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Intraarterial and Intraportal in vivo Catheterization of the Regenerating Rat Liver

Effects upon body and liver weights and DNA synthesis

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ABSTRACT

A method for concomitant partial hepatectomy and catheterization of the arterial and portal systems of the liver in the rat is described. Catheters were inserted into the gastroduodenal artery and the ileocolic vein. Continuous saline perfusion was performed during 36 hours. In catheterized rats recovery of liver and body weight lagged behind that of non-catheterized rats. The more extensive surgery and the presence of catheters also caused decreased incorporation of ^{3}H-thymidine into liver DNA 24 hours postoperatively. The variation in thymidine incorporation between animals was large. It was shown that by prelabelling liver DNA with ^{14}C-thymidine the rats can serve as their own controls during acute experiments involving ^{3}H-thymidine, thus reducing the inconsistancy of individual variation.

INTRODUCTION

Partial resection of 2/3 of the liver in rats induces hypertrophy and hyperplasia of the remaining liver tissue. This is characterized by changes in enzyme activities, in RNA- and DNA-synthesis, and in mitotic activity. Almost all cells are stimulated to synthesize DNA and pass through the cell cycle 1-2 times. Restoration of organ weight is usually seen within a week. The course of time for these events are well known (2,5,8,13,14).

In the present study we describe a model designed to analyze the effects of blood flow modifications on liver cell kinetics in unaesthetized rats. We evaluated the effects of concomitant catheterization of the hepatic artery and portal vein in connection with partial hepatectomy on body and liver weights, as well as the effect of catheterization upon incorporation of ^{3}H-thymidine and ^{14}C-thymidine into liver DNA, using each animal as its own control.

MATERIAL AND METHODS

Animals

Young, adult male Sprague Dawley rats weighing 170-210 g (SPF quality,

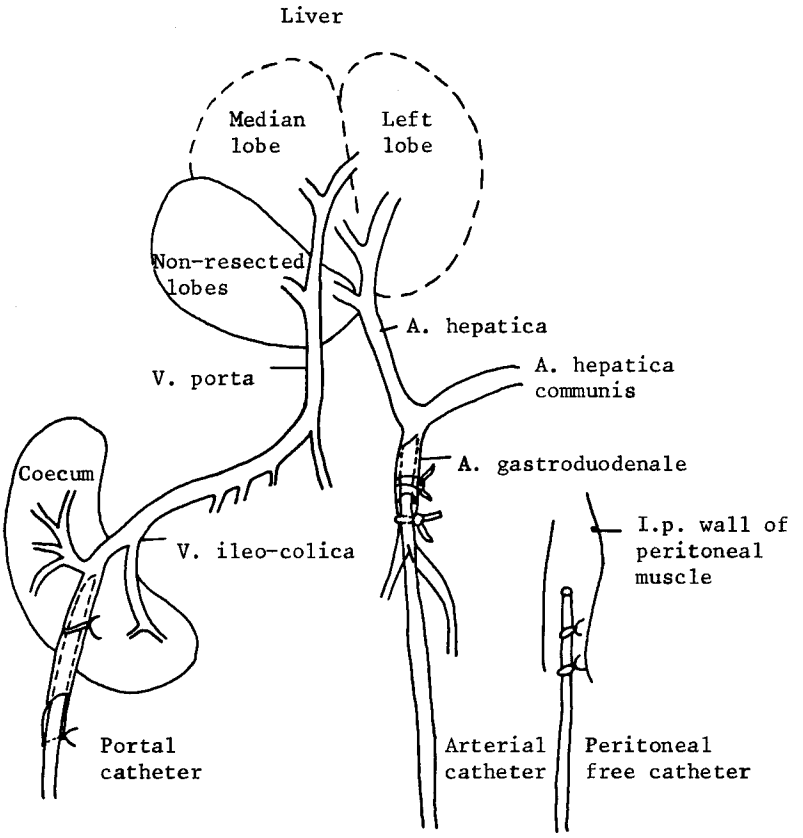

Fig. 1. Schematic view of catheters in the ileo-colic vein, in the gastroduodenal artery and of the free catheter emptying into the peritoneal cavity. For details of the arterial and portal systems refer to Green (7).

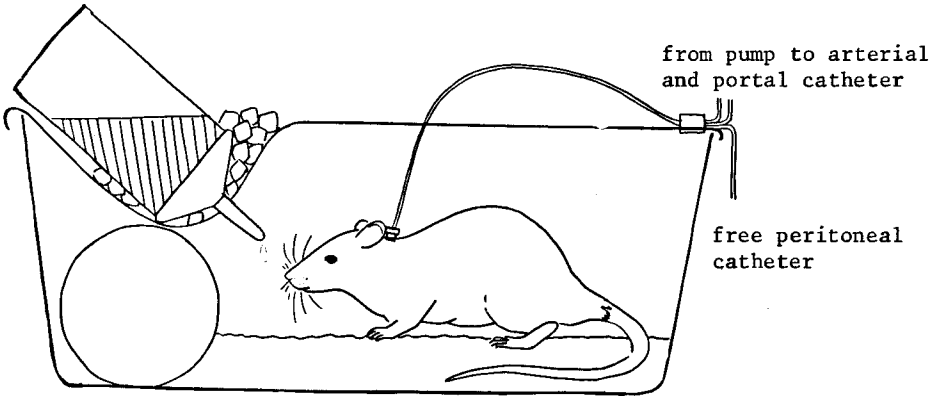

Fig. 2. Catheterized rat in cage.

Anticimex, Sweden) were used for the experiment. The animals were kept under constant conditions and had free access to standard laboratory diet before and after surgery.

Surgical procedures

The animals were anaesthetized with an ip injection of Mebumal, 30 mg/kg body weight. After midline incision and retraction of the liver, the operation was performed under a microscope (4 x). A polyethylene cannula (Intramedic PE 50) with a narrow tip was inserted into the gastroduodenal artery in the retrograde direction, according to Leivestad and Malt (12). The ileocolic vein was catheterized in the anterograde direction. To allow intraperitoneal infusion, the tip of a third catheter was positioned in the peritoneal cavity, fig. 1.

After catheterization the median and left lateral lobes of the liver were resected according to Higgins and Anderson (9). In separate experiments partial hepatectomy was accomplished before catheterization. The catheters were pulled subcutaneously to the neck and the abdominal wound was stitched. A small plastic vial attached to the neck secured the catheters from damage. From the vial to the top of the cage, the catheters were protected by a metal helix, fig. 2.

The intravascular catheters were connected to pumps (Brown-Melsungen, type 71100) which supplied 1 ml/24 hr of Fyskosal/Heparin (500 IE Hep/100 cc Fysk). The peritoneal catheter was filled with the same solution and closed at its free end.

Five rats subjected to partial hepatectomy only served as controls.

Partial hepatectomy alone was usually accomplished within 5 minutes. Catheterization required another 15-25 minutes. To minimize effects of diurnal variations, operative work was restricted to a 1-2 hour period in the morning.

One group of rats were detached from the pumps 36 hours after the operation. The metal helix was removed, the catheters were flushed, melted shut, coiled and put into plastic vials. This group and the control group of non catheterized rats were weighed daily. Seven days after operation the rats were killed and the livers removed and weighed.

Animals aimed for radionuclide studies were given radioactive thymidine 24 h urs after the operation via the peritoneal catheter or in the catheter in the ileocolic vein. Slow flushing with 5 µl at a time was accomplished with a repeating dispenser (Hamilton Comp) until 100 µl containing 185 kBq (5 µCi) of (methyl-^{14}C) thymidine (185 GBq, Amersham) and/or 100 µl containing 3.7 MBq (100 µCi) (methyl-^{3}H) thymidine (555-1110 GBq, Amersham) was administered. For double labelling, where each animal served as its own control, ^{14}C-thymidine was injected first and ^{3}H-thymidine one or two hours later.

One hour after the last injection the rats were killed and the livers were removed, and the different parts of the regenerating lobe were identified, frozen

on solid CO_2, and stored at -70°C until analysis.

To ensure that the two injections of thymidine did not interfere with each other, one group of animals received ^{3}H-thymidine only and another group ^{3}H-thymidine and ^{14}C-thymidine concomitantly. The same doses as described above were used.

To show metabolic effects on thymidine incorporation in liver DNA one group of animals was given ^{3}H-thymidine 24 hours after partial hepatectomy and one hour later ^{14}C-thymidine mixed with 0.001 or 0.2 mmol "cold" thymidine.

Analysis of DNA

Specimens taken from three different parts of the regenerating lobe were analyzed by two methods. DNA was extracted according to Schmidt-Tannhäuser as modified by Munro & Fleck (16) and quantified according to Burton (4). DNA was also isolated by proteinase-K according to Kasche-Amneus (10) and quantified by UV-spectrophotometry.

Radioactivity was analysed in a scintillation counter (Mark II) and DNA specific radioactivity determined. The two methods for DNA analyses were in good agreement. Only results from analyses performed according to the Schmidt-Tannhäuser method are reported here.

For calculation of the ratio between the specific ^{3}H- and ^{14}C-activity in DNA, the measured CPM/mg DNA was divided by the CPM of injected ^{3}H-thymidine and ^{14}C-thymidine, respectively.

RESULTS

The effects of surgery and catheterization upon total body and liver weights are given in Table 1. Differences between catheterized and non-catheterized rats were small, but recovery in rats with catheters lagged behind that of the control group.

Table 1. Effect of catheterization on body and liver weight 1-7 days after partial hepatectomy. Catheters were connected to the pumps for 36 hours. Mean ± standard deviation.

Number of rats	Body weight M ± SD								Liver weight M ± SD
	day								day
	0		1	2	3	4	5	6	7	7
5 with no cath.	202g ±5	100% ±2	90% ±2	92% ±3	95% ±4	97% ±4	100% ±4	-	107%[1] ±4	7.4g[2] ±0.3
4 with cath.	206g ±6	100% ±3	87% ±4	87% ±4	89% ±3	90% ±3	91% ±2	-	98%[1] ±3	6.4g[2] ±0.9

1) Difference significant, $0.05 > p > 0.02$ (Student's t-test)
2) $0.1 > p > 0.05$.

Neither pre- nor coadministration of ^{14}C-thymidine interferred significantly with the incorporation of ^{3}H-thymidine into liver DNA (Table 2).

Table 2. Effect of pre- or coadministration of ^{14}C-thymidine (10^{-4} mmol) on ^{3}H-thymidine (5×10^{-6} mmol) incorporation into liver DNA 24 hours after partial hepatectomy. Radionuclides were administered through a permanent catheter emptying into the peritoneal cavity. Mean ± standard deviation, relative values.

Number of animals	Hours after partial hepatectomy			CPM per mg DNA per injected CPM	
	injection of		killed	^{14}C	^{3}H
	^{14}C-thymidine	^{3}H-thymidine		M ± SD	M ± SD
4	-	24	25	-	2.43 ± 0.61
3	24	24	25	3.55 ± 0.55	2.90 ± 0.72
5	23	24	25	4.17 ± 0.72	3.08 ± 0.70

In Table 3, two groups of rats, having either an intraperitoneal catheter only (A_1) or having an intraperitoneal catheter as well as catheters in the gastroduodenal artery and ileocolic vein (B_1) are compared. Both groups were

Table 3. Effect of catheters in the gastroduodenal artery and the ileo-colic vein on thymidine incorporation into liver DNA 24 hours after partial hepatectomy. ^{14}C-thymidine or ^{3}H-thymidine was administered through a permanent catheter emptying into the peritoneal cavity. Mean ± standard deviation.

Catheters in		Radio-nuclide (number of rats)	Hours after partial hepatectomy		CPM per mg DNA per injected CPM	
			Injection of radionuclide	killed	M ± SD	SD % of M
peritoneal cavity	A_1	^{14}C-thymidine (5 rats)	23	25	4.17 ± 0.72*	17
peritoneal cavity gastroduodenal a. ileo-colic v.	B_1	^{14}C-thymidine (3 rats)	23	25	2.38 ± 0.49*	21
peritoneal cavity	A_2	^{3}H-thymidine (12 rats)	24	25	2.82 ± 0.68**	24
peritoneal cavity gastroduodenal a. ileo-colic v.	B_2	^{3}H-thymidine (7 rats)	24	25	1.75 ± 0.86**	49

* The difference is significant $0.01 > p > 0.002$
** " " " " $0.02 > p > 0.01$

given ^{14}C-thymidine through the intraperitoneal catheter. The experiment was repeated in two similar groups, A_2 and B_2, given ^{3}H-thymidine (Table 3). Rats with intravascular catheters had a larger variation in thymidine incorporation into DNA of the regenerating liver, and less incorporation compared to animals

Table 4. Variation in DNA labelling in groups of rats and in individual rats. ^{14}C-thymidine was administered 23 hours and ^{3}H-thymidine 24 hours postoperatively and the rats were killed one hour later.

Catheter in	Injection by catheter in	CPM per mg DNA per injected CPM				$^{3}H/^{14}C$ in DNA in each rat	
		^{14}C		^{3}H			
		M±SD	SD % of M	M±SD	SD % of M	M±SD	% of M
peritoneal cavity (5 rats)	peritoneal cavity	4.17±0.72	17	3.08±0.70	23	0.76±0.10	1
peritoneal cavity gastroduodenal artery ileo-colic vein (3 rats)	peritoneal cavity	2.38±0.49	21	1.85±0.54	29	0.89±0.14	16
ileo-colic vein (8 rats)	ileo-colic vein	2.19±0.66	30	2.10±0.90	43	1.03±0.16	16

Table 5. Effect of addition of "cold" thymidine on incorporation of radioactive thymidine in regenerating rat liver. All injections were made through a catheter in the ileo-colic vein. 0.001 or 0.02 mmol "cold" thymidine was mixed with the injected ^{3}H-thymidine. ^{14}C-thymidine was administered 23 hours and ^{3}H-thymidine with cold thymidine 24 hours postoperatively and the rats were killed one hour later.

"Cold" thymidine mmol/100 µl	CPM per mg DNA per injected CPM		$^{3}H/^{14}C$ in DNA
	^{14}C Mean ± SD	^{3}H Mean ± SD	Mean ± SD
A – (8 rats)	2.19 ± 0.66	2.10 ± 0.90	1.03 ± 0.16
B 0.001 (2 rats)	2.13 ± 1.79	0.79 ± 0.49 [1]	0.43 ± 0.13 [2]
C 0.2 (2 rats)	1.46 ± 0.56	0.36 ± 0.01 [3]	0.29 ± 0.04 [4]

1) Difference as compared to ^{3}H-activity in group A is not significant. $0.1 > p > 0.05$ (Student's t-test)
2) " " " " $^{3}H/^{14}C$ in group A is significant. $0.05 > p > 0.02$
3) " " " " ^{3}H-activity in group A is significant. $0.002 > p > 0.001$
4) " " " " $^{3}H/^{14}C$ in group A is significant. $0.001 > p$.

without intravascular catheters.

The specific activity of DNA was lower after intravascular administration of labelled thymidine than after intraperitoneal administration in animals without intravascular catheters (Table 4). The standard deviation of the DNA specific activity in the different groups of animals was 17-43% of the mean, whereas the standard deviation of the $^{3}H/^{14}C$ of DNA in each animal was 1-16% of the mean. Thus, with a pretreatment injection of labelled thymidine the level of incorporation into DNA for each animal could be determined.

The effect of an interfering substance could be demonstrated at the time of the second injection of labelled thymidine (Table 5). The addition of "cold" thymidine to the injected ^{3}H-thymidine decreased the incorporation of label into DNA in agreement with known metabolic effects (6,11).

DISCUSSION

The experimental use of the continuously perfused rat liver in vivo has been previously described (12). In this study we have shown that the portal and the arterial systems can be concomitantly perfused in rats in combination with partial hepatectomy. Partial hepatectomy stimulates thymidine phosphorylation and DNA synthesis in liver cells. Thus, the experimental system described is of interest for investigations of cell kinetics. Because of the dual blood flow the liver is more complicated than other models. It offers, however, interesting possibilities in the study of effects of blood flow restriction and compensatory mechanisms.

In the present study, infusion was interrupted 36 hours postoperatively but it could have been continued without difficulty. At later times following partial hepatectomy, however, operated rats seem to be no more sensitive to the experimental situation than are ordinary rats, which tolerate infusion quite well (12). A prolonged and complicated operative procedure and/or the presence of catheters affected body and liver weight as well as liver cell metabolism. Liver DNA synthesis was decreased or delayed and the variation between animals increased. These differences show that it is essential to use catheterized rats given sham injections as controls. In this study the inconsistancy of individual variation was decreased when animals were used as their own controls using the double labelling technique.

The choice of labelled thymidine in this series of experiments was related to the vast documentation of the fate of this precursor in the regenerating liver (13). It is well known that the diurnal rhythm of the rat is reflected in liver metabolism during the regenerative process (1) and that age and stress factors influence DNA synthesis (3). We could possibly have improved the synchrony of liver regeneration by using a regular feeding schedule (17) thus decreasing variation in thymidine incorporation between animals. We do not

think, however, that the variation between the rats due to catheterization would be decreased significantly. Therefore, the double labelling method should be a valuable tool in improving the significance of test results. It is possible that the interval between injections can be prolonged, but because of diurnal variation this must be investigated further. High concentrations of "cold" thymidine injected with ^{3}H-thymidine dilutes the radioactive molecules resulting in decreased labelling of DNA (11). Thymidine also disturbs deoxynucleotide metabolism and nucleic acid synthesis resulting in growth inhibition (6,11). The negative effect of added thymidine on the incorporation of ^{3}H-thymidine into DNA was obvious in this study. We consider DNA especially well siuted for the determination of final results of metabolic alterations because of its very low turnover. Other compounds with a low turnover such as certain RNAs and proteins should also be useful.

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