Effect of dietary state on hepatocyte size

B. D. UHAL* and K. L. ROEHRIG†

Department of Food Science and Nutrition, 122 Vivian Hall, 2121 Fyffe Road, The Ohio State University, Columbus, Ohio 43210, U.S.A.

(Received 12 October 1982)

In order to calculate cellular metabolite concentrations, it is necessary to determine accurately the cellular volume. The purpose of the experiments reported here was to determine the effect of dietary state on the volume of hepatocytes isolated from livers of fed, fasted (48 h) or fasted(48 h)-refed(48 h) male Sprague-Dawley rats. Cellular volumes of the essentially spherical hepatocytes were calculated from the diameters of the cells obtained easily and rapidly by using an optical micrometer. Fasting resulted in approximately a two-fold reduction in hepatocyte volume, and refeeding resulted in a return to 83% of the control value. The protein content/1000 cells also dropped two-fold upon fasting but returned to 70% of the control value upon refeeding. This rapid, simple method for determining hepatocyte volumes agrees well with data obtained by more laborious means.

Understanding the regulation of various metabolic processes in vivo requires knowledge of the concentrations of relevant substrates, products, and effectors in the cellular compartment where the system of interest is located. Determining localized concentrations by such techniques as classical centrifugal separations has been problematical because of the length of time required for such procedures.

Relatively recently, however, the digitonin fractionation technique for hepatocytes has come into wide use. This method, suggested by Zuurendonk and Tager (1974), has undergone a number of modifications but essentially consists of disrupting hepatocytes with digitonin and rapidly centrifuging the lysed cells through a hydrophobic layer such as bromododecane into perchloric acid. Since the mitochondrial membrane is more resistant to digitonin than the plasma membrane, the contents of the cytoplasm stay in the upper phase while the mitochondria are centrifuged into the bottom layer. The method is rapid and can be manipulated to give good separations of cytoplasm and mitochondria (Janski & Cornell, 1980). The substrates in each layer can then be easily assayed.

* Present address: Department of Biochemistry, St. Louis University, St. Louis, Missouri, U.S.A.
† To whom correspondence should be addressed.

©1982 The Biochemical Society
The amount of substrate present can be reported in several ways: \( \mu \text{mol/1000 cells}, \mu \text{mol/mg of protein}, \mu \text{mol/g of wet or dry wt. of liver or } \mu \text{mol/ml.} \) Each of these expressions might give quite different impressions of what is happening to substrate levels under different physiological conditions if the cell number, liver size, cell size, or protein content changes. This study was undertaken to determine the effect of dietary state on cell size and protein content in order to more accurately calculate the subcellular concentrations of various metabolites.

**Materials and Methods**

**Animals**

Male Sprague-Dawley rats were housed in galvanized, wire-bottom cages in a controlled-environment animal facility with a 7:00-P.M.-to-7:00-A.M. light cycle. Food and water were available ad libitum except during fasting periods, when only water was available. The composition of the diet has been previously described (Allred & Roehrig, 1973).

**Hepatocyte preparations**

Hepatocytes were prepared from perfused rat livers according to the method of Seglen (1976) as modified by Geelen et al. (1978) after anesthetizing the rats with diethyl ether. Type-II collagenase at a level of 0.75-1.5 mg/ml was used to separate the cells. Trypan blue dye exclusion was used to assess cell viability as suggested by Seiss and Wieland (1975). Cell yields after two washings in suspension buffer containing 10 mM glucose were at least 50%. Cells were diluted to a final concentration of \( 2.5 \times 10^7 \) cells/ml after being counted in a hemacytometer.

**Cell volume**

Cell diameter was measured with a calibrated microscope eyepiece. The diameter measurements were made on an average of 18 randomly chosen cells per preparation. After sitting in suspension buffer for a few minutes, the hepatocytes assumed a spherical shape. The cell volumes were calculated from the diameter using the formula for the volume of a sphere. Calculation of the cell volume by microscopic determination has been previously validated for adipocytes by Di Girolamo et al. (1971) and Di Girolamo and Mendlinger (1971).

**Protein determination**

For protein determinations by the method of Bradford (1976), cells were diluted 1:20, sonicated for 1 min at 0-4°C, and then diluted 1:400 before protein assays. Fraction V bovine serum albumin was used as the protein standard.

**Reagents**

Biochemicals were obtained from the Sigma Chemical Co., St. Louis.
Results and Discussion

In order to examine the effects of dietary state on liver cell size, isolated hepatocytes were prepared from ad-libitum-fed, fasted (48 h), or fasted (48 h)-refed (48 h) rats. The viability of these preparations as measured by dye exclusion was 93 ± 0.93, 90 ± 1.2, and 92 ± 1.0%, respectively. Fasting resulted in a 22% drop in the average diameter of the hepatocytes compared to the ad-libitum-fed controls, and refeeding the fasted rats for 48 h resulted in a return of the diameter to nearly the control value (Table 1). The relatively small change in diameter, however, led to a two-fold reduction in the volume of the liver cells from fasted animals because the volume changes with the cube of the radius. Thus, fairly small changes in diameter have a large impact on the volume of the cell. The diameter of the hepatocytes from fed animals in this study agrees well with textbook values of 20-30 μm for isolated hepatocytes and with the values reported by Weibel et al. (1969) for microscopic (light and electron) evaluation of thin sections of parenchymal cells. The volume also agrees well with the values reported by Tischler et al. (1977), who determined extracellular, whole-cell, and extramitochondrial volumes using radioactive inulin, sucrose, and water. The protein content of the hepatocytes from fed rats is very similar to the 22% observed by Harrison (1953) assuming that there are 10^8 parenchymal cells/g wet wt. of liver (Zahlten et al., 1973).

It has long been known that fasting rapidly decreases liver weight. In 1953, Harrison showed that the DNA content of the liver remains the same over a 6-day fast, meaning that there was no reduction in cell number. Thus, the cell volumes must be reduced, which is consistent with our observations and those of Cech et al. (1980), who compared fed, fasted, and diabetic rats. Other investigators, including Berry and Kun (1972), Parrilla et al. (1975), and Soboll et al. (1978), have reported volumes for the cytosol and mitochondria, but there is considerable variation in their values. Part of the problem may stem from the variability in the dry wt./wet wt. ratio which was used to calculate the cellular volume on a ml/g dry wt. basis. All of these

<table>
<thead>
<tr>
<th>Dietary state</th>
<th>Fed</th>
<th>Fasted</th>
<th>Fasted-refed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (μm/cell)a</td>
<td>22.8 ± 0.14b</td>
<td>17.8 ± 0.30</td>
<td>21.4 ± 0.45</td>
</tr>
<tr>
<td>Volume (10^-12 l/cell)c</td>
<td>6.2 ± 0.11</td>
<td>3.0 ± 0.15</td>
<td>5.2 ± 0.33</td>
</tr>
<tr>
<td>Protein (μg/1000 cells)</td>
<td>2.3 ± 0.09</td>
<td>1.2 ± 0.07</td>
<td>1.6 ± 0.02</td>
</tr>
</tbody>
</table>

aMeasured on 18 randomly chosen cells/preparation.
bValues are the means from five preparations/group ± S.E.M.
cCalculated from the diameters using the equation for the volume of a sphere.
investigators used a cytoplasmic-to-mitochondrial ratio of 10/1. Loud (1968) observed that the fraction changed depending upon the region of the liver from which the cells were obtained.

In order to calculate the cellular metabolite concentrations, it is necessary to be able to determine accurately the volume of the cells. For example, if the absolute amount of a metabolite were unchanged by fasting, the decrease in volume would result in a doubling of the concentration. The necessity for this information in relationship to hormone sensitivity and binding as well has been discussed by Cech et al. (1980).

The method reported here can be performed rapidly during the assessment of viability by dye exclusion and does not require waiting for data from more cumbersome and expensive procedures such as insulin and sucrose space determinations. Our data agree well with values derived from other techniques, and the method employed allows easy, rapid determination of cell size in each individual preparation under varying hormonal states without having to rely on literature values or occasional determinations on selected preparations.

Acknowledgements

This work was supported in part by a PHS grant GM29255 and by Hatch grant 666.

References


Harrison M (1953) Effect of starvation on the composition of the liver cell. Biochem. J. 55, 204-211.


