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Structural and Functional Integrity of Rat Liver Perfused in Backward and Forward Directions*

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Summary. The purpose of this study was to assess if reversal of the direction of isolated rat liver perfusion would cause significant alterations in hepatic functions and structure.

Five isolated rat livers were perfused forward and another five backward with oxygenated Ringer's solution for up to 90 min (hydrostatic pressure: ≤ 13 cm H_2O; flow rate: forward 3.88 ± 0.34 ml/min per gram and backward 3.76 ± 0.34 ml/min per gram). At the end of the experiment, livers were perfusion-fixed for morphological examination. The following results were obtained: No significant differences were noted between the forward and backward perfusions with respect to oxygen uptake, mean bile flow (forward 0.57 ± 0.12; backward 0.60 ± 0.14 ml/min per gram), average bile acid excretion (forward 2.39 ± 1.11; backward 2.83 ± 0.94 nmol/min per gram), hydroxylation pattern of bile acids, urea synthesis, release of lactic dehydrogenase, glucose secretion, and redox ratios. Light and electron microscopy, including morphometry of parenchymal and sinusoidal areas, revealed that the backward perfusion caused a greater degree of sinusoidal distension, but no other noteworthy differences. Hepatic ultrastructure was well preserved. We conclude that reversing the direction of perfusion does not alter structure and major hepatic functions significantly.

Key words: Isolated perfused rat liver – Backward perfusion – Hepatic functions – Hepatic morphology

Introduction

The isolated perfused rat liver is a well-established model in the study of hepatic function [8, 15, 17, 18, 24, 25, 30, 32, 35]. One unique potential of this model is

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that one can reverse the direction of perfusion and thereby reverse the relationship between the concentration gradient of oxygen and other substances in the perfusate and the hepatocytes along the length of the sinusoids. The reversed relationship between the acinar zones of the hepatic parenchyma and the intrasinusoidal concentration of perfused substances open the possibility to examine the functional heterogeneity of hepatocytes in different zones of the hepatic acinus which has been revealed by a number of studies [1, 3, 7, 9–13, 19, 31]. Usefulness of this experimental model has been shown recently with respect to the transport of taurocholate [9] and the metabolism of glutamine [13] and sulfobromophthalein [6]. If isolated livers perfused either backward (from the hepatic vein) or forward (from the portal vein) are used to examine the zonal heterogeneity of hepatic functions, it is essential to confirm that hepatic functions as a whole organ can be maintained without significant changes regardless of the direction of perfusion. Despite the considerable number of published studies using backward perfusion of the rat liver [2, 4, 16, 20, 29, 33, 34] no systematic comparison between backward- and forward-perfused livers has been made with respect to their functions and structural integrity. Therefore, we have compared several basic functions and morphology of the the livers perfused in a retrograde or forward direction.

Materials and Methods

Male Fisher 344 rats (body weight 240–300 g) fed a standard diet (Purina Rat Chow, St. Louis, MO, USA) were used. Chemicals of analytical grade were purchased from Mallinckrodt (Paris, KE), lactate, acetoacetate, and β-hydroxybutyrate from Sigma (St. Louis, MO, USA), pyruvate, NAD, NADH, NADP, and other biochemicals from Boehringer (Mannheim, FRG). Enzymes for the bioluminescence assays were partly obtained from Sigma (7α-hydroxysteroid dehydrogenase, 3α-hydroxysteroid dehydrogenase), and from Boehringer (bacterial diaphorase). The remaining enzymes, which were not commercially available, were supplied by Dr. Marlene DeLuca, Dep. of Chemistry, University of California, San Diego, CA (USA) (bacterial luciferase) [27] and by Dr. Ian A. MacDonald, Dep. of Medicine, Dalhousie University, Halifax, Canada (12α-hydroxysteroid dehydrogenase) [26].

All livers were perfused in situ with oxygenated Krebs Ringer bicarbonate buffer (KRB) (pH 7.4, 22°C) through the portal vein to clear the blood from the liver. A cannula was then introduced into the inferior vena cava to drain the effluent, and in situ perfusion was continued during surgical removal of the liver. The liver was then excised from the animal and placed in a plastic chamber in which it was perfused with KRB at 37°C without recirculation of the effluent. For the backward perfusion, the liver was perfused through the hepatic vein [14, 30]. The perfusate contained lactate (2.1 mmol/l), pyruvate (0.3 mmol/l), acetoacetate (0.08 mmol/l), β-hydroxybutyrate (0.1 mmol/l) and glucose (5 mmol/l). In some perfusions (three in each group) alanine (3 mmol/l) was added to the perfusate after 55 min of perfusion. The perfusion was continued for 90 min during which time bile was collected via a PE-50 polyethylene catheter placed in the common bile duct, and perfusate samples were obtained from the effluent at 10-min intervals. pH of the perfusate, pressure of perfusion, and inflow and outflow oxygen saturation were monitored continuously. Perfusate samples diluted 1:1 with HCO_4 (0.4 mol/l) were used to assess lactate, pyruvate, β-hydroxybutyrate, acetoacetate, and glucose concentrations using a fluorimetric method [22]. Undiluted samples were used to measure urea (test kit of Boehringer, Mannheim, FRG) and LDH activity (test kit, Sigma, St. Louis, MO, USA). Bile samples were weighed and then diluted (1:10–1:20). Bile acid concentrations were analyzed using bioluminescence assays for 3α-OH-, 7α-OH-, and 12α-OH-

bile acids which have been published recently [23, 26, 27]. Functional parameters of forward and retrograde perfusion groups were compared using the Student's *t*-test. After 90 min livers were fixed by perfusion with a mixture of 1.5% paraformaldehyde and 0.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) at a hydrostatic pressure of 13 cm H_2O. Fixed livers were cut into 100 µm thick blocks on a Sorvall TC2 tissue sectioner, washed in phosphate buffer, and postfixed in a mixture of 1% O_2OH and 1% potassium ferrocyanide in 0.1 M cacodylate buffer. Tissue blocks were then dehydrated and embedded in Epon for light and electron microscopy. Part of the tissue was embedded in JB-4 plastic for light microscopy. Sections for light microscopy were stained with toluidine blue and those for electron microscopy were doubly stained with uranyl acetate and lead citrate. The latter were examined in a Zeiss EM10 electron microscope. To estimate the effect of backward perfusion on the hepatic morphology, the area occupied by the sinusoids relative to the total tissue area in the acinar Zone 1 and Zone 3 was estimated by point counting using multiple square grids placed in a binocular microscope [21]. Zones 1 and 3 were defined as the parenchyma (including the sinusoids) within a distance of five hepatocytes from a portal triad or from a hepatic venule. Data were obtained from 20 areas each measuring 110 × 110 µm in Zones 1 and 3, respectively, in each of three forward- and three -backward perfused livers.

Results

The mean rat weight was 270.7 ± 30.1 g. Liver weight (forward: 9.62 ± 1.7 vs. retrograde: 9.73 ± 1.5) and the ratio of rat weight/liver weight (forward: 27.5 ± 3.6 vs. retrograde: 28.6 ± 3.9) were comparable between both groups. Functional perfusion parameters were not different significantly between the two groups (Table 1). Figure 1 shows urea synthesis and oxygen uptake during 90 min of perfusion. Addition of alanine to the perfusate at 55 min resulted in an increase in urea synthesis indicating that this function is well preserved. Figure 2 illustrates ratios of lactate/pyruvate and β-hydroxybutyrate/acetoacetate which were comparable between the two groups. Virtually no difference was seen between the two groups regarding mean bile flow, mean bile acid excretion, or rate of decrease in bile acid output with progression of time (Figs. 3, 4). The proportion of 7α-hydroxylated and 12α-hydroxylated bile acids in the total bile acids excreted were comparable in the two groups (Fig. 4) and they decreased with progression of time as described previously [28]. Release of LDH

Table 1. Perfusion parameters in forward and backward perfusions

Parameter	Forward	Backward
Average bile flow (µl/min/g)	0.57 ± 0.12 (4)	0.60 ± 0.14 (4)
Average bile acid excretion (nmol/min/g)	2.39 ± 1.11 (4)	2.83 ± 0.94 (4)
Flow rate (ml/min/g)	3.88 ± 0.34 (5)	3.76 ± 0.34 (5)
Oxygen consumption (µmol/min/g)	2.49 ± 0.09 (5)	2.45 ± 0.08 (5)
LDH release (µ/ml)	Not detectable	
Glucose release (µmol/g/min)	0.1–0.2	
Perfusion pressure (cm H_2O)	< 14	
Temperature (°C)	36–37	

Results are mean ± SD (number of rats studied in parentheses)

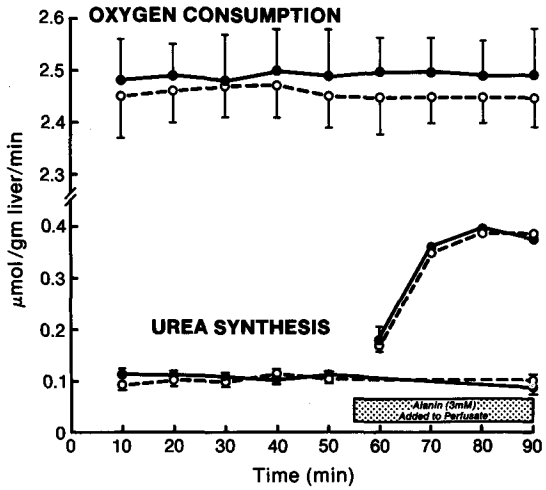

Fig. 1. Oxygen consumption ($n = 5$ for each group) and urea synthesis ($n = 3$ for each group). *Solid* lines (●———●) represent forward perfusion and *dashed* lines (○– – –○) represent backward perfusions. In experiments on urea synthesis addition of alanine at 55 min reveals well preserved synthetic capacity for urea

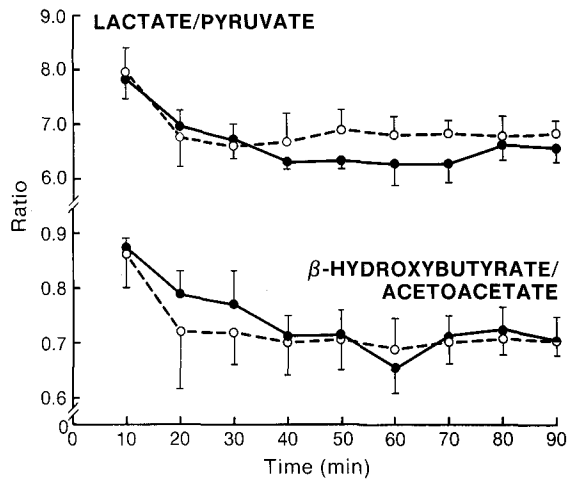

Fig. 2. Lactate/pyruvate β-hydroxybutyrate/acetotacetate ratio in forward (●———●) and backward (○– – –○) perfusions ($n = 5$ in each group)

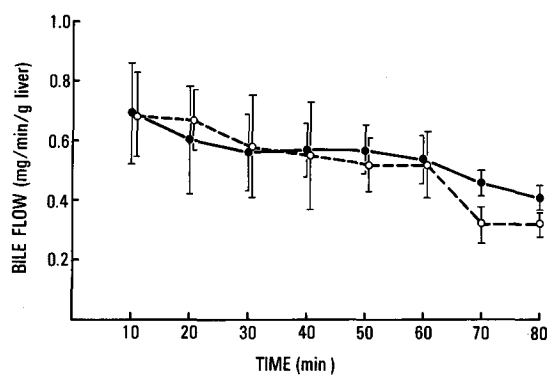

Fig. 3. Bile flow during forward (●———●) and backward (○– – –○) perfusions ($n = 5$ in each group)

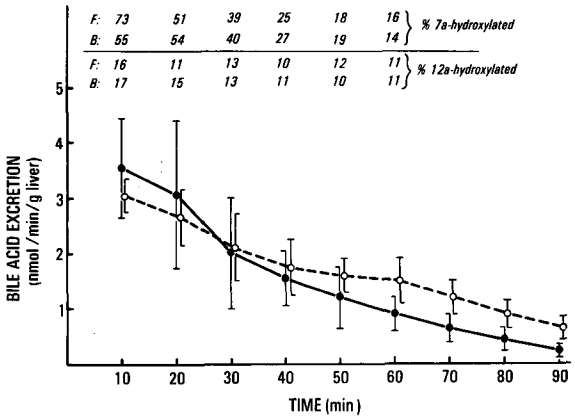

Fig. 4. Total bile acid excretion during forward (●———●) and backward (○– – –○) perfusions. 7α and 12α hydroxylated bile acids are expressed as % of total bile acids. *F* forward perfusion. *B* backward perfusion ($n = 4$ in each group)

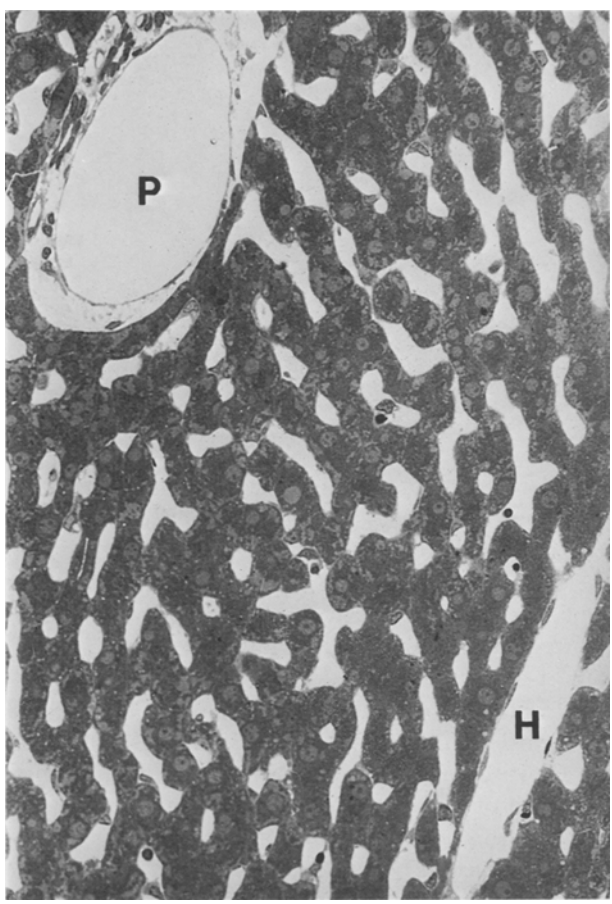

Fig. 5. Light-microscopic appearance of liver perfused in forward direction. Morphology of hepatic structure is well preserved except for slight distension of vascular channels. One-micrometer-thick section of Epon-embedded tissue stained with toluidine blue. *P* portal vein; *H* hepatic venule (× 362)

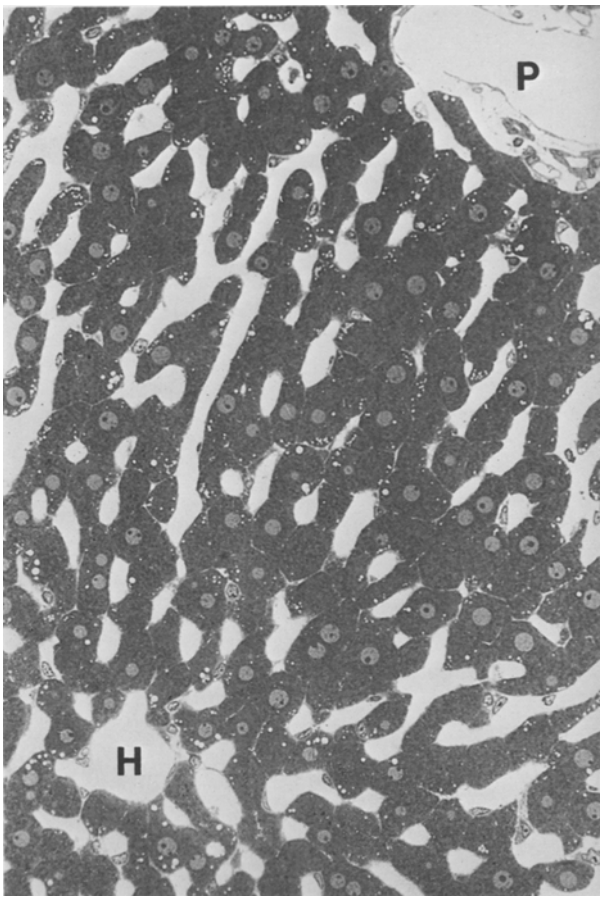

Fig. 6. Liver perfused in backward direction. Morphologically, it appears very similar to the one shown in Fig. 5 (× 435)

was not detectable throughout the course of perfusion. Thus, no significant metabolic differences were found between forward and backward perfusions.

Light microscopy revealed that perfused livers were morphologically well preserved and appeared indistinguishable from those perfusion-fixed in situ except for a greater degree of sinusoidal distension (Figs. 5, 6). Morphometry showed that the sinusoids comprised (mean ± SEM) 25.8 ± 1.6% in the acinar zone 1 and 29.2 ± 1.5% in zone 3 of the total tissue area in the forward perfusion, while it occupied 27.7 ± 3.4% in Zone 1 and 33.7 ± 5.5% in Zone 3 in the backward perfusion. There was no statistically significant difference between the forward and backward perfusions. Electron microscopy revealed good preservation of hepatic ultrastructure in which no noteworthy difference was seen between the backward and foreward perfusion. The endothelial lining was intact and space of Disse was not widened for the most part. In a few perivenular regions (Zone 3) of backward-perfused livers, sinusoids and space of Disse were more distended than elsewhere, but the endothelial lining was preserved. The endothelial cells and Kupffer cells showed no noteworthy alterations in their intracellular structure as compared with those in the in situ liver.

Discussion

Despite several early attempts at the use of backward liver perfusions for metabolic studies [2, 4, 16, 20, 29, 33, 34], none of them has established that hepatic structure and function in this model are comparable to those perfused forward. Although a recent study has demonstrated zonal differentiation of bile acid transport within the hepatic acinus [9], this study does not elaborate on the functional equivalence of livers perfused in opposite directions. Urea synthesis is the only function in the literature known to be equivalent in livers perfused in opposite directions [17, 24, 34]. The present study confirms the earlier observation and, in addition, shows that such basic functions as glucose secretion, oxygen consumption, redox ratios, release of LDH, bile acid excretion, and hydroxylation pattern of biliary bile acids are comparable between livers perfused in opposite directions. Although hepatic oxygen consumption during retrograde perfusion was found to be reduced in an earlier study [24], our results did not reveal any such alteration, and all the functional parameters were comparable to those of isolated livers perfused forward [8, 13, 14, 18, 24, 30, 35].

In this study, flow rate of perfusate was equivalent under the same hydrostatic pressure regardless of the direction of perfusion, indicating that the hepatic venous system is capable of accommodating the equivalence of perfusate flow.

These observations lead us to conclude that the backward perfusion of the liver is comparable to forward perfusion with respect to oxygen consumption, certain synthetic and secretory functions, and structural integrity. Both systems are equally sensitive to substrate addition. With respect to bile acid-related function, no difference was found concerning bile flow, bile acid exretion rate, and the hydroxylation pattern of biliary bile acids. Since a variety of functions are comparable between the two, this study confirms that the backward and forward perfusion models can be used for studies on interzonal difference in hepatocyte functions within the hepatic acinus. If any functional difference occurs upon the administration of a substance with a high uptake efficiency (hence, a steep concentration gradient is formed between the inflow and outflow points), it would not be attributable to the effect caused by changing the direction of perfusion. It would be attributable to the functional difference (whether it is intrinsic or due to sinusoidal concentration gradient) between Zone 1 and Zone 3 cells.

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