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EXPERIMENTAL STUDIES ON EXTRACORPOREAL LIVER PERFUSION

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The authors carried out extracorporeal liver perfusion on pigs with acute insufficiency of their own livers. During a 3-hour perfusion bile secretion, oxygen uptake and blood flow through the isolated liver approximated to physiological values. Ammonia and bromsulphthalein clearance took place during 20—30 minutes. It has been found, however, that in the animal subjected to the extracorporeal liver perfusion there gradually developed hypotension and metabolic acidosis.

Studies on eventual clinical use of extracorporeal perfusion of heterogenous liver in patients with acute hepatic failure have been carried out in the Department of Experimental Surgery, Polish Academy of Sciences, since 1966. This method, suggested 10 years ago, is still in the experimental stage requiring changes and improvement. Each group which undertakes the clinical trials of this type has to perform numerous experimental studies unless all difficulties and dangers concealed in this method have been realized. We believe that the perfusion of heterogenous liver performed without adequate experimental preparation and available of technical possibilities of immediate performance of various tests even in a patient of extreme hepatic insufficiency is a mistake. Perfusion done in unproper conditions on assumption that "there is no chance for this patient anyway" is in our opinion unjustified. The following most important problems have to be solved: 1) technique of a prompt removal of the liver from the animal, 2) removal of all blood from the liver, 3) achievement of sufficiently high pressure and sufficient flow in the perfused liver, 4) immediate evaluation of liver function, 5) reliable and immediate control of the patient subdued to liver perfusion.

Present paper deals with the evaluation of

1) secretory and biochemical function and of flow through the isolated pig liver,

2) biochemical and hemodynamic disturbances in animal which perfuses another, extracorporeal liver.

MATERIAL AND METHOD

The studies were carried out in 4 groups, using jointly 30 pigs of mean weight 25—30 kg. In group I twelve extracorporeal perfusions of pig liver were performed in pigs with experimental acute failure of their own liver. In group II (control) acute liver failure was induced in 6 pigs, no extracorporeal liver perfusion being, however, made.

Group I. Preparation of the recipient. Liver failure was induced as follows: under general eunarcon anesthesia superior mesenteric vein was anastomosed "side to side" with inferior vena cava, than hepatic artery, portal vein, common bile duct and remaining components of the hepato-duodeno-gastric ligament were ligated. This resulted in full stop of the blood inflow into own liver. The pig was prepared for perfusion by inserting cannula into aorta through the iliac artery, and into the inferior vena cava through the iliac vein. The cannulae were then connected with drains leading to the perfused liver present in a container.

Preparation of liver for perfusion. The liver was removed under sterile conditions from a pig anesthetized with eunarcon. Blood was rinsed out from liver vessels through the portal vein using 10—12 of Ringer solution (temp. 37°C) which container 50 ml/l of heparin and 300,000 u/l of penicillin, buffered to pH 7·8 with sodium bicarbonate. Mean time of liver ischemia, i.e. from the stop of the unflow of own blood and beginning of rinsing to the connection with recipient's circulation, was 15—20 min.

Scheme of the perfusion system (Fig. 1). During perfusion the blood was flowing from the aorta of recipient pig into isolated liver through a drain which just before the container was ramified: one branch led to the hepatic artery, the second one to the portal vein. Blood coming out from the liver through a tube inserted into the hepatic veins was flowing by force of gravity into the iliac vein of the pig. The container was placed 10 cm above the level of pig's inferior vena cava. Bile was collected into a calibrated vessel with a separate drain introduced into the common bile duct. The container was filled up with the fluid (No. 1/POLFA) of the temperature 39°C used for peritoneal dialysis. Constant temperature of the fluid was guaranteed by a thermostate.

The perfusion was carried out after previous administration to the pig of heparin (4 mg/kg). During the perfusion the blood pressure was measured in the carotid artery of the pig; in the liver placed in the container the blood pressure was measured in hepatic artery and portal vein. Blood outflow from the liver was also estimated. Besides, oxygen content, pH and pCO_2 were determined in the blood flowing into and outflowing from the isolated liver. The amount of

secreted bile and its bilirubin concentration were measured. Ammonium level was determined in the arterial and venous blood of the pig in the 5th, 10th, 15th, 30th, 45th min after the loading with ammonium citrate in a dose 30 mg/kg of body weight. In the same blood samples BSP retention in % in the 2nd, 5th, 10th, 15th, 30th min after intravenous administration of a dose 5 mg/kg was estimated. Prior to and after the perfusion hematocrit, serum SGOT, SGPT and alkaline phosphatase activities, creatinine, urea and protein levels were also determined.

The same biochemical determinations were performed in group II.

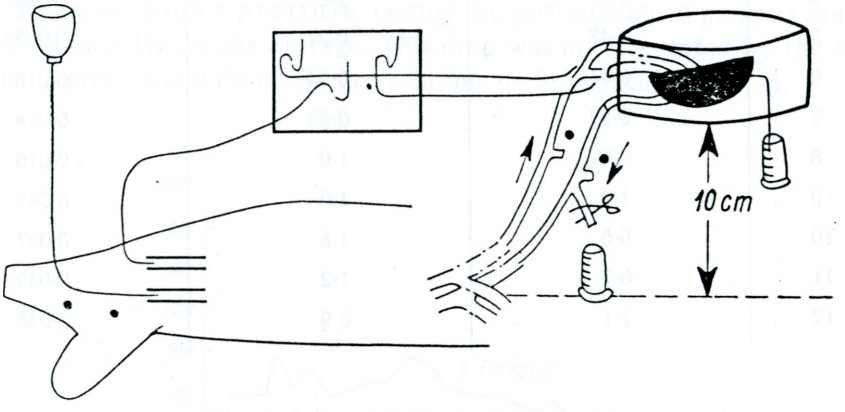

Fig. 1. Scheme of the perfusion system.

RESULTS

Group I. The perfusion lasted $2^1/_2$ hours on the average (2—4 hours) and its duration depended upon the condition of animal during experiment.

A. Function of perfused liver

a). Blood flow. The blood flow ranged from 0·27 ml/g of liver/min, mean value being 0·94 (Table I). The flow mostly depended upon the arterial blood pressure of the pig. The ratio of blood supplied to the isolated liver through hepatic artery and portal vein was 1 : 4. Blood pressure in the hepatic artery in the container ranged from 110/80 to 60/40 mm Hg, but in the portal vein from 15 to 5 mm Hg.

b). Liver oxygen consumption. Oxygen consumption ranged from 0·01 to 0·1 ml/g of liver/min, the mean value being 0·041 (Table I). It was generally dependent upon the blood flow rate through the liver.

c). Bile secretion. It ranged from 0·61 to 2·3 ml/100 g of liver/hour, the mean value being 1·15 ml. It was related, first of all, to the liver oxygen

Table I

Mean amounts of secreted bile, blood flow and oxygen consumption by *ex vivo* liver perfused for $2^1/_2$ hours

No. of experiment	Bile ml/100 g/hour	Blood flow ml/g/min	O_2 consumption ml/g/min
1	0·73	0·58	0·043
2	1·1	0·5	0·047
3	1·85	0·42	0·059
4	1·8	1·0	0·088
5	1·43	0·37	0·052
6	0·61	0·44	0·018
7	0·93	0·62	0·018
8	0·87	1·0	0·016
9	1·0	1·0	0·042
10	0·5	1·8	0·027
11	0·7	1·2	0·049
12	1·1	0·9	0·045

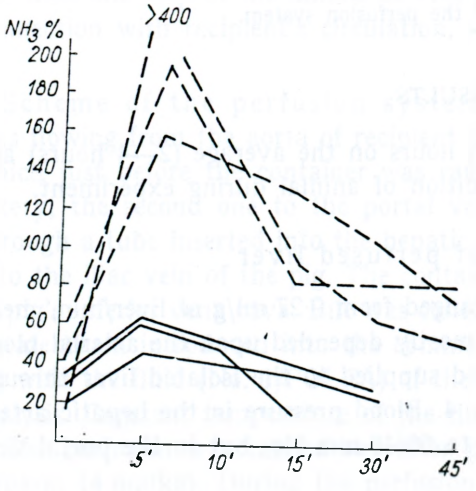

Fig. 2.

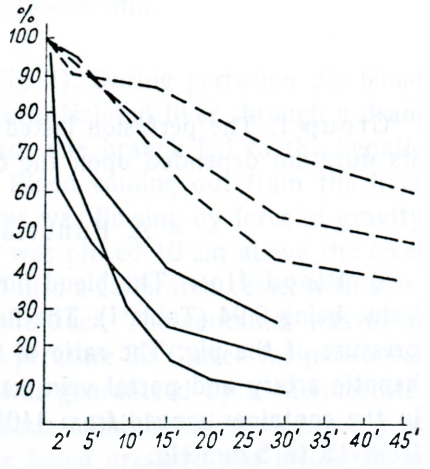

Fig. 3.

Fig. 2. Venous blood ammonium level during the first 45 min after intravenous loading with 30 mg/kg of ammonium citrate in 3 animals perfusing the extracorporeal liver (continuous line) and 3 animals of the control group (dashed line).

Fig. 3. Percentage of BSP retention in venous blood in the first 45 min after intravenous loading with 5 mg/kg. Continuous line indicates animals with extracorporeal liver, the dashed one — controls.

consumption and blood flow rate. Bile bilirubin concentration ranged from 2·0 to 22·2 mg%, 11·9 mg% on the average.

d). Ammonium and BSP clearance. Blood ammonium level returned to initial values 15 min after the loading with ammonium citrate (Fig. 2). BSP retention after 30 min was from 11 to 33% (Fig. 3). Ammonium and BSP clearance was of equal rapidity in all experiments. There was obvious dependence upon the liver blood flow rate and oxygen consumption.

B. Changes found in the pig which perfunded the extracorporeal liver

a). Arterial blood pressure. During the perfusion blood pressure dropped by 15—20 mm Hg on the average. This drop was not prevented by the transfusion of great amounts of Ringer solution or 5% glucose (Fig. 4).

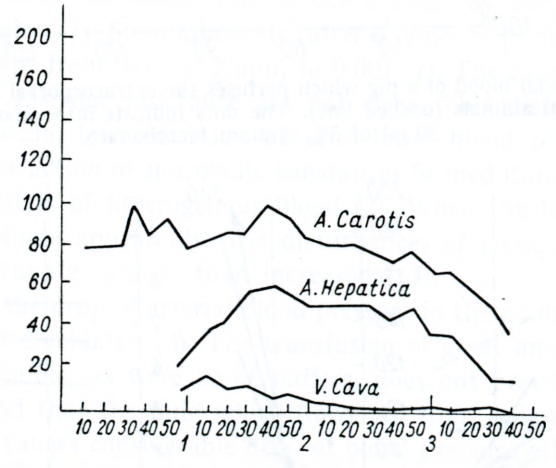

Fig. 4. Blood pressure in carotid artery, hepatic artery and portal vein in a pig during the first 3 hours of perfusion. Steady decrease of pressure.

b). Acid-base balance. Thirty minutes after beginning of the perfusion pH already dropped below 7·3, carbon dioxide partial pressure being 38—45 mm Hg. This required frequent intravenous administration of sodium bicarbonate in a general amount of 2·5—10 g (Fig. 5).

c). Hematocrit, serum protein, SGOT, SGPT and alkaline phosphatase. Within $2^1/_2$-hour perfusion hematocrit of venous blood dropped by 3—6%. Serum protein level decreased in the same time by 25—60% (Fig. 6). SGOT level markedly rose, the activity of alkaline phosphatase remained unchanged.

Group II (control). During 3-hour experiment arterial blood pressure negligibly decreased, 10 mm Hg/hour on the average. pH of arterial blood was within normal range and dropped only after the 3rd hour of experiment (Fig. 5).

Serum protein GOT and alkaline phosphatase levels, as well as hematocrit, were normal. Ammonium level in venous blood 5 minutes after the load with ammonium citrate was more than 150 γ%, and after 45 min markedly above the norm (Fig. 2). BSP retention after 45 min was more than 40% (Fig. 3).

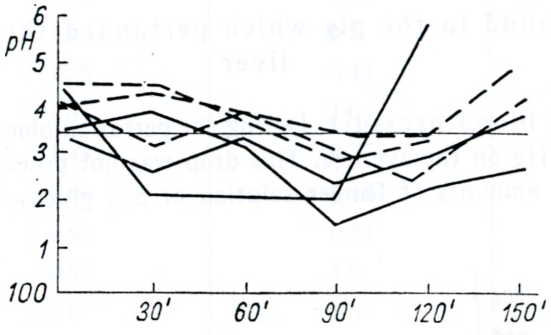

Fig. 5. pH of arterial blood of a pig which perfuses the extracorporeal liver (continuous line and of the control animals (dashed line). The dots indicate intravenous administration of) 30 ml of 5% sodium bicarbonate.

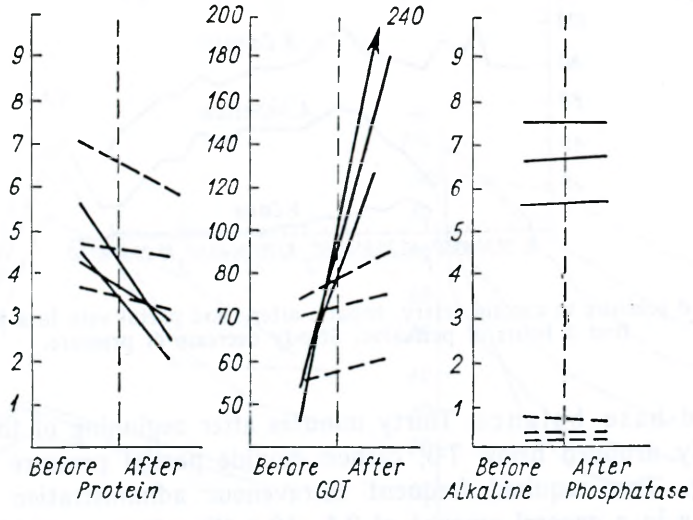

Fig. 6. Serum protein level, SGOT and alkaline phosphatase activities before and after liver perfusion. Dashed line refers to control animals.

DISCUSSION

Extracorporeal liver perfusion is used in the treatment of patients in the state of hepatic coma (1, 12). Wide clinical use of this method is, however limited, both because of technical reasons and hemodynamic disturbances,

occurring during the procedure. The latter comprise: 1) gradually increasing liver vascular resistance resulting in small venous outflow, 2) necessity of maintaining constant temperature of liver and blood which flows through it, 3) greatest possible abbreviation of the period liver ischemia during removal of the liver, 4) drop of arterial blood pressure and metabolic acidosis occurring in the perfusing animal.

The results of experimental studies indicate that the blood flow through isolated liver should be from 0·75 to 1·0 ml/g of liver/min (4, 13, 14). When the blood flow is below 0·5 ml/g/min the liver function is considerably impaired. Decreased flow through hepatic artery results in diminished bile secretion, diminished bilirubin clearance (14) and diminished blood lactic acid clearance (14, 14). Bile secretion is entirely stopped at the blood flow lower than 0·2 ml/min (10, 14). Reduced portal vein flow leads to disturbances in ammonium clearance (14).

Liver vascular resistance gradually rises during perfusion (10). This results in the diminution of the blood flow on the average by 30% during the first 5 hours of perfusion (7). Simultaneously, after 6 hours of perfusion liver oxygen consumption drops from 0·03 ml/g/min, to 0·007 (7). The causes of the steadily increasing liver vascular resistance are not clear. This disturbance seems to result from hypoxia, lowered blood pH and lower blood temperature (4) or, may be, from the action of spasmodic substances formed during the disintegration of the platelets of heterogenous blood (2). When the blood temperature is below 38°C, there appear also the disturbances of lactic acid metabolism. The lactate: pyruvate ratio is than increased (14).

The causes of the drop of arterial blood pressure in the animal which perfuses the liver are not elucidated (3). The transfusion of great amounts of blood or blood-replacing fluids, as were as of buffers, does not prevent this drop (8). It has been found that the fluid which flows out from isolated liver, ischemic for 30—60 min, causes considerable drop of blood pressure after transfusion to another animal (8) in spite of the fact that it is sterile and with only slightly lower pH.

The majority of liver-perfusing animals develop metabolic acidosis (2, 5, 11), particularly when the hepatic blood flow is below 0·5 ml/g/min. Acidosis increases hepatic vascular resistance and reduces the blood flow. Therefore, blood must be rinsed out from the liver taken for perfusion with the fluid of pH about 8·0, and the buffers administered to animal prior to and during perfusion (9).

In order to maintain great blood flow through extracorporeal liver we directed the blood directly from the iliac artery or aorta. Therefore, the mean flow was 0·9 ml/g/min. The flow through hepatic artery depended upon the arterial blood pressure. This concerned the portal blood flow to a lesser degree. In the majority of cases peripheral portions of the liver were ischemic and gradually became livid-red even when the blood flow was higher than 1 ml/g/min. When

the blood flow was within the range 0·5—1·0 ml/g/min the liver oxygen consumption was proportional to the blood flow. Bile secretion was proportional to the oxygen consumption and the blood flow. In contrast full clearance of ammonium occurred during 15 minutes, even when the blood flow was very low. BSP was removed from the blood at similar rate. All animals showed steady decrease of blood pressure during perfusion. First episode of blood pressure drop lasting a few minutes occurred after the beginning of perfusion. In the 2nd and 3rd hour of perfusion a second slow decrease of blood pressure occurred associated with the symptoms of metabolic acidosis. The transfusion of great amounts of blood and blood-replacing fluids as well as of sodium bicarbonate did not prevent the fall of blood pressure. Both the decreased blood pressure and acidosis occurred first of all in animals with blood flow below 0·5 ml/g/min. In such cases the liver was livid-red, mottled, its cut surface did not bleed. Such changes were observed when the period of liver ischemia prior to perfusion was longer than 30—40 min.

CONCLUSIONS

1. In the first 3 hours of perfusion bile secretion, oxygen consumption and blood flow through isolated pig liver perfused with arterial blood are close to the values generally accepted as the normal ones.

2. Total clearance of exogenous ammonium from the blood occurs within 30 min, the BSP retention does not exceed 30% after 20 min. The rate of ammonium and BSP clearance does not depend upon the blood flow.

3. The perfusing animal gradually developed arterial hypotension and metabolic acidosis. This happened as a rule when the blood flow was below 0·5 ml/g/min, and the oxygen consumption below 0·03 ml/g/min. This was associated with lowered blood serum protein level and rise of SGPT activities.

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