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Hepatic resuscitation after warm anoxia: one approach for increasing the donor pool for liver transplantation

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Abstract The deleterious effects of warm anoxia on the liver are seen to be irreversible if cooling and transplantation (LT) follow immediately after. The aim of our study is to demonstrate that livers subjected to anoxia may be suitable for LT if a period of resuscitation is interposed before the cooling process. Forty female Large White pigs were used. Preservation (Euro-Collins solution) and LT technique were the same in all 20 procedures. All donors underwent clamping of the porta hepatis at the end of harvesting dissection. In the so-called "resuscitated" groups (A_R and B_R), the clamp was released for a period of time before the liver was cooled. Then, all livers underwent 2 h of cold ischemia followed by LT. Ultrastructural study showed better maintenance of mitochondria and sinusoidal cell integrity in resuscitated livers after LT. Liver synthesis of total adenine nucleotides, graft function and recipient survival were found to be better in the "resuscitated" groups. In conclusion, anoxic livers may be retrieved for LT if a resuscitation period (i.e. aerobic perfusion) is allowed prior to cold preservation. Longer periods of warm anoxia are needed to further support these preliminary results.

Key words Anoxia · Cardiac arrest · Liver transplantation · Resuscitation · Warm ischemia

Introduction

The progress of clinical liver transplantation (LT) over the last 15 years is unquestionable. The efficiency of immunosuppressive drugs, refinements in surgical technique, and the introduction of the University of Wisconsin organ preservation solution (UW) are among the responsible factors [1, 2, 9]. Nevertheless, the real access of liver patients to transplantation is seriously limited by the shortage of available donor organs. In fact, the demand for organ donors has increased in recent years while the supply has reached a plateau or decreased [6]. Previous authors have suggested the possibility of resuscitating livers after warm ischemic injury [7]. This would make it possible to use cardiac arrest donors as a new source of organs for LT.

The deleterious effects of warm anoxia on the liver are seen to be irreversible if cooling and transplantation follow immediately after. The aim of our study is to demonstrate that livers subjected to anoxia may be suitable for LT if a period of resuscitation is interposed before the cooling process. We also attempt to study in depth the effects of warm ischemia (WI) followed by cold ischemia and subsequent LT.

Methods

Technical aspects

Forty female Large White pigs (15–20 kg) were used in 20 orthotopic LT procedures. At the end of harvesting dissection, all donors underwent a period of hepatic WI (clamping of the porta

Table 1 Time employed in each phase of the preservation-transplantation process (*WI* warm ischemia, *R* resuscitation, *CT* cold ischemia time, *ST* vascular suture time, *TT* total ischemia time)

Group	Wi (min)	R (min)	CT (min)	ST (min)	TT (min)
A	5	0	98 ± 12	53 ± 5	151 ± 16
A_R	5	5	91 ± 5	50 ± 5	140 ± 6
B	10	0	88 ± 14	55 ± 3	143 ± 13
B_R	10	10	85 ± 11	52 ± 3	137 ± 10

Table 2 Tissue TAN and ATP levels in anoxic liver grafts (*WI* warm ischemia, *R* resuscitation, *TAN* total adenine nucleotides (μmol/mg of liver tissue), *ATP* adenosine triphosphate, *P* end of preservation, *T* transplanted liver)

Group	WI + R (min)	% Baseline TAN		% Baseline ATP	
		P	T	P	T
A	5 + 0	78 ± 25	76 ± 16	73 ± 41	51 ± 34
A_R	5 + 10	75 ± 18	110 ± 78*[1]	46 ± 19	86 ± 19*[4]
B	10 + 0	54 ± 13	66 ± 17	70 ± 17	73 ± 30
B_R	10 + 10	78 ± 6*[2]	92 ± 25*[3]	81 ± 36	81 ± 5

*[1] $P < 0.01$ vs A; *[2] $P < 0.01$ vs B; *[3] $P = 0.08$ vs B; *[4] $P = 0.09$ vs A

Table 3 Graft function and survival with anoxic donor livers (*WI* warm ischemia, *R* resuscitation, *AST* aspartate aminotransferase, *BIL* bilirubin output)

Group	WI + R (min)	AST (24 h)	BIL (24 h) (cc)	Six-day survival
A	5 + 0	1736 + 774	64 + 35	1/5
A_R	5 + 10	1026 + 332	114 + 54*	5/5**
B	10 + 0	[a]	20 + 44	0/5[a]
B_R	10 + 10	1321 + 752	104 + 36*	3/5

* $P < 0.05$ vs B; ** $P < 0.05$ vs A

[a] All but one animal died within 24 h after LT

hepatis) followed, or not, by a period of resuscitation (R) (clamp release) before liver cooling. All donor livers were flushed with Euro-Collins solution and stored at 4 °C before LT.

Spontaneous porto-jugular bypass was established before the anhepatic phase. Heparin was not used. Arterial reconstruction was done with an end-to-end anastomosis between the donor celiac trunk and the recipient hepatic artery. Surgical loops (2 × 1) were used for this purpose. No biliary reconstruction was performed. A silicon tube was introduced into the donor bile duct and exteriorized through the abdominal wall for measuring the 24-h biliary output after each LT.

Animal groups

The animals were divided into four different groups, A, A_R, B, and B_R, according to the WI period and the presence or absence of the R period (Table 1). Aside from this, there were no differences among groups with respect to cold ischemia time (CT), vascular suture time (ST) or total ischemia time (TT = CT + ST) (Table 1).

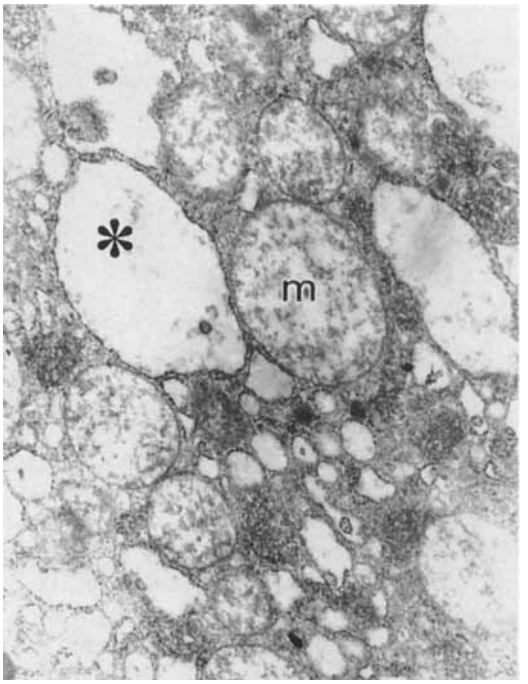


Fig. 1 Swollen mitochondria *(m)* and disorganized crests near dilated endothelial reticulum cisternae (*) can be seen in some hepatocytes subjected to 5 min of ischemia without resuscitation. TEM × 12000

Graft assessment

Liver biopsies were taken from each graft as follows: at baseline (B), the end of harvesting dissection; at the end of preservation (P), just before graft reperfusion; and in the transplanted liver (T), 1 h after graft reperfusion.

Morphological and ultrastructural studies

The morphological and ultrastructural studies were done by transmission electron microscopy. The samples were fragmented into small sections, fixed in 3 % glutaraldehyde for 2 h and placed in Milloning buffer (pH 7.3). For the ultrastructural study, the samples were postfixed in 2 % osmium tetroxide, dehydrated in a grade series of acetones and embedded in Araldite for thin sectioning. Afterwards, their contrast was enhanced with lead citrate and they were examined under a Zeiss 109 transmission electron microscope.

HPLC assay

ATP, ADP, and AMP levels in liver tissue were determined in each liver biopsy by HPLC assay. Total adenine nucleotide (TAN) levels were calculated by summing ATP + ADP + AMP.

Additional determinations

Plasma aspartate aminotransferase (AST) levels were compared 24 h after LT. Biliary output was collected and recorded for the same period of time in each recipient.

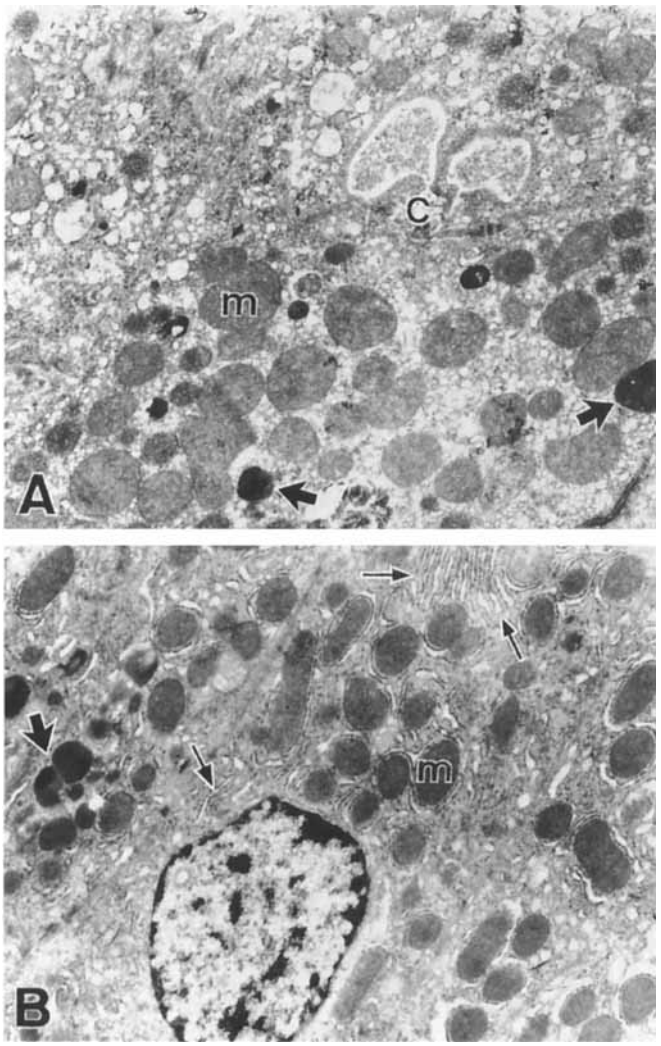


Fig. 2 **A** Group A(P). Microvesiculated hepatocytes, with swollen mitochondria and lysosomes. **B** Group A_R(P). Hepatocyte showing no noteworthy morphological changes after 5 min of resuscitation. (*m* mitochondria, *small arrows* rough endoplasmic reticulum, *large arrows* lysosomes, *C* hepatic canaliculi) TEM × 7000

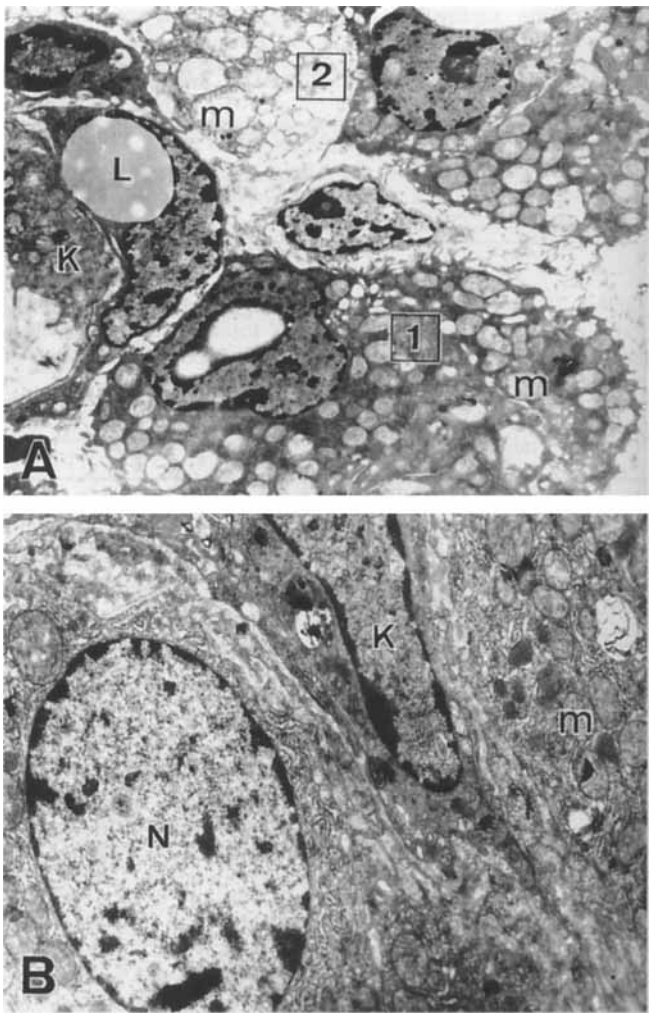


Fig. 3 **A** Group A(T). Dark-colored hepatocytes *(1)* exhibiting condensed cytoplasm, little vesiculation and small, light-colored mitochondria *(m)*, next to a light-colored, vacuolated hepatocyte *(2)* with swollen, ruptured mitochondria. (*L* Fat-storing cell with a large lipid droplet, *K* Kupffer cell) TEM × 3000 **B** Group A_R(T). Hepatocytes with typical morphology and normal mitochondria *(m)* next to a Kupffer cell *(K)*. (*N* Hepatocyte nucleus) TEM × 7000

Statistical study

The statistical analysis consisted of Student's *t*-test for parametric data and the Mann-Whitney test for nonparametric data. Fisher's exact test was used to compare survival rates among the groups. A *P* value of less than 0.05 was considered to be significant.

Results

The HPLC findings and rate of survival are summarized in Tables 2, 3. The ultrastructural study revealed that livers subjected to 5 min of anoxia without resuscitation at the end of preservation, group A(P), showed varying degrees of ultrastructural change. Two types of cellular lesion could be clearly distinguished. Some hepatocytes presented microvesiculation, dilatation of the smooth endoplasmic reticulum (SER) and swollen mitochondria (Fig. 1), having disorganized crests, different degrees of lysosome activation, and a light-colored nucleus with fragmented chromatin. Others presented a few peripheral vacuoles and several transversal cisternae in the SER, while the mitochondria and nucleus had undergone little change (Fig. 2 A). In both cases, the hepatocytes had a smooth sinusoidal pole, with no microvilli protruding toward Disse's space. The sinusoid endothelial cells presented a rounded nucleus, condensed mitochondria, a few vacuoles, and numerous micropinocy-

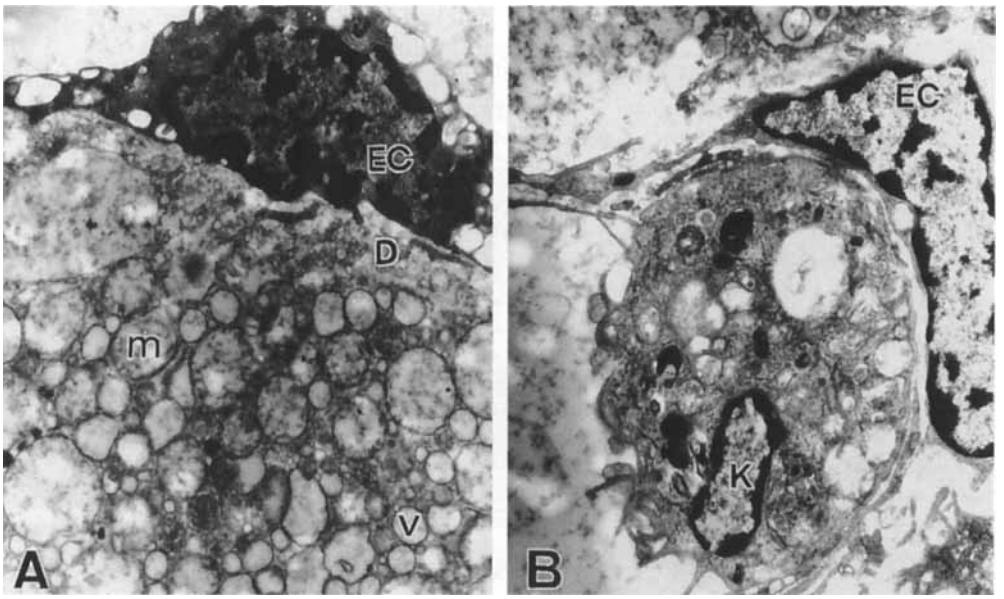
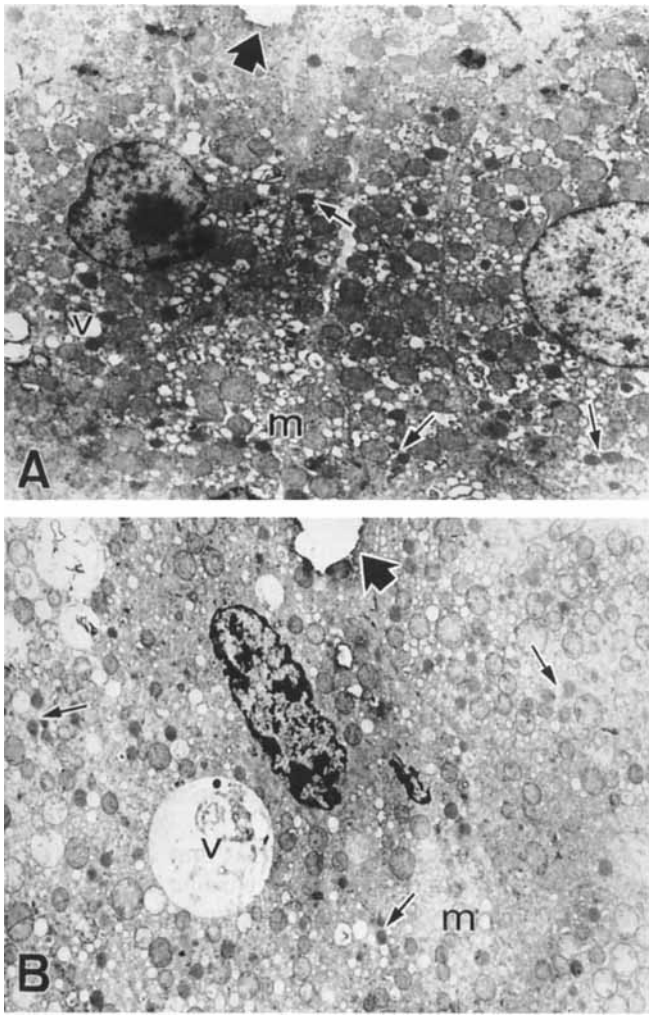


Fig. 4 A, B Group A(T). **A** Vacuolated endothelial cells *(EC)*, Disse's space *(D)* with numerous microvesicles and light-colored hepatocytes with vesicles *(v)*, and swollen mitochondria *(m)*. **B** Activated Kupffer cell *(K)* next to an endothelial cell *(EC)* having an elongated nucleus. TEM × 7000



totic vesicles. However, when the liver was subjected to a short period of resuscitation (5 min) prior to cooling, group $A_R(P)$, the changes observed in the hepatocytes were less marked, consisting of mild inflammation with increased SER (Fig. 2 B).

One hour after transplantation and revascularization of the graft with no resuscitation, group A(T), the differences between the two types of hepatocytes described above had become more marked (Fig. 3 A). In those of the first type, the vesiculation of the cytoplasm and inflammation of the mitochondria were more generalized, and rupture of the inner mitochondrial structure was observed. The second type of hepatocyte tended to greater cytoplasmic condensation; SER activity had decreased, while the content of the vacuoles appeared to have been concentrated in one or two larger ones. The hepatocytes had contracted thus widening the intercellular spaces and canaliculi. Disse's space was dilated and fat-storing and endothelial cells with tiny vacuoles were observed (Fig. 4 A). At this stage, the Kupffer cells were seen to be highly active (Fig. 4 B). In grafts that had undergone resuscitation after transplantation, group $A_R(T)$, the hepatocytes presented nearly normal morphology (Fig. 3 B).

When the liver remained anoxic for 10 min without post-ischemic resuscitation, group B(P), the tissue exhibited greater changes (Fig. 5 A). The hepatic canaliculi were markedly dilated, with distension of the hepatocel-

◀ **Fig. 5** **A** Group B(P). Dilated hepatic canaliculi *(large arrow)* and the hepatocellular border devoid of microvilli. The hepatocytes present vacuoles *(v)* of different sizes, swollen mitochondria *(m)* and a few lysosomes *(small arrow)*. TEM × 3000. **B** Group $B_R(P)$. Microvesiculated hepatocyte showing a large vacuole *(v)*

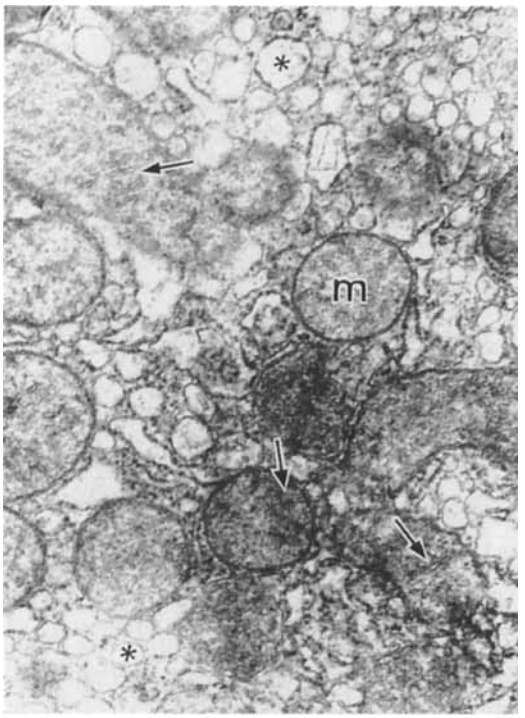


Fig. 6 Group B(P). Swollen mitochondria *(m)* with light-colored matrix and fragmented crests *(arrows)*, next to numerous smooth endoplasmic reticulum transversal cisternae (*). TEM × 20000

lular microvilli and widened intercellular spaces. The hepatocytes presented a few vacuoles and numerous transversal cisternae in the SER, swollen, light-colored mitochondria (Fig. 6), and changes in the nucleus. When anoxia was followed by 10 min of resuscitation, group B_R(P), the hepatocytes showed areas of normal structure, as opposed to others in which the vacuolation and the SER dilatation persisted (Fig. 5 B). Following revascularization of the ischemic organ, group B(T) (Fig. 7 A), the hepatocytes retracted, the cytoplasm became denser, and the microvesiculation was diminished; however, the canaliculi and the intercellular spaces remained dilated, and the hepatocytes presented vacuolation and dilatation of the SER. When the organ had undergone 10 min of resuscitation, group B_R(T), the structural changes produced in the tissue by the lack of oxygenation had not been totally normalized. The canaliculi remained dilated, although the dilatation of the hepatocellular SER was less marked and the vacuoles had fused into one or more of greater size, arranged in the direction of the sinusoid (Fig. 7 B). The endothelium remained stable, although a few cells had become rounded.

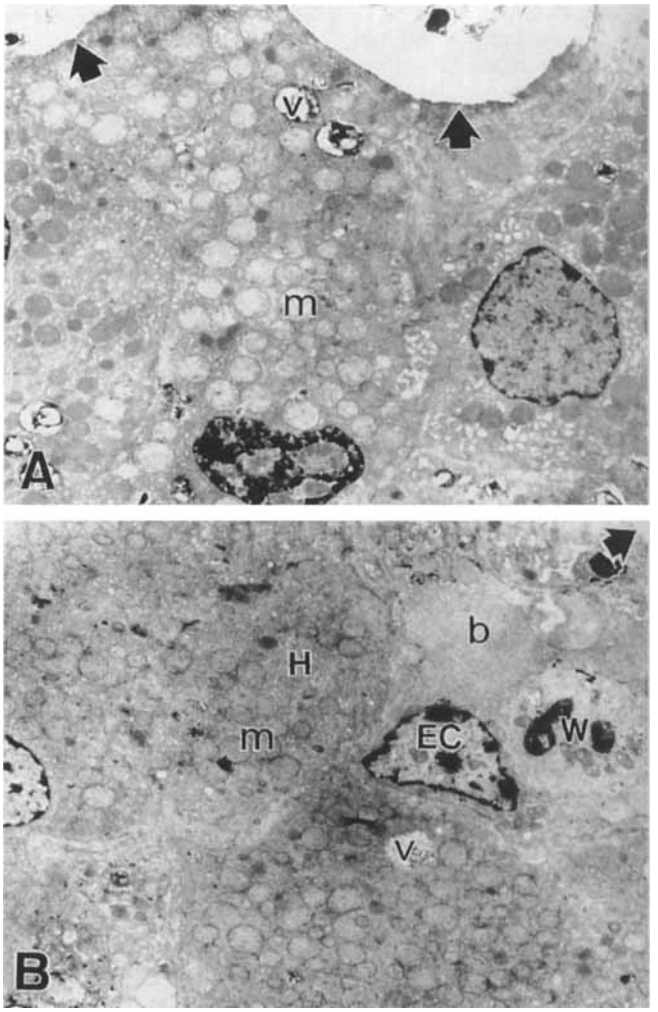


Fig. 7 **A** Group B(T). Following revascularization, the hepatocytes present few vacuoles arranged in the direction of the sinusoid and slightly swollen mitochondria *(m)*. Dilated hepatic canaliculi *(arrows)*. TEM × 3000. **B** Group B_R(T). Hepatocytes *(H)* next to a sinusoid surrounded by a normal endothelial cell *(EC)*; the lumen is occupied by a white cell *(W)* and a few bubbles *(b)*

Discussion

The number of organs available for liver grafting needs to be increased if transplantation is to be a realistic life-saving therapy for those patients with end-stage liver disease. Cardiac arrest donors and xenografts are the proposed new sources of organs to make this possible.

There exists a general consensus that if cold preservation follows immediately after cardiac arrest, the donor liver will not resume adequate function in the recipient. Meanwhile, there is widespread acceptance of the assertion that the liver can tolerate WI [4, 5], meaning that WI lesions may be reversible.

In a previous study, Schön et al. [7] have demonstrated the reversibility of WI by subjecting ischemic livers to machine perfusion. We have found no additional studies dealing with experimental LT after an insult of that kind. Shirakura et al. [8], working with dogs, also used a perfusion machine to resuscitate different organs retrieved from cardiac arrest donors prior to grafting. Nevertheless, the study does not report on LT.

We demonstrated that livers subjected to 5 min of anoxia could be fully recovered if a 5-min aerobic period was interposed before flushing and cold preservation. All A_R animals survived. Ultrastructural changes and tissue TAN levels were totally corrected soon after transplantation.

When the WI period was extended to 10 min, liver non-function occurring in the first 24 postoperative hours was common. Ten minutes of resuscitation (group B_R) was found to improve survival rates and biliary output, but the results cannot be considered optimal. In fact, TAN synthesis recovery did not reach statistical significance when compared to non-resuscitated livers (group B). The recovery of tissue TAN levels has been suggested as a viability prediction factor in LT [3]. Livers in both groups failed to fully resolve the ultrastructural lesions found at the end of preservation.

We conclude that 5 min of aerobic recovery is enough to resuscitate livers subjected to 5 min of WI. Livers that undergo 10 min of WI appear to require a longer period to achieve optimal graft function. Trials combining longer WI periods with longer periods of resuscitation should be performed to give consistency to these findings. We suggest that parallel results may be obtained with brief perfusion periods if an adequate machine is available.

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