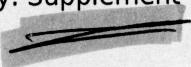


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The Function of the Isolated Perfused and the in Vivo Pig Liver

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The function of isolated perfused livers was compared with that of in vivo pig livers, studied by hepatic venous catheterization during halothane anaesthesia. In relation to the in vivo liver the isolated, perfused liver had an oxygen uptake of 50 per cent, the same ATP/ADP ratio, and a lower lactate/pyruvate ratio in hepatic venous blood. The galactose elimination rate was about 70 per cent. The bile flow was 50 per cent lower than in the in vivo pigs, but both uptake and excretion of Indocyanine Green were almost two times greater.

Key-words: Bile; galactose elimination; hepatic venous catheterization; Indocyanine Green; liver function; liver perfusion; oxydative metabolism

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Treatment of acute liver failure with pig liver perfusion is of great theoretical and practical interest, but so far the clinical results often have been disappointing (7, 8, 16). One reason for this may be that the isolated, perfused liver, due to unphysiologic working conditions, is in itself a failing organ and therefore incapable of supporting the patient with hepatic failure.

The aim of the present work was to compare the function of the isolated perfused pig liver with the liver function of anaesthetized pigs, studied by hepatic venous catheterization, in order to evaluate the functional integrity of the perfused liver.

MATERIAL AND METHODS

Preparation of the isolated pig liver. Pigs of Danish Landrace, weighing 40-55 kg, were fasted for 24 hours, with free access to water.

They were premedicated by 2 mg/kg of Sernylan® and brought to the operating theatre, where anaesthesia with Halothane® (0.8 per cent) and N₂O (70 per cent) in oxygen was induced. Tracheotomy was performed, and the abdomen was opened by a midline incision. The vessels of the liver were isolated, a tube (6 mm outer diameter) was introduced into the portal vein, and an infusion of oxygenated, heparinized (1500 units per litre), cold (10-15°C) Ringer-lactate solution was started at a pressure of 15 mm Hg. The vessels and the common duct were cut, and the liver was transferred to a 'liver bed', where the flushing was continued for another 8 to 10 minutes, at a rate of about 1 litre per minute. The common duct was cannulated, and the cystic duct ligated. A catheter (3 to 4 mm outer diameter) was inserted into the hepatic artery which was flushed with heparin-saline and clamped. A fenestrated, rigid tube (12 mm outer diameter)

Table I. Isolated perfused pig liver. Functional data of perfused pig livers. Mean values of observations made during the second hour of perfusion

Exp. No.	Liver weight Kg	Perfusion rate litres/min	Oxygen uptake mmol/min	Lactate pyruvate ratio hep. vein	Galactose elimination $\mu\text{mol}/\text{min}$	Bile flow $\mu\text{l}/\text{min}$	ICG clearance	
	ml/min	ml/min	ml/min	ml/min	ml/min	ml/min	ml/min	ml/min
16	1.10	1.00	0.96	—	—	134	129	54
24	1.33	1.82	1.54	—	605	314	—	—
26	1.60	1.59	—	21.9	875	231	409	278
31	1.57	1.15	1.81	19.7	599	467	291	227
32	1.45	1.58	1.62	26.0	660	62	256	37
35	1.30	1.47	—	14.4	526	241	—	—
36	1.00	0.67	1.08	16.1	449	169	124	81
46	1.39	1.26	1.63	10.2	529	190	425	119
50	1.26	1.11	1.10	13.6	583	—	—	—
105	1.00	1.26	1.87	26.8	470	142	296	134
108	1.17	1.11	1.19	8.4	563	257	302	239
140	1.14	1.30	1.50	13.4	564	180	338	267
142	1.03	0.91	1.13	18.4	407	175	154	72
144	0.93	0.74	0.36	18.7	449	165	241	211
146	0.89	0.97	0.98	12.4	—	—	—	—
158	1.21	1.02	1.59	6.3	459	350	308	323
171	1.25	0.94	1.60	8.5	444	235	288	247
Average	1.21	1.17	1.36	15.7	545	221	269	173
S.D.	0.21	0.31	0.41	6.3	117	99	93	93

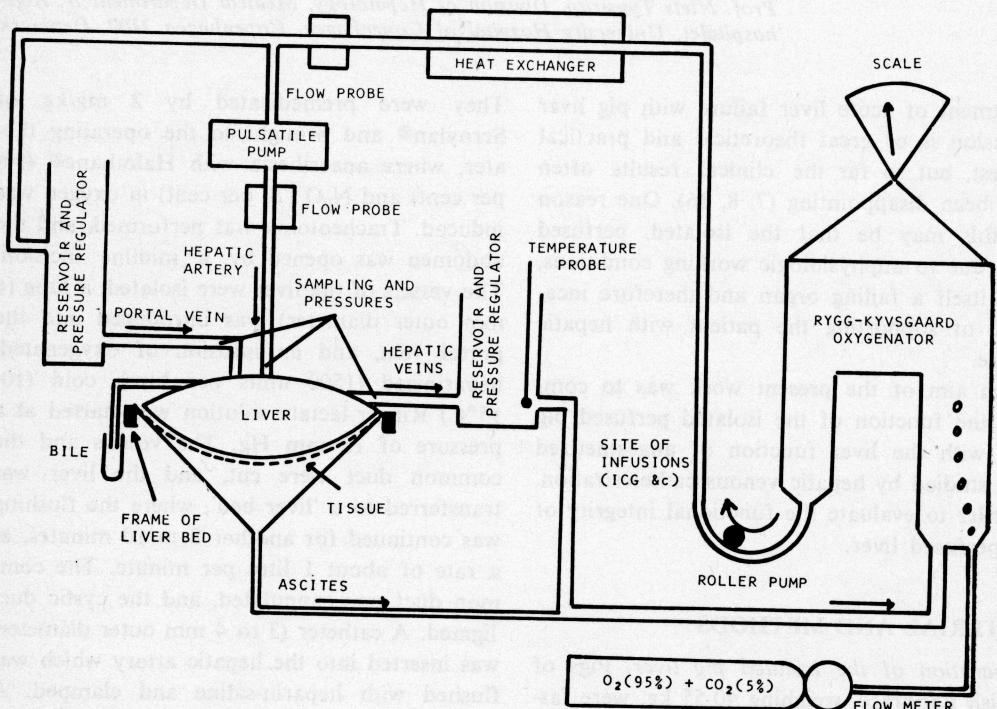
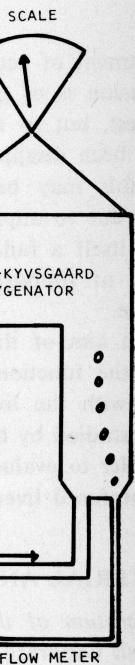


Fig. 1. The perfusion system.

ICG clearance plasma /min	Bile ml/min
129	54
-	-
409	278
291	227
256	37
-	-
124	81
425	119
-	-
296	134
302	239
338	267
154	72
241	211
-	-
308	323
288	247
269	173
93	93



was introduced into the cranial opening of the inferior caval vein. The caudal opening of the vein was ligated below the liver.

Then the liver, still resting on the liver bed, was transferred to the perfusion apparatus, and the tube in the portal vein, the tube in the inferior caval vein, and the catheter in the hepatic artery were connected with corresponding tubes of the perfusion system. During these procedures the flow in the portal vein was only interrupted for a few seconds.

The perfusion system is shown diagrammatically in Fig. 1. During the hepatectomy the apparatus had been primed with 2½ litres of fresh, heparinized (5000 units per litre) human blood, and 1½ litre of 0.9 per cent sodium chloride and sodium bicarbonate, to obtain a pH of 7.4. The perfusate was kept at 38°C by a heat exchanger and oxygenated in a bubble oxygenator by a mixture of 95 per cent oxygen and 5 per cent carbon dioxide.

After connection of the liver to the perfusion apparatus, the flow was adjusted to obtain a portal pressure of about 13 mm Hg, and a pulsatile arterial pressure with a mean pressure of about 80 mm Hg. The outflow pressure was kept close to zero.

The perfusion flow was measured by electromagnetic flow-meters (Nycotron, Oslo), calibrated before and after each experiment. During the first 10 to 15 minutes of the perfusion, the resistance of the liver was often increased, but after that period the flow could gradually be augmented and kept constant during the rest of the experiment.

When the flow was stabilized, priming doses were given of galactose (Kabi) (23 mmol), lithium lactate (18 mmol), and Indocyanine Green (ICG) (Hynson, Westcott & Dunning) (2.5 mg), followed by constant infusions of galactose (600 µmol/min), lactate (200 µmol/min), ICG (300 µg/min), and cholic acid (2 µmol/min).

At the end of the experiment 10 µCi of radioiodinated albumin (RISA) (Kjeller) was injected for determination of plasma volume, and in 6 cases tissue was taken for determination of adenine nucleotides.

To avoid the influence of variations taking

place during the equilibration period, the data obtained during the second hour of perfusion were used for this study.

The experiments selected for this study were characterized by the absence of definite technical defects, such as air-embolism, prolonged hypoxia during surgery, and high perfusion resistance. One-third of the perfusions performed were discarded on these grounds.

Catheterization procedure. The pigs were prepared and anaesthetized as above. Anaesthesia was maintained by Halothane® (0.3 per cent) and N₂O (70 per cent) in oxygen at a flow of 6 litres per min in a closed system with a CO₂ absorber. The hepatic veins were catheterized (Cournand catheter No. 7) from the surgically exposed internal jugular vein during X-ray control by an image amplifier. The correct position in a sufficiently large hepatic vein was secured by injection of contrast medium. Arterial and portal blood was taken by polyethylene catheters in the left external carotid artery and in the portal vein.

Portal venous flow was measured by an electro-magnetic flowmeter (Nycotron, Norway) placed around the main portal vein. After the experiment the portal vein was removed with the flowmeter in situ, and the flowmeter was calibrated with the blood of the pig in a thermostated perfusion system with a pump.

In 6 experiments (see Table II) portal flow was not measured, but was estimated as 60 per cent of hepatic blood flow, as this was the average ratio between portal and hepatic blood flow in the remaining experiments.

Priming doses and continuous infusions of galactose and ICG were given as above, except for a 3 times greater priming dose of galactose. An equilibration period of about 30 minutes was followed by an experimental period of about 60 minutes. After the experiment RISA was injected as above, and in 6 cases tissue was taken for determination of adenine nucleotides. Liver weight was determined after the experiment.

Analytical procedures and calculations. Lactate (12), pyruvate (6), galactose (10), ICG (19), and adenine nucleotides (3) were determined by the methods indicated. Tissue (about 5 g)

Table II. In vivo pig liver. Functional data of in vivo pig livers, studied by liver vein catheterization during anaesthesia. Mean values of observations made during about one hour, following an equilibration period of about 30 minutes

Exp. No.	Liver weight Kg	Hepatic blood flow litres/min	Portal blood flow litres/min	Splanchnic oxygen uptake mmol/min	Hepatic oxygen/ uptake mmol/min	Lactate/ yruvate ratio hep. vein	Galactose elimina- tion $\mu\text{mol}/\text{min}$	Bile flow $\mu\text{l}/\text{min}$	ICG clearance Plasma Bile ml/min ml/min
28	—	1.33	0.83	6.19	3.30	20.6	1 143	859	308 290
61	—	0.94	0.42	3.54	2.43	31.2	817	205	139 58
67	1.26	0.98	0.72	4.14	2.49	16.4	648	403	191 125
148	0.81	0.78	0.47*	4.46	2.98	22.7	992	355	171 70
164	1.08	0.91	0.55*	6.52	3.81	11.0	708	582	250 124
166	1.30	1.21	0.73*	4.11	2.03	30.1	704	—	220 —
190	1.11	1.53	0.92*	7.53	5.12	15.7	1 058	435	128 82
197	0.97	1.46	0.85	5.15	2.47	17.4	687	501	57 13
205	1.05	0.85	0.51*	3.58	1.77	22.6	546	276	147 48
208	0.85	1.01	0.61*	3.24	1.23	15.2	767	296	66 30
227	1.11	1.80	1.29	7.88	4.14	—	—	460	118 80
228	1.05	0.68	0.57	3.66	2.47	—	770	853	176 217
235	1.26	1.40	1.08	—	—	—	1 397	670	164 157
237	1.00	0.99	0.55	2.14	1.34	—	990	—	66 —
247	0.84	0.50	0.12	1.34	0.90	—	268	220	84 26
255	1.00	0.90	0.60	3.29	2.54	—	477	250	113 57
260	1.11	0.89	0.36	3.03	2.94	—	596	430	130 112
Average	1.05	1.07	0.66	4.36	2.62	20.3	786	453	149 99
S.D.	0.15	0.34	0.28	1.85	1.11	6.5	278	210	68 76

* Estimated as 60 per cent of hepatic blood flow.

for determination of adenine nucleotides was punched out of the liver after conclusion of the experiments and frozen less than 5 seconds after the removal by a clamp, cooled in liquid nitrogen. Blood oxygen content was measured by the van Slyke manometric apparatus. In the in vivo pigs correction was made for the content of N_2O (13). RISA was measured in a well-scintillation counter with 1 per cent counting accuracy. Pressures were measured with Elema electromanometers.

In the perfused liver oxygen uptake was calculated as $\text{PR} [\text{p}] - [\text{h}]$, where PR is perfusion rate, $[\text{p}]$ and $[\text{h}]$ are oxygen concentrations in portal and hepatic venous blood, respectively. Galactose elimination rate was calculated as $I_{\text{gal}} \pm \Delta[\text{p}]V$, where I_{gal} is infusion rate, $\Delta[\text{p}]$ is the mean change per minute in galactose concentration of the blood in the portal tube, and V is the volume of the perfu-

sate, determined by RISA and haematocrit. The hepatic plasma clearance of ICG (ICG 'uptake') was calculated as $(I_{\text{ICG}} \pm \Delta[\text{p}]V)/[\text{p}]$, symbols are used as above, but $[\text{p}]$ is plasma concentration and V plasma volume. The ICG bile clearance (ICG 'excretion') was calculated as $\text{BF} [\text{b}]/[\text{p}]$, where BF is bile flow, and $[\text{b}]$ is the concentration of ICG in the bile.

In the in vivo pig the hepatic blood flow (HBF) was calculated by the ICG infusion-extraction method (19), hepatic arterial flow (HAF) as the difference between HBF and portal flow (PF), measured by the flowmeter or estimated. Hepatic oxygen uptake was calculated as $\text{PF} ([\text{p}] - [\text{h}]) + \text{HAF} ([\text{a}] - [\text{h}])$, where $[\text{p}]$, $[\text{h}]$, and $[\text{a}]$ designate portal, hepatic venous, and arterial concentrations of oxygen, respectively. Splanchnic oxygen uptake was calculated as $\text{HBF} ([\text{a}] - [\text{h}])$, symbols as above. Galactose elimination rate was calculat-

ed as HBF (1).

Hepatic plasma

were calculated

RESULTS

Table I gives the data for all the livers. Amongst the 26 livers the relative variation in hepatic bile clearance was largest in the case of the in vivo livers (II), the smallest in the small livers (I). The largest is the average of the livers.

The average of the livers in the perfused and unperfused animals is 157 ml/min .

The comparison of the in vivo livers in the unperfused and in the greater weight of the livers due to an increase in the perfusion (1) shows no difference in the average (Fig. 2). The average of the unperfused and in vivo livers is 157 ml/min . The uptake of in vivo livers is terminations, in the unperfused livers, the mean value is 157 ml/min .

Table III. Comparison of the data in Tables I and II

Perfused liver
In vivo liver
Difference of
P*** of difference
Perfused/in vivo

* Mean value