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Cellular Transport of Lysosomal Enzymes

AN ALTERNATIVE HYPOTHESIS

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Hickman & Neufeld [(1972) *Biochem. Biophys. Res. Commun.* **49**, 992–999] have proposed that lysosomal enzymes reach the lysosomes by means of exocytosis and subsequent pinocytic reincorporation. The results leading to this conclusion are re-assessed and an alternative explanation is advanced that relates to the necessity for membrane recycling in endocytic cells.

Until recently there was general agreement that lysosomal enzymes enter the vacuolar system of cells in small primary lysosomes (de Duve & Wattiaux, 1966) that originate in the Golgi apparatus and smooth endoplasmic reticulum (Novikoff *et al.*, 1964). This view was called in question by observations made on the autosomal recessive human condition known as I-cell disease (or mucolipidosis type II), in which several lysosomal enzymes are absent or deficient from connective-tissue cells (Leroy *et al.*, 1972) and which may have as its primary defect an absent lysosomal sialidase (Thomas *et al.*, 1976). Hickman & Neufeld (1972) showed that cultured I-cell fibroblasts would pinocytose and retain exogenous lysosomal enzymes from normal cells, but that lysosomal enzymes secreted by I-cells were not taken up. They therefore proposed (Hickman & Neufeld, 1972) that the normal route by which lysosomal enzymes make their way from their site of synthesis to the vacuolar system is one that includes exocytosis to the extracellular environment and pinocytic recapture by neighbouring cells. Efficient capture of exocytosed enzymes was postulated to depend on interaction between a chemical moiety on the enzyme and a binding site on the plasma membrane; enzymes from I-cell fibroblasts, lacking this moiety, would fail to be pinocytosed, resulting in low intracellular concentrations.

The concept that lysosomal enzymes must first be secreted and then be reincorporated is intrinsically implausible. The present paper contends that the findings in I-cell disease do not require this concept and may originate in membrane recycling after pinocytosis.

Hypothesis

Actively pinocytosing cells must possess some mechanism to withdraw membrane from the vacuolar system as fast as membrane is entering. Removal of membrane, which probably takes place both before and after pinosome–lysosome fusion (Duncan & Pratten, 1977), could occur by budding of vesicles from pinosomes or secondary lysosomes either into the interior of the vacuole or into the cytoplasm. Intralysosomal budding would lead to the degradation of membrane components by lysosomal enzymes and may be the mechanism whereby some soluble cytoplasmic components such as cytosol enzymes are incorporated into lysosomes for degradation (Dean, 1975; Lloyd, 1976). Budding into the cytoplasm would lead to vesicles that could return to and be reincorporated into the plasma membrane. Such a recycling of membrane in vesicular form would explain how pinocytosing cells can continuously internalize membrane for long periods without exhausting the supply of plasma membrane (see Steinman *et al.*, 1976). Those vesicles derived from secondary lysosomes would contain lysosomal enzymes, and the proposed recycling process would seem to entail the continuous extracellular release of lysosomal enzymes. However, if the enzymes were bound to the lysosomal membrane, membrane recycling could occur without great loss of enzyme. The strength of binding need not be very great and might differ from one enzyme to another, explaining why many cell types release lysosomal enzymes into their environment and why the relative amounts of each enzyme exocytosed in this way do not simply reflect their intracellular concentration. Also, stimulation of pinocytosis is often found to be accompanied by increased exocytosis of lysosomal enzymes; this would be expected, on the proposed scheme, since an increased rate of pinocytosis would need to be accompanied by a corresponding increase in the rate of membrane recycling. On this scheme the failure of I-cell fibroblasts to retain their lysosomal enzymes would relate to a failure of the enzyme to bind to the membrane during membrane recycling, with consequent continuous loss of enzyme to the environ-

ment. There are many data indicating that the various lysosomal enzymes differ in the extent to which they are bound to the lysosomal membrane. One enzyme, β-glucosidase, seems to be unique, since it cannot be detached without the use of detergents (Beck & Tappel, 1968; Burton & Lloyd, 1976), and the observation that β-glucosidase is found in normal amount in I-cell fibroblasts (Leroy *et al.*, 1972) is consistent with the hypothesis advanced here, but not with that of Hickman & Neufeld (1972).

Discussion

One corollary of the present hypothesis, and equally of that of Hickman & Neufeld (1972), is that lysosomal enzymes might be present, albeit in low amount, in incoming pinocytic vesicles. The activity of these enzymes towards substrate present in the same vesicles would initially be very low, owing to the unfavourable pH, and would increase as the intravacuolar pH decreased towards that prevailing within lysosomes. It is not yet known whether the decrease of pH follows from fusion with lysosomes or occurs progressively as pinosomes fuse together and dehydrate during their inward migration; if the latter, digestion of exogenous substances might begin before any fusion with primary or secondary lysosomes had occurred.

Rat liver lysosomal enzymes injected intravenously into rats are cleared from the bloodstream by a mechanism that depends on the integrity of the enzymes' carbohydrate residues (Stahl *et al.*, 1976). Also, there is evidence that lysosomal enzymes from I-cell fibroblasts may differ from their normal counterparts by possessing additional sialic acid residues (Thomas *et al.*, 1976). These results, although constituting further evidence for the existence of specific binding sites for lysosomal enzymes on plasma membrane and for the nature of the deficiency in I-cell enzymes, are equally compatible with the hypothesis of Hickman & Neufeld (1972) and with that advanced here.

References

Beck, C. & Tappel, A. L. (1968) *Biochim. Biophys. Acta* **151**, 159–164

Burton, R. & Lloyd, J. B. (1976) *Biochem. J.* **160**, 631–638

Dean, R. T. (1975) *Biochem. Biophys. Res. Commun.* **67**, 604–609

de Duve, C. & Wattiaux, R. (1966) *Annu. Rev. Physiol.* **28**, 435–492

Duncan, R. & Pratten, M. K. (1977) *J. Theor. Biol.* in the press

Hickman, S. & Neufeld, E. F. (1972) *Biochem. Biophys. Res. Commun.* **49**, 992–999

Leroy, J. G., Ho, M. W., MacBrinn, M. C., Zielke, K., Jacob, J. & O'Brien, J. S. (1972) *Pediatr. Res.* **6**, 752–757

Lloyd, J. B. (1976) in *Proteolysis and Physiological Regulation* (Ribbons, D. W. & Brew, K., eds.), pp. 371–389, Academic Press, New York

Novikoff, A. B., Essmer, E. & Quintana, N. (1964) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **23**, 1010–1022

Stahl, P., Six, H., Rodman, J. S., Schlesinger, P., Tulsiani, D. R. P. & Touster, O. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4045–4049

Steinman, R. M., Brodie, S. E. & Cohn, Z. A. (1976) *J. Cell Biol.* **68**, 665–687

Thomas, G. H., Tiller, G. E., Reynolds, L. W., Miller, C. S. & Bace, J. W. (1976) *Biochem. Biophys. Res. Commun.* **71**, 188–195