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 [1-¹⁴C]acetate, [1-¹⁴C]decanoate and [1-¹⁴C]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.

Analytical and other results showed that the 1.2–1.8M-NaCl fractions were predominantly composed of chondroitin 4-sulphate and chondroitin 6-sulphate. The total uronic acid content in these fractions (expressed as a percentage of dry weight of cartilage) was fairly constant (3.4–4.5%) through childhood and adult life, but fell (to 2–3%) after the fifth decade. Changes were noted, however, in early life in the ease with which uronic acid-containing material was eluted from the Dowex 1 column. At birth up to 25% of the total uronic acid was eluted in the 1.2M fraction but during the first seven years this proportion fell to only 4%, reflecting an increase in the sulphation of the polymers. The amount of chondroitin 6-sulphate remained at about 55% of the total uronic acid containing material throughout life.

The predominant component of the fraction eluted by 2.5M-NaCl containing 8M-urea was an oversulphated keratan sulphate. The hexose content of this fraction (with D-galactose as standard), expressed as a percentage of dry weight of cartilage, ranged from negligible amounts in neonatal cartilage to 0.75% at 2 years and 1.6% at 17 years. Further increases up to a plateau of 2–2.5% during the fifth decade were noted but contents subsequently fell to about 1.5% after 65 years. Small quantities of keratan sulphate were observed in the early fractions from adult samples with a hexosamine/sulphate ratio closer to unity.

No consistent differences were observed in cartilages from different regions of the same bronchial tree.

The implications of these results will be discussed.

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Conversion of Lycopene into β -Carotene by Chloroplasts of Higher Plants

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In the normal red tomato the major carotenoid is lycopene (87%) with smaller amounts of β -carotene (7%) also present (Ferrari & Benson, 1961). Some doubt still exists whether the β -carotene present is formed from lycopene, though Decker & Uehleke

(1961) claimed that chloroplasts isolated from carrot leaves carry out the conversion, an observation supported by Wells, Schelble & Porter (1964) from studies in which γ -, δ - and β -carotenes were identified after administration of [^{14} C]lycopene to isolated tomato plastids or spinach chloroplasts. However, in normal red fruit (Goodwin & Jamikorn, 1952), high temperature inhibits the formation of lycopene but not of β -carotene, suggesting that β -carotene is not synthesized from lycopene. There is evidence to support the suggestion (Tomes, 1963) that in tomato fruit two pathways exist for β -carotene synthesis: one, temperature-sensitive, involving lycopene, and the other, temperature-insensitive, in which β -carotene is formed by some alternative pathway. Because of the availability of [^{14} C]lycopene, the product of another investigation, we report here studies on the conversion of lycopene into β -carotene by plastid preparations from tomato fruit and French bean (*Phaseolus vulgaris*) seedlings.

Radioactive lycopene was prepared by administration of 50 μ C of [^{14} C]mevalonic acid to whole half-ripened tomato fruit (120g.), which were then allowed to ripen for 4 days at 25° under artificial light. The red fruit were homogenized; lycopene was extracted by our standard procedures and recrystallized to constant specific radioactivity. The identification and purity of the lycopene were authenticated by thin-layer chromatographic behaviour and spectrophotometric studies.

[^{14} C]Lycopene (0.2mg.; 30 000 d.p.m./mg.) suspended in 0.5ml. of phosphate buffer, pH 7.2, containing 0.6% of Tween 80, was added to chloroplasts isolated by sucrose-density-gradient centrifugation (Leech, 1964) from French-bean leaves. After incubation at 25° in dim light for 15–24 hr., the β -carotene was extracted from the incubation mixtures, purified by standard procedures (see, e.g., Rogers, Shah & Goodwin, 1968), and recrystallized in the presence of unlabelled β -carotene after authentication by thin-layer chromatography and determination of spectra in three solvent systems.

It was found that incubation of [^{14} C]lycopene with disrupted chloroplasts resulted in incorporation of radioactivity into β -carotene. The specific radioactivity of the β -carotene isolated was in the range 12 000–13 000 d.p.m./mg. (representing incorporation of about 10% of the total radioactivity). On a specific radioactivity basis, similar results were obtained with plastids isolated from half-ripened tomato fruit, though total incorporation of ^{14} C in this case was only about 3%.

The incorporation of radioactivity into β -carotene appears to represent a small but real conversion from [^{14} C]lycopene, thus supporting the view that in plastids lycopene may be an intermediate in the biosynthesis of β -carotene.