Liver Function in the Pig

TOTAL HEPATIC AND PORTAL FLOW VALUES *IN VIVO*

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SUMMARY

The assessment of function of the isolated perfused liver remains complex. Much of this problem relates to an inability to compare function *in vitro* with that *in vivo*, because of a lack of knowledge of hepatic blood flow. This article documents measurement of total hepatic and portal blood flow *in vivo* in pigs, by means of the dye clearance method. The effects of starvation, glucose administration and anaesthesia are noted.

Although these techniques are simple and may be performed in the awake animal, there are wide variations in values which would necessitate individual flow readings being obtained for any comparative study.

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Use of the isolated perfused liver in the treatment of liver failure has lost favour because of its inadequate function and potential hazard.[1-3] Despite this, there are few studies which emphasise the functional deficit of the isolated liver in comparison with the *in vivo* state.[4,5] Assessment of transhepatic metabolism requires individual values for portal and arterial contribution to total flow, and while these values are readily available in the isolated perfused liver, there is little information on the distribution of hepatic blood flow *in vivo*, especially in the pig. In one study, Tygstrup *et al.*[6] measured total hepatic blood flow in 11 anaesthetised pigs, using the indocyanine green infusion-extraction method, and the electromagnetic flowmeter cuff for portal flow determinations.

In the present study, the bromsulphthalein (BSP) or indocyanine green (ICG) clearance was used to measure total hepatic blood flow, and para-amino-hippurate (PAH) infusion to determine the portal flow as described by Schambye[7] and modified by Katz and Bergman.[8] The combination of these methods has not previously been used in the pig.

Since function of the isolated perfused liver may be influenced by starvation, previous anaesthesia during removal from the animal, and the intra-operative administration of glucose rather than saline, the effect of these factors upon blood flow *in vivo* in the pig was also studied.

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MATERIALS AND METHODS

Operative Procedure

Twenty-two young pigs of either sex, weighing 18 to 22 kg, were anaesthetised after a 24-hour starvation period. Anaesthesia was induced with thiopentone sodium (total dose 2 - 3 mg/kg) injected into an ear vein. A cuffed endotracheal tube was passed and anaesthesia was maintained with oxygen and nitrous oxide given via a Magill-type circuit. The animals were allowed to breathe spontaneously to avoid possible interference by intermittent positive pressure ventilation with portal flow as described by Rabinovici.[9]

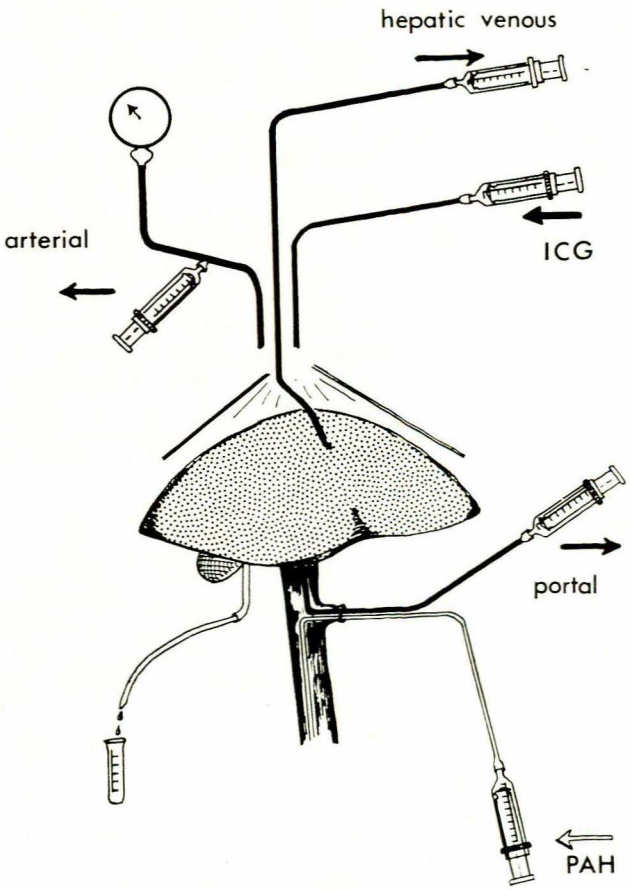

Fig. 1. The surgical preparation, showing positions of intravenous catheters. The direction of the arrows indicates the infusion or withdrawal of dye or blood samples.

Under clean but not sterile conditions, the animals were prepared for blood flow measurement (Fig. 1). Catheters were inserted into the right carotid artery for pressure measurement and arterial sampling, and into the right internal jugular vein for the administration of fluids and BSP or ICG. A catheter was introduced into the right external jugular vein and was manipulated into a hepatic vein (usually the left) by intra-abdominal palpation. With small pigs, the catheter could almost always be felt within the substance of the liver, and its position was confirmed by the appearance of a bloodless patch in the liver upon rapid infusion of saline. The catheter was then withdrawn 1 - 2 cm to prevent wedging.

The bile duct was catheterised and the cystic duct ligated. In order not to disturb the portal circulation, a small tributary draining the pancreas was used for two portal catheters. One, for sampling, was positioned at the hilum of the liver, 2 - 3 cm beyond the junction of the last tributary, and the other, for infusion of PAH, was sited 4 - 6 cm down the portal vein into the superior mesenteric vein, against the flow to ensure mixing with blood of intestinal and splenic origin. No pancreatic oedema or infarction was noted.

In 15 animals in groups I and II, anaesthesia was maintained and dye infusion begun as soon as the preparation was complete; in the 7 animals in group III, the abdominal and neck wounds were repaired with a single layer of nylon and the intra-abdominal catheters exteriorised through stab wounds in the lateral abdominal wall. The anaesthetic was discontinued and the animals were fully awake within 15 - 20 minutes; testing began 30 minutes after recovery. Total operating time was between 60 and 90 minutes in all cases.

Between 200 and 300 ml of intravenous fluid was given during the operation; in group I, saline was used, and in groups II and III, 10% invert sugar in Ringer's lactate. In addition, animals in groups II and III received a bolus of 25 g intravenous dextrose at the commencement of operation, in an attempt to replace glycogen lost during starvation. All animals were given 20 mEq sodium bicarbonate to counteract the mild metabolic acidosis which occurred after induction (base deficit 5 - 7 mEq/litre), and heparin 2 mg/kg to prevent clotting in the catheters.

Blood Flow Measurement

Total hepatic blood flow was measured by clearance of BSP or ICG. Bromsulphthalein was used initially but was replaced by ICG when no further supplies could be obtained. Preliminary studies showed similar results with the two dyes. The priming dose of BSP was 300 mg and infusion was continued at the rate of 5 mg/min. The priming dose of ICG was 2,5 mg, followed by infusion at 0,156 mg/min. Portal flow was measured by using PAH with a priming dose of 12,5 mg and subsequent infusion at 72 mg/min.

In groups I and II, when operative preparation was complete, the priming doses of dyes were given, and the constant infusion commenced for 30 minutes. During this time, three arterial samples were taken at 10-minute intervals to ensure stabilisation of blood levels. Thereafter infusion was continued for a further 30 minutes and simultaneous transhepatic samples were taken from the arterial, portal and hepatic venous catheters at 10-minute intervals. In group III, the dye infusion was commenced 30 minutes after discontinuation of the anaesthetic.

At the end of all experiments, the animals were sacrificed and the livers weighed after draining for a standard period of 15 minutes.[10]

Biochemical Analyses

Blood samples were analysed for BSP,[11] ICG,[12] and PAH[13,14] and no interference was found one with another. Acid-base status, glucose, osmolality and sodium and potassium were measured by standard techniques.[15] Individual flow values for each experiment were a mean of the three values obtained during the second 30-minute period, and for each group of experiments the mean of these individual values was taken. The final results were expressed in relation to liver weight.

RESULTS

Total and Portal Flow

The results of blood flow analyses in the 3 groups are shown in Table I. Mean total hepatic blood flow in starved anaesthetised animals given sodium chloride intra-operatively was 0,89 ± 0,01 ml/g/min. Administration of glucose increased this to 1,02 ± 0,03 ml/g/min ($P<0,001$). Under these conditions, there was no further significant increase in blood flow in the immediate postanaesthetic period (group III)—mean total flow 1,17 ± 0,07 ml/g/min. There was no significant change in the portal contribution of any group—this ranged from 74 ± 5% to 80 ± 2% of total flow.

TABLE I. MEAN TOTAL HEPATIC BLOOD FLOW RATES AND PORTAL CONTRIBUTION IN 3 GROUPS OF STARVED, ANAESTHETISED PIGS

	Hepatic blood flow	
	Total (ml/g/min)	Portal (%)
Group I (8 animals) 0,9% sodium chloride	**0,89 ± 0,01**	**74 ± 5**
Group II (7 animals) Glucose—25 g bolus plus 10% invert sugar in Ringer's lactate	**1,02 ± 0,03**	**80 ± 2**
Group III (7 animals) Glucose 25 g bolus plus 10% invert sugar in Ringer's lactate. (Awake for ½ h before study.)	**1,17 ± 0,07**	**77 ± 4**

Mean values are given with standard error of the mean. In total flow measurement, differences between groups I and II, and I and III are significant ($P<0,01$), but there is no significant difference between values in groups II and III, or in the portal contribution by any group.

Mean systemic arterial blood pressure was maintained between 90 and 110 mmHg and mean portal pressure ranged between 6 and 8 cmH_2O.

Acid-Base Studies

Mean pH values ranged from 7,30 to 7,35. Anaesthetised pigs breathing spontaneously frequently develop a respiratory acidosis, which probably contributed to the low pH. There was a minimal metabolic acidosis (mean base deficit 1 - 2 mEq/litre).

Glucose

The mean blood glucose levels through these studies are shown in Fig. 2, with the mean transhepatic difference shown after the first 30-minute study period. There was a significant release of glucose from the liver in animals in groups I and II, and in the initial period of study of group III. The terminal samples in group III showed a tendency for glucose uptake by the liver, although this was not significant.

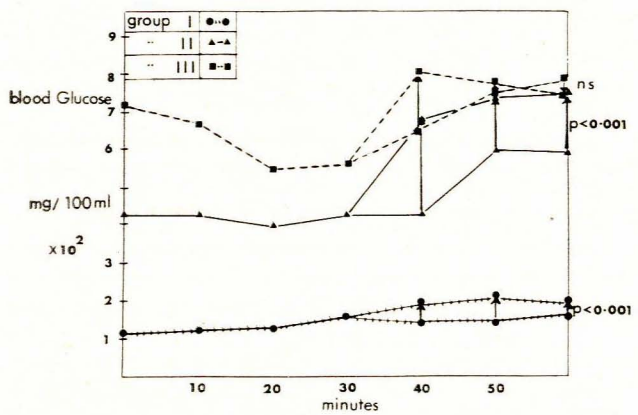

Fig. 2. The mean levels of osmolality in three groups of animals during measurement of blood flow. Mean arterial levels are shown for the first 30 minutes, and thereafter transhepatic values are shown. The direction of increase across the liver into the hepatic venous blood is indicated by the arrows.

Osmolality

The mean plasma osmolality values are shown in Fig. 3. There was a significant increase in osmolality in animals to which glucose was given and also a highly significant transhepatic increase in osmolality in these animals ($P<$ 0,001).

Other Observations

The amount of BSP required to attain stable blood levels in these pigs was greater than that recommended

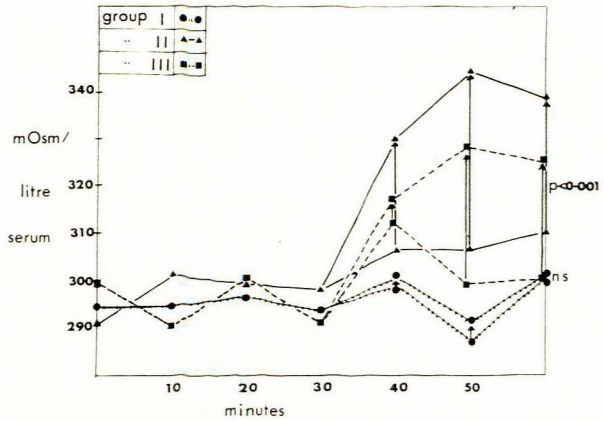

Fig. 3. The mean levels of blood glucose in three groups of animals during measurement of blood flow. Mean arterial levels are shown for the first 30 minutes, and thereafter transhepatic values are shown. The direction of change across the liver into the hepatic venous blood is indicated by the arrows.

for dogs of similar weight. In preliminary studies, when the dose of 100 mg was used, the first samples 10 minutes after injection showed a total plasma disappearance of the dye with appearance in the bile. Only by increasing the concentration to 300 mg could a measurable amount be constantly detected in the plasma.

In all instances when BSP was given, the volumes of samples collected at 15-minute intervals were increased to twice perfusion values. This was not observed with ICG administration. Bromsulphthalein was macroscopically obvious in the bile within 5 minutes of administration of the priming dose, but ICG was only obvious 15 - 20 minutes after first administration.

DISCUSSION

Liver blood flow has been measured throughout this century[16,17] but there are so many variables that it is probably essential to perform individual measurements in each experiment for accuracy. Hence a simple, non-invasive technique is needed.

Total transhepatic blood flow may be reliably determined by direct dye dilution techniques as used in this study. Portal flow has been measured in dogs using thermal or chemical methods[10,18] and with the electromagnetic flowmeter.[3,19] While the latter would seem ideal, there is the hazard of minimal portal vein occlusion if the cuff is to fit well. In addition, use in non-anaesthetised animals is hampered by variations with movement.

The values for total flow and portal contribution as documented in this study are in accordance with some values reported by others[3,18] but widely divergent reports exist;[5,20] portal to hepatic arterial interrelationships are said to vary widely with different circumstances[21-23] and autoregulation of total flow has also been described.[23,24] Two variables of importance in comparison of the isolated perfused liver with the *in vivo* state were investi-

gated in this study, viz. anaesthesia and the effect of glucose administration.

In the present study, no significant increase was noted in total or portal flow in the 90 minutes of study after anaesthesia with nitrous oxide and oxygen. Sodium thiopentone was used for induction in preference to halothane to avoid the reported decrease in splanchnic circulation caused by this agent.[25] Injection of sodium pentobarbital to dogs during blood flow studies has been reported to cause no effect.[26]

An increase in total hepatic blood flow in animals given intravenous glucose may have been on an osmotic or a metabolic basis, or the result of increased cardiac output. There was a transhepatic increase in osmolality in the two groups in which glucose was given, which differed significantly from values in animals given sodium chloride. It has been suggested that osmoreceptors exist in the portal system as a defence against fluid overload,[27] but there is little other information on portal plasma osmolality, despite possible variations during absorption.

In all three groups of animals, a transhepatic release of glucose was observed, though uptake would have been expected following starvation. Only in the last samples in the awake animals (group III) was there a trend towards hepatic uptake of glucose. Carbohydrate metabolism during and following anaesthesia has recently been extensively studied and reviewed.[28]

The capacity of the *in vivo* pig liver to clear BSP has been previously described[29] and requires further investigation. The higher dose required should be borne in mind when BSP is used as a measure of hepatic excretory function in the pig or *in vitro*.[30] The capacity may be even greater in the non-anaesthetised animal, since it has been suggested that thiopentone anaesthesia reduces biliary excretion of BSP.[21]

CONCLUSION

These studies were performed to investigate methods for study of total hepatic blood flow and the portal contribution in the pig as a baseline for comparative evaluation of the functional deficits of the isolated perfused pig liver. The use of two dyes to determine these values gave results in accord with those reported elsewhere, and the hazard of portal venous occlusion by an electromagnetic flowmeter cuff was avoided. In this model, the increase in total hepatic flow which was associated with glucose administration may have been on an osmotic or a metabolic basis, or the result of increased cardiac output. There was no apparent increase in flow during the initial period after anaesthesia.

These studies form an adequate baseline for evaluation of transhepatic function *in vivo* for comparison with the isolated perfused liver. With the variables involved, individual measurements should be made for each experiment and no generalisation can be made.

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