

# ARTERIOVENOUS SHUNT

## MEASUREMENTS IN EXTREMITIES

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There are two anatomic pathways for extremity blood flow—the capillary or nutrient flow and the arteriovenous anastomotic or shunt flow. In man these two pathways have been demonstrated both functionally and anatomically. An example of the functional demonstration of shunts was accomplished by measuring total arm flow with a plethysmograph while measuring capillary flow in a muscle of the same arm using radioiodide clearance (1). Postural changes, in this instance passively raising the legs, did not change muscle blood flow, but dramatically increased total arm blood flow suggesting that the increase was due to shunting. An example of the anatomic demonstration of shunts was accomplished by direct visualization of arteriovenous anastomoses in the leg of man using microangiographic techniques (2). Dysfunction of shunt pathway in the extremities has been attributed to the syndromes of various circulatory diseases (3–6). However, substantiating measurements have not been reported.

We have developed a method for measuring relative shunt blood flow in patients in order to study shunting more extensively. Radioactive human serum albumin microspheres, two to three times the diameter of capillaries, are injected intra-arterially. The shunted fraction of the dose is carried out of the extremity with the venous blood and becomes trapped in the microcirculation of the lungs. An external radiation detector is used to measure the radioactivity shunted to the lungs. The idea of this method was described earlier (7,8) and has been adapted to animal studies (9).

The purpose of this report is to describe the technique as it is now used routinely in patients and to report the results of a study of the precision and accuracy of the method. An additional purpose of this report is to describe experiments which demonstrate the lack of acute peripheral hemodynamic changes following the injection of microspheres. This part of the investigation was carried out in anesthe-

tized dogs. Also established was the quantity of microspheres required to alter peripheral hemodynamics so that the safety factor is determined.

### METHODS

**Materials and equipment.** Microspheres 15–30 microns in diam (3M-Brand albumin microsphere kits) were labeled on the morning of the studies with 30–100 mCi of  $^{99m}\text{Tc}$ . Just before the study, the suspending solution was removed and replaced with fresh suspending solution to reduce the unbound  $^{99m}\text{Tc}$  from 8–12% to about 2%. The labeling instructions and the suspending solution were included with the kits.

For the animal tests, albumin microspheres in bulk quantities were prepared for injection just before each experiment by mixing a weighed amount of the microspheres with a measured volume of saline solution containing 0.05% Tween 80. The microspheres were suspended with ultrasound. Concentrations of 5–100 mg/ml were prepared depending on the requirements of the particular experiment.

Heparinized saline was prepared by adding 1 ml (1,000 units) of heparin (Liquaemin Sodium "10", Organon) to a 30-ml vial of normal saline (Ambot Solution, Cutter). Twenty-gage Teflon catheter needles (Longdwell Catheter Needle, Teflon 6746, Becton, Dickerson and Co.) were used for catheterizing the arteries.

A dual 5-in.-probe, Ohio-Nuclear rectilinear scintillation scanner with a 1–5-scan reduction attachment and a digital scaler were used. One probe was also used in a stationary position to monitor the

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radioactivity over the chest as a function of time during the course of injections. For this part of the study, the spectrometer was adjusted to record only 140–160-keV photons. This calibration was necessary to eliminate detection of scatter photons. In some cases, a separate scintillation probe detector was used for the measurements during injections.

**Procedure in patients.** A dose of the suspending solution of the microspheres and several doses of microspheres were withdrawn from the vial containing the radioactive microspheres. This was made possible because the vial is double-ended. One end contained a porous filter which permitted withdrawal of solution only. Suspended microspheres were withdrawn from the other end. For each dose, volume was recorded and total radioactivity assayed and recorded. Usually 1 ml of suspending solution was used and 2–4-mCi doses of microspheres were used for each femoral artery injection, 1–2-mCi doses were used for each brachial artery injection, and a 0.5–1-mCi dose was used for an intravenous injection.

The patients were placed in the supine position and the probe positioned to detect radiations from the upper right lung. Studies were carried out at a comfortable room temperature.

A catheter was inserted into the artery of interest, usually the common femoral artery, and connected to a syringe containing heparinized saline by a three-way stopcock. The catheter was flushed with saline to prevent clotting.

The patients were then carefully instructed not to move during the following study period.

Counting over the chest was then begun. Counts were recorded every 30 sec. The injections were spaced at 3–5-min intervals. This permitted the collection of sufficient counts between injections to demonstrate a plateau in the counting rate that could be determined to within 2% accuracy. First the suspending solution was injected. This gave the background counting rate due to the unbound  $^{99m}\text{Tc}$  contained per milliliter of suspended solution. This was usually followed by a series of two arterial injections. In several cases, a second catheter was inserted into the contralateral artery and a second series of two arterial injections administered. The catheters were flushed with heparinized saline following each injection. The final dose of microspheres was administered in a superficial vein in an extremity in which shunting was not being measured. The counting rate after this injection was assumed to be proportional to 100% shunting. The catheters were then removed and pressure applied to the injection sites for about 5 min to stop bleeding. We avoided removing the

catheters until the counting rates plateaued because premature application of pressure at the injection site would delay passage of the shunted microspheres into the lungs.

Upon completion of the injections the patients were scanned using the 1–5 image reduction which permitted visualization of the extremities in a single image.

Each empty syringe was reassayed for residual radioactivity. Also the catheters and stopcocks were assayed to determine whether microspheres had adhered to them. Shunting was calculated as follows:

Shunting =

$$\frac{\text{Net counts/min/mCi injected intra-arterially}}{\text{Net counts/min/mCi injected intravenously}}$$

or

% shunting =

$$\frac{[C_i - C_{i-1} - C_1 (V_i/V_1)]A_i}{[C_n - C_{n-1} - C_1 (V_n/V_1)]A_n} \times 100,$$

where

$C_1$  = counting rate after injection of suspending solution — background,

$C_i$  = counting rate after  $i$ th arterial injection,

$C_n$  = counting rate after last or venous injection,

$V_1$  = volume of suspending solution,

$V_i$  = volume of  $i$ th arterial injection,

$V_n$  = volume of last venous injection,

$A_i$  = mCi of the  $i$ th arterial injection,

$A_n$  = mCi of the last or venous injection.

**Procedure in dogs.** Six mongrel dogs ranging from 25 to 37 lb were used in this study. They were anesthetized intravenously with 30 mg/kg sodium pentobarbital. The dog was intubated, and an intracath was inserted into the cephalic vein. Through the intracath an infusion of 1.4 mg sodium pentobarbital/ml normal saline was infused at a rate of 0.5–1.0 ml/min in an attempt to maintain a steady state of anesthesia, thus minimizing dynamic vasoactive effects of the anesthetic.

The femoral and iliac arteries were exposed retroperitoneally from 1.5 to 2.0 cm proximal to the branching of the deep femoral artery and distally to the sartorius muscle. All branches of the artery were ligated and cut distal to the deep femoral including the cranial femoral and the superficial circumflex iliac. The deep femoral artery was exposed for at least 2 cm and catheterized with PE 190 polyethylene tubing filled with heparinized saline. An electromagnetic flow probe was placed around the iliac artery just proximal to the branching of the deep femoral artery and was secured with sutures. The wound was

TABLE 1. DIFFERENCES BETWEEN PAIRS OF SHUNT MEASUREMENTS IN PATIENTS

Patient	% shunting			
	First measurement	Second measurement	Absolute average deviation from zero	Absolute difference between pairs
<b>In legs without evidence of shunting</b>				
Case 1, right leg	+0.3	+0.3	0.3	0.0
Case 1, right leg, repeated	+1.3	+0.1	0.7	1.2
Case 2, right leg	0.0	-0.3	0.2	0.3
Case 2, left leg	+0.2	-0.3	0.0	0.5
Case 3, right leg	+2.3	+4.6	3.4	2.3
Case 4, right leg	-1.7	-0.8	1.2	0.9
Case 4, left leg	-1.1	-0.3	0.7	0.8
Case 4, left leg, repeated	-2.7	-2.9	2.8	0.2
Case 5, right leg	-0.3	-1.4	0.8	1.1
Case 5, left leg	-0.9	+1.4	0.2	2.3
Case 6, right leg*	+0.1	+0.1	0.1	0.0
Mean and standard deviation			0.95 $\pm$ 1.13	0.87 $\pm$ 0.82
<b>In extremities with evidence of shunting</b>				
Case 6, left leg*	10.4	8.9	9.6	1.5
Case 7, right arm†	34.1	32.4	33.2	1.7

\* Case 6—A patient with congenital A-V fistula. † Case 7—A patient with hypertrophic pulmonary osteoarthropathy.

closed except for a small opening in the distal end of the femoral artery.

Umbilical tape was placed around the distal end of the artery, and using the tape for traction, a Cournand needle was inserted retrogradely into the artery so that the tip rested just distal to the flow probe.

The flow probe was connected to the Biotronex Lab 610 flowmeter and was immediately balanced by occluding the artery with the umbilical tape. The wound was then covered with saline wetted gauze. The catheter in the deep femoral artery was connected to the Biotronex Lab 9360 pressure transducer which led to the Biotronex Lab LB630 pressure meter. The pressure meter was then calibrated using a mercury manometer. The flow and pressure data were recorded on a strip-chart recorder.

An external radiation detector was placed over the apex of the right upper lobe of the dog's lung and the counts from the lung were recorded every minute.

The radioactive microspheres were injected into the femoral artery to measure the percent of the femoral arterial blood flowing through A-V anastomoses. Simultaneously, the blood pressure and the total-blood flow were being measured.

First an injection of the microspheres supernatant was made. Then three control injections of microspheres were made to determine the state of the microcirculation in the control situation. Each injection of microspheres was made 5 min apart to allow the counts from the lung, which were being measured in counts per minute, to reach a plateau. Control

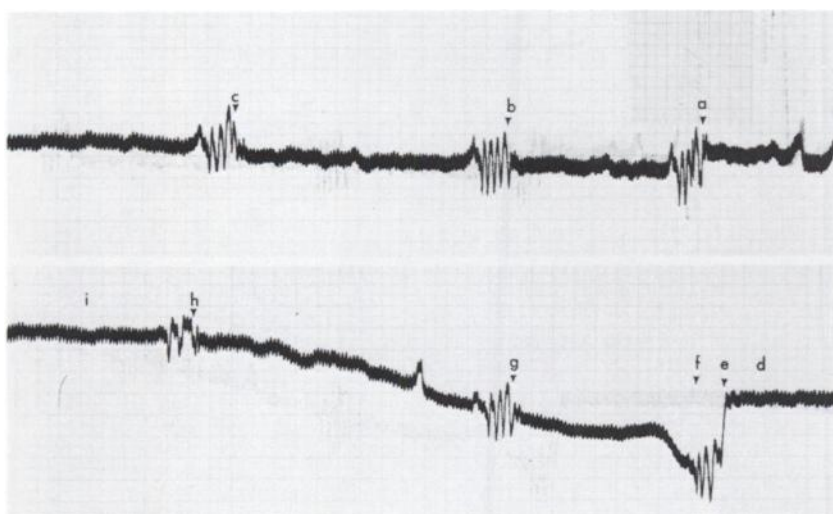
injections of heparinized saline were also administered.

Next a large dose of microspheres was administered. For this injection, 1 cc of the bulk microspheres suspension was mixed with 0.05 mg (0.1 cc) of the  $^{99m}\text{Tc}$ -labeled microspheres and the mixture injected as before.

From the collected data, capillary and shunt blood flow, percent shunting, and resistance values were calculated. The changes in total flow were graded as follows: 0—no effect; 1—minimum effect, detectable decrease in flow recovery in 1–2 min, no subsequent hyperemia; 2—intermediate effect, marked decrease in flow, subsequent hyperemia, recovery in 15–20 min; and 3—maximum effect, marked decrease in flow, subsequent marked hyperemia, no recovery during period of observation.

To conclude the experiment, a dose of microspheres was injected into the femoral vein to represent 100% shunting for the purposes of calculation. Finally the distal femoral artery was catheterized with a tube containing heparinized saline. Then the dog, after being heparinized with 2,000 U of Heparin, was bled for 20 sec into a calibrated cylinder. The flow into the cylinder was also recorded by the electromagnetic meter. This was used as a reference for the calibration of previous electromagnetic blood-flow measurements.

The dog was sacrificed at the end of the experiment using an intravenous injection of a saturated solution of potassium chloride. The distribution of the radioactivity in the leg was then imaged using



**FIG. 1.** Top curve. Tracing shows blood flow through femoral artery of anesthetized dog. Read right to left: a to b equals 5 min. Fluctuations at points a, b, and c correspond to injection of tracer doses (0.05 mg) of albumin microspheres at intervals of approximately 5 min. Responses are same as that of control injections of heparinized saline. Bottom curve. Blood-flow changes in femoral artery after injection of large dose (100 mg) of microspheres. Read right to left: d—control flow, e, f—injection of large dose, g, h—injection of tracer doses for measurement of shunting, i—hyperemia which is maximum 15–20 min following large dose.

a 3-in. rectilinear scanner. Using the scan as a guide, the leg was then removed and weighed. This allowed the blood-flow measurements to be expressed in units of ml/min/kg of perfused tissue.

Flow through the shunts or arteriovenous anastomotic flow (AVA) was calculated as follows:

$$\text{AVA flow} = \text{total flow} \times \% \text{ shunting.}$$

Nutrient flow was then determined as the difference:

$$\text{Nutrient flow} = \text{total flow} - \text{AVA flow.}$$

Additionally four mongrel dogs were anesthetized as before. Both femoral arteries were surgically ex-

posed. In one leg microspheres were injected into the lumen of the femoral artery (10 mg), the arterial wall (1 mg) and into muscle tissue (1 mg). In the opposite or control leg equal volumes of physiological concentrations of saline were injected into corresponding sites. One to 15 days later the dogs were sacrificed and tissue samples were removed. These were processed for histopathologic evaluation. In some samples granulation tissue and inflammatory infiltration was observed which corresponded to the surgery and the needle injuries. The responses to the microsphere injections were not significantly different from that of the control injections.

**TABLE 2. FEMORAL BLOOD-FLOW CHANGES IN DOGS AFTER INTRA-ARTERIAL DOSES OF MICROSPHERES**

Experiment	Diameter of microspheres (microns)	Unit dose (mg)	Number of measurements	Response of blood flow	Dose which affected blood flow with a response of magnitude 2 or greater mg/kg perfused tissue
15	30–45	0	1	0	67
		0.05	9	0	
		100	3	3	
16	15–30	0	1	0	68
		0.05	9	0	
		100	3	3	
17	15–30	0	2	0	14.5
		0.05	9	0	
		10	3	2–3	
22	15–30	0	2	0	—
		0.05	17	0	
		7	1	0	
		10	1	1	
		100	1	1	
23	15–30	0	1	0	14.1
		0.05	9	0	
		5	3	2	
24	15–30	0	1	0	18.4
		0.05	12	0–1	
		20	1	3	

## RESULTS

**Results of patient studies.** In a series of six patient studies, 11 paired measurements were made in legs without evidence of A-V shunting. Overall mean shunting in these studies was  $-0.09\%$ ; the absolute average deviation from the mean was  $0.95\%$  with a standard deviation of  $1.13\%$ . Assuming that shunting in these cases was truly zero, this indicates that  $95\%$  of patients without shunting should be within  $3.2\%$  of zero. The absolute difference between individual pairs of measurements was  $0.87\%$  with a standard deviation of  $0.82\%$ .

For comparison, two studies in patients with known shunting were  $33.2\%$  in the arm of a man with hypertrophic pulmonary osteoarthropathy with clubbing of the fingers and  $9.6\%$  in a woman with an angiographically demonstrated, congenital, arteriovenous fistula. The differences between paired measurements in these patients were  $1.7$  and  $1.5\%$ , respectively. These data are summarized in Table 1.

**Results of animal studies.** Figure 1A shows the tracing of blood flow during microsphere injections of  $0.05$  mg/kg of perfused tissue. The fluctuation of flow during injection corresponded to the movement of the plunger of the syringe in and out as the dose was injected and the syringe repeatedly flushed with blood. Postinjection flow was unchanged compared with preinjection flow. This was the same pattern as was observed with control injections of heparinized saline. Pressure was also unchanged.

Figure 1B shows the tracing when the integral dose exceeded  $14$  mg/kg of perfused tissue. This response was observed in five of the six dogs (Table 2). The failure of Dog 22 to respond to the larger doses was attributed to the length of the experiment. By the time the larger doses were injected the dog had been anesthetized for more than  $4$  hr and the vascular bed had apparently become nonresponsive. The typical response was a prompt postinjection drop in blood flow which recovered to the preinjection level within  $5$ – $6$  min. This was followed by a gradual increase in flow, reaching a maximum in  $10$ – $20$ -min postinjection. At this time, the flow was usually more than twice the preinjection level. When the integral dose was in the range of  $15$ – $30$  mg, the flow after  $20$  min had usually returned to the preinjection level. When the integral dose was in the range of  $100$ – $300$  mg, the flow remained higher than preinjection levels throughout the remaining period of observation. Blood pressure was not changed. No consistent pattern of change in percent shunting or shunt blood flow was observed. Capillary flow, on the other hand, was increased especially during periods when total flow was increased. This is seen in Fig. 2 in which the flow data have been replotted and the measure-

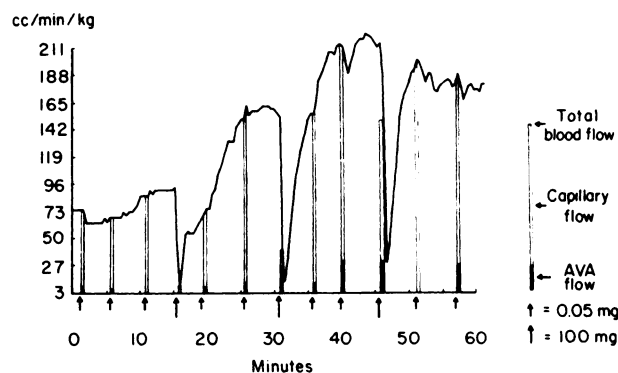
ments of AVA and nutrient flow shown by the bar graph.

## DISCUSSION

These experiments demonstrate that arteriovenous shunting in the extremities can be safely and precisely measured during intra-arterial injections of radioactive albumin microspheres. The tests are routinely carried out with  $1/300$  of the dose required to produce hemodynamic changes. No untoward effects of the procedure have been observed in any of the more than  $30$  patients studied to date. The only potential hazard of the technique appears to be that associated with the arterial puncture. Histopathology was not observed in dogs following intra-arterial, intramuscular, and arterial wall injections of the microspheres. The experience in both man and animals has indicated that antigenic reactions to the microspheres is unlikely (10).

Following the quantification of shunting the subjects can be scanned to provide pictures of the distribution of arterial blood flow to the extremity. Perfusion scannings of the extremities will be the subject of a subsequent report. Currently we are investigating the clinical usefulness of these perfusion scans in a variety of diseases. Previous studies have suggested applications in patients with arteriosclerosis (11) and clubbing of the fingers due to pulmonary disease (12,13).

Previously (6,13) we used  $30$ – $45$ -micron microspheres for shunt quantification. However, the smaller,  $15$ – $30$ -micron size is preferred. They are safer (14), and they are distributed to smaller arterioles so that their distribution pattern is more nearly representative of nutrient flow. Subjectively, scans with the  $15$ – $30$ -micron spheres appear more



**FIG. 2.** Read left to right: Graph shows total, capillary, and arteriovenous anastomatic (AVA) flows through femoral artery of anesthetized dog. Either tracer ( $0.5$  mg) or large ( $100$  mg) doses of microspheres are injected at approximately  $5$ -min intervals. Changes in AVA flow do not correlate with injection of microspheres. Capillary flow, on the other hand, immediately decreases with injection of large doses of microspheres and then increases during next  $15$ – $20$  min to maximum which is  $2$ – $3$  times the control value.

uniform than scans with larger spheres. Perhaps this is due in part to the larger number of particles injected. The 15–30-micron spheres are large enough to give zero shunting in the absence of shunts as illustrated by the data reported here. In the presence of shunting, more 15–30-micron particles are shunted than 30–45-micron particles (9) which makes them more sensitive for the detection of shunting. The advantages of the spheres over macroaggregates of albumin is the closer definition of particle size. Macroaggregates usually contain a fraction of particles small enough to pass capillaries so that even in the absence of shunting some particles pass through the extremity to give baseline values greater than zero (9,15).

#### SUMMARY

A method of quantifying percent of extremity blood flow shunted through arteriovenous anastomoses or vessels large enough to pass 15–30-micron microspheres is described and is carried out in conjunction with peripheral perfusion scanning. In patients without clinical evidence of shunting the values averaged  $-0.09\%$ ; 95% of the measurements were 3.2% or less. Differences between pairs of measurements averaged  $0.87 \pm 0.82\%$ . In patients with clinical evidence of shunting the values were 9.8% in a subject with congenital A-V fistula and 33.2% in a subject with hypertrophic pulmonary osteoarthropathy. Experimental studies in anesthetized dogs showed that the dose used clinically was 1/300 of that required to produce hemodynamic effects and that no histopathology due to microspheres was detected even when the entire dose was injected into an arterial wall.

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