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PRESERVATION OF PORCINE LIVER UNDER HYPOTHERMIA AND CONTINUOUS PERFUSION

I. BIOCHEMICAL AND FLOW ALTERATIONS DURING 3-HOUR PRESERVATION

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Studies on preservation of porcine liver under hypothermia during a 3-hour period at maintained perfusion with buffered and oxygenated Ringer's solution with added glucose, evidenced that as well the metabolic as the secretory function of the liver was in a considerable degree preserved after the period of storage.

Preparation of the recipient for cadaver liver transplantation lasts several hours. So much time is needed for tissue typing procedure and the crossmatch test for the detection of preformed cytotoxic antibodies in the recipients' serum. With positive results of these studies, that is when no major serological differences between the donor and recipient are present, hepatectomy is performed in the recipient as a preparatory step for orthotopic transplantation, or else the blood vessels in the abdomen are dissected for heterotopic liver transplant. All these procedures take much time, and the liver removed from the cadaver should be properly preserved for several hours to maintain its functional integrity.

The purpose of the presented experimental study was to elaborate a method for a 3-hour preservation of the liver and evaluation of its function in an artificial perfusion system.

The following questions present themselves:

1. What kind of biochemical changes occur in the preserved liver?
2. What kind of biochemical changes can be detected in the blood perfusing the preserved liver in an artificial perfusion system?
3. What is the flow through the central and peripheral parts of the liver during preservation and normothermic blood perfusion?

METHODS

The study was performed on 12 porcine livers perfused extracorporeally in a closed circuit system: pump-oxygenator-heat exchanger-liver (Fig. 1). The common bile duct was cannulated and the bile collected. Each experimental procedure was divided into two stages. In stage I, the liver was perfused for 3 hours with Ringer's solution at the temperature of 10°C, in stage II for the same period of time with 37°C blood.

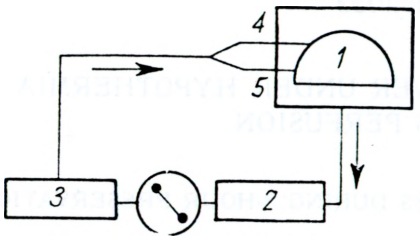

Fig. 1. Schematic drawing of perfusion-preservation system. 1 — liver, 2 — oxygenator, 3 — heat exchanger, 4, 5 — hepatic artery and portal vein.

In stage I, the liver removed from the pig was rinsed with 10 l of 4°C Ringer's solution of pH 7·8, then perfused for 3 hours with oxygenated Ringer's solution with glucose (3 g/l) at the temperature of 10°C and pH 7·8. Pump output was regulated so that the pressure was maintained in the hepatic artery at 25/10 mm Hg, in the portal vein at 5 mm Hg, and total flow at 0·2—0·4 ml/g of liver/min.

In stage II, the perfusion system was filled with 1500 ml of blood of hematocrit 25 at a temperature of 37°C. Perfusion of the liver was carried out under the pressure of: in the hepatic artery 120/80 mm Hg, and in the portal vein 15 mm Hg. Blood was oxygenated, its temperature maintained at 37—38°C in a period of 30 minutes. Stage II lasted for 3 hours. By the end of the 1st and 3rd hour in both stages the perfusion fluid, as well as the affluent and effluent blood were studied biochemically, and its pH, pO_2, pCO_2, lactic acid, potassium, sodium, total protein and creatinine level was determined. Also the volume and content of the excreted bile were investigated.

In stage I and II, radiositope studies were performed in the 3rd hour of the experiment. Liver perfusate and blood flow were measured with ^{133}xenon, liver function was evaluated with ^{131}I rose bengal.

RESULTS

Stage I. During the 3-hour liver preservations with 10°C Ringer's solution the pH and lactate level of the effluent showed to be dependent on the magnitude of flow. With the flow 0·2 ml/g of liver/min pH was 6·95—7·3, and lactate level on the average 50 mg% (30—98), with higher flows as 0·4 ml/g/min pH was

7·42—7·48 and lactate concentration 5 mg% (5—15) (Table I). Oxygen consumption was very low, and pO_2 difference between the affluent and effluent was on the average 74 mm Hg.

Table I

Biochemical studies of liver effluent in 3rd hour of preservation

	Flow in ml/g of liver/min	
	0·2	0·4
pH	6·95—7·3	7·42—7·48
Lactic acid mg%	50 (30—98)	5 (below 5—15)
SGOT u.		170 (40—428)
K^+ mEq/l		3·9 (2·9—6·2)
Creatinine mg%		0·7 (0·15—2·6)
Protein g%		0·17 (0—0·7)

Mean SGOT activity was 170 u. (40—428), potassium concentration 3·9 mEq/l (2·9—6·2), creatinine level 0·7 mg% (0·15—2·6). Weight increase did not exceed 10% after 3-hour preservation. During liver perfusion-preservation with 10°C Ringer's solution ^{133}xenon clearance and flow studies revealed a considerable decrease of flow in the marginal parts of the liver as compared with the central parts (Fig. 2). Radioisotopic studies with ^{131}I rose bengal showed a decreased take-up by the hepatocytes in the marginal regions of the liver (Fig. 2).

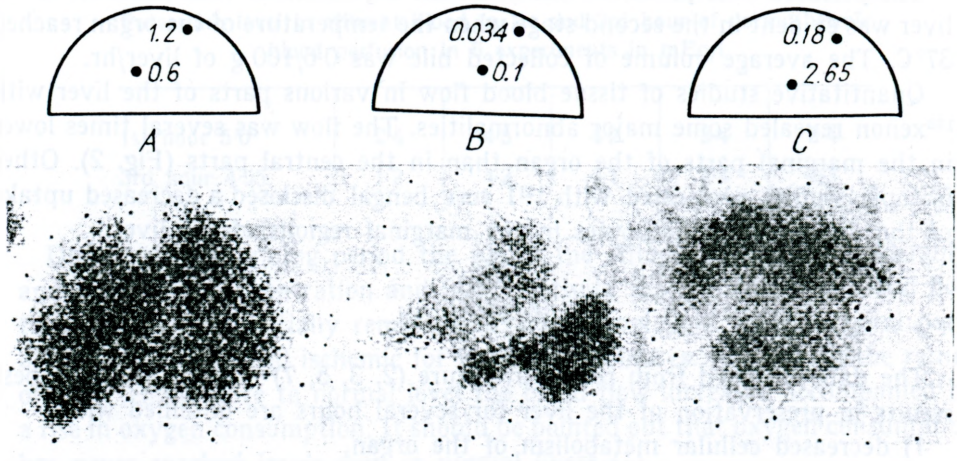

Fig. 2. Liver isotope studies. In upper part tissue blood flow studied with ^{133}xenon. Numbers in peripheral and central parts of organ indicate flow in ml/g/min. Lower parts of figure show liver scan with ^{131}I rose bengal. A — normal liver, B — liver after 3-hours preservation, C — liver after preservation and 3-hour normothermic blood perfusion.

Stage II. Perfusion of the liver with 37°C blood increased its temperature to normal level in about 30 minutes. At the beginning of blood perfusion only the central parts of the liver had the reddish pink color, the peripheral ones remaining ischemic. In the course of perfusion the marginal regions gradually turned bluish. The blood flow through the liver changes with a constant pressure of 120/80 mm Hg in the hepatic artery and 15 mm Hg in the portal vein, according to changes in liver vascular resistance from 0·45 to 1·5 ml/g of liver/min. Oxygen consumption increased from 0·004 to 0·0085 ml/g of liver/min with a slight drop of pH. Lactate level had a tendency to decrease, on the average from 105 mg% in the 1st hour to 93 mg% in the 3rd hour, SGOT from 80 to 76 u., potassium from 3·6 to 3·0 mEq/l, but creatinine concentration increased from 1·58 to 2·1 mg% (Table II). Also the blood protein concentration increased, in that stage, from 2·2 (0·78—3·3) to 2·31 (1·8—3·38) g%.

Table II

Biochemical studies of effluent blood in the 1st and 3rd hour of normothermic blood perfusion

Time of perfusion in hr	1	3
Lactic acid mg%	105 (22—250)	93·7 (22—170)
SGOT u.	80 (30—196)	76 (44—212)
K^+ mEq/l	3·6 (2·4—5·0)	3·0 (1·2—4·55)
Creatinine mg%	1·58 (0·25—6·9)	2·1 (0·25—7·6)
Protein g%	2·0 (0·78—3·3)	2·3 (1·8—3·38)
Bile ml/100 g of liver/hr	0	0·5

The perfused liver produced bile in all the experiments. That function of the liver was evident in the second stage when the temperature of the organ reached 37°C. The average volume of collected bile was 0·6/100 g of liver/hr.

Quantitative studies of tissue blood flow in various parts of the liver with ^{133}xenon revealed some major abnormalities. The flow was several times lower in the marginal parts of the organ than in the central parts (Fig. 2). Other isotopic studies performed with ^{131}I rose bengal disclosed a decreased uptake of the dye of the hepatocytes in the marginal regions of the liver.

DISCUSSION

The data obtained from recent literature (2, 3, 5, 7) indicate that the best results in preservation of the liver for several hours are obtained with:

1) decreased cellular metabolism of the organ,
2) adequate supply of oxygen and energetic substrates,
3) removal of cellular metabolic products and carbon dioxide (5).

This can be achieved by:

1) decrease of liver temperature to 4—10°C,

2) continuous low-flow perfusion of the liver with isoosmotic perfusate at the temperature of 4—10°C, and high oxygen partial pressure.

Hypothermia of the liver as the sole preservation factor is not adequate, irreversible changes in the hepatocytes usually occurring in such a case (7). The purpose of our studies was to elaborate a simple and inexpensive method for the preservation of the liver, consistent with all the above mentioned conditions. The preservation system consists of a simple roller pump, oxygenator and heart-exchanger.

The warm ischemia time was shortened to 5 minutes, with a cooling of the liver *in situ* through the portal vein with Ringer's solution. The same Ringer's solution buffered to pH 7·8 with the addition of glucose was used for 3-hour preservation. With the flow through hepatic artery and portal vein of 0·4 ml/g of liver/min there was no edema of the organ. The weight increase after a 3-hour preservation did not exceed 10 percent. The flow of 0·4 ml/g of liver/min protected hepatocytes against major changes. With lower flows the effluent was acidotic and had a high concentration of lactic acid. Liver oxygen consumption was usually very low during perfusion with Ringer's solution at the temperature of 10°C. This was due to decreased metabolism, and/or to an impaired oxygen uptake by the cells. An increase of SGOT activity in the perfusate indicated damage to the hepatocytes. Major biochemical changes were observed during rewarming of the organ, which corroborates the findings of other authors (3, 6, 7).

Table III

Potassium level in effluent blood in 1st and 3rd hour of normothermic blood perfusion in 6 experiments in mEq/l

1st hour 5·0	2·4	4·5	4·2	3·4	2·4
3rd hour 4·55	1·2	4·2	3·7	2·4	2·3

During the rewarming period the pH of the effluent dropped considerably, and lactic acid concentration and SGOT activity rapidly rose. The blood flow through the liver initially remained at a low level, and the peripheral parts of the organ remained ischemic for at least 30 minutes. Along with the return of liver temperature to normal level the blood flow increased accompanied by a rise in oxygen consumption. It should be pointed out that oxygen consumption has never reached levels seen in normal livers (1).

During normothermic liver blood perfusion lactic acid, potassium and SGOT levels slightly decreased. An increase in perfusing blood protein level could be explained by the rinsing liver protein production and also by a passive rinse from

the liver. The constant rise in creatinine level remains unexplained. According to some authors (4) this may result from the metabolic change of creatine to creatinine, a source of energy for the restoration of ATP in the liver tissue.

Our flow studies with isotopic methods revealed extremely low perfusion of the marginal parts of the liver at the time of preservation, and also during normothermic blood perfusion (6). Ischemia of some parts of the organ might be the cause of the observed biochemical changes in the effluent. The causes of peripheral ischemia may be diverse: low pressure in the hepatic artery and portal vein, RBC aggregation, precipitation of some plasma components as phospholipids (2).

Basing on the data obtained from our studies one can conclude that the metabolic function of the liver preserved for 3 hours may be maintained, at least to some extent, and considerably improved during blood perfusion, despite occurring biochemical abnormalities. Most convincing in this respect may be bile production by the tested liver, normal in volume and contents.

CONCLUSIONS

1. A liver preserved for a period of 3 hours by means of low-flow perfusion with 4—10°C Ringer's solution with glucose retains its biochemical activity and produces bile.

2. During normothermic blood perfusion of the previously preserved liver some biochemical changes are observed. These are: increase in lactate level, high SGOT activity, decrease in potassium concentration and rise of protein and creatinine levels.

3. Flow through the peripheral parts of the liver is impaired during preservation, as well as during blood perfusion.

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