138. *The Constitution of Yeast Ribonucleic Acid. Part IV. Syntheses of Uridylic and Guanylic Acids, Uridine-5-phosphate and Guanosine-5-phosphate.*

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Syntheses of the 3- and the 5-phosphoric esters of the nucleosides uridine and guanosine have been achieved by phosphorylation with phosphorus oxychloride in barium hydroxide and dry pyridine respectively. The hydroxyl groups were not protected, and the nature of the reagents determined which hydroxyl group of the ribose radical was esterified; barium hydroxide in water favoured 3-phosphorylation, dry pyridine that at position 5. Phenylphosphoryl dichloride has been investigated as a phosphorylating agent.

The preparation of uridine- and inosine-5-phosphates by phosphorylation in pyridine with phosphorus oxychloride was carried out by Levene and Tipson (*J. Biol. Chem.*, 1934, **106**, 113), who used the *iso*propylidene group to block the hydroxyls in positions 2 and 3 of the ribose radicals of the corresponding nucleosides. Bredereck, Berger, and Ehrenberg

(*Ber.*, 1940, **73**, 269) have just recorded the phosphorylation of 5-tritylcytidine and 2 : 3-diacetyladenosine with diphenylphosphoryl chloride in pyridine, whereby they obtained cytidine-**3**-phosphate and a small quantity of adenosine-5-phosphate. Jachimowicz (*Biochem. Z.*, 1937, **292**, 356), on the other hand, obtained adenosine-5-phosphate in poor yield by phosphorylating the nucleoside itself with phosphorus oxychloride in pyridine.

We have effected the partial synthesis of nucleotides by phosphorylation of the unprotected nucleosides, relying on variation in the reagents selected to bring about esterification of the different hydroxyl groups of the ribose radical. Phosphorylation of uridine by phosphorus oxychloride in pyridine yielded uridine-5-phosphate, identified as the brucine salt, which had the melting point and specific rotation already recorded (Levene and Tipson, *loc. cit.*). Phosphorylation of uridine with phosphorus oxychloride and baryta, a combination of reagents not hitherto used in this field, yielded a mixture of uridine-3-phosphate and uridine-5-phosphate, which were fractionated as their brucine salts. The salt of uridine-5-phosphate was identical with that obtained by phosphorylation of the nucleoside in pyridine, and the identity of brucine uridine-3-phosphate with the salt of uridylic acid prepared from yeast ribonucleic acid was shown by melting point, mixed melting point, and specific rotation. The constitutions assigned to both synthetic nucleotides were confirmed by comparison of the rates of liberation of free phosphate from them and from uridylic acid in hot N/10-acid; the curves obtained with uridylic acid and uridine-3-phosphate were identical, and the 5-phosphate was much more resistant. It is well known that the 5-phosphates are less readily hydrolysed than the 3-phosphates, and the curves very closely resembled those already published for uridine-3-and-5-phosphates (Levene and Tipson, *loc. cit.*). The 5-nucleotidase of Russell's viper venom (Gulland and Jackson, *Biochem. J.*, 1938, **32**, 597) did not dephosphorylate either uridine-3-phosphate or uridine-5-phosphate.

Phosphorylation of guanosine in pyridine with phosphorus oxychloride or phenylphosphoryl dichloride yielded a nucleotide which is regarded as *guanosine-5-phosphate* by analogy with the phosphorylations of uridine just described and of adenosine (Jachimowicz, *loc. cit.*). The yields were very small, probably on account of the poor solubility of guanosine in pyridine, but sufficient material was obtained to demonstrate that guanosine-5-phosphate is stable towards the 5-nucleotidase of Russell's viper venom, although readily dephosphorylated by purified bone monoesterase (Gulland and Jackson, *Biochem. J.*, 1938, **32**, 590). Phosphorylation of guanosine with baryta and phosphorus oxychloride or phenylphosphoryl dichloride produced guanosine-3-phosphate, the identity of which with authentic guanylic acid from yeast ribonucleic acid was proved by comparisons of specific rotations and of rates of dephosphorylation in acid solution, and by an elaborate method of mixed melting points of the brucine salts.

Whilst investigating the use of phenylphosphoryl dichloride as a phosphorylating agent, we prepared barium α-glycerophosphate from glycerol. Brigl and Müller (*Ber.*, 1939, **72**, 2121) have recently described experiments in which diphenylphosphoryl chloride is used as a phosphorylating agent.

We are continuing the phosphorylation of nucleosides, using baryta as the alkaline medium, and are investigating phenylphosphoryl dichloride as a reagent for the synthesis of dinucleotides and related compounds.

EXPERIMENTAL.

Preparation of Uridine.—A mixture of B.D.H. yeast nucleic acid (100 g.), aqueous ammonia (80 c.c., *d* 0·880), and water (420 c.c.) was heated in an autoclave for 3½ hours at an internal temperature of 140°. When cold, the viscous solution was left overnight in the refrigerator, stirred mechanically for some time to transform the precipitate of guanosine into a filterable condition, and filtered. The dark brown filtrate was neutralised to litmus with sulphuric acid and mixed with 20% lead acetate solution until no further precipitate formed. After some time the solid was collected, washed, and discarded, and 20% lead acetate and warm, saturated barium hydroxide solutions were added until precipitation was complete; excess of baryta had to be avoided. The bulky, yellowish-white precipitate of the lead salts of adenosine, cytidine and uridine was collected, washed thoroughly with cold water, and triturated with cold dilute

sulphuric acid until the solution was acid to Congo-red paper. After filtration, the precipitates of lead and barium sulphates were washed with water, and the combined solutions were shaken with barium carbonate until neutral in reaction, the barium sulphate and excess of carbonate being collected and washed. The combined solutions were evaporated to dryness under reduced pressure at 50°, and the viscous residue (25 g.) was dissolved in warm water (120 c.c.), mixed with potassium nitrite (60 g.), cooled in ice, mixed with glacial acetic acid (50 c.c.), and left overnight in a covered vessel. The mixture was diluted to 300 c.c., stirred mechanically, and neutralised by the addition of successive small quantities of barium carbonate. Excess of 20% lead acetate solution was added and the mixture was filtered. Warm barium hydroxide solution was added to the filtrate until a permanent precipitate was formed, and then lead acetate and baryta solutions were added alternately until precipitation was complete; excess of baryta had to be avoided. The lead salts of inosine and uridine were collected, washed thoroughly, decomposed by trituration with dilute sulphuric acid until acid to Congo-red, and filtered, the precipitate being again treated in a similar way. The three aqueous filtrates were combined, neutralised with barium carbonate, filtered, and concentrated to 25 c.c. at 40° under reduced pressure. Methyl alcohol (150 c.c.) was added, and after remaining overnight in the refrigerator, the solution was filtered from the inosine which had separated. The filtrate was concentrated under reduced pressure, and the syrup kept in an evacuated desiccator in presence of phosphoric oxide. After some days the gum crystallised when stirred with absolute alcohol, and the uridine was collected, washed with a little absolute alcohol, and crystallised from 95% alcohol. It formed large needles (6 g.), m. p. 163—164°.

In this preparation, it was essential that the lead acetate and barium hydroxide and carbonate were of analytical (A.R.) purity as otherwise great difficulty was experienced in crystallising the gum of crude uridine. These precautions have been adopted throughout the whole of the work now described.

Preparation of Natural Uridylic Acid.—Cytidylic acid was deaminated as described by Bredereck (*Z. physiol. Chem.*, 1934, **224**, 79), the uridylic acid being isolated as the brucine salt, which formed colourless needles, m. p. 179—181° with frothing and charring at 215°. It had $[\alpha]_D^{20^\circ}$ $-55{\cdot}0°$ in dry pyridine (*c* 1·00); Levene and Tipson (*J. Biol. Chem.*, 1934, **106**, 113) give $[\alpha]_D^{24^\circ}$ $-55{\cdot}9°$ in the same solvent.

The barium salt was prepared by extracting with chloroform a solution of the brucine salt in dilute aqueous ammonia, evaporating the aqueous layer to dryness under reduced pressure after the addition of a slight excess of baryta, dissolving the residue in warm water, precipitating the excess of barium with carbon dioxide, and concentrating the solution to small volume under reduced pressure. Barium uridylate separated in colourless needles (Found: N, 6·0; P, 6·8; Ba, 30·0. Calc. for $C_9H_{11}O_9N_2PBa$: N, 6·1; P, 6·8; Ba, 29·9%).

Phosphorylation of Uridine.

(i) *Phosphorus Oxychloride and Pyridine.*—Pyridine was dried by the distillation over barium oxide of a sample which had previously been treated with solid potassium hydroxide. Phosphorus oxychloride was freshly distilled before use.

Anhydrous uridine (0·5 g.) was dissolved in warm dry pyridine (20 c.c.), and the solution was stirred mechanically and cooled to $-20°$ whilst being protected from moisture. Phosphorus oxychloride (0·2 c.c.; 1·1 mols.) in dry pyridine (5 c.c.) was added slowly during 15 minutes to the stirred solution, which was maintained at $-20°$ for 1 hour and at $-10°$ for a further period of 1 hour. Aqueous pyridine (1 : 1; 10 c.c.) was added during 15 minutes to the bright red solution at $-30°$, and the solution was then removed from the cooling bath, left for 15 minutes, and mixed with ice-water (50 c.c.). The clear solution was shaken with silver sulphate until free from chloride, filtered, saturated with hydrogen sulphide to remove excess of silver, filtered through charcoal, aerated to remove hydrogen sulphide, and mixed with barium hydroxide solution until just alkaline to thymolphthalein. The precipitated barium phosphate was collected, and the solution evaporated to dryness under reduced pressure below 40° to remove pyridine. The white residue was dissolved in a little water, freed from excess of barium with carbon dioxide, filtered, concentrated to small volume, and poured into alcohol (4 vols.). The precipitated uridine-5-phosphate (50 mg.) was centrifuged, washed with alcohol and ether, and dried (Found : P, 6·8; Ba, 29·0. Calc. for $C_9H_{11}O_9N_2PBa$: P, 6·8; Ba, 29·9%). The brucine salt was prepared by adding to the aqueous solution of the barium salt that volume of dilute sulphuric acid required to precipitate the barium exactly, centrifuging the solution, neutralising exactly with alcoholic brucine solution, and evaporating to dryness under reduced pressure at 35°. The residue of brucine salt crystallised from 30% aqueous methyl alcohol in small,

colourless needles, m. p. 163—164° and decomp. with frothing at 198—200°, which dissolved in cold pyridine. Levene and Tipson (*J. Biol. Chem.*, 1934, **106**, 113) describe brucine uridine-5-phosphate as colourless crystals which soften at 163—165° and melt with foaming at 200°.

(ii) *Phosphorus Oxychloride and Baryta.*—Phosphorus oxychloride (7·6 g.) in ether (10 c.c.) was added in five portions during 15 minutes to uridine (2·0 g.) and powdered barium hydroxide (38 g.) shaken at 0° with water (25 c.c.) in a bottle containing agate balls. After the addition, shaking was continued for 2 hours at 0° and the solution was filtered; the residue was extracted with boiling water (100 c.c.) to remove all nitrogen and organically combined phosphorus, and then discarded. The united aqueous solutions were cooled in ice, mixed with a slight excess of sulphuric acid to remove all barium as sulphate, filtered, treated with successive small quantities of silver sulphate to remove chloride, filtered, freed from silver with hydrogen sulphide, filtered through charcoal, and aerated to remove hydrogen sulphide. The solution was almost neutralised with barium hydroxide and then shaken with barium carbonate and filtered, the residue extracted with warm water, and the neutral aqueous filtrates evaporated to dryness at 40° under reduced pressure. The residue was dissolved in warm water (20 c.c.), filtered from a little insoluble material, and poured into acetone (5 vols.). The precipitated barium salts (0·5 g.) were centrifuged, washed with alcohol and ether, and dried (Found: N, 6·1; P, 6·8; Ba, 29·5. Calc. for $C_9H_{11}O_9N_2PBa$: N, 6·1; P, 6·8; Ba, 29·9%). This material proved to be a mixture of the barium salts of uridine-3- and -5-phosphates and fractionation was effected through the brucine salts. The mixed barium salts (0·4 g.) were dissolved in warm water and freed from barium by the addition of the exact volume of sulphuric acid. After being centrifuged, the solution was neutralised to litmus by the addition of alcoholic brucine solution and evaporated to dryness under reduced pressure. The residue was dissolved in warm 33% aqueous methyl alcohol (30 c.c.) and when the solution was allowed to evaporate crystals separated and were collected. Further evaporation of the mother-liquors yielded a succession of crops of crystals with m. p.'s ranging from 175—180° for the first to 160—165° for the last.

The early crops were combined and recrystallised from 30% aqueous methyl alcohol. Brucine uridine-3-phosphate was obtained as colourless crystals, m. p. 179—181° and decomp. 215° with frothing, showing no depression of the m. p. on admixture with an authentic specimen. In dry pyridine it had $[\alpha]_D^{20^\circ}$ −54·9° (*c* 0·95). In the same solvent brucine uridylate (*ex* ribonucleic acid) had $[\alpha]_D^{20^\circ}$ −55·0° (*c* 1·00). The barium salt (Found: P, 6·8. Calc. for $C_9H_{11}O_9N_2PBa$: P, 6·8%) was prepared from the brucine salt as described in the case of the natural uridylate.

The later crops from the first fractionation were combined and purified by recrystallisation from 95% alcohol. Brucine uridine-5-phosphate, m. p. 163—164° and decomp. with frothing 195—200°, separated in colourless needles, which were shown by mixed m. p. to be identical with those obtained from the phosphorylaton of uridine in pyridine. It had $[\alpha]_D^{20^\circ}$ −69·7° in dry pyridine (*c* 0·97). Levene and Tipson (*loc. cit.*) record $[\alpha]_D^{24^\circ}$ −68·8° in the same solvent.

Rates of Dephosphorylation of Barium Uridine Phosphates.—The dried barium salts were weighed accurately, dissolved in water, and mixed with an equal volume of 1·027N/5-sulphuric acid, barium sulphate being removed by centrifugation. The total phosphorus was estimated in an aliquot portion (4·0 c.c.), and other portions were sealed in tubes which were heated by total immersion in a boiling water-bath. At appropriate intervals tubes were removed, and the free phosphate estimated in samples (4·0 c.c.) by the Bell–Doisy–Briggs colorimetric method.

A. Barium uridylate (*ex* ribonucleic acid), 36·80 mg. in 20 c.c. of water and 20 c.c. of sulphuric acid. Total P in 4·0 c.c. sample, 0·250 mg. P, 6·8%

B. Barium uridine-5-phosphate (phosphorus oxychloride in pyridine), 35·00 mg. in 20 c.c. of water and 20 c.c. of sulphuric acid. Total P in 4·0 c.c., 0·238 mg. P, 6·8%.

C. Mixed barium uridine-3- and -5-phosphates (phosphorus oxychloride in baryta), 46·00 mg. in 20 c.c. of water and 20 c.c. of sulphuric acid. Total P in 4·0 c.c., 0·315 mg. P, 6·8%.

D. Synthetic barium uridine-3-phosphate (phosphorus oxychloride in baryta), 17·90 mg. in 15 c.c. of water and 15 c.c. of sulphuric acid. Total P in 4·0 c.c., 0·160 mg. P, 6·7%.

Hours.	P, mg.	A, % hydrol.	P, mg.	B, % hydrol.	P, mg.	C, % hydrol.	P, mg.	D, % hydrol.
1·5	0·040	16·0	0·005	2·1	0·031	9·8	0·025	15·6
3	—	—	—	—	—	—	0·040	25·0
4	0·073	29·2	0·012	5·0	0·062	19·7	—	—
7	—	—	—	—	0·082	26·0	0·065	40·6
8	0·105	42·0	0·019	8·0	—	—	—	—
25	0·180	76·0	0·055	23·1	0·160	50·8	—	—
26	—	—	—	—	—	—	0·120	75·0

5-Nucleotidase and Uridine-3- and -5-phosphates.—Barium was precipitated with sodium sulphate from a solution of the mixed barium salts (20·383 mg.), and the resulting solution (5 c.c.), p_H 8·6 borate buffer (5 c.c.), and Russell's viper venom (10 mg.) were mixed and incubated at 37°, samples (2·0 c.c.) being taken. No liberation of phosphate occurred in 48 hours.

Phosphorylation of Guanosine.

(i) *Phosphorus Oxychloride and Pyridine.*—Phosphorus oxychloride (0·55 g.; 1 mol.) in anhydrous pyridine (5 c.c.) was dropped during 15 minutes into a vigorously stirred suspension of guanosine (1 g.), previously dried at 110°/0·5 mm., in an apparatus protected from moisture; guanosine was insoluble in pyridine and solution was not improved by previous heating. The mixture of reactants was stirred at 0° for 2 hours, mixed gradually with ice-cold 50% aqueous pyridine (10 c.c.) during 30 minutes, and then with ice-water (40 c.c.). The solution was rendered alkaline to thymolphthalein with a saturated solution of barium hydroxide and the precipitate was centrifuged. The centrifugate contained no organically combined phosphorus, and only unchanged guanosine was recovered from it. The precipitate was extracted five times with hot water (75 c.c.) until the white residue gave negative tests for purine (murexide) and pentose (phloroglucinol–hydrochloric acid). The combined extracts were neutralised with acetic acid and mixed with 20% lead acetate solution. The lead salt was collected, washed, decomposed by hydrogen sulphide in hot water, and the solution evaporated to dryness below 40°. The white residue was dissolved in hot water (2 c.c.) and, as it did not crystallise on cooling, was precipitated by the addition of acetone (30 c.c.), collected, washed with alcohol and ether, and dried. *Guanosine phosphate* (12 mg.) decomposed at 180—200° (Found: P, 8·6. $C_{10}H_{14}O_8N_5P$ requires P, 8·5%). It gave positive tests for purine and pentose, contained neither inorganic phosphate, barium nor chloride, and when precipitated from its acid aqueous solution with lead acetate solution, the filtrate gave no precipitate with basic lead acetate, showing the absence of guanosine.

The results of experiments at −20° and −10° were similar, the yields of nucleotide being slightly lower.

(ii) *Phenylphosphoryl Dichloride and Pyridine.*—Phenylphosphoryl dichloride (0·40 g.) in dry pyridine (2 c.c.) was added gradually to dried guanosine (0·5 g.) in dry pyridine (30 c.c.) cooled at 0° and stirred as before. After 2 hours the mixture was cooled to −15°, mixed gradually with 50% aqueous pyridine (10 c.c.) and then with a slight excess of barium hydroxide solution. The copious precipitate was collected and washed; the filtrate, which gave strong positive tests for free phenol, yielded only guanosine (30 mg.). The precipitate was dissolved in hot water, and the solution filtered from a little barium phosphate, neutralised with acetic acid, mixed with 20% lead acetate solution, and filtered whilst hot. The insoluble lead salt was washed with hot water, decomposed by hydrogen sulphide, and the solution concentrated to 5—10 c.c. below 35°, poured into alcohol (5 vols.), and placed in the refrigerator. Next day the precipitate of guanosine phosphate (10 mg.) was collected and washed with alcohol (Found: P, 8·5%). It gave positive tests for purine and pentose, and contained neither free phosphate nor free or combined phenol.

Addition of ammonia to the filtrate from the neutral lead acetate precipitation yielded a heavy precipitate from which guanosine was recovered.

Prolongation of the period of phosphorylation to 4 hours did not improve the yield.

Guanosine-5-phosphate. The nucleotide fractions from the phosphorylations in pyridine were combined and purified by conversion into the lead salt with neutral lead acetate solution, followed by regeneration with hydrogen sulphide. The resulting solution was concentrated and poured into acetone. *Guanosine-5-phosphate* was a white amorphous powder which did not crystallise and was sparingly soluble in cold water (Found in material dried at 110°: P, 8·5. $C_{10}H_{14}O_8N_5P$ requires P, 8·5%). A mixture of nucleotide (3·0 mg.), previously dissolved in a little sodium carbonate solution, M/10-magnesium sulphate (0·25 c.c.), p_H 8·6 borate buffer (2·5 c.c.), and purified bone phosphomonoesterase solution (1 c.c.) was diluted with water to 4 c.c. and incubated at 37°. After 24 hours 80% dephosphorylation had occurred. When a mixture of nucleotide (3·0 mg.), dissolved in sodium carbonate solution, Russell's viper venom (5 mg.), and p_H 8·6 borate buffer (2·5 c.c.) was diluted to 5 c.c. and incubated at 37°, no measurable dephosphorylation had occurred after 48 hours.

(iii) *Phosphorus Oxychloride and Baryta.*—Phosphorus oxychloride (3·3 g.) in ether (5 c.c.) was added to guanosine (1 g.), barium hydroxide (15 g.), and water (25 c.c.), shaken with agate balls in a glass bottle at 0° for 2 hours. The alkaline mixture was neutralised to litmus by the passage of carbon dioxide, and the solid contents of the bottle were collected and washed with

cold water (50 c.c.); the filtrate contained neither free nor combined phosphate and was discarded. The solid was extracted six times with hot water (75 c.c.), and the combined extracts were heated at 100° and mixed with neutral lead acetate solution. The hot liquid was filtered, and the residual lead salt washed with hot water (100 c.c.) and decomposed with hydrogen sulphide. The filtrate from the lead sulphide contained no chloride and was concentrated to small volume and poured into acetone (5 vols.). The precipitated product was purified by reconversion into the lead salt with neutral lead acetate, the final guanosine phosphate (0·1 g.) being a colourless powder (Found : P, 8·3%). The yield of nucleotide was not improved by prolonging the phosphorylation to 4 hours or by using a solution of the sodium salt of guanosine in place of the nucleoside itself.

(iv) *Phenylphosphoryl Dichloride and Baryta.*—Phenylphosphoryl dichloride (4·5 g.; 6 mols.) in ether (5 c.c.) was added slowly to a stirred mixture of guanosine (1 g.), powdered barium hydroxide (7·5 g.), and water (25 c.c.) at 0°. Two hours later carbon dioxide was passed into the solution until neutral, and the precipitate was collected; the filtrate contained free phenol. The subsequent treatment of the solid followed that described in the experiment using phosphorus oxychloride and baryta and yielded guanosine phosphate (10 mg.) (Found: P, 8·4%).

Guanosine-3-phosphate. The combined nucleotide fractions from the phosphorylations in baryta were purified by solution in water, precipitation with neutral lead acetate, decomposition of the lead salt in hot water with hydrogen sulphide, and concentration of the solution under reduced pressure. The colourless guanosine-3-phosphate which separated decomposed at 180—200° (Found in material dried at 110°: N, 19·4; P, 8·4. Calc. for $C_{10}H_{14}O_8N_5P$: N, 19·3; P, 8·5%). In 2% sodium hydroxide solution it had $[\alpha]_D^{20°}$ −58·0° (*c* 1·50); Levene and Bass ("Nucleic Acids," 1931, p. 226, Chemical Catalog Co., New York) give $[\alpha]_D^{25°}$ −57·0°. The brucine salt was prepared by neutralising an aqueous solution of the nucleotide with alcoholic brucine solution and recrystallising from aqueous alcohol the crystalline salt which separated; it formed white acicular crystals identical in microscopic appearance with those of authentic brucine guanylate. Comparison was made of the melting points of the brucine salts of the natural (A) and the synthetic (X) acid and of each of these salts with a mixture of equal amounts of both salts, this being the strictest comparison possible by the mixed melting-point method. The tubes were immersed in the bath at 200° and the heating was moderately rapid and uniform in all three comparisons; the successive temperatures are those of contraction, turning light brown, melting with evolution of gas, and charring.

	X.	A.
(1)	214°, 222°, 228—230°, 235—240°	213°, 222°, 230°, 238—240°
	X + A.	A.
(2)	213, 220, 230—232, 240	214, 222, 230, 238—240
	X + A.	X.
(3)	212, 220, 230, 235—240	213, 221, 230, 238—240

The rates of dephosphorylation of the natural and the synthetic acid in 0·1N-sulphuric acid were compared at 100°. A solution of guanylic acid (15·70 mg.) in water was diluted to 25 c.c., and the total P content of a sample (2·0 c.c.) was estimated colorimetrically as 1·07 mg., *i.e.*, P, 8·5%. A solution of the synthetic acid (16·65 mg.) was prepared in the same way and 2·0 c.c. contained 1·12 mg. P, *i.e.*, P, 8·4%. 2·0 C.c. quantities of each of these stock solutions were mixed with N/5-sulphuric acid (2·0 c.c.) and heated in sealed tubes in a boiling water-bath, tubes being removed at appropriate intervals for estimation of their content of free phosphate.

	Guanylic acid.		Synthetic acid.	
Hours.	P, mg.	% hydrol.	P, mg.	% hydrol.
1·5	0·427	40·0	0·470	42·0
4	0·727	68·0	0·750	67·0
9	0·889	83·0	0·940	84·0
27	1·02	95·5	1·05	94·0

When plotted, these hydrolysis curves are identical within the limits of experimental error.

With a view to providing yet further confirmation of the structure of the synthetic guanosine phosphate, if such were needed, we had hoped to split off the ribose-phosphate radical and convert it into a phospho-osazone, thus proving that the phospho-group does not esterify the hydroxyl in position 2. Unfortunately ribose-3-phosphate, prepared from xanthylic acid (for literature, see Gulland, J., 1938, 1722), lost its phosphoryl group quantitatively when

converted into an osazone, so that the projected method of confirmation was impracticable. This behaviour is analogous to that of glucose-3-phosphate, which loses its phosphoryl group when treated with phenylhydrazine (Raymond and Levene, *J. Biol. Chem.*, 1929, **83**, 619; 1930, **89**, 479; Josephson and Proffe, *Annalen*, 1930, **481**, 91), unlike glucose-4-phosphate (Raymond, *J. Biol. Chem.*, 1936, **113**, 375) and glucose-6-phosphate, which form phospho-osazones.

Phosphorylation of Glycerol.

Phenylphosphoryl Dichloride and Pyridine.—A cooled solution of phenylphosp horyl dichloride (5 g.; 1 mol.) in dry pryridine (5 c.c.) was added slowly to a stirred solution of dry glycerol (2 g.) in dry pyridine (25 c.c.), cooled in ice and protected from moisture. After 2 hours water (5 c.c.) was added, followed 15 minutes later by a further quantity (50 c.c.). Hot saturated baryta solution was added gradually to the stirred solution until it was just neutral to litmus, and after remaining overnight in the refrigerator the precipitate was discarded and the filtrate evaporated to 30 c.c. under reduced pressure at 40°. The aqueous solution, which was free from pyridine, was shaken with small quantities of silver sulphate to remove chloride, filtered, freed from excess of silver with hydrogen sulphide, neutralised with baryta solution, and evaporated to dryness at 40°. The residue was extracted with water (15 c.c.), and the solution poured into acetone (5 vols.), yielding barium α-glycerophosphate (1 g.), which was washed with acetone (Found in material dried at 100°: P, 10·2; Ba, 44·5. Calc. for $C_3H_7O_6PBa$: P, 10·1; Ba, 44·7%). It contained neither inorganic phosphate nor free or combined phenol. Estimation with periodic acid and arsenite (Fleury and Paris, *Compt. rend.*, 1933, **196**, 1416; Pyman and Stevenson, J., **1934**, 448) showed that 95% was α-glycerophosphate.

Investigation by *ad hoc* experiments which need not be detailed showed that the initial product of phosphorylation is phenyl glycerophosphate and that the phenyl group is labile. It can be removed completely from the ester by means of cold, dilute sodium hydroxide solution without simultaneous liberation of any inorganic phosphate.

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