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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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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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,

1967), which has subsequently been shown to reflect a loss of uronic acid from the matrix.

The tumour only exerts its degradative effect when it comes into contact with the cartilage. Often matrix degradation was recorded in the absence of any detectable damage to chondrocytes. Little or no degradation was noted when fibroblasts, constituting the pannus, were interposed between the tumour and the cartilage. Matrix degradation was also found to be associated with the presence of 'haloes' of staining around the chondrocytes, as observed in man by Sylvén (1947), indicating that the matrix is more concentrated around the chondrocytes.

Administration of either vitamin A or papain *in vivo* caused a similar degradation of the matrix, but only with papain treatment were 'haloes' commonly observed. Vitamin A has been shown to degrade cartilage by releasing acid protease and probably other lysosomal enzymes from chondrocytes (Fell & Dingle, 1963); papain has been shown to be capable of degrading the matrix directly, in a reversible manner (Potter, McCluskey, Weissmann & Thomas, 1960). β -Glucuronidase was demonstrated histochemically within the partly degraded matrix close to the edge of the tumour. This suggested the presence of other acid hydrolases. These results together suggest that the degradation of the matrix in the presence of the tumour is mediated mainly by lysosomal enzymes originating not from the chondrocytes but from a remote source such as the tumour.

A purified lysosomal preparation isolated from the tumour by differential centrifugation followed by density-gradient centrifugation with a glycogen-sucrose gradient was checked for purity, biochemically and by electron microscopy. At pH 5.0 *in vitro* this preparation caused degradation of xiphoid cartilage matrix that was enzymic in nature, in addition to that produced by enzymes released from the chondrocytes.

These findings suggest that the ability of the tumour to degrade the matrix may be mediated by the extracellular release and activity of lysosomal enzymes derived from the tumour.

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The Inhibition of Wheat β -Amylase

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β -Amylases were prepared from native-grown wheats, e.g. Atle Quern and Progress varieties, by a modification of existing methods (Ballou & Luck, 1941; Tipples & Tkachuk, 1965). All preparations contained three components, as shown by gel electrophoresis, a feature previously noted in the β -amylases of Canadian hard red spring wheats (Tipples & Tkachuk, 1965). The action of β -amylase on the starch extracted from the same wheat variety was followed. The effect of special thiol reagents, mercuric chloride and phenylmercuric chloride (PMC), on enzyme activity was also studied. The inhibition of β -amylase by PMC was affected by substrate concentration, and a competition between substrate and PMC for the enzyme was observed.

In the routine estimation of barley α -amylase in the presence of β -amylase (Bendelow, 1963) a saturated solution of PMC is usually added to the crude enzyme preparation to inhibit the β -amylase. This mixture is then added to a starch solution for the final enzyme assay. The validity of this sequence of steps will be discussed and an alternative procedure for the selective assay of α -amylase in the presence of β -amylase will be described.

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The Purification and Properties of Some Glycoside Hydrolases

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Enzymes are normally purified by a combination of the traditional techniques such as salt precipitation, ion-exchange and molecular-sieve chromatography. Several attempts have been made to adsorb an enzyme on to its substrate and these have met with limited success. Clarke & Stone (1965) removed a contaminating β -(1 $\rightarrow$ 3)-glucanase from a β -(1 $\rightarrow$ 4)-glucanase by adsorption of the former enzyme on to insoluble laminarin, and Halliwell (1961) adsorbed cellulase on to cellulose.

The chitinase from a *Streptomyces* sp. was found to bind strongly to chitin and recovery of the enzyme was effected by prolonged incubation

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