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Regulation of hepatic microcirculation in stepwise liver resection

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Abstract

Background : **After liver resection a small-for-size syndrome may result from the reduction of liver volume and additional liver damage caused by hepatic hyperperfusion. Therefore the influence of the extent of liver resection on liver perfusion is investigated.**

Material and methods : **A stepwise liver resection (removal of 30%, 70%, 90%, 95% and 97% of the liver) was performed under inhalation anaesthesia with isoflurane in 6 male Lewis rats. Besides systemic arterial and venous blood pressure the portal pressure and flow was measured and the sinusoidal perfusion was visualized. Sinusoidal diameter, intersinusoidal diameter and functional capillary density were determined.**

Results and conclusions : **A decrease in the portal flow but an increase in the portal pressure was observed. Sinusoidal diameter showed a steady but low increase when up to 70% of the liver was removed but a high increase after 90% or more of the liver was resected. This indicates a decompensation of a regulatory mechanism of sinusoidal perfusion.** (Acta gastroenterol. belg., **2007**, 70, **345-351**).

Key words : ?????.

Introduction

Extended liver resections are performed with increasing frequency. This surgical procedure is employed in living related liver transplantations and also for the removal of extended primary and secondary liver tumours.

An extended liver resection results in a massive reduction of the total hepatocellular volume and in addition in a marked reduction of the total vascular bed of the liver. Mesenteric blood flow drains into the portal vein and thus into the small-for-size liver. This leads to portal hypertension and hyperperfusion of the remnant liver.

Besides the surgical reduction of liver mass the additional damage inflicted by hepatic hyperperfusion (1) on the remnant liver leads to a further decrease of the functional liver mass. Thus hyperperfusion contributes to the onset of a life threatening small-for-size syndrome after extended liver resection or a small-for-size liver graft (2).

Surgical reduction of portal hyperperfusion by ligation of the splenic artery early after onset of symptoms led to a dramatic improvement of liver function in a case report (3). Reduction of portal pressure by Somatostatin leading to attenuation of shear stress in small-for-size livers was accompanied by a better liver function (4).

It is the aim of this study to understand the influence of the extent of liver resection on the regulation of liver perfusion and microcirculation. Elucidating this interaction could help to optimize treatment options in patients after extended liver resections in the future.

Material and method

Study design

In a rat model of stepwise liver resections hemodynamic parameters were recorded and the sinusoidal perfusion was visualized. Parameters describing sinusoidal perfusion were determined using image and video analysis software.

Animals

Six inbred male Lewis rats with a body weight of 275-295 g (Charles River®, Sulzfeld, Germany) were employed in this study. The animals were housed under standard animal care conditions and fed with rat chow *ad libitum*. All procedures and animal housing were carried out according to the German Animal Welfare Legislation. The animal experiment was approved by the Bezirksregierung Düsseldorf.

Operation

All operations were performed in inhalation anaesthesia using an isoflurane vaporizer (Sigma Delta, UNO, Holland) with an isoflurane concentration of 1,5-3% and an oxygen flow of 0,5 l/min. For the 30% partial hepatectomy (PH) the left lateral liver lobe was resected. Additional removal of the median lobe – amounting to 57% of the remaining liver after 30% PH – led to a 70% PH. To reach a 90 % PH the right superior and inferior lobe was resected, resulting in the removal of 67% of the remaining liver. 95% PH – which normally shows a 50% survival rate (5) – was performed by additional removal of the superior caudate lobe, thus removing 50% of the remaining liver. To reach a 97% PH – a lethal model (5)

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– the inferior caudate lobe was resected leaving only the paracaval liver tissue behind and resulted in the removal of 40% of the liver left behind after 95% PH. Liver resections were performed according to the technique described in detail by our group (5). Animals were sacrificed at the end of the procedure.

Monitoring

Vital parameters

Vital parameters were monitored constantly during the extended surgical procedure.

Body temperature was measured in the rectum of animals using a mon-a-therm®-Thermometer 12CH (A. Mallinckrodt®, Mexico).

Heart rate and respiratory rate were monitored using a small microphone attached to a rubber band (Kondensatormikrofon, Conrad® Electronic GmbH, Germany). Data acquisition was performed using the inline port of the on board sound of the ASUS computer motherboard (ASUS P4B533, Taiwan). Cool edit 2000 (Syntrillium Software, USA) was used for data analysis.

Continuous blood pressure monitoring was performed using a PE-catheter ×0,6 mm (Portex® PE-50 catheter, Portex Ltd., UK). All sensors were connected to a Sirecust® 403P-Monitor (Siemens®, Germany). Systemic arterial pressure was monitored putting a catheter into the right common carotid artery. Central venous pressure was measured in the caval vein introducing a catheter through the right ileolumbar vein.

Liver perfusion

Portovenous pressure was monitored with a catheter introduced into the ileocoelic vein.

Vascular blood flow was measured in the portal vein and the infrahepatic caval vein using a dopplersonographic device (Transonic® T106 small animal blood flow meter, Transonic® Systems Inc., USA).

Microcirculation

After opening the abdomen and after each liver resection step (30%, 70%, 90%, 95% and 97% PH) hepatic microcirculation was visualized employing an Orthogonal Polarization Spectral (OPS) Imaging System (Cytoscan, Cytometrics, Inc., Philadelphia, PA, USA). The OPS has a resolution of approximately 1 µm/pixel (6). The OPS imaging system was connected to a computer through a frame grabber card (Matrox Meteor II PCI frame grabber card). The probe was placed on the surface of the liver minimizing compression of the underlying liver parenchyma. At least two video sequences of about 30 seconds each were stored at all observation time points for subsequent offline analysis.

Image and video analysis

From each OPS video sequence one image was selected for determination of sinusoidal diameter, intersinusoidal diameter and functional capillary density (FCD) using CapiScope® (KK-Technology®, Bridleways, Devon, England) (see Table 1). Diameters were assessed in the midzonal region of a lobule. 30 data points were taken for each diameter. FCD was determined by outlining all sinusoids in the image.

Data analysis

Data are given as mean and in addition the standard deviation is provided. Statistical analysis (independent samples t-test) was performed using SPSS 11.0. A p value smaller than 0,05 was regarded to be statistically significant.

Results

Surgery

Total operation time of stepwise liver resection including monitoring and data capturing was in the range of 216 up to 265 minutes (Fig. 1A). Differences were not statistically significant.

Vital parameters

Body temperature

Body temperature of the rats was 34 +/- 0,5°C. Body temperature remained constant throughout the operation.

Respiratory rate

Respiratory rate of the rats was between 40 and 53 breath cycles per minute (Fig. 1B). A slight decline to a minimum of 31 breath cycles was observed after 95% and 97% PH.

Heart rate

Heart rate of the rats was between 330 and 480 beats beats/min throughout the operation (Fig. 1C). A major

Table 1. — Microcirculatory parameters measured and calculated in OPS video sequences using CapiScope®

sinusoidal diameter	• diameter of sinusoids in the midzonal region of a lobule • 30 sinusoids per picture
intersinusoidal diameter	• diameter of liver cell plates between two sinusoids in the midzonal region of a lobule • 30 intersinusoidal spaces per picture
functional capillary density	total length of perfused vessels per area of detection

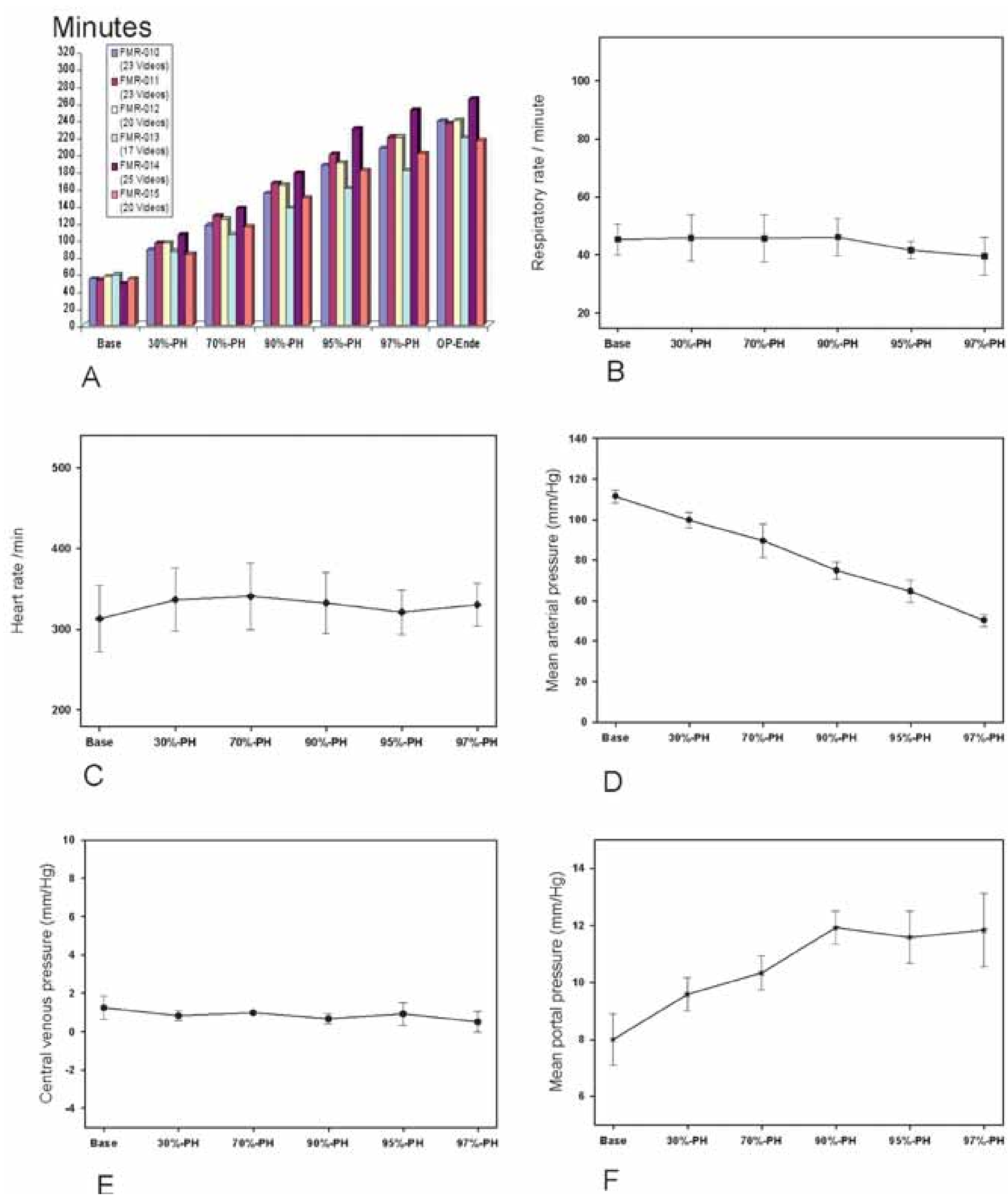

Fig. 1. — A Total operation time of stepwise liver resection in minutes, B respiratory rate, C heart rate, D systemic arterial pressure, E central venous pressure and F mean portovenous pressure during stepwise 30%, 70%, 90%, 95% and 97% liver resection.

increase in the heart rate during the later stages of the surgical procedure was not observed.

Mean systemic arterial pressure

Mean systemic arterial pressure of the rats declined constantly during the operation (Fig. 1D). Starting point at the beginning of the procedure was between 80 to 107 mm Hg and after 97% PH the mean arterial pressure was in the range of 30 to 40 mmHg.

Central venous pressure

Central venous pressure of the rats was in the range between 0,7 to 1,2 mm Hg (Fig. 1E). Central venous pressure remained constant during the operation. The animals experienced a severe drop in mean systemic arterial pressure at the same time a constant central venous pressure together with a constant heart rate showed that even at the end of the lengthy surgical procedure no hypovolemia – either due to blood loss or dehydration – developed in the animals.

Liver perfusion

Portovenous pressure

Starting point of mean portovenous pressure at the beginning of the procedure was 7 mm Hg. Pressure increased slowly but constantly up to 12,5 mmHg after 90% PH. After 95% and 97% PH mean pressure remained at this level but individual measurements among different rats showed a high variation (Fig. 1F).

Blood flow

Portal vein

Blood flow in the portal vein decreased in a linear fashion during the procedure starting at 15 ml/min in rats without liver resection and reaching 3,3 ml/min after 97% PH (Fig. 2A).

Infrahepatic caval vein

Blood flow in the infrahepatic caval vein decreased constantly during the procedure (Fig. 2B) starting at 27 ml/min in rats without liver resection and reaching 7 ml/min after 97% PH. Thus decrease of the flow in the caval vein went in parallel with the decrease of blood flow in the portal vein.

Microcirculation

Sinusoidal diameter

Sinusoidal diameter of the rats increased slowly but steadily during stepwise liver resection up to 70% PH (Fig. 2C). At the beginning of the procedure the sinusoidal diameter was between 5,2 and 5,7 µm. After 70% PH sinusoidal diameter was at 6,4 µm. A higher increase and a higher intra- and interindividual variation of the sinusoidal diameter could be observed during subsequent steps of liver resection reaching a median sinusoidal diameter up to 10,8 µm after 97% PH. The high variation of sinusoidal diameter indicates an uneven perfusion of the remaining liver. In addition it could hint towards a control mechanism regulating liver perfusion up to 70% PH, that decompensates after liver resections exceeding 70% of the liver.

Intersinusoidal diameter

Intersinusoidal diameter decreased constantly from a resection step to the next one until 90% of the liver was removed (Fig. 2D). Starting point at the beginning of the procedure was at 22,8 µm. Intersinusoidal diameter reached 18 µm after 90% PH. After 95% and 97% PH a higher variation could be observed.

Functional capillary density

Functional capillary density was in the same range at all time points, with a mean value of 43 cm/cm^2 before PH and 41 cm/cm^2 after 97% PH (Fig. 2E). Once more than 90% of the liver was removed a marked variation of the functional capillary density of the remnant liver was observed, demonstrating uneven sinusoidal perfusion.

Discussion

After classical liver resection residual normal non-cirrhotic liver tissue allows for a fast regeneration without clinically apparent impairment of liver function (1). After extended liver resection, with a remaining liver volume smaller than 30%, Yigitler *et al.* (7) reported, that patients required more clinical attention and their hospital stay was longer.

After extended liver resection some of the patients suffer from a small-for-size syndrome. Progressive cholestasis, coagulopathy, portal hypertension and ascites production are hallmarks of the small-for-size syndrome (1,8). Troisi *et al.* (1) noted that the small-for-size syndrome is primarily linked to portal hyperperfusion – a concept also shared by others (9). Portal hyperperfusion is also reported to result in graft dysfunction in small-for-size liver transplantations (10).

Reduction of liver volume during liver resection is associated with a reduction of the total vascular bed in the liver. Provided the amount of blood draining into the portal vein was constant this would lead to an increase in portal pressure as shown by Lee *et al.* (11) in the isolated perfused liver model (see Table 1). Despite the fact that blood flow in the mesentery and bowel draining into the portal vein decreased during liver resection, an increase in the portal pressure is reported in the literature (12) (see Table 1 for details). This was also confirmed by our data. We observed a linear increase in portal pressure when removing up to 90 % of the liver with a high variation of portal pressure among individual animals but no significant further increase of portal pressure across the whole group of animals after 95% and 97% PH.

Stepwise liver resection in addition with time consuming monitoring of the animals was a lengthy surgical procedure. Total operation time reached nearly four hours in all animals. Important vital parameters such as temperature, respiratory rate, heart rate and central venous pressure were monitored and remained constant during the whole procedure. Since central venous pressure is a sensitive parameter of hypovolemia due to blood loss but also dehydration (13) the fact that this parameter remained constant during the long surgical procedure argues against a relevant blood or volume loss. However we observed a linear decrease in the arterial pressure reaching a minimum of 30-40 mm Hg after 97% PH. Despite the low systemic arterial pressure when resecting more than 90% of the liver central venous pressure and heart rate, remained constant at all time points. Thus the hemodynamic situation did not fulfil all criteria of a hemorrhagic shock. A constant central venous pressure in combination with a drop in

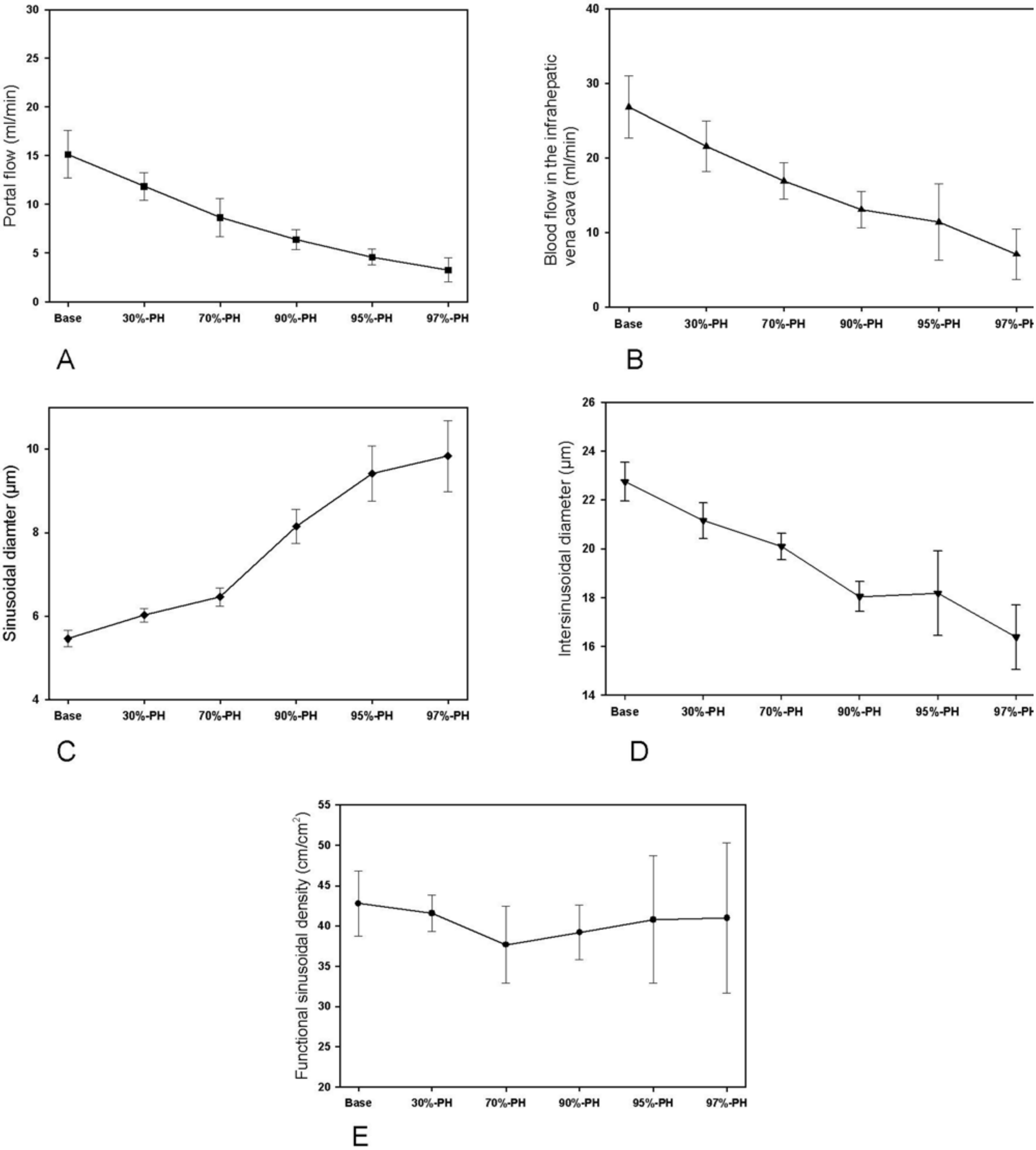

Fig. 2. — A Blood flow in the portal vein, B blood flow in the infrahepatic caval vein, C sinusoidal diameter D intersinusoidal diameter and E functional capillary density during stepwise 30%, 70%, 90%, 95% and 97% liver resection.

arterial pressure after extended hepatectomy and liver failure has also been reported in clinics (14).

An increase in portal pressure – as also observed in our experiment – is associated with an increased shear stress in liver vessels (9). Measuring shear stress directly is not within easy reach (15) and was not possible in our setting. Sato *et al.* (9) calculated shear stress based on the viscosity of the blood, flow velocity and the radius of the vessel. An increased portal pressure and thereby increased shear stress after PH is also associated with an increased sinusoidal diameter (16).

Orthogonal polarization spectroscopy enabled us to visualize sinusoids of the liver in-vivo. This device was first introduced into the market in 1999 (CytoScan®). In comparison to the more widely used *In Vivo Fluorescent Microscopy* the main advantage of this method is the easier handling of the device and the lower degree of damage inflicted on the organ.

Table 2. — Effect of 70% PH on liver perfusion

Parameter	Before PH	After 70% PH	Diff. in %	Author
Portal pressure	13 cm H_2O	18.3 cm H_2O	+40%	(25)
	13 cm H_2O	17.8 cm H_2O	+37%	(26)
	10.3 cm H_2O	14.1 cm H_2O	+37%	(27)
	7.5 mm Hg	11.1 mm Hg	+48%	(11)*
	8 cm H_2O	10.3 cm H_2O	+29%	Own data
Portal flow (ml/min)	14.8	14.8	0%	(11)*
	25.1	22.5	-10%	(12)
	15.1	8.7	-42%	Own data

* isolated perfused rat liver model.

The use of this device to visualize microcirculation in the liver has been established (17,18). Employing this device we measured a small but steady increase in mid-zonal sinusoidal diameter when resecting up to 70% of the liver. Sinusoidal diameter showed a pronounced increase and a higher intra- and interindividual variation in liver resections exceeding removal of 70% of the liver. This was paralleled by a high variation of the functional capillary density.

According to Wunder *et al.* (19) maintaining optimal, constant and homogenous sinusoidal perfusion is of outstanding importance to hepatocellular integrity. However the mechanism is discussed controversially. Sinusoidal perfusion and diameter is controlled by contractile cells in portal venules (20,21), stellate cells (22) and protrusion of endothelial nuclei into the vascular lumen (23). Different molecules like endothelin-1 and angiotensin are supposed to be involved in the regulation of sinusoidal perfusion (see Table 2).

Normal blood flow is uniformly distributed in the liver (24). Control of sinusoidal perfusion aims for a constant pressure and blood flow in this microcirculatory area. As Reynaert *et al.* (22) speculated hepatic stellate cells may not be able to perform a forceful contraction but only weaker contraction. This would explain a decompensation of this low power contractile system once wall tension – influenced by intrasinusoidal pressure and diameter – exceeds a certain level.

Our results especially the small increase in sinusoidal diameter up to 70% PH speak in favour of an active regulatory process keeping changes within close limits. Once portal pressure exceeds a given level after removing 90% or even more of the liver decompensation of this putative regulatory mechanism may occur. This could explain the marked increase of the sinusoidal diameter and the high variation both in the sinusoidal diameter and the functional capillary density of the liver upon removal of the additional 20% of liver mass. Especially the high variation hints towards a randomly distributed distension of sinusoids shunting blood away from less distended sinusoids. A higher diameter would also lead to a higher wall tension theoretically further extending the vessel. This could also explain the increasing variation of sinusoidal diameter in PH exceeding 90% compared with 70% PH.

Summary : Looking at the sinusoids in our model of stepwise liver resection we observed only a slight increase in sinusoidal perfusion and portal pressure subsequent to liver resections up to 70%. The sinusoidal perfusion seemed to be in a state of compensation. Once 90% partial hepatectomy was performed a higher increase in sinusoidal diameter and inhomogeneous sinusoidal perfusion were observed indicating decompensation of regulatory mechanisms.

The result of the inhomogeneous perfusion of the small remnant liver might contribute to the development of the clinically observed small for size syndrome in extended liver resections. The impact of porto-lineal shunting and vasoactive drugs on hepatic microcirculation and function after extended liver resections in rats will be tested in future experiments.

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