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-

acrylamide gel and immunodiffusion against multi-valent antisera raised against partially purified preparations; they show no increase in specific activity when subjected to further purification procedures. The pure forms of the enzyme have been run on a calibrated column of Sephadex G-100 at pH 6.9, and none has been found to have an elution volume significantly different from that of egg albumin (mol.wt. 45 000). The separated forms of cathepsin D from a given species have indistinguishable pH-activity profiles and give reactions of complete immunological identity in immunodiffusion against an antiserum raised against one of them. Isoelectric focussing of crude tissue extracts shows a pattern of isoenzymes very similar to that obtained in the final stage of purification. All forms of cathepsin D seem to contain some glucosamine, but no sialic acid was present in the one form of the chicken enzyme so far examined.

Specific antisera have been raised against cathepsin D in rabbits and sheep, by the injection of precipitin lines (Weston, 1969) or pure isoenzymes, and these sera have been found to be specific inhibitors of the enzyme in the range pH 5-7 (A. J. Barrett, P. D. Weston & J. T. Dingle, unpublished work). The action of chicken cathepsin D (1 unit/ml., as defined by Barrett, 1967) on both haemoglobin and the light protein-polysaccharide complex of cartilage is totally inhibited by less than 25 μ l of antiserum. Normal serum obtained before immunization does not inhibit cathepsin D, and the inhibition can be obtained with purified γ G globulin fractions of the antisera. In confirmation of the immunological evidence for the specificity of the antisera, we have shown that they do not inhibit papain or chicken liver acid phosphatase, β -glucuronidase, β -acetylglucosaminidase, β -glucosidase, arylsulphatase, hyaluronidase or cathepsin B. In immunodiffusion against the specific antisera, crude extracts of several organs of chicken as well as embryonic bone rudiments gave a single precipitin line fusing with that of liver, and containing acid proteinase (Weston, 1969).

As a model system in which to study the breakdown of articular tissues, we have used the autolytic breakdown of chicken cartilage matrix in the range pH 5-7. The release of the polysaccharide com-

ponent of the protein-polysaccharide complex was measured by chemical and radiochemical means, and the process was found to be powerfully inhibited by anti-(chicken cathepsin D) antisera (Weston, Barrett & Dingle, 1969); we take this as direct evidence for an important role of cathepsin D in this type of cartilage breakdown. Of even greater biological significance are experiments in which the antisera have been shown to inhibit the loss of matrix from bone rudiments grown in organ culture, in the presence of excess of vitamin A (P. D. Weston, A. J. Barrett, J. T. Dingle & H. B. Fell, unpublished work).

We consider that immunoenzymology, the use of antisera specific to enzymes, shows great promise as a tool with which to tackle many problems on the function of enzymes in biology and pathology. Not only do the antisera provide powerful and completely specific inhibition of an enzyme in culture, and possibly also *in vivo*, but they can be used to mark the location of the enzyme for both light and electron microscopy (Nakane & Pierce, 1967). It may also be possible to measure the rate of biosynthesis of the enzyme and of its translocation between cell and tissue compartments.

Antisera inhibitory to cathepsin D of rabbit and man, have now also been prepared, and we plan to use them in experiments designed to establish what part cathepsin D plays in the destruction of articular cartilage in rheumatoid arthritis.

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