Isolation and Primary Culture of Adult Human Hepatocytes
Ultrastructural and Functional Studies

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Summary. Biopsy tissue of adult human liver was gently dissociated with collagenase followed by Dispase. By repeated low g centrifugation, a large number of almost pure, viable hepatocytes was obtained. This is the first report of a successful procedure for obtaining adult human hepatocytes for study in tissue culture. The isolated cells have the typical morphology of liver parenchyma, and these characteristics persist throughout the period of culturing. Evidence of their function is indicated by albumin synthesis. This procedure is now being used to study human hepatocyte functions in vitro and the effects of a variety of agents including carcinogens and viruses.

Key words: Human – Isolated hepatocytes – Primary culture – Albumin synthesis – Ultrastructure

Primary cultures of hepatocytes, and the establishment of a continuously replicating line of liver cells has been achieved from small mammalian species (Berry and Friend 1969; Gerschenson et al. 1970). The hepatocytes in these studies were prepared for culture by first perfusing Ca\(^{2+}\)-free buffer solution followed by collagenase solution, through the liver, then mechanically dissociating the tissue (Seglen 1973). Rat liver tissue survives this treatment, and the dissociated cells are easily identified as hepatocytes by their morphology and classical assay for specific liver cell function (Wanson et al. 1979). Human liver, especially the small pieces obtained at biopsy, cannot be handled in this manner, and although numerous isolation procedures have been proposed, neither mechanical (Hopf et al. 1975) nor enzymatic disintegration (Belleman et al. 1977), applied to the tissue samples or to human liver pieces (Kaighn and Prince 1971; Okigaki et al. 1972; Wands and Isselbacher 1975; Schaefler and Kessler 1980), have proved to be satisfactory for...
the isolation of intact hepatocytes, as evaluated by correlated morphological and functional studies.

Adult human liver tissue has been maintained using explant culture technique in which peripheral outgrowth of cells has been observed around the small liver fragments (Le Guilly et al. 1970; Demoise et al. 1971; Sandström 1973; Lemonnier et al. 1976; Scotto et al. 1979). However, cell culture of isolated hepatocytes is more desirable as a simplified experimental model, since it permits one to work quantitatively with homogenous cell populations. In the present communication a technique is described for the isolation and concentration of dissociated adult human hepatocytes. Unlike reports by others, the cells in these preparations have the morphological characteristics of hepatocytes, and other cell types which often contaminate such preparations, like bile duct epithelium, Kupffer cells and stromal elements, are absent.

Materials and Methods

Biopsies of adult human liver weighing about 800 mg wet weight were obtained from patients, 30 to 70 years old undergoing cholecystectomy. The specimens were cut into smaller fragments in cold Ca²⁺-free Hanks' solution and stirred for 30 min at 4°C in a small flask. The cells and fragments were centrifuged at 50 g for 3 min and the pellet was resuspended in a flask in 10 ml of 0.05% collagenase IV (Worthington) in Hanks' solution. The suspension flask was agitated in a water bath at 90 oscillation/min for 20 min at 37°C. The centrifugation procedure was repeated, then the pellet was resuspended in 1000 PU/ml Dispase I (Godo-Shusei, Chiba, Japan) in Hanks' solution and the flask placed in the water bath and oscillated for 60 min at 37°C. The cell suspension was filtered through a double thickness of sterilized nylon stocking and the suspension of cells was centrifuged 3 more times at 50 g for 3 min. Each time the supernatant fluid was discarded, and the pellet resuspended in fresh Hanks' solution. Buffer and enzyme solutions were oxygenated with 95% O₂ and 5% CO₂ prior to use, and the pH was adjusted to 7.4 by NaHCO₃. Five to 6 x 10⁵ hepatocytes were inoculated into 35 mm Falcon plastic dishes in 2 ml of medium composed of 85% DM 160 (Kyokuto, Chiba, Japan) and 15% fetal bovine serum, to which were added 10⁻⁶ M insulin, 2 x 10⁻⁸ M hydrocortisone and 100 μg/ml of kanamycin. The plastic dishes were incubated at 37°C in a humidified atmosphere and gassed with 5% CO₂ and air. Albumin synthesis was determined by measuring radiolabelled albumin secreted into the medium and isolated by immunoprecipitation with a double antibody method. The precipitates were collected after standing in the cold at 4°C for 24 h and the radioactivity counted, along with appropriate controls, in a liquid scintillation counter as described by Vicrey et al. (1979). The cells were prepared for transmission and scanning electron microscopy by standard techniques. For transmission electron microscopy, cells were embedded in Epon 812 after fixation. For scanning electron microscopy, fixed cells were critically point dried and coated with gold in an ion sputter. For autoradiography, cultures were incubated for 12 h in 5 μCi/ml of [³H]thymidine (NEN), rinsed in Hanks' and fixed in Carnoy's solution. The cells were coated with Sakura NR-M2 nuclear track liquid emulsion and developed after 10 days.

Results

Yield of Viable Hepatocytes

The yield of hepatocytes was 2 x 10⁶ cells per g wet weight of the liver tissue. Repeated centrifugation of the cell suspension at 50 g facilitated the removal of the majority of cell contaminants, such as red blood cells, sinusoidal lining cells and cell debris. Thus, the final cell population consisted almost entirely of parenchymal