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A NEW METHOD FOR THE ISOLATION OF FRESH HEPATOCYTES FROM PERIPORTAL AND PERICENTRAL REGIONS OF THE LIVER LOBULE

U. K. Misra[a], H. Yamanaka[a], Z. Kizaki[a] F.C. Kauffman[b] and R. G. Thurman[a]

[a]Dept. of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, N.C., 27599-7365

[b]Department of Pharmacology, University of Maryland, Baltimore, Maryland 21201

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SUMMARY. A simple method which avoids the use of perfusion with calcium free buffer, hydrolytic enzymes and detergents has been developed to obtain fresh hepatocytes from periportal and pericentral regions of the liver lobule. Cylindrical plugs (200 x 500 microns) of periportal and pericentral areas of the rat liver lobule weighing about 1 mg were collected with a micropunch from fresh or perfused liver. Ninety percent of cells were intact as assessed from trypan blue staining. Glutamine synthetase activity was detected predominantly (ca. 85%) in plugs isolated from pericentral regions indicating that this method allows selective harvesting of pure sublobular zones of the liver lobule. Rates of oxygen uptake measured at 25°C by plugs from livers perfused in the anterograde direction were 56 ± 5 and 33 ± 7 μmol/g/h by periportal and pericentral plugs, respectively, values similar to data obtained from the intact organ. This method provides new opportunities to study the regulation of basic metabolic processes in cells from sublobular areas under nearly physiological conditions.

It is well known that subcellular organelles and many enzymes are distributed unevenly across the liver lobule (1,2). Moreover, metabolic processes such as glucose and urea synthesis and oxygen uptake are compartmentalized within the liver lobule (3-5). Recently, methods for isolation of hepatocytes from specific regions of the liver lobule have been described (6-8). However, those techniques employ perfusion with calcium free

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Send all correspondence to: R.G. Thurman, Department of Pharmacology, Faculty Laboratory Office Building, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7365.

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buffer and collagenase as well as the use of digitonin to destroy cells in periportal or pericentral regions of the liver lobule. Although this approach has provided populations of hepatocytes from sublobular zones, the harsh conditions used (6-8) may alter cellular integrity and metabolic regulation. For example, intracellular calcium, an important second messenger, is elevated in hepatocytes as a consequence of isolation (9,10). Accordingly, a new experimental approach to isolate fresh hepatocytes from periportal and pericentral regions of the liver lobule was developed without the use of detergents and hydrolytic enzymes.

METHODS

Liver Perfusion. Fed, female Sprague-Dawley rats(200-250 g) were treated with sodium phenobarbital (1 mg/l) in drinking water for at least 7 days to enhance the color contrast between periportal and pericentral regions of the liver lobule. Livers were perfused with Krebs-Henseleit bicarbonate buffer (pH 7.4, 37°C) saturated with 95% oxygen: 5% carbon dioxide in a nonrecirculating system as described previously (11). Perfusate was pumped into the liver at a rate of about 4 ml/g/min via a cannula placed in the portal vein, and left the liver via a cannula inserted into the inferior vena cava. The effluent perfusate flowed past a Teflon-shielded, Clark-type oxygen electrode and was discarded. Rates of oxygen uptake were calculated from the flow rate, the influent minus effluent oxygen concentration difference and the liver wet weight.

Glutamine Synthetase Activity. Cylinders of hepatocytes isolated with a micropunch (see Results and Fig. 1) were homogenized in 4 volumes of 20 mM Tris.HCl (pH 7.5) containing 2 mM dithiothreitol and centrifuged at 2,000 x g for 10 min. Glutamine synthetase activity in the supernatant was determined colorimetrically according to the method of Wellner and Meister (12) except that incubations were carried out for 60 min. Assays were linearly proportional with time and amounts of tissue up to 300 μg of protein.

Oxygen Uptake by Fresh Plugs. Cylinders from pericentral and periportal areas were collected in Krebs-Henseleit buffer containing 1% bovine serum albumin, pH 7.4, preequilibrated with 95% O_2:5% CO_2 at 4°C. Weighed plugs were transferred to Krebs-Henseleit buffer, pH 7.4, and oxygen uptake was measured with a Clark-type oxygen electrode at 25°C in a magnetically stirred reaction vessel of 0.6 ml capacity.

RESULTS AND DISCUSSION

Isolation of Fresh Plugs of Periportal and Pericentral Hepatocytes. Fresh periportal and pericentral regions of the

liver lobule were isolated based on the anatomy of the liver lobe. It is well known that columns of periportal and pericentral hepatocytes arise from the major vessels and radiate towards the liver surface (13). These columns end in spots on the liver surface that have long been used to identify periportal and pericentral regions (13).

Micro-punch technology is well developed and can be used to isolate small areas of tissue such as specific nuclei in the central nervous system or discrete areas of endocrine organs or the kidney (14). The purpose of these studies was to utilize this technology to isolate pure preparations of viable hepatocytes from periportal and pericentral regions of the liver lobule.

To achieve this goal, livers from rats pretreated with phenobarbital to enhance the color contrast between periportal (light) and pericentral (dark) spots on the liver surface (15) were perfused briefly (20 min) to remove blood and assess oxygen uptake by the whole organ. Subsequently, the major lobes were removed surgically and cut laterally along the major vessel (see Fig.1). Dark and light spots were then collected rapidly employing a micropunch (200 μm i.d.). Approximately 100 plugs can be harvested with this technique in about 10-15 mins.

Plugs prepared in this manner were cylinders with diameters of 0.2 mm and 0.2 to 0.5 mm long (Fig. 2A). Each plug weighed between 0.5 and 1 mg wet and contained approximately 14 to 35 x 10^3 parenchymal cells. More than 90% of the cells within plugs excluded trypan blue except for cells along the outer rim of the cylinder which were damaged uniformly (Fig. 2B). Cells in plugs isolated from periportal and pericentral regions of the liver lobule were histologically indistinguishable from those in the liver <u>in vivo</u> (Fig. 2C). In contrast to isolated hepatocytes (16) or hepatocytes isolated from periportal and pericentral regions of the liver lobule with detergent treatment (6-8), fresh hepatocytes within plugs maintain normal cell-to-cell communication and, possibly importantly, contain non-parenchymal cells (endothelial, and Kupffer and fat storing cells; Fig. 2C). Also, vectorial orientation of key enzymes such as Na^+/K^+ ATPase is maintained.

To ascertain the degree of purity of periportal and pericentral regions in plugs isolated as described above, we assayed glutamine synthetase which is localized exclusively in pericentral regions of the liver lobule (17). Over 85% of the glutamine synthetase was detected in plugs isolated from

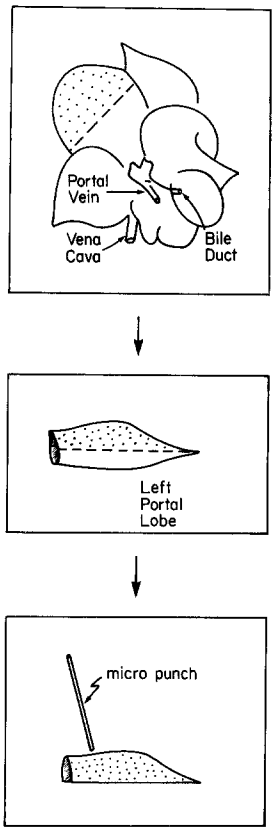

Figure 1. A schematic representation of the technique used in the isolation of periportal and pericentral plugs from liver lobule. A section (----) of a lobe from rat liver (upper panel) was surgically removed, placed on ice and sliced horizontally (middle panel). Periportal and pericentral regions were collected with a micropunch and transferred into ice-cold Krebs-Henseleit buffer containing 1% bovine serum albumin, pH 7.4 (lower panel). Dots represent dark (pericentral) areas.

pericentral regions whereas less than 15% was detected in cylinders from periportal areas (Fig. 3). Therefore, cross-contamination of periportal plugs with tissue from pericentral areas is minimal with this isolation procedure. Assuming that contamination of pericentral plugs with tissue from periportal areas is similar (i.e., about 15%), then it is safe to conclude that the purity of these preparations is very high.

To further assess viability, oxygen uptake of plugs isolated from periportal and pericentral regions of the liver lobule was studied. In plugs isolated from both regions, oxygen uptake was proportional to amount of protein as expected (Fig. 4). Plugs isolated from periportal areas consistently took up oxygen at rates about 50% higher than plugs isolated from pericentral

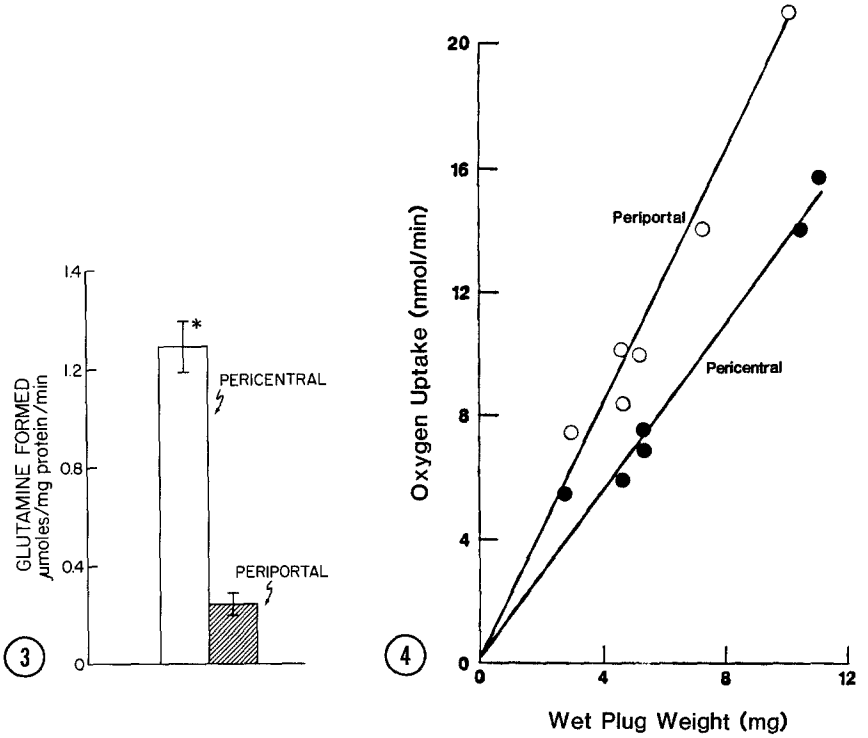

Figure 3. Glutamine synthetase activity in freshly isolated plugs from periportal and pericentral regions of the liver lobule. Plugs isolated as depicted in Fig. 1 were homogenized in 4 vol of 20 mM Tris.HCl buffer (pH 7.4) containing 2 mM dithiothreitol. The homogenate was centrifuged at 2000 x g for 10 min and glutamine synthetase activity was determined in the supernatant as described in Methods. Values are mean $\pm$ SEM from plugs from 4 rats. *, $p < 0.01$.

Figure 4. Effect of protein concentration on oxygen uptake by freshly isolated periportal and pericentral plugs from perfused liver. Plugs isolated as described in Fig. 1 were incubated at 25°C in Krebs-Henseleit buffer, pH 7.4, for 20 min and oxygen uptake was measured as described in Methods except that 5 mM succinate was present.

regions when livers were perfused in the anterograde direction (Fig. 4,5). In sharp contrast, when livers were perfused in the retrograde direction, oxygen uptake was significantly higher in plugs isolated from pericentral than from periportal areas (Fig. 5). These data are consistent with studies performed in this laboratory demonstrating that many metabolic activities including oxygen uptake move rapidly to the opposite side of the liver lobule when the direction of flow is reversed (3,5,18,19). Thus, the finding that fresh plugs of hepatocytes exhibit similar behavior as long as 60 to 120 min (i.e. they "remember") after isolation demonstrates convincingly that the direction of flow within the liver lobule plays an important role in regulating

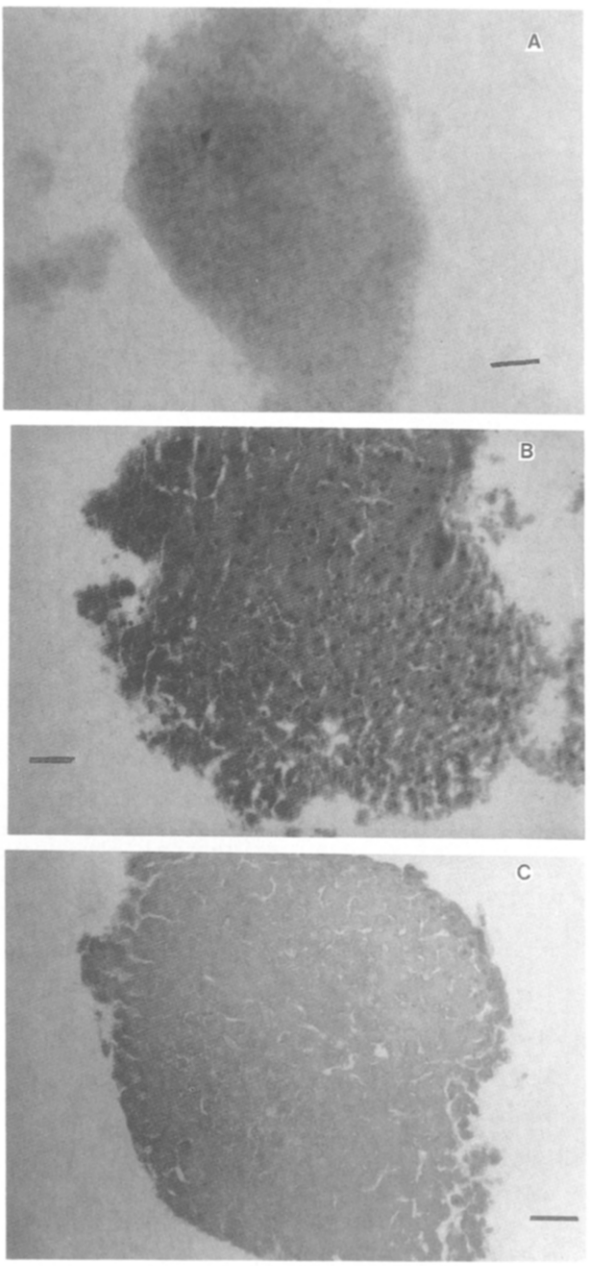

Figure 2. Photomicrographs of Fresh, Isolated Sublobular Regions. (A) Photomicrograph of unstained freshly isolated plugs. Each plug contains about 14-35 x 10^3 hepatocytes. (B) Hemotoxylin-Eosin stained freshly isolated plug demonstrating hepatic architecture. (C) Trypan blue stained section of freshly isolated plug from perfused liver. Note dye uptake only in peripheral layer of cells. Bar = 150 μm.

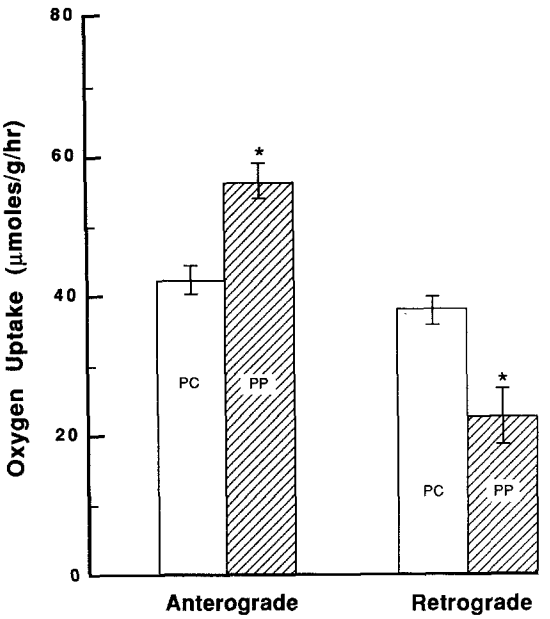

Figure 5. Effect of direction of perfusion on oxygen uptake by periportal (PP) and pericentral (PC) plugs. Livers were perfused in anterograde or retrograde directions in separate experiments for 20 min with Krebs-Henseleit buffer. Plugs were isolated as depicted in Fig. 1 and incubated in Krebs-Henseleit buffer, pH 7.4, at 25°C. Oxygen uptake was measured as described in Methods. Values are mean ± SEM for plugs from 4 rats per group. *, $p < 0.01$.

metabolism in subcellular zones of the liver. These data support the idea that local oxygen tension (18,19) is a major factor in regulating metabolism in subcellular zones.

This new methodology should expand research possibilities into mechanisms of this phenomenon as well as other important problems in hepatobiology and toxicology involving the liver lobule.

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