(Fisher, Whitehouse & Kent, 1967). In rat liver this enzyme is concentrated in a particulate fraction (A. J. Barrett & J. T. Dingle, cited by Fisher, Higham, Kent & Pritchard, 1966). Treatment of an aqueous suspension of a mitochondrial-lysosomal pellet, prepared by centrifuging at 10 000g for 20 min., freezing and thawing, sonication or Triton X-100 (0.1%, v/v) followed by recentrifugation, resulted in most of the β -xylosidase activity remaining in the supernatant, together with the β -galactosidase activity, assayed with *o*-nitrophenyl β -D-galactopyranoside (Sellinger, Beaufay, Jacques, Doyen & de Duve, 1960). This release of β -xylosidase was prevented when the pellet was treated in citrate-phosphate buffer, pH 5 (McIlvaine, 1921), whereas the release of β -galactosidase was unimpaired. It is suggested that the β -xylosidase is a membrane-bound enzyme that can, under certain conditions, be apparently solubilized.

The β -xylosidase has been partially purified by use of this effect (Higham, Kent & Fisher, 1968). It was obtained as a clear solution with a specific activity about 300 times that of the tissue and was not sedimented by centrifugation at 10⁵g for 60 min.

Some properties of this soluble preparation have been investigated. A single enzyme hydrolyses *o*-nitrophenyl and *p*-nitrophenyl β -xylosides and also *p*-nitrophenyl β -D-glucopyranoside as shown by the competitive inhibitor glucono-1,4-lactone, giving similar K_i values when measured against the three substrates (0.38, 0.25 and 0.25 mM respectively), and by mixed-substrate experiments (Thorn, 1949). This agrees with the suggestion by Beck & Tappel (1968) that the lysosomal membrane β -glucosidase (assayed with *p*-nitrophenyl glucoside) and β -xylosidase are one enzyme. It should be noted that the preparation had only slight β -glucosidase activity towards *o*-nitrophenyl β -glucoside.

The β -xylosidase had no action on synthetic xylose peptides containing the β -O-xylosyl-serine or -threonine linkage. Incubation of the enzyme with β -O-xylosides of serine, threonine, glycyl-serine, seryl-glycine and glycylseryl-glycine at pH 3.5, 5 and 7 at 37° for 24 hr. gave no release of xylose as judged by the methods of Park & Johnson (1949) and Benedict (1931). This is in marked contrast with an activity present in *Helix pomatia* digestive juices, which will hydrolyse these compounds (Fisher & Kent, 1968).

This study was supported by the Arthritis and Rheumatism Council and by the Medical Research Council. We are grateful to Dr M. Higham for the preparation of xylosyl-peptides.

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Acid Phosphatases in Rat Heart

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Since mammalian heart contains few lysosomes (Tappel, Sawant & Shibko, 1963), this tissue would not be expected to contain a large amount of acid phosphatase, a typical lysosomal enzyme (de Duve, 1963). To test this hypothesis, male adult Sprague-Dawley rats were killed and their hearts were chopped with scissors and ground in acid-washed sand with 1 ml. of 0.28 M-sucrose-EDTA, pH 7.2 (Cleland & Slater, 1953). The homogenate was filtered through muslin, 40 vol. (v/w) of sucrose-EDTA was added, and the whole was centrifuged at 2400 rev./min. in an MSE 18 High Speed centrifuge (69181 angle rotor) at 4° for 10 min. The pellet was discarded and the supernatant was centrifuged at 20 000 rev./min. in a Spinco model L preparative ultracentrifuge (N 50 rotor) for 30 min. The pellet was resuspended in sucrose-EDTA and frozen and thawed six times before the enzyme assays were performed. Acid phosphatase activity was measured by incubating samples of the pellet and of the supernatant at 38° for 20 min. in 100 mM-acetate buffer containing 5 mM-disodium *p*-nitrophenyl phosphate (Kilsheimer & Axelrod, 1957) or 10 mM- β -glycerophosphate as substrate. In the latter case, inorganic phosphorus was determined by the method of Chen, Toribara & Warner (1956). Sodium fluoride was used as an inhibitor, and the chlorides of Mg²⁺, Ca²⁺, Na⁺ and K⁺ were used as activators, at concentrations between 1 and 100 mM. Proteins were determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

With *p*-nitrophenyl phosphate as substrate the activity in the supernatant was 2243 nmoles of *p*-nitrophenol liberated/hr./mg. of protein, and was four times that of the pellet. Its pH optimum was between 5.6 and 5.8. The activity was slightly enhanced by 10 mM-Mg²⁺; it was unaffected by K⁺ or Na⁺, slightly inhibited by 10 mM-Ca²⁺ and only

partially inhibited by up to 100mM-NaF. With β -glycerophosphate as substrate, activity in the pellet was 77nmoles of P liberated/hr./mg. of protein. It was greatly stimulated by 10mM-Mg²⁺ but was unaffected by Ca²⁺, Na⁺ or K⁺; it was inhibited completely by 10mM-NaF. The pH optimum of the pellet enzyme was between 4.8 and 5.0. The supernatant contained no activity. These results were similar when centrifugation was performed at 45 000 rev./min. for 60 min.

It appears that rat heart contains several acid phosphatases, and their subcellular distribution is being investigated.

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Lysosomes in Hepatomas

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Few studies have been undertaken on the characterization of lysosomes of cancer cells. Therefore we started investigating the properties of these subcellular structures in several tumours. Results reported here have been obtained on five transplantable Morris hepatomas: 7316A, 7794A, 7794B, 5123A and HC. Three characteristic aspects of the organelles have been considered: their enzymic content, their sedimentation patterns in iso-osmotic sucrose and their behaviour in isopycnic centrifugation in a sucrose gradient and in a gradient made of glycogen dissolved in aqueous sucrose. The results have been compared with those obtained by de Duve and his co-workers on rat liver granules. The activities of the seven acid hydrolases we have assayed exhibit certain variations from one tumour to another, but are of the same order of magnitude as those found in rat liver. The distribution pattern of the enzymes after differential centrifugation is similar to that recorded for the liver enzymes: the hydrolases are mostly recovered in the mitochondrial fractions and show a peak of

specific activity in the light-mitochondrial fraction. The behaviour of acid phosphatase and acid deoxyribonuclease in gradient-centrifugation experiments suggests that the osmotic space of the lysosome is smaller in the hepatoma than in the liver and that the space freely accessible to sucrose is larger.

Latency of Endogenous and Exogenous Lysosomal Glucosidases

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Free and total maltase, cellobiase, *p*-nitrophenyl α -glucosidase and *p*-nitrophenyl β -glucosidase activities were measured at pH 5.0 in lysosome-rich liver fractions prepared from rats pre-injected with glucosidases of plant origin.

Male Wistar rats were starved overnight and injected intravenously with purified *Aspergillus niger* amyloglucosidase (Qureshi, 1967) (250mg./kg. body wt.), with almond emulsin β -glucosidase (Sigma Chemical Co., St Louis, Mo., U.S.A.) (125mg./kg.) or with iso-osmotic saline only. Rats were killed 4.5–5 hr. after injection, and their livers quickly removed and homogenized in cold 0.25M-D-mannitol. A large-granule fraction, sedimenting between 11 000g-min. and 225 000g-min., was prepared, resuspended in cold mannitol and assayed immediately for free and total glucosidases.

Six rats were injected with the fungal amyloglucosidase (A), five with emulsin β -glucosidase (B) and five with saline. Maltase activity in granules from rats given A was approximately three times that in those from rats given B or saline. However, the proportion of free to total maltase was the same (A, mean 10.4% $\pm$ s.d. 4.1%; B, 10.6% $\pm$ s.d. 4.7%; saline, 10.7% $\pm$ s.d. 2.9%), suggesting that injected A reaches the interior of liver lysosomes. This is consistent with the observed effect of the enzyme in depleting intralysosomal glycogen in Pompe's glycogenosis (Hug & Schubert, 1967).

Injections of A could simply have raised the endogenous maltase activity. However, *p*-nitrophenyl α -glucosidase activities were not significantly higher in the lysosomes of A-injected rats. The additional maltase activity that followed injection of A must, therefore, be due to an enzyme relatively more active on maltose than on *p*-nitrophenyl α -glucoside. Additional experiments showed that this corresponds to the substrate-specificity of A.

Beck & Tappel (1968) showed that the *p*-nitro-

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