The formation of uridine pyrophosphoglucuronic acid from uridine pyrophosphoglucose by extracts of a noncapsulated strain of pneumococcus*

The isolation of UPPG** and UPPGA from the noncapsulated strain of *Streptococcus pneumoniae* R19, derived from a strain of capsular type II, has been described[1]. The inability to demonstrate spectrophotometrically by the method of STROMINGER *et al.*[2] the conversion of UPPG to UPPGA by extracts of this organism has been found to be due to the presence in them of an extremely active DPNH oxidase (H. BERNHEIMER: unpublished observations). The present communication describes the formation of UPPGA from UPPG by appropriately prepared extracts of this strain of pneumococcus, the reaction being demonstrated by chromatographic techniques.

A noncapsulated strain of pneumococcus, R19 (kindly supplied by Dr. R. D. HOTCHKISS, The Rockefeller Institute for Medical Research, New York, N.Y., U.S.A.) derived from the capsulated type II strain, D39S, was employed. Glucuronic acid is a constituent of type II capsular polysaccharide. The organism was grown in 500 ml of Difco Brain-Heart-Infusion medium containing 0.1 % neopeptone. After incubation overnight, glucose was added to a final concentration of 1 % and the lactic acid formed on subsequent incubation was neutralized with 3 *N* NaOH according to the method of MACLEOD AND KRAUSS[3]. The bacteria were harvested by centrifugation at 1500 × *g* for 20 min at 4°, the packed cells suspended in 10 ml 0.067 *M* phosphate buffer, pH 6.6, and subjected to disruption in a Mickle disintegrator at 0° for 30 min in the presence of 2 drops of *n*-octanol and an amount of dry Ballotini equivalent to the volume of packed cells. The suspension of disrupted pneumococci was dialysed against several changes of distilled water at 0° for 48 h, the resultant cellular debris and precipitate were removed by centrifugation and solid $(NH_4)_2SO_4$ was added to the supernatant to a concentration of 3 *M* followed by equilibration at 0° for 18 h. The precipitate formed was then removed by filtration in the presence of Standard Super-Cel (Celite 519 A, Johns-Manville Corporation) and the filter cake was eluted with 10-ml portions of decreasing molarities of $(NH_4)_2SO_4$ as described by SMITH AND MILLS[4]. The amount of $(NH_4)_2SO_4$ in each fraction of eluate was adjusted to a final concentration of 3 *M* with the solid salt; the resulting precipitates were collected by centrifugation at 0°, dissolved in a minimum volume of water and dialysed overnight at 0°.

300 µl of the enzyme extract eluted between 2.0 *M* $(NH_4)_2SO_4$ and 1.5 *M* $(NH_4)_2SO_4$ were incubated with 2 ml of 0.1 *M* TRIS buffer, pH 9.1, 2 µmoles of chromatographically pure UPPG and 5 µmoles DPN for 30 min at room temperature. The reaction was stopped by the addition of $HClO_4$ to a final concentration of 1 % and the precipitated protein was eliminated by centrifugation. DPN and DPNH were removed from the deproteinised solution by passing it through a Dowex-50 H^+ column according to the method of SMITH AND MILLS[5] for the removal of AMP. The nucleotides remaining in the effluent from the column were adsorbed on to Norite and prepared for chromatography by the method of SMITH AND MILLS[5].

Chromatography of the nucleotides was carried out in the neutral ethanol–ammonium acetate solvent of PALADINI AND LELOIR[6] and a component was obtained having the same R_F value as UPPGA. After washing of the paper with absolute ethanol, this component was eluted from the chromatogram with water and the material was identified as UPPGA by methods

Material	$R_{glucose}$	
	Ethanol–ammonium acetate solvent	*Butanol–acetic acid–water solvent*
Glucuronolactone	1.04	1.80
Glucuronic acid	0.70	0.63
Galacturonic acid	0.66	0.55
Hydrolysed chromatogram extract	1.04	1.78

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** The following abbreviations have been used throughout: UPPGA = uridine pyrophosphoglucuronic acid; UPPG = uridine pyrophosphoglucose; DPN = diphosphopyridine nucleotide; DPNH = reduced diphosphopyridine nucleotide; AMP = adenosine-5′-monophosphate; UMP = uridine-5′-monophosphate; UPP = uridine pyrophosphate; TRIS = *tris*(hydroxymethyl)aminomethane.

described previously[5,7]. Further confirmatory evidence for identity of the uronic acid component of this nucleotide was obtained by hydrolysis of the eluted material with *N* HCl (100°, 15 min) followed by evaporation in vacuo and chromatography in the neutral ethanol–ammonium acetate solvent[6] and in the butanol–acetic acid–water solvent of PARTRIDGE[8]. The method of hydrolysis yields the lactone of glucuronic acid which can be separated readily from glucuronic and galacturonic acids in the solvents employed.

The evidence recorded indicates that the strain of pneumococcus under study is capable of oxidising UPPG to UPPGA with an enzyme system dependent upon DPN.

Although it had been found previously[1] that disruption of pneumococci with Ballotini in a Mickle disintegrator is accompanied by appreciable loss of enzymic activity, the presence of a small amount of *n*-octanol will reduce significantly the inactivation of several enzymes under the conditions employed.

Unfractioned extracts of pneumococcus produced in the Mickle disintegrator are capable of breaking down rapidly uridine pyrophosphoglycosyl compounds to UPP and UMP, presumably because of the presence of phosphatases and of organic pyrophosphatases. Fractionation with $(NH_4)_2SO_4$ employing Celite as an adsorbent appears to remove the major portion of such hydrolytic enzymes, for the breakdown of uridine pyrophosphoglycosyl compounds by the fractions so obtained is reduced significantly.

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Two-dimensional paper chromatography of higher fatty acids

Reversed-phase paper chromatography has been proved to be the most satisfactory method for the separation of longer-chain fatty acids[1–4]. The main disadvantage is the incomplete separation of various "critical pairs" of acids (*e.g.* palmitic–oleic, myristic–linoleic), the R_F values of which are the same.

KOBRLE AND ZAHRADNÍK[5] converted the unsaturated acids to the halogen derivatives with the reagent of Hanuš, but the separation from the saturated acids was rather incomplete. INOUYE *et al.*[6] prepared the mercuric acetate addition products of unsaturated fatty acid esters which were detected on the chromatograms by the sensitive color reaction with diphenylcarbazone. The esters of saturated acids give no color reaction and do not interfere. Oxidation of unsaturated acids with alkaline $KMnO_4$ and comparison of the spectrum of the total acids with that of the saturated acids left after oxidation was described by MICHALEC[7]. SCHLENK[8] has recently suggested a one-dimensional technique at low temperatures using papers impregnated with silicone for the separation of the saturated and unsaturated higher fatty acids.

Our modification using two-dimensional chromatography is a combination of the separation at laboratory and lower temperatures. Fig. 1 shows the results obtained with a synthetic mixture of saturated and unsaturated fatty acids while Fig. 2 shows the results obtained with human blood serum.