The mucopolysaccharidoses

C. A. PENNOCK and I. C. BARNES

From the Department of Child Health, Bristol University; and the Department of Chemical Pathology, Bristol Royal Infirmary

The group of inherited disorders referred to as the mucopolysaccharidoses, of which Hurler's syndrome is the best known example, have been expertly reviewed and classified by McKusick in 1965, 1969, and reclassified in 1972. The most recent classification (Table I) resulted from observations on skin fibroblasts made by Neufeld and her colleagues during their studies on the basic biochemical defects in the mucopolysaccharidoses. As a result of this work, and research by other groups, the actual enzyme defects in all the conditions shown in Table I have been identified, and this has made the antenatal diagnosis of these disorders a real possibility. These exciting developments which have occurred during the past four years are reviewed in the following paragraphs.

Mucopolysaccharide (glycosaminoglycan) structure and nomenclature

Mucopolysaccharides are the major chemical polymers of the ground substance and connective tissue (in association with collagen, reticulin, and elastin). The reports published in the past are often confusing since the terms mucopolysaccharide, mucoprotein, mucin, mucus, and glycoprotein have been used synonymously, but the position has been clarified by Jeanloz (1960) who introduced the term glycosaminoglycan (GAG). This more clearly defines the chemical nature of the mucopolysaccharides (GAGs) as being high molecular weight polymers of repeating dimers of an amino sugar usually linked to a hexuronic acid. The amino sugar is either glucosamine or galactosamine, both of which are usually either acetylated or sulphated at the amino group, and both of which may also be sulphated either at carbon 4 or carbon 6 of the hexose

ring. The hexuronic acid is either glucuronic acid (that is, glucose with a carboxyl group at carbon 5), or iduronic acid (the C-5 epimer of glucuronic acid). Several GAG chains of similar molecular weight may be linked through a short neutral carbohydrate linkage (usually galactose–galactose–xylose) to a protein core via a serine (or threonine) residue.

Full details of the chemistry of these polymers have been given in a review by Muir (1969) and are summarized in Table II.

Excretion of glycosaminoglycans

The term mucopolysaccharidosis was first suggested by Brante in 1952 after finding that the liver of two patients with Hurler's syndrome contained an excess of chondroitin sulphate B (dermatan sulphate), a designation that was made permanent by the demonstration of excess mucopolysacchariduria by Dorfman and Lorincz in 1957, and confirmed by Meyer and his colleagues in 1958. The study of urinary GAG excretion patterns gave the first indications of the biochemical pathogenesis of the mucopolysaccharidoses, and contributed greatly to our present understanding of the underlying defects.

(a) **Normal GAG excretion.** The first suggestion that GAGs might be present in normal urine is thought to have been made by Moerner in 1895 who isolated a sulphated polysaccharide, which was probably chondroitin sulphate (McGale, 1969). This finding was subsequently confirmed by Astrup (1947) and Kerby (1954) who isolated an alcohol-insoluble metachromatic material which was identified as chondroitin sulphate by DiFerrante and Rich in 1956. The latter subsequently showed that the main GAGs excreted in normal urine are chondroitin-4-sulphate and chondroitin-6-sulphate,

TABLE I
GENETIC MUCOPOLYSACCHARIDOSES (AS CLASSIFIED BY McKUSICK IN 1972)

Designation		Clinical Features	Genetics
MPS I H	Hurler syndrome	Early clouding of cornea, grave manifestations, death usually before age 10	Homozygous for MPS I H gene
MPS I S	Scheie syndrome	Stiff joints, cloudy cornea, aortic regurgitation, normal intelligence	Homozygous for MPS I S gene
MPS I H/S	Hurler-Scheie	Phenotype intermediate between Hurler and Scheie	Genetic compound of MPS I H and I S genes
MPS II A	Hunter syndrome, severe	No clouding of cornea, milder course than in MPS I H but death usually before age 15 years	Hemizygous for X-linked gene
MPS II B	Hunter syndrome, mild	Survival 30-50 years, fair intelligence	Hemizygous for X-linked allele for mild form
MPS III A	Sanfilippo syndrome A	Identical phenotype Mild somatic, severe central nervous system effects	Homozygous for Sanfilippo A gene
MPS III B	Sanfilippo syndrome B		Homozygous for Sanfilippo B (at different locus)
MPS IV	Morquio syndrome	Severe bone changes of distinctive type, cloudy cornea, aortic regurgitation	Homozygous for Morquio gene (probably more than one allelic form)
MPS V	Now vacant	Previously type IS	
MPS VI A	Maroteaux-Lamy syndrome classic form	Severe osseous and corneal change normal intellect	Homozygous for M-L gene
MPS VI B	Maroteaux-Lamy syndrome mild form	Severe osseous and corneal change normal intellect	Homozygous for allele at M-L locus
MPS VII	β -glucuronidase deficiency	Hepatosplenomegaly, dysostosis multiplex, white cell inclusions, mental retardation	Homozygous for mutant gene at beta glucuronidase locus

TABLE II
NOMENCLATURE AND STRUCTURE OF GLYCOSAMINOGLYCANS (PREVIOUSLY CALLED MUCOPOLYSACCHARIDES)

Old Name	New Name	Amino Sugar	Hexuronic Acid	Hexosamine Sulphate
Chondroitin	Chondroitin	GalNAc	GlcUA	None
Chondroitin sulphate A	Chondroitin-4-sulphate	GalNAc	GlcUA	O-SO ₄
Chondroitin sulphate C	Chondroitin-6-sulphate	GalNAc	GlcUA	O-SO ₄ *
Chondroitin sulphate B	Dermatan sulphate	GalNAc	GlcUA	O-SO ₄ *
Heparin	Heparin	GlcNAc	IdUA	N-SO ₄
Heparitin sulphate	Heparan sulphate	GlcNAc	GlcUA	O-SO ₄ *
Heparin monosulphate			IdUA	N-SO ₄
Corneal keratosulphate	Keratan sulphate I	GlcNAc	Gal	O-SO ₄ *
Skeletal keratosulphate	Keratan sulphate II	GlcNAc	Gal	O-SO ₄
Hyaluronic acid	Hyaluronic acid	GalNAc	GlcUA	None

Abbreviations: GlcNAc = N acetyl glucosamine; GalNAc = N acetylgalactosamine; GlcUA = glucuronic acid; IdUA = iduronic acid; Gal = galactose.

* Iduronic acid may also be sulphated at C-2.

probably derived from connective tissue break-down, with other GAGs present in trace amounts. Glycosaminoglycans of a similar pattern have been found in plasma by Calatroni, Donnelly, and DiFerrante (1969).

(b) **Excretion in the mucopolysaccharidoses.** The excretion pattern in the mucopolysaccharidoses is very different from the normal pattern. Dorfman and Lorincz (1957) isolated a large quantity of GAGs from the urine of a 6-year-old girl with Hurler's syndrome which was clearly abnormal, and was identified as a mixture of dermatan sulphate and

heparan sulphate. This abnormal excretion pattern was confirmed by Meyer *et al* in 1958. Pedrini, Lennzi, and Zambotti (1962) showed excessive keratan sulphate excretion in type IV (Morquio's disease), which was confirmed by Robins, Stevens, and Linker (1963), and shown to be a keratan sulphate-chondroitin sulphate-protein complex by Kaplan *et al* (1968). Lorincz (1958) also described a group of patients who excreted mainly heparan sulphate, and this was later confirmed by Sanfilippo *et al* (1963) and shown to be characteristic of a specific disease, now recognized as type III (Sanfilippo syndrome).

(c) **Laboratory diagnosis.** Laboratory diagnosis of the mucopolysaccharidoses has centred on the abnormal urinary excretion patterns, as defined by a number of different laboratories. Kaplan (1969) studied GAG excretion in 46 patients and found four different types.

- (i) Excretors of dermatan sulphate and heparan sulphate, with the former in excess, and the clinical features of type IH (Hurler) or type IS (Scheie).
- (ii) Excretors of equal amounts of these two GAGs and having features of type II (Hunter).
- (iii) Excretors of mainly heparan sulphate, type III (Sanfilippo).
- (iv) Excretors of dermatan sulphate alone, type VI (Maroteaux-Lamy).

Spranger (1970) reported similar findings to those of Kaplan but found that heparan sulphate was the only GAG present in type III and was also the predominant GAG in type I. He also confirmed the excess dermatan sulphate excretion in type VI and isolated chondroitin sulphate and keratan sulphate in excess from patients with type IV (Morquio). It is now clear that abnormal excretion is always demonstrable in the mucopolysaccharidoses, though the actual pattern of excretion in type II is difficult to distinguish from type I (Maroteaux, 1970; Pennock, 1973; Constantopoulos and Dekaban, 1975), and the excretion pattern may change with age and even be normal in type IV (Maroteaux and Lamy 1967; Linker, Evans, and Langer, 1970; Jenkins, Davies, and Harper, 1973). Current views on the excretion pattern in the different mucopolysaccharidoses are summarized in Table III.

Tissue culture and cellular metachromasia

The application of tissue culture to the study of the mucopolysaccharidoses was initiated by the discovery by Danes and Bearn (1965) that fibroblasts cultured from the skin of patients with the Hurler or Hunter syndromes show signs of disease in that they accumulate GAGs. This can be determined by staining with toluidine blue, which reveals 'metachromatic' granules, and by chemical analysis (Danes and Bearn, 1966a; Schafer *et al*, 1966). Matalon and Dorfman (1966) showed that metachromasia was the result of the intracellular accumulation of large amounts of GAGs, and fibroblasts cultured from patients with Hurler, Hunter, and Sanfilippo syndromes contained 5 to 10 times as much intracellular GAGs as did normal fibroblasts.

Upon studying fibroblasts from six families of patients with Hurler's syndrome, Danes and Bearn

TABLE III
URINARY GLYCOSAMINOGLYCAN EXCRETION IN
MUCOPOLYSACCHARIDOSSES

Mucopolysaccharidoses	Excretion Pattern
Types IH, IS and IH.S	Dermatan sulphate with heparan sulphate, former predominant
Type II A and B	Dermatan sulphate and heparan sulphate usually in roughly equal amounts but may be similar to type I
Type III A and B	Heparan sulphate (possible slight increase in dermatan sulphate)
Type IV	Keratan sulphate but often normal later in life
Type VI A and B	Dermatan sulphate
Type VII	?*

* Only two cases have been described, excretion was normal in one (Sly *et al*, 1973) and abnormal (dermatan sulphate in excess) in other (Beaudet *et al*, 1972).

(1966a) found metachromatic cells not only in cultures of the patients but also in those of both parents. The extent of metachromasia in the heterozygous carriers was comparable with that in the cells of the affected offspring. The finding of metachromasia in the fibroblasts of both parents of children with the Sanfilippo syndrome suggested that this disease, like Hurler's, is an autosomal recessive disorder. In three families of the X-linked Hunter syndrome, fibroblasts cultured from the skin of the father contained no metachromatic granules, whereas those of the heterozygous mother contained both metachromatic and ametachromatic cells.

The discovery of metachromasia in fibroblasts of patients with mucopolysaccharidoses originally gave promise that this phenomenon might be diagnostic for this group of disorders, but the situation became increasingly confusing with the appearance of metachromasia in a wide number of syndromes including Gaucher's disease (Danes and Bearn, 1968a), Marfan's syndrome (Matalon and Dorfman, 1968a), cystic fibrosis (Danes and Bearn, 1968b), and Fabry's disease (Matalon *et al*, 1969).

Pathogenesis of the mucopolysaccharidoses

It is clear from the preceding paragraphs that the pathological features of the mucopolysaccharidoses seem to stem from the intracellular deposition of GAGs in a number of tissues of the body. Studies have shown the accumulation of GAGs in cells that are not ordinarily involved in the synthesis of the connective tissue matrix, and to account for such widespread storage, a number of pathogenic mechanisms have been proposed.

(a) *Defective linkage* of the metabolite to another substance, such as the protein with which it normally forms a natural complex. This in turn may culminate in problems of degradation of the faultily linked complex.

(b) *Increased GAG synthesis.*

(c) *Defective catabolism*, in which the enzyme(s) required in the degradation of GAGs may be either partially or entirely absent.

GAG-protein complexes are synthesized by a series of reactions, which starts with the formation of the protein core at the rough endoplasmic reticulum and is followed by the sequential addition of the appropriate sugars and sulphate groups (Phelps, 1973). Interest grew in the *defective linkage* theory of the GAG and protein core when it was discovered that GAGs in the Hurler, Hunter, and Sanfilippo syndromes could be extracted very easily, even with water. This contrasted with the normal situation where, for instance, in the extraction of the dermatan sulphate-protein complex drastic means must be employed, such as alkali, high salt concentration, or extensive proteolytic digestion. This suggested that GAGs in the mucopolysaccharidoses were not present as normal multi-chain protein-polysaccharides. Following the original report by Brante (1952) on the unusual water solubility of hepatic GAGs in the Hurler syndrome, Brown (1957) reported that, in this disease, heparan sulphate from liver had a low molecular weight. Other investigators have also shown that both heparan sulphate and dermatan sulphate from urine and tissues, in MPS I, II, and III, are of lower molecular weight than the same GAGs isolated from normal tissues (Dorfman, 1964; Knecht, Cifonelli, and Dorfman, 1967; Constantopoulos, 1968; Gordon and Haust, 1971). These studies were interpreted as either indicating that the low molecular weight GAGs may be formed in the absence of protein synthesis, or that GAGs may be *synthesized in excess*. Rennett and Dekaban (1966a) found raised levels of the amino acids serine and tryptophan in urine from Hurler patients, and suggested that this could be related to abnormal bonding between mucopolysaccharide and protein.

To test these hypotheses, Matalon and Dorfman (1966) added labelled precursors of dermatan sulphate to cultures of dermal fibroblasts obtained from patients with Hurler's syndrome and subsequently analysed the dermatan sulphate produced. It was similar to that isolated from normal human umbilical cord and was synthesized in circumstances that promoted simultaneous synthesis of protein. Therefore, the biosynthesis of dermatan sulphate appeared neither to be impaired nor excessive in these fibroblasts. The suggestion that the mucopolysaccharidoses may be the result of the defective binding of GAG to protein finally became untenable when Matalon and Dorfman (1968b) showed that

GAG synthesis was inhibited by puromycin and cycloheximide (which inhibit protein synthesis). These substances also inhibited the incorporation of both sulphate-³⁵S and acetate-¹⁴C into the dermatan sulphate in dermal fibroblasts from patients with the Hurler syndrome as well as from normal individuals. It is, therefore, clear that protein synthesis must precede GAG synthesis and the *increased accumulation of GAGs in the mucopolysaccharidoses is neither due to abnormal protein synthesis nor to defective linkage*.

Electron microscopy of liver from patients with Hurler's disease reveals the presence of numerous intracytoplasmic partly empty vacuoles, which are usually limited by membranes and often fused with each other (Orizaga *et al*, 1963; Callahan and Lorincz, 1964, 1966). Van Hoof and Hers (1964) were convinced that the hepatic vacuoles represented lysosomes, and suggested that Hurler's disease was really a storage disease which resulted from the *absence of a lysosomal degradative enzyme*, a view subsequently confirmed by kinetic studies on cultured fibroblasts by Frattantoni *et al* (1968a).

The characteristics of lysosomal diseases have been given by McKusick (1972) and include the following features.

- (a) Evidence of storage always exists.
- (b) The stored material is usually heterogeneous and enclosed in membrane-bound vesicles.
- (c) Several tissues are usually affected.
- (d) The disease is usually progressive.

It is clear that the mucopolysaccharidoses fulfil all these criteria and, therefore, since the mid-60's, the emphasis has been on a search for the enzyme defect in the degradative pathways.

Lysosomal enzymes and GAG degradation

(a) **Hyaluronidase.** Early studies on degradation had largely been confined to the investigation of hyaluronidase, which Meyer, Dubos, and Smyth had discovered in pneumococci in 1937, and the presence of which was confirmed in mammalian tissues and equated with Duran-Reynals spreading factor by Chain and Duthie in 1940. Subsequent work (reviewed by Bollet, Bonner, and Nance, 1963) has shown that this enzyme is an endoglycosidase which will hydrolyse hexosaminyl-glucuronide bonds. Further degradation requires the participation of other glycosidases.

(b) **β Glucuronidase.** Oligosaccharides produced by mammalian hyaluronidase contain non-reducing terminal glucuronic acid residues linked in the β linkage. β glucuronidase is a widely distributed enzyme which was demonstrated in lysosomes

by de Duve *et al* (1955), and its ability to remove uronic acid residues from hyaluronic acid oligosaccharides was shown by Weissmann (1955) and Linker, Meyer, and Weissmann (1955). However, β glucuronidase does not hydrolyse the disaccharide, hyalobiuronic acid.

(c) **β -N-acetyl-hexosaminidases.** Removal of glucuronic acid from higher oligosaccharides results in the exposure of non-reducing β -N-acetylhexosaminyl residues. β -N-acetylhexosaminidases have been studied intensively because of their possible role in the degradation of GAGs, glycoproteins, and glycolipids. Weissmann, Hadjiioannou, and Tornheim (1964), for example, showed that highly purified β -N-acetyl-D-glucosaminidase of beef liver removes the non-reducing N-acetyl glucosamine residue from a disaccharide derived from hyaluronic acid. Buddecke and Werries (1964, 1965) have also found that purified β hexosaminidases are active on oligosaccharides derived from chondroitin sulphate and hyaluronic acid.

(d) **Sulphatases.** Additional enzymes must be required for degradation of the GAGs (dermatan sulphate, heparan sulphate, and keratan sulphate) which accumulate in the mucopolysaccharidoses; enzymes to cope with sulphate, iduronide bonds, and the protein linkage region (which includes galactosyl links and a xylosyl-serine link). Evidence for the existence of mammalian sulphatases active on chondroitin sulphate, or its metabolic products, was provided by Dohlman (1956) and Dziewiatkowski (1956) who found that subcutaneous administration of ^{35}S chondroitin sulphate to rats resulted in the excretion of inorganic radioactive sulphate. Subsequently chondroitin-4-sulphate sulphohydrolases were purified from rat liver by Tudball and Davidson (1969) and from the cell free supernatant of bovine arterial tissue homogenate by Held and Buddecke (1967).

(e) **β Galactosidase.** The role of β galactosidase in degradation of GAGs is unclear. In GAG other than keratan sulphate, galactose is restricted to the protein linkage region. If β galactosidase plays a significant role in the degradation of these polysaccharides then it must act only on the linkage fragments remaining after chain degradation, or as an endogalactosidase to separate GAG chains from protein. Interest grew in β galactosidase because of the similarity between Hurler's syndrome and the disease in which this enzyme is known to be deficient, namely GMI gangliosidosis. This has two phenotypically distinct forms, an infantile and late

juvenile form, of which the former has most in common with the mucopolysaccharidoses—rapidly progressive mental and physical retardation associated with hepatomegaly, severe bone deformities, and early death. There is neuronal accumulation of GMI ganglioside (which has galactose as a terminal sugar residue) and generalized accumulation of a keratan sulphate-like polysaccharide. The enzyme deficiency in this disorder has been clearly identified as β galactosidase (Okada and O'Brien, 1968).

Van Hoof and Hers (1968), in a study of acid hydrolases in 32 patients with mucopolysaccharidoses and 5 patients with GMI gangliosidosis, found complete absence of β galactosidase activity at pH 3.6 in the latter group and also found reduced levels in liver, brain, and skin, but not in kidney, spleen, and leucocytes, in 14 patients with Hurler's syndrome. Öckerman (1968) showed low β galactosidase activity in skin but found raised levels in plasma in Hurler's syndrome. That a deficiency of β galactosidase exists in Hurler's syndrome has been suggested by others (MacBrinn *et al*, 1969; Van Gemund *et al*, 1969; Thomas, 1969), and the deficiency appears to extend to Hunter and Sanfilippo's syndrome (Ho and O'Brien, 1969). However, a number of other workers have found normal β galactosidase levels in patients with mucopolysaccharidoses (Benson, Bowser-Riley, and Gianelli, 1970; Fluharty *et al*, 1970; Singer and Schafer, 1970; Gordon and Feleki, 1970). One explanation for the low levels in some patients may lie in the observation of Dorfman and Matalon (1972) that β galactosidase is inhibited by heparan sulphate, inhibition which is readily removed by increasing the concentration of chloride ions. A strong argument against β galactosidase being the deficiency is that the aberrant GAG metabolism of Hurler and Hunter fibroblasts is corrected by fibroblasts of the GMI gangliosidosis genotype, which are devoid of β galactosidase (Neufeld and Fratantoni, 1970). Thus, the role of the defective activity of β galactosidase shown in some patients with Hurler's syndrome remains obscure, though it is unlikely to be the primary defect.

(f) **Xylosidase.** In view of the presence of β -D-xylosylserine linkages in GAGs, β xylosidase might be expected to participate in the degradation of GAG-protein complexes. β xylosidase was shown by Fisher *et al* (1966) in rat liver and by Robinson and Abrahams (1967) in pig kidney. Studies of the rat liver lysosomal enzyme by Patel and Tappel (1969), and by Fisher and Kent (1969), indicate that a single enzyme is responsible for both β -D-xylosidase activity and β -D-glucosidase activity.

Thus, by the early 1970's sufficient data had accumulated for a possible degradative pathway for dermatan sulphate to be suggested by Dorfman and Matalon (1972).

Cross correction studies using fibroblast cultures

Running parallel with the studies of lysosomal enzymes in GAG degradation were investigations into GAG metabolism in fibroblast cultures. By incubating cultured fibroblasts from patients with mucopolysaccharidoses in medium containing ^{35}S labelled sulphate, Fratanoni *et al* (1968a) first confirmed that the defect in the mucopolysaccharidoses was one of degradation. After incorporation of labelled sulphate into newly synthesized GAGs, the cultures were washed and transferred to fresh medium without labelled sulphate, and the rate of leakage of radioactivity into the medium was measured and found to be significantly reduced when compared with normal control cultures. In the same year (1968b) they discovered that when Hurler and Hunter cells were mixed, mutual correction of the defect occurred, and the cells now behaved as normal cell cultures. Furthermore, the cells need not be mixed since the medium also contained material which would correct the abnormal storage of GAGs, so that the medium from either a normal individual's fibroblast culture, or from a patient with Hunter's syndrome, would correct the defect in Hurler cells. The diffusible substances were referred to as 'corrective factors'. The corrective factors were isolated and partially purified, and their enzymic nature suggested by Barton and Neufeld (1971) and Cantz *et al* (1970, 1972), also working in Neufeld's department. One interesting point, which emerged from the work of Wiesmann and Neufeld (1970) and Barton and Neufeld (1971), was that Hurler and Scheie fibroblasts behaved in a similar manner and shared the same 'corrective factor' deficiency. As a result of this observation, McKusick (1972) has reclassified the two disorders as mucopolysaccharidoses type IH and IS, respectively (the latter was formerly type V), in spite of the distinctly different clinical features. This is not quite so surprising on chemical grounds since the same GAG excretion pattern is seen in both conditions. Kresse *et al* (1971) and Neufeld and Cantz (1971) also showed that two groups of Sanfilippo patients existed whose fibroblast cultures would mutually cross-correct, and these have been designated type A and B, though they are indistinguishable clinically (McKusick, 1972). Kresse and Neufeld (1972) purified a Sanfilippo A corrective factor, Cantz *et al* (1970, 1972) purified a Hunter

corrective factor, and Barton and Neufeld (1972) purified a Maroteaux-Lamy corrective factor, but no factor could be isolated for Morquio's disease.

Enzyme deficiencies in the mucopolysaccharidoses

Since 1971, the work on corrective factors has been developed to such an extent that the enzyme deficiencies for all the mucopolysaccharidoses have been elucidated.

Hurler and Scheie syndromes—deficiency of α -L-iduronidase. The defect in Hurler and Scheie syndromes was identified by Matalon, Cifonelli, and Dorfman (1971) as being an α -L-iduronidase deficiency. They prepared enzyme extracts of fibroblast cultures and, on incubation with desulphated dermatan sulphate in the presence of glucuronidase and hyaluronidase, showed liberation of α -L-iduronic acid, which was identified by paper chromatography. Enzyme activity in Hurler cell cultures was greatly reduced. By a lengthy and difficult organic synthesis, Friedman and Weissmann (1972) prepared an artificial substrate (phenyl- α -L-iduronide) for α -L-iduronidase. Weissmann and Santiago (1972) used this substrate to identify α -L-iduronidase in rat lysosomes; Bach *et al* (1972) isolated Hurler corrective factor from normal urine and identified it as α -L-iduronidase; and Matalon and Dorfman (1972) confirmed the enzyme deficiency in Hurler liver, cultured fibroblasts and urine. Hall and Neufeld (1973) showed enzyme activity in amniotic fluid cells and Liem and Hooghwinkel (1975) in leucocytes.

Hunter syndrome—deficiency of L-iduronosulphate sulphatase. Developing the work of Cantz *et al* (1970, 1972) on the Hunter corrective factor, Bach *et al* (1973) showed that this factor, purified from human urine, removes about 2% of the sulphate residues from ^{35}S GAGs accumulated within Hunter fibroblasts, both from heparan sulphate and, to a lesser extent, dermatan sulphate. The radioactive product was identified as inorganic sulphate by paper chromatography. Other workers (Suzuki *et al*, 1968; Lindahl and Axelsson, 1971; Malmstrom and Fransson, 1971) have shown so-called 'oversulphated areas' in heparan and dermatan sulphate in which the iduronic acid is sulphated as well as the hexosamine residue, thus giving areas with two (or three) sulphate groups per dimer. Action of the Hunter corrective factor results in the disappearance of some 'oversulphated' areas (about one-third), and it was concluded that since the sulphate residue unique to such areas is that linked to

L-iduronic acid, the sulphatase is for sulphated iduronic acid. This was confirmed using the disaccharide 4-O- α -sulphoiduronosyl-D-sulpho-anhydromannose as substrate and measuring iduronic acid released by the combined action of Hunter factor and α -L-iduronidase. Whereas either enzyme alone releases only traces of iduronic acid, the two enzymes together released as much as 40% of the available iduronic acid. Acetone-powder extracts of fibroblasts derived from patients with the Hunter syndrome are deficient in this sulphatase.

The conclusion that Hunter's syndrome is caused by a deficiency of an L-iduronosulphate sulphatase was confirmed by the findings of Sjöberg *et al* (1973), and the same conclusion was reached by Bach *et al* (1973) and by Coppa *et al* (1973) who found a disulphated disaccharide containing iduronic acid in the urine of a Hunter patient. Recently, Lim *et al* (1974) have prepared a substrate for this enzyme, and are investigating the potential of the assay for cell extracts and for diagnostic purposes.

Sanfilippo syndrome type A—deficiency of heparin (heparan sulphate) sulphamidase. It had been shown by Dietrich (1970) that a heparin sulphamidase isolated from lymphoid tissues will catalyse the hydrolysis of amino-linked sulphate residues from heparin, and Friedman and Arsenis (1972, 1974) demonstrated similar activity in rat tissues, using amino-³⁵S-sulphated heparin as substrate. In 1973, Kresse was able to show a profound deficiency of heparin sulphamidase activity in cultured skin fibroblasts and peripheral leucocytes from patients with Sanfilippo syndrome type A, as measured with labelled heparin, and a partial deficiency was found in the cells of heterozygous carriers. Activity of the sulphamidase was also shown to be absent using a labelled heparan sulphate. Though heparan sulphate accumulates in fibroblasts of Sanfilippo A, heparin and heparan sulphate share typical structural features, and it is assumed that the degradative pathway for both substances has common steps. Thus, labelled heparin can be regarded as a satisfactory substrate. The results of Kresse were confirmed by Matalon and Dorfman (1974) who showed heparin sulphamidase deficiency in cultured skin fibroblasts and liver extracts of Sanfilippo A patients.

Sanfilippo syndrome Type B—deficiency of N-acetyl- α -D-glucosaminidase. Following the work of Kresse *et al* (1971) who differentiated the Sanfilippo syndrome into two distinct syndromes, O'Brien (1972) showed that the type B disorder was deficient in the enzyme N-acetyl- α -D-glucosami-

nase. Using an artificial substrate, enzyme activity was less than 10% of normal in frozen liver from two type B patients and was barely detectable in their fibroblast cultures. A partial deficiency was found in skin fibroblast from parents of one patient, indicating the heterozygous state of the parents. It was concluded that type B corrective factor isolated by Kresse *et al* (1971) is probably identical to N-acetyl- α -D-glucosaminidase.

Confirmation of a deficiency of this enzyme in type B patients was given by Figura and Kresse in 1972, and, as in previous reports, Sanfilippo type B fibroblasts were strikingly deficient in N-acetyl- α -D-glucosaminidase activity (6% of the activity found in normal fibroblasts). In the course of purification of urinary Sanfilippo B corrective factor, no separation of the factor and N-acetyl- α -D-glucosaminidase activity could be achieved, thus suggesting that the two were identical. Figura *et al* (1973) described three assays for the determination of N-acetyl- α -D-glucosaminidase activity in serum, and found that affected individuals had a residual enzyme activity in the range of 2 to 16% of that of normal control subjects, whereas their heterozygous parents had an activity diminished to 26 to 36%.

Morquio's syndrome—deficiency of N-acetylhexosamine-6-sulphate sulphatase. Matalon *et al* (1974b) proposed the enzyme deficiency in Morquio's syndrome. Labelled chondroitin sulphate, consisting of a mixture of 4- and 6-sulphates, was found to serve as a satisfactory substrate for normal fibroblast extracts, with release of labelled sulphate, but extracts of fibroblasts grown from patients with Morquio's disease showed a pronounced deficiency in capacity to cleave this substrate. With rat chondrosarcoma chondroitin sulphate (which contains only 4-sulphate linkage) as substrate a decrease in activity of Morquio's extracts was observed compared with normal extracts, but the decrease was not as great as with the mixed isomer. Since keratan sulphate, excreted in Morquio's disease, contains a C-6 sulphate, it seems likely that the deficient enzyme in Morquio's disease is an N-acetylhexosamine-6-sulphate sulphatase which may not be entirely specific and may exhibit some activity on the 4-sulphate.

Maroteaux-Lamy syndrome—deficiency of N-acetylgalactosamine-4-sulphate sulphatase (arylsulphatase B). In 1973, Stumpf *et al* demonstrated a deficiency of sulphatase B in tissues from three patients from two unrelated families with Maroteaux-Lamy syndrome; liver, kidney, spleen,

brain, and cultured fibroblasts all showed deficient activity. Fluharty *et al* (1974) confirmed a deficiency of arylsulphatase B in Maroteaux-Lamy fibroblasts, finding 10% of normal activity, and showed that the decreased GAG turnover in Maroteaux-Lamy fibroblasts was normalized by supplementation with an arylsulphatase B fraction from normal fibroblasts. Similar deficiencies were found by DiFerrante *et al* (1974), and also by Shapira *et al* (1975). In 1974, O'Brien, Cantz, and Spranger found that the non-reducing terminal moiety of $^{35}\text{SO}_4$ -dermatan sulphate which accumulated in fibroblasts cultured from the skin of patients with Maroteaux-Lamy syndrome was N-acetylgalactosamine-4-sulphate. This end group accounted for about 3% of the total radioactivity, and it was suggested that such fibroblasts were severely deficient in a sulphatase for such residues. Similar conclusions were reached by Matalon, Arbogast, and Dorfman (1974a), who showed that Maroteaux-Lamy fibroblasts showed a striking diminution in release of $^{35}\text{SO}_4$, both from (^{35}S) chondroitin-4-sulphate and from a heptasaccharide derived from this substance. Since there is a pronounced deficiency of arylsulphatase B activity in this disease, it was concluded that the natural substrate of arylsulphatase B is N-acetyl-galactosamine-4- SO_4 in chondroitin-4- SO_4 and dermatan sulphate. It has been suggested (Spranger *et al*, 1970; Stumpf *et al*, 1973; Fluharty *et al*, 1974; McKusick, 1972) that there may be two forms of Maroteaux-Lamy syndrome, and this view is supported by the observation of O'Brien *et al* (1974) that fibroblast lines from two patients with the clinical picture of Maroteaux-Lamy disease had normal activities of N-acetyl-galactosamine-4-sulphate sulphatase.

Mucopolysaccharidosis type VII—deficiency of β glucuronidase. In 1973, Sly *et al* presented clinical, radiological, and biochemical studies on a patient with a previously undescribed GAG storage disorder, and this is now classified as mucopolysaccharidosis type VII. Studies on cultured fibroblasts and white blood cells showed the disease to be associated with a nearly total deficiency of a lysosomal hydrolase, β -D-glucuronidase. These results were confirmed by Hall, Cantz, and Neufeld (1973) who were able to correct the abnormal GAG metabolism by addition of bovine β -glucuronidase to culture medium. Glaser and Sly (1973) and Beaudet *et al* (1975) found a deficiency of β -glucuronidase in serum, plasma, urine, white cell lysate, and cultured skin fibroblasts in patients with mucopolysaccharidosis type VII.

Our current understanding of the enzyme de-

TABLE IV

ENZYME DEFECTS IN MUCOPOLYSACCHARIDOSES*

Mucopolysaccharidoses	Enzyme deficiency
Type IH and IS	α -L-iduronidase
Type II	L-idurono sulphate sulphatase
Type III type A	Heparin (heparan sulphate) sulphamidase
Type III type B	N-acetyl- α -D-glucosaminidase
Type IV	N-acetyl hexosamine-6-sulphate sulphatase
Type VI	N-acetyl galactosamine-4-sulphate sulphatase (arylsulphatase B)
Type VII	β Glucuronidase

* Full details of mammalian enzymes required for the degradation of glycosaminoglycans have been given by Buddecke and Kresse (1974).

iciencies in the mucopolysaccharidoses is summarized in Table IV.

Prenatal diagnosis

Increasing understanding of the metabolic basis of the mucopolysaccharidoses has led to the possibility of their prenatal diagnosis. Nadler (1968) found toluidine blue metachromasia in amniotic fluid cells taken from a woman with a known history of rhesus incompatibility who was subsequently delivered of a male infant who developed Hurler's syndrome. This early promise that cellular metachromasia might be of value has since been shown to be unreliable. Indeed, in the first actual report of *in utero* diagnosis in 1969, Fratantoni *et al* were unable to show cellular metachromasia after three weeks of cell culture though it had appeared by the eighth week. The patient was subsequently delivered of a female infant with Hurler's syndrome which was confirmed by urine analysis. The actual intra-uterine diagnosis was based on increased cellular uptake and delayed release of labelled sulphate. They reported similar findings in a second patient who already had a son with Hurler's syndrome, and whose pregnancy was terminated at 25 weeks. However, diagnosis was not confirmed as cells from the abortus failed to grow in culture.

Commenting on the use of cultured cells for prenatal diagnosis, Matalon *et al* (1970) pointed out that there were three major disadvantages in their use.

(a) The time and expense required for establishment of tissue culture lines.

(b) The large number of diseases in which metachromasia of fibroblasts has been established.

(c) The difficulties in distinguishing heterozygous and homozygous individuals.

They developed a microprocedure for the determination of constituents of amniotic fluid and, in a

pregnancy which resulted in an infant with Hurler's syndrome, qualitative analysis showed GAG to be composed mainly of heparan sulphate, compared with hyaluronic acid in normal amniotic fluid. Similar results were obtained by Crawford *et al* (1973) who diagnosed Hurler's syndrome in a fetus by measurement of the GAGs in amniotic fluid.

In contrast to these results, Brock *et al* (1971) analysed the GAG content of amniotic fluid of an at-risk pregnancy and obtained normal levels. However, a female child was born whose continued excretion of massive amounts of GAGs made the diagnosis of a mucopolysaccharidosis virtually certain. Similarly, Matalon, Dorfman, and Nadler (1972) found normal levels of GAGs in two abnormal pregnancies. One studied at 10 weeks' gestation resulted in an infant with Sanfilippo syndrome, and the other, at risk for Hunter's syndrome, was later shown to have abnormal $^{35}\text{SO}_4$ cellular uptake and a termination was performed. It was concluded that the analysis of GAGs in amniotic fluid is unreliable for prenatal diagnosis on its own, and should be combined with analysis of the sulphate turnover of cultured amniotic cells.

In 1974, Harper *et al* published a detailed report on the study of a pregnancy at risk for the Sanfilippo syndrome in which previously described methods were used, as well as determination of enzyme activities associated with GAG catabolism. GAGs in amniotic fluid showed a striking increase compared with normal samples, and as there was insufficient time for culture of cells, the pregnancy was terminated at 25 weeks' gestation. Subsequent analysis confirmed the diagnosis of Sanfilippo syndrome type A. Tissue analysis showed an increased accumulation of GAGs, abnormal inclusions were seen in cultured fibroblasts and increased uptake of labelled sulphate occurred compared with skin fibroblasts of a normal fetus. In addition to these investigations, determination of heparin sulphamidase and N-acetyl- α -D-glucosaminidase was performed. Skin fibroblasts from the at-risk fetus did not release inorganic sulphate from heparin-N- 35 -sulphate in significant amounts, and the deficiency was also found in cultured amniotic fluid cells and in organs of the affected fetus (heart, lung, brain, adrenal glands). The results obtained established the diagnosis of Sanfilippo syndrome type A, which was confirmed by correction studies on cultured fetal skin fibroblasts.

Thus, the elucidation of the enzyme defects in the mucopolysaccharidoses has given new possibilities for the rapid, unequivocal diagnosis of these disorders. However, before enzyme assays can be used to their full advantage for prenatal diagnosis,

suitable assay methods must be developed which are applicable to the clinical situation, and more data must be obtained on variation in enzyme levels with time of gestation and with length of cell culture.

Attempts to correct biochemical defect

Methods of treatment suggested in the earlier literature included the use of steroids (Schiller and Dorfman, 1957; Wolfson *et al*, 1963; Rennert and Dekaban, 1966b), vitamin A alcohol, which promotes clearing of cellular metachromasia in fibroblasts cultures (Danes and Bearn, 1966b; Madsen and Linker, 1969) and vitamin C deficient diets (Schafer *et al*, 1966; DeJong, Robertson, and Schafer, 1968) but all met with little success. More recently, attempts have been made to introduce the missing enzyme by means of the infusion of whole blood, plasma, or leucocytes (DiFerrante *et al*, 1971; Knudson, DiFerrante, and Curtis, 1971; Dekaban, Holden, and Constantopoulos, 1972; Erickson, *et al* 1972). DiFerrante *et al* (1971) treated 2 Hurler and 5 Hunter patients with a plasma infusion and reported a decrease in excretion of large molecular weight GAGs and increased excretion of their degradative products. They also observed a striking decrease in hyperactive behaviour, a softening of the skin, and a dramatic improvement in the 'claw hands' of the Hurler patients. One month after treatment the patients' behaviour returned abruptly to that observed before treatment.

In none of the earlier reported studies had a quantification of the administered enzyme on the basis of its metabolic or enzymatic activity been attempted. However, Figura and Kresse (1974) investigated the cellular uptake of N-acetyl- α -D-glucosaminidase, the deficient enzyme in Sanfilippo syndrome type B, and studied its intracellular fate and metabolic effect in Sanfilippo B and normal fibroblasts. They showed the half life of the enzyme to be 34 hours after ingestion by Sanfilippo fibroblasts, and the cells maintained an effective and constant uptake of the deficient enzyme over a period of at least six days. They calculated that 150 ml serum would be needed for half-maximal correction of 1 g cell protein, and that, on this basis, the doses of enzyme applied in the clinical tests seem to be insufficient for a specific and successful therapy.

It is generally accepted that the transport of macromolecules from the exterior to the interior of animal cells occurs by pinocytosis, the engulfed plasma membrane forming a pinocytotic vesicle which finally merges with a lysosome. To account for the efficiency of uptake of certain lysosomal

enzymes into cultured fibroblasts, Hickman and Neufeld (1972) investigated skin fibroblasts cultured from patients with I-cell disease (mucopolisodosis II) which are conspicuously deficient in several lysosomal hydrolases, though the enzymes are found in surrounding medium. From their studies, it was suggested that the packaging of lysosomal enzymes requires their secretion followed by specific recognition and uptake, involving a recognition marker on the enzyme, a receptor on the fibroblast membrane and concentration of the enzyme against a gradient. Hickman, Shapiro, and Neufeld (1974) provided further evidence for a recognition marker, again using cultured fibroblasts.

These observations that catalytic activity and uptake into cells appear to be distinct and separable properties of lysosomal hydrolases is of importance to enzyme replacement therapy for lysosomal storage disorders. Administration of highly purified enzymes that fail to enter the affected cells must be considered a futile therapeutic endeavour. On the other hand, preparation of enzymes with the uptake marker (perhaps by chemical coupling of marker to protein) may permit targeting of the enzymes to lysosomes of the relevant cells. The possibility of such an approach has been suggested by Rogers and Kornfeld (1971) who showed that lysozyme and albumin were effectively taken up by rat liver if they were first modified by attachment of appropriate oligosaccharides. Enzyme replacement therapy has been attempted more frequently with regard to lipid storage diseases, and this subject has been reviewed by Brady, Gal, and Pentchev in 1974.

Conclusions

The importance of the enzyme discoveries is threefold.

(a) With suitable assay procedures they should enable an unequivocal diagnosis of the different mucopolysaccharidoses to be made. Until suitable enzyme assays are available, however, urine analysis will retain a significant role in diagnosis, coupled with fibroblast culture studies (labelled sulphate uptake and cross correction).

(b) Enzyme assays on cultured amniotic fluid cells will permit improved intrauterine diagnosis in early pregnancy.

(c) The discovery of the enzyme defects offers an opportunity for therapeutic replacement.

REFERENCES

- Astrup, P. (1947). On the determination of heparin in blood plasma and urine. *Acta Pharmacologica et Toxicologica*, **3**, 165-178.
- Bach, G., Eisenberg, F., Cantz, M., and Neufeld, E. F. (1973). The defect in the Hunter syndrome: deficiency of sulfiduronate sulfatase. *Proceedings of the National Academy of Sciences of the United States of America*, **70**, 2134-2138.
- Bach, G., Friedman, R., Weissmann, B., and Neufeld, E. F. (1972). The defect in the Hurler and Scheie syndromes: deficiency of α -L-iduronidase. *Proceedings of the National Academy of Sciences of the United States of America*, **69**, 2048-2051.
- Barton, R. W. and Neufeld, E. F. (1971). The Hurler corrective factor. Purification and some properties. *Journal of Biological Chemistry*, **246**, 7773-7779.
- Barton, R. W. and Neufeld, E. F. (1972). A distinct biochemical deficit in the Maroteaux-Lamy syndrome (mucopolysaccharidosis VI). *Journal of Pediatrics*, **80**, 114-116.
- Beaudet, A., DiFerrante, N., Ferry, G., Nichols, B., and Mullins, C. (1975). Variation in the phenotypic expression of β glucuronidase deficiency. *Journal of Pediatrics*, **86**, 388-394.
- Beaudet, A. L., DiFerrante, N., Nichols, B., and Ferry, G. D. (1972). β -glucuronidase deficiency: altered enzyme substrate recognition (abstract). *American Journal of Human Genetics*, **24**, 25a.
- Benson, P. F., Bowser-Riley, R., and Giannelli, F. (1970). β galactosidase in fibroblasts: Hurler and Sanfilippo syndromes. *New England Journal of Medicine*, **283**, 999-1000.
- Bollet, A. J., Bonner, W. M., and Nance, J. L. (1963). The presence of hyaluronidase in various mammalian tissues. *Journal of Biological Chemistry*, **238**, 3522-3527.
- Brady, R. O., Gal, A., and Pentchev, P. G. (1974). Evolution of enzyme replacement therapy for lipid storage disease. *Life Sciences*, **15**, 1235-1248.
- Brante, G. (1952). Gargoylism: a mucopolysaccharidosis. *Scandinavian Journal of Clinical and Laboratory Investigation*, **4**, 43-46.
- Brock, D., Gordon, H., Seligman, S., and Lobo, E. (1971). Antenatal detection of Hurler's syndrome. *Lancet*, **2**, 1324-1325.
- Brown, D. H. (1957). Tissue storage of mucopolysaccharides in Hurler-Pfaundler's disease. *Proceedings of the National Academy of Sciences of the United States of America*, **43**, 783-790.
- Buddecke, E. and Kresse, H. (1974). Mammalian enzymes degrading glycosaminoglycans. In *Connective Tissues. Biochemistry and Pathophysiology*, pp. 131-145. Ed. by R. Fricke and F. Hartmann. Springer-Verlag, New York.
- Buddecke, E. and Werries, E. (1964). Reinigung und Eigenschaften einer β -N-Acetyl-D-hexosaminidase aus Rindermilz. *Zeitschrift für Naturforschung*, **19b**, 798-800.
- Buddecke, E. and Werries, E. (1965). Untersuchungen zur Chemie der Arterienwand. VII. Reinigung und Eigenschaften der β -Acetyl-glucosaminidase aus der Aorta des Rindes. *Hoppe-Seyler's Zeitschrift für physiologische Chemie*, **340**, 257-272.
- Calatroni, A., Donnelly, P. V., and DiFerrante, N. (1969). The glycosaminoglycans of human plasma. *Journal of Clinical Investigation*, **48**, 332-343.
- Callahan, W. P. and Lorincz, A. E. (1964). Changes in liver ultrastructure associated with the Hurler syndrome (abstract). *Anatomical Record*, **148**, 267.
- Callahan, W. P. and Lorincz, A. E. (1966). Hepatic ultrastructure in the Hurler syndrome. *American Journal of Pathology*, **48**, 277-298.
- Cantz, M., Chrambach, A., Bach, G., and Neufeld, E. F. (1972). The Hunter corrective factor. Purification and preliminary characterization. *Journal of Biological Chemistry*, **247**, 5456-5462.
- Cantz, M., Chrambach, A., and Neufeld, E. F. (1970). Characterization of the factor deficient in the Hunter syndrome by polyacrylamide gel electrophoresis. *Biochemical and Biophysical Research Communications*, **39**, 936-942.
- Chain, E. and Duthie, E. S. (1940). Identity of hyaluronidase and spreading factor. *British Journal of Experimental Pathology*, **21**, 324-338.
- Constantopoulos, G. (1968). Hunter-Hurler syndrome: gel filtration and dialysis of urinary acid mucopolysaccharides. *Nature (London)*, **220**, 583-585.
- Constantopoulos, G. and Dekaban, A. S. (1975). Chemical definition of the mucopolysaccharidoses. *Clinica Chimica Acta*, **59**, 321-336.
- Coppa, G. V., Singh, J., Nichols, B. L., and DiFerrante, N. (1973). Urinary excretion of disulfated disaccharides in Hunter syndrome. Correction by infusion of a serum fraction. *Analytic Letters*, **6**, 225-233.
- Crawford, M., Dean, M., Hunt, D., Johnson, D., MacDonald, R., Muir, H., Payling Wright, E., and Payling Wright, C. (1973). Early prenatal diagnosis of Hurler's syndrome with termination of

- pregnancy and confirmatory findings on the fetus. *Journal of Medical Genetics*, **10**, 144–153.
- Danes, B. S. and Bearn, A. G. (1965). Hurler's syndrome: demonstration of an inherited disorder of connective tissue in cell culture. *Science*, **149**, 987–989.
- Danes, B. S. and Bearn, A. G. (1966a). Hurler's syndrome: a genetic study in cell culture. *Journal of Experimental Medicine*, **123**, 1–16.
- Danes, B. S. and Bearn, A. G. (1966b). Hurler's syndrome: effect of retinol (vitamin A alcohol) on cellular mucopolysaccharides in cultured human skin fibroblasts. *Journal of Experimental Medicine*, **124**, 1181–1198.
- Danes, B. S. and Bearn, A. G. (1968a). Gaucher's disease: a genetic disease detected in skin fibroblast cultures. *Science*, **161**, 1347–1348.
- Danes, B. S. and Bearn, A. G. (1968b). A genetic cell marker in cystic fibrosis of the pancreas. *Lancet*, **1**, 1061–1063.
- de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., and Appelmans, F. (1955). Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat liver tissue. *Biochemical Journal*, **60**, 604–617.
- DeJong, B. P., Robertson, W. V., and Schafer, I. A. (1968). Failure to induce scurvy by ascorbic acid depletion in a patient with Hurler's syndrome. *Pediatrics*, **42**, 889–903.
- Dekaban, A. S., Holden, K., and Constantopoulos, G. (1972). Effects of fresh plasma or whole blood transfusions on patients with various types of mucopolysaccharidosis. *Pediatrics*, **50**, 688–692.
- Dietrich, C. P. (1970). A heparin sulfamidase from mammalian lymphoid tissues. *Canadian Journal of Biochemistry and Physiology*, **48**, 725–733.
- DiFerrante, N., Hyman, B., Klisch, W., Donnelly, P., Nichols, B., and Dutton, R. (1974). Mucopolysaccharidosis VI (Maroteaux–Lamy disease). Clinical and biochemical study of a mild variant case. *Johns Hopkins Medical Journal*, **135**, 42–54.
- DiFerrante, N., Nichols, B. L., Donnelly, P. V., Neri, G., Hrgovcic, R., and Berglund, R. (1971). Induced degradation of glycosaminoglycans in Hurler's and Hunter's syndromes by plasma infusion. *Proceedings of the National Academy of Sciences of the United States of America*, **68**, 303–307.
- DiFerrante, N. and Rich, C. (1956). The mucopolysaccharide of normal urine. *Clinica Chimica Acta*, **1**, 519–524.
- Dohlman, C. H. (1956). The fate of the sulfate group of chondroitin sulfate after administration to rats. *Acta Physiologica Scandinavica*, **37**, 220–234.
- Dorfman, A. (1964). Metabolism of acid mucopolysaccharides. *Biophysical Journal*, **4**, Suppl., 155–165.
- Dorfman, A. and Lorincz, A. E. (1957). Occurrence of urinary acid mucopolysaccharides in the Hurler syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, **43**, 443–446.
- Dorfman, A. and Matalon, R. (1972). The mucopolysaccharidoses. In *The Metabolic Basis of Inherited Disease*, 3rd ed., pp. 1218–1263. Ed. by J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson. McGraw-Hill, New York.
- Dziewiatkowski, D. D. (1956). Some aspects of the metabolism of chondroitin sulfate-S35 in the rat. *Journal of Biological Chemistry*, **223**, 239–249.
- Erickson, R., Sandman, R., Robertson, W., and Epstein, C. (1972). Inefficacy of fresh frozen plasma therapy of mucopolysaccharidosis II. *Pediatrics*, **50**, 693–701.
- Figura, K. von and Kresse, H. (1972). The Sanfilippo B corrective factor: a N-acetyl- α -D-glucosaminidase. *Biochemical and Biophysical Research Communications*, **48**, 262–269.
- Figura, K. von and Kresse, H. (1974). Quantitative aspects of pinocytosis and the intracellular fate of N-acetyl- α -D-glucosaminidase in Sanfilippo B fibroblasts. *Journal of Clinical Investigation*, **53**, 85–90.
- Figura, K. von, Lögering, M., Mersmann, G., and Kresse, H. (1973). Sanfilippo B disease: serum assays for detection of homozygous and heterozygous individuals in three families. *Journal of Pediatrics*, **83**, 607–611.
- Fisher, D., Higham, M., Kent, P., and Pritchard, P. (1966). β -xylosidases of animal and other sources in relation to the degradation of chondroitin sulphate-peptide complexes. *Biochemical Journal*, **98**, 46P.
- Fisher, D. and Kent, P. (1969). Rat liver β -xylosidase: a lysosomal membrane enzyme. *Biochemical Journal*, **115**, 50–51P.
- Fluharty, A. L., Porter, M. T., Lassila, E. L., Trammell, J., Carrel, R. E., and Kihara, H. (1970). Acid glycosidases in mucopolysaccharidoses fibroblasts. *Biochemical Medicine*, **4**, 110–120.
- Fluharty, A. L., Stevens, R. L., Sanders, D. L., and Kihara, H. (1974). Arylsulfatase B deficiency in Maroteaux–Lamy syndrome cultured fibroblasts. *Biochemical and Biophysical Research Communications*, **59**, 445–461.
- Fratantoni, J. C., Hall, C. W., and Neufeld, E. F. (1968a). The defect in Hurler's and Hunter's syndromes: faulty degradation of mucopolysaccharide. *Proceedings of the National Academy of Sciences of the United States of America*, **80**, 699–706.
- Fratantoni, J. C., Hall, C. W., and Neufeld, E. F. (1968b). Hurler and Hunter syndromes: mutual correction of the defect in cultured fibroblasts. *Science*, **162**, 570–572.
- Fratantoni, J. C., Neufeld, E. F., Uhlendorf, B. W., and Jacobson, C. (1969). Intrauterine diagnosis of the Hurler and Hunter syndromes. *New England Journal of Medicine*, **280**, 686–688.
- Friedman, R. B. and Weissmann, B. (1972). The phenyl- α - and β -L-idopyranosiduronic acids and some other arylglycopyranosiduronic acids. *Carbohydrate Research*, **24**, 123–131.
- Friedman, Y. and Arsenis, C. (1972). The resolution of arylsulfatase and heparin sulfamidase activities from various rat tissues. *Biochemical and Biophysical Research Communications*, **48**, 1133–1139.
- Friedman, Y. and Arsenis, C. (1974). Studies on the heparin sulfamidase activity from rat spleen. Intracellular distribution and characterisation of the enzyme. *Biochemical Journal*, **139**, 699–708.
- Glaser, J. H. and Sly, W. S. (1973). β -glucuronidase deficiency mucopolysaccharidosis: methods for enzymatic diagnosis. *Journal of Laboratory and Clinical Medicine*, **82**, 969–977.
- Gordon, B. A. and Feleki, V. (1970). Acid hydrolases in the serum and liver in mucopolysaccharidoses types I and II. *Clinical Biochemistry*, **3**, 193–202.
- Gordon, B. A. and Haust, M. D. (1971). Hepatic acid mucopolysaccharides in the mucopolysaccharidoses type I, II, and III. *Clinical Biochemistry*, **4**, 147–155.
- Hall, C. W., Cantz, M., and Neufeld, E. F. (1973). A β glucuronidase deficiency mucopolysaccharidosis: studies in cultured fibroblasts. *Archives of Biochemistry and Biophysics*, **155**, 32–38.
- Hall, C. W. and Neufeld, E. F. (1973). α -L-iduronidase activity in cultured skin fibroblasts and amniotic fluid cells. *Archives of Biochemistry and Biophysics*, **158**, 817–821.
- Harper, P. S., Laurence, K. M., Parkes, A., Wusteman, F., Kresse, H., Figura, K. von, Ferguson-Smith, M., Duncan, D., Logan, R., Hall, F., and Whiteman, P. (1974). Sanfilippo A disease in the fetus. *Journal of Medical Genetics*, **11**, 123–132.
- Held, E. and Buddecke, E. (1967). Nachweis, Reinigung und Eigenschaften einer Chondroitin-4-Sulfatase aus der Aorta des Rindes. *Hoppe-Seylers Zeitschrift für physiologische Chemie*, **348**, 1047–1060.
- Hickman, S. and Neufeld, E. F. (1972). A hypothesis for I-cell disease: defective hydrolases that do not enter lysosomes. *Biochemical and Biophysical Research Communications*, **49**, 992–999.
- Hickman, S., Shapiro, L. J., and Neufeld, E. F. (1974). A recognition marker required for uptake of a lysosomal enzyme by cultured fibroblasts. *Biochemical and Biophysical Research Communications*, **57**, 55–61.
- Ho, M. W. and O'Brien, J. S. (1969). Hurler's syndrome: deficiency of a specific β galactosidase isoenzyme. *Science*, **165**, 611–613.
- Jeanloz, R. W. (1960). The nomenclature of mucopolysaccharides. *Arthritis and Rheumatism*, **3**, 233–237.
- Jenkins, P., Davies, G. R., and Harper, P. S. (1973). Morquio–Brailsford disease: a report of four affected sisters with absence of excessive keratan sulphate in the urine. *British Journal of Radiology*, **46**, 668–675.
- Kaplan, D. (1969). Classification of the mucopolysaccharidoses based on the pattern of mucopolysacchariduria. *American Journal of Medicine*, **47**, 721–729.
- Kaplan, D., McKusick, V. A., Trebach, S., and Lazarus, R. (1968). Keratosulfate-chondroitin sulfate peptide from normal urine and from urine of patients with Morquio syndrome (mucopolysaccharidosis IV). *Journal of Laboratory and Clinical Medicine*, **71**, 48–55.
- Kerby, G. P. (1954). The excretion of glucuronic acid and of acid mucopolysaccharides in normal human urine. *Journal of Clinical Investigation*, **33**, 1168–1174.

- Knecht, J., Cifonelli, J., and Dorfman, A. (1967). Structural studies on heparitin sulfate of normal and Hurler tissues. *Journal of Biological Chemistry*, **242**, 4652-4661.
- Knudson, A. G., DiFerrante, N., and Curtis, J. E. (1971). Effect of leukocyte transfusion in a child with type II mucopolysaccharidosis. *Proceedings of the National Academy of Sciences of the United States of America*, **68**, 1738-1741.
- Kresse, H. (1973). Mucopolysaccharidosis IIIA (Sanfilippo A disease): deficiency of a heparin sulfamidase in skin fibroblasts and leucocytes. *Biochemical and Biophysical Research Communications*, **54**, 1111-1118.
- Kresse, H. and Neufeld, E. F. (1972). The Sanfilippo A corrective factor. Purification and mode of action. *Journal of Biological Chemistry*, **247**, 2164-2170.
- Kresse, H., Wiesmann, U., Cantz, M., Hall, C., and Neufeld, E. F. (1971). Biochemical heterogeneity of the Sanfilippo syndrome: preliminary characterization of two deficient factors. *Biochemical and Biophysical Research Communications*, **42**, 892-898.
- Liem, K. O. and Hooghwinkel, G. J. M. (1975). The use of α -L-iduronidase activity determinations in leucocytes for the detection of Hurler and Scheie syndromes. *Clinica Chimica Acta*, **60**, 259-262.
- Lim, T. W., Leder, I. G., Bach, G., and Neufeld, E. F. (1974). An assay for iduronate sulfatase (Hunter corrective factor). *Carbohydrate Research*, **37**, 103-109.
- Lindahl, Y. and Axelsson, O. (1971). Identification of iduronic acid as the major sulfated uronic acid of heparin. *Journal of Biological Chemistry*, **246**, 74-82.
- Linker, A., Evans, L. R., and Langer, L. O. (1970). Morquio's disease and mucopolysaccharide excretion. *Journal of Pediatrics*, **77**, 1039-1047.
- Linker, A., Meyer, K., and Weissmann, B. (1955). Enzymatic formation of monosaccharides from hyaluronate. *Journal of Biological Chemistry*, **213**, 237-248.
- Lorincz, A. E. (1958). Acid mucopolysaccharides in the Hurler syndrome. *Federation Proceedings*, **17**, 266.
- MacBrinn, M. C., Okasa, S., Woolacott, M., Patel, V., Ho, M., Tappel, A. L., and O'Brien, J. S. (1969). β galactosidase deficiency in the Hurler syndrome. *New England Journal of Medicine*, **281**, 331-343.
- McGale, E. H. (1969). Protein-carbohydrate compounds in human urine. *Advances in Carbohydrate Chemistry*, **24**, 435-452.
- McKusick, V. A. (1969). The nosology of the mucopolysaccharidoses. *American Journal of Medicine*, **47**, 730-747.
- McKusick, V. A. (1972). *Heritable Disorders of Connective Tissue*, pp. 521-686. C. V. Mosby, St. Louis.
- McKusick, V. A., Kaplan, D., Wise, D., Hanley, W. B., Suddarth, S. B., Seveck, M. E., and Maumane, A. E. (1965). The genetic mucopolysaccharidoses. *Medicine*, **44**, 445-483.
- Madsen, J. and Linker, A. (1969). Vitamin A and mucopolysaccharidosis: a clinical and biochemical evaluation. *Journal of Pediatrics*, **75**, 843-852.
- Malmstrom, A. and Fransson, L.-A. (1971). Structure of pig skin dermatan sulfate. 2. Demonstration of sulfated iduronic acid residues. *European Journal of Biochemistry*, **18**, 431-435.
- Maroteaux, P. (1970). Différenciation biochimique des maladies de Hurler et de Hunter par fractionnement de l'héparine sulfate. *Revue Européenne d'Etudes Cliniques et Biologiques*, **15**, 203-205.
- Maroteaux, P. and Lamy, M. (1967). Studying the mucopolysaccharidoses. *Lancet*, **2**, 510.
- Matalon, R., Arbogast, B., and Dorfman, A. (1974a). Deficiency of chondroitin sulfate N-acetyl galactosamine-4-sulfate sulfatase in Maroteaux-Lamy syndrome. *Biochemical and Biophysical Research Communications*, **61**, 1450-1457.
- Matalon, R., Arbogast, B., Justice, P., Brandt, I., and Dorfman, A. (1974b). Morquio's syndrome: deficiency of a chondroitin sulfate N-acetyl hexosamine sulfate sulfatase. *Biochemical and Biophysical Research Communications*, **61**, 759-756.
- Matalon, R., Cifonelli, J., and Dorfman, A. (1971). α -L-iduronidase in cultured human fibroblasts and liver. *Biochemical and Biophysical Research Communications*, **42**, 340-345.
- Matalon, R. and Dorfman, A. (1966). Hurler's syndrome: biosynthesis of acid mucopolysaccharides in tissue culture. *Proceedings of the National Academy of Sciences of the United States of America*, **56**, 1310-1316.
- Matalon, R. and Dorfman, A. (1968a). The accumulation of hyaluronic acid in cultured fibroblasts of the Marfan syndrome. *Biochemical and Biophysical Research Communications*, **32**, 150-154.
- Matalon, R. and Dorfman, A. (1968b). The structure of acid mucopolysaccharides produced by Hurler fibroblasts in tissue culture. *Proceedings of the National Academy of Sciences of the United States of America*, **60**, 179-185.
- Matalon, R. and Dorfman, A. (1972). Hurler's syndrome, an α -L-iduronidase deficiency. *Biochemical and Biophysical Research Communications*, **47**, 959-964.
- Matalon, R. and Dorfman, A. (1974). Sanfilippo A syndrome. Sulfamidase deficiency in cultured skin fibroblasts and liver. *Journal of Clinical Investigation*, **54**, 907-912.
- Matalon, R., Dorfman, A., Dawson, G., and Sweeley, C. C. (1969). Glycolipid and mucopolysaccharide abnormality in fibroblasts of Fabry's disease. *Science*, **164**, 1522-1523.
- Matalon, R., Dorfman, A., and Nadler, H. (1972). A chemical method for the antenatal diagnosis of mucopolysaccharidoses. *Lancet*, **1**, 798-799.
- Matalon, R., Dorfman, A., Nadler, H., and Jacobson, C. (1970). A chemical method for the antenatal diagnosis of mucopolysaccharidoses. *Lancet*, **1**, 83-84.
- Meyer, K., Dubos, R., and Smyth, E. M. (1937). The hydrolysis of the polysaccharide acids of vitreous humor, of umbilical cord, and of streptococcus by the autolytic enzyme of pneumococcus. *Journal of Biological Chemistry*, **118**, 71-78.
- Meyer, K., Grumbach, M., Linker, A., and Hoffman, P. (1958). Excretion of sulfated mucopolysaccharides in gargoylism (Hurler's syndrome). *Proceedings of the Society for Experimental Biology and Medicine*, **97**, 275-279.
- Muir, H. (1969). The structure and metabolism of mucopolysaccharides (glycosaminoglycans) and the problem of the mucopolysaccharidoses. *American Journal of Medicine*, **47**, 673-690.
- Nadler, H. L. (1968). Antenatal detection of hereditary disorders. *Pediatrics*, **42**, 912-918.
- Neufeld, E. F. and Cantz, M. J. (1971). Corrective factors for inborn errors of mucopolysaccharide metabolism. *Annals of the New York Academy of Sciences*, **179**, 580-587.
- Neufeld, E. F. and Fratanoti, J. C. (1970). Inborn errors of mucopolysaccharide metabolism. *Science*, **169**, 141-146.
- O'Brien, J. S. (1972). Sanfilippo syndrome: profound deficiency of α -acetyl glucosaminidase activity in organs and skin fibroblasts from type B patients. *Proceedings of the National Academy of Sciences of the United States of America*, **69**, 1720-1722.
- O'Brien, J. F., Cantz, M., and Spranger, J. (1974). Maroteaux-Lamy disease (mucopolysaccharidosis VI), subtype A; deficiency of a N-acetyl galactosamine-4-sulfatase. *Biochemical and Biophysical Research Communications*, **60**, 1170-1177.
- Öckerman, P. A. (1968). Acid hydrolases in skin and plasma in gargoylism. Deficiency of β -galactosidase in skin. *Clinica Chimica Acta*, **20**, 1-6.
- Okada, S. and O'Brien, J. S. (1968). Generalised gangliosidosis: β -galactosidase deficiency. *Science*, **160**, 1002-1004.
- Orizaga, M., Haust, M. D., Bryans, A. M., and Frank, H. F. (1963). Electron microscopic study of liver in Hurler's syndrome. *Federation Proceedings*, **22**, 484.
- Patel, V. and Tappel, A. (1969). Identity of β -glucosidase and β -xylosidase activities in rat liver lysosomes. *Biochimica et Biophysica Acta*, **191**, 86-94.
- Pedrin, V., Lenz, L., and Zambotti, V. (1962). Isolation and identification of keratosulphate in urine of patients affected by Morquio-Ullrich disease. *Proceedings of the Society for Experimental Biology and Medicine*, **110**, 847-849.
- Pennock, C. A. (1973). Glycosaminoglycan excretion. M.D. Thesis, University of Bristol.
- Phelps, C. F. (1973). The biosynthesis of glycosaminoglycans. *Biochemical Society Transactions*, **1**, 814-819.
- Rennert, O. M. and Dekaban, A. S. (1966a). Amino acid metabolism in patients with Hurler's syndrome. *Metabolism*, **15**, 429-435.
- Rennert, O. M. and Dekaban, A. S. (1966b). Modification of urinary mucopolysaccharide excretion in patients with Hurler's syndrome. *Clinical Pharmacology and Therapeutics*, **7**, 783-787.
- Robins, M. M., Stevens, H. F., and Linker, A. (1963). Morquio's disease: an abnormality of mucopolysaccharide metabolism. *Journal of Pediatrics*, **62**, 881-889.
- Robinson, D. and Abrahams, H. E. (1967). β -D-xylosidase in pig kidney. *Biochimica et Biophysica Acta*, **132**, 212-214.
- Rogers, J. and Kornfeld, S. (1971). Hepatic uptake of proteins coupled to fetuin glycopeptide. *Biochemical and Biophysical Research Communications*, **45**, 622-629.
- Sanfilippo, S. J., Podosin, R., Langer, L. O., and Good, R. A. (1963).

- Mental retardation associated with acid mucopolysacchariduria (heparitin sulfate type). *Journal of Pediatrics*, **63**, 837-838.
- Schafer, I. A., Sullivan, J. C., Svecjar, J., Kofoed, J., and Robertson, W. (1966). Vitamin C-induced increase of dermatan sulfate in cultured Hurler's fibroblasts. *Science*, **153**, 1008-1010.
- Schiller, S. and Dorfman, A. (1957). The metabolism of mucopolysaccharides in animals: the effect of cortisone and hydrocortisone on rat skin. *Endocrinology*, **60**, 376-381.
- Shapira, E., DeGregorio, R., Matalon, R., and Nadler, H. (1975). Reduced arylsulfatase B activity of the mutant enzyme protein in Maroteaux-Lamy syndrome. *Biochemical and Biophysical Research Communications*, **62**, 448-455.
- Singer, H. S. and Schafer, I. A. (1970). White-cell β -galactosidase activity. *New England Journal of Medicine*, **282**, 571.
- Sjoberg, I., Fransson, L.-A., Matalon, R., and Dorfman, A. (1973). Hunter's syndrome: a deficiency of L-idurono-sulfate sulfatase. *Biochemical and Biophysical Research Communications*, **54**, 1125-1132.
- Sly, W. S., Quinton, B. A., McAlister, W. H., and Rimoin, D. (1973). β -glucuronidase deficiency: report of clinical, radiologic and biochemical features of a new mucopolysaccharidosis. *Journal of Pediatrics*, **82**, 249-257.
- Spranger, J. W. (1970). Biochemical definition of the mucopolysaccharidoses. *Zeitschrift für Kinderheilkunde*, **108**, 17-31.
- Spranger, J. W., Koch, F., McKusick, V. A., Natzschka, J., Wiedemann, H. R., and Zellweger, H. (1970). Mucopolysaccharidosis VI (Maroteaux-Lamy's disease). *Helvetica Paediatrica Acta*, **25**, 337-362.
- Stumpf, D. A., Austen, J. H., Crockev, A. C., and La France, M. (1973). Mucopolysaccharidosis Type VI (Maroteaux-Lamy syndrome). 1. Sulfatase B deficiency in tissues. *American Journal of Diseases in Children*, **26**, 747-755.
- Suzuki, S., Saito, H., Yamagata, T., Anno, K., Seno, N., Kawai, Y., and Furuhashi, T. (1968). Formation of three types of disulfated disaccharides from chondroitin sulfates by chondroitinases digestion. *Journal of Biological Chemistry*, **243**, 1543-1550.
- Thomas, G. H. (1969). β -D-galactosidase in human urine; deficiency in generalised gangliosidosis. *Journal of Laboratory and Clinical Medicine*, **74**, 725-731.
- Tudball, N. and Davidson, E. A. (1969). Isolation of a novel sulphatase from rat liver. *Biochimica et Biophysica Acta*, **171**, 113-120.
- Van Gemund, J. J., Giesberts, M. A., Gorsira, M. C., and Willighagen, R. G. (1969). Deficiency of 4-methylumbelliferyl- β -galactosidase activity in the liver of seven patients with Hurler's disease. *Maandschrift voor kindergeneeskunde*, **36**, 377-383.
- Van Hoof, F. and Hers, H. G. (1964). L'ultrastructure les cellules hépatiques dans la maladie de Hurler (gargoylisme). *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, **259**, 1281-1283.
- Van Hoof, F. and Hers, H. G. (1968). The abnormalities of lysosomal enzymes in mucopolysaccharidoses. *European Journal of Biochemistry*, **7**, 34-44.
- Weissmann, B. (1955). The transglycosylative action of testicular hyaluronidase. *Journal of Biological Chemistry*, **216**, 783-794.
- Weissmann, B., Hadjiioannou, S., and Tornheim, J. (1964). Oligosaccharase activity of β -N-acetyl-D-glucosaminidase of beef liver. *Journal of Biological Chemistry*, **239**, 159-163.
- Weissmann, B. and Santiago, R. (1972). α -L-iduronidase in lysosomal extracts. *Biochemical and Biophysical Research Communications*, **46**, 1430-1433.
- Wiesmann, U. and Neufeld, E. F. (1970). Scheie and Hurler syndromes: apparent identity of the biochemical defect. *Science*, **169**, 72-74.
- Wolfson, S. L., Davidson, E., Harris, J. S., Kahana, L., and Lorincz, A. E. (1963). Long-term corticosteroid therapy in Hurler syndrome. *American Journal of Diseases of Children*, **106**, 3-10.