COMMUNICATIONS

A Combined Electron-Microscope and Cytochemical Study of Acid Phosphatase Activity in Human Cervical Squamous Carcinoma

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The arrangement of the membranous structures comprising the Golgi complex is sufficiently distinctive and consistent from one cell type to another to permit easy identification. Its structure has been adequately reviewed by Beams & Kessel (1968), but its functions are still subject to much discussion. Though it appears to be directly involved with the secretory process, its existence in cells not known to be involved with secretion indicates still other functions essential to the internal economy of the cell.

With the development of the 'lysosome concept' by de Duve (1963) and Novikoff (1963) the Golgi complex took on a new importance. Early cytochemical evidence pointing to the Golgi complex as a site of high acid phosphatase activity was reviewed by Novikoff (1961). Numerous other observations supported the proposal that Golgi vesicles transfer acid hydrolases from their sites of formation to endocytic vacuoles.

These views concerning primary lysosomes have been subject to much discussion and modification. Novikoff and co-workers, though maintaining that endocytic vacuoles may be supplied with hydrolases from Golgi vesicles, drew attention to the possibility that the areas of the endoplasmic reticulum closely associated with the Golgi complex may also give rise to lysosomes. They symbolized this complex system by the initials GERL (Golgi: endoplasmic reticulum: lysosomes) (Novikoff, Essner & Quintana, 1964).

It has been observed in previous morphological studies that the Golgi complex is very prolific in cells obtained from invasive squamous-cell carcinoma of the human uterine cervix. We decided to determine if this proliferation was in any way associated with the production of primary lysosomes.

The sites of localization of acid phosphatase were investigated by using Gomori's technique modified for electron microscopy as reported by Rowden (1967). Reaction product was frequently found to overlie areas that had the configurations of the Golgi complex. Other observations of acid hydrolase activity in primary lysosomes will be presented.

Autophagy is frequently observed in carcinoma cells and could arise as part of a cellular response to

anoxia and lack of adequate nutrient supply caused by disproportionate growth of the epithelial cells in relation to their blood supply. The possible involvement of lysosomal enzymes with the observed autophagy will be discussed.

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Evidence for Proteolytic Activity in Liver Mitochondria

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It is known that protein breakdown occurs when preparations of liver mitochondria are incubated. The process is most rapid at acid pH, presumably on account of the acid cathepsin present in the accompanying lysosomes. However, appreciable breakdown occurs also at pH 7 and above (studied in detail by Alberti & Bartley, 1965) and the questions have arisen whether this is due to a separate enzyme and, if so, in which type of particle this occurs. Baird (1964) found that the distribution of amino acid-releasing activity between 'light' and 'heavy' mitochondrial fractions did not correspond exactly to that of either succinate dehydrogenase or acid phosphatase, but bore more relation to that of the lysosomal enzyme than to that of the mitochondrial enzyme.

Myoglobin is readily attacked by liver-mitochondrial preparations over a wide pH range and was used as substrate for particle proteinases. The proteolytic activity of 'heavy' mitochondrial fractions, measured from pH 4-8, fell sharply with no indication of a peak.

The proteolytic activity, measured at pH 7.0, of 'heavy' and 'light' mitochondrial fractions, obtained by differential centrifuging, was approximately the same, whereas proteolytic activity at pH 3.6 was always higher in the 'light' mitochondria. Mitochondria were further separated

from lysosomes by density-gradient centrifuging of liver particles obtained from mice and rats injected with Triton WR-1339 (Wattiaux, Wibo & Baudhuin, 1963). They showed a sharp decrease in proteolytic activity at pH 3.6 compared with unseparated particles; the activity at pH 7.0 was also diminished but never to the same extent. Treatment of the mitochondria with Triton X-100 removed most of the remaining acid proteinase activity; the activity at pH 7.0 was less affected by such treatment and was then of the same order as that at pH 3.6. These results appear to provide evidence for a proteinase in mitochondria, active at neutral pH. They accord with evidence of a different kind obtained by Alberti & Bartley (1969).

The reports of Penn (1960, 1961) that protein breakdown by liver particles is markedly stimulated by CoA could not be confirmed.

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Substrate Specificity of Mammalian Intestinal Dipeptidases and Tripeptidases

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Although several mammalian intestinal dipeptidases have been studied in recent years, relatively few have been purified, so that information is lacking about their substrate specificity and their relationship with dipeptidases in other tissues (see Lindberg, 1966). We decided to use starch-gel electrophoresis (Smithies, 1959) and to render the peptidases visible *in situ* on the gel, to obtain preliminary information about substrate specificity and tissue distribution of dipeptidases and tripeptidases.

Dipeptidases and tripeptidases from several tissues including intestine were compared in seven different mammalian species. After electrophoresis of the tissue extracts, three procedures were used to locate the peptidases *in situ* on the gels. The starch gel was covered with an agar gel containing a peptide substrate and one of the following mixtures: (a) L-amino acid oxidase, peroxidase and 3-amino-9-ethylcarbazole (Lewis & Harris, 1967);

(b) glutamate dehydrogenase, NAD, phenazine methosulphate and nitro blue tetrazolium; (c) aspartate aminotransferase, 2-oxoglutarate, glutamate dehydrogenase, NAD, phenazine methosulphate and nitro blue tetrazolium. Procedures (b) and (c) were used with some peptides containing glutamic acid and aspartic acid because the L-amino acid oxidase (from *Crotalus adamanteus*) was inactive with these amino acids.

Many of the dipeptides and some of the tripeptides were hydrolysed by more than one electrophoretically distinct protein in most of the tissues examined. For example, in human small intestinal mucosa, at least three dipeptidases were detected that hydrolysed glycyl-leucine, glycyl-valine, glycyl-phenylalanine, glycyl-tryptophan, phenylalanyl-leucine, leucyl-leucine, leucyl-alanine, leucyl-tyrosine and leucyl-glycine. In contrast, leucine amide and leucyl- β -naphthylamide were both hydrolysed by two other distinct proteins, a slowly migrating band that was more active with leucine amide, and a more rapidly migrating band that was more active with leucyl- β -naphthylamide.

Although the same tissues from different mammalian species had characteristic peptidase patterns, there was a striking similarity in the peptidase patterns of different tissues within a given species.

Some tripeptides were hydrolysed by one or more of the dipeptidase bands, e.g. two of the tripeptidases splitting tyrosyl-tyrosyl-tyrosine had similar electrophoretic mobilities to two of the four dipeptidases splitting glycyl-tyrosine.

We thank the Medical Research Council of Ireland for a grant and the Agricultural Institute, Dublin, for a scholarship to J. O. D.

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Lysosomes of the Locust Digestive Caeca

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The electron-cytochemical localization of acid phosphatase by lead-salt and azo-dye methods, with tissue cubes from the anterior digestive caeca of the desert locust, has already been investigated (Bowen, 1968). In the present study the localization produced by using these techniques is compared with that obtained by using frozen sections as recommended by Holt & Hicks (1961). The Gomori-type lead-salt method with tissue cubes exhibits a varying degree of non-lysosomal staining,