43. *The Constitution of Yeast Ribonucleic Acid. Part V. Synthesis of Yeast Adenylic Acid.*

By Geoffrey R. Barker and J. Masson Gulland.

Yeast adenylic acid has been synthesised by phosphorylation of adenosine.

The method devised by Gulland and Hobday (J., 1940, 746) for phosphorylating nucleosides at the 3-position of the carbohydrate radical with phosphoryl chloride and baryta has now been applied to the conversion of adenosine into yeast adenylic acid (adenosine 3-phosphate). The identity of the product with an authentic sample was demonstrated by comparison of their specific rotations and rates of dephosphorylation by acid, and by the failure of the 5-nucleotidase of Russell's viper venom (Gulland and Jackson, *Biochem. J.*, 1938, **32**, 597) to effect dephosphorylation.

Experimental.

A mixture of adenosine (1 g.), finely powdered (60 mesh) barium hydroxide (30 g.), and water (25 c.c.) in a pulversing mill containing agate balls was cooled in ice and salt, and the mill and freezing mixture were shaken mechanically. A solution of phosphorus oxychloride (3·8 g.) in ether (5 c.c.) was added during an hour and the shaking was continued for a further 2 hours. The mixture was diluted with water and neutralised to litmus by passage of carbon dioxide, and the precipitate collected and washed free from chloride with water (ppt. A).

The filtrate was freed from chloride by the addition of a little acetic acid and silver acetate solution, and after removal of the silver chloride, the solution was concentrated to about 25 c.c. under reduced pressure. The addition of neutral lead acetate produced no precipitate, indicating that no nucleotide was present, but the addition of ammonia caused the formation of a slight precipitate, from which a small quantity of adenosine was recovered.

Precipitate A was extracted six times with boiling water (75 c.c. each), and the combined extracts were

concentrated to small volume under reduced pressure and mixed with excess of lead acetate. The precipitated lead salt was collected, suspended in hot water (150 c.c.), and decomposed with hydrogen sulphide, and the solution was filtered from lead sulphide, concentrated to small volume under reduced pressure, and poured into alcohol. After standing overnight in the refrigerator, the nucleotide (0·14 g.) was centrifuged, and the products from several experiments were combined, reconverted into the lead salt, and the filtrate resulting from its decomposition as before was concentrated and poured into alcohol. The yeast adenylic acid was collected and washed with alcohol and ether (Found in material dried at 110° : N, 20·6; P, 8·9. Calc. for $C_{10}H_{14}O_7N_5P$: N, 20·2; P, 8·9%). In 2% sodium hydroxide solution (*c* 0·664), it had $[\alpha]_D^{20}$ — 60·9°; Levene (*J. Biol. Chem.*, 1920, **41**, 483) gives — 59·5°, Embden and Schmidt (*Z. physiol. Chem.*, 1929, **181**, 135) — 56·0°, both in the same solvent.

A mixture of this acid (10 mg.), Russell's viper venom (2 mg.), and p_H 8·6 borate buffer (5 c.c.) was diluted to 10 c.c. with water, containing toluene, and incubated at 37°. Samples (2 c.c.) were withdrawn at intervals for the colorimetric estimation of inorganic phosphate, but none was liberated during 2 hours.

The acid (9·081 mg.) was dissolved in water (15·0 c.c.), and portions (2·0 c.c.) were mixed with an equal volume of N/5-sulphuric acid, sealed in glass tubes, and heated at 100°. Tubes were withdrawn at intervals, and the free phosphate in their contents determined colorimetrically. The percentage dephosphorylation, calculated on the basis of the inorganic phosphate liberated and the total phosphorus content of the initial solution, was compared with that which occurred in an identical experiment with authentic yeast adenylic acid.

Time, hrs.	1	2	4	8
Dephosphorylation of synthetic adenylic acid, % ...	35·3	58·4	77·4	91·5
,, ,, yeast ,, ,, , % ...	33·2	58·8	74·7	90·6

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UNIVERSITY COLLEGE, NOTTINGHAM. [*Received, January 2nd*, 1942.]