Lysosomes and the connective tissue diseases

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Lysosomes are small intracellular organelles present in most or all cells of animals of widely different evolutionary development. In general their diameter may vary from 0.2 to 0.5 μm so that they overlap the dimensions of mitochondria. In the original differential centrifugation studies they were isolated as the 'light mitochondrial' fraction. It is difficult to give an exact size to these organelles because the term 'lysosome' covers a wide range of structures from the small primary lysosomes budded off from the Golgi apparatus, to secondary lysosomes caused by the fusion of primary lysosomes with endocytotic vacuoles, to complex fusion-structures such as heterolysosomes and autophagic vacuoles. Indeed, de Duve (1969) suggested that lysosomes are only a part of the intracellular vacuolar system. Consequently the term 'lysosome' includes granules of various sizes, specific densities, and enzymatic content.

Possibly these structures have different complements of enzymes in the various tissues of the same animal. The lysosomes of polymorphonuclear cells, the azurophil, and possibly the specific granules (Goldstein, 1974) are peculiar in both their relatively great size and enzymic complement. Seemingly, however, the main cellular content of hydrolytic enzymes, particularly those acting optimally at an acidic pH, is within the lysosomes and is one of the characterising features of these organelles. They may be defined as small intracellular organelles that contain acid hydrolases bounded by a semi-permeable membrane which is said to control the latency of the intralysosomal enzymes and to allow the organelles to behave as osmometers (de Duve, 1959). By electron microscopy they are seen to be bounded by a single membrane (Daems *et al.*, 1969; Schellens *et al.*, 1977).

Lysosomes can be seen when living cells are examined by phase-contrast microscopy, when their greater refractility distinguishes them from the smaller mitochondria or chondriosomes. Indeed, it is now apparent that the lipochondria of the older microscopists probably correspond to lysosomes (Lane, 1968; Munro *et al.*, 1964). Such bodies were seen to congregate around the food vacuole in amoebae shortly after the uptake of food. Cor-

respondingly, in phagocytic cells primary lysosomes become attached to endocytic vacuoles and release their hydrolytic enzymes into these vacuoles, digesting the endocytosed matter (Cohn and Fedorko, 1969).

Methods for studying lysosomes

BIOCHEMICAL

The term 'lysosome' was coined by de Duve (de Duve *et al.*, 1955; de Duve, 1969) for particles isolated by homogenisation and differential centrifugation that contained acid hydrolases in a latent form. The latency of the enzymic activities was the most surprising feature of this organelle. Now these structures are usually separated from mitochondria by recentrifuging the crude 'mitochondrial' pellet to equilibrium in a medium that has a gradient of different specific gravities. The lysosomes equilibrate at a specific gravity of about 1.22 whereas the mitochondria accumulate at a specific gravity of about 1.19 (Beaufay, 1969). Occasionally the specific gravity of the lysosomes is deliberately altered by using their ability to concentrate material of low density such as Triton WR-1339 or material of high density such as Dextran 500, iron, or gold (Dean and Barrett, 1976; Dean, 1977b).

If lysosomes were completely stable possibly the activities of none of their characteristic enzymes could be demonstrated biochemically because all would be sequestered behind the semi-permeable membrane of the organelle. Treatment with acetate at pH 5.0 at 37°C, or more crudely with a surfactant such as Triton X-100, modifies or otherwise disrupts the lysosomal membrane and releases the enzymes, which then express their full biochemical activity. When there is no 'free' activity, so that the activity of the intralysosomal enzymes is totally latent, the membrane is considered fully stable. This type of finding led to the idea that these organelles were 'suicide particles' by analogy with the suicide pastilles that could safely be held in the mouth until the casing was broken. However, it was then found that in different physiological and pathological conditions more or less of the enzymic activities were present in the fluid in which the purified lyso-

somal fraction was isolated. This gave rise to many studies (for example, de Duve, 1959; Peters *et al.*, 1975) in which the 'free' activity was measured separately from that activity which still remained latent, or bound, within the organelles and which could be measured only after the particles had been physically or chemically disrupted. This 'free-to-bound' ratio seemed to relate to physiological or pathological activity in the cells.

ELECTRON MICROSCOPY

Lysosomes and related structures are recognised by the characteristic single outer membrane bounding a structure that may be electron-dense and contains acid hydrolase activity. The application of electron microscopy to the study of lysosomes has been fully reviewed by Daems (Daems *et al.*, 1969; Schellens *et al.*, 1977)

CYTOCHEMISTRY

Lysosomes can be recognised by their concentration of acridine orange so that they appear as vivid red granules when viewed by fluorescence microscopy (Robbins and Marcus, 1963; Allison and Young, 1969). They can usually be stained histochemically by a suitable method for acid phosphatase activity (Holt, 1959; Bitensky, 1963a, b) because the lysosomes of most but not of all cells contain active acid phosphatases. The presence of lysosomal enzymes, in characteristic particles, can also be visualised by immunohistochemical methods (Weston and Poole, 1973).

Quantitative cytochemical methods applicable to the activity of many characteristically lysosomal enzymes are now well established (Bitensky and Chayen, 1977). Two of these techniques allow the assessment of 'freely manifest' and of 'bound', or latent, activity, approximating to the free-to-bound ratios used by biochemists. These cytochemical techniques are considerably more sensitive than the biochemical tests for assessing the function of the lysosomal membranes. For example, they have shown that thyrotrophin, acting on thyroid follicle cells, increases the instability of the lysosomal membranes as a prelude to lysosomal involvement in the production of thyroid hormones.

The cytochemical bioassay of thyrotrophin (Bitensky *et al.*, 1974a), 10 000 times more sensitive than the radioimmunoassay, is now the microbioassay recommended by the World Health Organization. The cytochemical ratio of freely available to total activity is altered in many pathological states and during physiological conditions which apparently depend on lysosomal intervention (Bitensky, 1963b; Bitensky *et al.*, 1973). One of the advantages of cytochemical investigations is that the

lysosomal activity in histologically identified cells can be measured in individual cells by microdensitometry.

Contents of lysosomes and role in inflammation

Lysosomes in different cell-types contain different enzymes and bioactive molecules, and possibly the lysosomes of any one cell type are not homogeneous. Nevertheless, the lysosomes of most if not all cells contain an array of hydrolytic enzymes including those that will hydrolyse proteins, nucleic acids, polysaccharides, and phospholipids. The pH optima of all these enzymes, with a few exceptions, are in the acidic range. Barrett and Dean (1976; Barrett and Heath, 1977) have listed all the lysosomal enzymes that have been encountered. The possible role of the proteinases in the degradation of the protein matrix of cartilage in rheumatoid arthritis has been much studied (Dingle, 1962; Weissmann, 1966). The phospholipases are thought to have a potential function in the liberation of unsaturated fatty acids that initiates the formation of prostaglandins (Anderson *et al.*, 1971; Zurier, 1974). Prostaglandins alone can cause resorption of bone (Robinson *et al.*, 1975) and their production can be blocked at a very early stage by glucocorticoids (Kantrowitz *et al.*, 1975) which influence lysosomal function (see later).

Lysosomes of many types of cell also contain amino-acid arylamidases capable of generating bradykinin or other inflammatory amines from their inactive precursors (Hopsu-Havu *et al.*, 1966). Apart from such enzymes lysosomes of polymorphonuclear leucocytes contain a number of inflammatory mediators (Goldstein, 1974) including cationic proteins (Janoff and Zweifach, 1964), an activator of plasminogen (Lack and Ali, 1964), a protease that causes changes in the permeability of capillaries (Movat *et al.*, 1964; Uriuhara *et al.*, 1965), as well as haemolysins (Desai and Tappel, 1965). Thus, not surprisingly, when the contents of lysosomes isolated biochemically were injected under the skin or into joints (Weissmann *et al.*, 1969) they proved highly inflammatory. Repeated intra-articular injection of lysates of lysosomes isolated from rabbit leucocytes produced hypertrophy and hyperplasia of the cells lining the synovium, round cell infiltration of the synovium close to blood vessels, the formation of a pannus, and erosion of cartilage (Weissmann *et al.*, 1969; Page-Thomas, 1969). Moreover, the fact that many anti-inflammatory agents appreciably stabilise lysosomal membranes (Weissmann, 1968, 1969; Chayen *et al.*, 1972; Bitensky *et al.*, 1974b) also indicates a strong lysosomal component in inflammation.

Intracellular digestion

There is now a wealth of information about the intracellular digestion, mediated by lysosomes, of material endocytosed by cells (Dean and Barrett, 1976). There is also clear evidence from electron microscopy that lysosomes participate in the intracellular digestion of parts of the cell such as mitochondria. This process, known as autophagy, is related to the digestion of secretory granules (crinophagy)—seen, for example, in the parathyroid and pituitary glands—which apparently regulates the amount of hormone available for secretion. Crinophagy can be induced in the appropriate cells in the anterior lobe of the pituitary gland if secretion of the trophic hormone is blocked (Farquhar, 1969). However, autophagy and possibly crinophagy may represent pathological roles of the intracellular lysosomal digestive system.

Over and above these morphologically identifiable processes, lysosomes in normal cells are probably concerned with the regular degradation of cellular components and the turnover of cellular proteins. Thus the normal rate of degradation of intracellular proteins in the liver is retarded by causing a specific inhibitor of carboxyproteinases, supplied in liposomes, to be incorporated into liver lysosomes (Dean, 1975). Further evidence of lysosomal participation in the normal turnover of cellular proteins is given by Dean (1977a; La Badie *et al.*, 1976). The indirect evidence that lysosomes are concerned in the turnover of other chemical components of cells comes from the study of inborn errors of metabolism (see later; also M. F. Dean (Dean, 1978) at page 120).

Extracellular digestion

The extracellular release of lysosomal enzymes into the lacunar space has been implicated in the destruction of bone by osteoclasts (Vaes, 1969). There is evidence that lysosomal cathepsin D released from cells participates in the early stages of the breakdown of connective tissues (Dingle, 1971). Dingle (1968) suggested that the release of lysosomal enzymes from cells could be as packages released by exocytosis.

The earlier work of Fell and coworkers (Fell and Thomas, 1960) is still the clearest evidence that lysosomal enzymes released from living cells can degrade cartilage and other connective tissue. In particular, studies on cells in tissue or organ culture have shown that addition of vitamin A (which labilises lysosomes) or of non-digestible material (which accumulates within lysosomes) results in the release into the culture medium of lysosomal enzymes and in the loss of metachromasy in the cartilage (Fell and Dingle, 1963; Dingle *et al.*, 1969; Dingle, 1969).

Other, more circumstantial, evidence will be discussed later. Nevertheless, these studies indicated that degradation of the matrix of connective tissue could result from the extracellular release of lysosomal enzymes from still-viable cells.

Nature of lysosomal enzymes

INTRALYSOSOMAL pH

There has been some concern over the fact that most of the enzymes located within lysosomes act optimally at pH values around pH 5 or even lower, and that some are totally inactive at what is considered to be the 'physiological pH'—namely, about pH 7.

Several attempts have been made to explain how the pH within these organelles can be so different from that of their environment. Some have adduced evidence for an active proton pump (de Duve *et al.*, 1974; Mego, 1973) to explain why the measurable intralysosomal pH is appreciably lower than the pH of the surrounding medium. Goldman (1976) found it to be 1.6 units of pH lower; Reijngoud and Tager (1973) found it to increase from pH 4.9 to 6.9 as the pH of the external medium was raised from 5.0 to 8.5. Moreover, Koenig (1974) showed that lipoproteins make up about half of the total protein complement of lysosomes isolated from rat liver and kidney.

The influence of these acidic, ionisable phosphate moieties could play some part in maintaining a relatively acidic hydrogen-ion activity (see below) at the active sites of the intralysosomal enzymes. Dean and Barrett (1976) considered that such fixed anions in the interior of the organelles could be sufficient to account for the maintenance of the acidic intra-organelle pH that has been recorded (Goldman, 1976) without recourse to active proton pumps. Among the fixed anions they listed were sialic acid on the inside of the membrane, acidic lipoproteins and phospholipids, and an excess of amino-acids produced by hydrolysis of peptide bonds.

pH OF EXTRACELLULAR DIGESTION

Many workers are disquieted by the fact that lysosomal enzymes act optimally at acidic pH values while the digestion of extracellular matrix occurs, ostensibly, at physiological pH values—namely, around pH 7.0. Because purified cathepsin D had no detectable action on a protein-polysaccharide complex or proteoglycan subunit at physiological pH, Woessner (1973) concluded that it and, presumably, other cathepsins with acidic optimal activities were unlikely to have any significant effect on the matrix of cartilage at pH values greater than pH 6.0. Any degradation of cartilage must therefore be due to the influence of neutral proteases. Such proteases

have been found in the granules of polymorphonuclear leucocytes (Goldstein, 1974) and in other cells. Lysosomal carboxypeptidase B and cathepsin C have been shown to have pH optima of 6.2 and 5.6 respectively (Barrett and Heath, 1977).

Some lysosomal endopeptidases, such as acrosin (pH optimum: 8) and cathepsin G (pH optimum: 7.5), act optimally at around neutral pH, as do some other enzymes such as aspartyl glucosyl aminase (E.C. 3.5.1.26) which hydrolyses the amide link in aspartamido-(N-acetyl-) glucosamine, and which may be relevant to the breakdown of cartilage-matrix (Barrett and Heath, 1977). Furthermore, even such endopeptidases as cathepsin B, which acts optimally at pH values of between 3.5-6.0, may still have significant activity at higher pH values, even at pH 7.0.

The lowest mean pH of sterile inflammatory exudates has been measured as 7.049 (Edlow and Shelden, 1971), leading Goldstein (1974) to conclude that the pH was far above the range at which acid cathepsins could be active. Similarly the pH of fluid from rheumatoid joints was 7.1-7.28 (Barnett *et al.*, 1961; Cummings and Nordby, 1966). However, these arguments are somewhat academic. There is sufficient neutral lysosomal protease activity to account for degradation of tissue matrix at neutral pH, and polymorphonuclear leucocytes, when involved, contain an array of neutral hydrolytic lysosomal enzymes (Barrett and Heath, 1977). Moreover, the whole argument is based on the pH—namely the hydrogen-ion concentration in solution—which operates when solubilised enzymes act on their substrate in solution.

Small (1954) emphasised that at surfaces we are concerned not with pH but with hydrogen-ion activity. McLaren and Packer (1970) reviewed the whole question of how pH optima can be modified when the enzyme becomes bound to a solid matrix or when a soluble enzyme acts to digest a solid matrix, as it must when digesting extracellular connective tissue components. Several examples are now known of enzymes, such as chymotrypsin or trypsin, whose pH optimum may be changed as much as 2 pH units depending on whether they act in solution or attached to a solid matrix (McLaren and Packer, 1970).

Dean and Barrett (1976) reviewed work on enzymes linked to insoluble model membranes in which extensive changes in the pH optima and even enzymatic characteristics have been recorded. They gave as one example a lysosomal proteolytic activity that acts optimally at pH 6.5 when bound to a membrane but at pH 4.5 when solubilised. It is by no means inconceivable that isolated and purified lysosomal enzymes which act optimally at an acidic

pH of, for example, 4.5 may in fact act optimally close to neutrality when adsorbed on to extracellular connective-tissue components or even when present within the matrix of the lysosome. A typical example of the former is given by Quarles and Dawson (1969). They studied the action of isolated phospholipase D acting on micelles of phospholipids and showed that the pH optimum could be shifted 1.5 pH units towards neutrality, depending on the nature of the micelles. They pointed out that the pH in the bulk phase (pH_B) was related to the pH in the surface phase (pH_S) adjacent to a negatively charged interface by an equation derived from the Boltzmann equation:

$$\text{pH}_S = \text{pH}_B + (\epsilon\psi/2.3KT)$$

where K is the Boltzmann constant, T is the absolute temperature, ϵ is the electronic charge, and ψ is the potential of the surface phase (Davies and Rideal, 1961). Although it is difficult to calculate ψ there is every reason to expect a strong shift towards neutrality.

Nature of lysosomal membranes

Electron microscopy shows that lysosomes are bounded by a single membrane, and biochemical investigations on the osmotic properties of lysosomes (Dean and Barrett, 1976) added weight to the view that these organelles are encased in a semi-permeable membrane. Some biochemical studies indicated that sulphhydryl groups in the membrane are important in the labilisation of these membranes by thiols and certain metals (Lucy, 1969) and by vitamin A (Lucy and Lichti, 1969). Lysosomal membranes can be labilised by some steroids and by retinol and stabilised by other steroids (Dean and Barrett, 1976). Dingle and Barrett (1968) isolated a peculiar phospholipid component of lysosomal membranes that may play a significant role in some of the cytochemical tests made on these organelles (Bitensky and Chayen, 1977).

Proof that changes in the permeability of lysosomal membranes, which are sometimes reversible, may play a significant part in cellular physiology and pathology depended on the adequate measurement of lysosomal membrane function. These changes were shown first by cytochemistry (Bitensky, 1963b; Allison and Mallucci, 1964, 1965; Bitensky and Cohen, 1965; Bitensky *et al.*, 1963) but have now been shown biochemically by Peters and Seymour (1976) and by Burton and Lloyd (1976). Both cytochemically and biochemically, the effects measured are estimates of the permeability or stability of the lysosomal membranes. Although there are dissenting voices (Baccino and Zuretti, 1975; Koenig, 1969),

the weight of evidence favours the concept that reversible changes do occur in the permeability of lysosomal membranes under different physiological and pathological conditions and when tissues are subjected to drugs such as steroids (Weissmann, 1969; Allison, 1968a; Bitensky *et al.*, 1974b) or dimethyl sulphoxide (Misch and Misch, 1975).

Pathogenetic role of lysosomes

Lysosomes were first thought of as 'suicide capsules'. When they burst they would release their cytolytic enzymes into the cytosol and digest the cell in which they were present. Although there was some evidence that this might occur in morphogenesis (Weber, 1969), it is now clear that this is remarkably rare and occurs only in a few particular situations. One such situation was shown to be the uptake of toxic particles by macrophages (Allison *et al.*, 1966; Allison, 1971). Silica was taken up by these cells, lysosomes fused with the phagosome and released lysosomal enzymes into the so-formed secondary lysosomes. The toxic particles, such as those of silica, reacted with the membranes of the lysosomes (Allison, 1968a, b), allowing the lysosomal enzymes to escape into the cytoplasm. Macrophages that had ingested such particles were killed within 24 hours whereas those that ingested non-toxic particles (such as diamond dust or silica coated with either aluminium or polyvinyl-pyridine-N-oxide), incapable of disrupting the lysosomal membranes, were not killed.

It now seems likely that the common effect of lysosomes is to release their enzymes into the external milieu of the cell. Dingle (1968) suggested that perturbation of the lysosomal membranes allowed the lysosomes to fuse with similarly perturbed regions of the plasma membrane and so to extrude packages of lysosomal enzymes by a form of exocytosis. Lysolecithin, produced locally, could mediate this phenomenon (Lucy, 1970). An alternative mechanism, which might be called 'regurgitation during feeding', has been postulated by Weissmann *et al.* (1971a, b). This suggests that the phagocytic vacuole in the leucocyte may remain open at the external surface of the cell and that the lysosomes fuse with the inner boundary of such vacuoles and release their enzymes into these vacuoles, which, being still open to the outside medium, allow the loss of lysosomal enzymes into the environment of the cell.

Evidence from electron micrographs is consistent with such a mechanism (Zucker-Franklin and Hirsch, 1964; Henson, 1971). Indeed, there is considerable biochemical evidence for the selective release of lysosomal enzymes from leucocytes and from macrophages (Cardella *et al.*, 1974; Schorlem-

mer *et al.*, 1977) when these cells are exposed to a variety of stimuli, including immune complexes. This can occur even in the absence of any signs of loss of viability of the cells, monitored by the release of cytosolic enzymes such as lactate dehydrogenase. A particular example of the release of nine lysosomal enzymes from cells which remain viable is the study by Hösli and Vogt (1977) on fibroblasts grown from patients with cystic fibrosis.

A system of particular interest in inflammation is the activation of complement by lysosomes. Various workers (Goldstein and Weissmann, 1974; Schorlemmer and Allison, 1976) have shown that when mononuclear leucocytes are stimulated by agents that induce chronic inflammation they secrete hydrolytic enzymes. These enzymes degrade connective tissue and interact with the complement system to generate mediators of inflammation. The cleavage products of complement can then further stimulate macrophages (Schorlemmer *et al.*, 1976) to perpetuate the inflammatory response. Many workers have shown that this activation of complement is by the alternative pathway resulting in the cleavage of C3 and the generation of C3b, which causes the secretion of hydrolases from these cells. This work, and the concepts arising out of it, have been reviewed by Schorlemmer *et al.* (1977).

Given that lysosomal enzymes can be exteriorised from the cell, conceivably they could have the following influences.

Firstly, body fluids contain many hydrolytic and degradative enzymes which occur in a latent form, often as a proenzyme or in combination with inhibitors. For example, there is much work on procollagenases and proelastases (Vaes, 1972; Woessner, 1977; Sellers *et al.*, 1977). These can be activated by proteolytic enzymes (Birkedal-Hansen *et al.*, 1976; Day, 1976; Eeckhout and Vaes, 1977). Glynn (1977) and Reynolds *et al.* (1977) suggested that such activation of collagenase, possibly by lysosomal enzymes extruded from still viable cells, could participate in the degradation of cartilage in osteoarthritis and rheumatoid arthritis. Similarly, cathepsin B can inactivate the circulating α_1 -proteinase inhibitor, allowing proteases, previously latent because of the presence of this inhibitor, to be free to act on connective tissue. (For the possible relationship between this circulating protease inhibitor and emphysema see Galdston *et al.*, 1973; Goldstein, 1974.) Equally lysosomal enzymes can act on kallidin-10 to form bradykinin (Hopsu-Havu *et al.*, 1966; Goldstein, 1974).

Secondly, if the release of lysosomal enzymes were to occur in the synovium or in the joint they could attack the connective tissue matrix, provided that the pH or hydrogen-ion activity was suitable.

It could cause a cascade phenomenon of inflammation by the alternative pathway of complement activation; it could influence the permeability of the capillaries; and, by the release of chemotactic substances known to be present in lysosomes (Goldstein, 1974), it could exacerbate the cellular infiltration in the synovium or joint. We have seen that the injection into joints of lysates of lysosomes apparently has all these effects. It is also known that when cells ingest certain substances they release lysosomal enzymes (Dingle *et al.*, 1969). Page-Thomas (1969) showed that the injection into a joint of disazo dyes or polysaccharides produced an arthritis, apparently due to excessive synthesis of lysosomes and lysosomal enzymes. The ingestion of immune complexes has a similar effect in the secretion of lysosomal enzymes from leucocytes and macrophages (Weissmann *et al.*, 1971b; Cardella *et al.*, 1974).

A different aspect of the extrusion of matter from lysosomes concerns the handling of immune complexes by these organelles. Antigens appear to be taken up into lysosomes by endocytosis and to be 'processed' by them (Weissmann, 1964; Allison, 1968c). The fate of these proteins seems to be determined by the state of the lysosomal membranes. As reviewed by Chayen and Bitensky (1971), the more stable lysosomes (as may occur in polymorphonuclear leucocytes) are said to be capable of more complete digestion of these antigens than are less stable lysosomes, as found in macrophages (Allison, 1968c). Treatment which labilised lysosomal membranes prolonged the 19S antibody response (Weissmann, 1967), implying that the antigen had not been degraded under these conditions (Uhr, 1964). Weissmann (1964; 1966) argued that this type of phenomenon could play some part in autoimmunity. The hypothesis requires cells that are undergoing autophagy and endocytosis. If the lysosomal membranes are labile there will be incomplete degradation with retention of antigenicity. The selective release of these incompletely degraded antigens, whether by 'regurgitation,' exocytosis, or any other mechanism, will result in an immune response either to the original cell or to the exogenous immune complex. Similar hypotheses have been propounded by Page-Thomas (1969) and by Hollander (Hollander *et al.*, 1965; Rawson *et al.*, 1965; Restifo *et al.*, 1965). Such hypotheses would account for the chronic nature of rheumatoid arthritis, as discussed by Chayen and Bitensky (1971).

Possible role of lysosomes in certain diseases of connective tissues

STORAGE DISEASES

Many diseases caused by inborn errors of meta-

bolism are now known. They have been reviewed fully by Hers and van Hoof (1973). In many a single specific lysosomal enzyme is absent. The fact that undigested matter accumulates in giant, swollen lysosomes, from which phenomenon the name 'storage diseases' is derived, indicates that lysosomes may well be involved in the normal turnover of these substances. In Tay-Sachs disease there is a deficiency of lysosomal hexosaminidase A which would normally cleave the terminal N-acetyl- β -D-galactosamine from ganglioside G_{M2}. This results in huge lysosomes filled with cytoplasmic membranous bodies presumably of ganglioside material (O'Brien, 1973).

Type II glycogenosis, also called type II glycogen-storage disease or Pompe's disease, is ascribed to a complete defect of α -glucosidase; this results in the intralysosomal accumulation of intact glycogen which causes the lysosomes to swell to up to 8 μ m-diameter (Hers and de Barsy, 1973). Cardiac and skeletal muscle hypertrophy follow. In three of the mucopolysaccharidoses (Hurler's, Hunter's, and Sanfilippo's disease) there may be a *complete* defect of one of the degradative enzymes—for example, iduronidase (which liberates sulphate ions from dermatan sulphate) in Hurler's disease, sulphatase, or β -glucuronidase—or *partial* defects of α - or β -galactosidases (Hers, 1973). Gaucher's disease, with its hepatosplenomegaly and bone erosion, is associated with a nearly complete defect of glucocerebrosidase (β -glucosidase). Gaucher cells are characterised by lysosomes filled with membrane-bound inclusions (Brady and King, 1973).

These and other lysosomal defects discussed by Hers and van Hoof (1973) indicate how important lysosomes may be in the normal turnover of materials in life, so that defects in their metabolic activity become apparent in these diseases. Surprisingly no genetic disease due to deficiencies of lysosomal proteolytic activity seem to have been recorded. Whether this is because such defects are lethal or because the wide array of proteolytic potency of lysosomes can cover-up the effect of a single deletion is not determined.

GOUT AND CRYSTAL SYNOVITIS

Long, needle-shaped crystals of monosodium urate monohydrate are found in the fluid from joints during acute attacks of gout (see J. T. Scott (Scott, 1978) at p. 205, and P. A. Dieppe (Dieppe, 1978) at p. 214). It was known in the last century that crystals occurred at the sites of gouty necrosis and that the subcutaneous injection into animals of microcrystals of various urates, xanthine, hypoxanthine, and creatinine—and even of calcium carbonate—provoked acute inflammatory responses

and the phagocytosis of the crystals by mononuclear and polymorphonuclear leucocytes (Chayen and Bitensky, 1971). These effects were rediscovered by Seegmiller and Howell (1962) and by McCarty (1968). Seegmiller and Howell (1962) suggested that the urate crystallises in tissue or synovial fluid and that the crystals are phagocytosed by leucocytes. They assumed that in this process lactic acid was produced locally. This would lower the pH and so cause further precipitation of urate from the exudate, which would be in equilibrium with the hyperuricaemic blood. The last step is probably an over-simplification because Watts *et al.* (1971) found urate crystals in tissues of patients in whom the blood levels of urates were well below saturation.

McCarty (1968) emphasised that inflammation induced by crystals either of urates, as in gout, or of pyrophosphate, as in pseudogout or chondrocalcinosis, depended on the presence of polymorphonuclear leucocytes. Phelps and McCarty (1966) and Chayen and Bitensky (1971), however, reported plenty of urate crystals in some synovial fluids in which very few of these cells could be found. For all that, the general view is that the crystals are phagocytosed by polymorphonuclear leucocytes into an endocytic vacuole. The cells degranulate, injecting their lysosomal enzymes into this vacuole. These enzymes then leak from the cells either by the regurgitation mechanism discussed previously or by some other selective release, very much as when such cells ingest particles of silica (as discussed above). Indeed, there is probably no call to invoke selective release of lysosomal contents from viable cells in this instance and possibly the fluids that contained crystals but very few polymorphonuclear leucocytes could have been sampled at a time when these cells had already disintegrated, before the inflow of fresh cells.

Probably, therefore, the inflammation associated with gout, chondrocalcinosis, and possibly the other calcium phosphate synovitis (Dieppe *et al.*, 1976) is brought about by the lysosomal contents of leucocytes that have ingested these particles. When such crystals are deposited in muscle they do not produce an inflammatory response but may be associated with muscle pain, as in the cases of xanthinuria reported by Chalmers *et al.* (1969) and possibly when urates of various forms crystallise in the muscle of patients with gout (Watts *et al.*, 1971).

RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a systemic disease of unknown origin. The earliest joint changes are in the synovium, where the established lesion is characterised by villous enlargement, hyperplasia of the lining cells, infiltration by inflammatory cells, deposi-

tion of fibrin interstitially, and the formation of pannus and erosion of cartilage of the joint surface (Lack, 1969). The chronic inflammation and erosion of cartilage point to lysosomal participation. This view is strengthened by the fact that an experimental arthritis can be induced by injecting lysates of lysosomes into the joint (Weissmann *et al.*, 1969) and that it can be produced experimentally (Page-Thomas, 1969) by injecting various substances that interfere with lysosomes, such as those which rupture lysosomes, or disazo dyes which, by inhibiting some lysosomal enzymes, may cause excessive production of other enzymes, as has been found in many of the storage diseases.

If lysosomes participate in the pathogenesis of rheumatoid arthritis it is necessary to identify which cells are involved. In gout there seems little doubt that the lysosomal dysfunction is in the polymorphonuclear leucocytes. These are unlikely to be the source of lysosomal enzymes and inflammatory substances in the rheumatoid joint because, were they the source, the erosive influences would be liberated into the synovial fluid and the erosion of the cartilage would begin uniformly over the whole surface of the cartilage. Ball (1968) has shown that erosion begins at the articular cartilage junction. This location implicates the synovium itself, which originates at this site—at which it also shows its most florid appearance in completely normal joints. Muirden (1972) showed that the severity of joint erosion did not correlate with the degree of inflammatory cell infiltrate in the synovium but with the amount of hyperplasia of the synovial lining cells. Ghadially and Roy (1967) found a striking increase in the number of lysosomes and autophagic vesicles, particularly in Type A synoviocytes, in rheumatoid synovial tissue (Lack, 1969).

From this evidence it is possible that the lysosomes of rheumatoid synoviocytes are abnormal and capable of extruding their contents on to the cartilage, to which these cells are already in close apposition. But, as Page-Thomas (1969) has remarked: 'The fibrous nature of synovial membrane and the cellular heterogeneity of the diseased tissue have deterred workers from serious biochemical studies of subcellular fractions'. Thus this was an ideal system for analysis by the techniques of quantitative cytochemistry (Bitensky *et al.*, 1973; Bitensky and Chayen, 1977).

Cytochemical analysis of the functional state of the lysosomal membranes, based on a comparison of the free and latent activities of two intralysosomal enzymes, showed that the lysosomes of non-rheumatoid synoviocytes contained between 28% and 60% latent activity, the lower values being found in recently traumatised joints. The total

activity found in rheumatoid synoviocytes was at least as great, or greater, than that found in non-rheumatoid synoviocytes. But the striking finding was that not more than 5% of this activity was latent, showing that the lysosomal membranes in the rheumatoid cells were relatively functionless, at least with regard to these substrates (Chayen *et al.*, 1971). Glucocorticoids administered to synovial tissue maintained *in vitro* improved the amount of latent activity up to 21%. A similar improvement in latent activity was found in eight out of 13 patients who had received at least 5 mg of prednisolone daily. It was not found in patients receiving less prednisolone (Bitensky *et al.*, 1974b). These levels of prednisolone correlated well with the findings of Chamberlain and Keenan (1976). In a double-blind trial patients receiving prednisolone 3 mg/day gained little clinical benefit whereas some clinical improvement was apparent in those treated with 5 mg/day.

Conclusion

Since lysosomes were first described as a biochemical entity in 1955 (de Duve *et al.*, 1955) they have been a favoured candidate for a pathogenic role in connective tissue diseases. Today it is clear that they have important functions in these diseases. Their importance in intracellular metabolic processes is highlighted by the damage caused by the deletion of a single intralysosomal enzyme, as in the storage diseases. Their abundance in polymorphonuclear leucocytes and macrophages and their behaviour in response to inflammatory stimuli implicate lysosomal enzymes in acute and chronic inflammation. Such a function seems clear in gout and probably in other crystal synovitides.

There is strong evidence that extracellular lysosomal enzymes are concerned in the normal turnover of bone (Vaes, 1969) and of the extracellular components of connective tissue (Reynolds, 1969; Dingle, 1969). How much they participate in the pathogenesis of rheumatoid arthritis and osteoarthritis still awaits full clarification. But there is strong circumstantial evidence that their part is a major if not decisive one.

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