a mixture of adenosine-2′ and -3′ benzyl hydrogen phosphate (Brown and Todd, *loc. cit.*) was set aside with 0·5N-sodium hydroxide at 30° overnight, complete debenzylation to a mixture of adenylic acids *a* and *b* occurred. Under the same conditions adenosine-5′ benzyl hydrogen phosphate was quite unaffected. The reaction between phenyldiazomethane and adenylic acids *a* and *b* affords a mixture of the dibenzyl and monobenzyl esters of the respective acids; these esters are each degraded, with rearrangement, to a mixture of adenylic acids *a* and *b* by dilute sodium hydroxide. These experiments show that complete accord exists between the reactions of the adenylic acid derivatives and those of the glycerol phosphates.

The above considerations can be applied directly to the polynucleotides. It has long been known that ribonucleic acids suffer rapid degradation to their component mononucleotides under a variety of mildly alkaline conditions (Steudel and Peiser, *Z. physiol. Chem.*, 1922, **120**, 292; Levene, *J. Biol. Chem.*, 1919, **40**, 415; 1923, **55**, 9; Jones and Perkins, *ibid.*, 1925, **62**, 557; Calvery, *ibid.*, 1927, **72**, 27; Loring, Roll, and Pierce, *ibid.*, 1948, **174**, 729). In none of these cases has any fission product larger than a mononucleotide been isolated; claims to the isolation of larger fragments (Jones *et al.*, *ibid.*, 1916, **25**, 93; 1917, **29**, 111, 123; **31**, 39; Thannhauser and Dorfmüller, *Z. physiol. Chem.*, 1915, **95**, 259) have generally been relinquished or refuted by other workers on the general grounds that the products described were separable mixtures of mononucleotides (Levene, *J. Biol. Chem.*, 1919, **33**, 229). In the same way, mild acid hydrolysis of ribonucleic acid yields mononucleotides, although further degradation, especially of the purine nucleotides, occurs. The early work in this field was hindered by difficulties in separation and identification of the products, but latterly application of chromatographic analysis, together with physical methods of identification, have amply confirmed the earlier observations.

Deoxyribonucleic acids are not degraded to small molecules by mild treatment with alkali (Steudel and Peiser, *loc. cit.*); this observation has provided a method for their analytical separation from ribonucleic acids (Schmidt and Thannhauser, *J. Biol. Chem.*, 1945, **161**, 83).

If the simple straight-chain polynucleotide sequence (VI) in which the individual nucleoside residues are represented briefly as $C_{2'}$–$C_{3'}$–$C_{5'}$ be considered, it can be seen that alkaline degradation will proceed through an intermediate (VII), followed by ready fission of the triester groups *exclusively* at the P–O–$C_{5'}$ linkage. The liberated cyclic nucleoside-2′ : 3′ phosphates will then give rise to a mixture of nucleoside-2′ and -3′ phosphates. No



such mechanism can apply in the case of the deoxyribonucleic acids where the absence of a hydroxyl at $C_{2'}$ precludes the essential formation of a cyclic structure, and this accounts for their more normal stability towards alkali and acid. The isolation of thymidine diphosphate and cytosine deoxyriboside diphosphate after relatively vigorous acid hydrolysis of deoxyribonucleic acid (Levene and Jacobs, *J. Biol. Chem.*, 1912, **12**, 411; 1938, **126**, 63; Thannhauser and Ottenstein, *Z. physiol. Chem.*, 1921, **114**, 39; Dekker and Todd, unpublished observations) may be regarded as confirmation of our view that no special mechanism operates in this case and that the fission of the polynucleotide proceeds in accordance with the known behaviour of phosphodiesters towards acids (Kosolapoff, *op. cit.*); analogous ribonucleoside diphosphates have never been obtained from ribonucleic acids by chemical hydrolysis, nor are they, in our view, to be expected.

An understanding of the mechanism of alkaline hydrolysis of ribonucleic acid does not of itself define the position of the internucleotide linkages, although it excludes several possible combinations. In addition to structure (VI) in which the polymeric linkage is shown joining the 3′- and 5′-positions of the nucleoside residues, other structures containing a $C_{(2')}$–$C_{(5')}$ or $C_{(2')}$–$C_{(3')}$ (VIII) repeating unit or mixed $C_{(2')}$–$C_{(2')}$ and $C_{(3')}$–$C_{(3')}$ units in sequence (IX) would all show lability to alkali on the basis of the mechanism under discussion. A $C_{(5')}$–$C_{(5')}$ linkage, however, cannot occur anywhere in the molecule as this would be stable to alkali and would lead to the appearance of dinucleotides in ribonucleic acid hydrolysates. Gulland and Smith (*J.*, 1948, 1532) prepared a compound which they considered to be diuridine-2′ : 2′ phosphate and which was stable to alkali and acid under conditions which bring about hydrolysis of yeast ribonucleic acid. The synthetic route used in preparing this substance has since been shown (Brown, Haynes, and Todd, *loc. cit.*) to yield 5′-substituted nucleosides so that the compound of Gulland and Smith was in reality diuridine-5′ : 5′ phosphate. Its stability is therefore in accord with our expectation that the cyclisation necessary for rearrangement and easy fission cannot occur between the 5′- and either the 2′- or the 3′-position in the same ribose residue. Consideration of the stereochemistry of the ribofuranoside ring structure suggests that such a cyclisation would not be likely to occur, although Gulland (*J.*, 1938, 1722) at one time considered the possibility of a $C_{(5')} \longrightarrow C_{(3')}$ migration in seeking to explain the non-occurrence of 5′-nucleotides in chemical hydrolysates of ribonucleic acids in face of evidence for 5′-phosphoester linkages from enzyme experiments. One difference between the poly-3′ : 5′-diester structure (VI) and structures (VIII) and (IX) is that in (VI) rupture

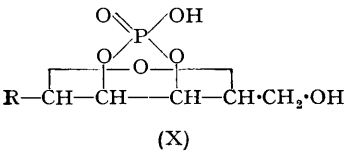
of the chain to yield mononucleotides could, in theory, occur simultaneously at many points, whereas in (VIII) and (IX) it must occur stepwise starting from the end of the chain bearing a free hydroxyl at $C_{(2')}$ or $C_{(3')}$. Such considerations will, of course, only apply to hypothetical unbranched polynucleotide chains and would have to be modified in, for example, branched-chain structures based on (VI).

Mainly as a result of experiments using enzymes, evidence for the occurrence of 5′-phosphoester linkages in nucleic acids has gradually accumulated, although general acceptance of their presence was prevented in the past by the complete failure, until recently, of attempts to isolate nucleoside-5′ phosphates from ribonucleic acid hydrolysates. The evidence rested upon the action of phosphatases, in particular the specific 5′-nucleotidase (Takahashi, *J. Biochem. Japan*, 1932, **16**, 463; Klein and Rossi, *Z. physiol. Chem.*, 1935, **236**, 201; Gulland and Jackson, *J.*, 1938, 1492). The enzyme preparations used have in general contained more than one component. The Russell's viper venom used by Gulland and Jackson had in addition to 5′-nucleotidase and phosphodiesterase activity also a weak non-specific phosphomonoesterase activity; however, they showed that acting on yeast ribonucleic acid the venom alone liberated 25% of the bound phosphorus as inorganic phosphate and this was increased to 75% by the addition of bone phosphomonoesterase. These earlier indications of 5′-phosphoester linkages have now been confirmed by Cohn and Volkin (*Nature*, 1951, **167**, 483) who treated ribonucleic acid successively with ribonuclease and intestinal phosphatase in presence of arsenate to inhibit the phosphomonoesterase. The enzymic hydrolysate was analysed chromatographically on an ion-exchange resin and shown to contain large amounts of the 5′-phosphates of adenosine, guanosine, uridine, and cytidine, identified by direct comparison with synthetic specimens (Michelson and Todd, *loc. cit.*). Using the same phosphatase system (Klein, *Z. physiol. Chem.*, 1933, **218**, 164) Klein and Thannhauser (*ibid.*, p. 173; 1934, **224**, 252; 1935, **231**, 96) and Volkin, Khym, and Cohn (*J. Amer. Chem. Soc.*, 1951, **73**, 1533) have isolated four deoxyribonucleotides from deoxyribonucleic acid. Experiments with purified 5′-nucleotidase (Heppel and Hilmoe, *J. Biol. Chem.*, 1951, **188**, 665) show that these are almost certainly 5′-deoxyribonucleotides (Carter, *J. Amer. Chem. Soc.*, 1951, **73**, 1573). Further evidence for the existence of 5′-phosphoester groupings in ribonucleic acids comes from the observations of Allen (*Fed. Proc.*, 1951, **10**, 155) and of Cavalieri, Kerr, and Angelos (*J. Amer. Chem. Soc.*, 1951, **73**, 2567) on the periodate titration of products obtained by treatment of ribonucleic acid with ribonuclease.

On the available evidence, then, there can be no doubt that $C_{(5')}$ is involved in the main internucleotide linkage both of ribonucleic and of deoxyribonucleic acids. In the case of the latter, the main linkage must be the $C_{(3')}$–$C_{(5')}$, but a decision between $C_{(2')}$ and $C_{(3')}$ as the second point of attachment in ribonucleic acids cannot yet be made with certainty. It is hoped that further evidence on this point will accrue from a study of dinucleoside phosphates and dinucleotides at present being prepared in this laboratory. The production of both nucleoside-2′ and -3′ phosphates on alkaline hydrolysis and the interconversion possibility precludes this method, at least in its present form, from yielding information as to whether $C_{(2')}$ or $C_{(3')}$ or both positions are involved along with $C_{(5')}$. It should, moreover, be remembered that the designations *a* and *b* for the isomeric adenine mononucleotides were arbitrarily chosen and we do not know with certainty which is a 2′- and which a 3′-phosphate. At the moment, however, it seems advantageous to identify the second point of attachment of the main internucleotide linkages in ribonucleic acids as $C_{(3')}$, *i.e.*, to regard the "backbone" of the molecule as a sequence of nucleosides linked together by phosphoric acid groups between $C_{(3')}$ and $C_{(5')}$ as in (VI) by analogy with deoxyribonucleic acids where a $C_{(2')}$ link cannot occur and by consideration of the physical evidence, admittedly small (cf. Astbury, *Nucleic Acid Symp. Soc. Exp. Biol.*, 1947, **1**, 66). Such a structure would, on the arguments developed in this memoir, be hydrolysed readily in alkali to a mixture of isomeric 2′- and 3′-nucleotides, and with enzymes preferential rupture of the secondary alkyl linkages, *i.e.*, at P–O–$C_{(3')}$ rather than P–O–$C_{(5')}$, would yield 5′-nucleotides as it does with the deoxyribonucleic acids. It is, of course, clear that in the discussion which follows the same would hold good for precisely similar structures in which $C_{(2')}$ and $C_{(3')}$ were interchanged.

Although deoxyribonucleic acids appear to be largely straight-chain polynucleotides, many lines of evidence indicate that ribonucleic acids have a branched-chain structure. Electrometric titration data have been interpreted by invoking branched structures involving in particular phosphotriester groups to account for the presence of secondary phosphoryl dissociations (Fletcher, Gulland, and Jordan, *J.*, 1944, 33; Gulland and Jordan, *Nature*, 1948, **161**, 561; Cavalieri, Kerr, and Angelos, *loc. cit.*). There is nevertheless no general agreement as to the proportion of monoesterified phosphate in the nucleic acids studied (Zittle, *J. Biol. Chem.*, 1946, **166**, 491; Allen and Eiler, *ibid.*, 1941, **137**, 757; Wiener, Duggan, and Allen, *ibid.*, 1950, **185**, 163; Vandendriessche, *Compt. rend. Trav. Lab. Carlsberg*, 1951, **27**, 341). The action of ribonuclease on ribonucleic acids gives rise to fragments of widely varying molecular weight (and of which up to 85% may be dialysable) without liberation of inorganic phosphate. Schmidt, Cubiles, Swartz, and Thannhauser (*J. Biol. Chem.* 1947, **170**, 759) found that in addition to larger fragments only pyrimidine nucleotides are liberated by ribonuclease, an observation which has been confirmed by Carter and Cohn (*J. Amer. Chem. Soc.*, 1950, **72**, 2604). Loring and Carpenter (*J. Biol. Chem.*, 1943, **150**, 381) obtained both purine and pyrimidine nucleotides from a nucleic acid in this way. Markham and Smith (*Research*, 1951, **4**, 344) report that a large proportion of the pyrimidine nucleotide present in turnip yellow mosaic virus ribonucleic acid and other ribonucleic acids is liberated in two fractions by ribonuclease in about 10 minutes and that these fractions, by chemical hydrolysis or by the further action of ribonuclease, yield uridylic and cytidylic acids respectively. These two fractions have been provisionally identified by paper chromatography with the cyclic 2′ : 3′-phosphates of uridine (X; R = uracil) and cytidine (X; R = cytosine) prepared by Mr. D. Magrath in this laboratory; further experiments are in progress in collaboration with Drs. Markham and Smith to confirm the identity.

This production of large amounts of pyrimidine nucleotides following ribonuclease treatment of ribonucleic acids suggests that these nucleotides are derived from side chains in the molecule and that these chains or branches must occur at frequent intervals. It suggests further that the side-chains are short—probably for the most part containing only one nucleotide residue. If we consider a backbone structure (VI) for the



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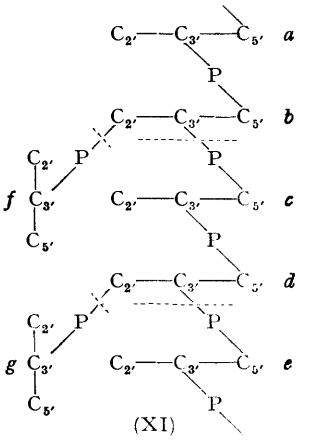

(XI)

nucleic acid, then, omitting for the moment branching by inclusion of triester linkages, it is clear that the attachment of the branches to the main chain must occur at $C_{(2')}$ and that this must be linked through the phosphate group to $C_{(3')}$ (or $C_{(2')}$) in the first nucleoside residue in any branch. Only by such means could the alkali-lability of the nucleic acid be maintained; linkage of $C_{(2')}$ in the main chain to $C_{(5')}$ in the first residue of the side-chain would give an alkali-stable structure (since cyclisation would be impossible in such a structure, *both* $C_{(2')}$ and $C_{(3')}$ in the nucleoside residue in the main chain at the branching point being occupied). The general structure which we envisage for ribonucleic acids is that shown diagrammatically in (XI).

In this structure, the branches are shown as containing one nucleotide only; longer chains, if they occur, would be accommodated by extending them by the normal $C_{(5')}$–$C_{(3')}$ sequence as in the main chain. The attachment of a certain number of branches on phosphorus, *i.e.*, the incorporation of phosphotriester linkages, would present no problem as the grouping involved would be alkali-labile, provided, again, that the $C_{(5')}$–$C_{(3')}$ sequence were followed in the branching chain. Dilute alkali would convert a molecule of type (XI) into a mixture of the mononucleotides in accordance with the observed facts. If we assume that *f* and *g* are pyrimidine nucleoside residues, then their removal as a first step in ribonuclease action by fission at the P–O–$C_{(2')}$ linkage (probably initially as cyclic phosphates) is in accordance with our prediction. The increased periodate uptake shown by ribonuclease-treated ribonucleic acid already mentioned would be explained by simultaneous or subsequent fission of some of the $C_{(3')}$–O–P linkages in the main chain, as indicated by the broken line in (XI). It will be observed (*a*) that fragments would be produced bearing at one end a nucleoside residue attached by phosphate through the 5′-position only, *i.e.*, oxidisable by periodate, (*b*) that subsequent treatment of the products with alkali should yield in addition to mononucleotides a quantity of free nucleosides, and (*c*) that, although they have not as yet been isolated, some nucleoside diphosphates should occur in *alkaline* hydrolysates of ribonuclease-treated ribonucleic acids. It will further be noted that enzymic hydrolysis of such a structure as (XI) with ribonuclease followed by intestinal phosphatase should yield the four 5′-nucleotides from the main chain, and in addition the side chain groups *f* and *g* should yield 2′- or 3′-nucleotides. It is of considerable interest that in addition to 5′-nucleotides and some unidentified materials Cohn and Volkin (*Nature, loc. cit.*; *Fed. Proc.*, 1951, **10**, 173) have shown that such treatment does give also the *b* isomers of uridylic and cytidylic acids in considerable amount. Present knowledge of ribonuclease specificity does not, of course, permit the conclusion that in the main or "backbone" chain of (XI) only $C_{(3')}$–O–P linkages would undergo fission by its action; $C_{(5')}$–O–P linkages may also be split without affecting our general thesis. The $C_{(3')}$–O–P fission has been singled out for special comment above because, only if it does occur, can immediate increase in periodate uptake following ribonuclease action be explained.

There can be no question of finality about any nucleic acid structure at the present time, since it is clear that there is no available method for determining the nucleotide sequence. If the view of Schmidt and Thannhauser (Ann. Report, Nat. Res. Council Comm. on Growth, U.S.A., 1950, 194) be accepted that ribonuclease attacks pyrimidine nucleotide linkages preferentially, then it is possible to speculate as to the location of pyrimidine nucleotides in the main polynucleotide chain [*e.g.*, *b* and *d* in (XI)] but such speculations go rather beyond present evidence. There are, too, some possible variants of structure (XI) which could equally be considered since the choice between $C_{(2')}$ and $C_{(3')}$ as a linking point in any given residue is arbitrary. Nevertheless, we feel it desirable to indicate that structures of type (XI) are capable of explaining a surprising number of the known facts about the hydrolytic behaviour of ribonucleic acids, without invoking any linkages between nucleoside units other than simple phosphate groups between hydroxyl groups in the ribofuranose residues. At present they represent a convenient working hypothesis which we hope to test in further synthetic studies now in progress.

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