378. *The Constitution of Yeast Ribonucleic Acid. Part III. The Nature of the Phosphatase-resistant Group.*

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The group in yeast ribonucleic acid which resists dephosphorylation by mixed bone phosphomonoesterase and Russell's viper venom is associated with the cytidylic and adenylic radicals. Cytidylic acid, or an isomer, and adenine were isolated from the nucleotide fraction after dephosphorylation had come to a standstill, and other products were guanine, guanosine, and uridine. Sweet-almond emulsin also effected only 75% dephosphorylation, and examination of the products suggests that the course of the reaction is the same as that with mixed bone and venom enzymes.

The action of mixtures of phosphatases on yeast ribonucleic acids results in the liberation of 75% of the phosphorus as inorganic phosphate, one phosphorus atom per tetranucleotide remaining in a state of organic combination (Gulland and Jackson, J., 1938, 1492). The isolation and identification of this phosphatase-resistant compound should throw light on the mode of union of the nucleotides, and a partial investigation has now been made into the nature of the products of the fission of Boehringer yeast ribonucleic acid with a mixture of the phosphomonoesterase of mutton bone and the phosphodiesterase, phosphoamidase and 5-nucleotidase of Russell's viper venom (Gulland and Jackson, *Biochem. J.*, 1938, **32**, 590, 597; Falconer, Gulland, Hobday, and Jackson, this vol., p. 907).

Fission of the nucleic acid under conditions similar to those described in the earlier paper was allowed to proceed to a standstill, as shown by periodical estimation of inorganic phosphate in aliquot samples, and the resulting mixture was worked-up for the isolation of its acidic, phosphorus-containing nucleotides on the one hand, and its non-acidic nucleosides on the other. An insoluble precipitate of guanine was collected from the reaction mixture, and the nucleoside portion was fractionated into guanosine and uridine.

The nucleotide portion was split into two fractions, both containing organically combined phosphorus. These were a barium salt (A), which has not yet been obtained pure but yielded adenine on acid hydrolysis, and a *nucleotide* (B), having the composition of cytidylic acid but possibly being isomeric with it. Comparison of the low rates of acid hydrolysis of (A) and (B) with those of adenylic and guanylic acids indicated that they are pyrimidine nucleotides. Investigation of the behaviour of (A) and (B) towards mutton bone phosphomonoesterase showed that both suffered dephosphorylation, contrary to expectation; this point requires further study. Russell's viper venom liberated no phosphate from (A) in 72 hours, but (B) slowly underwent dephosphorylation; this is the first occasion in which liberation of phosphate has been effected by this venom in the case of a derivative of a nucleic acid, and in terms of current views may possibly indicate that the phosphoryl radical is substituted for a hydrogen atom of the amino-group or of the hydroxyl at C_5 of cytidine.

Bredereck (*Ber.*, 1938, **71**, 408) and Bredereck, Caro, and Richter (*ibid.*, p. 2389) observed that emulsin preparations readily dephosphorylate yeast ribonucleic acid. In our hands hydrolyses with enzyme preparations from sweet-almond cake came to a standstill when about 75% dephosphorylation had occurred, and the addition of fresh enzyme did not cause further fission. The course of these hydrolyses thus seems to be similar to those effected by bone monoesterase and Russell's viper venom. Acid hydrolysis of the nucleotide fraction

yielded adenine and cytidine, whereas the nucleoside fraction contained guanosine and uridine.

It therefore seems to be established that the phosphatase-resistant group is associated with the cytidylic and adenylic radicals. The complete elucidation of these and similar dephosphorylations may occupy some time, and it seems desirable to record the present position in a preliminary communication.

Experimental.

Hydrolysis with Bone Phosphomonoesterase and Russell's Viper Venom.—A mixture of Boehringer yeast ribonucleic acid (5 g.), just dissolved in the minimum quantity of dilute sodium hydroxide solution, Clark and Lubs' borate buffer at p_H 8·6 (200 c.c.), purified phosphomonoesterase solution (100 c.c.), and Russell's viper venom (0·1 g.) was diluted to 500 c.c. with water and incubated at 37° in presence of toluene, samples (0·5 c.c.) being taken at appropriate intervals for estimation of inorganic phosphate by the Bell–Doisy–Briggs colorimetric method. During the early part of the hydrolysis the p_H tended to become more acid and was adjusted by the addition of sodium hydroxide.

When the liberation of phosphate had ceased (75%), the precipitate which had separated was collected, washed thoroughly with water, and identified as guanine; it dissolved in hydrochloric acid, was reprecipitated with ammonia, gave the murexide reaction, but contained no pentose.

Barium hydroxide solution was added to the reaction mixture so as to adjust the alkalinity to p_H 9·5, the barium phosphate removed, and an equal volume of industrial methylated spirit added to the clear solution to precipitate a colourless barium salt (A), which was collected. The filtrate was neutralised with acetic acid and mixed with excess of neutral lead acetate; the resulting lead salt was collected and washed [see (B) below], and the filtrate made alkaline with ammonia. The lead salts of the nucleosides thus precipitated were collected and washed.

Nucleoside fraction. The lead salts were suspended in water and decomposed by hydrogen sulphide, and after the filtrate from lead sulphide had been concentrated under reduced pressure at 40° to $\frac{1}{3}$ of its volume, it was left at 0° overnight. The white, gelatinous precipitate which separated was recrystallised from hot water and identified as guanosine; it was hydrolysed to guanine by acids, furfural being formed from the pentose.

The filtrate from the guanosine (above) gave no precipitate with picric acid and hence contained no adenosine. It was concentrated under reduced pressure to a syrup, which when stirred with industrial methylated spirit yielded uridine (Found: N, 11·4. Calc. for $C_9H_{12}O_6N_2$: N, 11·5%); it contained pentose, and when treated successively with bromine and phenylhydrazine yielded diphenylhydrazinouridine, m. p. 210° (decomp.) (Levene and La Forge, *Ber.*, 1912, **45**, 608; Levene, *J. Biol. Chem.*, 1925, **63**, 653). Concentration of the mother-liquors, followed by trituration with alcohol–ether, yielded only a further quantity of uridine (Found: N, 10·1%), which also formed diphenylhydrazinouridine.

Nucleotide fraction. The barium salt (A), forming the major part of this fraction, contained a small amount of barium phosphate and was partially purified by solution in warm water and reprecipitation with alcohol (Found: N, 9·3; P, 3·5%). When its solution in 2N-sulphuric acid was heated at 100° for 2 hours, filtered from barium sulphate, and neutralised with sodium hydroxide, adenine separated and was identified as the characteristic picrate, m. p. 278°, and the hydrochloride.

The lead salt was suspended in water and decomposed with hydrogen sulphide, and the filtrate was concentrated to small volume under reduced pressure and mixed with alcohol. The resulting precipitate of *nucleotide* (B), decomp. unsharply about 220°, contained pentose but not inorganic phosphate [Found: N, 11·4; P, 10·0; N in NH_2 (van Slyke), 4·7. $C_9H_{14}O_8N_3P$ requires N, 13·0; P, 9·3; N in NH_2, 4·3%].

Hydrolyses at 100° with 2N-sulphuric acid (10 c.c.) were carried out with the barium salt (A) (62 mg.), the nucleotide (B), and guanylic and adenylic acids (5 mg. of each), samples (0·5 c.c.) being withdrawn for estimation of inorganic phosphate. The results are tabulated below:

	Percentage liberation of phosphate.			
Time, mins.	A.	B.	Guanylic acid.	Adenylic acid.
30	2·5	6	23	20
60	5·0	12	50	48
90	7·5	20	80	92
210	14	30	100	100

Mixtures of (A) (16·4 mg., freed from barium with sodium sulphate) and (B) (11·5 mg.) respectively, and p_H 8·6 borate buffer (10 c.c.), M/10-magnesium sulphate solution (1 c.c.), and purified phosphomonoesterase solution (10 c.c.) were diluted with water to 50 c.c. (toluene) and incubated at 37°, samples (0·5 c.c.) being taken for estimation of phosphate. In 48 hours the dephosphorylation of (A) was 70% and of (B) 74%.

Dephosphorylation of (B) (10·1 mg.) was effected by Russell's viper venom (20 mg.) when a mixture (50 c.c.) with p_H 8·6 borate buffer (10 c.c.) and M/10-magnesium sulphate solution (1 c.c.) was incubated at 37° in presence of toluene. After 72 hours 37% dephosphorylation had occurred.

Hydrolysis with Sweet-almond Emulsion Preparations.—A mixture of Boehringer yeast nucleic acid (8 g.), dissolved in the minimum quantity of sodium carbonate solution, p_H 4·9 acetate buffer (400 c.c.), and 2% emulsion (200 c.c.) (Bredereck, *Ber.*, 1938, **71**, 408) was diluted to 1 l. with water (toluene) and incubated at p_H 4·9 and 37°. Samples (0·5 c.c.) were removed for estimation of inorganic phosphate, the course of the reaction being as follows :

Time, hrs.	19	72	96	120	168	188	240	264
Dephosphorylation, %	15	22	42	51	64	67	70	70

The mixture was centrifuged, and mixed with 25% lead acetate solution. The lead salts of the nucleotide fraction were collected, and the filtrate treated with more lead acetate and ammonia to precipitate the lead salts of the nucleoside fraction.

Nucleotide fraction. The lead salt was washed with water and decomposed with hydrogen sulphide, and the filtrate was concentrated to small volume under reduced pressure and heated at 100° for 3 hours with 2% sulphuric acid (25 c.c.). Silver sulphate was then added to precipitate purines, and after removal of the precipitate (see below) the solution was freed from sulphate and silver ions with barium hydroxide and hydrogen sulphide, concentrated to a syrup, and stirred with alcohol to precipitate cytidine.

The silver precipitate was washed and extracted with dilute hydrochloric acid, and the resulting solution was filtered from silver chloride and mixed with aqueous picric acid. Adenine picrate separated and formed characteristic needles, m. p. 278°, after recrystallisation.

Nucleoside fraction. The lead salts were decomposed with hydrogen sulphide in water, and after concentration of the solution guanosine separated in crystalline form and was collected. The filtrate contained uridine, which was obtained slightly impure by evaporation of the solution under reduced pressure and stirring of the residue with alcohol.

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