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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Received November 25th, 1957

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.

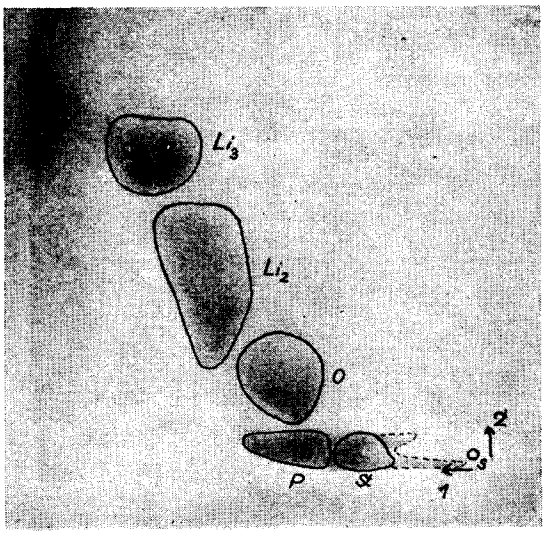

Fig. 1. Synthetic mixture of higher fatty acids. St, stearic acid; P, palmitic acid; O, oleic acid; Li_2, linoleic acid; Li_3, linolenic acid. S represents the starting point; direction 1, 93% acetic acid at 20°; 2, 85% formic acid–acetic acid–water (50:50:5) at —8° (16 h).

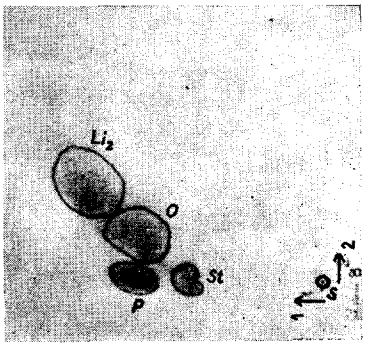

Fig. 2. Human blood serum. Symbols as in Fig. 1.

Experimental

A strip of Whatman No. 3 paper (15 × 15 cm) was impregnated with a solution of 5% paraffin oil in ether. On the starting point 2 cm from the bottom of the paper a solution of the sample was spotted. In the first run, the ascending chromatography was carried out with 93% acetic acid as mobile phase. The temperature was maintained at about 20° during 5-h development. The chromatogram was then dried at 80–90°. The second chromatography was run at —8° for 16–20 h with a mixture of 85% formic acid–acetic acid–water (50:50:5 (v/v/v)) as solvent. The separated compounds were identified with cupric acetate and $K_4Fe(CN)_6$[3] or with mercuric acetate and diphenylcarbazone[9].

Isolation of higher fatty acids from blood serum

To 1 ml of blood serum, 9 ml of ethanol–ether mixture (3:1) were added. After 10 min standing, the mixture was centrifuged and 5 ml of clear supernatant were evaporated nearly to dryness in a test tube. 2 ml of a saturated solution of KOH in methanol were added and after stirring the mixture was saponified 2 h in hot-water bath. After cooling, 3 ml of distilled water were added and the cloudy solution acidified with H_2SO_4. Free fatty acids were extracted with 5 ml ether and after evaporation, the residue was dissolved in 0.2 ml chloroform, and 0.05 ml of this solution were spotted.

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Received December 18th, 1957