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Lysosomes and Mucopolysaccharidoses

By H. G. HERS and F. VAN HOOFF. (*Laboratoire de Chimie Physiologique, Université de Louvain, Belgium*)

A series of conditions are known in human pathology in which there is a tissular accumulation of both mucopolysaccharides and glycolipids. Some of them have been classified as mucopolysaccharidoses, others as gangliosidoses or lipidoses, and they could be adequately designated as 'lipo-mucopolysaccharidoses'. The clinical syndrome includes variable degrees of dwarfism, hepatosplenomegaly, skeletal deformities and mental retardation (for a more extensive bibliography see Dorfman, 1966; Hers & Van Hoof, 1969). In the Hurler syndrome, there is an excessive urinary excretion of chondroitin sulphate B, heparitin sulphate and keratan sulphate, either alone or in association. In the pseudo-Hurler disease, also called Tay-Sachs disease with visceral involvement or generalized gangliosidosis, there is a tissue accumulation of the GM₁ ganglioside, which bears a terminal galactosyl unit, and of a mucopolysaccharide rich in galactose. Several other clinical types of mucopolysaccharidoses have also been reported.

As storage diseases in which the depot is chemically heterogeneous, the mucopolysaccharidoses must be regarded as potential inborn lysosomal diseases. This group of affections (Hers, 1965) includes all conditions resulting from the congenital abnormality of one lysosomal protein; when this protein is an enzyme, the loss of its activity causes the intravacuolar storage of all the compounds that would normally require the missing enzyme for their degradation in the process of either autophagy or of heterophagy (for a review on the physiology of lysosomes, see de Duve & Wattiaux, 1966). Like other digestive enzymes, the lysosomal acid hydrolases usually do not display a high substrate specificity and most of them are able to open one type of linkage, whatever the molecule in which it is present; therefore the loss of their activity easily explains the chemical heterogeneity of the depot that is so characteristic of many mucopolysaccharidoses and lipidoses. As a rule, the inborn lysosomal diseases are progressive and affect most tissues of the organism. Mechanical disturbance due to the lysosomal enlargement, discharge of lysosomal enzymes in the cytoplasm or dysfunction of the lysosome itself may be the cause of the pathological manifestation.

The involvement of the lysosomes in the pathology of mucopolysaccharidoses is clearly indicated by the ultrastructure of the tissues. In the liver of both Hurler (Van Hoof & Hers, 1964) and pseudo-

Hurler patients (Van Hoof & Hers, 1967), the stored material is concentrated in large vacuoles for which there is now a general agreement that they are enlarged lysosomes. Vacuoles have also been observed in other tissues, for instance in the brain (Aleu, Terry & Zellweger, 1965), where their 'zebra' appearance is presumably due to the abundance of glycolipids accumulated in that tissue.

Two mechanisms have been considered for the entry of mucopolysaccharides in the lysosomal system. A first possibility is that this material is synthesized in excess by the fibroblasts, released in the circulating fluids and then either excreted in the urine or taken up by the tissues through endocytosis. In this view, the lysosomes themselves would be initially normal and would accumulate mucopolysaccharides in the same way that they accumulate dextran when present in the circulating fluids. This theory does not explain the simultaneous deposition of glycolipids and of mucopolysaccharides.

The second interpretation is the congenital defect of one acid hydrolase normally involved in the degradation of both mucopolysaccharides and glycolipids. In this view, the mucopolysaccharidoses would be typical inborn lysosomal diseases, similar to type II glycogenosis, in which a defect of acid α -glucosidase explains the intralysosomal deposition of glycogen. In relation with this hypothesis, the activity of a large number of hydrolases has been assayed in the liver of patients with mucopolysaccharidosis (Van Hoof & Hers, 1968). It has been found that, in agreement with the concept of inborn lysosomal disease, many patients display either a complete absence or a great diminution of one acid hydrolase. Further, there is a large excess of several acid hydrolases in the liver of nearly all patients; this excess is presumably the consequence of the lysosomal enlargement and its mechanism is still badly understood. The patients have been tentatively classified in four groups, according to the main enzymic abnormality which could be discovered in their liver:

(1) A complete and generalized deficiency of α -fucosidase was observed in three Italian patients, who had also in common a great excess of fucose-containing glycolipids and polysaccharides in their tissues. The ultrastructure of their liver (Van Hoof & Hers, 1967) was also highly characteristic owing to the presence of two types of vacuoles, one similar to those currently found in the Hurler disease and with an empty appearance, the other containing membranous material probably of glycolipidic nature. The α -galactosidase (730% of the mean normal activity), the β -xylosidase (620%) and the α -glucosidase (460%) were the most hyperactive acid hydrolases. The names fucosidosis and mucopolysaccharidosis F have

been proposed to designate the disease affecting these three patients.

(2) In the pseudo-Hurler disease, a complete absence of acid β -galactosidase, at least when measured at pH 3.6, was observed in the liver and in all the tissues that were assayed. There was, however, a residual activity at higher pH values, which, in agreement with the electrophoretic study of Robinson, Price & Dance (1967), presumably reflects the presence of isoenzymes. The same deficiency has also been reported by Okada & O'Brien (1968) and by Dacremont & Kint (1968). It explains the accumulation of the GM₁ ganglioside and of galactose-rich mucopolysaccharides in the tissues of the patient. The α -galactosidase (620% of the mean normal activity), the *N*-acetylhexosaminidase (590%) and the α -fucosidase (500%) were markedly hyperactive. In relation to the complete defect of acid β -galactosidase, the pseudo-Hurler disease has been called mucopolysaccharidosis Gal.

(3) In the liver, skin and brain but not in all tissues of most patients with the usual types of Hurler syndrome there is an important diminution in the activity of acid β -galactosidase, which is decreased to a value ranging from 2 to 25% of the normal. The separation of several isoenzymes with β -galactosidase activity either by gel filtration (Öckerman & Hultberg, 1968) or on electrophoresis (Ho & O'Brien, 1969) has indicated that only one of the isoenzymes could be deficient in this type of disease. The causal relationship between the defect of this isoenzyme and the storage of mucopolysaccharides that are not particularly rich in galactose remains to be established. The livers of these patients contain an excess of *N*-acetylhexosaminidase (820% of the mean normal value), of α -fucosidase (450%) and of β -glucuronidase (410%). The disease characterized by this partial defect in acid β -galactosidase is called mucopolysaccharidosis Gal-.

(4) In contrast with the decreased activity of the acid β -galactosidase in the mucopolysaccharidoses Gal and Gal- and with its normal activity in most other patients, a great excess of this enzyme was found in the liver of two patients who also, as shown by Loeb, Tondeur, Toppet & Cremer (1969), distinguished themselves by a somewhat different aspect of their liver under the electron microscope. More recently, we have observed the same excess of β -galactosidase in two other patients studied by Spranger, Wiedemann, Tolksdorf, Graucob & Caesar (1968) and who also displayed the same structural aspect of their liver. It appears probable to us that these patients are affected by a separate type of disease, which we have called the mucopolysaccharidosis Gal+. The enzymic deficiency responsible for this disease needs still to be discovered. It may be mentioned that cultured

fibroblasts from the two last patients showed inclusions similar to those previously described by Leroy & DeMars (1967) and considered by them as characteristic of what they called 'I cell disease' (Dr J. Spranger, personal communication). All cases showing this type of inclusions in cultured fibroblasts do, however, not belong to the Gal+ group.

Other patients affected by mucopolysaccharidosis do not enter our classification because they do not display a typical abnormality in the activity of the lysosomal enzymes. One can hope, however, that further research involving the determination of a larger number of acid hydrolases will allow the recognition of other aetiological defects.

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Cathepsins in Pathology: an Immunoenzymic Study

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In considering the role of cathepsins in pathology one is concerned with defects in the balance between synthesis and breakdown of proteins and protein-carbohydrate complexes. It is suspected that such disturbances of the normally precise regulation of turnover are important in the net loss of muscle protein that occurs in muscular dystrophy, and of the protein-polysaccharide complex of cartilage in rheumatoid arthritis. In these diseases, synthesis

has been found to proceed at not less than normal rates, and breakdown is accelerated; there is evidence that lysosomal proteolytic enzymes are involved in these processes (Iodice & Weinstock, 1969; Dingle, 1966).

The cathepsins are a heterogeneous group of enzymes that hydrolyse peptide bonds under acidic conditions; their properties have recently been reviewed (Barrett, 1969). Cathepsins A, B, C and D occur in lysosomes; cathepsin E may be a derivative of cathepsin D. Probably all of the cathepsins, and some related enzymes that for historical reasons are not called cathepsins, are involved in the hydrolysis of proteins in tissues. Control of the rate of breakdown is probably mediated through the activity of the endopeptidases, however, since they initiate the degradation by hydrolysing bonds in intact molecules of the protein substrate. Cathepsins B, D and E are endopeptidases, whereas cathepsins A and C are exopeptidases. Cathepsin A acts on the peptides produced by cathepsin D and thus has a synergistic effect in a test system *in vitro* (Iodice, Leong & Weinstock, 1966).

Examination of extracts of many whole tissues shows that most of their proteolytic activity is due to an enzyme that rapidly hydrolyses haemoglobin at acid pH and is unaffected by cysteine and iodoacetamide; this enzyme is lysosomal, and it is now clear that it is cathepsin D (Barrett, 1967). There is already strong, but indirect, evidence that cathepsin D may be involved in the degeneration of cartilage (reviewed by Barrett, 1968), and for this reason my colleagues and I have begun a more detailed study of its biological role.

The first part of the project involved the thorough purification and characterization of cathepsin D from each of the three species in which we are interested, chicken, rabbit and man. We now have a purification scheme that can be used with livers from any of these species (A. J. Barrett, unpublished work); it involves homogenization of the tissue, removal of particulate material aggregated with calcium phosphate and autolysis. The preparation is then purified by fractionation with acetone, column chromatography on DEAE-cellulose and CM-cellulose and isoelectric focussing. With rabbit liver, which is not a good source of the enzyme, additional column chromatography with Sephadex G-100 and hydroxyapatite is required. From chicken liver, two main forms, or isoenzymes, of cathepsin D are resolved by isoelectric focussing, whereas three forms are obtained in a pure state from the human tissue. The forms of cathepsin D in rabbit liver are much more numerous, and as yet only very small quantities of any one isoenzyme have been completely purified. The final preparations of cathepsin D from chicken and man are homogeneous in isoelectric focussing in poly-