60 min., the pH7.5 peptidase was recovered in the 0.6–0.8M range of the sucrose gradient. In other fractionations, with an M.S.E. BXV zonal rotor, two peaks of peptidase activity were found. The major peak was in the same region of the gradient; the minor one was in the less dense fractions. In some experiments fractions were resedimented and examined by electron microscopy. The patterns of distribution of the pH5.5 peptidase, the pH7.5 peptidase, alkaline phosphatase and arylamidase in the gradient fractions were strikingly similar. Electron micrographs of the enzyme-rich fractions revealed both vesicular and tubular elements, the latter having the appearance of microvilli. Negative staining revealed that the surface of each microvillus was uniformly covered with discrete granules, resembling the elementary particles on the microvilli of the intestinal mucosa, described by Oda & Seki (1966). There is histochemical evidence for the localization of alkaline phosphatase in microvilli (Goldfischer, Essner & Novikoff, 1964). It is suggested that the peptidase may be localized in the microvilli of the renal tubule.

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Goldfischer, S., Essner, E. & Novikoff, A. B. (1964). *J. Histochem. Cytochem.* **12**, 72.

Mahadevan, S. & Tappel, A. L. (1967). *J. biol. Chem.* **242**, 2369.

Oda, T. & Seki, S. (1966). *Electron Microscopy*, **2**, 387 (6th International Congress for Electron Microscopy, Kyoto).

Shibko, S. & Tappel, A. L. (1965). *Biochem. J.* **95**, 731.

Wong-Leung, Y. L. & Kenny, A. J. (1968). *Biochem. J.* (Preceding communication).

A Comparison of Enzymes in Rat Kidney that Hydrolyse L-Leucinamide, L-Leucyl-*p*-Nitroanilide and [¹²⁵I]Iodinated Insulin B Chain

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L-Leucinamide is the commonly used substrate for leucine aminopeptidase (LAP), a metal-activated enzyme that has been shown to hydrolyse the oxidized chains of insulin (Hill & Smith, 1957). L-Leucyl-*p*-nitroanilide and L-leucyl- β -naphthylamide are substrates for the arylamidases (Patterson, Hsiao & Keppel, 1963). A partially purified arylamidase from pig kidney has been shown to hydrolyse the B chain of insulin (Pfleiderer & Celliers, 1963). We therefore tried to ascertain the contribution of these enzymes to the hydrolysis of iodinated insulin B chain, with which we studied rat and rabbit kidney peptidases (Wong-Leung & Kenny, 1968; Wong-Leung, George, Aparicio & Kenny, 1968).

The supernatant fraction of a kidney homogenate, obtained after removal of the microsomal pellet, contained activity that hydrolysed L-leucinamide and was generally similar in its properties to a commercial sample of pig LAP. Thus the two preparations were activated by Mg²⁺, inhibited by EDTA and by pyrophosphate and were labile during incubation at pH5.3. The rat enzyme, however, was 78% inhibited by *p*-chloromercuribenzoate (0.5 mM) whereas the commercial LAP was unaffected. In contrast, the microsomal fraction, which attacked iodinated insulin B chain rapidly, hydrolysed L-leucinamide very slowly. Indeed, the distribution of LAP activity between supernatant and microsomes was in the ratio 20:1, whereas the ratio of the distribution of the iodinated insulin B chain peptidase activity was 1:10.

Arylamidase was present in the microsomal fraction of the rat kidney. In this respect it was like the pH7.5 peptidase that hydrolysed iodinated insulin B chain. In addition, the two activities had similar distribution patterns when fractionated on sucrose density gradients. However, the properties of the two enzymes were clearly distinguished. Unlike the pH7.5 peptidase, arylamidase activity was (a) inhibited by Mg²⁺ or Ca²⁺, (b) activated by orthophosphate and (c) relatively unaffected by EDTA. When the microsomal suspension was brought to 60° for 5 min. the arylamidase retained 95% activity, but only 25% of the activity of the pH7.5 peptidase remained.

These results show that the microsomal pH7.5 peptidase that hydrolyses iodinated insulin B chain is not identical with either LAP or arylamidase.

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Hill, R. L. & Smith, E. L. (1957). *J. biol. Chem.* **228**, 577.
Patterson, E. K., Hsiao, S. H. & Keppel, A. (1963). *J. biol. Chem.* **238**, 3611.

Pfleiderer, G. & Celliers, P. G. (1963). *Biochem. Z.* **339**, 186.
Wong-Leung, Y. L., George, S. G., Aparicio, S. G. R. & Kenny, A. J. (1968). *Biochem. J.* (Preceding communication).

Wong-Leung, Y. L. & Kenny, A. J. (1968). *Biochem. J.* (Preceding communication).

The Role of Lysosomal Enzymes in Malignant Growth

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Initial studies *in vivo* of the effect of an invasive rat tumour (Guerin T8) on xiphoid cartilage revealed a degradation of the matrix at its infiltrating edge. These changes were reflected by a decrease and disappearance of the metachromatic staining of cartilage with toluidine blue (Poole & Williams,