When the digestions were performed at pH 3·4, a number of peptide bonds were more susceptible to cleavage by the fundic enzymes. Bonds 1–2, 11–12 and 23–24 were now hydrolysed to completion by pepsin A, and human pepsin 3 displayed an increased activity at bonds 1–2, 11–12 and 24–25. Human pepsin 5 and swine pyloric pepsin exhibited the same respective specificities at the higher pH.

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The Hydroxy Acids of *Pseudomonas aeruginosa* 8602

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The fatty acids of the phenol-extractable lipopolysaccharides of *Pseudomonas aeruginosa* differ from those of the lipids extractable by the Folch procedure (Hancock & Meadow, 1967; Roberts, Gray & Wilkinson, 1967). The major fatty acid components of the lipopolysaccharide were analysed by g.l.c. of their methyl esters, and 78% of the fatty acids were found to be polar compounds not present in the Folch-extractable lipids. They were separated from the non-polar components by t.l.c. of their methyl esters in light petroleum (b.p. 40–60°)–diethyl ether (60:40, v/v) (Eng, Lee, Hayman & Gerstl, 1964), which resolved them into two bands. One band contained 3-hydroxydodecanoate (3-OH $C_{12:0}$) (36·6% of the total fatty acids) and 3-hydroxydecanoate (3-OH $C_{10:0}$) (6% of the total fatty acids), which were identified by g.l.c. of their methyl esters before and after acetylation. The second band contained a single component (36·6% of the total fatty acid), which was identified by g.l.c., response to acetylation, hydrogenation, oxidation and mass spectroscopy as 2-hydroxydodecanoate (2-OH $C_{12:0}$). 3-Hydroxymyristic acid (3-OH $C_{14:0}$), a component of most Gram-negative bacterial lipopolysaccharides, was absent.

Mild alkaline methanolysis of the lipopolysaccharide caused preferential release of the 2-OH $C_{12:0}$ and 3-OH $C_{10:0}$ acids, suggesting that they are linked to the lipid A core as *O*-esters (Kasai, 1966). The 3-OH $C_{12:0}$ acid was released only after acid hydrolysis suggesting an amide link between it and the rest of the lipid. This differential hydrolysis was used to separate the 2-OH $C_{12:0}$ and 3-OH $C_{12:0}$ acids from 60g. of freeze-dried bacterial cell walls. After purification by t.l.c. and g.l.c., about 200mg. of each acid was obtained. Optical-rotatory-dispersion studies showed positive Cotton effects for both the methyl esters with maxima at 223nm. for the 2-OH $C_{12:0}$ acid and at 227nm. for the 3-OH $C_{12:0}$ acid. They were thus shown to be L-(+)-2-hydroxydodecanoate and D-(−)-3-hydroxydodecanoate (Sjöberg, 1962).

Comparison of the incorporation, by washed cell suspensions of *P. aeruginosa*, of [1-^{14}C]acetate, [1-^{14}C]decanoate and [1-^{14}C]dodecanoate into the hydroxy acids showed them to be formed directly from the corresponding long-chain unsubstituted fatty acids. A cell-free system catalysing the hydroxylation of decanoic acid and dodecanoic acid to their corresponding 3-hydroxy derivatives has been studied.

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Age Changes in the Glycosaminoglycan Composition of Human Bronchial Cartilage

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A previous study (Saltzman, Siezer & Green, 1963) of human main-stem bronchial cartilage showed a decrease in galactosamine content and in galactosamine/glucosamine ratio between the fourth and the eighth decades of life. The aim of the present study was to investigate variation in glycosaminoglycan composition over a more comprehensive age range and in cartilage obtained from various parts of the bronchial tree.

Bronchial trees were removed from normal lungs of subjects ranging in age from birth to the eighth decade. Cartilage was dissected from the lower four tracheal rings, the main-stem bronchus and as far as was possible along its divisions to the upper, middle and lower lobes.

Dry cartilage was subjected to proteolytic digestion (Scott, 1960) followed by ethanol precipitation. After preliminary purification (Wusteman, Dodgson, Johnson & Bell, 1968) the glycosaminoglycan mixture was fractionated on Dowex 1 (X2, 200–400 mesh, Cl^- form) resin with a stepwise gradient of 1·2, 1·4, 1·6 and 1·8M-NaCl and then 2·5M-NaCl containing 8M-urea.