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Regional Blood Flows by the Microsphere Method: Reproducibility in Portal Hypertensive Rats and Influence of a Portal Vein Catheter (42689)

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Abstract. To determine the reproducibility of splanchnic blood flow measurements by the microsphere method in rats with portal hypertension and the effects of laparotomy with portal vein cannulation, eight groups of 10 rats were studied. Cardiac output and regional blood flows were measured twice, 10 min apart, in pentobarbital anesthetized or awake, sham-operated or portal vein-ligated rats, with or without portal cannulation. Variability between the two successive measurements was not affected by portal hypertension or portal cannulation, and was not different in the splanchnic territory and in other organs. Laparotomy with portal cannulation had no significant effect in sham-operated rats. In awake portal hypertensive rats, cardiac output (53.9 ± 3.0 vs 45.8 ± 2.9 ml · min^{-1} · 100 g body wt^{-1}, $P < 0.01$) and splanchnic blood flow (12.31 ± 0.72 vs 9.34 ± 0.85 ml · min^{-1} · 100 g body wt^{-1}, $P < 0.01$) were lower in portal vein cannulated rats compared with those of non-cannulated animals. In anesthetized portal hypertensive rats blood flows were unaffected by portal cannulation, but arterial pressure (100.2 ± 4.3 vs 119.9 ± 3.4 mm Hg, $P < 0.01$) and heart rate (366.5 ± 10.0 vs 405.5 ± 7.4 beats · min^{-1}, $P < 0.01$) were elevated. Anesthesia also decreased portal pressure (14.8 ± 0.5 vs 12.0 ± 0.4 mm Hg, $P < 0.05$) in portal hypertensive rats. We conclude that the microsphere method remains reproducible in portal hypertensive rat models. Laparotomy with portal cannulation can alter systemic and splanchnic hemodynamics in portal hypertensive rats; these effects can also be changed during pentobarbital anesthesia. Regional blood flow measurements in portal hypertensive rats should be performed in animals without portal cannulation and preferably in the awake state.

The radioactive microsphere method has allowed new insights in the pathophysiology of portal hypertension (1–7). Although this technique has been adapted to the measurement of regional blood flows in small animals, the results obtained in different laboratories and on different experimental models still show numerous discrepancies. The influences of some experimental conditions have been studied, predominantly the effects of anesthetics on both systemic and splanchnic circulations (6, 8–10). The microsphere method gave promising results by opening the possibility of serial determinations of splanchnic blood flows during various physiologic or pharmacological manipulations (2, 9, 17, 18). Reproducibility has been evaluated by the use of repeated microsphere injections in the same animal (11–15) and was considered good for cardiac output and regional blood flows, except for the liver, stomach, and spleen and, in spontaneously hypertensive rats, poor in the intestines as well (11, 16). Moreover, the reproducibility of the method has not been tested in portal hypertensive rat models where significant modifications of the portal tributary circulation (4, 19, 20) could interfere with the basic assumption that spheres, once trapped in a tissue, do not subsequently migrate (15, 21).

Precise evaluation of hepatoportal hemodynamics also requires simultaneous determination of the portal pressure and, in a portal-hypertensive setting, measurement of portosystemic collateral circulation. Both have been achieved only by surgical opening of the abdominal cavity (2, 5, 19, 22, 23). Even when minimal and subsequently closed, this could induce hemodynamic alterations which have not been studied in conscious rats after recovery from anesthesia nor in a portal hypertensive model.

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This study was thus designed to determine first the reproducibility of liver and portal venous territory blood flows as measured by the microsphere method, in sham-operated or portal vein-ligated rats, anesthetized or awake (after recovery from anesthesia and surgery), and second the effect of laparotomy with the introduction of a portal catheter in these models.

Materials and Methods. Eighty male Sprague–Dawley rats (Charles River, Saint-Aubin-les-Elbeuf, France) were divided into eight groups of 10. Rats were allowed free access to food and water until 14–18 hr before the hemodynamic study, when food was withdrawn. Four groups had been previously rendered portal hypertensive by partial ligation of the portal vein. Under either anesthesia, the abdomen was incised and the portal vein exposed. A polyethylene catheter of 0.96 mm external diameter was passed alongside, and 3-O silk was used to ligate both the catheter and the portal vein. The catheter was then removed and the abdomen closed with catgut. Four groups were sham-operated; they underwent the same procedure but the portal vein was only exposed, not ligated, then the abdomen closed. All rats were studied 3 to 4 weeks after the procedure.

The portal hypertensive and sham-operated rats were studied either anesthetized (n = 40) or awake (n = 40). Anesthesia was induced by intraperitoneal injection of pentobarbital sodium, 5 mg/100 g body wt. The trachea was intubated in all anesthetized rats to ensure free ventilation. Rats were studied 30–40 min after induction, when hemodynamics were stable. For the studies in awake animals, ether anesthesia was initially used during the catheter insertion phase. Rats were then carefully immobilized in a restraint apparatus as previously described (6, 8) and allowed to reawaken. The studies were then performed after at least 30 min of consciousness, quiet rest, and hemodynamic stability.

In all rats the femoral artery was cannulated and connected to a multichannel recorder (Honeywell et Philips, Epinay-sur-Seine, France) for measurements of arterial pressure and heart rate and reference sample withdrawal. Another catheter was passed into the left ventricle via the right carotid. Correct position was confirmed by pressure tracings. The rectal temperature was monitored using a thermistor probe and kept at 37 ± 0.5°C by heat lamps. In 40 rats (20 anesthetized and 20 awake) a portal venous catheter was also introduced: a 1.5- to 2-cm midline abdominal incision was made. With minimal mesenteric manipulation, a polyethylene catheter (0.7 mm external diameter) was inserted at the junction of two small ileal veins and advanced under direct observation up to the confluence of the superior mesenteric and splenic veins. All catheters, previously rinsed with heparin, were connected to pressure transducers with zero reference level set at the midposition of the rat. The abdomen and all incisions were closed with catgut after local application of 2% lidocaine gel, intended to minimize postoperative pain.

^{113}Sn- or ^{141}Ce-labeled microspheres (15 ± 3 μm, sp act 10 mCi/g; New England Nuclear, Boston, MA) were suspended in 0.9% NaCl with one drop of 0.05% Tween 80 and sonicated for 10 min before injection. A precounted aliquot of 80 μl (60,000 microspheres) was injected and flushed with 700 μl saline over 45 sec into the left ventricle. Starting 5 sec before the microsphere injection, the reference sample was drawn into a motor-driven syringe at a rate of 0.7 ml/min for 1 min.

A "placebo" solution of saline was infused at a rate of 0.1 ml/min and arterial pressure and heart rate were monitored for 10 min before the second microsphere injection was made.

Immediately after the second microsphere injection, the animals were killed with an overdose of pentobarbital sodium and individual organs were dissected out. Radioactivity of each organ and the reference blood sample were counted in a scintillation counter (Compteur Gamma 4000, Intertechnique, Plaisir, France). Energy windows were set at 70–210 keV for ^{141}Ce and 280–1000 keV for ^{113}Sn. Appropriate corrections for isotope counting overlap were made using ^{113}Sn and ^{141}Ce standards.

The following requirements were met before data analysis: (a) adequate microsphere mixing, assured by a difference of <10% be-

tween left and right kidneys; (b) stability of mean arterial pressure (<10 mm HG variation) and heart rate (<30 beats/min variation) between the two injections. Any animal not meeting these requirements was rejected from analysis.

Cardiac output (CO) was calculated by the formula: CO (ml/min) = radioactivity injected (cpm)/reference sample radioactivity (cpm) × 0.7 (ml/min). Cardiac index (CI) was derived from the formula: CI (ml/100 g body wt) = CO/100 g body wt. Regional blood flows were calculated according to the formula: organ blood flow (ml/min) = (organ radioactivity (cpm)/radioactivity injected (cpm)) × CO (ml/min). Portal venous territory blood flow was taken as the sum of the spleen stomach, small bowel, colon, and mesentery with pancreas. Splanchnic tributary blood flow was defined as the sum of portal venous territory and liver arterial blood flows. Portal venous territory and splanchnic blood flows are expressed in ml/100 g body wt. Total peripheral resistance (TPR) and portal territory vascular resistance (PTVR) were calculated according to the following formulas:

$$\text{TPR (dyn} \cdot \text{sec} \cdot \text{cm}^{-5}) \cdot 10^3 = \text{Mean arterial pressure (mm Hg)} \times 80/\text{CO (ml/min)}.$$

$$\text{PTVR (dyn} \cdot \text{sec} \cdot \text{cm}^{-5}) \cdot 10^3 = (\text{Mean arterial pressure (mm Hg)} - \text{corresponding group mean portal pressure (mm Hg)}) \times 80/\text{portal tributary blood flow (ml/min)}.$$

Results are given as mean ± SE. Systemic and regional hemodynamic values in the eight groups were compared by one-way analysis of variance for the presence or absence of a portal venous catheter, independently for each group of portal hypertensive or sham-operated, anesthetized or awake rats. First and second determinations were compared using paired *t* test. Coefficient of variability was defined as (2^{nd} injection measurement − 1^{st} injection) · $100/1^{st}$ injection measurement. Two-way analysis of variance was used for comparison of coefficients of variability between groups; the mean and SE of the coefficient of variability were calculated for each organ in each group. Portal pressures were compared between anesthetized and awake groups using one-way analysis of variance.

Results. Hemodynamic results for the eight groups of rats are shown in Tables I–IV.

In sham-operated rats, anesthetized or awake, there was no significant difference between groups with or without a portal catheter, or between first and second determinations of cardiac output and regional blood flows.

In anesthetized portal hypertensive rats, laparotomy with portal cannulation induced no alteration in cardiac output or regional blood flows, but significantly elevated total peripheral resistance, arterial pressure, and heart rate.

In awake portal hypertensive rats, total peripheral resistance was also elevated in portal vein-cannulated rats, in comparison with their noncannulated counterparts. Laparotomy with the presence of a portal catheter did not alter mean arterial pressure and heart rate, but significantly lowered cardiac index, brain, intestinal, liver, and total splanchnic blood flows.

Variability in liver, portal venous territory, and total splanchnic blood flow measurements was not significantly different from that of kidney and brain. However, liver and portal territory blood flow variability tended to be higher in all groups with a portal catheter as compared to those without a portal catheter; because of large interindividual differences in variability, this did not reach the level of statistical significance. Anesthesia did not significantly alter variability in either sham-operated or portal vein-stenosed rats.

Portal pressure was significantly higher in awake than in anesthetized portal hypertensive rats. Portal pressure was not significantly different in awake and anesthetized sham-operated rats.

Discussion. This study was designed to answer two questions: (a) What is the variability of liver and portal venous territory blood flow measurements by the microsphere method in normal and portal hypertensive rats? (b) Can laparotomy with portal cannulation influence regional arterial blood flows

TABLE I. HEMODYNAMICS IN ANESTHETIZED SHAM-OPERATED RATS

	Without portal vein catheter (n = 10)			With portal vein catheter (n = 10)		
	1st determination	2nd determination	Coefficient of variability (%)	1st determination	2nd determination	Coefficient of variability (%)
Systemic hemodynamics						
Mean arterial pressure, mm Hg	117 ± 4	117 ± 3		125 ± 3	117 ± 7	
Heart rate, beats · min^{-1}	400 ± 8	392 ± 9		392 ± 9	399 ± 7	
Cardiac index, ml · min^{-1} · 100 g body wt^{-1}	34.2 ± 2.1	33.7 ± 2.2	8.3 ± 2.5	30.8 ± 1.5	32.4 ± 1.3	18.0 ± 3.9
Total peripheral resistance, dyn · sec · cm^{-5} · 10^3	91.3 ± 5.1	92.6 ± 5.2		108.1 ± 5.7	96.3 ± 5.1	
Splanchnic hemodynamics						
Portal pressure, mm Hg				7.4 ± 0.5	7.5 ± 0.4	
Stomach	0.51 ± 0.05	0.60 ± 0.07	23.9 ± 6.7	0.45 ± 0.05	0.60 ± 0.07	42.2 ± 13.3
Intestine	2.09 ± 0.24	2.52 ± 0.44	20.8 ± 5.2	1.08 ± 0.22	1.88 ± 0.25	28.4 ± 7.3
Colon	1.17 ± 0.09	1.18 ± 0.09	23.8 ± 4.9	0.99 ± 0.09	0.97 ± 0.07	17.7 ± 5.0
Spleen	3.47 ± 0.44	3.20 ± 0.42	22.2 ± 4.2	1.77 ± 0.26	2.30 ± 0.33	221.4 ± 145.6
Mesentery-pancreas	1.03 ± 0.09	0.93 ± 0.08	21.5 ± 6.4	0.93 ± 0.06	0.94 ± 0.12	23.8 ± 4.9
Portal tributary, ml · min^{-1} · 100 g body wt^{-1}	6.33 ± 0.46	6.39 ± 0.35	17.5 ± 4.4	5.76 ± 0.54	6.10 ± 0.39	26.3 ± 6.7
Portal tributary vascular resistance, dyn · sec · cm^{-5} · 10^3	462.6 ± 48.8	455.7 ± 48.0		544.0 ± 51.5	478.8 ± 50.4	
Liver	0.41 ± 0.08	0.42 ± 0.06	32.3 ± 9.8	0.38 ± 0.06	0.45 ± 0.05	32.9 ± 10.1
Splanchnic tributary, ml · min^{-1} · 100 g body wt^{-1}	7.57 ± 0.53	7.39 ± 0.40	14.9 ± 4.6	6.84 ± 0.52	7.32 ± 0.36	25.1 ± 7.4
Extrasplanchnic hemodynamics						
Kidneys	7.88 ± 0.58	7.92 ± 0.82	13.9 ± 2.5	7.13 ± 0.70	7.09 ± 0.61	27.0 ± 6.5
Brain	0.87 ± 0.09	0.78 ± 0.09	18.8 ± 2.7	0.91 ± 0.09	0.90 ± 0.08	27.0 ± 6.4

Note. Values are means ± SE. Organ blood flows are expressed in ml · min^{-1} · g tissue wt^{-1}. Portal tributary blood flow is equal to the sum of stomach, intestine, colon, spleen, and mesentery-pancreas blood flows. Liver is equal to hepatic arterial blood flow. Splanchnic tributary flow is equal to the sum of portal tributary and hepatic arterial blood flows.

TABLE II. HEMODYNAMICS IN ANESTHETIZED PORTAL VEIN-STENOSED RATS

	Without portal vein catheter (n = 10)			With portal vein catheter (n = 10)		
	1st determination	2nd determination	Coefficient of variability (%)	1st determination	2nd determination	Coefficient of variability (%)
Systemic hemodynamics						
Mean arterial pressure, mm Hg	100 ± 4	101 ± 4		120 ± 3*	116 ± 3	
Heart rate, beats · min^{-1}	366 ± 10	368 ± 10		405 ± 7*	399 ± 8	
Cardiac index, ml · min^{-1} · 100 g body wt^{-1}	42.5 ± 2.4	40.4 ± 1.9	8.3 ± 2.0	41.3 ± 2.9	45.1 ± 3.4	17.9 ± 4.0
Total peripheral resistance, dyn · sec · cm^{-5} · 10^3	62.9 ± 3.3	66.8 ± 3.5		77.4 ± 4.1**	69.0 ± 3.6	
Splanchnic hemodynamics						
Portal pressure, mm Hg				12.0 ± 0.4	11.9 ± 0.4	
Stomach	0.79 ± 0.11	0.64 ± 0.06	17.7 ± 5.1	0.59 ± 0.07	0.66 ± 0.07	40.6 ± 8.2
Intestine	1.92 ± 0.15	1.74 ± 0.15	26.6 ± 7.2	1.97 ± 0.18	2.04 ± 0.15	26.0 ± 9.9
Colon	1.65 ± 0.11	1.63 ± 0.16	13.9 ± 3.8	1.65 ± 0.26	1.66 ± 0.23	28.1 ± 6.8
Spleen	1.68 ± 0.21	1.65 ± 0.15	28.9 ± 6.4	1.57 ± 0.27	1.64 ± 0.21	63.1 ± 29.4
Mesentery-pancreas	0.93 ± 0.12	0.94 ± 0.10	19.9 ± 5.0	1.14 ± 0.18	1.17 ± 0.16	29.1 ± 6.3
Portal tributary, ml · min^{-1} · 100 g body wt^{-1}	7.67 ± 0.48	7.08 ± 0.45	21.0 ± 6.1	7.73 ± 0.80	8.12 ± 0.73	29.4 ± 9.2
Portal tributary vascular resistance, dyn · sec · cm^{-5} · 10^3	306.6 ± 32.3	336.0 ± 35.5		372.2 ± 39.2	343.2 ± 36.2	
Liver	0.81 ± 0.01	0.81 ± 0.01	27.6 ± 8.7	0.81 ± 0.10	0.90 ± 0.10	27.9 ± 10.0
Splanchnic tributary, ml · min^{-1} · 100 g body wt^{-1}	10.00 ± 0.75	9.07 ± 0.60	17.4 ± 6.4	9.16 ± 1.17	10.59 ± 1.09	24.5 ± 9.5
Extrasplanchnic hemodynamics						
Kidneys	7.89 ± 0.52	6.86 ± 0.53	20.1 ± 5.1	7.34 ± 0.52	8.00 ± 0.53	21.2 ± 5.8
Brain	1.03 ± 0.09	0.85 ± 0.07	22.2 ± 7.0	1.06 ± 0.08	1.22 ± 0.13	24.2 ± 5.8

Note. Values are means ± SE. Organ blood flows are expressed in ml · min^{-1} · g tissue wt^{-1}. Portal tributary blood flow is equal to the sum of stomach, intestine, colon, spleen, and mesentery-pancreas blood flows. Liver is equal to hepatic arterial blood flow. Splanchnic tributary flow is equal to the sum of portal tributary and hepatic arterial blood flows.

* Significantly different from rats without portal vein catheter ($P < 0.01$).

** Significantly different from rats without portal vein catheter ($P < 0.05$).

TABLE III. HEMODYNAMICS IN AWAKE SHAM-OPERATED RATS

	Without portal vein catheter (n = 10)			With portal vein catheter (n = 10)		
	1st determination	2nd determination	Coefficient of variability (%)	1st determination	2nd determination	Coefficient of variability (%)
Systemic hemodynamics						
Mean arterial pressure, mm Hg	108 ± 3	114 ± 5		108 ± 3	114 ± 2	
Heart rate, beats · min^{-1}	369 ± 11	362 ± 9		367 ± 11	370 ± 14	
Cardiac index, ml · min^{-1} · 100 g body wt^{-1}	41.5 ± 2.1	43.4 ± 1.7	14.0 ± 2.7	44.9 ± 2.2	41.2 ± 2.4	15.8 ± 3.2
Total peripheral resistance, dyn · s · cm^{-5} · 10^3	68.4 ± 3.4	67.9 ± 4.1		64.1 ± 3.4	73.5 ± 3.9	
Splanchnic hemodynamics						
Portal pressure, mm Hg				8.0 ± 0.3	8.3 ± 0.3	
Stomach	0.70 ± 0.11	1.21 ± 0.12	39.2 ± 7.6	0.51 ± 0.08	0.76 ± 0.18	49.5 ± 19.4
Intestine	1.93 ± 0.16	2.13 ± 0.16	22.4 ± 5.3	1.67 ± 0.19	1.81 ± 0.18	25.6 ± 7.0
Colon	1.24 ± 0.10	1.24 ± 0.09	14.2 ± 2.6	1.25 ± 0.10	1.30 ± 0.10	18.6 ± 4.6
Spleen	3.04 ± 0.30	2.85 ± 0.42	36.3 ± 11.5	2.98 ± 0.28	3.93 ± 0.71	59.0 ± 12.3
Mesentery-pancreas	1.13 ± 0.08	1.06 ± 0.12	18.5 ± 4.8	1.14 ± 0.11	1.27 ± 0.17	29.8 ± 13.8
Portal tributary, ml · min^{-1} · 100 g body wt^{-1}	6.06 ± 0.54	6.29 ± 0.41	13.3 ± 4.0	5.66 ± 0.56	6.23 ± 0.51	24.5 ± 7.2
Portal tributary vascular resistance, dyn · sec · cm^{-5} · 10^3	455.9 ± 41.6	435.7 ± 35.9		469.3 ± 49.8	446.1 ± 47.1	
Liver	0.67 ± 0.04	0.63 ± 0.05	16.8 ± 3.9	0.78 ± 0.10	0.79 ± 0.12	23.9 ± 4.3
Splanchnic tributary, ml · min^{-1} · 100 g body wt^{-1}	8.01 ± 0.59	7.93 ± 0.53	11.1 ± 3.3	7.92 ± 0.60	8.32 ± 0.67	20.3 ± 6.4
Extrasplanchnic hemodynamics						
Kidneys	7.30 ± 0.49	6.97 ± 0.72	17.0 ± 4.5	7.13 ± 0.49	6.55 ± 0.70	18.8 ± 4.8
Brain	1.44 ± 0.16	1.46 ± 0.09	11.6 ± 4.2	1.35 ± 0.16	1.51 ± 0.09	44.6 ± 15.1

Note. Values are means ± SE. Organ blood flows are expressed in ml · min^{-1} · g tissue wt^{-1}. Portal tributary blood flow is equal to the sum of stomach, intestine, colon, spleen, and mesentery-pancreas blood flows. Liver is equal to hepatic arterial blood flow. Splanchnic tributary flow is equal to the sum of portal tributary and hepatic arterial blood flows.

TABLE IV. HEMODYNAMICS IN AWAKE PORTAL-HYPERTENSIVE RATS

	Without portal vein catheter (n = 10)			With portal vein catheter (n = 10)		
	1st determination	2nd determination	Coefficient of variability (%)	1st determination	2nd determination	Coefficient of variability (%)
Systemic hemodynamics						
Mean arterial pressure, mm Hg	102 ± 3	104 ± 2		101 ± 3	101 ± 3	
Heart rate beats · min^{-1}	349 ± 11	341 ± 10		356 ± 15	342 ± 13	
Cardiac index, ml · min^{-1} · 100 g body wt^{-1}	53.9 ± 3.0	55.6 ± 3.6	16.4 ± 3.6	45.8 ± 2.9*	44.8 ± 2.9	15.9 ± 3.2
Total peripheral resistance, dyn · sec · cm^{-5} · 10^3	52.9 ± 3.1	52.7 ± 11.5		62.7 ± 3.0**	63.1 ± 2.7	
Splanchnic hemodynamics						
Portal pressure, mm Hg				14.8 ± 0.5	14.2 ± 0.6	
Stomach	0.94 ± 0.12	0.88 ± 0.09	47.4 ± 17.1	0.70 ± 0.15	0.65 ± 0.09	20.5 ± 4.3
Intestine	2.10 ± 0.19	2.37 ± 0.21	30.0 ± 10.5	1.56 ± 0.16**	1.89 ± 0.17	38.6 ± 10.6
Colon	1.92 ± 0.25	2.06 ± 0.31	20.5 ± 8.8	1.46 ± 0.26*	1.14 ± 0.09	29.4 ± 6.5
Spleen	2.40 ± 0.47	2.56 ± 0.31	38.1 ± 14.6	2.12 ± 0.52	1.75 ± 0.36	38.1 ± 7.2
Mesentery-pancreas	1.48 ± 0.05	1.52 ± 0.18	25.9 ± 9.2	1.50 ± 0.14	1.56 ± 0.24	26.7 ± 5.8
Portal tributary ml · min^{-1} · 100 g body wt^{-1}	8.72 ± 0.68	9.58 ± 0.83	23.2 ± 10.0	7.17 ± 0.67*	7.44 ± 0.74	24.1 ± 5.3
Portal tributary vascular resistance, dyn · sec · cm^{-5} · 10^3	288.6 ± 27.5	271.0 ± 27.5		356.9 ± 30.0**	349.7 ± 33.2	
Liver	1.24 ± 0.09	1.23 ± 0.09	13.7 ± 4.4	0.83 ± 0.10*	0.91 ± 0.08	29.3 ± 7.0
Splanchnic tributary, ml · min^{-1} · 100 g body wt^{-1}	12.31 ± 0.72	13.07 ± 1.01	16.4 ± 5.8	9.34 ± 0.85*	9.80 ± 0.84	21.9 ± 4.7
Extrasplanchnic hemodynamics						
Kidneys	7.96 ± 0.57	7.45 ± 0.91	26.1 ± 6.1	6.98 ± 0.51	7.09 ± 0.66	21.1 ± 2.4
Brain	1.91 ± 0.24	1.50 ± 0.14	25.2 ± 4.8	1.24 ± 0.11*	1.30 ± 0.11	30.3 ± 5.4

Note. Values are means ± SE. Organ blood flows are expressed in ml · min^{-1} · g tissue wt^{-1}. Portal tributary blood flow is equal to the sum of stomach, intestine, colon, spleen, and mesentery-pancreas blood flows. Liver is equal to hepatic arterial blood flow. Splanchnic tributary flow is equal to the sum of portal tributary and hepatic arterial blood flows.

* Significantly different from first determination in rats without a portal vein catheter ($P < 0.01$).

** Significantly different from first determination in rats without a portal vein catheter ($P < 0.05$).

in these models? The study design also allowed determination of the effects of pentobarbital anesthesia on portal pressure.

We did not reevaluate the specific hemodynamic patterns observed in portal vein stenosed rats (1, 3–5, 7, 24), nor the effects of anesthesia on splanchnic hemodynamics (6, 8, 9), as both issues seem now settled. The present results are in keeping with previous work, showing that, in both sham-operated and portal vein-stenosed rats, pentobarbital sodium anesthesia diminished cardiac output and increased the fraction of the cardiac output perfusing the splanchnic organs (8). We noted, however, that the present cardiac index and splanchnic blood flows are in all groups shifted in a parallel manner to higher values than in our previous works (6, 8). This raises the question of seasonal variation and stresses the constant need for control groups.

Variability of repeat measurements of portal venous territory and liver blood flows by the microsphere technique was ~20% in all groups, independent of portal hypertension or the use of pentobarbital anesthesia and of the same order of magnitude as variability of brain or kidney blood flows. Low reproducibility of splanchnic organ blood flow measurements in earlier studies, where only 20,000 spheres were injected, can probably be explained by numbers of spheres below 400 in some of these tissues (15).

Microsphere trapping largely depends on microcirculatory anatomy and regulation which could be altered by pathophysiological modifications known to occur in portal hypertension (19, 20). Benoit *et al.* (19) demonstrated a fourfold increase in arteriovenous shunting of 15-μm microspheres in portal hypertensive rats. However, 15-μm spheres infused during vasoconstriction have been demonstrated to move to different layers of the intestinal wall or to be released into venous blood up to the eighth minute after subsequent vasodilation or sudden increase in perfusion pressure (21, 25, 26). Thus our results suggest that intestinal blood flow measurements using repeated microsphere injections remain reproducible in portal vein-stenosed rats; this cannot, however, be extended without caution to the analysis of the repartition of blood flow between different intestinal layers or to the study of vasodilators.

Previous studies on systemic and splanchnic effects of laparotomy were conducted only in anesthetized animals, and with contradictory results. In cats, McNeil (27) showed that laparotomy increased mean arterial pressure and peripheral and mesenteric resistance. In dogs, laparotomy altered cardiac output and intestinal blood flow in one study (28) and not in the other (29), but mean arterial pressure and total peripheral resistances were not mentioned. In anesthetized portal hypertensive rats, Groszmann *et al.* (5) indicated that transient laparotomy with spleen exposure did not alter portal venous inflow, but systemic hemodynamic data are not given. To our knowledge, no previous work in awake animals has examined the effect of laparotomy on regional blood flows. In the present work, only in portal hypertensive rats did laparotomy with introduction of a portal vein catheter induce significant hemodynamic alterations. Several factors could account for this finding: elevated portal pressure could exaggerate blood losses during laparotomy. More important, adrenoceptor regulation and sensitivity could be altered in portal hypertension and could be responsible for abnormal responses to stress (22, 30, 31). Several other humoral factors could also account for the specific sensitivity of portal vein-stenosed rats to laparotomy with portal cannulation, including serotonin (2) and regulatory peptides (19). The present work does not allow definite conclusions about these possibilities.

Pentobarbital anesthesia altered the response to laparotomy with portal cannulation in portal hypertensive rats: in anesthetized rats, arterial pressure, heart rate, and systemic vascular resistance were higher in the presence of a portal vein catheter; cardiac output and splanchnic blood flows were unchanged. In awake portal hypertensive rats, systemic vascular resistance also increased, but arterial pressure was unchanged, due to a simultaneous decrease in both cardiac output and portal territory blood flow. Although we did not measure plasma catecholamines, renin activity, and angiotensin, it is reasonable to speculate that surgical stress and

blood loss could account for increased vascular resistance. In hemorrhaged dogs, Zimpfer *et al.* demonstrated that pentobarbital could modify the ability to maintain arterial pressure and induce different patterns of catecholamine and renin–angiotensin system activation (10). In the same way, portal vein-stenosed anesthetized rats could, in response to surgical stress, have blunted arterial pressure regulation through abnormal compensatory neural and humoral mechanisms, and abnormal arterial baroreceptor and chemoreceptor reflexes (10), while awake ones could retain effective arterial pressure homeostasis.

In previous works (6, 8), we had measured portal pressure in separate groups of rats, to eliminate potential derangements in splanchnic hemodynamics; portal pressure was found to be lower (by 10%) in anesthetized portal vein-stenosed and bile duct-ligated rats than in awake ones, although this difference was not significant. In the present study, anesthesia induced a significant 18% reduction in portal pressure in portal vein-stenosed rats, in accordance with most clinical observations that portal pressure falls during anesthesia (32). Since the absolute portal tributary blood flows were not different in awake and anesthetized animals, pentobarbital decreased total portal venous resistance in portal vein-stenosed rats. Surgical portal vein stenosis has a constant diameter; thus, pentobarbital probably decreased porto-portal (collaterals across the stenosis) and/or portosystemic collateral resistance.

In conclusion, in a portal hypertensive rat model, the microsphere method remains reproducible. Laparotomy with portal vein cannulation induced significant systemic and splanchnic hemodynamic changes only in portal vein-stenosed rats. Pentobarbital anesthesia altered this response to a surgical stress and also reduced portal pressure in portal hypertensive rats. This has direct implications for future works on portal hypertensive models since reference regional blood flow analyses, portal pressure measurements, and all other procedures with laparotomy should be performed in separate groups and preferably in awake animals where arterial pressure hemeostasis is preserved.

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