obtained with adult-rabbit white-muscle myosin were shown by myosin from skeletal muscle of the rat, mouse, pigeon and hen.

Subfragment 1 (Mueller & Perry, 1962) prepared from myosin by papain digestion (S. Lowey, personal communication) of rabbit white muscle yielded three bands in an 8M-urea gel. One of these had an electrophoretic mobility identical with that of the fast band obtained with adult myosin.

None of the bands obtained from whole sarco-plasm, actin, troponin and tropomyosin coincided with those from myosin studied under identical electrophoretic conditions.

Although it cannot be concluded finally from these studies that the low-molecular-weight components are integral parts of the myosin molecule, their consistent nature and the relation between them and different myosin types suggest that they are. It is suggested that myosin consists of iso-enzymes that differ in the electrophoretic mobility of their small subunits and in the extent of methylation of the histidine in the main chains (Trayer, Harris & Perry, 1968). On this hypothesis, white-muscle myosin would contain more of the isoenzyme composed of electrophoretically fast small subunits than myosin isolated from red, foetal and cardiac muscles.

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The Sites of Action of Human and Swine Pepsins on the B Chain of Oxidized Insulin

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Five different pepsins have been found in swine (Ryle, 1965) and at least five in man (Etherington & Taylor, 1967, 1969). The major fundic enzymes, swine pepsin A and human pepsin 3, have analogous features, as have swine pepsin C and human pepsin 5 (Taylor, 1968). Functionally, swine pepsin C (Ryle & Porter, 1959) differs from swine pepsin A in that it can cleave only six of the ten bonds in the insulin B chain that are susceptible to pepsin A at pH 2.0 (Sanger & Tuppy, 1951) (see Fig. 1).

Human and swine pepsins sometimes display a second pH optimum against proteins above pH 3, and many synthetic substrates are hydrolysed maximally near pH 4 (see Taylor, 1968). We have, therefore, investigated the sites of action of purified swine and human pepsins at both pH 1.7 and pH 3.4.

Swine pepsin A was purified from a crystalline preparation, and swine pyloric pepsin, which was chromatographically similar to pepsin C, was obtained from a pyloric pouch secretion. Human pepsins 3 and 5 were separated from normal gastric juice.

At pH 1.7 human pepsin 3 failed to cleave bonds 1-2 and 23-24, but was otherwise similar to pepsin A. Swine pyloric pepsin differed from swine pepsin C by its ability to hydrolyse bonds 1-2 and 23-24; but hydrolysis at bond 11-12 was negligible. The specificity of human pepsin 5 was essentially similar to the specificity of swine pyloric pepsin except for its failure to attack bond 1-2.

Fig. 1. Cleavage by swine pepsin A and pepsin C of the oxidized B chain of insulin. CySO₃H, cysteic acid.

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.