WALDEMAR OLSZEWSKI, WOJCIECH ROWIŃSKI, KRYSTYNA OLSZEWSKA,
AGNES DANIELEWICZ-KOTOWICZ, JERZY W. BOROWICZ *

BIOCHEMICAL AND ULTRASTRUCTURAL CHANGES IN DOG AND PIG LIVERS PROCURED FOR TRANSPLANTATION

Surgical Research and Transplantation Laboratory, Experimental and Clinical Medical Center, Laboratory for Electron Microscopy, Polish Academy of Sciences, Warsaw

*Buffered Ringer's solution, "intracellular type" electrolyte solution, allogeneic blood were used for cooling of the dog and pig liver before transplantation. Biochemical studies of the wash-out fluids revealed only moderate increase in K * and glucose concentration and slightly higher aminotransferase activity. However ultrastructural alterations could be demonstrated in the endothelial lining of liver sinusoids. The type and extent of changes did not depend on the type of fluid used for cooling.*

Major biochemical and morphological changes accompany the procurement of donor liver for transplantation. Among many reasons warm ischemia, rapid cooling, and wash-out of blood from the organ with electrolyte solution should be mentioned. The signs of cellular changes in the organ are release of large amounts of glucose, glycogen, and high activity of transaminases and LDH found in the wash-out fluid. Had the temperature of the core of the liver reached 6—8°C the destruction process slowes down and the organ can be safely preserved for another 2 or more hours. These findings indicate that the initial period of warm ischemia and cooling may be critical for the liver, and changes which develop at that time may bear upon the rate of success of transplantation and survival of recipients.

The purpose of the present study was to evaluate the extent of these changes and to answer the following questions:

1) which intracellular substances and enzymes appear in the wash-out fluid during liver cooling,

2) what ultrastructural alterations develop in the liver sinusoids and hepatocytes during cooling,

3) which of the four cooling fluids: buffered Ringer's solutions, "intracellular type" electrolyte solution, allogeneic plasma, or allogeneic blood proves to be the least damaging organ protective.

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* With technical assistance of J. Pawlak.

MATERIAL AND METHODS

The studies were carried out in 6 dogs and 4 pigs. The animals were sacrificed with an intravenous bolus of barbiturate, the abdomen opened, the portal vein and suprahepatic portion of the IVC cannulated. All the tributaries of the vein were ligated to avoid any splanchnic blood contamination during the wash-out procedure. The liver was washed out with 1500 ml fluid at 4°C and the pressure of 25—30 cm H_2O. All fluid was collected in a cylinder, samples were taken for biochemical investigations. Four livers were washed out with buffered Ringer's solution pH 7·4, two with "intracellular type" electrolyte solution (KH_2PO_4 2·05 g/l, K_2HPO_4· 3 H_2O 9·7 g/l, KCl 1·12 g/l, $NaHCO_3$ 0·84 g/l, glucose 25·0 g/l, $MgSO_4$ 7·38 g/l), two other with fresh allogeneic plasma buffered to 7·4, two with allogeneic blood diluted to the hematocrit of 25. Biochemical investigations of the wash-out fluid included: glucose, K^+, Na^+, lactate and pyruvate concentration and aminotransferase and LDH activity. At 30 minutes of the experiment with the wash-out completed, liver specimens were taken for electron microscopy. The speciments were fixed in 2·5% glutaraldehyde and 2% OsO_4 in Millonig buffer pH 7·4, then dehydrated and embedded in Epon 812. For studies JEM 7A electron microscope was used.

RESULTS

A. Biochemical studies. The summarized results have been presented in Table 1. There was an evident increase of K^+ and glucose concentration in the wash-out fluid. With the "intracellular type" of fluid a decrease in K^+ concentration was observed. Osmolality of fluid increased considerably in all but "intracellular type" wash-out fluid. Aspartate aminotransferase activity increased only slightly above the normal levels.
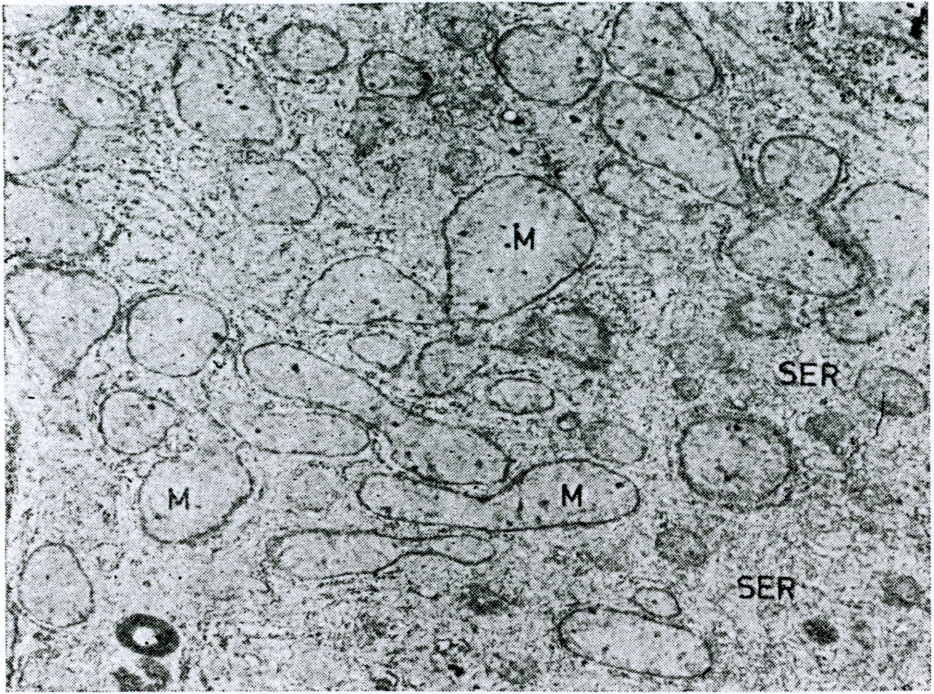
*B. Ulstrastructural studies*

a. Hepatocytes

1. Ringer's solution wash-out. Scarcity of rough endoplasmic reticulum, swelling and irregular outlines of mitochondria with diminution of cristae (Fig. 1, 2).
2. "Intracellular type" of fluid. Ultrastructural findings similar to those seen in livers washed out with Ringers solution (Fig. 3).
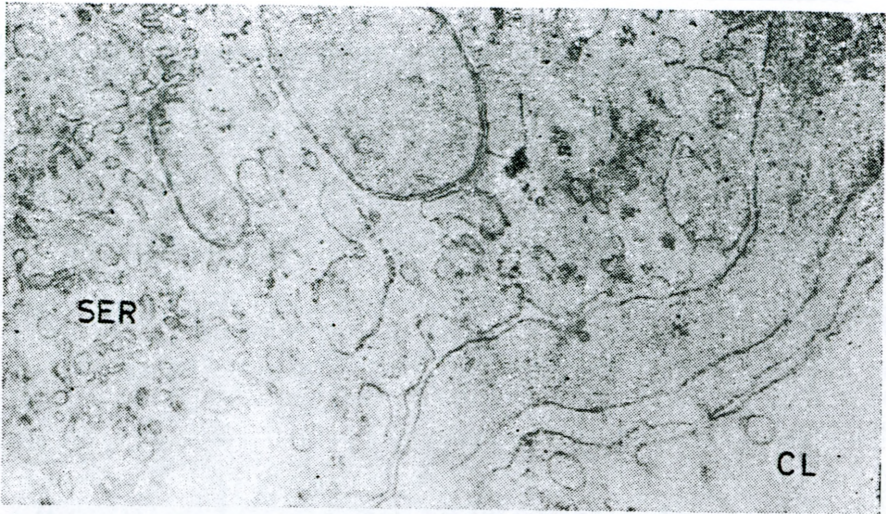
Fig. 1. Fragment of a hepatocyte from the liver of a dog, washed with Ringer's solution. Irregular shape and size of mitochondria (M). Abundant smooth endoplasmic reticulum (SER). Magn. × 13·000.
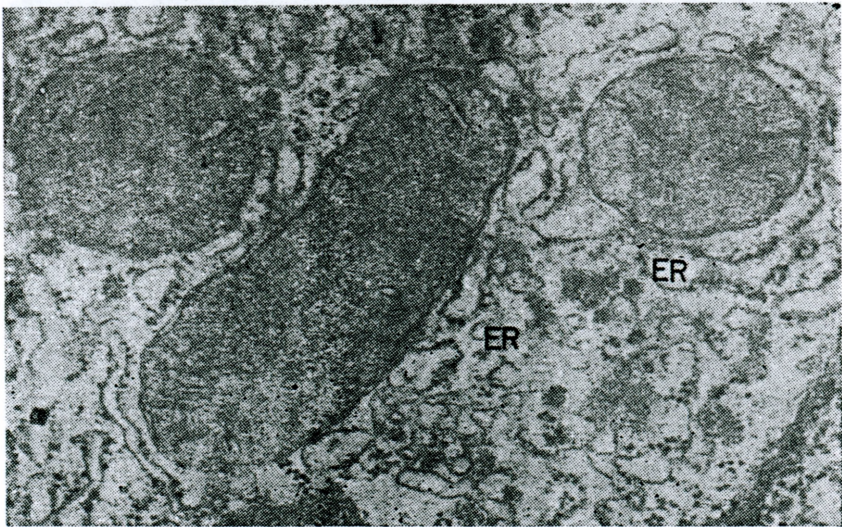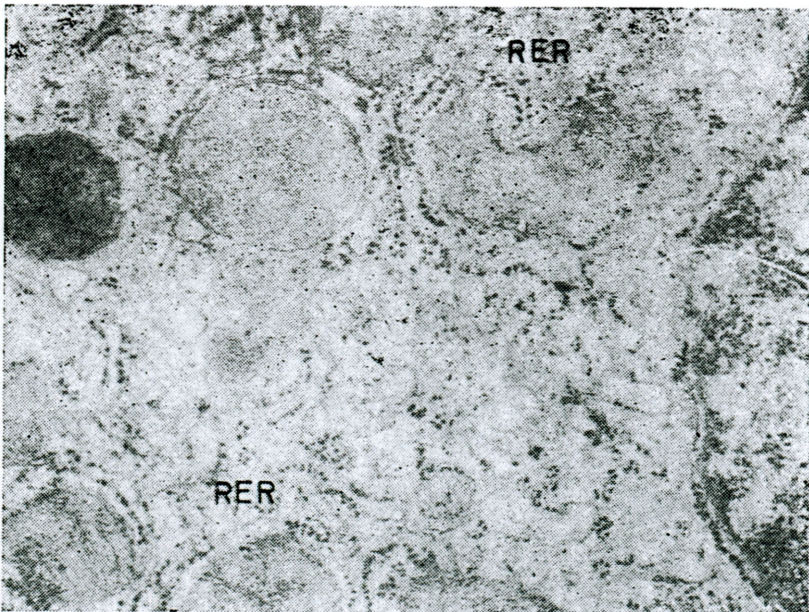

Fig. 2. Fragment of cytoplasm of a hepatocyte from the liver of a pig, washed with Ringer's solution. Dilated cisterns of the endoplasmic reticulum (ER) and diminished number of ribosomes. Magn. × 24·500.
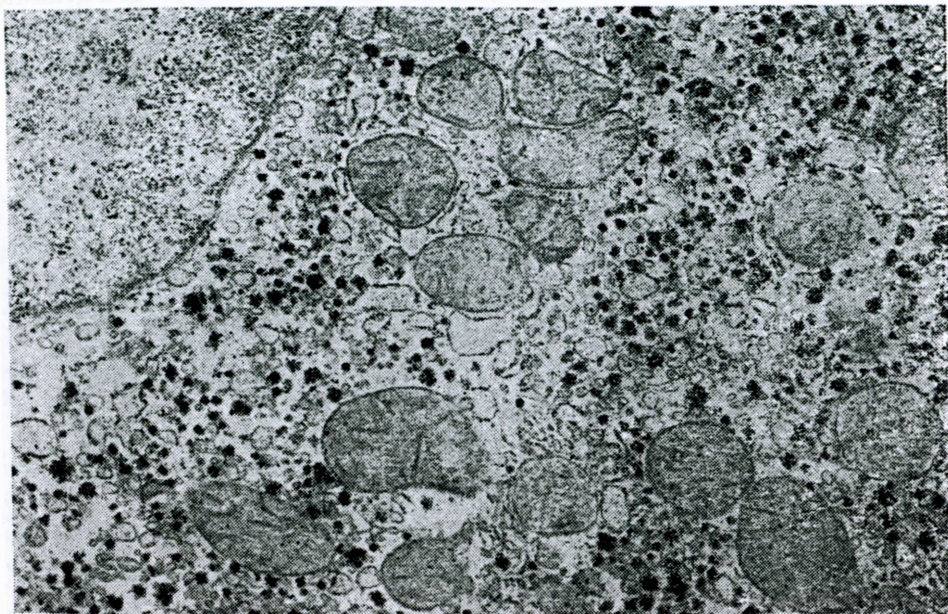
Fig. 3. Fragment of a hepatocyte and sinusoid (CL) of pig liver washed with "intracellular type" fluid. Abundant smooth endoplasmic reticulum (SER), and complete absence of rough reticulum. Magn. × 26·000.
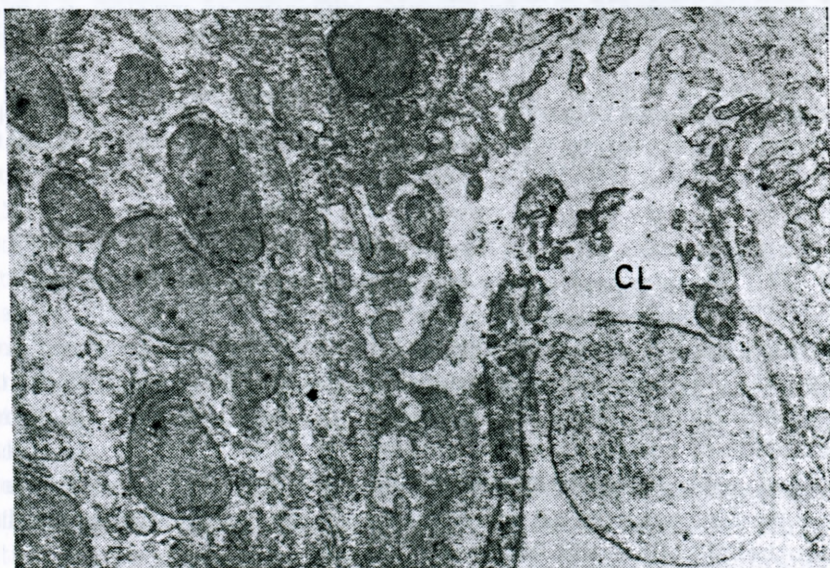

Fig. 4. Fragment of a hepatocyte from pig liver, washed with plasma. Diminished amount of rough endoplasmic reticulum (RER). Magn. × 27·000.

Fig. 5. Fragment of a hepatocyte from pig liver, washed with allogenic **blood**. Norma appearance of the hepatocyte. Magn. × 13·500.

Fig. 6. Fragment of a sinusoid (CL) from the liver of a dog, washed with Ringer's solution. Damaged endothelial lining. Magn. × 20·000.

3. Normal mitochondria. Slight decrease in number of glycogen granules (Fig. 4).

4. Allogeneic blood. Normal pattern of endoplasmic reticulum and mitochondria. No changes in the nuclei (Fig. 5).
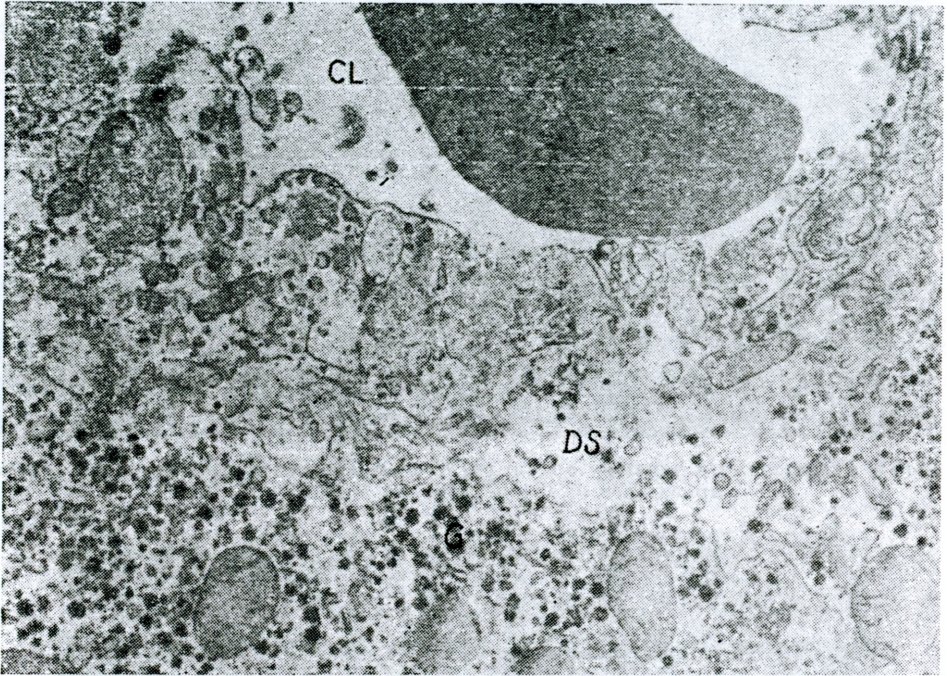

Fig. 7. Fragment of a sinusoid (CL) from pig liver, washed with blood. Damaged endothelial lining. the dilated Disse spaces (DS) and intercellular space contain glycogen (G) granules and free intracytoplasmic structures. A small defect of the cell membrane of the hepatocyte is visible. Magn. × 14·000.

C. Sinusoids

The same pattern of alterations was found in all livers irrespective of the type of fluid used for cooling and wash-out. There was dilatation of the interendothelial junctions, disruption of endothelial cell membrane, desquamation of endothelial and Kupfer cells. Disse's spaces were dilated, often filled with glycogen granules, loose mitochondria and lysosomes. In livers washed out with allogeneic blood platelets adherent to the damaged endothelial cells could be seen. (Fig. 6, 7).

Table 1. Changes in glucose, K+ concentration, AspAT activity, and osmolality in the wash-out fluids used for cooling of the liver, at 30 minutes of cooling

Type of fluid	Ringer's solution		"Intracellular type"		Allogeneic plasma		Allogeneic blood	
Liver of	dog	pig	dog	pig	dog	pig	dog	pig
Number of experiments	3	1	1	1	1	1	1	1
Glucose mg%	+43 +200 +135	+60	+50	+125	+50	+55	+155	+120
AspaAt u/ml	+4	+6	+19	+5	+3	+16	+38	+26
K+ mEq/l	0 +6 +2 +1·9	+0·9	−31	−45	+2·2	+3·1	+1·05	+1·1
Osmolality mOsm/l	+25	+35	+4	−2	+16	+21	+19	+28

DISCUSSION

The process of liver preservation for transplantation can be divided into 3 phases:

Phase 1. Cooling of the liver in-situ and surgical dissection of the organ.

Phase 2. *Ex-vivo* hypothermic preservation with or without continuous perfusion.

Phase 3. Surface hypothermia during the implantation procedure. The present study was designed to investigate the alterations developing in Phase 1. Two morphological elements of the liver may undergo degenerative changes at the time of preservation, namely hepatocytes and the endothelial cells with Kupfer cells. According to the recent knowledge hepatocytes belong to a type of cells most sensitive to ischemia and hypoxia. No data can be found in the literature dealing with the problem of ischemia alterations of the capillary wall and its cellular lining. It is commonly known that revascularization of the transplanted liver is immediately followed by the increased vascular resistance of that organ and alterations in its blood flow. That last observation may indicate that the endothelial lining and consequently the microcirculation are primarily affected by ischemia, whereas hepatocyte destruction is a secondary phenomenon.

The results of our studies would support this view, as there was only slight increase in aminotransferase activity in the wash-out fluid, thus negating major destructive changes in the hepatocyte membrane. Also the ultrastructural studies did not reveal any major alterations in hepato-

cytes. On the other hand major changes were demonstrated in the liver
sinusoids, with desintegration of endothelial cells, their desquamation
into the vascular lumen, and acute dilatation of Disse's spaces. Whether
these alterations were due to ischemia and/or to mechanical damage by
the wash-out fluid can not be decided basing on the experimental findings.
Irrespective of this it should be pointed out that serious alterations develop
in the liver already in the initial period of preservation, what may bear
upon the restoration of function of the transplant as well as on recipient
survival.

CONCLUSIONS

1. In the initial period of preservation only moderate amounts of K^+
and glucose, and slight increase in aminotransferase activity can be de-
tected in the wash-out fluid, what points against major changes in hepa-
tocytes.

2. However major ultrastructural alterations develop at that time in
liver sinusoids.

3. The type and extent of alterations do not depend on the type of
fluid used for cooling.