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.

Alberti, K. G. M. M. & Bartley, W. (1965). *Biochem. J.* **95**, 641.

Alberti, K. G. M. M. & Bartley, W. (1969). (unpublished).

Baird, G. D. (1964). *Biochim. biophys. Acta*, **93**, 293.

Penn, N. W. (1960). *Biochim. biophys. Acta*, **37**, 55.

Penn, N. W. (1961). *Biochim. biophys. Acta*, **53**, 490.

Wattiaux, R., Wibo, M. & Baudhuin, P. (1963). In *Ciba Found. Symp.: Lysosomes*, p. 176. Ed. by de Reuck, A. V. S. & Cameron, M. P. London: J. and A. Churchill Ltd.

Substrate Specificity of Mammalian Intestinal Dipeptidases and Tripeptidases

By J. O. DOLLY and P. F. FOTTELL. (*Department of Biochemistry, University College, Galway, Irish Republic*)

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.

Lewis, W. H. P. & Harris, H. (1967). *Nature, Lond.*, **215**, 351.

Lindberg, T. (1966). *Acta physiol. scand.* **69**, suppl. 285.

Smithies, O. (1959). *Biochem. J.* **71**, 585.

Lysosomes of the Locust Digestive Caeca

By I. D. BOWEN and W. A. L. EVANS. (*Department of Zoology, University College of South Wales and Monmouthshire, Cardiff*)

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,

whereas the azo-dye method produces a strictly lysosomal localization of acid phosphatase. Enzyme distribution demonstrated by the method of Holt & Hicks (1961) closely resembles that obtained by the azo-dye method, except for occasional cytoplasmic staining. The use of frozen sections, however, results in some loss of ultrastructure.

Combined electron-cytochemical and ultrastructural studies permit a tentative classification, based on that of de Duve & Wattiaux (1966), of the vacuole population of the epithelial cells. The fate of the primary lysosomes, which appear to arise from Golgi vacuoles, falls into three categories: (1) secretion into the gut lumen, where it is likely that the lysosomal enzymes are released for digestive purposes, (2) fusion with each other to form larger vacuoles and (3) direct fusion with secondary lysosomes. Primary lysosomes are also concerned with the pinching off of small portions of cytoplasm during apocrine secretion.

There is no evidence of heterophagy in the digestive epithelial cells and many of the secondary lysosomes are obviously autophagic in origin, containing remains of mitochondria and endoplasmic reticulum. The autophagic secondary lysosomes or autolysosomes contain acid phosphatase at all stages of development and no autophagosomes as such have been observed. It is possible to distinguish between early and mature autolysosomes. The early forms in general contain easily recognizable cytoplasmic organelles since lysis at this stage is minimal. Such vacuoles are usually surrounded by two membranes that are presumably derived from the sequestration membranes, whereas the mature autolysosomes are invariably surrounded by a single membrane, as are the telolysosomes and residual bodies.

- Bowen, I. D. (1968). *J. R. micr. Soc.* **88**, 279.
 de Duve, C. & Wattiaux, R. (1966). *Annu. Rev. Physiol.* **28**, 435.
 Holt, S. J. & Hicks, R. M. (1961). *J. biophys. biochem. Cytol.* **11**, 47.

Lysosomal and Extralysosomal Localization of Acid Phosphatases in Rat Sensory Ganglia

By M. KALINA and J. J. BUBIS (introduced by P. B. GAHAN). (*Department of Cell Biology and Histology, Medical School, Tel-Aviv University, Tel-Aviv, Israel*)

Classification of sensory ganglia into small 'dark' and large 'light' neurones is well known (Crosby, Humphrey & Lauer, 1962).

Demonstration of acid phosphatase activity in rat trigeminal and spinal ganglion cells, with

β -glycerophosphate as a substrate (Gomori, 1952), showed a particulate-granular localization of the reaction product in the small cells. Activity in the large neurones appeared as a network of fine filaments (Kalina & Bubis, 1968). The β -glycerophosphatase reaction in the large neurones was sensitive to aldehydes but was activated by 0.1 mM-molybdate; the reaction in the small cells was not affected. Activity of acid phosphatase when demonstrated with naphthol AS/B1 phosphate as a substrate (Burstone, 1958) showed a granular type of reaction in both types of cells. Similar results were also obtained in mouse, rabbit and human preparations of sensory ganglia.

Observation under the electron microscope showed the localization of β -glycerophosphatase to be only in lysosomes. In the large cells the reaction product appeared in the cisternae and vesicles of the Golgi apparatus and also attached to the outer surface of the endoplasmic-reticulum membranes.

The results suggested the possibility that more than one acid phosphatase was present in the small and the large neurones and that the enzymes were differently localized within the cells. This possibility was tested by a chromatographic study on acrylamide gels. A homogenate of trigeminal or spinal ganglia was separated on the gel into seven to nine bands of β -glycerophosphatase, but only three of them showed the naphthol AS/B1 phosphatase activity. Gel electrophoresis of Triton-treated cytoplasmic fractions obtained by differential centrifugation showed that the lysosomal fraction contained four bands that exhibited β -glycerophosphatase activity, two of which had naphthol AS/B1 phosphatase activity. The microsomal fraction contained three β -glycerophosphatase-reacting bands and only a trace of naphthol AS/B1 phosphatase activity. Without Triton treatment no migration occurred.

These results indicate the existence of multiple forms, or isoenzymes, of acid phosphatase in the sensory ganglia of rat and other species. These forms are probably differently localized in the small and the large neurones. It is possible that the different localization, although its meaning is unclear, is of biological significance and may suggest that the two groups of cells differ in function.

- Burstone, M. S. (1958). *J. Histochem. Cytochem.* **6**, 322.
 Crosby, E. C., Humphrey, T. & Lauer, E. W. (1962). *Correlative Anatomy of the Nervous System*. New York: MacMillan Co.
 Gomori, G. (1952). *Microscopic Histochemistry*. Chicago: University of Chicago Press.
 Kalina, M. & Bubis, J. J. (1968). *Histochemie*, **14**, 103.