Arteriovenous anastomotic blood flow in the mesenteric organs

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Arteriovenous anastomotic (AVA) blood flow in the mesenteric organs of the dog was quantitated by injecting 20-μ radioactive spheres into the superior mesenteric artery (SMA). Microspheres bypassing capillaries via A-V channels larger than 20 μ traversed the portal vein and lodged in the liver. An average of approximately 3% of the injected radioactivity was recovered in the liver. Systemic epinephrine, nonepinephrine, histamine, and pitressin did not significantly alter this partition of blood flow between capillaries and AVAs. Anesthetized dogs undergoing laparotomy and needle puncture of the SMA demonstrated significantly fewer patent AVAs than did awake animals with chronic inlying SMA catheters. The conclusion is that a very small fraction of the mesenteric blood flow passes through AVAs in the normal dog.

arteriovenous anastomoses; mesenteric circulation; radioactive microspheres; vasoactive drugs

THICK-WALLED PRECAPILLARY COMMUNICATIONS, arteriovenous anastomoses (AVAs), provide a mechanism whereby arterial blood reaches veins and returns to the heart without perfusing capillaries. These intriguing vascular conduits provide fertile ground for physiologic speculation. However, simple questions such as the presence or absence of AVAs in particular organs remain in dispute (12). Information on the quantity of blood passing through precapillary arteriovenous anastomoses and the mechanisms for their control is generally lacking. Few acceptable data are available regarding arteriovenous anastomoses in the splanchnic viscera.

The purpose of this investigation was to determine the fraction of blood passing through arteriovenous communications of different sizes (20 μ+, 30 μ+, 40 μ+) in the mesenteric organs of the intact dog.

Microspheres injected into the superior mesenteric artery either lodge in the arterioles of the splanchnic viscera or, bypassing these, enter the portal vein and proceed to the liver. Experiments were done comparing the arteriovenous anastomotic blood flow, the passage of microspheres, in the organs supplied by the superior mesenteric artery under control conditions with that occurring under the influence of vasoactive drugs, anesthesia, and laparotomy.

METHODS

The yttrium 169-tagged polystyrene spheres used in this study were obtained from the Minnesota Mining and Manufacturing Company. The spheres had a density of approximately 1.3. Low molecular weight dextran was used for suspension and injection. Separation into groups of relatively discrete diameter was accomplished by sieving and screening. Three different mean diameters were employed, 20 μ, 30 μ, and 40 μ. Small samples were taken for estimation of size distribution from individual batches, and at least 100 spheres were measured from each size group. Table 1 gives data for the three sizes used.

Initial radioactivity was 2.5–5.5 mc/g. Spheres (100–400 mg) were suspended in 10 ml of low molecular weight dextran. The estimated numbers of spheres injected ranged from 50,000 to 500,000 in the 20-μ experiments. The estimated numbers of spheres injected ranged from 50,000 to 500,000 in the 20-μ experiments. An important property of the plastic microspheres is their solubility in boiling nitric acid. This permits accurate estimation of activity in entire organs and in large tissue samples.

All studies were done using mongrel dogs fasted for 18–24 hr. Anesthesia, when used, consisted of intravenous pentobarbital in a dose of approximately 30 mg/kg body wt. Anesthetized dogs had endotracheal tubes, but mechanical respiratory assistance was not employed.

Injections were made from disposable 2.5-ml plastic syringes via no. 20 needles. The rubber-stoppered bottle containing the spheres suspended in low molecular weight dextran was shaken and 0.5–1.0 ml of the suspension withdrawn. Injections were made rapidly against the flowing blood stream to promote turbulence and thereby improve mixing.

Sphere injections into the superior mesenteric artery of anesthetized animals were made by needle puncture of the surgically exposed vessel with the dog lying in the right lateral position. Waking animals were prepared...
MESENTERIC ARTERIOVENOUS ANASTOMOSES

TABLE 1. Size distribution of spheres from different groups

<table>
<thead>
<tr>
<th>Mean Diameter, ( \mu )</th>
<th>Range, ( \mu )</th>
<th>SD, ( \mu )</th>
</tr>
</thead>
<tbody>
<tr>
<td>39.3</td>
<td>29–48</td>
<td>3.7</td>
</tr>
<tr>
<td>30.8</td>
<td>26–39</td>
<td>4.0</td>
</tr>
<tr>
<td>20.0</td>
<td>14–26</td>
<td>1.6</td>
</tr>
</tbody>
</table>

FIG. 1. Retrograde sphere injection via an inlying arteria catheter.

3–4 days in advance by threading a PE-90 tube through a peripheral mesenteric artery branch retrograde into the main mesenteric artery (see Fig. 1). The catheter was filled with heparin, occluded, and the tip kept in the subcutaneous position for later injection. The incision was closed and the animal allowed to recover. These latter injections were done with the dogs awake.

Drugs were administered intravenously with a constant-rate infusion pump according to the following protocol: 1) histamine, 1 \( \mu \)g/kg per min for 10 min; 2) epinephrine, 1 \( \mu \)g/kg per min for 10 min; and 3) vasopressin, 0.01 CU/kg per min for 10 min. At the end of 10 min, with the infusion still running, the radioactive microspheres were injected.

The suitability of the liver as a sieve for spheres arriving via the portal vein was determined by direct needle injection of spheres into the vessel, sacrifice of the animal, and analysis of the liver and lungs for radioactivity, the assumption being that spheres bypassing the liver would lodge in the pulmonary microcirculation.

A few studies were done in which the spheres were injected into the surgically exposed femoral artery and the entire hindlimb and lungs digested and analyzed for radioactivity. Spheres reaching the systemic venous circulation lodged in the lungs.

Shortly after sphere injection the dog was sacrificed by an intravenous overdose of sodium pentobarbital. The tip of the catheter was checked for position. The splanchnic viscera were then removed, along with the liver. The organs were separated and trimmed free of fat, mesentery, and omentum. Organs evaluated for isotope content were: stomach, small intestine, colon, pancreas, spleen, and omentum plus mesentery. When it was established that essentially no radioactivity reached the stomach or spleen, these organs were subsequently discarded.

Each tissue sample was weighed and digested in boiling nitric acid. Aliquots (5 ml) of each digest were pipetted into test tubes, weighed, and counted for 5–10 min in a well-type crystal scintillation detector. The radioactivity in each sample was calculated as follows:

\[
\text{counts/} \min \times \frac{\text{total weight of digest}}{\text{weight of counted aliquot of digest}} = \text{total counts in tissue digested}
\]

The total radioactivity injected into the SMA was determined simply by summing the activities of the various tissues. Hepatic radioactivity was then expressed as a fraction of the total, a term which indicated what percent of the spheres had bypassed the splanchnic capillary bed and reached the portal vein.

The activity recovered in each organ was also expressed as a fraction of the total in order to give a measurement of distribution of the microspheres among the various organs supplied by the superior mesenteric artery. After it became clear that organ distribution was reasonably constant, experiments were done simply digesting all the splanchnic viscera as a single specimen, the liver as a second, and calculating only what fraction of spheres had reached the liver.

RESULTS

Portal vein injections (the liver). Table 2 lists the data for experiments in which spheres were injected into the portal vein. The results indicate that a tiny fraction, less than 0.1%, of the microspheres in the size ranges used here passed from the portal vein through the liver and thence into the lungs. The conclusion is that essentially all of the portal vein-hepatic vein conduits in the normal

<table>
<thead>
<tr>
<th>Exp No</th>
<th>Sphere Size, ( \mu )</th>
<th>Total Activity in Lungs, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,305</td>
<td>40</td>
<td>0.02</td>
</tr>
<tr>
<td>5,185</td>
<td>30</td>
<td>0.10</td>
</tr>
<tr>
<td>6,075</td>
<td>20</td>
<td>0.02</td>
</tr>
<tr>
<td>6,135</td>
<td>20</td>
<td>0.02</td>
</tr>
</tbody>
</table>
dog arc at some point less than 20 μ in diameter. The validity of using the liver as a "sieve" for particles bypassing the gastrointestinal microvasculature via AVAs and reaching the portal vein was thus established.

Femoral artery injections (the hindlimb). Because pre-capillary arteriovenous anastomoses exceeding 20 μ in diameter definitely do exist in the hindlimb of the dog (1, 10) it was chosen as a test for the general usefulness of the technique employed. Arteriovenous anastomatic blood flow was measured in the hindlimb by upstream needle injection of spheres into the exposed femoral artery of anesthetized dogs and subsequent determination of radioactivity in the leg and the lungs. The results are given in Table 3. Almost 30% of injected 30-μ spheres passed through arteriovenous anastomoses and lodged in the lungs in one experiment, and a like percent of the 20-μ spheres in two other experiments. It seems fair to conclude that where arteriovenous anastomoses are present, the plastic radioactive microspheres can and do pass through them.

Superior mesenteric artery injection (the splanchnic organs). The method used here provides information regarding arteriovenous anastomatic blood flow in the entire distribution of the superior mesenteric artery rather than the intestine alone. In a number of individual experiments, distribution of radioactivity among the splanchnic organs was determined. These data are provided in Table 4. The intestine proper retained a mean of 76.4% of the injected spheres. This proportion of the total superior mesenteric artery blood flow therefore perfused the small intestine. The mean value for the mesentery plus omentum was 12.0%. The proportion of the mesenteric artery flow perfusing the colon showed considerable variability. The average for all animals in which this value was obtained was 8.6%. The pancreas, which has a dual blood supply from both the celiac and superior mesenteric arteries, retained a variable small share of the injected microspheres, a mean of 1.3%. The stomach and spleen each received less than 0.1% of the total injected radioactivity and therefore were ignored in later experiments. The reasonably consistent distribution of spheres among the splanchnic organs provides indirect evidence that mixing in the superior mesenteric artery was adequate.

Table 5 gives the fraction of radioactivity recovered in the liver following superior mesenteric artery injection of 40-μ spheres. The data suggest that a greater percent of the spheres bypassed the capillary bed when injected via an inlying arterial catheter in an awake dog than when injected at laparotomy in an anesthetized animal. In no instance, however, did more than 0.35% of the radioactivity reach the liver. None of the drugs tested (epinephrine, norepinephrine, pitressin) caused appreciable alteration of hepatic recovery of radioactivity, the conclusion again being that arteriovenous communications larger than 40 μ in diameter definitely do exist in the hindlimb of the dog.

Table 6 provides similar data for 30-μ spheres. Average liver recovery in the control dogs was greater than for the 40-μ spheres. Again none of the drugs substantially altered the fraction of flow through A-V pathways exceeding 30 μ in diameter. The smallest liver recovery in the catheter-injected group exceeded the largest of the needle-injected group. Despite small differences in hepatic recovery of radioactivity, the conclusion again can be stated that an extremely small portion, an average of less than 1%, of blood flow in the superior mesenteric artery distribution passes through arteriovenous pathways which exceed 30 μ in diameter.

Table 7 provides results of the experiments done with 20-μ spheres. When injected by needle puncture of the superior mesenteric arteries of anesthetized dogs at

### Table 3. Percent of radioactivity lodging in lungs following femoral artery injection of microspheres

<table>
<thead>
<tr>
<th>Exp No.</th>
<th>Sphere Size, μ</th>
<th>Activity in Lungs, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5, 255</td>
<td>30</td>
<td>94.4</td>
</tr>
<tr>
<td>5, 265</td>
<td>30</td>
<td>93.3</td>
</tr>
<tr>
<td>6, 303</td>
<td>20</td>
<td>86.8</td>
</tr>
<tr>
<td>6, 045</td>
<td>20</td>
<td>86.6</td>
</tr>
<tr>
<td>6, 075</td>
<td>20</td>
<td>4.5</td>
</tr>
</tbody>
</table>

### Table 4. Mean organ distribution of microspheres injected into superior mesenteric artery

<table>
<thead>
<tr>
<th></th>
<th>Mean, %</th>
<th>SE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestine</td>
<td>76.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Colon</td>
<td>8.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Mesentery and omentum</td>
<td>12.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

### Table 5. Percent of radioactivity reaching liver after superior mesenteric artery injection of 40-μ spheres

<table>
<thead>
<tr>
<th>Injection</th>
<th>No. of Exp</th>
<th>Percent of Spheres to Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Needle</td>
<td>Mean, %</td>
</tr>
<tr>
<td>Control</td>
<td>Catheter</td>
<td>6</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Catheter</td>
<td>3</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Needle</td>
<td>1</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>Needle</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 6. Percent of radioactivity reaching liver after superior mesenteric artery injection of 30-μ spheres

<table>
<thead>
<tr>
<th>Injection</th>
<th>No. of Exp</th>
<th>Percent of Spheres to Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Needle</td>
<td>Mean, %</td>
</tr>
<tr>
<td>Control</td>
<td>Catheter</td>
<td>3</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Catheter</td>
<td>1</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Catheter</td>
<td>1</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>Needle</td>
<td>1</td>
</tr>
<tr>
<td>Histamine</td>
<td>Needle</td>
<td>1</td>
</tr>
</tbody>
</table>
laparotomy, an average of less than 1% of the spheres reached the liver in control animals. Histamine, pitressin, and norepinephrine results were not notably different from the control mean.

With injection of 20-μm spheres via a previously placed superior mesenteric artery catheter, sufficient spheres reached the liver so that some conclusions could be derived. Table 7 also lists these results. In the average control animal 2.89% of the injected spheres bypassed the mesenteric capillaries via A-V channels larger than 20 μm and lodged in the liver. This value was significantly larger than the mean found in the needle-injected 20-μ group, at the 95% confidence level. The conclusion is that approximately 3% of superior mesenteric artery blood flow reaches the portal vein by way of A-V channels larger than 20 μm in diameter. Krogh (7) and others have reported that intestinal capillaries seldom exceed 9–10 μm in diameter. Arterial to venous passage of 20-μ particles, therefore, demonstrates the presence of arteriovenous anastomoses.

This study had as one of its aims to determine the effects of certain vasoactive agents on arteriovenous anastomoses in the gut. Therefore, findings that relatively few A-V channels exceeding 20 μm in diameter were present in the canine intestine makes it rather tenuous to speculate about minor differences from control values observed during administration of drugs. From the data obtained in these experiments, no firm conclusions regarding humoral effects can be stated.

The most consistent variation in the fraction of blood flow passing through AVAs was between the groups of dogs with inlying catheters and those receiving spheres by direct puncture of the superior mesenteric artery. The former animals were awake and the only apparent deviation from normal was a relatively recent laparotomy. The other group was anesthetized. The perivascular nerves were traumatized during dissection of the superior mesenteric artery and some of the abdominal viscera were exposed to the ambient environment. One or more of these factors led to a significant decrease in A-V flow. Future investigations of A-V anastomoses will best be carried out in awake animals and untraumatized organs.

### Table 7. Percent of radioactivity reaching liver after superior mesenteric artery injection of 20-μm spheres

<table>
<thead>
<tr>
<th>Injection</th>
<th>No. of Exp</th>
<th>Percent of Spheres to Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Needle</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td>Catheter</td>
<td>5</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Needle</td>
<td>1</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Catheter</td>
<td>4</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Needle</td>
<td>1</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Catheter</td>
<td>4</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>Needle</td>
<td>2</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>Catheter</td>
<td>4</td>
</tr>
<tr>
<td>Histamine</td>
<td>Needle</td>
<td>3</td>
</tr>
<tr>
<td>Histamine</td>
<td>Catheter</td>
<td>4</td>
</tr>
</tbody>
</table>

**Discussion**

**Literature review.** Mall (9) published in 1887 the results of his classic investigations of the intestinal vasculature of the dog. Using an injection-cast technique with controlled pressure arterial infusion, he was able to identify no precapillary arteriovenous communications.

An extensive study of the intestinal microcirculation in a variety of species, including man and dog, was reported in 1932 by Spanner (13). The methods employed were intra-arterial injection of contrast material and in vivo observation of the intestinal surfaces. He described AVAs in the villi of man and herbiverous animals. The intestinal villi of carnivores and ungulates lack these structures, but were purported to have numerous submucosal AVAs approximating 20 μm in diameter. Lindseth (8) has criticized Spanner’s work on the grounds that the injection pressures may have been too high (they were not reported) and that in vivo observations of the surface of a three-dimensional microcirculatory system are easily misinterpreted. Lindseth concluded that Spanner probably overestimated the frequency of intestinal AVAs. Our data would support this latter conclusion.

Jacobsen and Noer (6) injected both India ink and latex at near physiologic pressures and studied the vessels by serial section and by transillumination of the tissue. These workers found no arteriovenous anastomoses in the intestine of the dog or the opossum.

The most authoritative study of AVAs in the canine intestine was reported by Lindseth and Grim and Lindseth (5). These investigators injected a known quantity of radioactive glass microspheres into a small artery supplying an isolated in situ loop of canine intestine. They collected the venous drainage and determined the fraction of spheres passing through arteriovenous channels of various sizes. No. 44-μm spheres could be recovered in the venous outflow. Of the 20-μm spheres, approximately 3% appeared in the effluent, that is, 3% of the total blood flow went through A-V channels larger than 20 μm. The authors defined this as arteriovenous anastomotic blood flow. The close agreement with the present work lends credibility to both studies. A significant fraction, 14–17%, of 12-μm spheres was recovered in the venous blood. This was interpreted as representing arteriovenous bridge or thoroughfare channel flow.

Folkow ct al. (4) observed that the cat kept that continued sympathetic nerve stimulation led to diminution in mucosal blood volume with a concomitant increase in submucosal vascular filling. At the same time the total intestinal blood flow did not decrease much. They speculated that opening of the submucosal arteriovenous anastomoses could account for these observations. Perhaps the cat and dog have species differences, but one must be cautious in drawing conclusions from injections of contrast material. Clearly, the density of ink represented blood volume, not blood flow. While the arteriovenous anastomoses could provide an explanation for the observations, an equally plausible hypothesis would
be that the arterioles leading to the mucosa constricted while those in the submucosa and muscularis dilated.

Zweifach (15) has made extensive in vivo microscopic observations of the mesentery of various species. Pertinent to the present discussion, he described AVAs which exceeded 20 μm in diameter.

The human stomach, colon, and small intestine were studied by Boulter and Parks (2) after perfusion with warm formalin followed by injection of staining substances to coat the intravascular lining. He noted frequent simple-type arteriovenous anastomoses of 20–25 μm in diameter in the stomach. Similar structures were also described in the small and large intestine, but were said to be far less frequent.

Methodological considerations. A possible source of error in the technique reported here could arise from factors inherent in the plastic microspheres. Some undefined physical or chemical property might lead to rapid agglutination of fibrin or adherence of cellular elements to the surface of the sphere, rendering it a larger bolus. Spheres in microscopic tissue sections appear consistently to be wedged into vessels of their own diameter (Fig. 2), tending to negate the likelihood that elements of the blood adhere to the sphere surfaces.

Another possibility is that the spheres, leaving the catheter tip of the needle in a stream, might be incompletely mixed with the mesenteric blood. That mixing defects are probably not significant is evidenced by the observation of the relative consistency of sphere distribution among the mesenteric organs.

A further potential problem is imperfect uniformity of sphere size. Since almost no spheres reached the liver, this was of little consequence in the two larger groups. Among the spheres having a mean diameter of 20 μm, however, a few were as small as 14 μm and therefore could pass through A-V communications of appreciably less than 20-μm diameter. Errors arising on this account would tend to give an overestimation of the blood flow through channels defined as larger than 20 μm.

The technique described here provides estimates of flow through A-V channels of some arbitrary size. An A-V channel could be the same diameter as a capillary but not permit metabolic exchange between blood and interstitial fluid because of a thick muscular wall. This would be a functional precapillary shunt, but would not be detected by the microsphere method. However, most AVAs so far described have exceeded 20 μm in diameter, and many have been reported as being larger than 100 μm (12).

The method used in the present study has distinct merits when compared with other means for evaluating arteriovenous anastomoses (5, 14). First, and most important, the awake animal is in a nearly normal state. Discrete variables can be introduced without the additional consideration of anesthesia and trauma to the organs under study. The observation of decreased AVA flow during laparotomy and anesthesia emphasizes the importance of this aspect of the technique. Secondly, quantitative data are obtained. Most other studies of this subject have been primarily descriptive in character.

Prinzmetal and co-workers (11) previously used the lungs and liver as sieves for collecting spheres from the veins. This is an important methodological consideration in that venous cannulation for collection of effluent blood can lead to serious distortions in AVA flow (13). Prinzmetal et al. did not have the advantage of soluble microspheres nor of uniform size, and were therefore limited to observations regarding the maximum diameter of the

FIG. 2. Sphere (20 μ) lodged in intestinal mucosal arteriole.
spheres which reached the lungs or liver. Our technique of digesting entire organs or organ segments in order to quantitate the radioactive micropsheres present has apparently not been reported before.

Functional considerations. The sparsity of arteriovenous anastomoses found in the splanchnic viscera of the normal dog makes it doubtful that they play a quantitatively significant role in the mesenteric circulation. Discrepancies in anatomic descriptions of previous investigators who used injection or histologic techniques tend to negate the credibility of their reports. The results of the study presented here lead to further reservations about the accuracy or usefulness of anatomic studies in assessing this aspect of the microcirculation.

Submucosal arteriovenous anastomoses could play an important role in diverting blood to or away from the intestinal mucosa. Teleologically it would seem reasonable that the mucosa should receive more blood during active secretion or absorption than in resting circumstances. Arteriovenous anastomoses could serve to raise capillary pressure for secretion and reduce it for absorption, and to increase or decrease mucosal blood flow, as appropriate. With less than 5% of the total blood flow passing through A-V precapillary shunts, it is improbable that they do, in fact, play an important part in such local vascular control in the mesenteric viscera.

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REFERENCES