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The isolated perfused porcine liver: assessment of viability during and after six hours of perfusion

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Abstract Isolated liver perfusion was developed for the study of liver physiology and preservation. The recent development of new perfusion devices and appropriate liver preservation solutions prompted us to reconsider liver perfusion for the specific purpose of evaluating viability in terms of biochemical changes, paying special attention to modifications in the histological ultrastructure. Twenty-two isolated pig livers were perfused with autologous blood. Arterio-portal perfusions were carried out using an extracorporeal perfusion circuit with a hollow fibre membrane oxygenator. Four groups of pig livers were studied using three different liver flushing solutions [Ringer's lactate, ELOHES, and University of Wisconsin (UW)] and two different oxygenation modalities. Liver function tests and histological studies were done. Our results revealed that a high partial oxygen pressure (PO_2) level was deleterious to the ultrastructural elements of hepatocytes, in particular to the mitochondria. It was also associated with deficient metabolic performance, i.e., poor bile production and lack of aerobic metabolism. Normal blood gas values could be obtained with the use of air for liver oxygenation. Flushing of the liver with Ringer's lactate or a macromolecular solution such as ELOHES was associated with severe liver cell injuries, as reflected by a marked rise in liver enzymes and histological lesions. Satisfactory results were obtained when UW solution was used for liver harvesting. We conclude that an appropriate liver preservation solution, normal blood gas values, and normal physiological arterio-portal pressure and blood flow are essential for appropriate liver function with preservation of liver architecture and of hepatocyte ultrastructures. Total bilirubin in bile and Factor V are sensitive indicators of good liver function.

Key words Liver preservation, ex vivo perfusion · Preservation, ex vivo perfusion, liver · Ex vivo perfusion, liver, pig

Introduction

Isolated liver perfusion has been used to assess liver physiology [13] to treat patients with hepatic coma and for liver preservation prior to transplantation [2, 4, 5, 7]. In these studies, different flushing solutions and oxygenation modalities were reported. The aim of this present study was to compare liver function after 6 h of perfusion using three different liver flushing solutions and two oxygenation modalities.

Materials and methods

Twenty-two livers were procured from Landrace pigs weighing 25–30 kg under general anesthesia. Pre-anesthesia included an intramuscular injection of ketamina UVA1000, 4 mg/kg, midazolam, 5 mg/kg, and atropine, 0.5 mg. Induction was carried out by intravenous injection of propofol, 40 mg/kg, after which endotracheal intubation was achieved. After intubation, 5 mg of tracrium and 0.05 mg of fentanyl citrate were given intravenously. Anesthesia was maintained with a continuous intravenous injection of propofol, 190 mg/h. Heparin was given at a dose of 5000 IU.

Experimental groups

Four experimental groups were included in the study and defined by the type of solution used for liver flushing and by the composition of the gas used for liver oxygenation. In group 1 ($n = 6$), the liver flushing solution used was Ringer's lactate, and the composition of the gas was nitrogen 76 %, oxygen 19 %, and carbon dioxide 5 %. In group 2 ($n = 5$), the flushing solution was ELOHES 6 % (Hydroxyethylamidon) and the gas composition was also nitrogen 76 %, oxygen 19 %, and carbon dioxide 5 %. In group 3 ($n = 6$), Ringer's lactate was used and the gas composition was oxygen 95 % and carbon dioxide 5 %. In group 4 ($n = 5$), University of Wisconsin (UW) solution was used to flush the liver and the composition of the gas was nitrogen 76 %, oxygen 19 %, and carbon dioxide 5 %. Perfusion time was 6 h in all groups.

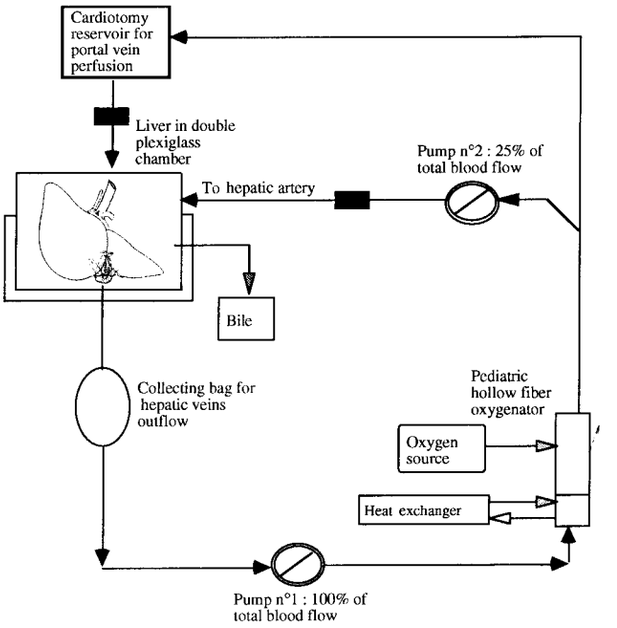

Fig. 1 Ex vivo perfusion circuit (■ pressure control)

Hepatectomy

An aseptic technique was used throughout. The peritoneal cavity was entered by means of a long midline incision. Liver harvesting was performed after dissection of the portal vein down to the superior mesenteric vein. The lesser omentum was opened and the right gastric vessels ligated. The common bile duct was sectioned and cannulated after ligation of its distal part just above the pancreas. This allowed exposure of the gastroduodenal artery, which was ligated and sectioned. Complete dissection of the celiac trunk was achieved, after successive ligation of the left gastric and splenic arteries, down to the aorta after securing pancreatic and diaphragmatic branches [3].

The infrarenal aorta and the portal vein were then cannulated. The superior mesenteric artery and vein and the splenic vein were then ligated. The liver was flushed through the portal vein using 500 ml of either Ringer's lactate in groups 1 and 3 or ELHOES 6 % in group 2 (Laboratoire Biosedra, Louviers, France) at 4 °C. At the same time, autologous blood was collected through the aortic cannula into a cardiotomy reservoir to which another 5000 IU of heparin was added. In group 4, 1 l UW solution was used to flush the liver through the portal and aortic cannulas after ligation of the celiac aorta. In this group, blood for the perfusate was taken from another pig under the same anesthetic conditions to avoid hyperkalemia related to UW perfusion. A total volume of 1200–1500 ml of blood was thus collected. The liver was then removed en bloc with an aortic segment for the arterial cannula, and the suprahepatic vena cava was removed until the right atrium.

While the liver was kept at 4 °C, the cystic duct was ligated on the back table and the gall bladder washed and cannulated. A 16 F Bard cannula was secured in the aortic segment for hepatic artery perfusion, and arterial branches of this aortic segment were also secured. A 24 F portal cannula was then secured, and a 28 F Bard cannula was also secured in the retro-hepatic inferior vena cava through its proximal portion for hepatic outflow. The supra-hepatic vena cava was then ligated. The liver was subsequently weighed. In group 4, the UW solution was washed out just prior to perfusion.

The perfusion circuit

The perfusion circuit (Fig. 1) included a cardiotomy reservoir for portal perfusion and a hollow fibre membrane pediatric oxygenator with a heat exchanger (Dideco, Mirandola, Italy). Two roller pumps (Cobe) were used. The first one pumped blood through the oxygenator and supplied the total blood flow rate to the circuit, which was set to 1 ml/g liver mass/min. The second pump was used for arterial perfusion at a rate of 0.25 ml/g liver mass/min. The liver was suspended in a plastic bag and the portal vein and aortic cannula were connected. Venous drainage was carried out by gravity through the cannula placed in the retrohepatic vena cava. This cannula was connected to a collecting bag from which the blood was pumped once again to the oxygenator through the first pump. As the blood entered the oxygenator, it was heated by a heat exchanger to 38 °C. Oxygenated blood at the exit of the oxygenator was then directed through a Y joint to the arterial pump on one side and to the cardiotomy reservoir on the other side. The portal pressure was adjusted to maintain an equilibrium between the hepatic blood inflow and outflow, according to the predefined pump rates.

Analytical studies

Blood samples were taken from the pigs before liver harvesting and from the perfusate prior to perfusion, 5 min after the beginning of perfusion, and then hourly for 6 h. Serum electrolytes, glucose, urea, and white blood cells and platelets were measured. Liver function tests included aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), and total serum bilirubin (conjugated and unconjugated). Bile flow was measured throughout the procedure and

Table 1 Changes in electrolytes during isolated liver perfusion (*ND* not done)

	Group	Pig	Perfusate	5 min	1 h	2 h	3 h	4 h	5 h	6 h
Osmolality	1	ND	ND	379 ± 21	389 ± 21	394 ± 21	406 ± 29	434 ± 48	417 ± 49	436 ± 56
(mOsm/kg)	2	296 ± 17	722 ± 321	441 ± 30	432 ± 34	430 ± 52	437 ± 65	447 ± 60	452 ± 61	458 ± 52
	3	ND	ND	ND	ND	ND	ND	ND	ND	ND
	4	303 ± 26	296 ± 20	342 ± 85	302 ± 6	311 ± 17	320 ± 17	315 ± 10	325 ± 15	326 ± 6
Potassium	1	4.2 ± 1	5.9 ± 0	4.3 ± 1	4.5 ± 2	4.3 ± 2	3.6 ± 2	3.6 ± 2	4.3 ± 3	4.9 ± 3
(mmol/l)	2	3.4 ± 0	7.6 ± 4	6.1 ± 2	6.8 ± 3	6.8 ± 3	7.8 ± 3	10.2 ± 5	10.5 ± 5	12.9 ± 6
	3	4.4 ± 2	ND	4.7 ± 1	5.9 ± 3	6.4 ± 3	6.3 ± 5	7.1 ± 5	7.7 ± 5	7.6 ± 7
	4	3.8 ± 0	10.2 ± 9	6.3 ± 3	5.7 ± 1	6.4 ± 2	6.6 ± 2	5.9 ± 1	6.1 ± 1	6.3 ± 2
Bicarbonate	1	24 ± 4	ND	10 ± 5	9 ± 7	4 ± 3	5 ± 5	8 ± 6	9 ± 8	9 ±12
(mmol/l)	2	21 ± 5	13 ± 6	13 ± 8	11 ± 9	13 ± 10	17 ± 11	14 ± 11	13 ± 9	14 ± 10
	3	23 ± 5	ND	8 ± 9	7 ± 5	7 ± 6	5 ± 6	8 ± 7	6 ± 5	6 ± 5
	4	25 ± 3	22 ± 9	20 ± 4	20 ± 6	19 ± 7	18 ± 7	18 ± 6	16 ± 6	16 ± 5
Protein	1	56 ± 5	ND	27 ± 8	33 ± 10	30 ± 7	28 ± 7	28 ± 7	28 ± 6	33 ± 10
(g/l)	2	52 ± 5	49 ± 28	43 ± 30	27 ± 4	25 ± 5	26 ± 5	26 ± 4	27 ± 6	30 ± 5
	3	45 ± 17	ND	23 ± 6	24 ± 5	25 ± 5	27 ± 4	27 ± 5	28 ± 6	29 ± 6
	4	53 ± 2	53 ± 6	49 ± 2	48 ± 2	49 ± 2	50 ± 1	50 ± 2	51 ± 1	51 ± 1
Glucose	1	9 ± 4	ND	42 ± 17	41 ± 19	39 ± 20	38 ± 20	38 ± 22	39 ± 22	40 ± 23
(mmol/l)	2	8 ± 2	11 ± 5	23 ± 17	23 ± 17	22 ± 17	16 ± 11	22 ± 17	22 ± 16	23 ± 15
	3	11 ± 3	ND	51 ± 12	50 ± 16	46 ± 11	43 ± 8	44 ± 10	46 ± 13	44 ± 13
	4	10 ± 3	10 ± 4	20 ± 3	30 ± 6	23 ± 3	17 ± 1	15 ± 2	15 ± 4	16 ± 5

total bile bilirubin content was measured. Venous and arterial blood samples were taken for blood gas analysis and measurement of oxygen consumption. Liver weight was recorded before and after perfusion. Results were reported as mean ± standard deviation. Statistical analysis was done using Student's *t*-test to detect significant differences between the control results (pig value before liver harvesting) and those after 6 h of perfusion. They were considered significant when *P* was below 0.05. For liver enzymes, values were reported as enzyme enrichment/100 g liver mass/l. Oxygen consumption was calculated as follows: (A sat O_2 – V sat O_2) × Hgb × 1.3 × (Portal blood flow + Arterial blood flow)/liver weight, where A sat O_2 represents arterial oxygen saturation (%), V sat O_2 is venous oxygen saturation (%), Hgb is hemoglobin (g/dl), and blood flow is given as ml/g liver mass/min and liver weight expressed in grams.

Histological study

Liver biopsies were taken before harvesting, before perfusion, after 5 min of perfusion, and then hourly for light and electron microscopic study. For light microscopy, the liver biopsies were fixed in Bouin's liquid, embedded in paraffin, and stained in 3-μ sections with hematoxylin-phloxin-saffron. For electron microscopy, 1 mm^3 liver pieces were fixed in 1 % OsO_4 0.15 M NaCacodylate/HCl, pH = 7.4, for 1 h at 4 °C, dehydrated in graded ethanols, and embedded in epoxy resin. Semi-thin sections (1 *v*) were stained with methylene blue – azur II in order to allow lobular topography recognition of the lesion sites. Ultra-thin sections (80 nm) were contrasted with methanolic uranyl acetate and lead citrate and observed with a CM120 Philips electron microscope.

Results

Electrolyte balance

Table 1 shows the effects of perfusion on the electrolyte balance.

Potassium

All groups showed hyperkalemia, which reached its highest values in group 2. The rise in the potassium level was significant for group 3 ($P = 0.021$) and group 2 ($P = 0.01$). *P* values for groups 1 and 4 were 0.58 and 0.059, respectively.

Sodium

Hyponatremia was noted in group 2. The other groups showed hypernatremia that was related to correction of acidosis with sodium bicarbonate.

Chloride and calcium

Chloride and calcium levels were normal during the 6 h of perfusion.

Bicarbonate

All but group 4 showed a marked fall in bicarbonate concentration after the start of perfusion. Bicarbonate loss was statistically significant for group 1 ($P = 0.014$) and group 3 ($P = 0.000$). *P* values for groups 2 and 4 were 0.114 and 0.06, respectively.

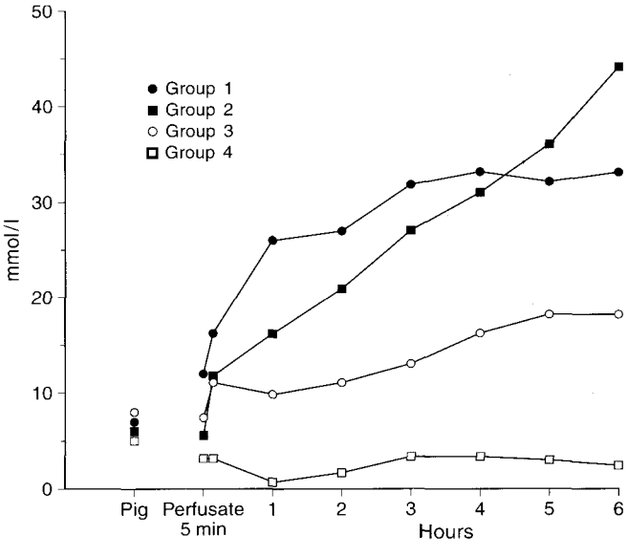

Fig. 2 Lactate level during liver perfusion

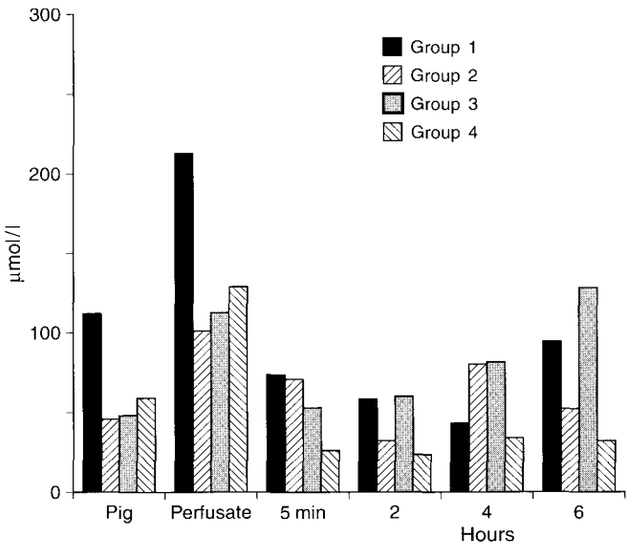

Fig. 3 Blood ammonia level

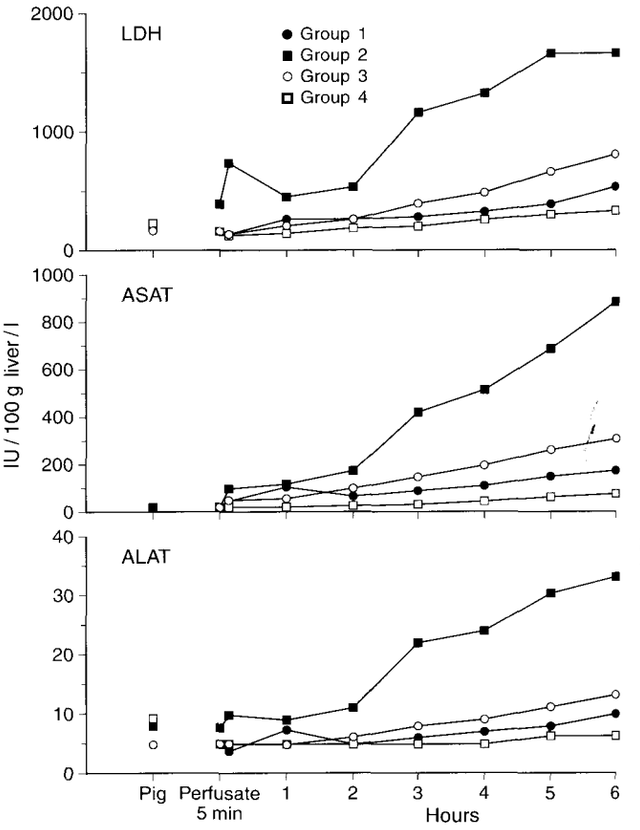

Fig. 4 Changes in the liver enzymes ASAT, ALAT, and LDH levels after liver perfusion

Protein

Minimal variation in protein level was recorded during perfusion.

Glucose

Hyperglycemia was present in all perfusions. It started at the beginning of perfusion and was associated with a fall in liver glycogen content, as seen in electron microscopy. Only group 4 showed a decrease in the glucose level after the 3rd h of perfusion. The rise in perfusate glucose level was significant in group 1 ($P = 0.009$) and group 3 ($P = 0.000$). P values for groups 2 and 4 were 0.094 and 0.117, respectively.

Lactate

All but group 4 showed a marked rise in lactate level (Fig. 2). After 6 h of perfusion, the lactate level (mmol/l) was: group 1, 33.3 ± 7; group 2, 43.6 ± 13; group 3, 17.6 ± 4, and group 4, 2.3 ± 1. The change in perfusate lactate level was significant for group 1 ($P = 0.000$) and group 2 ($P = 0.002$). For groups 3 and 4, the P levels were 0.266 and 0.062, respectively.

Ammonia

The serum ammonia level showed no significant variations during perfusion. After 6 h of perfusion, its highest level was noted in group 3 and its lowest level in group 4 (Fig. 3).

Table 2 Serum bilirubin level (*ND* not done)

	Group	Pig	Perfusate	5 min	1 h	2 h	3 h	4 h	5 h	6 h
Serum bilirubin (μmol)	1	10 ± 6	ND	11 ± 11	31 ± 44	23 ± 24	13 ± 8	13 ± 7	14 ± 7	33 ± 47
	2	7 ± 2	59 ± 90	67 ± 106	19 ± 8	17 ± 7	17 ± 7	17 ± 6	17 ± 4	19 ± 7
	3	4 ± 1	ND	3 ± 2	4 ± 2	6 ± 5	11 ± 12	14 ± 17	17 ± 19	20 ± 19
	4	7 ± 4	6 ± 5	5 ± 2	7 ± 3	8 ± 3	9 ± 4	11 ± 4	12 ± 4	12 ± 4

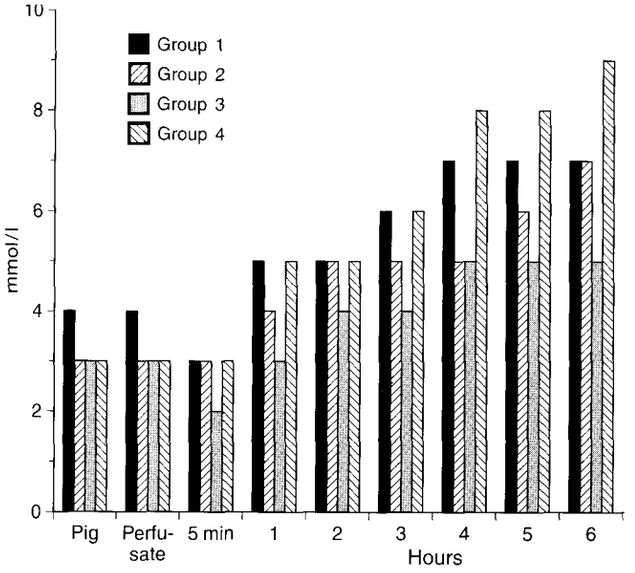

Fig. 5 Urea level during liver perfusion

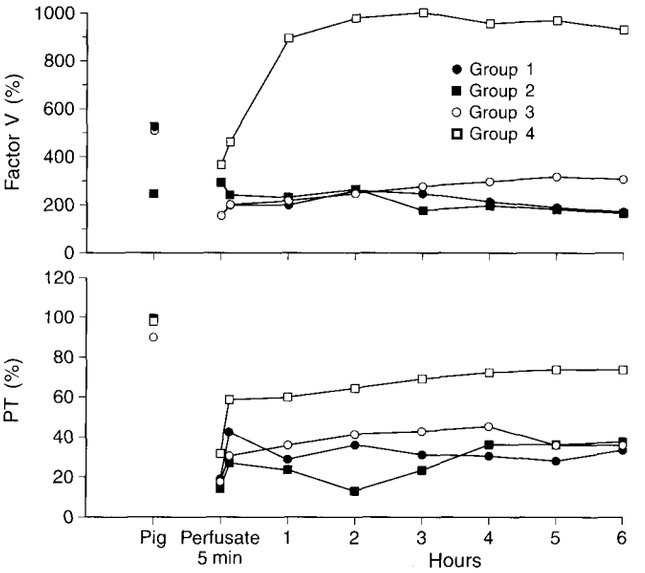

Fig. 6 Prothrombin time and Factor V levels

Liver function tests

Liver function tests and enzyme enrichment in the perfusate during the 6 h of perfusion are shown in Fig. 4 and Table 2. All but group 4 showed a marked rise in LDH and ASAT levels. After 6 h of perfusion, ASAT reached the following levels (in IU/100 g liver mass/l): group 1, 165 ± 117; group 2, 876 ± 609; group 3, 312 ± 268; group 4, 76 ± 82. The rise in ASAT was significant in all but group 4 (group 1, $P = 0.008$; group 2, $P = 0.01$; group 3, $P = 0.009$; group 4, $P = 0.184$).

The rise in ALAT and AP was moderate in all groups and was lowest in group 4. The values of ALAT (IU/100 g liver mass/l) after 6 h of perfusion were: group 1, 10 ± 6; group 2, 33 ± 22; group 3, 13 ± 8; and group 4, 6 ± 1. The highest level for enzyme production was in group 2. A significant rise in ALAT was recorded for group 2 ($P = 0.018$) and group 3 ($P = 0.032$); in groups 1 and 4, P values were 0.075 and 0.184, respectively.

The urea level increased steadily and was highest in group 4 (Fig. 5). This rise was not significant in group 4 (P values were: group 1, 0.013; group 2, 0.016; group 3, 0.011; group 4, 0.197). PT reached the following values after 6 h of perfusion (%): group 1, 34 ± 20; group 2, 39 ± 13; group 3, 37 ± 17; group 4, 74 ± 32. The changes in PT were not significant in group 4 ($p = 0.261$), in all other groups a significant fall of PT was observed with a P value of 0.000. The mean value (± SD) of the pig Factor V (%) in our experiments (measured before liver harvesting) was 431 ± 293. After 6 h of perfusion, it reached 345 ± 431 in group 1, 113 ± 95 in group 2, 337 ± 130 in group 3, and 930 ± 693 in group 4. Changes in Factor V were not significant in all groups $P = 0.486$ in group 1, 0.632 in group 2, 0.181 in group 3, and 0.352 in group 4. The highest level for PT and Factor V were recorded in group 4 (Fig. 6).

The serum bilirubin level was normal in only group 4; all of the other groups showed hyperbilirubinemia. Changes in total bilirubin level were only significant for group 2 ($P = 0.006$). For the other groups, the P value was: group 1, 0.266; group 3, 0.071, and group 4, 0.158. The mean (± SD) bile production after 6 h of perfusion was: group 1, 3 ± 3; group 2, 2 ± 2; group 3, 61 ± 14; and group 4, 3 ± 3 ml/h. Figure 7 shows the bile production at every hour of the perfusion. Total bilirubin in bile (μmol/l) was measured in the bile collected from the gall bladder prior to liver harvesting; its level was 345 ± 219. During perfusion, bile was collected

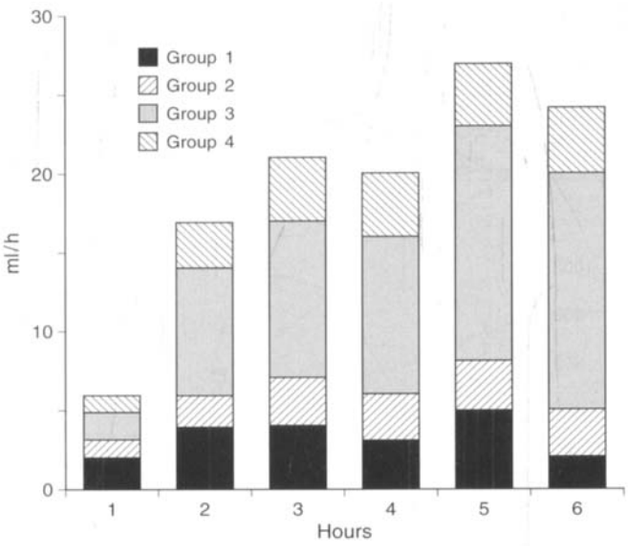

Fig. 7 Bile flow

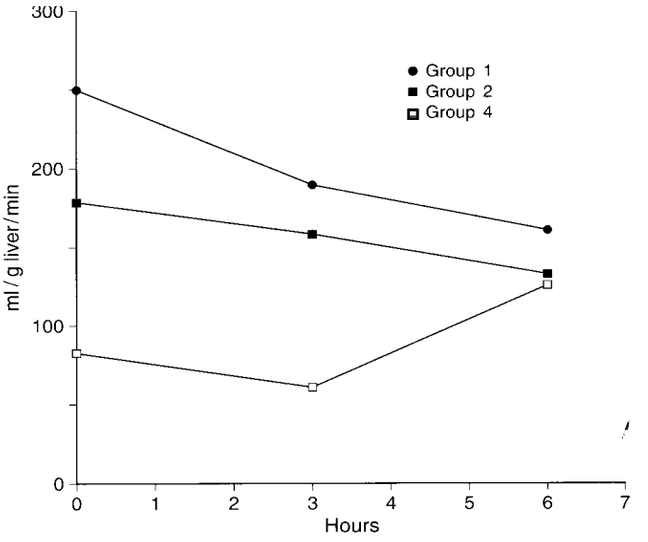

Fig. 9 Oxygen consumption

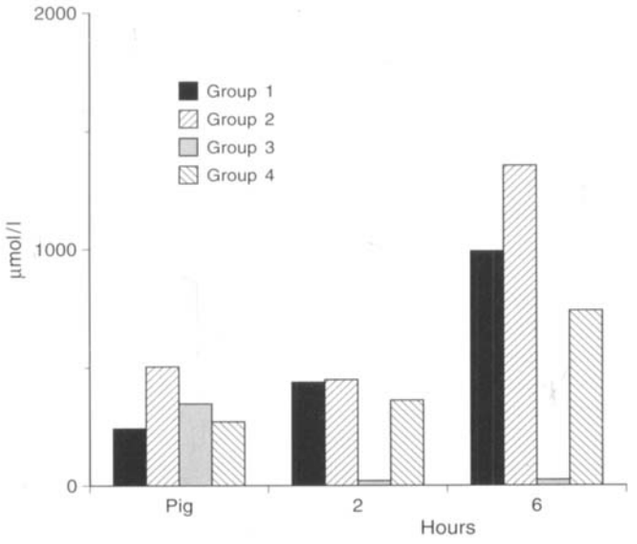

Fig. 8 Total bilirubin content in bile

hourly; total bilirubin is presented in Fig. 8. After 6 h of perfusion, it reached the following levels: group 1, 1114 ± 350; group 2, 1347 ± 514; group 3, 16 ± 2; and group 4, 733 ± 334. Oxygen consumption was similar in all perfusions (Fig. 9).

Hemodynamic study

Hemodynamic aspects of liver perfusion are reported in Table 3. The mean portal blood pressure varied from 11 ± 2 to 20 ± 5 cm H_2O; higher portal pressure was recorded for group 4. The mean arterial line pressure was 37 ± 18–95 ± 78 mm Hg. The variation in arterial line pressure was correlated to the mean arterial blood flow. Weight gain at the end of perfusion was: group 1, 132 ± 43 g; group 2, 170 ± 12 g; group 3, 188 ± 53 g; group 4, –78 ± 7 g.

White blood cells and platelets

Depletion of white blood cells (WBC) and platelets was noted in all perfusions. The rate of depletion of both WBCs and platelets was rapid and identical in all perfusions (Fig. 10).

Histological study

Group 1 (Ringer's lactate, N_2 76 %, O_2 19 %, CO_2 5 %)

Scattered hepatocyte single cell necrosis and increasing Kupffer cell hypertrophy with lysosomal overload were constant features from the beginning of perfusion, indicating the exacerbated renewal of isolated hepatocytes. Nevertheless, the lobular architecture was well preserved until 4 h of perfusion, when mediolobular sinusoid dilatation occurred, leading to mediolobular parenchymal necrosis after 6 h of perfusion (Fig. 11 a). Sinusoidal dilatation coincided with ultrastructural hepatocytic changes in the smooth endoplasmic reticulum, which appeared segregated in the cytoplasm with a microvesicular or macrovacuolar configuration without disturbing the structures involved in the biliovascular hepatocytic polarity, i.e., the biliary canaliculi and

Table 3 Hemodynamic aspects of isolated liver perfusion in groups 1, 2, and 4 [*PP* portal pressure (cm H_2O), *ABF* arterial blood flow (ml/g liver mass/min), *MABP* mean arterial blood pressure (mm Hg), *TBF* total blood flow (ml/g mass/min)]

	Group	5 min	1 h	2 h	3 h	4 h	5 h	6 h
PP	1	13 ± 3	13 ± 4	12 ± 4	13 ± 4	14 ± 5	13 ± 5	13 ± 5
cm H_2O	2	11 ± 2	12 ± 3	13 ± 3	14 ± 3	13 ± 2	13 ± 1	12 ± 1
	4	16 ± 7	18 ± 5	17 ± 4	17 ± 3	19 ± 2	19 ± 4	20 ± 5
ABF	1	0.245 ± 0.03	0.243 ± 0.03	0.239 ± 0.03	0.223 ± 0.04	0.236 ± 0.04	0.248 ± 0.06	0.244 ± 0.06
ml/g per min	2	0.212 ± 0.5	0.238 ± 0.04	0.284 ± 0.11	0.288 ± 0.12	0.285 ± 0.13	0.285 ± 0.13	0.23 ± 0.04
	4	0.138 ± 0.01	0.197 ± 0.06	0.198 ± 0.06	0.198 ± 0.06	0.173 ± 0.06	0.173 ± 0.06	0.178 ± 0.05
MABP	1	81 ± 28	92 ± 21	81 ± 28	78 ± 22	78 ± 26	77 ± 21	78 ± 21
mm Hg	2	74 ± 39	49 ± 9	64 ± 13	74 ± 28	119 ± 101	99 ± 71	94 ± 67
	4	72 ± 61	53 ± 23	51 ± 27	52 ± 32	45 ± 20	46 ± 19	64 ± 37
TBF	1	0.971 ± 0.07	0.981 ± 0.06	0.909 ± 0.17	0.901 ± 0.15	0.879 ± 0.19	0.885 ± 0.18	0.922 ± 0.23
ml/g per min	2	0.859 ± 0.16	0.925 ± 0.09	0.943 ± 0.09	0.943 ± 0.09	0.943 ± 0.09	0.943 ± 0.09	0.93 ± 0.11
	4	0.896 ± 0.13	0.973 ± 0	0.973 ± 0	0.969 ± 0.01	0.969 ± 0.01	0.974 ± 0.01	0.974 ± 0.01

the membrane junctional complexes or the microvilli in Disse's spaces. The endothelial lining continuity was generally maintained in spite of sinusoid enlargement, but some focal endothelium gaps appeared filled with isolated platelets, and stellate cell processes were seen consolidating the sinusoidal wall. Later, after 6 h of perfusion, while the periportal parenchyma showed remarkable integrity (Fig. 11 b), diffuse mediolobular endothelial destruction allowed red blood cell extravasation in the widened Disse's spaces, and the adjacent hepatocytes appeared degenerative or necrotic (Fig. 11 c).

Group 2 (ELOHES 6 %, N_2 76 %, O_2 19 %, CO_2 5 %)

The topographical and chronological pattern of liver architecture preservation was similar to that in group 1. The mediolobular tropism of the lesions was histologically detectable after 1 h of perfusion at the microvacuolar aspect of the hepatocytes. The lesions, consisting of progressive mediolobular sinusoidal dilatation, were delayed after 4 h of perfusion and led to zonal destruction of the hepatocyte trabeculae after 6 h of perfusion (Fig. 12 a). The periportal and centrolobular parenchyma remained the same after 6 h of perfusion (Fig. 12 b). At the ultrastructural level, the earliest hepatocytic change involved the rough endoplasmic reticulum (RER), which showed dilated and vacuolar cisternae. Extending cytoplasmic alteration was indicated by mitochondrial swelling, peroxisomal overload, and loss of cytoplasmic electron density, which might have led to single cell hepatocyte balloonizing necrosis in mediolobular areas where sinusoid dilatation took place (Fig. 12 c). At 1 h in the periportal and centrolobular areas, the endothelial sinusoid lining remained continuous and appeared strengthened by stellate cell processes developed in Disses's spaces, while the sinusoidal lumen was narrowed by hypertrophic Kupffer cells. In the mediolobular areas, the sinusoidal widening was coincident with focal endothelial discontinuity that did not compromise the hepatocyte trabecular architecture until after 4 h of perfusion (Fig. 12 c).

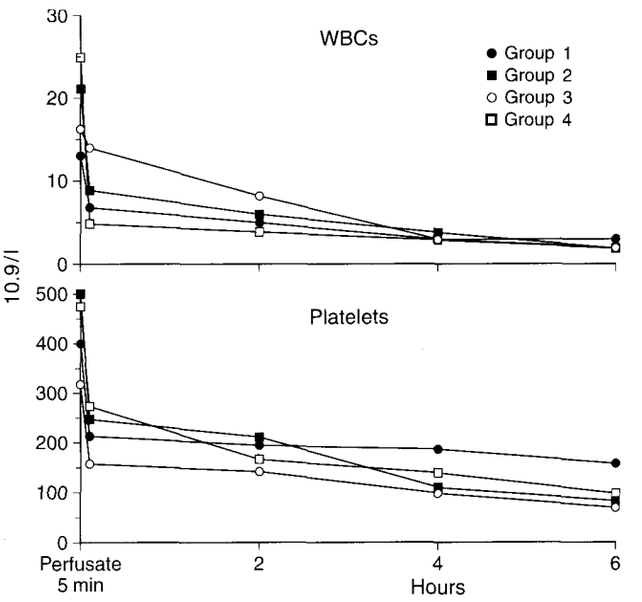

Fig. 10 White blood cell and platelet depletion during perfusion

Group 3 (Ringer's lactate, O_2 95 %, CO_2 5 %)

Long-term blood perfusion in such gasometric conditions was highly damaging: severe, congestive sinusoidal dilatation with hepatocytic trabeculae compression was diffuse in the whole lobule after 3 h of perfusion (Fig. 13 a). At that time, extravasation of red blood cells and platelets in the widened Disses's spaces was common and hepatocytic lesions were prominent, ranging

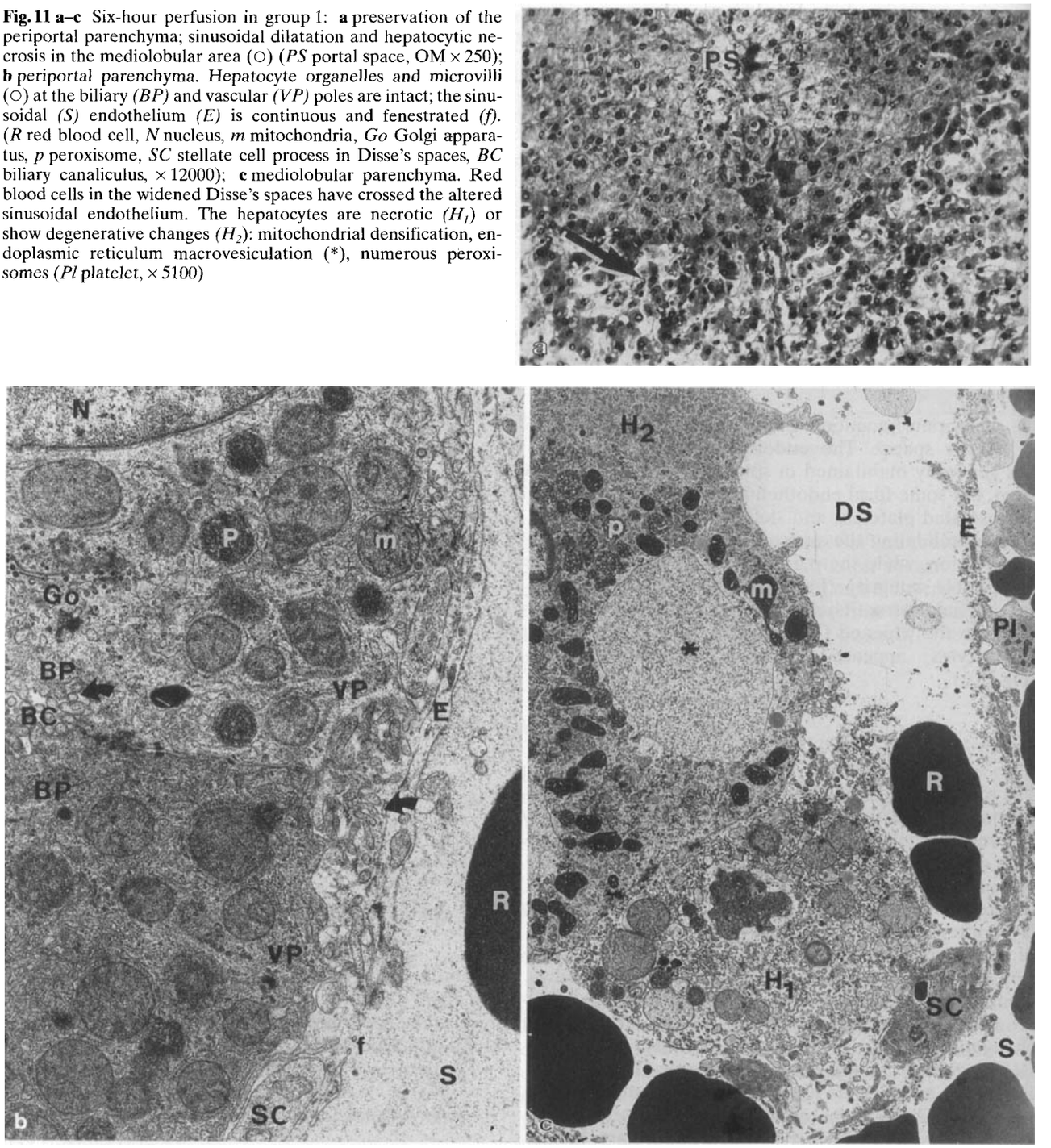

Fig. 11 a–c Six-hour perfusion in group 1: **a** preservation of the periportal parenchyma; sinusoidal dilatation and hepatocytic necrosis in the mediolobular area (○) (*PS* portal space, OM × 250); **b** periportal parenchyma. Hepatocyte organelles and microvilli (○) at the biliary *(BP)* and vascular *(VP)* poles are intact; the sinusoidal *(S)* endothelium *(E)* is continuous and fenestrated *(f)*. (*R* red blood cell, *N* nucleus, *m* mitochondria, *Go* Golgi apparatus, *p* peroxisome, *SC* stellate cell process in Disse's spaces, *BC* biliary canaliculus, × 12000); **c** mediolobular parenchyma. Red blood cells in the widened Disse's spaces have crossed the altered sinusoidal endothelium. The hepatocytes are necrotic *(H_1)* or show degenerative changes *(H_2)*: mitochondrial densification, endoplasmic reticulum macrovesiculation (*), numerous peroxisomes (*Pl* platelet, × 5100)

from abrasion of the vascular pole with loss of the microvilli and clasmatosis, micro- and macrovesicular cytomembrane segregation, and mitochondrial alteration (Fig. 13b) to end-stage balloonizing cell necrosis (Fig. 13c) among red blood cell influx.

Group 4 (UW solution, N_2 76 %, O_2 19 %, CO_2 5 %)

Long-term (6 h) integrity of the liver architecture was preserved after flushing with UW solution (Fig. 14a, b). After 1 h of perfusion, the hepatocyte ultrastructure

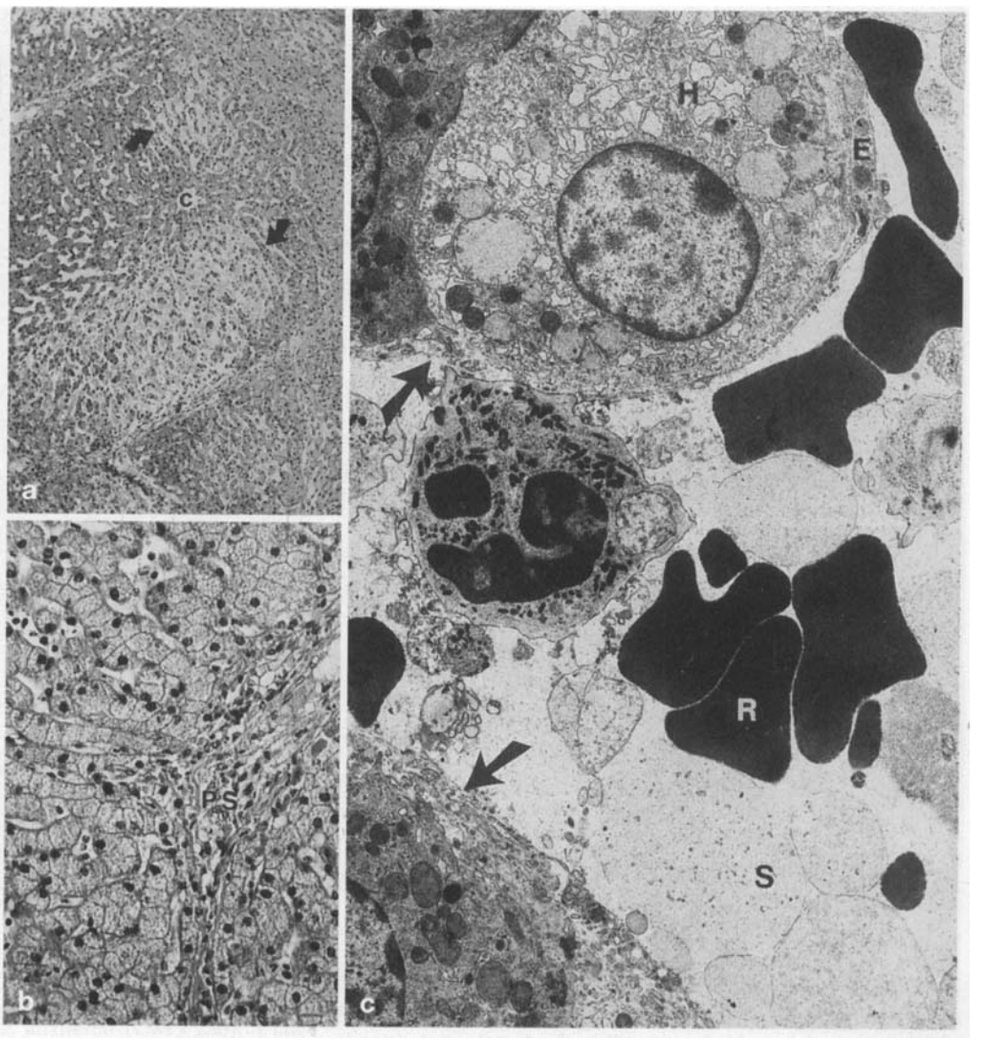

Fig. 12a,b Six-hour and **c** 4-h perfusion in group 2: **a** mediolobular tropism of the lesions (○) (**c** centrolobular vein, OM × 100); **b** integrity of the periportal parenchyma (*PS* portal space, OM × 400); **c** mediolobular area. A balloonized necrotic hepatocyte *(H)* is protruding in the widened sinusoid *(S)* bordered by a discontinuous (○) endothelium *(E)* (*R* red blood cell, × 4600)

was normal all over the lobule: the cytoplasmic membranes of adjacent hepatocytes were intimately joined, the RER formed narrow cisternae encircling the mitochondria, and glycogen B particles were regularly distributed among the smooth endoplasmic reticulum. At the vascular pole, numerous microvilli were projected into Disse's spaces where discrete stellate cell processes spread. Some lipopigments surrounded the biliary canaliculi, the sinusoidal lumen was normal in size in relation to the red blood cell diameter, the endothelial sinusoidal lining was regular and continuous, and Kupffer cell hypertrophy was moderate. Afte 6 h of perfusion, mediolobular lesions could be detected at the ultrastructural level. Focal discontinuities along the endothelial sinusoidal lining were frequently filled with platelets. Edema widened the subjacent Disse's spaces and disjoined adjacent hepatocytes since the corresponding hepatocyte vascular pole showed few microvilli and clasmatotic process. Macrovacuolar change in the endoplasmic reticulum was obvious and glycogen load was scarce, but a major part of the RER cisternae remained normally distributed among the unaltered mitochondria (Fig. 14c).

Discussion

Evaluation of the viability of an isolated perfused liver is difficult. Three parameters are easily measured and were proposed for this purpose: blood flow, oxygen consumption, and bile flow. Blood flow was measured for groups 1, 2, and 4 and showed that group 4 had stable blood flow near the physiological value (1 ml/g liver mass/min). This was achieved by a higher portal pressure than in groups 1 and 2. Oxygen consumption is a property of living cells. Only group 4 showed an increase in oxygen utilization up to the 6th h of perfusion;

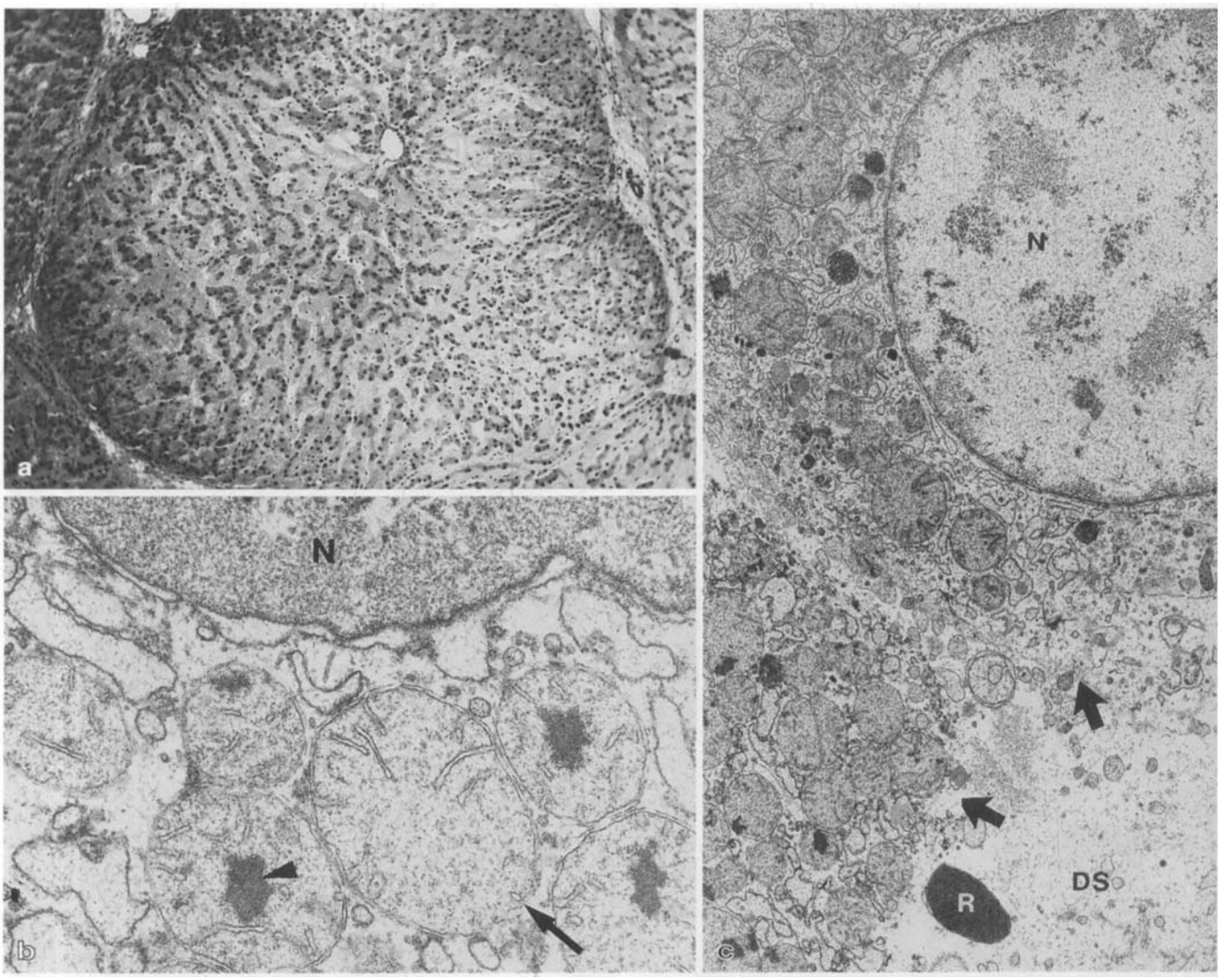

Fig. 13a–c Six-hour perfusion in group 3: **a** diffuse lobular sinusoidal dilatation with hepatocytic trabeculae necrosis (OM × 100); **b** mitochondrial alteration: membrane rupture (○) with matrix clarifying and protein condensation (▶) (× 29000); **c** ballooning hepatocyte necrosis (× 11000)

the decrease in oxygen consumption in groups 1 and 2 could be related to the lowered metabolic function of the liver in this closed circuit rather than to lesser viability of liver cells. Nevertheless, oxygen consumption was a poor discriminator of liver injury, which is in concordance with some research [13] but not with others [10].

Bile production is an indicator of the metabolic performance of the liver. Only functioning liver cells can produce bile with a normal bilirubin concentration. It appears to be the easiest parameter to study and it gives consistent information about liver function in the isolated, perfused liver model [7, 14, 15]. Unlike in the isolated perfused rat liver [9], satisfactory bile flow in the pig liver can be obtained without the infusion of bile salts. Bile production was quite similar in all but group 3, which showed a higher bile flow. However, this group 3 "bile" was very poor in total bilirubin content. This finding could be correlated to the intracellular injuries, mainly mitochondrial, related to the high partial oxygen pressure level in this group. A correlation between oxygen consumption an bile production was found by Abouna et al. [1]. Others showed that a decrease in bile production is related to the degree of liver damage [4]. Our results show that the total bilirubin level in bile is a sensitive indicator of good liver function.

A liver enzyme study revealed that group 2 exhibited the highest rise in ASAT, ALAT, and LDH, and this was attributed to the use of ELOHES for liver harvesting. Group 4 exhibited the lowest increase in enzyme level and had a normal PT. LDH and ASAT levels are more sensitive indicators of cell injuries, as the rise in ALAT

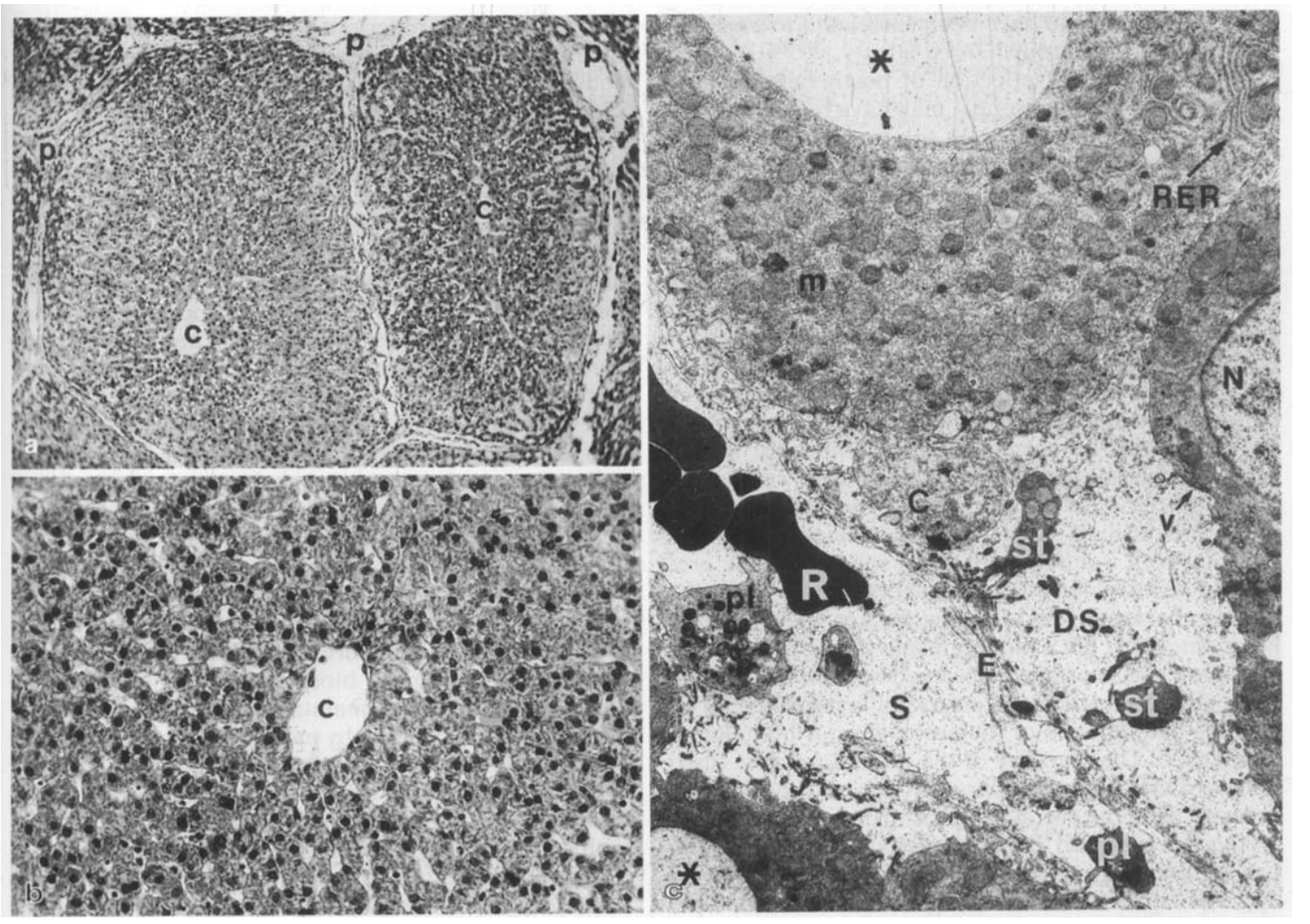

Fig. 14 a–c Six-hour perfusion in group 4: **a** preservation of the lobular architecture (OM × 100); **b** The relative distribution of the hepatocyte trabeculae and the sinusoidal network in the centro- and mediolobular areas is unaltered (OM × 250); **c** late changes of the hepatocyte-sinusoid exchange unit in the mediolobular area (* cytomembrane macrovesiculation, *RER* normal organization of the rough endoplasmic reticulum cisternae, *C* hepatocytic clasmatosis, *v* loss of hepatocyte microvilli, *DS* widened Disse's spaces, *st* thickened stellate cell processes, *S* sinusoidal lumen, *E* endothelium, *pl* platelet in front of focal endothelial discontinuity, × 4000)

level is very low in the pig liver, even with severe liver damage [1]. Other workers have found the ASAT level to be a useful index of viability [14, 15]. The highest rise in potassium level was recorded in group 2, possibly reflecting cell membrane injury in this group [12]. This phenomenon is a useful indicator of nonviable liver cells that continue to release potassium after revascularization [4]. The rise in Factor V during perfusion is attributed to the capacity of the liver to produce up to fivefold the human level of Factor V [17]. Only group 4 had an increasing level of Factor V and PT.

The interpretation of glucose metabolism is complicated by a lack of knowledge about glucose metabolism in the isolated perfused liver. The rapid rise in the glucose level after liver perfusion was constant in all perfusions. This was related to the breakdown of liver glycogen [11]. Our study revealed depletion of glycogen content upon electron microscopic examination. During the preliminary study, the addition of insulin to the perfusate was only associated with hypokalemia and did not modify the glucose level in the perfusate.

The lactate level was normal in group 4, a good indicator of proper liver function, whereas a decline in lactate level is suggestive of aerobic metabolism [12, 18]. The metabolism of ammonia and nitrogenous substances is represented by the rise in urea synthesis, which is an important criteria in the assessment of liver function. Decreased blood urea has been used as an indicator of impaired liver function [18]. In other studies, an increase in urea production was attributed to alteration of the control mechanism secondary to prolonged hypothermia [16]. In our study, an increase in the urea level was noted in all groups; the highest level was recorded for

group 4, which could have been correlated to better liver function in this group.

Weight gain is a reflection of postischemic edema. The loss of weight in group 4 may have been secondary to the use of a hypertonic UW solution for liver harvesting [4]. Rapid depletion of white blood cells and platelets was attributed to their sequestration into the perfusion circuit [14].

The hourly histological follow-up allowed us to establish the differential chronology of the lesional pathway according to the nature of the flushing medium and the gasometric conditions of blood perfusion, while electron microscopy revealed early changes in the hepatocyte cytostructure and the sinusoidal components. Ringer's lactate (group 1) and ELOHES (group 2) were shown to be harmful in their own right to liver cells. The histological damage in group 3 was not observed in the other groups; it was, thus, considered as specific to the high-oxygen content, making an additional group with a different flushing solution in the presence of high-oxygen content unnecessary.

Liver harvesting and flushing did not affect the lobular architecture, but a moderate and transient sinusoidal dilatation was constantly observed in the centrolobular areas after 5 min of blood perfusion, irrespective of the flushing medium and of the autologous or homologous blood used. The recovery of the normal, relative distribution of the hepatocyte parenchyma and the sinusoidal network from the portal to the centrolobular areas in all of the 1-h-perfused livers suggests a correlation, between this event and the establishment of ex vivo hemodynamic equilibrium at the beginning of the blood perfusion. The histological study in our model could also be used to compare lesions developed during liver xenoperfusion and to determine whether these changes could be attributed to perfusion damage or to the humoral xenoreaction.

Different authors have described several devices they have used with an ex vivo liver perfusion circuit, including a pressurized chamber for liver perfusion. However, this does not seem to be essential for good liver performance [8]. We believe that the rate of bile flow does not depend on diaphragmatic massage and that the hepatic venous outflow is appropriate whenever hemodynamic stability is achieved. The use of dialysis during liver perfusion to improve liver function after warm ischemia has been reported [6]. In our study, we had no warm ischemia and the duration of cold ischemia was less than 1 h. Nevertheless, the use of hemodialysis will only correct electrolyte imbalances that reflect liver injury during perfusion. Thus, hemodialysis will only hide the manifestations of liver injury during perfusion.

We conclude that optimal liver perfusion can be achieved under the following conditions: (1) UW solution is used to flush the liver during harvesting, (2) normal physiological pressure and blood flow are maintained, and (3) normal blood gas values (PO_2, PCO_2, and O_2 saturation) are maintained. Assessment of liver function should be done by measuring such sensitive indicators as the total bilirubin content in bile, which seems to reflect liver cell injury rather than bile flow, and Factor V, the level of which increases significantly with appropriate liver perfusion. Histological study is a sensitive method for assessing liver cell injury. Electron microscopy is needed to determine the type of ultrastructural changes that occur during perfusion.

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