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-Mg^{2+} but was unaffected by Ca^{2+}, 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 45000rev./min. for 60min.

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

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Lysosomes in Hepatomas

By R. Wattiaux, S. Wattiaux-De Coninck, J. M. Van Dijck and H. P. Morris. (*Laboratory of Physiological Chemistry, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgium, and Howard University, Washington, D.C., U.S.A.*)

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

By J. B. Lloyd.* (*Department of Biochemistry, University of Miami, Miami, Fla., U.S.A.*)

Free and total maltase, cellobiase, *p*-nitrophenyl α-glucosidase and *p*-nitrophenyl β-glucosidase activities were measured at pH5·0 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 4·5–5hr. after injection, and their livers quickly removed and homogenized in cold 0·25M-D-mannitol. A large-granule fraction, sedimenting between 11000*g*-min. and 225000*g*-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% ± S.D. 4·1%; *B*, 10·6% ± S.D. 4·7%; saline, 10·7% ± 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-

* Present address: Department of Biochemistry, University College, Cardiff.

phenyl β-glucosidase activity of rat liver lysosomes is associated with the membrane fraction. I found this activity was fully available in fresh granules from *A*- or saline-injected rats. Such non-latency would result either if the enzyme was located on the cytoplasmic face of the lysosome membrane or if the lysosome membrane provided no barrier to substrate entry. The latter possibility was excluded by the finding that lysosomes from rats injected with *B* had six times the nitrophenyl β-glucosidase activity of those from saline- or *A*-injected rats, and that the additional activity was 90% latent.

Cellobiase activity, detected only in granules from rats injected with *B*, was almost wholly latent, a result consistent with the ability of cellobiose to afford osmotic protection to rat liver lysosomes (J. B. Lloyd, 1969).

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Lysosomal Uptake of Actinide Elements

By D. M. Taylor and C. J. Danpure. (*Department of Biophysics, Institute of Cancer Research, Sutton, Surrey*)

A number of metals are known to concentrate in lysosomes of liver and other cells (Witschi & Aldridge, 1968). Some of these metals are known to produce tumours at various sites and lysosomal involvement in carcinogenesis has been suggested (Allison, 1968). Colloidal plutonium has been shown to concentrate in rat liver lysosomes (Rahman & Lindenbaum, 1964), and this communication reports a similar lysosomal accumulation of plutonium, americium and actinium in rat liver and/or testes after injection of ^{239}Pu, ^{241}Am or ^{227}Ac in soluble form.

Male Marshall–August hybrid rats were injected intravenously with 0·5–2 μc of [^{239}Pu]plutonium citrate, [^{241}Am]americium citrate or [^{227}Ac]actinium nitrate and killed from 1 hr. to 90 days later. Immediately before injection the solutions were filtered through a 10 nm.-pore-diameter filter to remove polymeric material (Turner & Taylor, 1968). The livers and testes were homogenized in 0·25 M-sucrose and fractionated by differential centrifugation (Worwood & Taylor, 1969), or by centrifugation through a sucrose continuous density gradient.

At 1 hr. after injection most of the plutonium or americium in liver or testes was in the soluble fraction (105000*g* supernatant), but at longer time-intervals most of the plutonium or americium was in the lysosomes, as shown by the coincidence of the metal and arylsulphatase A and B distribution profiles. Similar results were obtained in more limited studies of actinium distribution in liver.

These results show that lysosomal uptake of plutonium, americium and actinium occurs in liver and/or testes within a relatively short time after injection of the metals in a soluble form. Retention of these elements is prolonged in both tissues and plutonium can induce testicular (Bensted, Taylor & Sowby, 1965) and hepatic tumours (Taylor, Dougherty, Shabestari & Dougherty, 1969).

The mechanisms concerned in the lysosomal uptake of these elements are not understood. However, for plutonium at least, it does not appear to be due entirely to endocytosis of colloidal plutonium formed in the blood or extracellular fluids. Plasma plutonium is bound to transferrin (Turner & Taylor, 1968) and during the first few hours after injection the plutonium in the soluble fraction of liver is bound to a protein of molecular weight about 300000 (Boocock, Popplewell & Taylor, 1968).

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