Table 2. Effect of vacuolating agents (see Table 1 for doses and route of administration) on the rate of uptake of 125I-labelled bovine serum albumin by 17.5-day rat yolk sac cultured in vitro

<table>
<thead>
<tr>
<th>Vacuolating agent</th>
<th>Batch of 125I-labelled bovine serum albumin</th>
<th>No. of expts.</th>
<th>Endocytic Index (µl/h per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>I</td>
<td>3</td>
<td>15.4 ± 1.2</td>
</tr>
<tr>
<td>None (control)</td>
<td>II</td>
<td>4</td>
<td>25.3 ± 5.7</td>
</tr>
<tr>
<td>Triton WR-1339</td>
<td>I</td>
<td>3</td>
<td>13.8 ± 2.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>II</td>
<td>3</td>
<td>22.9 ± 2.8</td>
</tr>
<tr>
<td>Polyvinylpyrrolidone</td>
<td>II</td>
<td>3</td>
<td>28.5 ± 6.3</td>
</tr>
</tbody>
</table>

Index of 125I-labelled bovine serum albumin in tissue from animals treated with vacuolating agents; no difference from controls was seen. In all experiments the amount of substrate contained in the tissue became constant after 1–2 h, indicating that the rate-determining step in the overall process of uptake and digestion is uptake. Thus there is no evidence that lysosomal enzymes are inhibited.

These experiments show, first, that it is possible to simulate the morphological features of lysosomal storage disease in rat yolk sac by administration of appropriate substances to the mother in vivo and, secondly, that these pre-treated yolk sacs show no alteration in either pinocytic or digestive capacity. The substances used so far have all been uncharged molecules and thus unlikely to exert inhibitory effects on lysosomal enzymes, but it is interesting that cells with a grossly distended vacuolar system may pinocytose as readily as normal cells and that, for the vacuolating agents studied, the alteration of the intralysosomal milieu by large amounts of a foreign compound appears not to affect the efficiency of digestion. These results contrast with the finding of Wagner et al. (1971) that pinocytosis of sucrose by Chang liver cells in culture led to a 'blockade' of uptake when the cells had become highly vacuolated.

We thank the M.R.C. for their support of this work and Professor A. R. Gemmell of the Department of Biology, Keele University, for the generous provision of electron-microscope facilities.


Analytical Subcellular Fractionation of Guinea-Pig Myocardium with Special Reference to the Localization of the Adenosine Triphosphatases

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Previous subcellular fractionation studies on heart muscle have usually been preparative in nature. With this approach, particular organelles are isolated to a high degree of homogeneity, usually with little regard to yield, e.g. mitochondria (Green et al., 1955),
myofibrils (Perry, 1952) and microsomal fractions (Gibson & Harris, 1968). On the other hand, analytical subcellular fractionation (Claude, 1950; Schneider & Hogeboom, 1951; de Duve, 1971) attempts to resolve the various organelles with quantitative recovery of the various components, and is therefore more suitable for application to pathological tissue.

Left ventricular myocardium from male guinea pigs was homogenized in 0.25M-sucrose, containing 1mM-EDTA and 0.1% ethanol, with a loose-fitting Dounce homogenizer. The homogenate was centrifuged (800g, 10min) and the supernatant (PNS fraction) was fractionated by isopycnic centrifugation on a continuous sucrose gradient in the Beaufay automatic zonal rotor as described by Peters et al. (1972). Optimal and linear kinetics were established for enzymes that have been used in other tissues to locate the position of the organelles in sucrose gradients (Peters et al., 1972).

Fractionation of myocardium in sucrose alone gave poor resolution of the subcellular organelles. Addition of 20mM-KCl (Canonico & Bird, 1970) to the homogenization medium and gradient improved the separation, but significantly inhibited the marker enzymes, and decreased the lysosomal integrity. Addition of 50i.u. of sodium heparin/ml (Stagni & de Bernard, 1968) to the sucrose considerably improved the resolution and maintained lysosomal integrity.

Table 1 shows the pH optima of the assays, and enzyme content of the myocardial homogenate. With the exception of DNA and catalase, about one-half of each enzyme was found in the PNS fraction. Approximately one-quarter of the DNA was found in the PNS fraction, indicating that there was some disruption of the nuclei during homogenization.

Fig. 1 shows the distribution profile of marker enzymes for the principle subcellular organelles. Cytochrome c oxidase (mitochondria) was concentrated over a narrow density span with a modal density of about 1.17. Glutamate and malate dehydrogenases and adenylate kinase (not shown) also showed a sharp peak at a density of 1.17, but significant amounts of activity, presumed to be soluble, remained with the sample layer. A large proportion of catalase (peroxisomes?) was found with the soluble proteins with about 25% forming a peak at a density of 1.18. Approx. 25% of the catalase in the PNS

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>pH optimum</th>
<th>Specific activity (munits/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome c oxidase</td>
<td>7.0</td>
<td>527 ± 120</td>
</tr>
<tr>
<td>Catalase</td>
<td>7.0</td>
<td>10.0 ± 3.1</td>
</tr>
<tr>
<td>5’-Nucleotidase</td>
<td>9.0</td>
<td>15.9 ± 7.2</td>
</tr>
<tr>
<td>Neutral α-glucosidase</td>
<td>7.0</td>
<td>0.259 ± 0.085</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>5.8</td>
<td>0.975 ± 0.235</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>7.4*</td>
<td>724 ± 65</td>
</tr>
<tr>
<td>Mg²⁺-dependent ATPase</td>
<td>7.2*</td>
<td>133.6 ± 32.2</td>
</tr>
<tr>
<td>Na⁺ + K⁺-activated Mg²⁺-dependent ATPase</td>
<td>7.2*</td>
<td>58.2 ± 14.5</td>
</tr>
<tr>
<td>Basal ATPase</td>
<td>7.2*</td>
<td>29.7 ± 12.6</td>
</tr>
<tr>
<td>Ca²⁺-activated ATPase</td>
<td>7.2*</td>
<td>228 ± 25</td>
</tr>
<tr>
<td>NaN₃-insensitive Ca²⁺-activated ATPase</td>
<td>7.2*</td>
<td>96.8 ± 34</td>
</tr>
<tr>
<td>DNA (mg/g of myocardium wet wt.)</td>
<td></td>
<td>1.29 ± 0.43</td>
</tr>
<tr>
<td>Protein (mg/g of myocardium wet wt.)</td>
<td></td>
<td>116 ± 27</td>
</tr>
<tr>
<td>Protein (mg/g of DNA)</td>
<td></td>
<td>81.2 ± 14.7</td>
</tr>
</tbody>
</table>

* pH of assay.

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Fig. 1. Isopycnic centrifugation of supernatant (800g, 10 min) from guinea-pig myocardial homogenate

The plots show frequency-density distribution for various enzymes. ■ represents, over an arbitrary abscissa interval, the enzyme remaining in the sample layer. The % recovered activity for each enzyme is: (a) cytochrome c oxidase, 120%; (b) 5'-nucleotidase, 87%; (c) Ca\(^{2+}\)-activated ATPase, 89%; (d) Na\(^{+}\)+K\(^{+}\)-activated Mg\(^{2+}\)-dependent ATPase 82%; (e) catalase 81%; (f) N-acetyl-β-glucosaminidase, 72%; (g) lactate dehydrogenase, 84%; (h) neutral-α-glucosidase, 108%.

fraction was latent. No significant uricase, l-hydroxy acid or D-amino acid oxidase activity was detected in homogenates of guinea-pig myocardium. Thus although catalase is at least partially particle-bound and exhibits latency, the biochemical criteria for peroxisomes (de Duve & Baudhuin, 1966) are not fulfilled.

N-Acetyl-β-glucosaminidase (lysosomes) showed a broad distribution pattern around a modal density of 1.16. A similar distribution was found for β-galactosidase (not shown). The latent activity of N-acetyl-β-glucosaminidase in the PNS fraction was 55–60% and thus the biochemical criteria for the presence of lysosomes are established. Lactate de-
hydrogenase was found almost entirely with the soluble fractions, indicating that this enzyme is cytoplasmic in the myocardial cells. Non-specific binding of lactate dehydrogenase to the various organelles was not demonstrated, as has been found in other tissues (de Duve et al., 1962). Neutral α-glucosidase (microsomal fractions) showed a broad band of particulate activity at a modal density of 1.14, with significant amount of activity remaining in the sample layer. 5'-Nucleotidase (plasma membrane) showed a sharp peak with a modal density of 1.12.

The ATPases (adenosine triphosphatases) were assayed by using [γ-32P]ATP, essentially by the method of Avruch & Wallach (1971). The Na⁺+K⁺-activated Mg2+-dependent ATPase (Na⁺, K⁺, Mg2⁺, 75 mM, 15 mM, 2 mM respectively) showed a single peak, with a modal density of 1.12 and has the same distribution as 5'-nucleotidase, suggesting that this ATPase is localized to the plasma membrane (sarcolemma). Only trace amounts of Na⁺+K⁺-activated Mg2+-dependent ATPase was found in the mitochondrial fractions. The Ca2+-activated ATPase (Ca2⁺, 10 mM) showed a bimodal distribution, one peak corresponding to the mitochondrial marker (p = 1.17), and the other having a modal density of 1.12. There was about 80% inhibition of the ATPase activity of the mitochondrial peak in the presence of 10 mM-NaNO₃ (Chandler et al., 1967). However, there was only about 20% inhibition of the activity of the peak at 1.12.

Localization of the NaN₃-insensitive Ca2⁺-activated ATPase is uncertain; it is clearly particle bound, and appears to have a similar distribution to the plasma-membrane marker. Other workers have suggested at least a partial localization of the Ca2⁺-activated ATPase to the sarcoplasmic reticulum (Martonosi & Feretos, 1964; Greaser et al., 1969; MacLennan, 1970).

A single-step fractionation procedure for guinea-pig myocardium is described, which resolves the lysosomes, mitochondria, microsomal fractions, peroxisomes and plasma-membrane fragments. The technique is applicable to milligram quantities of tissue and should provide a useful tool for the study of normal and pathological tissue, in both man and experimental animals.

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Analytical Subcellular Fractionation Studies on Human Liver Biopsies

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Assay of serum enzyme activities plays an important role in the investigation of liver disease. Enzymes commonly assayed include 5'-nucleotidase, γ-glutamyl transpeptidase, alkaline phosphatase and leucyl-β-naphthylamidase. There is, however, little detailed