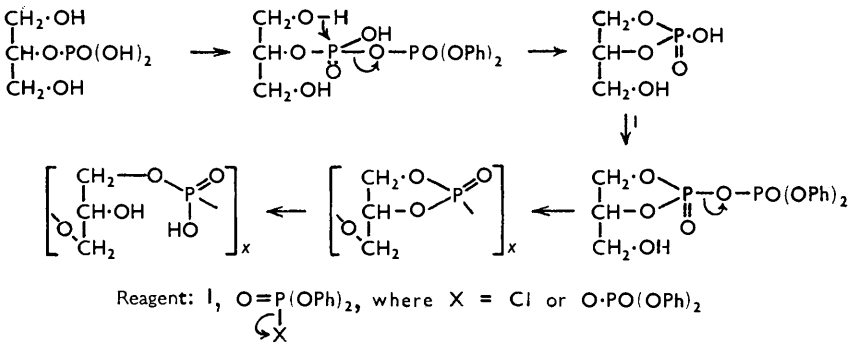
# 270. *Polynucleotides. Part I. Synthesis and Properties of Some Polyribonucleotides.*

By A. M. Michelson.

Polyglycerophosphoric acid and a number of polyribonucleotides from dimers to fractions with an average chain length ~12 have been prepared from the corresponding monophosphates by the action of diphenyl phosphorochloridate or tetraphenyl pyrophosphate. Chemical and enzymic degradation shows that they are linear polymers analogous to those of natural origin, except that in the polynucleotides some of the linkages are 2′–5′ rather than 3′–5′. The hyperchromic effect, the effect on the light absorption of basic dyes, and changes in dissociation constants shown by the synthetic polynucleotides indicate ordered structures with restricted rotation about the internucleotide linkages. Biological activity is shown by some of the polymers.

Beginning a new approach to the synthesis of polynucleotides we have examined the formation of polymers from, first, glycerophosphoric acid and then the readily available ribonucleoside-2′ or -3′ phosphates.

Treatment of glycerol 2-[mono(tri-*n*-octylammonium) hydrogen phosphate] (or the mixture of 1- and 2-phosphates obtained by phosphorylating glycerol with a limited amount of polyphosphoric acid [1]) with diphenyl phosphorochloridate [2] or tetraphenyl pyrophosphate [3] in the presence of base, a method first used by Forrest, Mason, and Todd [4] for the synthesis of a cyclic phosphate of riboflavin, gave glycerol-1 : 2 cyclic phosphate.[5] Addition of more diphenyl phosphorochloridate, or tetraphenyl pyrophosphate, and tri-*n*-butylamine to the concentrated solution then caused rapid polymerisation, to give a good yield of polyglycerophosphoric acid, isolated as the calcium salt. The course of the reaction, which is essentially an acylation through a mixed anhydride intermediate, may be represented as in the annexed formulæ.




No direct phosphorylation of hydroxyl groups by the diphenyl phosphorochloridate has been observed under the conditions employed, largely because attack at the anion is much faster than at an alcoholic hydroxyl group, most of the reagent being thus consumed immediately. The final cleavage, in aqueous solution, of one of the bonds of the phosphate triester to form the diester can occur in three ways, leading to 1 : 3 glycerol–phosphate linkages, 2 : 3 linkages, or degradation of the polymer. The hydrolysis of uridine-3′ dimethyl phosphate at all pH's (presumably *via* the methyl cyclic phosphate) mainly to

[1] Church, *Proc. Roy. Soc.*, 1864, **13**, 520.
[2] Brigl and Müller, *Ber.*, 1939, **72**, 2121.
[3] Khorana and Todd, *J.*, 1953, 2257.
[4] Forrest, Mason, and Todd, *J.*, 1952, 2530.
[5] Ukita, Bates, and Carter, *J. Biol. Chem.*, 1955, **216**, 867.

monoesters of uridine-2′ and uridine-3′ phosphate with only a trace of uridine-2′ : 3′ cyclic phosphate [6] indicates that degradation should be slight. Nevertheless this and other considerations will impose a practical limit on the size of polymers which can be prepared by the methods described in this communication.

The crude polymer was a mixture of polyglycerophosphoric acids of different chain lengths, ending in a glycerol cyclic phosphate residue. Dilute acid at room temperature [5] opened this cyclic phosphate to give polymers ending in a phosphate group. Some fractionation was achieved by dialysis, giving a residue with an average chain length of 6·4 glycerol phosphate units. Further dialysis against 2M-sodium chloride gave a final residue (about 20% of the material) with an average chain length of 12·1 units. Paper chromatography indicated that this material contained a considerable amount of higher polymers. Chain length was determined by estimating the inorganic phosphate liberated from the acid-treated polymers by prostatic monoesterase purified by Kerr and Chernigoy's method,[7] the ratio of terminal phosphate to total phosphate giving the average length. Hydrolysis of polyglycerophosphoric acid by 0·1N-hydrochloric acid at 100° caused complete degradation to glycerol, glycerol monophosphates, and glycerol diphosphates. For chromatographic comparison a mixture of glycerol diphosphates was prepared by treatment of glycerol with polyphosphoric acid, followed by acid-hydrolysis; isolation and purification of the product, obtained in good yield, was facilitated through the inverse solubility of the dibarium salt of glycerol-1 : 2(or 3) diphosphate, and final purification was by ethanolic precipitation of the acid barium salt.

Hydrolysis by 0·2N-sodium hydroxide also completely degraded polyglycerophosphoric acid to glycerol and glycerol mono- and di-phosphates. The formation of such products by the action of both acid and alkali is in accord with the general mechanisms advanced by Brown and Todd [8] for the acid-isomerisation of nucleoside-2′ and -3′ phosphates and alkaline degradation of ribonucleic acids. Neither ribonuclease nor the mixture of monoesterase and diesterase present in rattlesnake (*Crotalus atrox*) venom affected the polymers, in agreement with their high specificity. Prostate monoesterase liberated a small amount of inorganic phosphate from the untreated poly(glycerophosphoric acid) owing to partial opening of the terminal cyclic phosphate during working-up. The dephosphorylation of polymers ending in a phosphate group became slower with increase in average chain length.

The synthesis of polynucleotides through the nucleoside cyclic phosphates was even more successful, giving quantitative yields.[9] Dilute solutions of the tri-*n*-octylammonium or tri-*n*-decylammonium salt of the nucleoside-2′(or 3′) phosphate in anhydrous dioxan with slightly more than 1 mol. of tetraphenyl pyrophosphate or diphenyl phosphorochloridate and 2—2·5 mol. of tri-*n*-butylamine yielded the cyclic phosphate quantitatively in one hour at room temperature. Adenosine-2′ : 3′ phosphate and uridine-2′ : 3′ phosphate were thus prepared [10] and isolated as calcium salts. The preparations behaved as single entities on paper chromatography and paper electrophoresis, and were identical in such behaviour with authentic specimens.[11] Acid-hydrolysis yielded the corresponding nucleotides, as did hydrolysis with alkali [11] or prolonged incubation at 37° in a buffer solution at pH 10. While adenosine-2′ : 3′ cyclic phosphate was resistant to ribonuclease, the uridine derivative was converted into the 3′-phosphate.[12]

Further addition of diphenyl phosphorochloridate to a concentrated solution of the nucleoside cyclic phosphate then caused rapid polymerisation. In practice, the cyclic

[6] Brown, Magrath, and Todd, *J.*, 1955, 4396.
[7] Kerr and Chernigoy, *J. Biol. Chem.*, 1957, **228**, 495.
[8] Brown and Todd, in " The Nucleic Acids," ed. Chargaff and Davidson, Academic Press, New York, 1955, Vol. I, p. 409.
[9] Michelson, *Nature*, 1958, **181**, 303.
[10] Michelson, *Chem. and Ind.*, 1958, 70.
[11] Brown, Magrath, and Todd, *J.*, 1952, 2708; Dekker and Khorana, *J. Amer. Chem. Soc.*, 1954, **76**, 3522.
[12] Brown, Dekker, and Todd, *J.*, 1952, 2715.

phosphate was generally made in concentrated solution, the reagents being added slowly with stirring in order to prevent precipitation of the nucleotide. Concomitant polymerisation was then unavoidable, and the reaction was completed by the second addition of reagents. The crude product was then precipitated by ether or *cyclo*hexane and purified by precipitation from water as the free acid or the calcium or magnesium salt. Apart from polyuridine-2′(or 3′) phosphate, however, these salts were of little use owing to their very low solubilities in water, particularly with the higher polymers. In this way polyadenylic acid, polyguanylic acid and polyuridylic acid were prepared, as was a co-polymer of the four major monoribonucleotides (adenylic, cytidylic, guanylic, and uridylic acid), which was a synthetic ribonucleic acid similar in many respects to a purified sample of commercial yeast nucleic acid. Polymerisation of mixtures of adenylic and guanylic acids, and of adenylic and uridylic acid, in various proportions gave the mixed polynucleotides, and the versatility of the method was demonstrated by using dilute solutions of the nucleoside-2′ : 3′ cyclic phosphates and allowing the reaction to proceed for a limited time, to give products containing relatively large amounts of smaller oligonucleotides. Co-polymerisation of a mixture of adenylic and uridylic oligonucleotides prepared separately in this way then gave polymer differing from the co-polymerised adenylic and uridylic acids, in that the nucleotides occurred as tracts of purines and pyrimidines rather than in a random fashion. That all of the mixed polymers were truly co-polymers was readily proved by paper electrophoresis.

While co-polymerisation of adenylic and uridylic acid leads to a more or less random distribution of the two nucleotides, some selectivity due to chemical processes may occur. For example, an adenylic–uridylic linkage may be formed in the initial stages more readily than an adenylic–adenylic or uridylic–uridylic linking, owing to the different reactivities of the intermediate phosphate anhydride and of the 5′-hydroxyl groups in the nucleotides. The latter difference would be most significant for guanosine (and possibly cytidine) derivatives, which would tend to appear as the head unit in any chemical co-polymerisation of 2′(or 3′)-nucleotides, or as the tail unit if a 5′-nucleotide derivative were polymerised, owing to the low reactivity of the sugar hydroxyl groups.[13] Adenylic and uridylic acid were chosen to demonstrate the differences between random and non-random distribution because their reactivities were more similar than those of other pairs. However, selectivity may also be caused by $\pi$-electron interaction between the bases, so that, while for the moment a random arrangement is assumed, chemical co-polymerisation could, under suitable conditions, lead to a non-random distribution of purines and pyrimidines.

Under the polymerisation conditions generally used, about 20% of the crude product dialysed against water to give a residue with an average chain length of 5—6 nucleotides. Further dialysis against 2M-sodium chloride gave a final dialysis residue (35—40% of the original mixture) with an average molecular weight of 3500—4500. While the average chain length of this fraction was 10—12, the molecular species varied from polymers containing approximately 6 units, to those with up to about 20 nucleotides, and further fractionation should give material of higher average molecular weight. For comparison with the synthetic materials, a specimen of commercial yeast nucleic acid was similarly purified; the ribonuclease-resistant "cores" (*i.e.*, polypurine nucleotides terminating with a pyrimidine-3′ phosphate) were also prepared. Among other products separated from the mixture of oligonucleotides resulting from the action of pancreatic ribonuclease on ribonucleic acid, was the dinucleotide of adenylic and cytidylic acid (A3′P5′C3′P), isolated as the highly crystalline free acid.

The properties of the synthetic polymers indicated that their general structure was analogous to that ascribed to natural oligonucleotides.[8] Mild acid-treatment opened the terminal cyclic phosphate to give polynucleotides with tail units containing a 2′(or 3′)-phosphate. More vigorous acid-degradation gave either purines and/or pyrimidine

[13] Michelson, *Tetrahedron*, 1958, **2**, 333.

nucleoside-2′ and -3′ phosphates depending on the polymer. Aqueous sodium hydroxide caused complete breakdown to the respective mixtures of 2′- and 3′-mononucleotides only; no alkali-stable 5′-phosphorylated material was observed. Treatment with rattlesnake venom gave the expected nucleosides and nucleoside-2′(or 3′) : 5′ diphosphate (the tail nucleotide) [14] after prolonged incubation with rather large quantities of enzyme. Average chain length was determined in all cases by spectrophotometric estimation of the relative amounts of nucleoside and nucleoside diphosphate, paper chromatography or paper electrophoresis being used for the separation. Base ratios of the mixed polymers were likewise determined in this way and by separation of the mononucleotides obtained on alkaline degradation. Where applicable, chain lengths were again determined by removal of the terminal phosphate group by purified prostatic phosphatase. The extremely low solubility of some of the polymers in acid solutions—dissolution is necessary to open the terminal cyclic phosphate—precluded this as a general method, the enzyme preparation being inactive against 2′ : 3′-cyclic phosphates.

While polyadenylic and polyguanylic acid were resistant to the action of pancreatic ribonuclease, some 65% of the internucleotide linkages of polyuridylic acid were cleaved to give a mixture of uridylic acid, and di-, tri-, and tetra-nucleotides containing 2′–5′-linkages exclusively. These oligonucleotides were separated on sheets of paper, and the terminal phosphate group of each was removed with monoesterase to give a series of polyuridylic acids terminating with a nucleoside, that is, U2′P5′U; U2′P5′U2′P5′U; U2′P5′U2′P5′U2′P5′U. The structure and composition of each of these was verified by alkaline hydrolysis to nucleotides and tail nucleoside, estimation of which gave the chain length, confirmed by the ratio of inorganic phosphate liberated by the monoesterase to total phosphate. Spectroscopic examination and determination of total phosphate in the alkaline hydrolysate also gave a molar ratio from which the structure could be determined. While these derivatives were not isolated in the solid state, it is believed that their identity is established.

A mathematical interpretation of the data obtained from the action of ribonuclease on polyuridylic acid is simplified in view of the known specificity of the enzyme. If " A " is a uridine-3′ phosphate unit in the polymer and " B " a uridine-2′ phosphate, then in a chain " $n$ " units long containing " A " in the molar proportion $1 - p$ and " B " in the molar proportion $p$, fragments of length $r$ can be produced in three ways, if it is assumed that A and B are distributed at random:

(1) When the chain begins $B^{r-1}A$: the probability that a chain begins so is $p^{r-1}(1 - p)$.

(2) Whenever the combination $AB^{r-1}A$ occurs in the chain: the probability of this event for each starting position is $p^{r-1}(1 - p)^2$ for $(n - r)$ possible starting positions, giving a probability that a fragment of length $r$ arises from this cause equal to $(n - r)p^{r-1}(1 - p)^2$.

(3) When the chain ends $AB^r$. The probability is $p^r(1 - p)$. These probabilities are all independent and the joint probability is:

$$p^{r-1}(1 - p) + p^r(1 - p) + (n - r)p^{r-1}(1 - p)^2 = p^{r-1}(1 - p)\,[1 + p + (n - r)(1 - p)]$$

The proportion by weight of fragments of length $r$ is then:

$$\frac{rp^{r-1}(1 - p)}{n}\,[1 + p + (n - r)(1 - p)]$$

When $1 + p - r(1 - p) = 0$, *i.e.*, $r = (1 + p)/(1 - p)$ and therefore $p = (r - 1)/(r + 1)$, then the expression is independent of " $n$," that is, the proportion of fragments of length $r$ units is constant, independently of the length of the original chain. From the above

[14] Markham and Smith, *Biochem. J.*, 1952, **52**, 558.

expressions it is possible to calculate the value of $p$ which gives a constant proportion of fragments of given length $r$, the proportion of $r$ in each case being $rp^{r-1}(1-p)^2$, *e.g.*: $p = \frac{1}{3}, r = 2$; $p = \frac{1}{2}, r = 3$; $p = \frac{3}{5}, r = 4$; etc.

Thus by measuring the proportions of di-, tri-, and tetra-nucleotides formed by the enzyme from fractions of the polymer of different average chain lengths, an estimate of $p$ can be made from the fragment which shows least variation in proportion. The assumption of random distribution of 2′–5′- and 3′–5′-linkages in the synthetic polyuridylic acid may not be valid, as the possibility that formation of a particular linkage depends to some extent on the adjacent linkages cannot be ruled out.

The above expressions may also be applied to ribonucleic acids. For example, if the nucleic acid has a molar ratio of pyrimidines (A) to purines (B) equal to 1, then the proportion of trinucleotide produced by the action of ribonuclease, independently of the state of degradation of the polymer, is $\frac{r(n-r+3)}{n2^{r+1}} = 3/16$ if random distribution occurs, giving a possible method for demonstrating the non-randomness of the purines and pyrimidines in certain ribonucleic acids and biosynthetic copolymers.

When the polymerisation was carried out in dilute solution, products containing relatively large amounts of small oligonucleotides were obtained. Examination of such products from adenosine-2′ : 3′ cyclic phosphate by Dr. L. A. Heppel of the National Institutes of Health, Bethesda, Maryland, indicated the presence of di-, tri-, and tetra-nucleotides terminating with a cyclic phosphate. Treatment of the dinucleotide with dilute acid gave A2′(or 3′)P5′A2′(or 3′)P identical in behaviour on paper chromatography, and with the same electrophoretic mobility, as a biosynthetic specimen of the dinucleotide. Spleen phosphodiesterase [15] split approximately 50% of the material to adenylic acid, the remaining A2′P5′A2′(or 3′)P being resistant to the enzyme. Removal of the terminal phosphate from the dinucleotide by the action of monoesterase gave a mixture of adenosine-2′ adenosine-5′ phosphate and adenosine-3′ adenosine-5′ phosphate, the latter being cleaved by spleen diesterase to adenosine-3′ phosphate and adenosine. (Since the original material had been treated with acid, a process causing isomerisation of internucleotide linkages under certain conditions,[16] the actual proportions of 2′–5′- and 3′–5′-compounds cannot be referred to the dinucleotide mixture obtained from the polymerisation.) Treating the higher polymers of adenylic acid with spleen diesterase yielded products analogous to those obtained by the action of ribonuclease on polyuridylic acid, that is, some mononucleotide, together with enzyme-resistant oligonucleotides containing 2′–5′-internucleotide linkages exclusively. A final point examined by Dr. Heppel [17] was the action of the synthetic polymers as primers for polynucleotide phosphorylase. The synthetic polyadenylic acid greatly stimulated enzymic polymerisation of adenosine diphosphate but was not incorporated into the biosynthetic polymer, as were normal primers containing a free 3′-hydroxyl group available for esterification.

In view of the instability of the terminal cyclic phosphate in oligonucleotides containing such a group, even on storage in the solid state,[18] and since some ring opening was invariably observed in the crude preparations of low molecular weight, causing a somewhat confusing pattern on paper chromatography, no attempt was made to separate the initially formed homologues on a preparative scale. The polyadenylic and polyuridylic acids of low molecular weight were converted into the corresponding oligonucleotides terminating with a 2′(or 3′)-phosphate group by the standard treatment with 0·1N-hydrochloric acid at room temperature. Paper chromatography gave series of adenylic and uridylic oligonucleotides ranging from di- to penta-nucleotide. The structure of each

[15] Heppel and Hilmoe in " Methods in Enzymology," ed. by Colowick and Kaplan, Academic Press, New York, 1955, Vol. II, p. 565.
[16] Brown, Magrath, Neilson, and Todd, *Nature*, 1956, **177**, 1124.
[17] Singer, Heppel, and Hilmoe, *Biochim. Biophys. Acta*, 1957, **26**, 447.
[18] Heppel, personal communication.

member of the series was determined by the methods previously mentioned and described in detail by Heppel, Ortiz, and Ochoa.[19] Unlike the oligonucleotides obtained by the action of enzymes on polymers of higher molecular weight, the homologues prepared in this way contained both 2′ : 5′- and 3′ : 5′-phosphodiester linkages, as expected. Treatment of each oligonucleotide with prostatic phosphatase gave the corresponding nucleotide derivative containing $n$ nucleosides and $n - 1$ phosphate residues. That the various members described formed a homologous series was further indicated by plotting chain length against log $[(1/R_F) - 1]$, a function of the partition coefficient,[20] for the series $(AP)_n$, $(AP)_{n-1}A$, $(UP)_n$, and $(UP)_{n-1}U$. Straight lines, characteristic of homologous series, were obtained (Fig. 1).

The synthetic polynucleotides having been established as linear polymers containing nucleosides combined through 2′ : 5′- and 3′ : 5′-phosphodiester linkages, the next stage

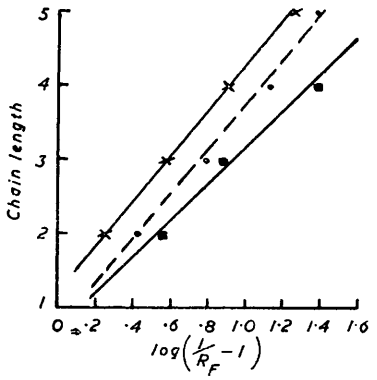



FIG. 1.

× $(UP)_{n-1}U$, ● $(AP)_{n-1}A$, ■ $(AP)_n$

was to prepare polymers with specific head and tail residues. Since ribonucleic acid may be regarded as a polynucleoside-3′ phosphate, or as a polynucleoside-5′ phosphate, depending on whether the terminal phosphate is attached to the secondary or the primary sugar hydroxyl group,[21] it was of particular interest to synthesise oligonucleotides containing a terminal 5′-phosphate. While the obvious route would involve polymerisation of a 5′-nucleotide (or derivative thereof) the success of the present methods encouraged a more circuitous approach.

Treatment of uridine-2′(or 3′) : 5′ diphosphate [22] with excess of diphenyl phosphorochloridate (or tetraphenyl pyrophosphate) yielded $P^1$-uridine 2′ : 3′-(cyclic phosphate) 5′-($P^2P^2$-diphenyl pyrophosphate) quantitatively.[23] Incubation of an aqueous solution at pH 9·5 cleaved the pyrophosphate linkage under conditions such that internucleotide linkages were not broken.[24] Reaction of uridine-2′(or 3′) : 5′ diphosphate and excess of adenosine-2′(or 3′) phosphate in the usual way, followed by the mild pyrophosphorolytic treatment, gave polymers of adenylic acid terminating with uridine-5′ phosphate at the head and adenosine-2′ : 3′ phosphate at the tail. A similar process with uridylic acid gave polyuridylic acid with a terminal 5′-phosphate at one end and a terminal 2′(or 3′)-phosphate at the other, after acid treatment to open the cyclic phosphate. Molar ratios obtained by alkaline hydrolysis to nucleotide and nucleoside-2′(or 3′) : 5′ diphosphate followed by separation and estimation gave a measure of the actual incorporation of 5′-phosphate residue into the polymers. Since ratios of 1 : 10—15 were obtained for material with an average chain length of approximately 7 units, only half of the material actually possessed

[19] Heppel, Ortiz, and Ochoa, *J. Biol. Chem.*, 1957, **229**, 679.
[20] Bate-Smith and Westall, *Biochim. Biophys. Acta*, 1950, **4**, 427.
[21] Todd, *Chem. and Ind.*, 1956, 802.
[22] Hall and Khorana, *J. Amer. Chem. Soc.*, 1955, **77**, 1871.
[23] Michelson, *J.*, 1958, 1957.
[24] Michelson, *Chem. and Ind.*, 1957, 1669.

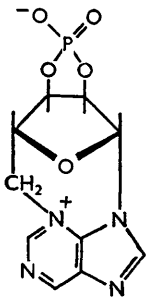
a terminal 5′-phosphate group. Suitable ratios of the starting materials and alteration of the reaction conditions would probably give more favourable results and, in principle, purification and separation of the smaller oligonucleotides should be possible. The introduction of specific head residues is not limited to nucleosides bearing a 5′-phosphate group; presumably any nucleoside-2′(or 3′) phosphate derivative blocked at the 5′-hydroxyl group would behave similarly and it is clear that polymers terminating with pyrophosphate could be obtained by hydrogenolysis of the diphenyl derivatives.

Attention was then directed to the tail of the molecule. Treatment of uridine-2′ : 3′ cyclic phosphate with diphenyl phosphorochloridate in the presence of excess of benzyl alcohol gave the monobenzyl esters [25] of uridine-2′ and -3′ phosphate as the major products. Hydrolysis with pancreatic ribonuclease [8] indicated that approximately 75% of the mixture was uridine-3′ benzyl hydrogen phosphate, the remaining uridine-2′ benzyl hydrogen phosphate being resistant to the enzyme. In addition to the simple nucleotide benzyl esters, a second product, tentatively identified as a monobenzyl dinucleotide, was also observed. Application to the synthesis of adenosine-2′(or 3′) uridine-5′ phosphate using 2′ : 3′-di-*O*-benzyluridine [26] as the alcohol was not entirely successful, as considerable polymerisation of the adenylic acid occurred in addition to incorporation of the uridine moiety. Further difficulties were encountered in the hydrogenolysis of the benzyl groups, and the preparation was therefore abandoned. More success was obtained with acylation of 3′-*O*-acetylthymidine,[27] the acetyl group being removed from the final products by incubation at pH 9·6 at room temperature for 3 days. The characteristic colour reactions [28] of 2-deoxyribose derivatives facilitated identification of the numerous products shown by paper chromatography; again considerable homopolymerisation occurred in addition to incorporation, even when excess of the thymidine derivative was used. Identification of the homologous series APT, APAPT, APAPAPT was made by the usual methods; these compounds represent a class of nucleotide derivatives not so far found in Nature. A final experiment involved the polymerisation of adenosine-2′(or 3′) phosphate in the presence of both uridine-2′(or 3′) 5′-diphosphate and 2′ : 3′-di-*O*-acetyluridine,[29] followed by very mild alkaline treatment. Although incorporation did occur, to give polyadenylic acid terminating with uridine-5′ phosphate at the head and uridine at the tail, *i.e.*, a polynucleoside-5′ phosphate, this was so only in a statistical sense, and the results are not entirely satisfactory.

In view of the extensive work on the use of mixed anhydrides as synthetical reagents, it was of some interest to examine the action of acid anhydrides other than tetraphenyl pyrophosphate on nucleoside cyclic phosphates. When adenosine-2′ : 3′ cyclic phosphate was treated with *O*-benzylphosphorous *OO*-diphenylphosphoric anhydride,[30] approximately 50% of the adenylic acid was converted into adenosine 2′ : 3′-(cyclic phosphate) 5′-(benzyl phosphite), identified by conversion into a mixture of adenosine-2′ : 5′ and -3′ : 5 diphosphates.[31] The remaining 50% was isolated as polymers containing a 5′-benzyl phosphite group at the head, and a cyclic 2′ : 3′-phosphate at the tail. Average chain lengths of these polymers could not be satisfactorily determined, possibly because of enzyme inhibition, and the polymers were not further examined. Oxidation and hydrogenolysis would presumably give polymers with a terminal 5′-phosphate group. Treatment of uridine-2′ : 3′ cyclic phosphate with methanesulphonyl chloride gave one product only, presumably 5′-methanesulphonyluridine-2′ : 3′ cyclic phosphate (from its behaviour on paper chromatography and paper electrophoresis). Alkaline hydrolysis yielded 5′-methanesulphonyluridine-2′(or 3′) phosphate, isolated as the calcium salt, which showed

[25] Brown and Todd, *J.*, 1953, 2040.
[26] Michelson and Todd, *J.*, 1956, 3459.
[27] *Idem*, *J.*, 1953, 951.
[28] Buchanan, *Nature*, 1951, **168**, 1091.
[29] Kenner, Todd, Webb, and Weymouth, *J.*, 1954, 2288.
[30] Corby, Kenner, and Todd, *J.*, 1952, 3669.
[31] Michelson, *J.*, 1958, 2055.

the expected changes in chromatographic and electrophoretic behaviour. No polymerisation was detected. The action of methanesulphonyl chloride on adenosine-2′ : 3′ cyclic phosphate likewise yielded what was presumably the unstable covalent 5′-methanesulphonyl derivative. Heating this intermediate in anhydrous dioxan caused cyclisation to 3 : 5′-*cyclo*adenosine-2′ : 3′ cyclic phosphate (I) which, like the analogous 2′ : 3′-*OO-iso*propylidene-3 : 5′-*cyclo*adenosine toluene-*p*-sulphonate[32] and 2′-deoxy-3′-*O*-acetyl-3 : 5′-*cyclo*adenosine toluene-*p*-sulphonate,[33] showed a bathochromic shift of the ultraviolet absorption maximum from 259 to 272 mμ. As expected, the electrophoretic mobility of this compound was considerably less than that of adenosine-2′ : 3′ cyclic phosphate.



It is well known that the intensity of ultraviolet absorption increases on degradation of a polynucleotide. This hyperchromic effect is relatively large for ribonucleic acid fragments with a high guanine content, and Magasanik and Chargaff[34] suggested that it might be associated particularly with guanylic acid residues, but others

TABLE 1.

| Compound | Average chain length | $\lambda_{max.}$ in 0·2N-NaOH Polymer | Products | Alkaline hyperchromicity (%) |
|---|---|---|---|---|
| Poly U | 11·9 | 261 | 261 | 8·6 |
| Poly U | 6·6 | 261 | 261 | 6·5 |
| Poly U | 3·5 | 261 | 261 | 4·8 |
| Poly A | 13·6 | 257·5 | 259·5 | 36·7 |
| Poly A | 7·7 | 258 | 259·5 | 31·8 |
| Poly A | 3·3 | 258·5 | 259·5 | 19·6 |
| Adenosine-2′(3′) phosphate | — | — | 259·5 | 0 |
| Diadenylic | — | 258·5 | 259·5 | 15·1 |
| Triadenylic | — | 258 | 259·5 | 22·8 |
| Tetra-adenylic | — | 258 | 259·5 | 30·7 |
| Penta-adenylic | — | 258 | 259·5 | 32·9 |
| Poly G | 12·5 | 260—266 | 260—266 | 0 |
| Poly G | 5·4 | 260—266 | 260—266 | 0 |
| Poly G | 3·2 | 260—266 | 260—266 | 0 |
| A2′P5′U | — | 261 | 260 | 12·8 |
| A2′(3′)P5′T | — | 261 | 261 | 12·7 |
| A3′P5′C3′P (from RNAase digest) | — | 262 | 262 | 7·2 |
| Poly AU (4·5 : 1) Random | 5·2 | 259 | 260 | 21·1 |
| Poly AU (4·5 : 1) Random | 11·5 | — | — | 32·0 |
| Poly AU (1·4 : 1) Random | 8·3 | 259·5 | 261 | 13·4 |
| Poly AU (1·4 : 1) Random | 13·1 | — | — | 22·0 |
| Poly AU (0·8 : 1) Random | 6·8 | 260 | 260 | 9·1 |
| Poly AU (3·1 : 1) Tracts | 5·3 | 259 | 260 | 22·9 |
| Poly AU (0·12 : 1) Tracts | 4·3 | 261 | 261 | 8·5 |
| Poly AG (2·0 : 1) Random | 6·1 | 258 | 259·5 | 19·0 |
| Poly AG (1·9 : 1) Random | 3·3 | 258 | 260 | 18·1 |
| Poly ACGU | 12·5 | — | — | 14·3 |
| Poly ACGU | 5·2 | 260 | 261 | 11·0 |
| Poly ACGU | 3·2 | 260 | 261 | 10·2 |
| Yeast RNA | 12·2 | — | — | 12·0 |
| Yeast RNA | 5·1 | 260 | 261 | 12·7 |
| RNAase resistant core | 6·2 | 259 | 260 | 10·9 |
| Dialysate | — | 262 | 262 | 4·7 |
| P5′U poly A | 11·9 | — | — | 32·0 |
| P5′U poly A | 6·7 | 258 | 259·5 | 30·5 |
| P5′U poly A | 4·0 | 258 | 259 | 23·1 |
| P5′U poly U | 5·4 | 260 | 261 | 7·8 |
| P5′U poly U | 3·4 | 260 | 261 | 7·7 |

attributed it mainly to guanylic and cytidylic[35] or polypurine segments.[36] The increase in intensity of ultraviolet absorption at $\lambda_{max.}$ on hydrolysis, by 0·2N-sodium hydroxide at 37°

[32] Clark, Todd, and Zussman, *J.*, 1951, 2952.
[33] Andersen, Hayes, Michelson, and Todd, *J.*, 1954, 1882.
[34] Magasanik and Chargaff, *Biochim. Biophys. Acta*, 1951, **7**, 396.
[35] Mihalyi, Bradley, and Knoller, *J. Amer. Chem. Soc.*, 1957, **79**, 6387.
[36] Reddi, *Biochim. Biophys. Acta*, 1958, **27**, 1.

for 24 hr., then at 20° for 24 hr., of a number of nucleotide derivatives is recorded in Table 1. Appreciable hyperchromic effects were obtained even for dinucleotides, in agreement with observations by de Garilhe and Laskowski [37] and by Sinsheimer [38] on oligodeoxynucleotides from natural sources. The low values for the dialysable products obtained from ribonucleic acid by ribonuclease are due largely to the fact that a considerable proportion of this material is mononucleotide.

The results, and those of de Garilhe and Laskowski,[37] show that the effect is a function, not of any particular tract of nucleotides, but of the total molecule, determined not only

TABLE 2.

| Compound | 0·01N-HCl | Hyperchromicity in $H_2O$ at pH 7 | 0·2N-NaOH |
|---|---|---|---|
| Diadenylic | 0 | — | 15·1 |
| Triadenylic | 6·8 | — | 22·8 |
| Tetra-adenylic | 13·2 | — | 30·7 |
| Penta-adenylic | 16·4 | — | 32·9 |
| Polyadenylic (13·6) | 22·9 | — | 36·7 |
| Polyguanylic (3·2) | 9·0 | 10·3 | 0 |
| Polyguanylic (5·4) | 14·6 | 16·5 | 0 |
| Polyguanylic (12·5) | 18·0 | 23·0 | 0 |
| Poly AG (6·2) | 14·7 | 36·7 | 19·0 |
| A2′P5′U | 12·3 | 16·0 | 12·8 |
| Polyuridylic (3·5) | 4·8 | — | 4·8 |
| Polyuridylic (6·6) | 7·5 | — | 6·5 |
| Polyuridylic (11·9) | 7·5 | — | 8·6 |

by the composition but also by the order of bases in the polymer. The influence of chain length is shown in the polyadenylic acid series, where a limiting value of 35% is reached at chain lengths of 5—6. Since polyguanylic acid showed an unexpected zero hyperchromicity, the variation of hyperchromicity with pH was examined (Table 2). The

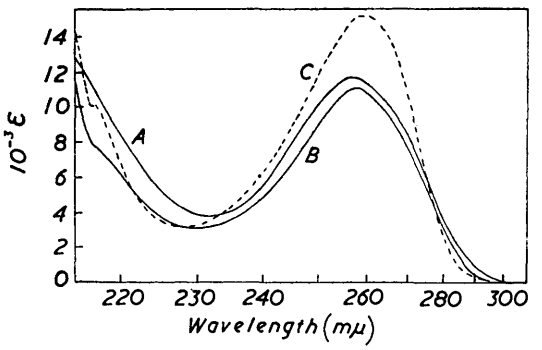



FIG. 2. *Polyadenylic acid (average chain length 13·6 units). A, in* 0·01N-HCl; *B, in* 0·01N-NaOH; *C, alkaline hydrolysis products in* 0·2N-NaOH.

effect of pH also varies both with the molar ratios of the bases and their distribution. Some full spectra are reproduced in Figs. 2—4. In water at pH 7, the spectrum of polyadenylic acid (13·6) was virtually identical with the alkaline spectrum ($H_2O$, $\lambda_{max.}$ 257·5 mμ, ε 10,750; $\lambda_{min.}$ 229·5 mμ, ε 3000), but that of polyuridylic acid (11·9) in water at pH 7 was identical with the acid spectrum ($\lambda_{max.}$ 260·5 mμ, ε 9400; $\lambda_{min.}$ 231 mμ, ε 2300). The hypochromicity of many of the polymers may be readily determined by comparing the ε values [39] with those of the mononucleotides (cf. Table 3). Good agreement with the hyperchromicities determined experimentally by degradation of the polymers was obtained in such cases.

Unlike the case with biosynthetic polymers of high molecular weight,[40] there was no

[37] de Garilhe and Laskowski, *J. Biol. Chem.*, 1956, **223**, 661.
[38] Sinsheimer, *J. Biol. Chem.*, 1954, **208**, 445.
[39] Chargaff and Zamenhoff, *ibid.*, 1948, **173**, 327.
[40] Warner, *ibid.*, 1957, **229**, 711.

TABLE 3.

| Compound | Average chain length | 0·01N-HCl $\lambda_{max.}$ (mμ) | 0·01N-HCl $10^{-3}\varepsilon$ | 0·01N-HCl $\lambda_{min.}$ (mμ) | 0·01N-HCl $10^{-3}\varepsilon$ | 0·01N-NaOH $\lambda_{max.}$ (mμ) | 0·01N-NaOH $10^{-3}\varepsilon$ | 0·01N-NaOH $\lambda_{min.}$ (mμ) | 0·01N-NaOH $10^{-3}\varepsilon$ |
|---|---|---|---|---|---|---|---|---|---|
| Poly U | 11·9 | 260·5 | 9·4 | 231 | 2·3 | 260 | 7·15 | 242 | 5·9 |
| Poly U | 6·6 | 260·5 | 9·4 | 230·5 | 2·1 | 260 | 7·25 | 242 | 5·9 |
| Poly U | 3·5 | 260·5 | 9·54 | 230 | 2·2 | 260 | 7·3 | 242 | 5·9 |
| Poly A | 13·6 | 256·5 | 11·72 | 232 | 3·8 | 257·5 | 11·05 | 229 | 3·1 |
| Poly A | 7·7 | 256·5 | 12·75 | 231 | 3·65 | 257·5 | 11·75 | 228 | 2·9 |
| Poly A | 3·3 | 257 | 13·8 | 230 | 3·4 | 258 | 12·65 | 228 | 3·0 |
| Adenosine-2′(3′) phosphate | — | 257 | 14·4 | 229 | 3·2 | 259·5 | 15·1 | 227 | 2·6 |
| Diadenylic | — | — | 14·25 * | — | — | 258·5 | 13·1 | 229 | 2·9 |
| Triadenylic | — | — | 13·3 * | — | — | 258 | 12·3 | 229·5 | 3·0 |
| Tetra-adenylic | — | — | 12·54 * | — | — | 258 | 11·5 | 230 | 2·9 |
| Penta-adenylic | — | — | 12·22 * | — | — | 258 | 11·4 | 231 | 2·7 |
| Poly G | 12·5 | 257 | 10·1 | 229 | 2·85 | 261 | 11·1 | 232 | 4·6 |
| Poly G | 5·4 | 257 | 10·5 | 229 | 2·9 | 260—265 | 11·1 | 232 | 4·3 |
| Poly G | 3·2 | 257 | 11·0 | 229 | 3·0 | 260—266 | 11·1 | 232 | 4·3 |
| A2′P5′U | — | 258 | 21·8 | 231 | 5·6 | 261 | 20·0 | 236 | 8·8 |
| A3′P5′C3′P (from RNAase digest) | — | 266 | — | 235 | — | 262 | — | 232 | — |
| Poly AU (4·5 : 1) | 5·2 | 257 | 11·6 | 230·5 | 3·1 | 259 | 10·55 | 230 | 3·25 |
| Poly AU (1·4 : 1) | 8·3 | 258 | 10·75 | 30 | 2·8 | 259 | 9·65 | 233 | 4·3 |
| Poly AU (0·8 : 1) | 6·8 | 258 | 11·1 | 30 | 2·8 | 259 | 9·8 | 234 | 5·0 |
| Poly AU (3·1 : 1) | 5·3 | 258 | 12·7 | 30 | 3·2 | 259 | 11·1 | 230 | 3·9 |
| Poly AU (0·12 : 1) | 4·3 | 260 | 9·95 | 230 | 2·4 | 260 | 8·1 | 240 | 5·9 |
| Poly AG (2·0 : 1) | 6·1 | 257 | 12·0 | 230 | — | 258 | 11·6 | 230 | — |
| Poly ACGU | 5·2 | 258 | 10·2 | 230·5 | 3·5 | 260 | 9·9 | 232·5 | 4·2 |
| Poly ACGU | 3·2 | 258 | 11·3 | 230 | 3·9 | 260 | 10·6 | 231 | 4·9 |
| Yeast RNA | 5·1 | 259·5 | 10·2 | 231·5 | 3·35 | 260 | 9·7 | 233 | 5·2 |
| RNAase resistant core | 6·2 | 257 | 11·1 | 230 | 3·5 | 259 | 10·5 | 231 | 4·4 |
| P5′U Poly A | 6·7 | 257 | 11·3 | 231 | 3·2 | 258 | 10·0 | 229 | 2·6 |
| P5′U Poly A | 4·0 | 257 | 13·4 | 230 | 3·55 | 258 | 11·7 | 229 | 2·7 |
| P5′U Poly U | 5·4 | 260 | 8·8 | 230 | 2·2 | 260 | 6·4 | 243 | 5·2 |
| P5′U Poly U | 3·4 | 260 | 8·8 | 230 | 2·2 | 260 | 6·4 | 242 | 5·1 |
| Uridine-2′(3′) phosphate | — | 262 | 10·0 | 230 | 2·0 | 261 | 7·7 | 242 | 5·3 |
| Guanosine-2′(3′) phosphate | — | 257 | 12·2 | 228 | 2·6 | 260—265 | 11·1 | 230 | 4·3 |

* At 260 mμ.

TABLE 4.

| | Average chain length | Optical density ratios: In 0·01N-HCl 280/260 mμ | In 0·01N-HCl 250/260 mμ | In 0·01N-NaOH 280/260 mμ | In 0·01N-NaOH 250/260 mμ |
|---|---|---|---|---|---|
| P5′U Poly A | 6·7 | 0·28 | 0·86 | 0·27 | 0·61 |
| P5′U Poly A | 4·0 | 0·26 | 0·86 | 0·23 | 0·81 |
| P5′U Poly U | 5·4 | 0·34 | 0·78 | 0·26 | 0·87 |
| P5′U Poly U | 3·4 | 0·32 | 0·79 | 0·23 | 0·87 |
| Poly U | 11·9 | 0·36 | 0·77 | 0·29 | 0·87 |
| Poly U | 6·6 | 0·34 | 0·77 | 0·27 | 0·87 |
| Poly U | 3·5 | 0·32 | 0·77 | 0·27 | 0·87 |
| Poly G | 12·5 | 0·64 | 0·90 | 0·61 | 0·85 |
| Poly G | 5·4 | 0·64 | 0·90 | 0·62 | 0·84 |
| Poly G | 3·2 | 0·65 | 0·93 | 0·62 | 0·86 |
| Adenosine-2′(3′) phosphate | — | 0·22 | 0·85 | 0·15 | 0·80 |
| Diadenylic | — | — | — | 0·22 | 0·80 |
| Triadenylic | — | — | — | 0·24 | 0·805 |
| Tetra-adenylic | — | — | — | 0·26 | 0·81 |
| Penta-adenylic | — | — | — | 0·285 | 0·83 |
| Poly A | 13·6 | 0·31 | 0·90 | 0·29 | 0·84 |
| Poly A | 7·7 | 0·28 | 0·87 | 0·27 | 0·82 |
| Poly A | 3·3 | 0·25 | 0·84 | 0·25 | 0·81 |

decrease in $\varepsilon_{max.}$ when equal amounts of polyuridylic and polyadenylic acid (both of chain length ~12 units) were mixed, even when the solution was made $10^{-4}$M with respect to magnesium. Lack of macromolecular interaction was confirmed by paper electrophoresis

—the mixture separated into two discrete spots identical with polymer markers, again quite unlike the behaviour of the mixed biosynthetic polymers.[40]

The small hypsochromic shift noted by Warner [40] has also been observed with some of the synthetic polymers, particularly the polyadenylic acids. Variation of optical-density ratios with increase in chain length is shown in Table 4, and Figs. 5 and 6 illustrate the anomalous ultraviolet absorption of adenosine-2′ uridine-5′ phosphate and triadenylic acid compared with the spectra obtained on alkaline degradation.

Polymerisation can also cause pronounced changes in the apparent p$K_a$ values of the bases, particularly with polyguanylic acids. Thus spectrophotometric titrations indicated

FIG. 3. *Polyguanylic acid (average chain length* 12·5 *units). A, in* 0·01N-HCl; *B, in* 0·01N-NaOH; *C, in* $H_2O$, pH 7.

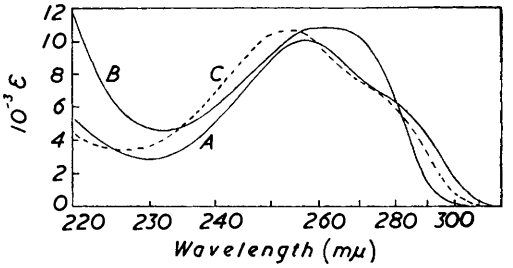



FIG. 4. *Polyuridylic acid (average chain length* 12·4 *units). A, in* 0·01N-HCl; *B, in* 0·01N-NaOH.

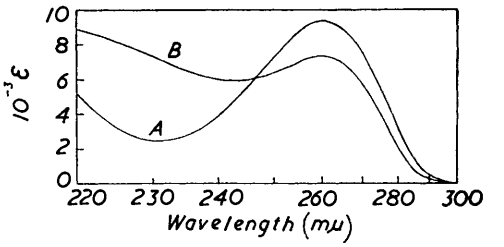



FIG. 5. *Action of alkali on adenosine-2′ uridine-5′ phosphate. A, in* 0·2N-NaOH, *before hydrolysis; B, products.*

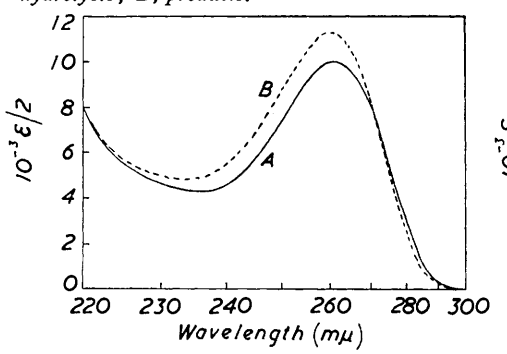
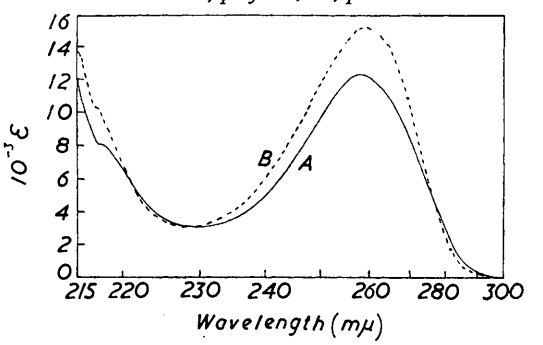



FIG. 6. *Action of alkali on triadenylic acid. A, in* 0·2N-NaOH, *polymer; B, products.*




that the apparent p$K$ of 2·32 for guanosine-2′(or 3′) phosphate was shifted to 2·63 for polyguanylic acid with an average chain length of 5·4 units, while in the alkaline region shifts from 9·33 to 10·76 (for polymer with an average chain length of 5·4 units) and to 11·20 (for polyguanylic acid, average length 12·5 units) were noted (Fig. 7).

If, as seems reasonable, even in the relatively small synthetic polymers, the purine and/or pyrimidine bases are to some extent stacked in layers above each other, interaction of π-electrons of adjacent rings [41] causes essentially a new electronic species with an ultraviolet absorption characteristic of the entire molecule, rather than a simple summation of the independent absorption of the component mononucleotides. This interaction of the π-electron systems, evident in the altered absorption spectrum, would also have an effect on the ionisable groups of the purines and pyrimidines, since they participate directly in the chromophoric systems. Also, perturbation of these groups, by hydrogen bonding or

[41] Laland, Lee, Overend, and Peacocke, *Biochim. Biophys. Acta*, 1954, **14**, 356; Scott, in " Physical Techniques in Biological Research," ed. Oster and Pollister, Academic Press, New York, 1955, Vol. I, p. 144.

ionisation, would affect the π-electron interaction of the total chromophoric system. Variation in ionic strength or other factors changing the macromolecular configuration and superstructure (*e.g.*, bonding between helices or different parts of a single helix) would likewise affect this basic interaction and hence the absorption. Indeed the influence of the macromolecular superstructure of undenatured nucleic acids seems to account for some 50% of the total hypochromicity, as shown on denaturation by mild acid, alkaline, or heat treatment.[42]

Certain planar basic dyes are bound by nucleic acids; these dyes also fail to obey Beer's law in aqueous solution, owing to molecular aggregation in the more concentrated solutions. The effect of the synthetic polymers on the absorption spectra of dilute solutions of Rosaniline, Toluidine Blue, and Acridine Orange is shown in Figs. 8—10. Equimolar amounts of dye and polymer phosphate were used in order to avoid the inter-

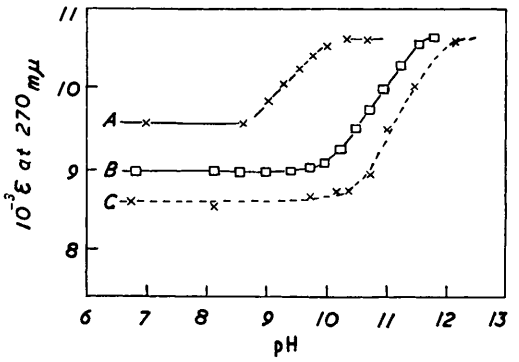



FIG. 7. *Spectrophotometric titration at* 270 mμ *of* (*A*) *guanosine-2′(3′) phosphate,* (*B*) *polyguanylic acid* (*average chain length* 5·4 *units*), *and* (*C*) *polyguanylic acid* (*average chain length* 12·5 *units*).

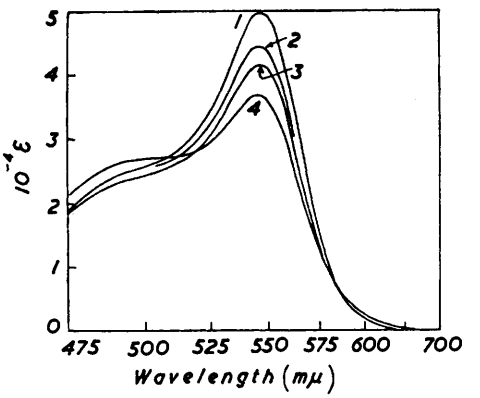



FIG. 8. *Action of polynucleotides* [2 × $10^{-5}$M-(P)] *on the absorption spectrum of Rosaniline* (2 × $10^{-5}$M) *in* 0·01M-*ammonium acetate at* pH 6·8. 1, *Dye alone;* 2, 3, *and* 4, *with added polyuridylic, polyguanylic, and commercial yeast ribonucleic acid respectively.* (All polymers with an average chain length of ~6 units.)

planar reaction between dye and purine ring systems observed between a large excess of adenosine and riboflavin,[43] and to avoid " monomeric " binding [44] to phosphate groups, which would presumably arise if a large excess of polymer were employed. In all cases the effects of the synthetic polymers were smaller than those of yeast ribonucleic acid, possibly because of distortion due to the irregular distribution of 2′–5′-internucleotide linkages in the synthetic materials. Marked quenching of the fluorescence of Acridine Orange was also observed, but again, while comparable with that due to yeast ribonucleic acid, the effect was smaller for the synthetic polymers. The low hypochromicity of polyuridylic acid at neutrality may be compared with its much smaller effects on the various dyes, and with the negligible shift in apparent p*K*. Polyglycerophosphoric acid

[42] Lawley, *Biochim. Biophys. Acta*, 1956, **21**, 481.
[43] Weber, *Biochem. J.*, 1950, **47**, 114.
[44] Lawley, *Biochim. Biophys. Acta*, 1956, **19**, 328.

produced no spectral changes or quenching of fluorescence, indicating that the phosphate binding sites must be suitably spaced to produce metachromasy in combined dyes.

The physical properties show that the synthetic polymers, although relatively small, have configurations involving stacking of purines and/or pyrimidines parallel to each other, in aqueous solution. To some extent, even dinucleotides can be regarded as having only a limited freedom of movement about the internucleotide linkage under normal conditions.

FIG. 9. *Action of polynucleotides* [3 × $10^{-5}$M-(P)] *on the absorption spectra of Acridine Orange* (3 × $10^{-5}$M) (*left*) *and Toluidine Blue* (3 × $10^{-5}$M) (*right*), *in* 0·01M-*ammonium acetate at* pH 6·8. 1, *Dye alone;* 2, 3, 4, *and* 5, *with added polyuridylic, polyadenylic, polyguanylic, and yeast ribonucleic acid respectively.*

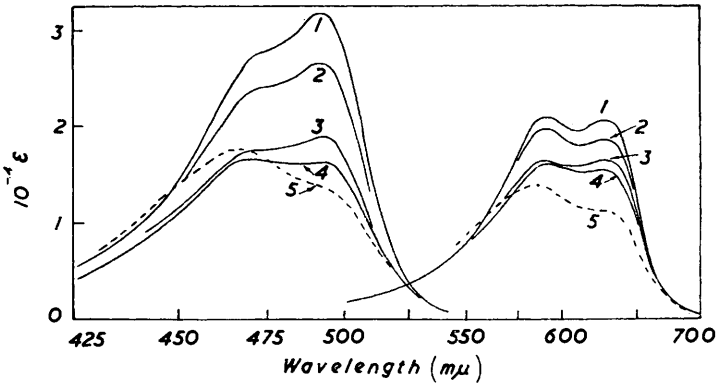
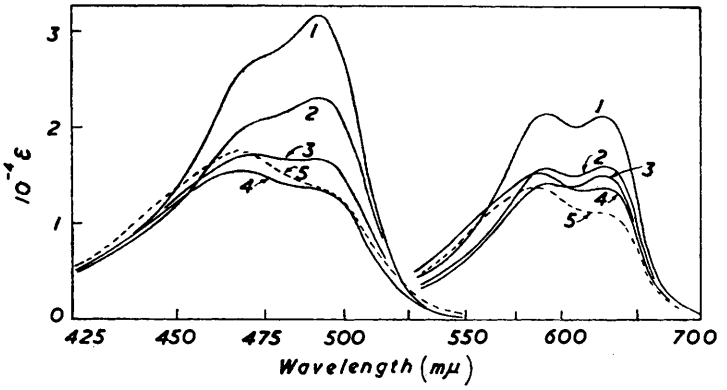



FIG. 10. *Action of polynucleotides* [3 × $10^{-5}$M)-(P)] *on the absorption spectra of Acridine Orange* (3 × $10^{-5}$M) (*left*) *and Toluidine Blue* (3 × $10^{-5}$M) (*right*), *in* 0·01M-*ammonium acetate at* pH 6·8. 1, *Dye alone;* 2, 3, 4, *and* 5, *with added synthetic "ribonucleic acid," polyadenylic-guanylic acid, ribonuclease-resistant "core" material from yeast nucleic acid, and yeast ribonucleic acid respectively.*




*Biological Properties of the Polymers.*—Dr. M. McCarty of the Rockefeller Institute or Medical Research, New York, has demonstrated an immunochemical relation between natural and synthetic polyglycerophosphoric acid. Precipitation occurred with the synthetic material and antiserum (prepared with heat-killed group A *Streptococci*) selected because of its reactivity with a bacterial substance, identified as natural polyglycerophosphoric acid. Certain other antisera, with which the synthetic material did not give precipitates, showed specific inhibition of the reaction with the bacterial antigen. Drs. D. Shugar and H. Tomerska of the Polish Academy of Sciences, Warsaw, have found that the synthetic polyguanylic acid is highly active (considerably more so than natural nucleic acids) in inducing streptolysin S formation in *Streptococci*, while Drs. G. B. Brown and J. J. Biesele of the Sloan-Kettering Institute for Cancer Research, New York, report that,

although polyuridylic acid had no action on tissues in culture, polyadenylic acid was an effective inhibitor.

## Experimental

*Polyglycerophosphoric Acid.*—A solution of glycerol 2-(disodium phosphate) (10 g.) in water (250 c.c.) was run through a column of IR-120 resin ($H^+$ form, 3 × 20 cm.). To the eluate and washings was added tri-*n*-octylamine (15 c.c.), and the mixture evaporated to dryness under reduced pressure, dioxan, ethanol, and toluene being added from time to time. The viscous residue was further dried by evaporation with more dry dioxan and toluene and finally dissolved in dioxan (35 c.c.). Diphenyl phosphorochloridate [2] (8·5 c.c., 1·25 mol.) followed by tri-*n*-butylamine (18·9 c.c., 2·5 mol.) were added, with shaking, under anhydrous conditions and the clear solution was kept at room temperature for 1½ hr. More diphenyl phosphorochloridate (8·5 c.c.) and tri-*n*-butylamine (18·9 c.c.) in dioxan (35 c.c.) were then added and the solution was kept at room temperature for a further 3 hr. After concentration under reduced pressure until a slight turbidity appeared water (30 c.c.) was added, and the mixture left at 0° overnight. Two volumes of ethanol (approx. 250 c.c.) were added, followed by an ethanolic solution of calcium chloride (3·5 g.). After ½ hr. the white precipitate of *calcium polyglycerophosphate* was collected by centrifugation, washed with acetone, then ether, and dried (4·5 g.). A further 2·3 g. were obtained from the mother-liquors by addition of acetone. The crude material was purified by reprecipitation or by dialysis against water [Found, in the dialysate specimen, dried at 90°/$10^{-3}$ mm. for 24 hr.: P, 17·85. Found in dialysis residue: P, 17·9. $(C_3H_6O_5PCa_{\frac{1}{2}})_x$ requires P, 17·9%].

*Paper Chromatography.*—Ascending chromatograms on Whatman No. 1 paper were used with solvent systems: *A*, ethanol–M-ammonium acetate (5 : 2); *B*, *tert.*-amyl alcohol–formic acid–water (3 : 2 : 1); and *C*, propan-2-ol–ammonia (*d* 0·88)–water (14 : 1 : 5). Results were:

| | $R_F$ | | |
|---|---|---|---|
| | *A* | *B* | *C* |
| β-Glycerophosphate | 0·23 | 0·54 | 0·26 |
| Glycerol-1 : 2 cyclic phosphate | 0·50 | — | 0·66 |
| Glycerol-1 : 2(3) diphosphate | 0·02 | 0·34 | 0·02 |
| Polyglycerophosphoric acid | 0 to 0·12 | 0·05 to 0·30 | 0 to 0·12 |
| Polyglycerophosphoric acid treated with 0·1N-HCl at 100°/2 hr. | 0·03 & 0·24 | 0·37 & 0·56 | 0 & 0·28 |
| Polyglycerophosphoric acid treated with 0·1N-NaOH at 37°/24 hr. | 0·02 & 0·23 | 0·34 & 0·54 | 0·05 & 0·24 |

*Action of Ribonuclease.*—Calcium polyglycerophosphate (3 mg.) and crystalline ribonuclease (0·3 mg.) were dissolved in water (0·5 c.c.), and the solution adjusted to pH 7·2 with dilute ammonia, and incubated at 37° for 24 hr. Paper chromatography showed that no degradation had occurred. Rattlesnake venom had no action on either β-glycerophosphoric acid or the polymer. Prostate monoesterase had no action on the polymer until the terminal cyclic phosphate had been opened.

*Action of Acid on Polyglycerophosphoric Acid to open the Terminal Cyclic Phosphate Group.*—Calcium polyglycerophosphate (500 mg.) was left in 0·1N-hydrochloric acid (5 c.c.) at room temperature for 3 hr., then neutralised with 2N-ammonia (0·25 c.c.). Calcium chloride was added followed by ethanol (30 c.c.). The precipitated *calcium salt* was collected by centrifugation, washed with ethanol, then ether, and dried (475 mg.) (Found in material dried at 90°/$10^{-3}$ mm. for 24 hr.: P, 17·65%. Average chain length 6·4 units). Dialysis against 2M-sodium chloride gave a residue (110 mg.) with an average chain length of 12·1 glycerophosphate units.

*Determination of Chain Length by Purified Prostate Phosphatase.*—Inorganic and total phosphate were estimated after incubation at 37° of solutions of the polymer and of glycerophosphate in 0·05M-ammonium citrate at pH 5, with 0·1 c.c. of a stock solution of purified prostate phosphatase per c.c. of substrate solution. (Phosphatase solution *ca.* 0·5 mg./c.c. Substrate 2 mg./c.c.)

After ½ hr. the β-glycerophosphate was completely dephosphorylated, and liberation of inorganic phosphate from the polymer reached a limit at 12 hr.

*Glycerol Monophosphate.*—A mixture of glycerol (5 c.c.), phosphoric acid (10 g.), and

phosphoric anhydride (10 g.) was kept at 60° for 1 hr. with exclusion of moisture and occasional stirring. The syrup was then heated in water (100 c.c.), on a water-bath for 30 min. Aqueous lithium hydroxide was added to pH 8·3, and the filtered solution passed through a column of IR-120 ($H^+$ form) to remove cations. Aqueous barium hydroxide was added to the eluate and water washings, to pH 7·4, and the precipitated barium phosphate removed by centrifugation. The supernatant liquid and washings were concentrated to 300 c.c., and the solution was boiled gently for 5 min. and filtered hot. The filtrate was concentrated to 100 c.c., two volumes of ethanol were added, and the precipitated barium glycerophosphate was collected, washed with ethanol, then acetone, and dried (7·75 g.) (Found, in material dried at 90°/$10^{-3}$ mm. for 12 hr.: P, 10·4. Calc. for $C_3H_7O_6PBa$: P, 10·1%).

*Glycerol Diphosphate.*—A mixture of glycerol (5 c.c.), phosphoric acid (20 g.), and phosphoric anhydride (25 g.) was kept at 60° for 2 hr., then dissolved in water (100 c.c.) and kept at 100° for 30 min. Aqueous lithium hydroxide was then added to pH 8·3, and the mixture left at 0° overnight, then filtered. The filtrate was concentrated to 100 c.c., excess of barium bromide added, the mixture boiled for 5 min., and the precipitated solid collected from the hot suspension. This crude salt was dissolved in the minimum quantity of 2N-hydrobromic acid and diluted to 50 c.c., and the pH adjusted to 1. Ethanol (3 vols.) was added and the precipitated *glycerol barium hydrogen diphosphate* purified by reprecipitation from the acidic solution with ethanol (10·8 g.) (Found: P, 14·6. Calc. for $C_3H_8O_9P_2Ba,2H_2O$: P, 14·7%).

*Uridine Phosphates.*—The free acid (1 mol.) was dissolved in methanol, tri-*n*-octylamine (1 mol.) added, and the solution evaporated to dryness. The residual solid foam was dried by evaporation with anhydrous dioxan and toluene and stored over $P_2O_5$ *in vacuo* at room temperature.

*Adenosine and Guanosine Phosphates.*—Tri-*n*-decylamine (1 mol.) and the free acid (1 mol.) were boiled in 1 : 1 methanol–ethanol, until dissolution was effected. The solution was then evaporated and dried as above to give a white solid foam.

*Uridine-2′ : 3′ Cyclic Phosphate.*—(a) *With diphenyl phosphorochloridate.* Uridylic acid(-2′ and -3′) (100 mg.) was converted into the anhydrous monotri-*n*-octylamine salt which was dissolved in anhydrous dioxan (5 c.c.) and benzene (5 c.c.). Diphenyl phosphorochloridate (0·105 g., 1·25 mol.) was then added followed by tri-*n*-butylamine (0·184 c.c., 2·5 mol.), and the clear solution kept at room temperature for 2 hr., with occasional shaking. Solvent was then removed under reduced pressure, and *cyclo*hexane (10 c.c.) added to the residue with shaking. The precipitated gum was dissolved in ethanol (5 c.c.) and neutralised to pH 7·4 with 2 : 6-lutidine, and ethanolic calcium chloride was added. The precipitated calcium salt was centrifuged off, washed with absolute ethanol, then ether, and dried. Reprecipitation by dissolution in a little water and addition of 1 : 1 ethanol–acetone gave the pure *calcium uridine-2′ : 3′-cyclic phosphate* (90 mg.). Paper chromatography of the initial reaction mixture showed that complete reaction occurred, the cyclic phosphate being the sole product, chromatographically identical with an authentic sample [Found, in material dried at 50°/$10^{-3}$ mm. for 12 hr.: N, 8·4; P, 9·4. Calc. for $(C_9H_{10}O_8N_2P)_2Ca$: N, 8·6; P, 9·5%].

(b) *With tetraphenyl pyrophosphate.* Tri-*n*-octylammonium uridine-2′(3′) phosphate (from 50 mg. of the nucleotide) in dioxan (2 c.c.) was added to tetraphenyl pyrophosphate [3] (94 mg., 1·25 mol.), followed by tri-*n*-butylamine (0·092 c.c., 2·5 mol.), and the clear solution was left at room temperature for 2 hr. Paper chromatography showed complete conversion into the cyclic phosphate. Ethanolic calcium chloride was added, and the precipitated calcium salt purified as described above (yield 95%).

*Adenosine-2′ : 3′ Cyclic Phosphate.*—Yeast adenylic acid (200 mg.) as the anhydrous tri-*n*-decylamine salt was dissolved in anhydrous dioxan (15 c.c.) and to the solution, stirred under anhydrous conditions, was added diphenyl phosphorochloridate (0·195 g., 1·25 mol.) in benzene (1 c.c.). The solution was kept at room temperature for 2 hr., then evaporated under reduced pressure, the residue shaken with *cyclo*hexane (20 c.c.), and the precipitated gum dissolved in ethanol (10 c.c.), neutralised to pH 7·5 with 2 : 6-lutidine, and treated with ethanolic calcium chloride. The precipitated calcium salt was centrifuged off and purified in the usual manner. (200 mg.) [Found, in material dried at 50°/$10^{-3}$ mm. for 12 hr: N, 19·9; P, 9·2. Calc. for $(C_{10}H_{11}O_6N_5P)_2Ca$: N, 20·1; P, 8·9%]. Tetraphenyl pyrophosphate gave similar results.

*Polyadenylic Acid.*—Diphenyl phosphorochloridate (0·65 c.c.) and tri-*n*-butylamine (1·25 c.c.) were added to a solution of mono(tri-*n*-decylammonium) adenosine-2′ : 3′ cyclic phosphate

[from 0·75 g. of adenosine-2′(3′) phosphate] in dioxan (10 c.c.) with stirring under anhydrous conditions. After 3 hr. at room temperature, solvent was removed under reduced pressure, and ether (50 c.c.) added to the residue with shaking. The precipitate was collected, washed with ether, and dried. The crude material was dissolved in water (10 c.c.), ammonia was added to pH 8·0, then ethanol and 2N-hydrochloric acid to precipitate the polyadenylic acid which was centrifuged off, washed with ethanol, then ether, and dried (0·72 g.).

A similar yield was obtained when the diphenyl phosphorochloridate was replaced by an equivalent amount of tetraphenyl pyrophosphate.

*Polyuridylic Acid.*—Mono(tri-*n*-octylammonium) uridine-2′(3′) phosphate (from 0·75 g. of the nucleotide) in dioxan (10 c.c.) was treated with diphenyl phosphorochloridate (0·70 c.c.) and tri-*n*-butylamine (1·40 c.c.) to prepare the cyclic phosphate. Further quantities of diphenyl phosphorochloridate (0·70 c.c.) and tri-*n*-butylamine (1·40 c.c.) were added and the solution was kept at room temperature for 3 hr. Solvent was removed under reduced pressure, ether (50 c.c.) added to the residue, and the precipitated material dissolved in water (10 c.c.), the pH being adjusted to 8·7 with ammonia. The solution was extracted twice with ether to remove amines, and the polyuridylic acid was isolated as the calcium salt (0·73 g.) by the addition of calcium chloride and ethanol. Alternatively the polymer was obtained as the magnesium salt by the addition of ethanolic magnesium chloride.

A similar run from uridine-2′(3′) phosphate (100 mg.), as the mono(tri-*n*-octylammonium) salt in dioxan (1 c.c.), and tetraphenyl pyrophosphate (376 mg.) and tri-*n*-butylamine (0·37 c.c.) gave a 100% yield of polymer.

*Polyguanylic Acid.*—An aqueous solution of disodium guanosine-2′(3′) phosphate (0·5 g.) was run through a small column of IR-120 ($H^+$ form), and to the eluate and water washings was added tri-*n*-decylamine (0·7 c.c., 1 mol.). The mixture was evaporated to dryness with ethanol, dioxan, and dimethylformamide under reduced pressure to give the anhydrous salt. This was dissolved in dioxan (4 c.c.), diphenyl phosphorochloridate (0·45 c.c.) and tri-*n*-butylamine (0·9 c.c.) were added with shaking under anhydrous conditions, and the mixture was shaken for a further 2 hr. at room temperature. To the solution of guanosine-2′ : 3′ cyclic phosphate were added more diphenyl phosphorochloridate (0·5 c.c.) and tri-*n*-butylamine (0·9 c.c.), and the mixture was evaporated to *ca.* 5 c.c. under reduced pressure, and kept at room temperature for 3 hr., then worked up in the usual way after precipitation of the crude product with a mixture of ether (15 c.c.) and *cyclo*hexane (15 c.c.), final washing being with ether. The crude product (0·4 g.) was purified by the addition of ethanol containing hydrochloric acid to a solution of the ammonium salt (250 mg.). A similar run from 3 g. of sodium guanylate yielded 1·5 g. of polymer.

*Polyadenylic-cytidylic-guanylic-uridylic Acid.*—Tri-*n*-octylamine (0·144 c.c.) was added to a mixture of uridine-2′(3′) phosphate (20 mg.), adenosine-2(3′) phosphate (35 mg.), guanosine-2(3′) phosphate (15 mg.), and cytidine-2(3′) phosphate (30 mg.) in aqueous ethanol, and the solution evaporated to dryness. The residue was dissolved in ethanol and re-evaporated to dryness under reduced pressure and finally dried by repeated evaporation to dryness of the dioxan solution, giving a solid foam. This was dissolved in dioxan (1 c.c.). Diphenyl phosphorochloridate (0·09 c.c.) and tri-*n*-butylamine (0·19 c.c.) were added and the mixture was shaken under anhydrous conditions for 1 hr. Fresh diphenyl phosphorochloridate (0·09 c.c.) and tri-*n*-butylamine (0·19 c.c.) were then added and the mixture was kept at room temperature for 4 hr. *cyclo*Hexane (10 c.c.) and ether (10 c.c.) were added with shaking, the precipitated material was washed with ether, dried, and dissolved in water (5 c.c.) with the addition of ammonia to pH 8·5, and the solution extracted twice with ether. Acetic acid was added to the aqueous layer to pH 3·5 and the solution kept at room temperature for 48 hr. in order to remove diphenyl phosphate residues from the cytidine amino-groups. Ethanol (15 c.c.) containing 2N-hydrochloric acid (0·2 c.c.) was then added and the white precipitate centrifuged off, washed with ethanol and ether, and dried (58 mg.).

The supernatant liquids yielded a further 35 mg. of calcium polynucleotide on treatment in the usual way.

A similar run from a mixture of the cyclic phosphates from 250 mg. of each nucleotide and diphenyl phosphorochloridate (1·0 c.c.) and tri-*n*-butylamine (1·8 c.c.) yielded 0·6 g. of copolymer, the dialysis residue of which had a molar ratio of adenine 3·67, guanine 2·17, uracil 2·42, and cytosine 1·0. The dialysate had adenine 2·3, guanine 1·7, uracil 1·3, and cytosine 1·0.

*Polyadenylic-guanylic Acid.*—A mixture of the mono(tri-*n*-decylammonium) salts of adenosine-2′(3′) phosphate (0·5 g. of the free acid) and guanosine-2′(3′) phosphate (from 0·5 g. of the disodium salt) in anhydrous dioxan (10 c.c.) was treated in the usual way with diphenyl phosphorochloridate (0·9 c.c.) and tri-*n*-butylamine (1·8 c.c.) to give the cyclic phosphates. Addition of the same quantity of reagents and reaction for 4 hr. at room temperature gave the mixed polymer which was isolated as the free acid (600 mg.). Dialysis against water gave a dialysis residue (380 mg.) which had a molar ratio adenine 2, guanine 1.

*Polymers containing Various Proportions of Uridylic and Adenylic Acids.*—These were prepared in the usual way from various ratios of uridylic and adenylic acid. Acid precipitation gave polymers richer in adenylic acid than the starting ratio when a large excess of adenylic acid was not used, owing to the greater solubility of polymers containing more uridylic than adenylic acid.

*Poly AU* (1 : 2).—To a solution of uridine-2′(3′) phosphate (200 mg.) and adenosine-2′(3′) phosphate (100 mg.) as the mono(tri-*n*-octylammonium) salt and mono(tri-*n*-decylammonium) salt respectively in dioxan (5 c.c.) were added tetraphenyl pyrophosphate (1·11 g. 2·5 mol.) and tri-*n*-butylamine (1·08 c.c., 5 mol.), and the solution was then evaporated to *ca.* 3 c.c. under reduced pressure and left at room temperature for 3 hr. A 1 : 1 mixture (10 c.c.) of ether and *cyclo*hexane was added, and the precipitate centrifuged off, washed with ether, and dried. The crude material was dissolved in water (4 c.c.), neutralised with aqueous ammonia, and extracted twice with ether. Three volumes of ethanol were added, followed dropwise by 2N-hydrochloric acid until precipitation was complete. The precipitate was centrifuged off, washed with alcohol, then ether, and dried (180 mg.). Degradation showed that the molar ratio of this material was adenine 1, uracil 1·25.

*Polyadenylic-uridylic Acid (Tracts).*—Adenosine-2′(3′) phosphate (250 mg.) and uridine-2′(3′) phosphate (250 mg.) were each separately converted into polymers of low molecular weight in dioxan (20 c.c.) as previously described. After 2 hr. at room temperature (paper chromatography showed largely di-, tri-, and tetra-nucleotides) the two solutions were combined and evaporated under reduced pressure to *ca.* 10 c.c. A further quantity of diphenyl phosphorochloridate (0·3 c.c.) and tri-*n*-butylamine (0·6 c.c.) was added with shaking, and the mixture kept at room temperature for 3 hr. Solvent was then removed under reduced pressure, and *cyclo*hexane (50 c.c.) added to the residue with shaking. The precipitate was washed with ether (25 c.c.) and dissolved in water (5—10 c.c.), 2 : 6-lutidine being added to neutrality. After extraction with ether, ethanol and 2N-hydrochloric acid were added to the aqueous layer to precipitate the polymer, which was washed with alcohol, acetone, and ether, and dried (385 mg.).

The mother-liquors were neutralised with 2 : 6-lutidine and alcoholic calcium chloride was added. The calcium salts were centrifuged off, washed with alcohol, then ether, and dried (145 mg.).

*Dialysis of Polymers.*—A neutral solution of each polymer (*ca.* 2 mg.) in water (2 c.c.) was dialysed in Visking tubing against two changes of water (20 c.c.), then against two changes of 2M-sodium chloride (20 c.c.) for 24 hr. each. The final dialysis residue was then diluted to 40 c.c., and the absorption of each fraction measured at 260 mμ. The results are expressed as a percentage of the total absorption. Because of the increase in hypochromicity of polynucleotides with molecular size, the actual weight percentages would be somewhat larger for the dialysis residues. It should also be emphasised that the results refer to the crude products representing a quantitative yield, before fractionation. Determination of chain length indicated that dialysis against water left a residue with an average chain length of 5—6 units. Dialysis against 2M-sodium chloride gave a residue of average chain length 10—12 mononucleotides:

| | Dialysate | | |
|---|---|---|---|
| | Water | 2M-NaCl | Residue |
| Poly U | 31·8 | 45·7 | 22·5% |
| Poly A | 24·9 | 45·5 | 29·6% |
| Poly G | 15·8 | 38·0 | 46·2% |
| P5′U Poly A | 19·9 | 46·6 | 33·5% |
| Poly AU (1·42 : 1) | 19·8 | 46·6 | 33·6% |
| Poly AU (4·54 : 1) | 20·7 | 47·0 | 32·3% |
| Poly ACGU | 18·5 | 43·3 | 38·2% |
| Yeast RNA | 14·8 | 29·8 | 55·4% |

Dialysis of concentrated solutions of the polymers against three changes of 5—10 volumes of water on a preparative basis gave the following yields (g.), slight losses occurring during the isolation:

| Compound | Amount | Dialysate | Dialysis residue |
|---|---|---|---|
| Calcium polyuridylate | 0·90 | 0·29 | 0·575 |
| Polyadenosine-2′(3′) phosphate | 0·90 | 0·34 | 0·515 |
| Polyguanosine-2′(3′) phosphate | 1·75 | 0·405 | 1·18 |
| Calcium 5′-phosphouridine-2′(3′) phosphopoly uridylate | 0·50 | 0·205 | 0·265 |
| 5′-Phosphouridine-2′(3′) phosphopolyadenosine-2′(3′) phosphate | 0·65 | 0·24 | 0·37 |
| Polyadenylicguanylic acid | 0·60 | 0·20 | 0·38 |
| Poly ACGU | 0·50 | 0·185 | 0·28 |
| 5′-(Benzyl phosphite) polyadenosine-2′(3′) phosphate | 0·90 | 0·46 | 0·15 |
| Calcium polyglycerophosphate | 5·00 | 1·70 | 2·64 |

*Purification of Commercial Yeast Ribonucleic Acid.*—The nucleic acid (10 g.) was dissolved in water with the addition of 2N-ammonia to pH 7·8, and the solution placed in Visking tubing and dialysed against five volumes of water for 24 hr. This process was repeated three times and the dialysis residue treated with 2N-hydrochloric acid and ethanol to precipitate the purified nucleic acid which was collected, washed with ethanol and ether, and dried (5·8 g.). From the concentrated combined dialysates, a similar procedure gave 2·7 g. of material. Hydrolysis to mononucleotides, and separation and estimation by the usual methods, gave the following molar ratios for the purified nucleic acid; adenine 1·0, guanine 0·98, cytosine 0·80, uracil 1·0; average chain length 5·2.

*Ribonuclease-resistant Cores from Yeast Nucleic Acid.*—To an aqueous solution of nucleic acid (10 g. in 300 c.c.) at pH 8 with ammonia, was added ribonuclease (10 mg.), and the solution was incubated at 37° for 48 hr., dilute ammonia being added occasionally to maintain pH 7·2. A further quantity of ribonuclease (5 mg.) was added after 24 hr. The solution was then dialysed against several changes of water, and the ribonuclease-resistant cores were precipitated by 2N-hydrochloric acid and ethanol, collected by centrifugation, washed with ethanol, then ether, and dried (2·4 g.). Estimation by the usual methods gave an average chain length of 6·1 units and molar ratios of adenine 1·0, guanine 1·15, cytosine 0·20, uracil 0·23.

*Adenylic-cytidylic dinucleotide* (A3′P5′C3′P) was isolated from the dialysates obtained as above, by ion-exchange chromatography on Dowex 1 × 2. Recrystallisation from water gave needles, m. p. 212° (decomp.) (Found, in material dried at 90°/$10^{-3}$ mm.: N, 17·2; P, 9·6. $C_{19}H_{26}O_{14}N_8P_2$ requires N, 17·2; P, 9·5%).

*Adenosine-2′ Uridine-5′ Phosphate.*—Prepared as previously described,[45] the crystalline material had $\lambda_{max.}$ 261 mμ (ε 21,600), $\lambda_{min.}$ 231 mμ (ε 4600) in water at pH 7.

*Viscosity.*—An average of 10 readings of 1% solutions of the sodium salts in an Ostwald viscometer gave the following results: water 76·8 sec.; yeast ribonucleic acid 79·6 sec., polyadenylic acid 79·3 sec.

*Action of Alkali on Polymers.*—Treatment with 0·2N-sodium hydroxide at 37° for 24 hr. gave the expected nucleotides or mixtures of nucleotides, separated by paper chromatography and paper electrophoresis.

*Action of Acid on Polymers.*—Treatment of polyuridylic acid with 0·1N-hydrochloric acid at 37° for 48 hr. caused hydrolysis to the mixed 2′- and 3′-mononucleotides. The polypurine nucleotides were broken down to purines by the action of N-hydrochloric acid at 100°.

*Action of Rattlesnake Venom on Polymers.*—To the substrate (approx. 2½ mg.) in glycine buffer (0·3 c.c. of 0·25M-solution at pH 9·0) were added 0·1M-magnesium chloride (0·1 c.c.) and rattlesnake (*Crotalus atrox*) venom (0·2 c.c. of a solution of 20 mg. of venom in 1 c.c. of 0·1M-potassium chloride), and the mixture was incubated at 37° for 2 days. Paper electrophoresis on Whatman No. 1 paper in 0·02M-disodium hydrogen phosphate at 10 v/cm. for 1 hr. separated the nucleoside (adenosine and uridine were also separated in this system) and nucleoside-2′(3′) : 5′ diphosphate which were eluted with 0·1N-hydrochloric acid and estimated spectroscopically to give the average chain length. Alternatively paper chromatography in ethanol–M-ammonium acetate (5 : 2) was used for the separation.

[45] Michelson, Szabo, and Todd, *J.*, 1956, 1546.

The crude venom seemed to be somewhat more active against polyuridylic acid than against polyadenylic or polyguanylic acid.

*Estimation of Chain Lengths by using Purified Prostate Monoesterase.*—Stock monoesterase (0·8 c.c. of an aqueous solution containing 1 mg./c.c.) was added to a solution of the substrate (16 mg.) in 0·05M-citrate buffer at pH 5 (8 c.c.), and the mixture incubated at 37°. Aliquot parts were removed at timed intervals, and total and inorganic phosphate were estimated by Allen's method.[46] For polyuridylic and polyguanylic acid, the procedure was modified so as to include a preliminary acid treatment to open the terminal cyclic phosphate. To a solution of calcium polyuridylate (16 mg.) in water (2 c.c.) was added 0·2N-hydrochloric acid (2 c.c.), and the solution kept at room temperature for 3 hr. Then 2N-sodium hydroxide (0·2 c.c.) was added, followed by 0·1M-citrate at pH 5 (4 c.c.) and stock phosphatase solution (0·8 c.c.), and the solution was incubated as above. Dephosphorylation of the terminal phosphate was complete in 6—12 hr.

*Action of Ribonuclease on Polyuridylic Acid.*—Ribonuclease (1·8 mg.) was added to a solution of calcium polyuridylate (250 mg.) in water (10 c.c.), and the whole was adjusted to pH 7·4 with aqueous ammonia and kept at 37° for 24 hr., 0·2N-ammonia being added to keep the pH between 7·1 and 7·4. Ethanolic calcium chloride (100 mg.) was then added, followed by ethanol (40 c.c.), and the precipitated calcium salt was centrifuged off, washed with alcohol and ether, and dried (250 mg.). A portion of this material was chromatographed on Whatman No. 1 paper (descending) with *tert.*-amyl alcohol–formic acid–water (3 : 2 : 1) as solvent; the bands of uridylic acid, U2′P5′UP, U2′P5′U2′P5′UP, U2′P5′U2′P5′U2′P5′UP, etc., were cut out, eluted with 0·1N-hydrochloric acid, and estimated spectroscopically: mononucleotide 49%, dinucleotide 27%, trinucleotide 12·5%, tetranucleotide 7%, higher polymers 4·5%. This indicates about 65% of 3′–5′- and 35% of 2′–5′-internucleotide linkages. Another specimen gave some 60% of uridylic acid.

Polyadenylic and polyguanylic acid were unaffected by ribonuclease. Mixed purine–pyrimidine polymers were broken down only slightly, owing to the variety of enzyme-resistant linkages.

*Polyadenylic Acids of Low Molecular Weight.*—Adenosine-2′(3′) phosphate (250 mg.) was converted into the cyclic phosphate in dioxan (20 c.c.) in the usual way. Further quantities of diphenyl phosphorochloridate (0·2 c.c.) and tri-*n*-butylamine (0·4 c.c.) were added with shaking; the clear solution was kept at room temperature for 2 hr., then poured into *cyclo*hexane (75 c.c.), and the precipitated material centrifuged off, washed three times with ether, and dried (230 mg.; average chain length 3·6).

*Polyuridylic Acids of Low Molecular Weight.*—A solution of uridine-2′(3′) phosphate (250 mg.) as its mono(tri-*n*-octylammonium) salt in dioxan (20 c.c.) was converted into the cyclic phosphate by diphenyl phosphorochloridate (0·21 c.c.) and tri-*n*-butylamine (0·45 c.c., 2·5 mol.) at room temperature for 1 hr. Fresh diphenyl phosphorochloridate (0·21 c.c.) and tri-*n*-butylamine (0·45 c.c.) were then added. After 2 hr. at room temperature the clear solution was poured into *cyclo*hexane (75 c.c.), and the oily precipitate dissolved in 80% ethanol, 2 : 6-lutidine being added to neutrality. An ethanolic solution of calcium chloride (200 mg.) was then added and the calcium salt was centrifuged off, washed with ethanol, then ether, and dried (260 mg.; average chain length 3·3).

*Opening of Terminal Cyclic Phosphates.*—(A) *Polyuridylic acids of low molecular weight.* The calcium salts (100 mg.) were dissolved in 0·1N-hydrochloric acid (5 c.c.) and kept at room temperature for 4 hr. and the solution was then neutralised with 2 : 6-lutidine. Calcium chloride (100 mg.) in ethanol (20 c.c.) was then added, and the precipitated calcium salt collected by centrifugation, washed with ethanol, then ether, and dried (100 mg.).

(B) *Polyadenylic acids of low molecular weight.* The polyadenylic acids (100 mg.) were dissolved in water (5 c.c.) with the addition of ammonia. 0·2N-Hydrochloric acid (5 c.c.) was then added, and the solution kept at room temperature for 4 hr. (some material was precipitated). After neutralisation of the solution with 2 : 6-lutidine to pH 7·5, magnesium chloride (100 mg.) in ethanol (30 c.c.) was added, and the precipitated magnesium salt collected, washed with ethanol, then ether, and dried (120 mg.).

*Separation of Polymers of Low Molecular Weight.*—Acid-treated polyadenylic, polyuridylic acid of low molecular weight, and ribonuclease-treated polyuridylic acid were run on Whatman

[46] Allen, *Biochem. J.*, 1940, **34**, 858.

No. 1 paper (descending) with *tert.*-amyl alcohol–formic acid–water (3 : 2 : 1) as solvent. Appropriate zones were eluted with water, and the eluates neutralised with ammonia and concentrated to small volume under reduced pressure.

*Treatment with Purified Prostate Monoesterase.*—Samples were treated at 37° for 3 hr. with the monoesterase at pH 5 in 0·05M-ammonium citrate. The uridylic acids lost the terminal phosphate group rather more slowly, as shown by paper chromatography at 1½ hr.

*Stability of* P¹-*Nucleoside*-5′ P²P²-*Diphenyl Pyrophosphates.*—Treatment of uridine-5′ phosphate and uridine-2′(3′) : 5′ diphosphate with tetraphenyl pyrophosphate gave *P*¹-uridine-5′ *P*²*P*²-diphenyl pyrophosphate and *P*¹-uridine 2′ : 3′-(cyclic phosphate) 5′-*P*²*P*²-(diphenyl pyrophosphate) quantitatively. These were hydrolysed to the corresponding phosphates with 0·1N-hydrochloric acid at room temperature. Instability to alkali was even more marked. Incubation at 37° at pH 9—10 caused complete break-down to uridine-5′ phosphate and uridine-2′(3′) : 5′ diphosphate respectively, within 48 hr.

5′-*Phosphouridine*-2′(3′) *Phosphopolyuridine*-2′(3′) *Phosphate.*—Diphenyl phosphorochloridate (0·5 c.c.) and tri-*n*-butylamine (1·00 c.c.) were added to a solution of mono(tri-*n*-octylammonium) uridine-2′(3′) phosphate (from 0·5 g. of the nucleotide) and bis(tri-*n*-octylammonium) uridine-2′(3′) : 5′ diphosphate (from 0·25 g. of the dibarium salt) in dioxan (7 c.c.), and the solution was kept at room temperature under anhydrous conditions for 1 hr. More diphenyl phosphorochloridate (0·5 c.c.) and tri-*n*-butylamine (1 c.c.) were added and after 3 hr. at room temperature the mixture was worked up as for polyuridylic acid, to give the calcium salt (0·5 g.).

5′-*Phosphouridine*-2′(3′) *Phosphopolyadenosine*-2′(3′) *Phosphate.*—This was prepared as described above, from adenosine-2′(3′) phosphate (0·5 g.) instead of uridine-2′(3′) phosphate, and with working up as for polyadenylic acid (0·5 g.). In each case an aqueous solution of the polymer was adjusted to pH 9—9·5 and kept at 37° for 24 hr. to convert the terminal *P*′-uridine-5′ *P*²*P*²-diphenyl pyrophosphate into a uridine-5′ phosphate unit.

*Uridine*-2′(3′) *Benzyl Phosphate.*—A solution of uridine-2′(3′) phosphate (0·25 g.) as the tri-*n*-octylamine salt, in dioxan (3·3 c.c.), was treated with diphenyl phosphorochloridate (0·21 c.c.) and tri-*n*-butylamine (0·45 c.c.) to convert it into uridine-2′ : 3′ cyclic phosphate. Benzyl alcohol (0·25 c.c.) followed by diphenyl phosphorochloridate (0·21 c.c.) and tri-*n*-butylamine (0·45 c.c.) were then added, and the solution was kept at room temperature overnight under anhydrous conditions. The crude benzyl esters were worked up as usual and isolated as the barium salts. In addition to a trace of uridylic acid, the product, largely uridine-2′(3′) benzyl phosphate, also contained a compound which was probably a benzyl dinucleotide. The crude product (50 mg.) was separated on a sheet of Whatman No. 1 paper, and the appropriate fraction of uridine-2′(3′) benzyl phosphate eluted with water. The action of ribonuclease (control: RNAase on uridine-2′ : 3′ cyclic phosphate), followed by separation on paper and ultraviolet estimation, showed that ~74% was uridine-3′ benzyl phosphate, the remainder being uridine-2′ benzyl phosphate unaffected even by prolonged treatment (24 hr.) with ribonuclease at 37°.

*Paper Chromatography.*—$R_F$ values are as follows for ascending chromatograms on Whatman No. 1 paper, the solvent being ethanol–M-ammonium acetate (5 : 2): Uridine-2′(3′) phosphate 0·15; uridine-2′3′ cyclic phosphate 0·47; uridine-2′(3′) benzyl phosphate 0·71; U2′(3′)P5′U2′(3′) benzyl phosphate 0·47; RNAase-treated uridine-2′ : 3′ cyclic phosphate 0·15; RNAase-treated uridine-2′(3′) benzyl phosphate 0·15 and 0·71.

*Uridine*-2′(3′) *Thymidine*-5′ *Phosphate.*—A solution of uridine-2′ : 3′ cyclic phosphate [from 250 mg. of uridine-2′(3′) phosphate] in dioxan (3·3 c.c.) was added to 3′-*O*-acetylthymidine (0·25 g. anhyd.) in dioxan (3 c.c.), followed by diphenyl phosphorochloridate (0·21 c.c., 1·25 mol.) and tri-*n*-butylamine (0·45 c.c., 2·5 mol.), and the clear solution was left at room temperature overnight. Solvent was removed under reduced pressure, and the residue shaken successively with *cyclo*hexane (10 c.c.) and ether (10 c.c.). The residue was then dissolved in 50% ethanol (20 c.c.), lithium hydroxide was added to pH 9·6, and the solution kept at this pH and at room temperature for 3 days; paper chromatography then showed that deacetylation was complete. Acetic acid was added to pH 8·4, ethanol removed under reduced pressure, and the aqueous solution diluted and run through Dowex 2 × 8 ($Cl^-$ form; 7 × 1·5 cm.) which was washed with water. The aqueous forerun contained the excess of thymidine and was discarded. The column was eluted with 0·01N-hydrochloric acid to remove uridylic acid and uridine-2′(3′) thymidine-5′ phosphate. After neutralisation of the appropriate fractions with ammonia

these were concentrated and further fractionated on sheets of Whatman No. 1 with ethanol–M-ammonium acetate as solvent (descending). The yield of dinucleoside phosphate was low.

*Adenosine-2′(3′) Thymidine-5′ Phosphate.*—A mixture of adenosine-2′ : 3′ cyclic phosphate (from 250 mg. of adenylic acid) and 3′-*O*-acetylthymidine (250 mg.) in dioxan (10 c.c.) was treated with diphenyl phosphorochloridate (0·2 c.c.) and tri-*n*-butylamine (0·4 c.c.), and worked up as for uridine-2′(3′) thymidine-5′ phosphate.

Paper chromatograms marked for ultraviolet absorbing spots and developed with the cysteine spray [28] showed a series of derivatives APT, APAPT, APAPAPT, etc. A portion of the crude solution was therefore run on sheets of Whatman No. 1 (descending) with ethanol–M-ammonium acetate (5 : 2) as solvent, and appropriate zones were cut out and eluted.

Rechromatography of each zone in *tert.*-amyl alcohol–formic acid–water (3 : 2 : 1), to remove contamination with polyadenylic acids, gave a series of samples which on hydrolysis with 0·2N-sodium hydroxide followed by chromatography (to separate the adenylic acid and thymidine, estimated in the usual way) were shown to be APT, APAPT, APAPAPT, APAPAPAPT.

*Polyadenylic Acids with a Terminal 5′-(Benzyl Phosphite) Group.*—Diphenyl phosphorochloridate (0·8 c.c.) was added to a solution of mono(tri-*n*-decylammonium) adenosine-2′(3′) phosphate (from 1 g. of the nucleotide) in dry dioxan (20 c.c.), and the solution was left at room temperature for 1 hr., then evaporated to half volume under reduced pressure. *O*-Benzyl-phosphorous *OO*-diphenylphosphoric anhydride (from 1·5 g. of benzyl hydrogen phosphite) in dioxan (10 c.c.) was then added, followed by 2 : 6-lutidine (1 c.c.) with stirring. The solution was set aside at room temperature overnight. Solvent was removed under reduced pressure, and ether (50 c.c.) added to the residue with shaking. The precipitated solid was dissolved in a little dioxan, and tri-*n*-butylamine added to neutrality. Methyl cyanide (25 c.c.) was next added and the precipitated polymer centrifuged off, washed with methyl cyanide and ether, and dried (0·5 g.) (Found, in dialysis residue, isolated in the usual way as the free acid and dried at 40°/$10^{-3}$ mm. for 12 hr.; N, 15·9; P, 11·7. Found, after drying at 80°/$10^{-3}$ mm. for 12 hr.: N, 16·6; P, 12·0%).

*5′-Methanesulphonyluridine-2′(3′) Phosphate.*—To a solution of uridine-2′ : 3′ cyclic phosphate (from 0·25 g. of uridylic acid) in dioxan (3·3 c.c.) was added methanesulphonyl chloride (0·1 c.c., 1·5 mol.) followed by tri-*n*-butylamine (0·4 c.c.), and the clear solution was left overnight at room temperature. Solvent was then removed under reduced pressure, and *cyclo*hexane (10 c.c.) added to the residue. The precipitated oil was shaken with ether (10 c.c.), the residue dissolved in 0·3N-lithium hydroxide (15 c.c.), and the solution extracted three times with ether, the extracts being discarded. The aqueous layer was kept at room temperature overnight, then neutralised with hydrochloric acid and evaporated to dryness under reduced pressure. The residue was dissolved in 80% ethanol (20 c.c.), and ethanolic calcium chloride added. The precipitated *5′-methanesulphonyluridine-2′(3′) calcium phosphate* was collected by centrifugation, washed with alcohol, then ether, and dried (290 mg.) (Found, in material dried at 90°/$10^{-3}$ mm. for 24 hr: N, 6·2; P, 6·8. $C_{10}H_{13}O_{11}N_2PSCa$ requires N, 6·4; P, 7·0%); in 0·05N-HCl: $\lambda_{max.}$ 261 mμ, $\lambda_{min.}$ 230 mμ, density ratios 280/260 mμ 0·31; 250/260 mμ 0·77; in 0·05N-NaOH; $\lambda_{max.}$ 261 mμ; $\lambda_{min.}$ 240 mμ; density ratios 280/260 mμ 0·29; 250/260 mμ 0·81.

*Action of Methanesulphonyl Chloride on Adenosine-2′ : 3′ Cyclic Phosphate.*—Mono(tri-*n*-decylammonium) adenosine-2′(3′) phosphate (from 250 mg. of the nucleotide) was converted into the cyclic phosphate in the usual way, in dioxan (10 c.c.). Methanesulphonyl chloride (0·1 c.c., 2 mol.) and tri-*n*-butylamine (0·5 c.c.) were then added and the clear solution was left at room temperature for 6 hr., then at 0° overnight. Solvent was removed under reduced pressure, ether (10 c.c.) added, and the precipitated material collected by centrifugation, washed with ether, dried, then dissolved in dioxan (20 c.c.) with tri-*n*-butylamine to neutrality. The clear solution was heated at 100° for 2 hr. and, after cooling, the precipitated cyclo*adenosine-2′ : 3′ cyclic phosphate* was collected, washed and dried (160 mg.) (Found: N, 17·1; P, 7·5. $C_{10}H_{10}O_5N_5P,5H_2O$ requires N, 17·4; P, 7·7%). Light absorption in EtOH: $\lambda_{max.}$ 272 mμ, $\lambda_{min.}$ 236 mμ; density ratios 280/260 mμ 1·17; 250/260 mμ 0·58.

*Paper Chromatography.*—Ascending chromatograms on Whatman No. 1 paper were used with solvents *A*, *B*, and *C*. Results are tabulated.

| | *A* | *B* | *C* |
|---|---|---|---|
| Adenosine-2′(3′) phosphate | 0·12 | 0·41 | 0·25 |
| Adenosine-2′ : 3′ cyclic phosphate | 0·42 | 0·44 | 0·59 |
| 5′-Methanesulphonyladenosine-2′ : 3′ cyclic phosphate | 0·52 | 0·45 | 0·63 |
| *cyclo*Adenosine-2′ : 3′ cyclic phosphate | 0·28 | 0·38 | 0·31 |
| Uridine-2′(3′) phosphate | 0·18 | 0·44 | 0·25 |
| Uridine-2′ : 3′ cyclic phosphate | 0·51 | 0·43 | 0·51 |
| 5′-Methanesulphonyluridine-2′ : 3′ cyclic phosphate | 0·58 | 0·48 | — |
| 5′-Methanesulphonyluridine-2′(3′) phosphate | 0·22 | 0·48 | 0·25 |
| Adenosine | 0·60 | 0·63 | — |
| Uridine | 0·71 | 0·67 | 0·49 |
| Guanosine-2′(3′) phosphate | 0·07 | 0·33 | — |
| Guanosine-2′ : 3′ cyclic phosphate | 0·47 | — | — |
| Cytidine-2′(3′) phosphate | 0·11 | 0·41 | — |
| Cytidine-2′ : 3′ cyclic phosphate | 0·54 | — | — |
| APAP | 0·04 | 0·22 | — |
| APAPAP | 0·01 | 0·12 | — |
| APAPAPAP | 0 | 0·04 | — |
| APAPAPAPAP | 0 | 0·01 | — |
| UPUP | 0·09 | 0·24 | — |
| UPUPUP | 0·03 | 0·14 | — |
| UPUPUPUP | 0·01 | 0·04 | — |
| UPUPUPUPUP | 0 | 0·01 | — |
| Uridine-2′(3′) : 5′ diphosphate | — | 0·27 | — |

| | | *A* | *B* | *C* |
|---|---|---|---|---|
| APA | | 0·22 | 0·27 | — |
| APAPA | | 0·07 | 0·14 | — |
| APAPAPA | | 0·02 | 0·07 | — |
| APAPAPAPA | | 0·0 | 0·04 | — |
| UPU | | 0·34 | 0·36 | — |
| UPUPU | | 0·16 | 0·21 | — |
| UPUPUPU | | 0·07 | 0·11 | — |
| UPUPUPUPU | | 0·02 | 0·05 | — |
| All polymers | 2M-NaCl dialysis residue | 0·0 | 0·0 | 0 |
| | $H_2O$ dialysis residue | 0·0 | 0·0— | 0 |
| | $H_2O$ dialysate | — | 0·13 | — |
| | | — | 0 to 0·20 | — |
| Thymidine | | 0·85 | 0·73 | — |
| U2′(3′)P5′T | | 0·52 | 0·49 | — |
| A2′P5′U | | 0·30 | 0·30 | — |
| A2′(3′)P5′T | | 0·46 | 0·44 | — |
| APAPT | | 0·16 | 0·24 | — |
| APAPAPT | | 0·05 | 0·15 | — |
| APAPAPAPT | | 0·02 | 0·08 | — |

*Paper Electrophoresis.*—Movement towards the anode is tabulated, for Whatman No. 1 paper, with (I) M/50-$KH_2PO_4$; (II) M/50-$Na_2HPO_4$; both at 10 v/cm. for 2 hr. Results (cm.) were as shown.

| | I | II |
|---|---|---|
| Adenosine-2′(3′) phosphate | 5·6 | 8·9 |
| Adenosine-2′ : 3′ cyclic phosphate | 5·2 | 5·9 |
| 5′-Methanesulphonyladenosine-2′ : 3′ cyclic phosphate | 4·9 | 5·9 |
| *cyclo*Adenosine-2′ : 3′ cyclic phosphate | 2·6 | 3·1 |
| Uridine-3′ phosphate | 7·2 | 10·5 |
| Uridine-2′ : 3′ cyclic phosphate | 6·8 | 7·8 |
| 5′-Methanesulphonyluridine-2′(3′) phosphate | 7·2 | 9·8 |

Movement of the polymer dialysis residues in solvent (I) at 25 v/cm. for 75 min. and solvent (III), 0·05M-ammonium formate, at 13 v/cm. for 2 hr., is tabulated, for Whatman No. 1 paper:

| | I | III |
|---|---|---|
| Adenosine-2′(3′) phosphate | 9·2 | 6·3 |
| Polyadenylic acid | 11·3 | 7·1 |
| Polyadenylic-uridylic acid (4·5 : 1) | 11·4 | 7·1 |
| Polyadenylic-uridylic acid (1·4 : 1) | 11·7 | — |
| Polyadenylic-uridylic acid (1 : 1·25) | 12·1 | — |
| Polyuridylic acid | 12·5 | 10·1 |
| Uridine-2′(3′) phosphate | 10·0 | 8·4 |
| Synthetic " ribonucleic acid " | — | 6·8 streak |
| Yeast ribonucleic acid | — | 6·8 streak |

Electrophoretic separation in solvent (III) at pH 3·5 at 17 v/cm. for 2 hr. showed no apparent combination between polyadenylic (8·2 cm.) and polyuridylic acid (10·1 cm.) [cf. mixture of polymers (8·1 and 10·1 cm.); copolymer of adenylic and uridylic acids (9·4 cm.)].

*Analyses* (Mr. W. Byrne).—In view of the nature of the compounds, no general attempt was made to obtain completely anhydrous materials.

| Compound | Average chain length | After drying at 60°/$10^{-3}$ mm. 12 hr. Found N (%) | Found P (%) | Reqd. in anhyd. material N (%) | P (%) | Molar ratios N : P Found | Reqd. |
|---|---|---|---|---|---|---|---|
| Poly A | 9·1 | 19·4 | 8·6 | 21·3 | 9·4 | 5·0 | 5·0 |
| Poly A | 7·7 | 19·3 | 8·4 | 21·3 | 9·4 | 5·1 | 5·0 |
| Poly A | 3·3 | 19·5 | 8·3 | 21·3 | 9·4 | 5·2 | 5·0 |
| Poly A (dried at 100°) | — | 20·0 | 9·1 | 21·3 | 9·4 | 4·9 | 5·0 |
| Poly G | 5·4 | 18·1 | 8·2 | 20·3 | 9·0 | 4·9 | 5·0 |
| Poly G | 3·2 | 17·4 | 8·1 | 20·3 | 9·0 | 4·8 | 5·0 |
| Poly U Ca | 8·2 | 8·6 | 9·1 | 8·6 | 9·5 | 2·1 | 2·0 |
| Poly U Ca | 6·6 | 7·7 | 8·4 | 8·6 | 9·5 | 2·0 | 2·0 |
| Poly U Ca | 3·5 | 7·6 | 8·2 | 8·6 | 9·5 | 2·05 | 2·0 |
| Poly U Ca (dried at 100°) | — | 8·4 | 9·3 | 8·6 | 9·5 | 2·0 | 2·0 |
| Poly ACGU | 5·2 | 15·7 | 8·7 | — | — | 4·0 | — |
| Poly ACGU | 3·2 | 16·9 | 9·1 | — | — | 4·1 | — |
| Yeast RNA | 5·1 | 15·5 | 9·1 | — | — | 3·8 | — |
| RNAase resistant cores | 6·2 | 17·1 | 8·5 | — | — | 4·5 | — |

Analyses are tabulated but results for other polymers prepared were as follows. Poly AU (4·5 : 1): N, 17·6; P, 8·9. Poly AU (4·5 : 1); N, 18·5; P, 9·5. Poly AU (1·4 : 1): N, 16·6; P, 9·2; Poly AU (0·8 : 1): N, 14·8; P, 9·5. Poly AU (0·8 : 1): N, 14·6; P, 9·3. Poly AU (3·1 : 1): N, 16·5; P, 8·7. Poly AU (0·12 : 1)Ca: N, 8·5; P, 8·3. P5′U Poly A (13·5 : 1): N, 18·8; P, 9·2. P5′U Poly A (10·4 : 1): N, 18·6; P, 9·0. P5′U Poly U Ca (16 : 1): N, 7·6; P, 9·1. P5′U Poly U Ca (12·5 : 1): N, 7·4; P, 9·1%.

*Action of Polynucleotides on the Absorption Spectra of Rosaniline, Toluidine Blue, and Acridine Orange.*—Stock solutions of the dyes ($10^{-4}$M), polynucleotides ($10^{-4}$M with respect to phosphorus), and ammonium acetate (0·1M; pH 6·8) were mixed and diluted to give solutions for spectroscopic examination which were $2 \times 10^{-5}$M with respect to Rosaniline and polynucleotide and $3 \times 10^{-5}$M in the case of Toluidine Blue and Acridine Orange. All solutions were 0·01M with respect to ammonium acetate. No metachromasy was observed with adenosine-2′(3′) phosphate, adenosine-2′ uridine-5′ phosphate, adenylic-cytidylic dinucleotide (A3′P5′C3′P), or polyglycerophosphoric acid (average chain length ~12). The effects of the other polymers (average chain length ~6) are shown in Figs. 8—10. Spectra were measured on a Perkin-Elmer recording spectrophotometer (Spectracord model 4000).

*Quenching of the Fluorescence of Acridine Orange.*—The fluorescence of Acridine Orange ($3 \times 10^{-5}$M) in 0·01M-ammonium acetate at pH 6·8 in the presence of $3 \times 10^{-5}$M(P)-polynucleotide was measured in a Beckman fluorimeter. Average chain length of the polymers both natural and synthetic was ~6 in all cases except for polyglycerophosphate (12·2). The results are expressed as a percentage of the fluorescence of Acridine Orange in the absence of polymer.

| Substance | Fluorescence |
|---|---|
| Acridine orange alone | 100 |
| Polyglycerophosphate | 99·5 |
| Adenosine-2′(3′) phosphate | 99 |
| A2′P5′U | 99 |
| A3′P5′C3′P | 98 |
| Poly U | 86 |
| Poly ACGU | 75 |
| Poly A | 62 |
| Poly G | 52 |
| Poly AG | 46 |
| RNAase resistant core | 37 |
| Yeast RNA | 30 |

*Spectrophotometric Titrations.*—Determinations of pH were made on an E.E.L. direct reading pH meter and of ultraviolet absorption density on a Beckman DU spectrophotometer. Apparent p$K$'s were calculated by using the expression $pK_\alpha = pH - \log (\varepsilon_{HA} - \varepsilon)/(\varepsilon - \varepsilon_A)$, with the following results. Guanosine-2(3′) phosphate, 2·32 and 9·33; polyguanylic acid (average chain length 5·4), 2·63 and 10·76; polyguanylic acid (average chain length 12·5), 11·20. Acid titrations were made at 290 mμ and alkaline ones at 270 mμ to utilise maximum optical changes. With polyadenylic-guanylic acid (2·0 : 1) over the range pH 8—13 at 290 mμ

a shift to p$K$ 10·5 was noted (owing, however, to the ill-defined slope of the curve, possibly due to the various combinations in the mixed polymer, this is not necessarily accurate). The polyuridylic acids gave only slight changes in the apparent p$K$'s associated with the 6-position of the pyrimidine ring, measurements being made between pH 7 and 13 at 260 mμ. Similar measurements on adenosine-2′ uridine-5′ phosphate at 270 mμ (near the isosbestic point of adenosine) indicated no significant change in p$K_a$ (9·65).

Thanks are offered to Dr. L. A. Heppel, to Mr. L. E. Hudson for mathematical assistance, to Dr. A. K. Mills for interest and encouragement, and to the Directors of Arthur Guinness Son & Co. (Dublin) Ltd. for permission to publish this work.

ARTHUR GUINNESS SON & CO. (DUBLIN) LTD.,
DUBLIN, IRELAND.

[*Received, August 20th,* 1958.]