

## The use of quantitative scintigraphy in the measurement of portal-systemic shunting in rats

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Portal-systemic shunting was studied in 54 portal hypertensive rats both *in vivo* and *in vitro* using radioactive microspheres. The animals underwent partial portal vein ligation around needles of varying diameter to produce a wide range of shunting. Two to four weeks later, quantitative lung-liver scintigraphic and whole body images were obtained *in vivo* following ileocolic vein injection with  $^{99m}\text{Tc}$ -MAA. After sacrifice, the lung and liver activities were determined by the gamma camera, a dose calibrator, and a well counter. Portal-systemic shunting ranged from 0.1–97.6%. When shunting was compared *in vivo* and *in vitro*, an excellent correlation was found ( $r=0.99$ ,  $p<0.001$ ). A subgroup of 24 animals had consecutive injections of  $^{99m}\text{Tc}$ -MAA and  $^{51}\text{Cr}$ -labeled  $15\text{ }\mu\text{m}$  microspheres, which, although different in size, yielded similar results ( $r=0.89$ ,  $p<0.001$ ). We conclude that in small laboratory animals a wide range of shunting can be measured accurately *in vivo* by quantitative scintigraphy.

**Key words:** portal hypertension, portal vein ligation, portal-systemic shunting, microspheres,  $^{99m}\text{Tc}$ -MAA, quantitative scintigraphy

### INTRODUCTION

PARTIAL PORTAL VEIN LIGATION (PVL) in the rat has been proposed as an animal model for investigating the pathophysiology of portal hypertension and its consequences such as portal-systemic shunting (PSS).<sup>1</sup> Previously described reference methods for measuring PSS in small laboratory animals utilizing injections of radioactive microspheres into the portal system<sup>2,3</sup> require sacrifice of the animal for the quantitation of radioactivity in the dissected isolated organs, and do not permit serial studies. We therefore developed an alternative method for *in vivo* measurement of PSS in rats by quantitative planar scintigraphy after intraportal injection of  $^{99m}\text{Tc}$ -MAA and compared the new method with the *in vitro* procedures. Furthermore, we compared

shunt percentages obtained by injection of either MAA (10–90  $\mu\text{m}$  diameter) or  $15\text{ }\mu\text{m}$  polystyrene microspheres in order to relate our results to those of other investigators.<sup>2,3</sup> It is the purpose of this paper to present the results of these three experiments.

### MATERIALS AND METHODS

#### a) Animals

Male Sprague-Dawley rats were supplied by Zivic-Miller Laboratories, Zelienople, PA. Animals were housed in cages with wire mesh floors, and fed a standard rat chow. Food, but not water, was withdrawn 7–10 hours before PSS measurements and blood sampling.

#### b) Partial portal vein ligation

Partial portal vein ligation (PVL) was performed in 70 rats, as previously described.<sup>1,3</sup> Briefly, in animals weighing 150–250 g the portal vein was surgically exposed under ether anesthesia. A single ligature of 4-0 silk was placed around the portal vein between

Received March 24, 1992, revision accepted March 24, 1992.

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the confluence of the gastroduodenal and the portal vein and the bifurcation of the portal vein into its right and left branches. A blunt-tipped injection needle was then held alongside the portal vein, and the ligature was snugly tied around both the needle and the vein. Thereupon the needle was slipped out, thus allowing the portal vein to re-expand to the calibre of the needle used. The viscera were replaced and the abdomen was closed in two layers.

In order to produce a wide range of PSS the outer needle diameter was varied from 0.53 mm (25 gauge) to 1.27 mm (18 gauge) (Table 1). The smaller animals (150 g) were assigned to the 23 and 25 gauge groups because of the relationship between animal size and mortality seen at higher degrees of acute portal vein stenosis.<sup>4,5</sup> Nevertheless, we still observed a mortality of 80% in the 25 gauge group, 50% in the 23 gauge group, and 10% in the 22 gauge group; death usually occurred during the first 24 hours after PVL. Thus 54 animals remained alive for the measurement of PSS.

### c) Measurement of PSS

Animals were studied two to four weeks (22 days on average) after the initial surgery. Under pentobarbital anesthesia (50 mg/kg intraperitoneally) and after an equilibration period of 30 minutes, a mid-line incision was made, and the superior mesenteric vein was exposed. The bowel was covered with gauze moistened with saline pre-warmed to 37°C. Rectal temperature was monitored and kept at 37±0.5°C using an electric heating pad.

**Table 1** Portal-systemic shunting in the various groups of animals studied

Group	Needle Gauge	Diameter mm <sup>a</sup>	n <sup>b</sup>	%PSS <sup>c</sup>
1	18 G	1.27	6	1.5±0.8 <sup>d</sup> (0.1–4.8)
2	20 G	0.91	15	20.0±5.3 <sup>d</sup> (0.8–60.1)
3	21 G	0.82	10	42.3±7.9 <sup>e</sup> (6.0–79.0)
4	22 G	0.72	12	45.2±4.0 <sup>e</sup> (22.2–68.3)
5	23 G	0.65	8	54.5±4.9 <sup>f</sup> (35.9–68.1)
6	25 G	0.53	3	69.1±14.6 <sup>g</sup> (49.3–97.6)

<sup>a</sup> Outer needle diameter measured by microcalipers.

<sup>b</sup> Number of rats surviving partial portal vein ligation. Due to the high degree of portal vein stenosis mortality was 80% in the 25 G group.

<sup>c</sup> Means±SEM and range of the shunt percentages obtained *in vitro*. The degree of PSS increased as a function of portal vein stenosis, i.e. with decreasing needle diameter. ANOVA yielded a F-ratio of 10.88 ( $p<0.001$ ). Multiple comparisons were carried out by Duncan's Range Test ( $\alpha=0.05$ ).

<sup>d</sup> significantly different from groups 3, 4, 5, 6

<sup>e</sup> significantly different from groups 1, 2, 6

<sup>f</sup> significantly different from groups 1, 2

<sup>g</sup> significantly different from groups 1, 2, 3, 4

The first 30 out of the 54 rats had quantitative lung-liver images *in vivo* (vide infra) with subsequent *in vitro* counting of the isolated organs. One mCi (equivalent to approximately 200,000 particles) of <sup>99m</sup>Tc-MAA (TechneScan MAA, Mallinckrodt, St. Louis, MO) dissolved in 0.1 ml of isotonic saline was placed in a 1.0 ml plastic syringe, briefly vortexed and injected over a period of 15 seconds through a 27 gauge needle into the distal superior mesenteric vein near its junction with one of the ileocolic tributaries, i.e. at a distance of 1–2 cm from the ileocaecal valve; this will be hereafter termed 'ileocolic injection'.

Twenty four rats had consecutive ileocolic injections of both particle types through a 23 gauge butterfly needle. We first injected 10 µCi (equivalent to approximately 50,000 particles) of <sup>51</sup>Cr-labeled Nen-Trac microspheres, 16.5±0.1 µm diameter, specific activity 40 mCi/g (New England Nuclear, Wilmington, DE). Those microspheres were suspended in 0.1–0.2 ml 0.9% saline with 0.01% Tween-80 added to prevent aggregation, and were sonicated for 5 minutes prior to each use. Upon vortexing the syringe briefly, each injection was made over a period of 15 seconds. The injection of the microspheres was followed by that of MAA as described above, and the tubing was flushed with 0.4 ml of saline.

### d) Imaging procedure

Immediately after the injection of the gamma-emitting particles, whole-body images were recorded for 3 minutes with a mobile gamma camera (Technicare Sigma 420, Cleveland, OH), using a parallel-hole collimator. A Siemens Micro-Delta computer system was used for data acquisition. Thereafter the animals were sacrificed by exsanguination from the abdominal aorta. This procedure did not result in dislocation of the beads, as verified by comparing pre- and post-sacrifice images in three animals.

### e) In vitro procedures

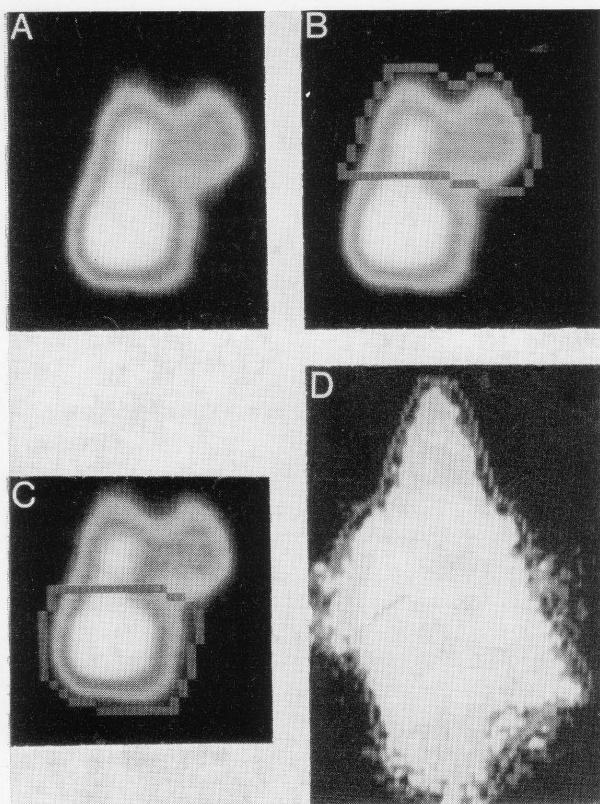
The lungs and the liver were excised, placed into pill cups and quantitatively imaged as isolated organs for another 3 minutes. Thereafter organ activities were measured by a dose calibrator (Capintec CRC-30 BC radioisotope calibrator) and, after dissection of the organs into suitably small pieces, by a well counter (Packard Auto-Gamma 5660, Downers Grove, IL). In order to rule out a possible influence of the difference in volume and/or water content of the organs counted, phantoms were constructed by measuring a point source before and after the addition of known volumes of water. Count rates were independent of volume up to a filling height of 3 cm in the well counter and up to a volume

of 25 ml in the dose calibrator. For all organs counted the filling height or volume was kept below these values.

In the experiments with injection of both  $^{51}\text{Cr}$  and  $^{99\text{m}}\text{Tc}$ , two different methods were used: (i) dual radionuclide well-counting with corrections for spill-over of  $^{51}\text{Cr}$  into the  $^{99\text{m}}\text{Tc}$  channel by using radioactive standards, and (ii) determination of the  $^{99\text{m}}\text{Tc}$ -activity (which was 100-fold higher than the  $^{51}\text{Cr}$ -activity) by the dose calibrator and well-counting of  $^{51}\text{Cr}$  after complete decay of  $^{99\text{m}}\text{Tc}$ .

#### f) Data handling

For the determination of PSS *in vivo*, net organ activities were obtained by drawing regions of interest (ROIs) over the lungs and the liver (Fig. 1 A–C). After increasing the intensity, a whole-animal ROI was drawn (Fig. 1 D). All ROIs were drawn without knowledge of the isolated organ counts and independently by three of the authors (R.E.S., T.M.,



**Fig. 1** A. Quantitative lung-liver image obtained after ileocolic injection of 1 mCi  $^{99\text{m}}\text{Tc}$ -MAA. Regions of interest were drawn around the lungs (B) liver (C), and whole body (D) where the intensity of the readout mechanisms was turned up so that activity in the whole animal could be visualized and counted. In this rat the average counts (obtained by 3 independent observers) over the lungs were 58,093 and over the liver 64,213, resulting in 47.5% PSS which agreed with the *in vitro* figure of 48.1%.

and W.N.T.), and the average of these observations was used.

The percentage of portal-systemic shunting was calculated as previously described by Chojkier and Groszmann (1981):

$$\% \text{PSS} = \frac{\text{cpm}(\text{lungs})}{[\text{cpm}(\text{lungs}) + \text{cpm}(\text{liver})]} \times 100 \quad (1)$$

This formula, which was initially designed for intrasplenic injection of microspheres, yields %PSS independent of the amount of the injected dose. Furthermore, since virtually 100% of the particles injected via an intraportal route are entrapped in the liver and/or lungs only, the degree of shunting could be calculated from the lung activity and the dose alone, provided the efficiency of the camera were known:

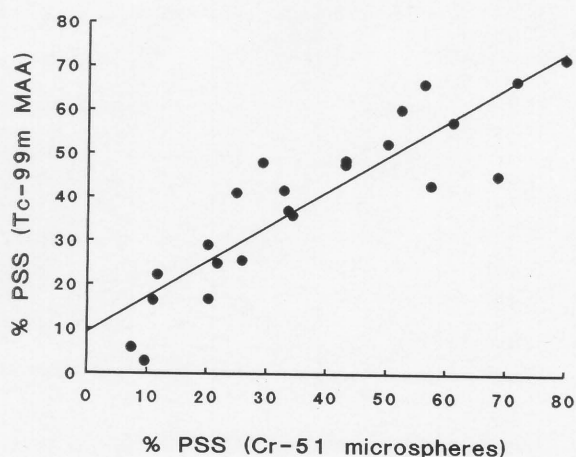
$$\% \text{PSS} = \frac{\mu\text{Ci}^*(\text{lungs})}{\mu\text{Ci}(\text{dose})} \times 100 \quad (2)$$

where  $\mu\text{Ci}^*$  represents the theoretical activity of an imaged isolated organ derived from the ROI cpm multiplied by a conversion factor. For this purpose, the efficiency of the camera was determined by imaging a known source under the same conditions as the animal.

Both formulas were also used for the data obtained *in vitro*, where the  $\mu\text{Ci}$  results yielded by the dose calibrator were utilized.

#### g) Statistical analysis

Quantitative data are expressed as mean values  $\pm$  SEM. Subgroups of rats were compared by one-way analysis of variance (ANOVA) and Duncan's range test for multiple comparisons, using the NCSS statistical package on a microcomputer. Linear



**Fig. 2** Comparison of percent shunting obtained by consecutive injections of  $^{99\text{m}}\text{Tc}$ -MAA and  $^{51}\text{Cr}$  15- $\mu\text{m}$ -microspheres. Linear regression analysis yielded a significant relationship:  $n=23$ ,  $r=0.89$ ,  $y=0.80x+9.15$  ( $\pm 8.77 \text{ Sy.x}$ ).

regression analysis was used to compare the relationships between the variables studied. A p-value of less than 0.05 was considered statistically significant.

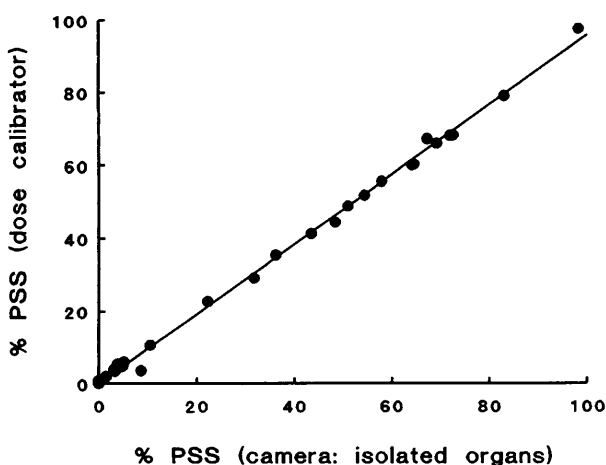
## RESULTS

Ileocolic injection of  $^{99m}\text{Tc}$ -MAA was successful in 53 out of the 54 rats studied. As expected the degree of portal systemic shunting was inversely related to the grade of the portal vein stenosis (see the Table 1). A significant linear relationship was found ( $r = -0.97$ ,  $p < 0.005$ ). When mean shunt percentages were plotted against the outer needle diameter. Consecutive injection of both particle types ( $^{99m}\text{Tc}$ -MAA and  $^{51}\text{Cr}$  microspheres) was successful in 23 out of the 24 rats and also yielded a significant correlation of percent shunting (Fig. 2).

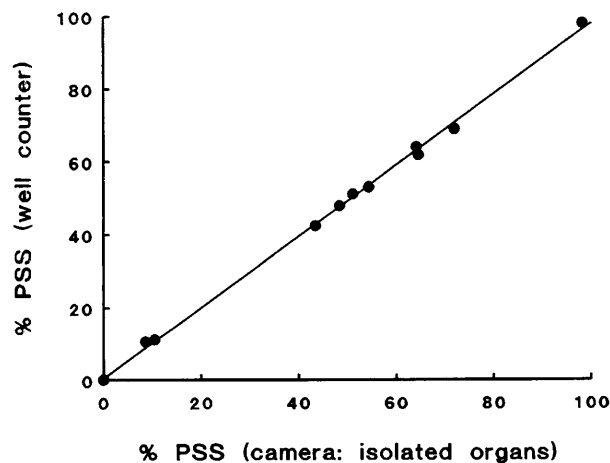
Data for comparing the shunt percentages obtained from the isolated organs were available in 29 rats and are shown in Fig. 3 A-C. After the excellent correlation between shunt percentages meas-

ured by well counter and dose calibrator became evident in the first 20 animals (Fig. 3B), the latter was solely used throughout the subsequent experiments.

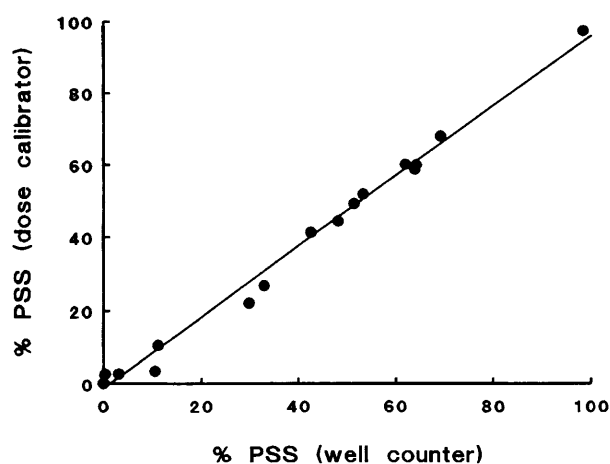
The correlations between shunt percentages obtained *in vivo* by quantitative scintigraphy and those obtained by counting the isolated organs are shown in Fig. 4A and 4B. One animal was excluded from the analysis because part of the dose was lost by extravasation. The average of the ROI determinations by three independent observers yielded a r-value of 0.99 ( $y = 0.97x + 2.40$ ;  $Sy.x = 5.08$ ) for %PSS when calculated by formula (1). Thus, the degree of PSS seen *in vitro* could be predicted from the averaged scintigraphic findings with an accuracy of approximately  $\pm 10\%$ . The individual r-values obtained by each observer were 0.98 ( $y = 0.96x + 3.29$ ;  $Sy.x = 5.94$ ), 0.97 ( $y = 0.99x + 1.49$ ;  $Sy.x = 7.58$ ), and 0.94 ( $y = 0.90x + 3.67$ ;  $Sy.x = 10.30$ ) respectively. A similar relationship between the findings *in vivo* and *in vitro* was obtained when %PSS was calculated as



A

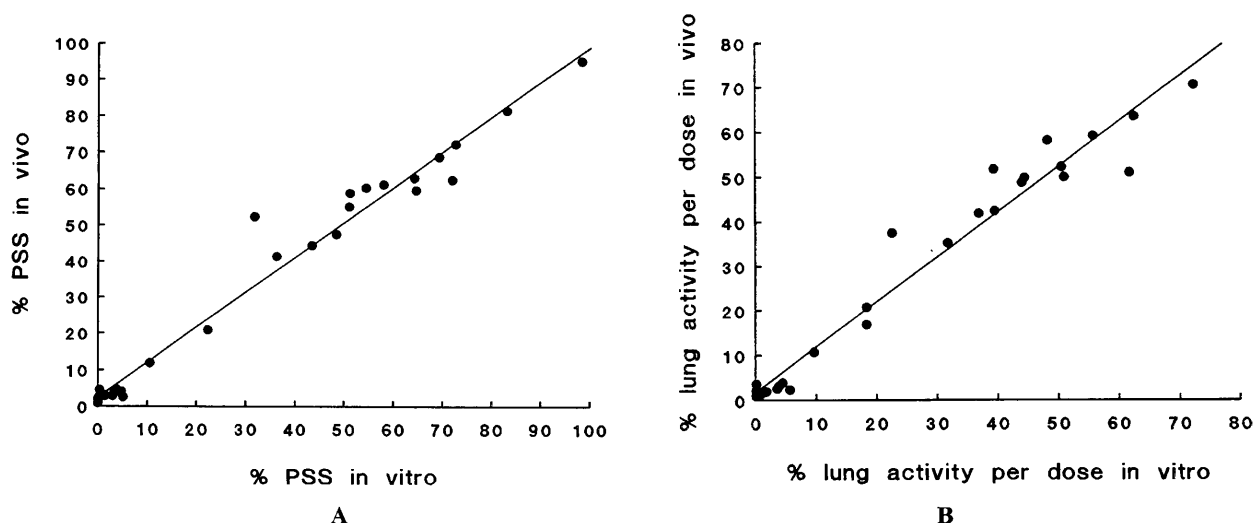


C



B

**Fig. 3** Comparison of percent shunting obtained *in vitro* from isolated organs by different methods of counting: dose calibrator versus gamma camera (A), dose calibrator versus well counter (B), and well counter versus gamma camera (C). The well counter was used for 12 animals of the 'imaging group' ( $n=29$ ) and for 8 animals of the 'dual microspheres group' ( $n=23$ ), which explains the different numbers in each comparison. All three relationships were highly significant and close to the identity line: (A)  $n=29$ ,  $r=0.999$ ,  $y=0.96x+0.05$  ( $\pm 1.43 Sy.x$ ); (B)  $n=20$ ,  $r=0.996$ ,  $y=0.97x-1.18$  ( $\pm 2.67 Sy.x$ ); (C)  $n=12$ ,  $r=0.999$ ,  $y=0.98x+0.55$  ( $\pm 1.21 Sy.x$ ).



**Fig. 4** Comparison of quantitative organ imaging *in vivo* and counting of isolated organs *in vitro*. A. Calculation by formula (1): percent shunting derived from the average lung and liver ROIs in the rat body images versus percent shunting calculated from isolated organ images ( $n=28$ ,  $r=0.99$ ,  $y=0.97x+2.40$ ,  $Sy.x=5.08$ ). B. Calculation by formula (2): theoretical lung activities (derived from lung ROIs in the rat body images) per injected dose versus lung activities *in vitro* (measured by the dose calibrator) per injected dose ( $n=28$ ,  $r=0.98$ ,  $y=1.01x+1.72$ ,  $Sy.x=4.94$ ).

lung counts per dose according to formula (2) (Fig. 4B).

Comparison of %PSS obtained by formula (1) versus formula (2), both calculated from the *in vitro* data, yielded the following correlation:  $n=28$ ,  $r=0.94$ ,  $y=0.76x+1.30$ ,  $Sy.x=8.12$ . When all the individual organ counts (lung and liver ROIs) expressed as percentages of the whole-animal counts in the scintigrams were plotted against the isolated organ activities per injected dose, a slightly weaker correlation was found ( $n=56$ ,  $r=0.92$ ,  $y=1.04x+7.10$ ,  $Sy.x=12.57$ ).

## DISCUSSION

The present study was designed to evaluate portal-systemic shunting (PSS) by a simple yet accurate method in a widely-used animal model of portal hypertension, the PVL rat. Our main findings are that (i) measuring PSS in rats by quantitative scintigraphy is feasible, (ii) substituting  $15\text{ }\mu\text{m}$  microspheres with MAA does not affect the results, and (iii) variation of the degree of portal vein stenosis produces a wide range of PSS in the PVL rat model.

Tc-99m labeled MAA was chosen primarily in order to permit high counting rates necessary for imaging, its 'physiologic' specific gravity, similar to that of whole blood (1.05) as opposed to 1.3 for polystyrene microspheres, and a smaller tendency to aggregate. A theoretical drawback is their larger and quite variable size with  $>90\%$  ranging from 10 to

$90\text{ }\mu\text{m}$  diameter ('typically'  $10\text{--}40\text{ }\mu\text{m}$ ), whereas polymeric resin spheres are available at very uniform sizing ( $16.5\pm0.1\text{ }\mu\text{m}$  diameter). However, considering the correlation we found between  $^{99\text{m}}\text{Tc}$ -MAA and  $^{51}\text{Cr}$ -microspheres, a major influence of the particle size on the results of PSS measurements seems unlikely. Finally, an additional advantage of the  $^{99\text{m}}\text{Tc}$ -MAA method is related to the short half life of the radionuclide, which would facilitate subsequent biochemical investigations of animal tissues while avoiding radioactive contamination of laboratory equipment.

Using ileocolic vein injections as was done in the present study, Blei et al.<sup>2</sup> reported approximately 85% PSS in PVL rats studied 10 days after ligation around a 21 gauge needle,<sup>2</sup> as opposed to  $42.3\pm7.9\%$  in the 21 gauge group of the present study. It should be noted that in their study PSS was evaluated 2–10 days after PVL as opposed to 10–30 (on average 22) days in the present study. Although PSS from the mesenteric and splenic beds are closely related during the first week post PVL,<sup>2</sup> mesenteric shunting appears to decrease with longer duration of the portal vein stenosis, probably as a consequence of the development of porto-portal collaterals as has been shown in a similar rat model of prehepatic portal hypertension, i.e. staged complete portal vein ligation.<sup>6</sup> Our results are further confirmed by recent reports in PVL rats, which demonstrated, following ileocolic injection,  $60\pm9\%$  PSS after 7 days and  $51\pm9\%$  after 28 days.<sup>7,8</sup>

In conclusion, in small laboratory animals, portal-systemic shunting can be measured *in vivo* by quantitative lung-liver imaging after ileocolic administration of  $^{99m}\text{Tc}$ -MAA with an accuracy of approximately  $\pm 10\%$ . Both the MAA and  $15\ \mu\text{m}$  polystyrene spheres are equally suitable for the measurement of extrahepatic PSS in portal hypertensive rats. The animal model herein characterized should prove useful as a tool to study the effects of PSS on hepatic encephalopathy and/or endocrinopathy. Given the short half-life of the tracer and the biodegradability of albumin serial studies with repeated imaging are conceivable in animals with evolving collateralization, and possible pharmacologic manipulation of the portal hypertension.

#### ACKNOWLEDGMENTS

This work was in part supported by grants NIAAA 04425, 06601, and 06971, NIDDK 32556, and by the Department of Radiology and Central Imaging Services, Inc., Presbyterian University Hospital, Pittsburgh, PA 15213. R. E. S. was the recipient of a Postdoctoral Research Exchange Grant of the Max Kade Foundation, New York, New York 10007.

The authors wish to thank Mr. James Ruszkiewicz for his excellent technical assistance and to Ms. Judith Holden for her excellent manuscript preparation.

#### REFERENCES

1. Van Thiel DH, Gavaler JS, Slone FL, et al: Is feminization in alcoholic men due in part to portal hypertension: a rat model. *Gastroenterology* 78: 81–91, 1980
2. Blei AT, O'Reilly DJ, Gottstein J: Portal-systemic shunting and the hemodynamic effects of nitroglycerin in the rat. *Gastroenterology* 86: 1428–1436, 1984
3. Chojkier M, Groszmann RJ: Measurement of portal-systemic shunting in the rat by using gamma-labeled microspheres. *Am J Physiol* 240: G371–G375, 1981
4. Myking AO, Halvorsen JF: A method for graded portal vein stenosis in rats: survival related to degree of stenosis. *Eur Surg Res* 5: 454–457, 1973
5. Myking AO, Halvorsen JF: Two stage occlusion of the portal vein in the rat: survival related to weight variation and the interval between partial and total occlusion. *Eur Surg Res* 7: 366–374, 1975
6. Burge DM, Holbrook AG, Karran SJ: Noninvasive assessment of portosystemic shunting in extrahepatic portal hypertension in rats. *J Pediatr Surg* 22: 211–214, 1987
7. Geraghty JG, Angerson WJ, Carter DC: Autoradiographic study of the regional distribution of gastric blood flow in portal hypertensive rats. *Gastroenterology* 97: 1108–1114, 1989
8. Geraghty JG, Angerson WJ, Carter DC: Portal venous pressure and portosystemic shunting in experimental portal hypertension. *Am J Physiol* 257: G52–G57, 1989