306. *The Constitution of Yeast Ribonucleic Acid. Part XII. Synthesis of Cytidine-2′ Phosphate.*

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Cytidine-2′ phosphate has been synthesised from benzylidene cytidine by procedures which follow the general pattern of the synthesis of uridine-2′ phosphate previously described; phosphorylation was, however, effected by means of phosphorus oxychloride instead of diphenyl chlorophosphonate. The reagent attacked both amino- and sugar hydroxyl groups, the former preferentially, thus necessitating selective fission of the phospho-amide linkages. The stabilities of cytidine-2′ phosphate and cytidylic acid toward alkali are almost identical, and this confirms the view previously expressed that the facile alkaline fission of yeast ribonucleic acid is not due to a marked and inherent lability of the phospho-ester linkage in position 2′, but is a property of the polynucleotide.

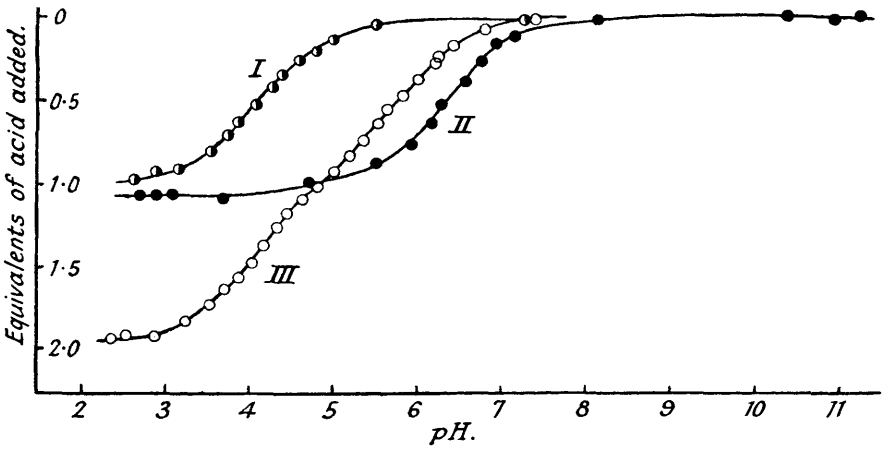
In a previous paper (Gulland and Smith, *J.*, 1947, 338) we reported the synthesis of uridine-2′ phosphate, and from its stability toward alkali concluded that there is no inherently greater lability of a phosphoryl group in the 2′, as compared with the 3′, position, and that the problem of the positions of the internucleotide linkages of yeast nucleic acid was thus reopened. We now describe the synthesis of *cytidine-2′ phosphate*, and a study of its properties reveals so close a parallelism with those of uridine-2′ phosphate that our previous conclusions receive further strong support. The general scheme of this synthesis followed the earlier work in that the 3′ and 5′ hydroxyl groups of the sugar were blocked by the benzylidene radical during phosphorylation of the 2′ position, but the presence of the amino-group introduced complications which necessitated a modification of the phosphorylation technique.

Beautifully crystalline cytidine was obtained by dephosphorylation of cytidylic acid with boiling aqueous pyridine. Its m. p. 211—214° (decomp.), unchanged by repeated recrystallisation, differed from that recorded by Levene and La Forge (*Ber.*, 1912, **45**, 608), namely sintering at 220° and decomposition at 230°; we have found no other record of the m. p. of cytidine for comparison, but the figure given above has been obtained repeatedly with different preparations. The two compounds were undoubtedly identical, since the specific rotation of our material was the same as that given by Levene and La Forge, and the m. p. of the sulphate and picrate agreed with those recorded by other workers (Levene and Jacobs, *Ber.*, 1910, **43**, 3150; Davoll, Lythgoe, and Todd, *J.*, 1946, 833).

Interaction of cytidine, benzaldehyde, and zinc chloride yielded a zinc-containing complex which could not be fractionated, but dry hydrogen chloride proved an excellent condensing agent, and a good yield of 3′ : 5′-*benzylidene cytidine* (I) was obtained. It was demonstrated that no change had occurred in the furanose ring structure of the sugar by the recovery of cytidine after hydrolysis of the benzylidene derivative with dilute acid. The titration curve of benzylidene cytidine (figure, curve I) was almost identical with that of cytidine, the pK' of the amino-group being 4·1 for the benzylidene derivative as compared with 4·2 for cytidine (Levene and Simms, *J. Biol. Chem.*, 1925, **65**, 519), and this afforded proof that the benzaldehyde had condensed with the sugar radical and not with the amino-group in the form of a Schiff's base. Determination of the position of the benzylidene group on the sugar presented difficulties. Since cytidine-5′ phosphate has not been described, the method of comparing the properties of the 2′-, 3′-, and 5′-phosphates which proved the structure of 3′ : 5′-benzylidene uridine (Gulland and Smith, *loc. cit.*) could not be applied here. For the present, therefore, knowledge of the structure of 3′ : 5′-benzylidene cytidine, and hence of the position of the phosphoryl in cytidine 2′-phosphate, rests on the analogy with the benzylidene derivatives of uridine and of guanosine (Gulland and Overend, this vol., p. 1380), on the experimentally demonstrated difference between cytidine-2′ phosphate and cytidylic acid (see below), and on the parallel behaviour of the cytidine and uridine phosphates towards alkali and acid.

The first phosphorylating agent to be examined was diphenyl chlorophosphonate, and analysis of the product by the action of one molecular proportion of this on benzylidene cytidine in dry pyridine at — 20° showed that it was a mixture of benzylidene cytidine diphenyl phosphate and a smaller quantity of benzylidene cytidine tetraphenyl diphosphate. Two properties revealed that phosphorylation had occurred preferentially at the amino-group and that the major constituent of the mixture was the diphenyl-N^6 phosphate. First, the material was insoluble in dilute mineral acid. Secondly, investigation of the behaviour of this product during attempts to remove the phenyl residues and convert it into cytidine-2′ phosphate showed that the bulk of the phosphoryl radical was converted into inorganic phosphate by mild acid or alkaline hydrolysis followed by hydrogenolysis of the surviving phenyl radical. This change could not have occurred if the phosphoryl radical had esterified a sugar hydroxyl, as will be clear from a study of the method of preparation and properties of uridine-2′ phosphate (Gulland and Smith, *loc. cit.*) and of cytidine-2′ phosphate (see below).

Attention was then turned to double phosphorylation, to the preparation of *3′ : 5′-benzylidene cytidine tetraphenyl*-N^6 : *2′ diphosphate* (II) from which the benzylidene radical and *N*-phosphoryl might be removed simultaneously by acid hydrolysis, and in which hydrogenolysis might then remove the phenyl radicals from the phosphoryl remaining in the 2′-position. Condensation of



Electrometric titrations of benzylidene cytidine (I), *the deaminated nucleotide* (II), *and cytidine-2′ phosphate* (III).

benzylidene cytidine with 2·2 mols. of diphenyl chlorophosphonate yielded the compound (II), thus confirming that phosphorylation of both the amino- and the hydroxyl group occurred and providing a parallel with the work of Levene and Tipson (*J. Biol. Chem.*, 1937, **121**, 131) on the tritylation, tosylation, and phosphorylation of adenosine or its derivatives. The compound (II) was hydrolysed with hot N/4-hydrochloric acid in methyl alcohol, and the resulting product hydrogenated at room temperature and atmospheric pressure in presence of Adams's platinum catalyst. Free ammonia was detected in the solution, from which was isolated the barium salt of a deaminated nucleotide, possibly 2-keto-2 : 3-dihydropyrimidine-3-(ribofuranosido-2′ phosphate).

The nature of the nitrogenous ring structure of this compound has not yet been fully elucidated, but the absence of an amino-group was clearly indicated by analysis and confirmed by electrometric titration (figure, curve II), in which the only dissociation group between pH3 and 11 was that of a secondary phosphoryl. It was shown that preferential hydrogenolysis of the phenyl radicals without removal of the amino-group was not possible under the conditions used; thus, hydrogenation of an equimolecular mixture of phenyl phosphate and cytidylic acid caused the absorption of that volume of hydrogen theoretically required for one phenyl group, but only half of the phenyl phosphate had been converted into inorganic phosphate whereas the theoretical quantity of ammonia corresponding to the amino-group was present.

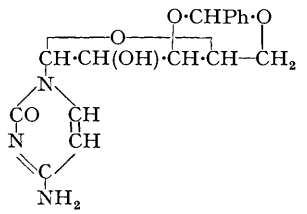
The conditions and mechanism of the aminolysis are not clear. Although Levene and Jorpes (*ibid.*, 1929, **81**, 575) did not observe the liberation of ammonia when cytidylic acid was hydrogenated using Adams's platinum catalyst, we obtained a quantitative yield of ammonia

by hydrogenating cytidylic acid at room temperature for 1½ hours in hydrogen at one atmosphere pressure. Brown and Johnson (*J. Amer. Chem. Soc.*, 1924, **46**, 702) hydrogenated cytosine at 75° for 7 hours at a pressure of 2 atmospheres in presence of platinum, and the product readily split in the acid medium into dihydrouracil and ammonia. In the present case the product of a similar reaction would contain an enolic hydroxyl group similar in its dissociation constant to that of dihydrouracil which has pK' 11·5 (Levene, Bass, and Simms, *J. Biol. Chem.*, 1926, **70**, 229); the figure, curve II, shows, however, that this group was absent.

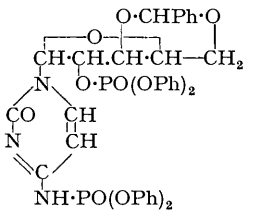
It thus became evident that in this synthesis a method of phosphorylation which did not involve hydrogenolysis would be preferable, and attention was turned to the use of phosphorus oxychloride. Attempts to protect the amino-group in the form of a Schiff's base with benzaldehyde and with *p*-nitrobenzaldehyde were unsuccessful, but better prospects were offered by simultaneous phosphorylation of the amino- and sugar hydroxyl groups followed by preferential hydrolysis of the *N*-phosphoryl radical; Levene and Tipson (*loc. cit.*) had limited success with a similar procedure in the synthesis of adenosine-5′ phosphate.

Benzylidene cytidine was phosphorylated in dry pyridine at — 20° in presence of 3·2 mols. of phosphorus oxychloride, the formation of a derivative of di- or possibly even tri-cytidine monophosphate being avoided by addition of benzylidene cytidine in small portions to the oxychloride, so as to ensure that the latter was constantly present in excess. After hydrolysis of the contents of the reaction mixture with cold water, determination of the amount of uncombined phosphoric acid showed that 3 mols. of phosphorus oxychloride had reacted. It is considered that the primary product was 3′ : 5′-benzylidene cytidine 2′ : N^6 : N^6-trisdichlorophosphinate or the corresponding N^1 : N^6-derivative. Hydrolysis of the product with N/4-hydrochloric acid at 100° for 2 hours removed the benzylidene radical and set free rather more than the theoretical quantity of phosphoric acid to correspond with two *N*-phosphoryl groups. After being isolated as the lead salt, cytidine-2′ phosphate (III) was obtained in reasonably good yield in the form of its crystalline *barium* salt, from which the crystalline nucleotide was prepared. Electrometric titration (figure, curve III) showed the presence of an amino-acid and a secondary phosphoryl group, thus proving that this compound was not a phosphoamide and that preferential hydrolysis of both *N*-phosphoryl groups had occurred. Comparison of the crystalline forms, m. p., acid-hydrolysis curves, and specific rotations of the nucleotides and of the rotations of their barium salts revealed clearly that synthetic cytidine-2′ phosphate and cytidylic acid are different, and since preferential participation by the 5′-hydroxyl in the benzylidenation and its consequent blocking seem almost certain these differences provide strong support that the synthetic nucleotide is the 2′-phospho-ester, since the long-held assumption that cytidylic acid is the 3′-derivative has now been proved (Gulland and Smith, *loc. cit.*).

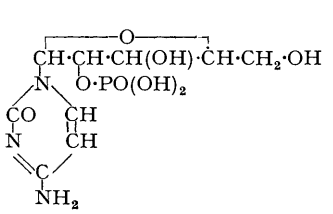
Cytidine-2′ phosphate resembled uridine-2′ phosphate in being completely stable to cold 1% sodium hydroxide for 3 days, conditions which decompose yeast ribonucleic acid into nucleotides. Comparison of the hydrolysis curves in hot acid and alkali shows a strong analogy between cytidine-2′ and -3′ phosphates and the corresponding uridine compounds; the -2′ phosphate is more stable to acid than is the -3′ phosphate, and the stabilities in alkali are closely similar, the -2′ phosphate being slightly the more labile, but the difference is far too minute to justify any suggestion of complete fission of the 2′ phosphoryl linkage in preference to the 3′ linkage (for the significance of this observation, see Gulland and Smith, *loc. cit.*).



(I.)



(II)



(III.)

Experimental.

Cytidine.—A solution of cytidylic acid, m. p. 228—232° (36 g.), in 50% aqueous pyridine (1600 c.c.) was refluxed for 51 hours, by which time about 95% of the total phosphorus had been converted into inorganic phosphate. Pyridine was removed by repeated evaporation under reduced pressure after additions of water. Addition of hot barium hydroxide solution until alkalinity to phenolphthalein was reached and filtration from barium phosphate removed inorganic phosphate. After concentration of the filtrate and washings, excess of barium hydroxide was removed by passing carbon dioxide, boiling, and

filtration. The filtrate and washings (400 c.c.) were mixed with absolute industrial alcohol (1600 c.c.), and the small amount of precipitated barium cytidylate was filtered off and washed with 80% alcohol. After concentration of the filtrate and washings and repeated evaporation with water to remove alcohol, N-sulphuric acid (164 c.c., equivalent to 95% of the cytidylic acid used) was added, and the solution was concentrated to 200 c.c., filtered through charcoal, brought to boiling point, and boiling absolute industrial alcohol (about 800 c.c.) added until there were signs of precipitation. Cytidine sulphate (22·0 g.) separated in long needles from the solution as it cooled, and was recrystallised by solution in boiling water (180 c.c.) and addition of boiling absolute industrial alcohol (700 c.c.).

Cytidine sulphate, m. p. 230—233° (22 g.), was dissolved in water (1 l.) and sulphate ions precipitated by a slight excess of barium hydroxide. After filtration, washing, and concentration of the combined filtrate and washings, excess of barium hydroxide was removed by passage of carbon dioxide, boiling, and filtration. The filtrate and washings were concentrated (60 c.c.), brought to boiling point, and boiling absolute industrial alcohol (540 c.c.) added. On cooling, cytidine (17 g.) crystallised; it recrystallised from 90% alcohol (35 pts.) in long needles, m. p. 211—214° unchanged by repeated recrystallisation, $[\alpha]_D^{17°}$ + 29·7° (*c*, 9·1) in water (Found: C, 44·3; H, 5·3; N, 17·2. Calc. for $C_9H_{13}O_5N_3$: C, 44·4; H, 5·4; N, 17·3%). The picrate separated from 95% alcohol as yellow crystals, m. p. 183—186°, after sintering at 180°. Levene and La Forge (*loc. cit.*) give the optical rotation of cytidine as $[\alpha]_D^{21°}$ + 29·63°; Levene and Jacobs (*loc. cit.*) give the m. p. of the picrate as 185—187° and of the sulphate as 233°, and Davoll, Lythgoe, and Todd (*loc. cit.*) give the m. p. of the picrate as 183°.

3′ : 5′-*Benzylidene Cytidine.*—Cytidine (8 g.) dried at 100° over phosphoric oxide in a vacuum for 4 hours, was suspended in freshly distilled benzaldehyde (80 c.c.) protected from atmospheric moisture. A rapid stream of dry hydrogen chloride was passed through the stirred suspension for 3 hours; the thick white suspension which formed at first became much less viscous at the end of the condensation. The suspension was poured into ether (800 c.c.) and washed thoroughly with saturated sodium carbonate solution (about 80 c.c.) followed by successive small quantities of water. Crude *benzylidene cytidine* (8·1 g.) was collected, washed with ether, and recrystallised twice from water (10 parts) from which it (6·8 g.) separated in colourless needles, m. p. 193—195° (Found: C, 57·8; H, 5·2; N, 12·5. $C_{16}H_{17}O_5N_3$ requires C, 58·0; H, 5·2; N, 12·7%).

Cytidine was regenerated by hydrolysis of benzylidene cytidine (1 g.) at 100° for 1 hour with N/4-sulphuric acid (100 c.c.). After removal of the benzaldehyde by extraction with ether and of the sulphuric acid by means of barium hydroxide, the solution was evaporated to a syrup which was dissolved in hot absolute alcohol (10 c.c.). On cooling, cytidine (0·6 g.) separated in long colourless needles and was recrystallised from 90% alcohol; m. p. 211—214° alone or mixed with an authentic sample; $[\alpha]_D^{15°}$ + 29·4° (*c*, 10·5).

Conversion of Benzylidene Cytidine into a Mixture of Benzylidene Cytidine Diphenyl-N⁶ Phosphate and Tetraphenyl-N⁶ : 2′ Diphosphate.—A solution of freshly distilled diphenyl chlorophosphonate (0·63 c.c.) (Brigl and Müller, *Ber.*, 1939, **72**, 2121) in dry pyridine (10 c.c.) was added during 15 minutes to a mechanically stirred solution of dry benzylidene cytidine (1 g.) in dry pyridine (20 c.c.), cooled in an ice–salt mixture and protected from moisture; stirring was continued for 2 hours in the freezing mixture and then for 1 hour at room temperature. The faintly yellow solution was again cooled in the freezing mixture, 50% aqueous pyridine (5 c.c.) was added during 15 minutes, and stirring was continued for 1 hour. The solution was poured into ice-water (200 c.c.), and the pyridine and water removed under reduced pressure, the volume being reduced to 10 c.c. After 2 successive additions of alcohol followed by evaporation under reduced pressure, the solution (30 c.c.) was cooled in a freezing mixture and poured very slowly into ice–water (100 c.c.). After further standing in the refrigerator, the colloidal suspension was flocculated by addition of saturated sodium chloride solution (2 c.c.). The solid (1·2 g.) was collected, washed with ice–water, dried in a vacuum desiccator over phosphoric oxide, dissolved in alcohol (30 c.c.), and reprecipitated as described above. The product (1·1 g.) was a white amorphous powder, insoluble in water and dilute acid (Found: N, 7·1, 7·1; P, 5·7, 5·8. Calc. for $C_{28}H_{26}O_8N_3P$: N, 7·5; P, 5·5. $C_{40}H_{35}O_{11}N_3P_2$ requires N, 5·3; P, 7·8%).

This material (0·5 g., equivalent to 28 mg. of phosphorus) was hydrolysed with hot N/2-alcoholic sodium hydroxide for 1 hour and hot N/2-alcoholic hydrochloric acid for a further 1 hour in order to remove one phenyl residue and the benzylidene residues, and the remaining phenyl radical was removed by hydrogenolysis in presence of Adams's catalyst. The bulk of the phosphorus (23 mg.) was then present as inorganic phosphate. A similar result was obtained when the preliminary alkaline hydrolysis was omitted.

3′ : 5′-*Benzylidene Cytidine Tetraphenyl-N⁶ : 2′ Diphosphate.*—Benzylidine cytidine (1 g.) was phosphorylated with 2·2 mols. of diphenyl chlorophosphonate (1·4 c.c.), and the product (1·9 g.) isolated in exactly the same manner as in the previous experiment. This *compound* was a light-brown amorphous powder which could not be crystallised; it dissolved in alcohol and chloroform but not in water or dilute acid (Found: C, 59·8; H, 4·6; N, 5·4; P, 7·9. $C_{40}H_{35}O_{11}N_3P_2$ requires C, 60·4; H, 4·4; N, 5·3; P, 7·8%).

Hydrolysis and Hydrogenation of 3′ : 5′-*Benzylidene Cytidine Tetraphenyl-N⁶ : 2′ Diphosphate. Isolation of a Deaminated Nucleotide.*—A mixture of benzylidene cytidine tetraphenyl diphosphate (1·8 g.), methyl alcohol (40 c.c.), and 1·25N-hydrochloric acid (10 c.c.) was refluxed gently for 3 hours. After the hot solution had been shaken with silver oxide (precipitated from 9·8 c.c. of 1·25 N-silver nitrate) and filtered from silver chloride to remove most of the hydrochloric acid, the filtrate and washings were evaporated under reduced pressure to about 10 c.c.; some material which came out of solution was redissolved by adding methyl alcohol (20 c.c.). The solution was shaken at room temperature with hydrogen at atmospheric pressure, Adams's catalyst being added in five portions; 903 c.c. of hydrogen were absorbed (Calc. for hydrogenolysis of 4 phenyl groups, 845 c.c.). The catalyst was filtered off and washed with warm 60% methyl alcohol. After the remainder of the chloride ions had been precipitated quantitatively with silver acetate solution and the acetic acid removed by repeated evaporation under reduced pressure, the solution was diluted with water (200 c.c.) and made alkaline to phenolphthalein with

barium hydroxide solution. The presence of ammonia was recognised by odour and confirmed with Nessler's reagent. Barium phosphate was filtered off, and excess of barium hydroxide was removed from the filtrate and washings by passing carbon dioxide, boiling, and filtering. The filtrate was evaporated under reduced pressure to 25 c.c., and the solid precipitated by addition of absolute industrial alcohol (50 c.c.) was collected by centrifuge, washed with alcohol and ether, and dried. When redissolved in water (25 c.c.) and reprecipitated as before, the deaminated *nucleotide* (0·15 g.) formed a white amorphous powder which failed to crystallise (Found : N, 5·8; P, 6·6; Ba, 32·3. $C_9H_{11}O_8N_2PBa$ requires N, 6·3; P, 7·0; Ba, 30·9%). On hydrolysis with N/10-sulphuric acid, 20% of the phosphorus was liberated as inorganic phosphate in 7 hours.

Hydrogenation of an Equimolecular Mixture of Phenyl Phosphate and Cytidylic Acid.—Disodium phenyl phosphate (5 g.) was dissolved in N-sulphuric acid (50 c.c.), and absolute industrial alcohol (500 c.c.) added. Sodium sulphate was filtered off, and the filtrate and washings evaporated under reduced pressure to 100 c.c.

6·75 C.c. of this solution (equivalent to 48 mg. of phosphorus and 139 c.c. of hydrogen for hydrogenolysis of the phenyl group) and cytidylic acid (0·5 g., equivalent to 26·3 mg. of ammonia from the amino-group) were mixed, and the solution diluted to 30 c.c. with water. When shaken at room temperature in one atmosphere of hydrogen, 0·2 g. of Adams's catalyst being added in two portions, this solution absorbed 135 c.c. of hydrogen in 1½ hours. After removal of the catalyst, analysis of aliquot portions showed the presence of inorganic phosphate (25 mg. of phosphorus) and free ammonia (25·9 mg.). Deamination thus occurred preferentially.

Cytidine-2′ Phosphate.—Preliminary experiments, not described, were carried out to determine the number of phosphoryl groups entering into organic combination, the number removed by acid hydrolysis, and the optimum duration of hydrolysis. A solution of dry benzylidene cytidine (3 g.) in dry pyridine (30 c.c.) was added in 6 portions during ½ hour to a mechanically stirred solution of freshly distilled phosphorus oxychloride (2·8 c.c.) in dry pyridine (10 c.c.), cooled in ice–salt and protected from atmospheric moisture. The reaction mixture was maintained at a low temperature for 3 hours, the temperature gradually rising to 0° and the colour changing through deep yellow to orange-red. The solution was again cooled to −18°, and 50% aqueous pyridine (20 c.c.) was added in 6 portions during ½ hour; a brown gum separated, and the mixture was kept in the freezing mixture for a further 1 hour. After the addition of water (500 c.c.) pyridine was removed under reduced pressure and the solution (180 c.c.) was mixed with N-sulphuric acid (60 c.c.) and refluxed for 2 hours, poured while hot into a boiling suspension of finely powdered silver sulphate (14 g., equivalent to 98% of the chloride ions present) in water (500 c.c.), and, after being shaken vigorously for ½ hour, freed from silver chloride by filtration. Sulphate and phosphate were removed from the combined silver-free filtrate and washings by making alkaline (phenolphthalein) with hot barium hydroxide solution and collecting and washing the precipitate with hot water. The filtrate and washings were evaporated under reduced pressure, and during the evaporation sulphuric acid was added until the solution was just acid to phenolphthalein; barium sulphate was removed and the solution extracted with ether to remove any remaining benzaldehyde. The solution (pH 7—7·5) was concentrated to 40—50 c.c., and filtered 25% lead acetate solution (at pH 7—7·5 with ammonia) added slowly until no more precipitate formed; excess of lead acetate dissolved the lead salt. The lead salt was collected, washed well with water, suspended in water (200 c.c.), and decomposed by passing hydrogen sulphide for 2 hours. After boiling, filtration from lead sulphide, and aeration, the filtrate was evaporated under reduced pressure and mixed with barium hydroxide solution until alkaline to phenolphthalein, excess of barium hydroxide being then removed by passage of carbon dioxide, boiling, and filtration. After evaporation under reduced pressure to 60 c.c., the solution was filtered and mixed with absolute industrial alcohol (120 c.c.). The precipitated barium salt was collected, washed with alcohol, dissolved in water (60 c.c.), reprecipitated, and washed as before; it was crystallised from boiling water (12 parts). The *barium* salt formed small white shining needles (Found in material dried at 100°/0·1 mm. : C, 23·4; H, 2·9; N, 9·1; P, 6·7; Ba, 29·7. $C_9H_{12}O_8N_3PBa$ requires C, 23·6; H, 2·6; N, 9·2; P, 6·8; Ba, 29·9%. Loss from air-dried material : 14·6, 16·5% of dry weight; $4H_2O$ requires 15·7%). The air-dried substance had $[\alpha]_D^{26°}$ + 5·5° (*c*, 1·39 in water).

Cytidine-2′ phosphate was prepared from the barium salt (0·4 g.) by removing the barium quantitatively from the aqueous solution, evaporating under reduced pressure to 10 c.c., adding hot absolute alcohol (20 c.c.) to the boiling solution, and allowing the mixture to cool slowly. The crystals (0·2 g.) were collected and recrystallised by dissolving in boiling water (10 c.c.) and adding hot absolute alcohol (20 c.c.) as before.

Cytidine-2′ Phosphate formed anhydrous white irregular prisms (Found : C, 33·3; H, 4·4; N, 13·0; P, 9·8. $C_9H_{14}O_8N_3P$ requires C, 33·4; H, 4·4; N, 13·0; P, 9·6%). It melted with vigorous decomposition at 240—242° (placed in bath at 230°, diameter of m. p. tube 1 mm., rate of heating 4° per minute), and had $[\alpha]_D^{18°}$ + 21·4° (*c*, 1·05 in water).

For comparison, the following properties of cytidylic acid and of its barium salt are quoted (Levene, *J. Biol. Chem.*, 1919, **39**, 80; 1920, **41**, 484; Thannhauser and Dorfmüller, *Ber.*, 1918, **51**, 467; *Z. physiol. Chem.*, 1919, **104**, 65; Bredereck and Richter, *Ber.*, 1938, **71**, 718). Acid : rectangular plates, m. p. (decomp.) 227°, 230°, 230—233°; $[\alpha]_D^{50°}$ + 48·5°, 47·5° (*c*, 2·0); $[\alpha]_D^{20°}$ + 49·1°. Air-dried salt in aqueous solution : $[\alpha]_D^{20°}$ + 14·0° (*c*, 4·0).

Hydrolysis in 0·1N-Sulphuric Acid at 100°.—(*a*) Cytidylic acid, m. p. 230—233°, was recrystallised thrice from 50% alcohol (Found : C, 33·4; H, 4·4; N, 12·7; P, 9·4. Calc. for $C_9H_{14}O_8N_3P$: C, 33·4; H, 4·4; N, 13·0; P, 9·6%). It (56·2 mg.) was dissolved in water, 0·975 N-sulphuric acid (10·28 c.c.) was added, and the solution diluted to 100 c.c. After removal of 2 control samples the remainder of the solution was transferred to a 250 c.c. " Pyrex " flask and brought rapidly to boiling. The flask was stoppered and immersed in a boiling water-bath up to the level of the liquid inside. At intervals samples were removed, cooled quickly to room temperature, and analysed for inorganic phosphate (Briggs, *J. Biol. Chem.*, 1922, **53**, 13); duplicate determinations had a maximum variation of 4%.

(*b*) The crystalline barium salt of cytidine-2′ phosphate (106·8 mg.) was dissolved in water, and

0·975N-sulphuric acid (12·72 c.c.) was added. Barium sulphate was filtered off and the solution diluted to 120 c.c. and treated exactly as above. Duplicate analyses had a maximum variation of 3%.

	Average dephosphorylation, %.							
Hours at 100°	0	2	4	6	8	20	22	24
Cytidylic acid	0	14	27	36	45	78	83	87
Cytidine-2′ phosphate	0	2	4·5	6·5	8·5	18	19·5	21

Hydrolysis in 0·1N-*Sodium Hydroxide at* 100°.—(*a*) A solution of cytidylic acid (63 mg.) in 0·998N-sodium hydroxide (12·4 c.c.) was diluted to 120 c.c. with water. The solution was treated exactly as described for the acid hydrolysis above; a greenish tinge in the colour developed for the phosphate determinations decreased the accuracy, the maximum variation being 7%.

(*b*) A solution of cytidine-2′ phosphate (70 mg.) in 0·998 N-sodium hydroxide (13·5 c.c.) was diluted to 130 c.c. with water. The solution was treated exactly as described above; here again a greenish tinge decreased the accuracy, the maximum variation being 8%.

	Average dephosphorylation, %.							
Hours at 100°	0	1	2	3	4	5	7	8
Cytidylic acid	0	2	3·5	5·5	7	9	14	16·5
Cytidine-2′ phosphate	0	2	4	6·5	8	11	16	18·5

Electrometric Titrations.—These were carried out according to the procedure described by Gulland, Jordan, and Taylor (*J.*, 1947, 1131).

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