REVISTA ESPAÑOLA DE FISIOLOGIA, 29, 27-32. 1973

Extracorporeal Hepatic Perfusion With Isolated Dog Liver

H. Ortiz-Hurtado, J. Teijeira, J. Goiti, L. González, J. Voltas and C. M. G. Durán

Departamento de Cirugía
Facultad de Medicina y Clínica Universitaria
Universidad de Navarra
Pamplona (Spain)

(Received on 20 October 1972)

H. ORTIZ-HURTADO, J. TEIJEIRA, J. GOITI, L. GONZALEZ, J. VOLTAS and C. M. G. DURAN. *Extracorporeal Hepatic Perfusión with Isolated Dog Liver.* Rev. esp. Fisiol., 29, 27-32. 1973.

Extracorporeal liver perfusion was carried out in 36 experiments. The isolated dog liver was used. Liver function during the experiments was measured with three parameters: B.S.F. clearance, oxigen consumption and biliary output. Six different types of perfusion modifications were introduced. At the end of each experiment, the microscopic appearance of the liver was studied.

The results showed that the viability of the dog liver under our conditions of perfusion was 60-120 minutes. If the period of cold ischemia was avoided, the viability was prolongued to 240-300 minutes.

The use of extracorporeal liver perfusion as a support treatment in acute liver failure, is based on the experimental work of OTTO *et al.* (9). These authors used the extracorporeal perfusion of a dog liver to reduce the rise in ammonia induced in a dog with an Eck fistula. They observed that the perfused isolated liver, metabolized ammonia, had a billiary output and cleared Bromosulphataleine (BSF). Clinically it has been applied since 1967.

Whithin the field of liver extracorporeal perfusion, one of the observations reported by several authors is the difficulty encountered when the dog is selected as the recipient animal. Whether a homologous (10) or heterologous (3, 5, 6) liver is used, the cause for this difficulty has not yet been established.

The present experimental work was carried out with the aim to study the behaviour of the perfused isolated dog liver in an attempt to elucidate the above problem.

Materials and Methods

36 adult mongrel dogs weighing between 20 and 25 kg were used. The day before operation 1,500 ml of 5 % glucose in water were administered.

General anaesthesia was induced with Thyopentone (20 mg/kg), Flaxedil (3 mg per kg) and Phenergan (1 mg/kg). The animals were intubated and ventilated with

a Bird Mark 8 respirator with a 320 ml per minute flow of oxygen (40 %) and air (60 %). During surgery 500 ml of 5 % glucose in saline were administered. Sodium bicarbonate was given according to the measured blood *p*H.

In 24 animals the operation was a standard hepatectomy (7). In the other 12 animals, the liver blood vessels were cannulated within the animal without sectioning the hepatic ligaments. The afferent cannula was placed in the supradiaphragmatic inferior vena cava through an 8th intercostal space thoracotomy.

In 30 experiments the liver was mantained under hypothermic ischaemia (20° C) for 60 minutes before starting the perfusion. In the other six experiments, the perfusion was started as soon as the portal vein was cannulated.

The perfusion circuit represented in Figure 1 (upper) was used in the first 6 experiments and in the other 30 the circuit is shown in Figure 1 (lower). In both circuits a Kay-Cross oxygenator and two Sygmamotor TM2 pumps were used. The perfusion chamber was similar to that described by ABOUNA (1). The total primming volume was 1,600 ml. The composition, minute flow and pressures are described elsewhere (7).

In 30 experiments the following parameters were studied every 20 minutes: Arterial and venous *p*H, excess base (BE), pCO_2, pO_2 and oxygen consumption. In the other 6 experiments the same parameters were collected every hour. The Siggaard-Andersen technique (11, 12) was used with a *p*H meter PHM 27. Every hour, BSF retention was determined with a Zeiss PMQ II photocolorimeter. At the end of each experiment a liver section was taken for microscopy.

Results

The 36 animals were divided in 6 groups of 6 experiments each:

Series I. The liver was extracted from the donor and perfused with the circuit described in Figure 1 (lower). Acid Base balance was determined every hour.

The results obtained are represented in Figure 2. BSF retention was mantained within normal limits in the first 60 minutes of perfusion. The oxygen consumption suggested an adequate function of the hepatocite during that period of time. After 90 minutes of perfusion both parameters followed a trend incompatible with a nor-

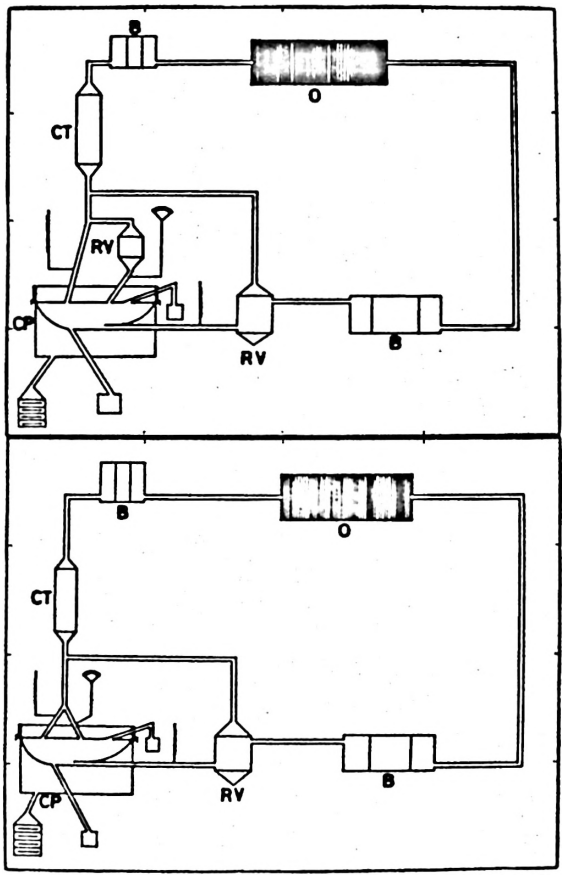

Fig. 1. *Diagram of the perfusion apparatus.* CP: Perfusion chamber. O: Oxygenator. B: Pump. CT: Heat exchanger. RV: Venous reservoir. The upper circuit makes possible the separation of the afferent flow to the liver. A portal line reservoir results in a continous venous flow.

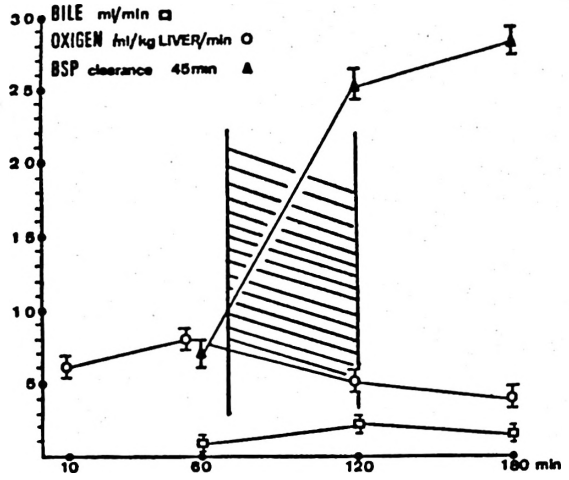

Fig. 2. *Isolated dog's liver perfussion.*
Series I: The shaded area corresponds to the established limits of viability.

mal liver function and after 120 minutes they were incompatible with life. During the 2 hours of the experiment no important changes in billiary secretion were detected.

Macroscopically the liver presented in all experiments a progressive and not homogenous change in colour. Patchy dark coloured areas appeared on the liver surface. The microscopy of these areas showed the presence of platelet aggregates that blocked the lumen of the afferent vein at the portal space and sinusoids.

Series II. Every 20 minutes the blood *p*H was corrected and the heparinization doses were increased 3 times in an attempt to correct the metabolic acidosis and the platelet aggregates observed in the previous series.

The results did not show any significant change from those in the first series. There was no acidosis but the platelet aggregates were present.

Series III. The histological controls of the two previous groups did not show any alteration in the vascular tree. However, assuming a possible mechanical repercusion of the initial high pump flow it was decreased to 0.1 ml/g/minute. No difference with the previous series was detected.

Series IV. These experiments were performed with the circuit represented in Figure 1 (upper). It was devised to observe the influence of a continous afferent venous flow on the organ viability.

The data obtained were better than those with the initial circuit and the time limit of viability was prolonged to 90-120 minutes.

Series V. The liver was not extracted from the donor and was perfused without any previous hypothermic ischaemic period in order to determine the influence of ischaemia on the organ viability. As function parameters the billiary secretion and oxygen consumption were considered. The degree of BSF retention could not be used as it was impossible to measure the BSF present in the ascitic fluid accumulated in the peritoneal cavity.

The results presented in Figure 3 showed a progressive rise in the oxygen con-

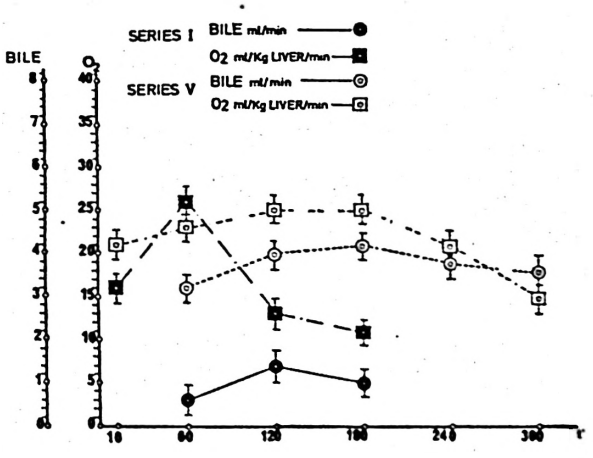

Fig. 3. *Comparative results between series I and series V.*
In the series V the liver was not extracted from the donor and was perfused without any previous hypothermic ischaemic period.

sumption during the first three hours of perfusion, maximal in the second hour, and only returning to the basal values at the fourth hour.

The billiary secretion increased progressively with the highest values on the third hour and starting to decrease gradually between the forth and fifth hour.

The macroscopic appearence of the liver was similar to the observed in the previous perfusions. However the colour change started at 120 minutes and progressed more slowly. After five hours it was similar to the pattern observed after two hours of the previous perfusion technique.

The limit of viability for this technique, determined by the already established parameters, could be fixed between three and four hours.

Series VI. The *in situ* perfused liver was subjected to a period of ischaemic hypothermia. The aim of these series was to determine the influence between ischaemia and the liver surrounding medium on the organ viability.

The oxigen consumption data showed no significant change between this series and the first one. The billiary secretion however was higher in the first two hours.

Discussion

With the technique and functional parameters used in this study it can be stated that the viability limit of an isolated perfused dog liver is 90 minutes. These results are similar to those reported by BELINSKAYA (2). This author using histological controls found that after 120 minutes of perfusion, circulatory changes, parenchymal oedema and hepatic cell necrosis appeared.

There are a large number of factors that might influence unfavorably the viability of the isolated organ. However in our study the decisive factor has been the hypothermic ischaemic period. When avoided, it raised significantly the time of organ viability. Our results differ from those of VAN WICK (15) who mantained livers after two hours of hypothermic ischaemia. This discrepancy can be explained by the species difference since when we have used pig livers our results have been similar to those of this author (8).

The cause for the limited viability of the dog liver with our perfusion system cannot be determined exactly. It might be related to the sphincter mechanism present in the afferent vascular system of the dog liver (4, 14). In this animal a higher tendency to closure when under hypothermic ischaemia has been widely documented in organ transplantation (13).

Our results seem to indicate that the failure of the homologous perfusion in dogs with hepatic failure, reported by some authors (10) might not be related to an immunological problem. Instead it could be due to the passage to the receptor of acid metabolites from the necrosis of the perfused organ.

References

1. ABOUNA, G. M.: *Lancet*, **2**, 1216, 1968.
2. BELINSKAYA, A. M., PLIKH, M. B., GONGHAROV, A. I.: *Eksper Khir. Anestesiol.*, **4**, 13, 1970.
3. BIBLER, D. D., CONDON, R. E., NYHUS, L. M.: *Surg. Forum*, **16**, 286, 1965.
4. DEYSACH, L. J.: *Amer. J. Physiol.*, **132**, 713, 1941.
5. DRAPANAS, T., ZEMEL, R., VANG, J. O.: *Ann. Surg.*, **164**, 522, 1966.
6. HAM, J., PIROLA, R. C., DAVIDSON, G. M., YARROW, B., ELMSLIE, R. G.: *Surg. Gynec. Obst.*, **127**, 543, 1968.
7. ORTIZ-HURTADO, H., ZORNOZA, G., PÉREZ-CABAÑAS, I., MARTÍNEZ-LECEA, F., FIDALGO, B., VOLTAS, J.: *Rev. Med. Univ. Navarra*, **15**, 33, 1971.
8. ORTIZ-HURTADO, H., FIDALGO, B.: *Rev. Clinc. Esp.* (In press.)
9. OTTO, J. J., POMDER, J. C., CLEARY, J. H., SENSENIG, D., WELCH, C. J.: *Surgery*, **43**, 301, 1958.

10. SEN, P. K., BHALERAO, R. A., PARULKER, G. P., SAMSI, A. B., KINARE, S. G.: *Surgery*, 59, 774, 1966.
11. SIGGAARD-ANDERSEN, O., ENGEL, K.: *Scand. J. Clin. Lab. Invest.*, 12, 177, 1960.
12. SIGGAARD-ANDERSEN, O.: *Scand. J. Clin. Lab. Invest.*, 14, 598, 1962.
13. STARZL, T. E.: «Donor hepatectomy and liver preservation». «Hepatic Transplantation», Saunders Co. Philadelphie, 1969, p. 41.
14. THOMAS, W. D., ESSEX, H. E.: *Amer. J. Physiol.*, 158, 303, 1949.
15. VAN WICK, J., TAIT, J. B., EISEMAN, B.: *Surgery*, 50, 374, 1965.