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PRESERVATION OF PORCINE LIVER UNDER HYPOTHERMIA AND CONTINUOUS PERFUSION

II. FUNCTIONAL EVALUATION OF THE PRESERVED LIVER BY *IN VIVO* PERFUSION

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Porcine liver was stored during a 3-hour period under hypothermia (+4°C) at maintained continuous perfusion. The function of stored liver was examined by means of connecting it to the systemic circulation of another pig with previously induced acute liver insufficiency. Studies thus carried out evidenced that the stored liver resumed its fundamental as well biochemical as secretory activity after having been connected to the host's organism. Anatomopathological examinations of stored livers did not reveal any features of degeneration or the presence of necrotic foci.

In the previous communication biochemical and flow changes in the 3-hour preserved liver were discussed. Preservation was carried out under hypothermia and continuous low-flow perfusion. The present study was aimed at an evaluation of function of the liver, preserved in the same way, by *in vivo* perfusion. In pigs, acute insufficiency of their own liver was produced by ligation of the arteries and portal vein in the hilum, the splanchnic blood being directed to the vena cava through the porto-caval shunt.

METHODS

The study was carried out on 18 pigs weighing 17 to 50 kg. Each experimental procedure was divided into two stages. In stage I the liver taken from the pig was preserved for a period of 3 hours under hypothermia in a perfusion system. The conditions of preservation and detailed description of the preservation system have been presented in the previous publication (5, 6).

In stage II the preserved liver was connected with another pigs circulation and perfused for 3—5 hours.

In general anesthesia with halothane (halothane concentration 3·5 to 1 Vol%) the abdomen was opened with special attention paid to the hemostasis of the wound. A side-to-side porto-caval shunt was performed, and the whole hepato-duodenal ligament with all its structures clamped. With no blood supply to the liver an experimental state of acute liver insufficiency was created. The preserved liver placed in a special container was connected with the iliac vessels of the recipient pig.

Arterial blood flowed from the iliac artery of the recipient to the hepatic artery and portal vein of the donor's liver under the pressure 70—90 mm Hg. The effluent blood was directed from the hepatic veins and vena cava to the iliac vein. At the beginning of perfusion the recipient pig received 4 mg/kg of heparin. Further dosage of heparin depended on thrombin coagulation time. Pressures in the portal vein, hepatic artery, carotid artery and central veins were recorded continuously during the whole experiment with a Elema-Mingograph manometer.

The perfusion lasted for 3 to 5 hours. Liver blood flow, partial pressure of oxygen in the affluent and effluent blood and also the amount of produced bile were measured. Oxygen consumption was calculated from the formula:

$$\text{Oxygen consumption (ml/g/min)} = \frac{\text{A-V saturation difference (in vol\%)} \times \text{liver blood flow (in ml)}}{100 \times \text{liver weight (in g)}}$$

Also other studies were performed, as coagulation and fibrinolysis, acid-base balance, aminotransferase activity, protein electrophoresis and blood glucose level. For the acid-base balance measurements Astrup's apparatus was used. Tissue blood flow was measured with ^{133}xenon, and hepatocytes function evaluated with ^{131}I rose bengal.

In the following study we present the description of macroscopic appearance of the liver, blood flow and oxygen consumption data, bile volumes, acid-base balance changes, results of isotopic and microscopic investigations, and some more important biochemical findings.

RESULTS

In the first moments after unclamping mostly the parahilar parts of the liver were filled with blood, all the others being less perfused. After a period of 30 minutes the whole organ was filled with blood, and its temperature rose from 10 to 37°C. At the same time the temperature of the pig went down to 35°C. Infrared light was used to maintain the recipient's temperature and protect

it against deeper hypothermia. The pig's arterial pressure had a tendency to drop constantly, with an initial decrease of 20 mm Hg to the mean 80—90 mm Hg, then in course of perfusion it remained lower by 20—30 mm Hg from the initial values.

The changes in acid-base balance observed during 3-hour perfusion indicate a tendency toward respiratory acidosis. Carbon dioxide partial pressure went up, so did the bicarbonate level. The pH showed a slight decrease. In one case metabolic acidosis was noted. Along with the duration of perfusion arterial pH became more acidotic. It occurred together with a decrease in bicarbonate concentration and pCO_2 drop. Metabolic acidosis was controlled by infusion of 5% $NaHCO_3$. In one case metabolic alkalosis was present during the whole 5-hour perfusion period (Fig. 3).

High anti-heparin serum activity was observed in all experiments. This was checked with thrombin coagulation time. With a shortened coagulation time additional doses of heparin were given. Already after 1-hour perfusion major changes in the fibrinolytic system could be observed. There was a decrease in fibrinogen level, platelet count and activation of fibrinolysis. Fibrinolysis was controlled with intravenous infusion of EACA and EMCHA. All data concerning coagulation and fibrinolysis disturbances were published elsewhere (4).

Biochemical studies revealed an increase in aminotransferase activity (Table I), alkaline phosphatase, lactic dehydrogenase, and glutamic dehydrogenase in the blood serum (8, 9). The bromsulphtalein retention test demonstrated a 27% retention after 45 minutes.

Table I

Aminotransferase activity changes during the perfusion

Enzyme	Activity	
	initial	terminal
AspAT	58 u/ml	220 u/ml
AlAT	20 u/ml	32 u/mi

Blood flow studies through the isolated liver are summarized in Table II. Thirty minutes after the beginning of perfusion the whole liver was perfused with blood, but major abnormalities in flow distribution were observed as well. The central parts of the lobes and parahilar region were reddish-pink, whereas the peripheral parts remained reddish-blue. As the perfusion went on in time the number of reddish-blue spots gradually decreased, more of the organ being

well perfused. The blood flow through the liver ranged from 0·36 to 1·3 ml/g/min depending on the arterial pressure of the pig and the liver's vascular resistance. The flow values immediately after restoration of the flow ranged from 0·45 to 1·3 ml/g/min, on the average 0·75 ml/g/min, with the pig's arterial pressure of 90 mm Hg and portal vein pressure of 15 mm Hg. During the next few hours no changes in blood flow were observed unless there was a fall in the pig's systemic pressure. In three experiments, with perfusion lasting for 6 hours, a fall in the blood flow was seen by the end of the procedure amounting to 0·48—0·6 ml/g/min.

Table II

Blood flow, oxygen consumption and bile production by a preserved liver perfused *in vivo*

No.	Flow ml/g of liver/min						Oxygen consumption ml/g/min			Bile ml/100 g of liver/min
Time	0	1	2	3	4	5	1	3	5	
1	0·96	1·00	0·96	0·96			—	—	—	0·45
2	0·61	0·69	0·92	0·92			0·006	0·013		0·45
3	0·45	0·61	0·61	0·61			0·014	0·018		0·82
4	1·10	0·63	0·63	0·63			0·008	0·008		0·26
5	0·63	0·70	0·74	0·49			0·006	0·028		0·78
6	0·60	0·60	0·60	0·72	0·48	0·48	0·025	0·012		1·20
7	0·36	0·66	0·96	1·09	0·60	0·96	0·029	0·087	0·073	3·54
8	1·30	0·57	0·66	0·64	0·86	0·60		0·018	0·029	0·29

During perfusion liver oxygen consumption ranged from 0·006 to 0·087 ml/g/min, increasing along with the duration of the experiment. In the first hour the mean oxygen consumption was 0·015 ml/g/min, in the third 0·029 ml/g/min, in the fifth (in three cases) 0·038 ml/g/min.

Liver bile production started soon after filling of the liver with blood, and was dependent on the blood flow and oxygen consumption. The production ranged from 0·26 to 3·54 ml of bile/100 g of liver/hr, on the average 1·06 ml/100 g of liver/hr. The bilirubin concentration was 7 to 42 mg%. The quantitative data of blood flow, oxygen consumption and bile production are presented in Table II.

The isotopic studies revealed that the blood flow through the central parts of the lobes was several times higher than that through the peripheral parts. The studies of hepatocyte activity in various parts of the liver showed a high ^{131}I rose bengal uptake in the central parts of the organ (Fig. 1).

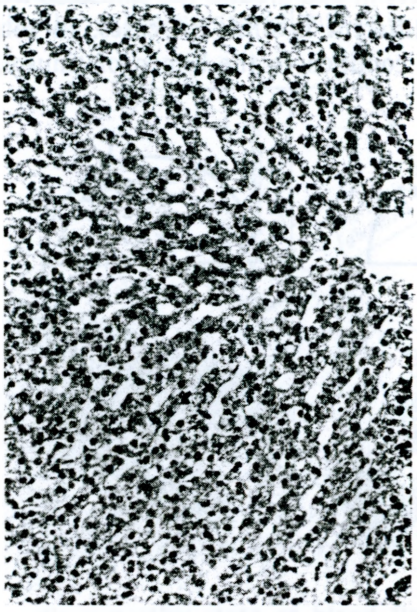

Fig. 1 a

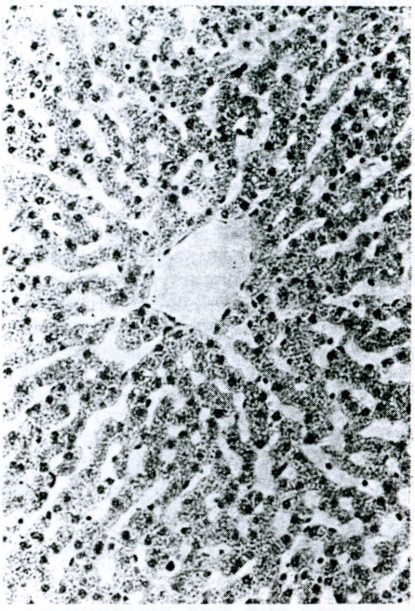

Fig. 1 b

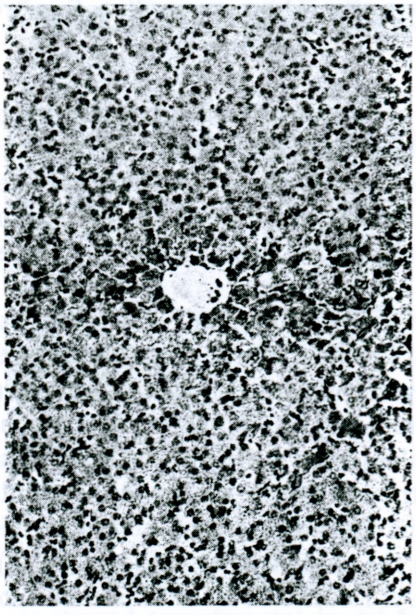

Fig. 1 c

Fig. 1. Histological examination of liver. a — normal liver H. E., × 150. b — preserved liver H. E., × 150. c — Liver after preservation and perfusion. Many lymphocytes are seen in sinusoids. H. E., × 150.

Histological studies. Six liver specimens were evaluated after staining with hematoxylin-eosin. No degenerative and necrotic changes could be seen. On the other hand major infiltrations of lymphocytes in and around the sinusoids were found. The number of lymphocytes was different in different cases. In some specimens lymphocyte accumulations were confined to the lumen of sinusoids (Fig. 2).

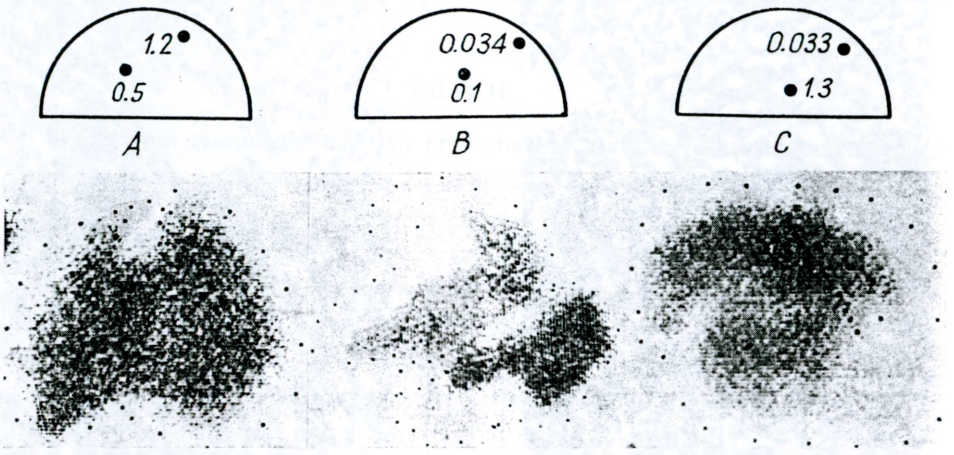

Fig. 2. Isotopic study of liver. In upper part liver tissue flow measured with ^{133}xenon. Numbers indicate flow in peripheral and central parts of liver in ml/g/min. In lower part liver scan after ^{131}I rose bengal. A — normal liver, B — liver after 3-hour preservation, C — liver preserved and perfused for 3 hours by pig.

The presented results indicate that liver preserved for 3 hours under hypothermia and continuous low-flow retains its biochemical and excretory function. Perfusion of the preserved liver with the blood of another pig with acute liver insufficiency created a situation analogous to that seen in animals after orthopic liver transplantation. At the same time all the technical inconveniences of surgery were avoided.

The mean blood flow through the liver was 0·75 ml/g/min, which was less than could be expected in normal livers. That low flow depended on the changes in the pigs arterial pressure and on the vascular resistance of the liver. Oxygen consumption of the perfused liver changed from 0·015 ml/g/min in the 1st hour to 0·038 ml/g/min in the 5th hour. These values were a little lower than those obtained from a normal organ. Nevertheless they were very similar to the results from other series of experiments performed in our laboratory on normothermic liver perfusion. They also did not differ from the data of other authors (1, 2, 3).

Functional impairment of the preserved liver resulted in an increased fibrinolytic activity of serum, rise in aminotransferase activity, and retention of bromsulphtalein 27% after 45 minutes.

Passive hyperemia of the liver seen in some cases was probably due to mechanical factors like local pressure on the tissue in the container. It was limited to the subcapsular regions.

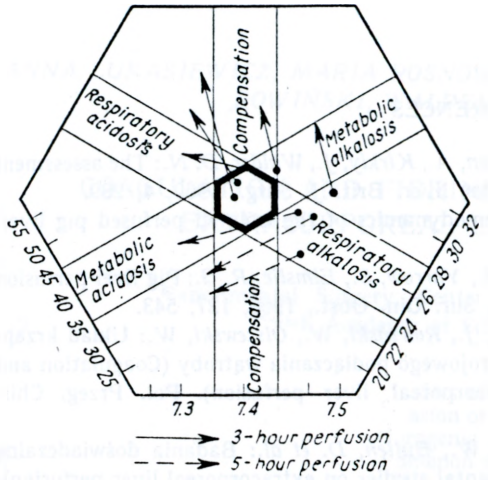

Fig. 3. Acid-base balance in different perfusion procedures. Hexagon in middle of drawing corresponds to normal values. Areas situated at 3:00 and 9:00 hours indicate intermediate changes, metabolic and respiratory. Areas on both sides of central rectangular are reserved for changes with tendency for equilibration.

Comparative data of blood flow, oxygen consumption and bile production by the perfused liver during and after preservation were summarized in Table III. Oxygen consumption and bile production were much higher during normothermic perfusion.

Table III

Blood flow, oxygen consumption, and bile production by preserved liver perfused *in vitro* in artificial system and *in vivo* by pig

	Time in hr	Flow ml/g/min	Oxygen consumption ml/g/min	Bile production ml/100 g of liver/h
Artificial perfusion system	3	0·88	0·020	0·46
	3	0·74	0·013	0·61
Perfusion *in vivo*	6	0·70	0·041	1·896

CONCLUSIONS

1. Perfusion of the preserved liver *in vivo* by a pig with acute liver insufficiency is a good experimental model for studies of metabolic and excretory function of the isolated organ. It limitates the situation seen after liver transplantation, at the same time avoiding the technical problems of transplantation.

2. Liver preserved for 3 hours by hypothermic low-flow perfusion with oxygenated Ringer's solution retains its biochemical and excretory functions, which has been proved during blood normothermic perfusion *in vivo*.

3. Histological studies of perfused liver specimens did not reveal any degenerative or necrotic changes, even after 3 hours of preservation.

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