ISOLATION OF RAT HEPATOCYTES WITH EDTA AND 
THEIR METABOLIC FUNCTIONS IN PRIMARY CULTURE

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SUMMARY

Isolated hepatocytes from adult rat liver were prepared after dissociation of the liver with EDTA. The morphological appearance, viability (94.5%) and yield (1.76 x 10^7 cells/g liver) compare well with those of previously described methods using collagenase. Differentiated functions of the hepatocytes in primary culture such as albumin secretion (10.9 μg/mg cell protein/d) and triglyceride synthesis and secretion are maintained. Induction of triglyceride synthesis and secretion by oleic acid takes place to an extent similar to that observed in vivo and liver perfusion. Particles with a lipid composition resembling circulating very low density lipoproteins are secreted into the medium. These characteristics demonstrate the ability of hepatocytes isolated with EDTA and subsequently used in primary culture to retain complex and highly differentiated functions of the intact liver.

Key words: rat; hepatocyte isolation; culture; EDTA.

INTRODUCTION

The quality of primary culture of hepatocytes depends greatly on the quality and reproducibility of the first step in the isolation of the cells. This step consists of the disruption of the collagen fibril network holding the cells together. So far, dissociation of this network has been carried out almost exclusively with bacterial collagenases. Until today, such techniques prevailed. Unfortunately, the use of collagenase presents a few disadvantages such as the presence of noncollagen proteases which are difficult to eliminate (6) or batch-to-batch variation in the activity of the enzyme resulting probably from storage conditions during shipping. Recently, important differences in the yield and viability of the cells have been reported by Queral et al. (19).

In this report we present a preparation method based on that described by Berry et al. (5), using EDTA as the dissociating agent followed by separation of the viable cells on a Percoll density gradient. This last step has been shown to separate hepatocytes with an improved viability (8). Such preparations of hepatocytes in culture maintain specific and differentiated liver functions: albumin secretion, triglyceride synthesis and secretion, and respond to an increased free fatty acid concentration in the medium by an increase in synthesis and secretion of triglycerides as observed in perfuso (1,17) and in rat hepatocytes in primary culture (9).

MATERIALS AND METHODS

Materials. All reagents used were of analytical grade. Surgical instruments, tubing, and glassware were sterilized before each experiment.

Methods. The animals (Charles River, France) were male Sprague-Dawley (175 to 400 g body weight), and female Wistar (150 to 180 g body weight) rats and were fed ad libitum until the time of killing.

The perfusion medium consisted of 140 mM NaCl, 5 mM KCl, 0.8 mM MgSO₄, 1.6 mM Na₂HPO₄, 0.4 mM KH₂PO₄, 2 mM EDTA, and 25 mM NaHCO₃, pH 7.4. Just before use, the buffer was equilibrated with O₂:CO₂, 95:5, vol/vol (buffer A.). After cell dissociation, hepatocytes were washed in a modified buffer A containing 1 mM CaCl₂ but no EDTA or NaHCO₃ (buffer B.). The Percoll solution consisted of 9 vol of Percoll (Pharmacia Fine Chemicals, Upsala, Sweden) and 1 vol of 10 X buffer B without calcium (final density 1.135 g/ml). All buffers were sterilized by filtration through 0.22 μm Millipore filters before use.

Surgery. Liver perfusion was performed in situ under light ether anesthesia. After cannulation of the portal vein and the inferior vena cava close to the heart, the inferior vena cava was ligated just above the renal vein. Buffer A, prewarmed at 37°C, was then infused through the portal vein at a constant flow rate of 3 to 4 ml/g liver/min using a peristaltic pump for 30 to 40 min. Rat liver weight was estimated before the experiment as 4.5% of body weight. The effluent perfusate was not recirculated. After this time, softening of the liver was evident and the buffer began to ooze through the liver capsule.

Cell isolation. The softened liver was excised and cut gently into small pieces with a scalpel blade. The pieces were put in a beaker containing 10 ml of buffer B. An additional 10 ml of buffer B was added while gently swirling the beaker, and the cell suspension was filtered through two layers of sterile gauze. The remaining liver fragments were washed four to five times following the same procedure. The cells in suspension were sedimented for 2 min at 50 g, and washed twice after
TABLE 1

YIELD, VIABILITY, AND ALBUMIN SECRETION RATE OF RAT HEPATOCYTES ISOLATED WITH EDTA

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>Sex</th>
<th>n</th>
<th>Body Weight</th>
<th>Yield</th>
<th>Cell Viability</th>
<th>Albumin Secretion Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprague-Dawley</td>
<td>male</td>
<td>8</td>
<td>115-450 g</td>
<td>1.94 ± 0.29</td>
<td>93 ± 3%</td>
<td>N.D.</td>
</tr>
<tr>
<td>Wistar</td>
<td>female</td>
<td>14</td>
<td>150-180 g</td>
<td>1.76 ± 0.12</td>
<td>96 ± 1%</td>
<td>10.9 ± 2.2</td>
</tr>
</tbody>
</table>

*Results are given as mean ± SEM. Cell viability is based on Erythrosine B exclusion (0.04% solution). Albumin secretion is determined by immunoelectrophoresis as described in (14).

Cell culture. Cells were counted in a hemocytometer and plated on 60 or 100-mm petri dishes at 4.10^6 cells per dish or 10.10^6 cells per dish, respectively, and maintained at 37°C in a humidified atmosphere (95% air:5% CO2). Twenty-four hours later the medium containing the unattached cells was changed and replaced with fresh DME as above without FBS.

Morphology. The attached hepatocytes were observed under phase contrast microscopy.

Metabolic activities. After the indicated time period, the medium from six 60-mm Petri dishes was pooled. Loose cells and debris were pelleted at 1500 g for 5 min. The medium was concentrated to 2 ml by ultrafiltration under nitrogen using an Amicon Model 52 apparatus and PM 30 membranes. Five microliter aliquots of the

**Fig. 1.** Hepatocytes in primary culture observed 5 h after plating under phase contrast microscopy. X360.