Thomas M. Donnelly, DVM, Column Editor

Failure to observe fluoroscopic contrast agent in mouse hind limb

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A research team planned to use contrast fluoroscopy to evaluate vascular growth in the mouse hind limb ischemia model. Ischemia induces both hypoxia and inflammation that trigger angiogenesis. This tightly controlled process is associated with pathological conditions such as tumor growth and ischemic diseases. Hence, understanding angiogenesis is of major clinical and therapeutic interest. Surgically induced hind limb ischemia can be used to study the role of anti-inflammatory cytokines[1], angiogenic factors[2], circulating bone marrow–derived endothelial progenitor or hematopoietic stem cells[3,4], and current or investigational new drugs[5,6] on the development of new vessels from preexisting blood vessels.

The mouse hind limb ischemia model involves ligation of the proximal femoral artery, including the superficial and the deep branch as well as the distal saphenous artery. After a period of 2–4 weeks, investigators calculate angiographic score, capillary density, and footpad perfusion[7,8] in the ischemic versus normal limb. Methods to quantitate angiogenesis include microangiography using barium sulfate injected in the abdominal aorta, followed by image acquisition with a digital X-ray transducer and computerized quantification of vessel density; assessment of capillary densities by immunostaining with an antibody directed against fibronectin and morphometric quantification; and laser Doppler perfusion imaging to assess tissue perfusion in the legs.

The researchers planned to perform microangiography using high-definition fluoroscopy, as a method to quantitate angiogenesis. As this was a novel approach, they first needed to do a pilot study to evaluate visualization of arteries in the hindlimb. As part of the pilot study, the researchers anesthetized a mouse with an intraperitoneal injection of 100 mg/kg ketamine and 5 mg/kg xylazine. After an appropriate level of anesthesia was attained, they shaved, aseptically prepped, and placed the mouse on a plastic surgery table. Surgery involved incising the thorax and removing the sternum. The surgeons then euthanized the mouse by exsanguination via cardiac puncture. They exposed the thoracic aorta and ligated it with 5-0 silk suture material. Caudal to the ligation, the surgeons cannulated the aorta with PE50 tubing that was held in place with a 5-0 silk ligature. The mouse, still on the plastic surgery table, was then placed directly on the ventral detector of an OEC Series 9600 C-Arm Fluoroscope (OEC Medical Systems, Salt Lake City, UT) for imaging.

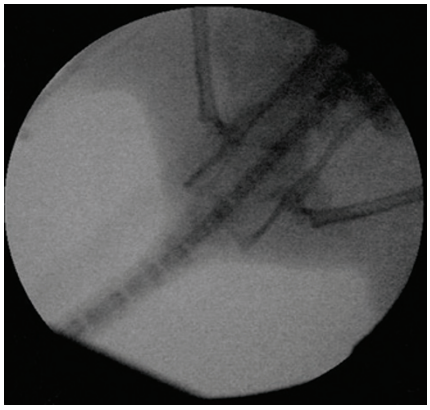

FIGURE 1 | Photoflurograph of the pelvis, lumbosacral and coccygeal vertebrae, and the left and right femurs of an outbred mouse.

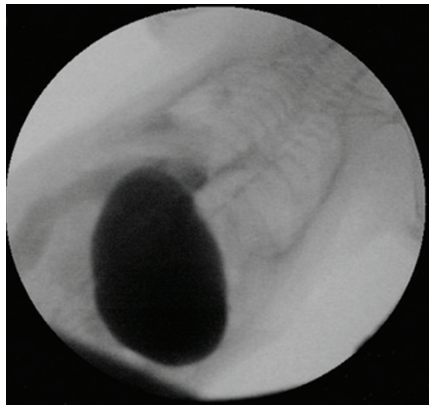

FIGURE 2 | Photoflurograph of the abdominal and thoracic cavities of the same mouse in Figure 1. An ovoid radiodense mass is occupying more than half the abdominal cavity.

Initially, 250 µl of 300 mg/ml Omnipaque (iohexol), a nonionic radiographic contrast medium, was infused into the aortic catheter. The fluoroscope acquired serial images as the contrast agent was injected. Surprisingly, no arteries were visualized in the hind limb by the end of the injection (**Fig. 1**). The researchers hypothesized more contrast agent was required to compensate for the volume retained in the 12-cm catheter. After several subsequent infusions totaling more than 1.25 ml of contrast agent, the hind limb vasculature was still not visible. The researchers then decided to reposition the fluoroscope over the thorax and abdomen of the mouse (**Fig. 2**).

What is the radiodense ovoid mass in **Figure 2**? What is the most likely explanation for the appearance of **Figure 2**?

What's your diagnosis?

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