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STUDIES OF LYSOSOMAL FUNCTION: I. METABOLISM OF SOME COMPLEX LIPIDS BY ISOLATED HEPATOCYTES AND KUPFFER CELLS

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SUMMARY

The enzymatic hydrolysis of four complex lipids was measured in extracts of rat hepatocytes and Kupffer cells. Sphingomyelin, glucocerebroside, ceramide trihexoside, and G_{M1}-ganglioside were hydrolyzed by extracts of both cell types. Hepatocytes contain 90% or more of the complex lipid hydrolases present in liver. The activities of ten additional acid hydrolases are located predominately in hepatocytes with only a small fraction of the total activity present in Kupffer cells.

INTRODUCTION

An understanding of the functions and interrelationships of hepatocytes and Kupffer cells is necessary to appreciate the complexity of the disorders that cause lysosomal storage in liver. Anatomic studies have been useful in identifying different cell types in the liver and suggesting their functional roles (1,2). Various physiologic and biochemical approaches have been used to confirm these observations and uncover new functions specific to certain cells (2,3,4). Recently, the physical separation of hepatocytes and Kupffer cells has been improved and is useful in posing some questions of specific cell function (5,6). One application of this technique is the localization of receptors on cell surfaces. The classical work of Ashwell and Morell (7) locating the receptor for galactose terminal glycoproteins on the hepatocyte has been confirmed *in vitro* in a preparation of intact hepatocytes (8). The relative importance of hepatocytes and Kupffer cells in the clearance of circulating native

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and desialylated lysosomal enzymes has been demonstrated (9). In addition, the operation of hepatic binding protein in the clearance of desialylated lysosomal enzyme by hepatocytes has been suggested (9). Because of the observation that clearance of a lysosomal enzyme was unequal between hepatic cell types and because the pattern of storage of material is not uniform among lysosomal storage disorders, we were stimulated to study the lysosomal enzymes of the two cell types. Four enzymes involved in known deficiency states producing lysosomal storage were chosen for evaluation. Each is normally responsible for the hydrolysis of a specific complex lipid.

METHODS AND MATERIALS

Preparation of Hepatocytes. Hepatocytes were prepared by a modification of the method of Berry and Friend (5). The apparatus and technique of liver perfusion were as described by Hems et al (10), as modified by Krebs et al (11). Calcium-free Krebs-Henseleit buffer was used as the perfusate which contained 0.03% collagenase; hyaluronidase was not added. The gas phase was 95% O_2/5% CO_2. Male Osborne-Mendell rats (250-300 g) were anesthetized by intraperitoneal injection of sodium pentobarbital. The abdomen was opened and the liver perfused at a rate of 20-25 ml/min for 25 minutes at room temperature. The liver weighing 10-12 grams was cut into small pieces and transferred to 100 ml Krebs-Henseleit solution, pH 7.4. The mixture was gently swirled for 2 minutes at 21°C. The cell suspension was filtered several times through nylon mesh 0.5 mm X 0.3 mm. Cells were sedimented by gravity for 5 minutes at 4°C. The supernatant containing non-parenchymal cells was pipetted off leaving hepatocytes in 8-10 ml of solution. The sediment was diluted with 30 ml of fresh Waymouth's medium 752/1 (12) to which had been added bovine serum albumin to a final concentration of 1.3%. Cells were counted by hemocytometer.

Preparation of Kupffer Cells. Modifications of the method described by Munthe-Kaas and Seglen (13) were as follows: (a) The initial cell suspension was prepared in Krebs-Henseleit solution. (b) A single solution of Metrizamide was used at a final concentration of 16% (W/v) (16 g Metrizamide, 0.24 g HEPES, 0.5 g KCl, 0.018 g $CaCl_2 \cdot 2H_2O$, 5.5 ml 0.1 M NaOH and H_2O to 100 ml), pH 7.6 at 37°C. Tubes containing 13 ml of this solution onto which were layered 25 ml portions of the supernatant obtained from the hepatocyte isolation were placed in a SW 27 Beckman rotor and centrifuged at 4°C for 45 min at 3000 X g. The purified non-parenchymal cells which included Kupffer cells were recovered by pipette at the Metrizamide-buffer interface. The cell suspension was washed with 50 ml Krebs-Henseleit buffer, pH 7.4 and centrifuged at 500 X g for 3 min. Cells were resuspended in 4 ml Waymouth's media 752/1 with 1.3% bovine serum albumin and counted by hemocytometer.

Enzyme Assays. Glucocerebrosidase and sphingomyelinase were determined as reported previously using ^{14}C-radiolabelled substrate (14, 15). Activity of the detergent extracted cell sonicates is expressed as nanomoles substrate hydrolyzed per hour.

G_{M1}-ganglioside β-galactosidase was determined by the method of Tanaka and Suzuki using the tritium labeled substrate (16).

TABLE I

Activity of Complex Lipid Hydrolases in Isolated Hepatic Cells

	Hepatocytes		Kupffer Cells	
	U/10^6 Cells	U/gram*	U/10^6 Cells	U/gram*
Glucocerebrosidase	83 ± 6	7055	12 ± 0.5	180
Sphingomyelinase	86 ± 5	7510	23 ± 5	345
Ceramide trihexosidase	23 ± 2	1855	4 ± 0.4	60
G_{M1}-β-galoctosidase	3 ± 1	255	0.9 ± 0.3	13

Assays carried out in triplicate on preparations of cells from four different rats. Activity units (U) are expressed in nanomoles hydrolyzed per hour ± S.E. *Units per gram liver calculated on basis of 85 X 10^6 hepatocytes and 15 X 10^6 Kupffer cells per gram liver (28).

Ceramide trihexosidase was determined by a previously reported method using ceramide trihexoside labeled in the terminal galactose (17).

Ten additional lysosomal acid hydrolases were determined fluorometrically using 5 mM solutions of the appropriate 4-methylumbelliferyl substituted glycopyranosides or phosphate (products by Koch-Light Laboratories Ltd., Buckinghamshire, England or Sigma Chemical Co., St. Louis, Missouri). Assays were carried out in citrate-phosphate buffer, pH 4.5 at 37°C. Activities are expressed in nanomoles substrate hydrolyzed per hour. Glucose production and ATP content was measured by the method of Lamprecht et al (18).

RESULTS AND DISCUSSION

The preparation of hepatocytes contains less than 1% visible Kupffer cells under the light microscope. More than 80% of the cells excluded trypan blue. The cells produce 0.8-1.0 μmol glucose/min from pyruvate per gram wet weight of hepatocytes. Also, the hepatocytes contain 2.5-3.0 μmol ATP per gram wet weight. Both these values are similar to those reported for hepatocytes in other studies (19,20).

The Kupffer cell preparation contained less than 0.5% hepatocyte contamination and 95% of the cells excluded trypan blue. By the methods of latex microsphere phagocytosis (20) and Fc and complement receptors (21), approximately

TABLE II

Activity of Lysosomal Acid Hydrolases in Isolated Hepatic Cells.

	Hepatocytes		Kupffer Cells	
	U/10^6 Cells	U/gram	U/10^6 Cells	U/gram
β-hexosaminidase	63 ± 6	5372	11 ± 1	165
α-galactosidase	32 ± 2	2677	15 ± 4	225
β-glucosidase	4.6 ± 0.6	391	2 ± 0.8	32
α-glucosidase	110 ± 17	9350	17 ± 1	250
β-galactosidase	75 ± 3	6375	36 ± 12	180
Acid phosphatase	1039 ± 147	88315	353 ± 111	5280
α-mannosidase	149 ± 24	12665	48 ± 10	720
α-arabinosidase	3 ± 0.3	255	2 ± 0.7	32
β-xylosidase	0	0	0	0
β-fucosidase	0	0	0	0

Activity units (U) are expressed in nanomoles hydrolyzed per hour ± S.E.

30% of the cell population was determined to be authentic Kupffer cells with the balance being endothelial cells.

The results of the assays for complex lipid hydrolysis are shown in Table I. The enzymes that are responsible for the catalysis of the reactions are contained predominantly in hepatocytes in each case. Less than 5% of the total activity in a gram of liver is contained in Kupffer cells. The concentration of each enzyme per 10^6 cells is 4-6 fold greater in hepatocytes. However, the total Kupffer cell volume is only about 1/20 of hepatocytes (22). One would interpret this to mean that the Kupffer cell concentration of hydrolases would be greater. This would follow only if the lysosomal content of the two cell types were equal. In fact, it is not. Kupffer cells contain about half of the total lysosomal volume of the liver (22). Thus, the lysosomal concentration of these enzymes is not likely to be greater in Kupffer cells. In order to investigate

if this distribution of lysosomal enzymes were a general phenomenon, we studied ten additional lysosomal acid hydrolases. The results are summarized in Table II. In every case, the enzymes are located predominantly in hepatocytes with 90% or more of the total enzyme found in this cell type. This is in contrast to an earlier interpretation which suggested an equal distribution of enzymes (23).

It has been suggested that acid hydrolases are concentrated in Kupffer cells (24). This seems appropriate since these cells are responsible for phagocytosis and the presumed catabolism of various macromolecules. Our results do not support this contention, but more simply that Kupffer cells have a larger portion of their cell volume occupied by lysosomes. Teleologically, this would serve a similar purpose. However, the location of most of the total enzymatic activities in hepatocytes may indicate a less primary role for Kupffer cells in the metabolism of macromolecules and implies a more complex situation. Several possibilities exist. Macromolecules phagocytosed by Kupffer cells may not be completely digested within these cells, but might be transfered to hepatocytes for further metabolism. The appearance of infused carcinoembryonic antigen first in Kupffer cells and later in hepatocytes suggests such a possibility (25). Furthermore, the observation of a lag in lipid metabolism and appearance of fat droplets in Kupffer cells some time after phagocytosis of cell membranes may indicate the participation of more than one cell in the catabolic process (26). An alternative hypothesis to explain the abundance of lysosomal enzymes in hepatocytes is that these lysosomal enzymes are synthesized in hepatocytes and excreted to Kupffer cells in the fashion of the secretion-reuptake hypothesis suggested by Neufeld for fibroblasts (27). The disclosure of the interrelationships between these cells is critical to an understanding of the dynamics of lysosomal storage of uncatabolized by-products of cell metabolism.

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