

Iodine-123-HIPDM Lung Imaging in Pulmonary Vein-Banded Pulmonary Hypertension

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To evaluate the use of N,N,N'-trimethyl-N-[2-hydroxy-3-methyl-5 iodobenzyl]-1, 3 propanediamine iodine-123 (HIPDM) in lung imaging for the diagnosis of individual pulmonary vein stenosis, 14 single-pulmonary vein-banded rats underwent lung imaging. After i.v. injection of 250–300 μ Ci [123 I]HIPDM lung images were recorded at 2 min by a gamma camera interfaced with a PDP computer. Banded lung demonstrated higher [123 I]HIPDM radioactivity than that of nonbanded lungs. The activity ratio of banded-to-nonbanded lungs ranged from 4.0 to 1.22 (average 1.62). Technetium-99m-macroaggregated albumin (MAA) lung images showed decreased perfusion in banded lung, and normal perfusion in the nonbanded lung. Post-mortem microscopic changes in pulmonary arteries and veins were compatible with the elevated pulmonary artery and venous pressure in vein-banded lung. Whether the high uptake of [123 I]HIPDM in banded lungs relates to endothelial receptors for HIPDM is unknown. Nevertheless, the finding of high lung uptake in banded lung in [123 I]HIPDM lung imaging may be potentially used to diagnose individual pulmonary vein stenosis.

J Nucl Med 1990; 31:668–673

Pulmonary circulation is interposed between the right and left heart. All circulating blood passes through the lung which acts as a “biochemical filter.” During the transpulmonary pass, the blood interacts with the very large, metabolically active endothelial surface to perform metabolic functions such as the removal, biosynthesis, and release of vasoactive hormones (1,2). The lung uptake accumulates and metabolizes a number of endogenous and exogenous substances. Endogenous substances include the biogenic amines: prostaglandin and norepinephrine. Exogenous substances include aromatic lipophilic and basic amines. Amphetamine is removed by a carrier-mediated transport process (2).

Received Jun. 30, 1989; revision accepted Dec. 8, 1989.

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Iodine-123- (123 I) labeled monoamine N-isopropyl p-iodoamphetamine (IMP) (3) and 123 I-labeled N,N,N'-trimethyl-N-[2-hydroxy-3-methyl-5 iodobenzyl]-1, 3 propanediamine (HIPDM), two amphetamine analogues, are radiopharmaceuticals known for their use in cerebral flow studies (4). After intravenous (i.v.) injection, both tracers rapidly localize and more than 90% accumulates in the lung (3,4). Because of its unique and high level of localization in the lung [123 I]HIPDM also may be used as a pulmonary imaging agent.

Passive pulmonary venous hypertension is one of the most common mechanisms for secondary pulmonary hypertension in humans (e.g., secondary to left ventricular failure and mitral valve disease). The pulmonary vein-banded rat model is readily reproducible and shows lung hemodynamic and histologic findings in vessels anticipated from human studies (5,6). Iodine-123-HIPDM lung imaging may have potential for the development of noninvasive diagnosis, and for monitoring passive pulmonary venous hypertension. A portion of this work was published earlier in abstract form (7).

MATERIALS AND METHODS

Fourteen male white Sprague-Dawley rats weighing 300–350 g formed the basis for this study. The operative procedures of pulmonary-vein constriction (5,6), discussed below, were performed by one of the coauthors (CMC).

Operative Procedures of Pulmonary-Vein Constriction

Animal Preparation. After the weight was measured and recorded, anesthesia was given intraperitoneally utilizing 5 mg/100 g pentobarbital supplemented with 0.2 mg/100 g ketamine. Each rat underwent endotracheal intubation by the method of Schaeffer et al. (8), and was placed on a rodent ventilator (Harvard #680, South Natick, MA) with a total volume of 1 ml/100 g of body weight and a rate of 80 breaths/min.

Operative Procedures to Induce Pulmonary-Vein Stenosis

After thoracotomy, the lungs were gently retracted posteriorly. The pulmonary veins were isolated and a #4 silk tie

placed around it. Utilizing a #24 Jelco plastic sheath as a sizer, a knot was tied encompassing both the Jelco and the pulmonary vein; Jelco was then removed. The ribs were approximated with a single #2-0 silk tie. The subcutaneous muscular layer and skin were sutured and closed (6,8).

Preparation of Radiopharmaceuticals

Iodine-123-HIPDM Preparation. The radiolabeling of HIPDM was achieved by a single exchange reaction. A solution of HIPDM (1 mg in 1 ml of 0.07 N HCl) and 100 μ l 0.1 N HCl solution containing 8 mCi of Na^{123}I in a sealed 10-ml serum vial was heated in a boiling-water bath for 30 min. The reaction mixture was analyzed by thin layer chromatography (TLC). The radiochemical incorporation was >95%. The mixture was diluted with 1 ml of 0.9% saline and sterilized by passage through a 0.22-micron filter (4).

Technetium-99m-MAA Preparation. Macroaggregated albumin (MAA) kits were obtained from a commercially available source (Macrotec, Squibb Diagnostics, Princeton, NJ). Technetium-99m was labeled with MAA in preparation for clinical perfusion lung imaging.

Imaging Procedures

Pulmonary vein constricted rats were imaged at 4–6 wk as follows: after intraperitoneal anesthesia with 5 mg/100 g pentobarbital, each rat was placed under a scintillation camera with a pinhole collimator. The camera was interfaced with a PDP-11 computer.

Technetium-99m-MAA Pulmonary Perfusion Imaging Procedures. The lung images and imaging data were obtained from the thoraces of the rats after 5–10 min i.v. injection 200–250 μ Ci of $^{99\text{m}}\text{Tc}$ -MAA.

Iodine-123-HIPDM Pulmonary Imaging Procedures. This imaging was performed 48–72 hr after the $^{99\text{m}}\text{Tc}$ -MAA lung study. Five to ten minutes after i.v. injection of 200–250 μ Ci of ^{123}I -HIPDM images and imaging data were obtained from the thoraces of the rats.

Differential Pulmonary Uptake of ^{123}I -HIPDM and $^{99\text{m}}\text{Tc}$ -MAA

Both differential pulmonary uptake of ^{123}I -HIPDM and differential pulmonary perfusion using $^{99\text{m}}\text{Tc}$ -MAA and counts of treated/nontreated lung were computed as follows:

1. A region of interest (ROI) of each lung (same size of rectangle) was made and counts of each side of ROI were obtained.
2. The pulmonary perfusion percent of treated and nontreated lung was calculated.
3. The pulmonary activity ratios of treated-to-nontreated lung also were computed.

Lung Tissue Preparation

The animals were killed after intraperitoneal injection with pentobarbital, 50 mg/100 g (a lethal dose). After breathing ceased, the heart and lungs were removed en bloc and the tracheobronchial tree was perfused at low pressure (20 cm H_2O) with 10% neutral buffered formalin. Sections of lung were stained with H.E stain and Prussian blue and elastic trichrome combination stains.

RESULTS

Iodine-123-HIPDM Pulmonary Imaging

The images of the treated lung in 14 rats consistently demonstrated much higher activity than that in nontreated lung in sequential images of pulmonary-vein constriction (Fig. 1). The activity ratio of treated-to-nontreated lung confirmed ^{123}I -HIPDM activity to be significantly higher in the treated lung ($p < 0.001$) (Table 1). Thirteen out of 14 rats exhibited a treated/nontreated ratio in the range of 1.2–2.4. Figure 2 shows the method of computation of the differential pulmonary activity for treated versus nontreated lung.

Technetium-99m-MAA Pulmonary Perfusion Imaging

Five of 14 rats underwent the pulmonary perfusion study. The lung images of these five rats showed decreased perfusion in the treated lung as compared with nontreated lung. Differential pulmonary perfusion of the treated versus nontreated lung ranged from 29%–37% to 62%–71%, respectively. Figure 3 demonstrates mis-matched ^{123}I -HIPDM and $^{99\text{m}}\text{Tc}$ -MAA lung images.

Concurrent pulmonary contrast angiogram showed delayed washout of contrast media through the vein-banded lung. In the treated lung, there was a “prune tree” or the “tree-in-winter” appearance of the pulmonary artery, consisting of narrowing of the pulmonary artery. No contrast in the smaller arteries or dilated pulmonary vein was seen (Fig. 4).



FIGURE 1
Iodine-123-HIPDM lung image 6 wk post-pulmonary vein stenosis on the left showing markedly increased uptake in the left lung computation of nontreated (right) and treated (left) lung activity ratio and differential pulmonary activity.

TABLE 1
Differential Pulmonary [^{123}I]HIPDM Uptake and Treated/
Nontreated Pulmonary Uptake Ratio

Ratio no.	Treated	Nontreated	Treated/Nontreated ratio
1	80%	20%	4.00
2	55.5%	44.5%	1.25
3	57%	43%	1.33
4	60%	40%	1.50
5	57%	43%	1.33
6	58%	42%	1.38
7	55.9%	44.1%	1.27
18	55.3%	44.7%	1.24
19	60.8%	39.2%	1.55
10	61%	39%	1.56
11	57.1%	42.9%	1.33
12	57%	43%	1.33
13	55%	45%	1.22
14	70.6%	29.4%	2.40
\bar{x}	60.0%	40.0%	1.62
s.d.	7.0	7.0	0.75

$p < 0.001$ by paired t-test.

Pathologic Examination

Postmortem microscopic changes in pulmonary arteries and veins were compatible with elevated resistance in veins banded in this model (Fig. 5). For pulmonary arteries, thickening of muscular layers, intimal proliferation, and perivascular edema are shown with elastic trichrome stain. The pulmonary vein is shown dilated with arterIALIZATION and perivascular fibrosis. Bronchial vessels are prominent. In addition, prominent iron pigment in macrophages demonstrated by Prussian blue stain, is seen in the treated lung.

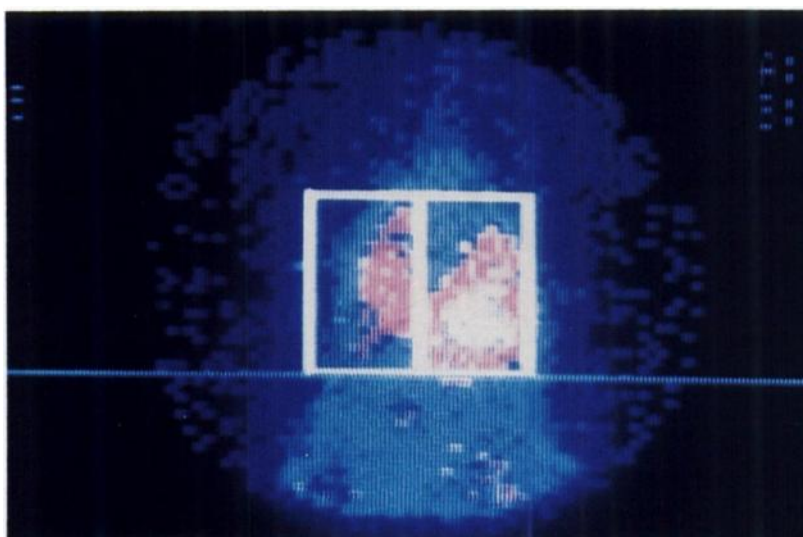
DISCUSSION

Stenosis of individual pulmonary veins, either congenital or acquired, may occur at any point in the extrapulmonary course (9). This stenosis may lead to significant alteration of pulmonary structure and function.

Currently, a reliable, noninvasive method for determining pulmonary artery pressure is virtually absent. Methods for assessing pulmonary hypertension, include symptoms of pulmonary hypertension, physical examination, ECG, chest roentgenogram (CXR), and ultrasound (9). Pulmonary hypertension symptoms of dyspnea, fatigue, syncope, angina-like pain, hemoptysis, and hoarseness may not develop until the resting pulmonary arterial pressure is ~ 2 or more times normal; earlier indicators of pulmonary hypertensive disorders are desired. The physical finding of the closure sound of the pulmonic valve (P2) gives specific pulmonary-artery-pressure information, but this change is subtle in mild pulmonary hypertension and interpretation is complicated by variations in chest wall thickness and the amount of lung overlying the pulmonary artery. In severe pulmonary hypertension, the ECG shows right ventricular hypertrophy; however, when the ECG is normal, pulmonary hypertension, especially of mild or moderate degree, is not ruled out. CSR, as evidence of width of the descending branch of the right pulmonary artery, hilar width, and hilar thoracic index is useful as a noninvasive estimate of pulmonary artery pressure, but imperfect (10). The echocardiogram is useful in some instances in assessing pulmonary hypertension, but it is also not always reliable. Ultimate diagnosis of pulmonary artery pressure depends on contrast angiography, which is invasive. More than 90% of i.v. injection of [^{123}I]IMP or [^{123}I]HIPDM is extracted in the lung during transpulmonary passage (3,4,11), with ho-

FIGURE 2

Computation of nontreated (right) and treated (left) lung activity ratio and differential pulmonary activity. Treated/nontreated lung ratio (left/right) is 1.88. Treated (left) and nontreated (right) differential pulmonary activity is 65.3% and 34.7%, respectively.



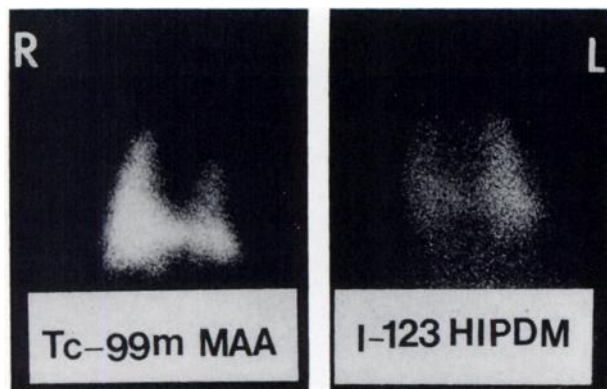


FIGURE 3
Technetium-99m-MAA lung perfusion image and [123 I]-HIPDM lung image 6 wk after left pulmonary vein stenosis: showing markedly decreased ^{99m}Tc -MAA perfusion on the left lung as compared to the right lung. Differential treated (left) and nontreated (right) pulmonary perfusion is 29.8% and 72.2%, respectively, and the treated (left) and nontreated (right) lung activity ratio is 0.42. In the [123 I]-HIPDM lung image, there is marked increase in uptake in the left lung.

mogeneous distribution throughout both lungs. These radiopharmaceuticals have been utilized as lung imaging agents (12–14). The lung images of 16 patients with chronic obstructive pulmonary disease showed decreased uptake in the upper one-half or one-third of the lung (11) and absent perfusion pulmonary mass (13).

Noninvasive radionuclide using [123 I]-HIPDM scintigraphy to detect pulmonary hypertension has not been

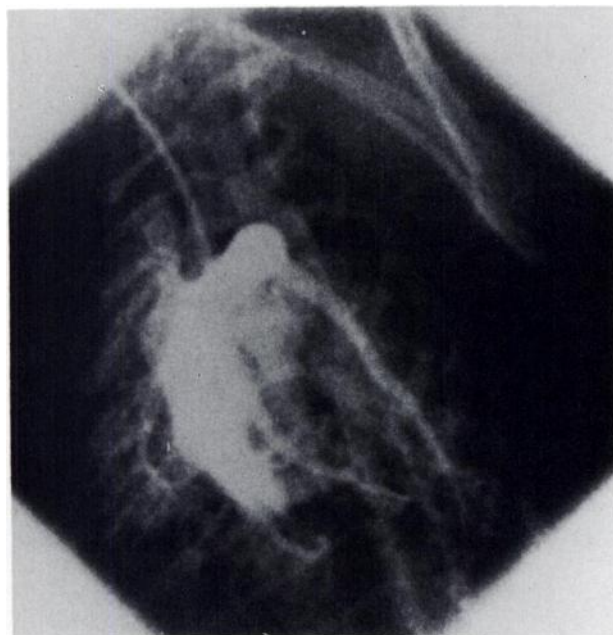


FIGURE 4
Contrast angiography 6 wk postbanding left pulmonary vein: the left pulmonary artery is small with poor filling of the small vessels due to severe pruning—consistent with pulmonary hypertension.

evaluated in humans. Our animal model of pulmonary hypertension demonstrated significantly high [123 I]-HIPDM uptake in treated lung. The mechanism and site of increased accumulation of [123 I]-HIPDM in the lung is unknown and subject to further study.

Circulating bioamines such as serotonin or norepinephrine are cleared by the vascular endothelium of mammalian lung (15). The capability of the bioamine to localize is considered to be an indicator of pulmonary endothelial integrity (16). Bend et al. (2) hypothesized that a carrier-mediated transport system might be involved as one of the components of amphetamine extraction. Iodine-123-IMP localization in the endothelium of small vessels of the lung has been documented by microautoradiography (13). As with propranolol, it is well known that [123 I]-IMP binds to the lung endothelial cells (3,17,18). The [123 I]-IMP lung extraction decreased progressively from 90% to 62% as the amount of propranolol gradually increased from 0 to 20 mg; propranolol competes with [123 I]-IMP for the same lung endothelial binding sites (19). Preloading doses of imipramine also depressed [123 I]-IMP lung uptake (20). This suggests that lung uptake of ^{123}I -IMP is by the way of a saturable mechanism (3,15–19). In the evaluation of [125 I]-HIPDM lung uptake using an isolated perfused lung model by Slosman et al. (21), a carrier-mediated transport system was not confirmed because of the lack of effects of ouabain or a Na^+ free medium.

However, Miniati et al. (22) reported that HIPDM is bound to subcellular organelles in the lung, suggesting it is lysosomotropic. How increased uptake of [123 I]-HIPDM was induced in the lungs of our experimental animals is unclear. We speculate that the induction of pulmonary hypertension by constriction of pulmonary veins may activate “receptors” or increase the number of receptors, allowing for more tracer localization in the lung. This is, however, subject to further investigation.

Pulmonary-vein stenosis is expected to be accompanied by pulmonary edema, with a consequent decrease in pulmonary flow. Technetium-99m-MAA lung perfusion has been reportedly poor or absent in affected lung, and the search for a definitive technique has been considered an important goal of investigators (9). Our five animal experiments reinforced findings that the vein-banded lungs show significantly decreased ^{99m}Tc -MAA perfusion.

Absent or poor perfusion in ^{99m}Tc -MAA lung imaging is, however, nonspecific and may occur in a variety of pulmonary vascular and parenchymal lung disease. Combined ^{99m}Tc -MAA and [123 I]-HIPDM lung image are subject to further study. Mis-matched ^{99m}Tc -MAA and [123 I]-HIPDM pulmonary imaging findings, may be used for pulmonary hypertension secondary to pulmonary vein stenosis.

In conclusion, while pulmonary accumulation of

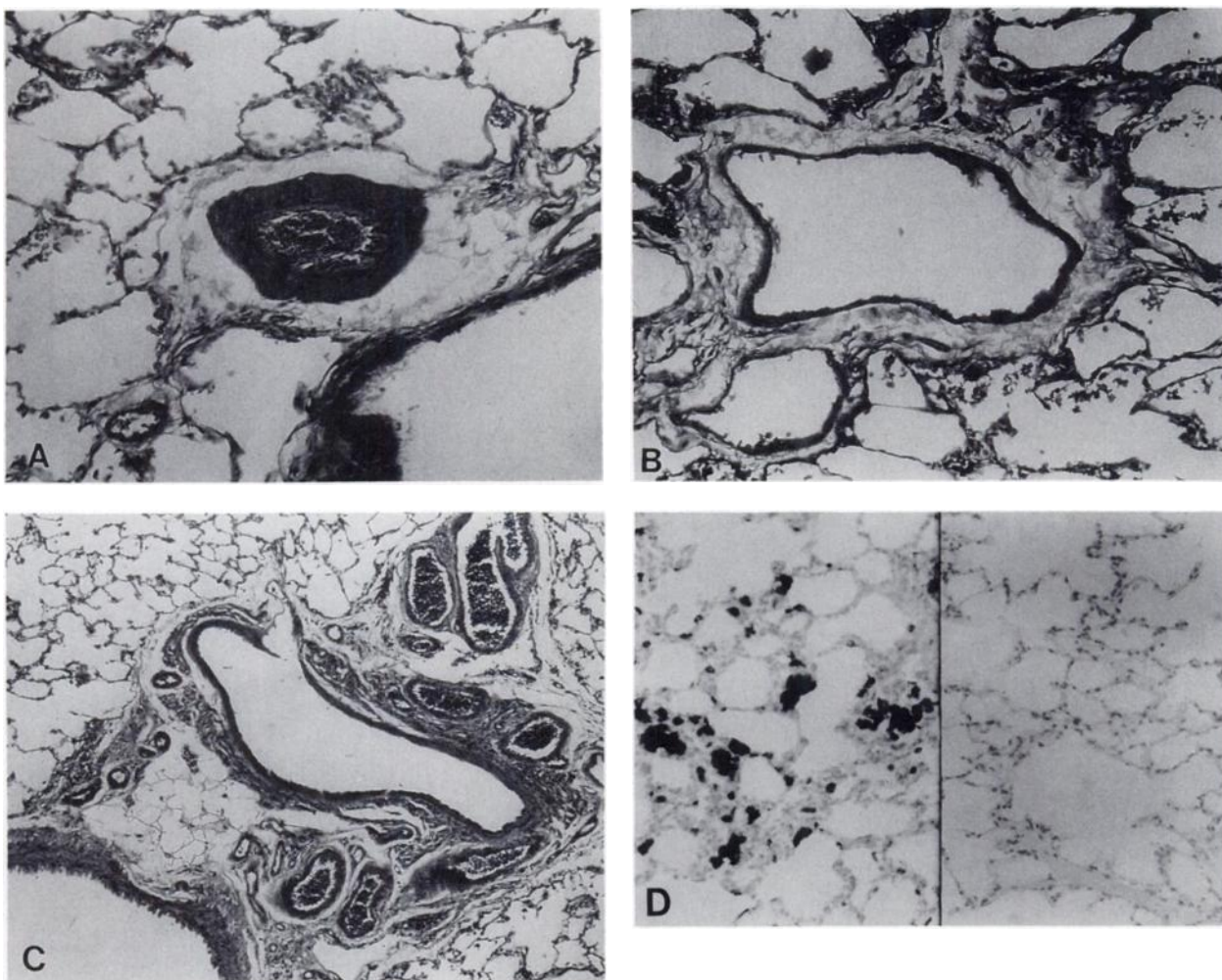


FIGURE 5

Microsections with elastic trichrome stain. (A) Section of pulmonary artery along the airway showing intimal proliferation, muscular layer thickening, and perivascular edema. (B) Section of pulmonary vein showing dilatation of pulmonary vein with arterialization and perivascular fibrosis. (C) Section of bronchial vessels showing very prominent bronchial vessels. (D) Lung section of iron (Prussian blue) from banded-lung (left) and nonbanded-lung (right): notice the iron deposition in the banded-lung section.

HIPDM is considered a drawback in cerebral flow studies, [^{123}I]HIPDM may have potential as a pulmonary imaging agent. Increased [^{123}I]HIPDM uptake in the lung, unrelated to pulmonary flow, may relate with nonspecific or specific endothelial localization, subject to further study. Current noninvasive diagnosis of pulmonary hypertension is sometimes difficult and non-specific. This radionuclide pulmonary study, using [^{123}I]HIPDM combined with $^{99\text{m}}\text{Tc}$ -MAA, may be used for the early detection of pulmonary artery hypertension.

ACKNOWLEDGMENTS

This study was supported in part by the American Heart Association, Kentucky Affiliate and USPHS NIH Research Grant HL-25089.

The authors would like to express their appreciation to Melissa Shryock for her technical help and to Dr. R. Kryscio, a biostatistician, for his valuable statistical consultation.

REFERENCES

1. Gills CN. Pharmacological aspects of metabolic processes in pulmonary microcirculation. *Ann Rev Pharmacol Toxicol* 1986; 26:183-200.
2. Bend JR, Serabjit-Singh CJ, Philpot RM. The pulmonary uptake, accumulation and metabolism of xenobiotics. *Ann Rev Pharmacol Toxicol* 1985; 25:97-125.
3. Touya JJ, Rahimian J, Grubbs DE, et al. A noninvasive procedure for in vivo assay of lung amine endothelial receptor. *J Nucl Med* 1985; 26:1302-1307.
4. Kung HF, Tranposch K, Blau M. A new brain perfusion imaging agent I-123-HIPDM. *J Nucl Med* 1983; 24:66-72.
5. Cottrill CM, Fitz R, O'Connor WN. Sequellae of lobar pulmonary venous congestion in the rat. Presented at the Southeastern Pediatric Cardiology Society Annual Meeting, Sept. 11-13, 1986, Atlanta, Georgia.
6. O'Connor W, Fitzer R, Cottrill C. Morphology of the lungs following unilateral lobar surgical vein stenosis in the rat [Abstract]. *Lab Invest* 1987; 56:56A.
7. Shih WJ, Cottrill CM, O'Connor W, et al. I-123 HIPDM

- metabolic lung imaging in pulmonary-vein-banded pulmonary hypertension: comparison with Tc-99m MAA and contrast angiographic studies [Abstract]. *J Nucl Med* 1988; 29:765.
8. Schaefer DF, Brachett DJ, Douns P, Tomplins P, Wilson MF. Laryngoscopic endothelial intubation of rats for inhalation anesthesia. *J Appl Physiol* 1984; 56:533-539.
 9. Belcourt CL, Roy DL, Nanton MA. Stenosis of individual pulmonary veins: radiologic findings. *Radiology* 1986; 161:109-112.
 10. Reeves JT, Groves BM. Approach to the patient with pulmonary hypertension. In: Weir EK, Reeve JT, Eds. *Pulmonary hypertension*. Mt. Kisco, NY: Futura Publishing Co., Inc.; 1984:1-44.
 11. Pistolesi M, Fazio F, Marini G, et al. Lung uptake of I-123 HIPDM in man: an index of lung metabolic function. *J Nucl Med Allied Sci* 1983; 27:180.
 12. Shih WJ, Coupal JJ, Dillon ML, Kung HF. Application of I-123 HIPDM as a lung imaging agent. *Eur J Nucl Med* 1988; 14:21-24.
 13. Shih WJ, Coupal JJ, DeLand FH, Domstad PA, Brandenburg S, Dillon ML. Demonstration of pulmonary mass defect by I-123 HIPDM as lung imaging. *Clin Nucl Med* 1986; 9:632-633.
 14. Itoh K, Oshima M, Tadokora M, et al. High lung uptake of I-123 IMP and its application to pulmonary scintigraphy. *J Nucl Med* 1988; 29:985-986.
 15. Nicholas TE, Strum JM, Angelo LS, Junod AF. Site and mechanism of uptake of ^3H -I-norepinephrine by isolated perfused rat lungs. *Circ Res* 1974; 35:670-680.
 16. Junod AF. 5-hydroxytryptamine and other amines in the lungs. In: Fischer AP, ed. *Handbook of physiology. The respiratory system*. Bethesda, MD: American Physiological Society; 1985:337-349.
 17. Rachimian J, Glass EC, Touya JJ, et al. Measurement of metabolic extraction of tracers in the lung using multiple indicator dilution technique. *J Nucl Med* 1984; 25:31-37.
 18. Pang JA, Blackburn JP, Butland RJA, et al. Propranolol uptake by dog lung: effect of pulmonary artery occlusion and shock lung. *J Appl Physiol* 1982; 49:393-401.
 19. Akber SF. A noninvasive assessment of pharmacological interaction of amines in the lung. *Nucl Med Commun* 1987; 8:889-893.
 20. Moretti JL, Holman BL, Delmon L, et al. Effect of antidepressant and narcoleptic drugs on n-isopropyl-p-iodomphetamine biodistribution in animals. *J Nucl Med* 1987; 28:354-359.
 21. Slosman DO, Brill AB, Polla BS, Alderson PO. Evaluation of (iodine-125) N,N,N',-trimethyl-propanediamine lung uptake using an isolated perfused lung model. *J Nucl Med* 1987; 28:203-208.
 22. Miniati M, Paci A, Ciarimboli G, et al. Lung subcellular distribution of the pneumophilic compound HIPDM. *Fed Am Soc Exp Biol J* 1989; 3(4):A1147.