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IMPROVED TECHNIQUE FOR RODENT MICROARTERIOGRAPHY

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We have developed new techniques in rodent microarteriography which provide excellent two-plane views of the rat femoral artery. These techniques employ routine mammography film and equipment for imaging as well as standard intravenous catheters for vascular access. The use of mammographic equipment provides high quality, finely detailed, orthogonal X-ray images. The elective outpatient nature of mammography allows microsurgical investigators convenient access to the necessary equipment without interfering with

patient care. The standard intravenous catheters permit easy and inexpensive vascular access via the contralateral femoral artery. The technique avoids the more invasive thoracic and abdominal aortic approaches without compromising X-ray image quality. Additionally, the same rat can be imaged multiple times if care is taken to repair the vascular access route.

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Microarteriography is a commonly used method for the evaluation of microvascular anastomoses. Several investigative groups,¹⁻³ including our own,⁴ have described various techniques in rodent microarteriography. We have developed a new method of rodent microarteriography that offers distinct advantages over previously described techniques. Our technique focuses on the femoral artery, the most studied microvascular model. It specifically examines the artery between the inguinal ligament and the inferior epigastric artery, since this area is commonly under investigation. This method utilizes common radiological machinery and film as well as inexpensive and easily obtained catheters. It provides biplanar visualization of the common femoral artery thereby allowing proper evaluation of stenotic lesions. Finally, it allows for multiple investigations of the same rat over time by utilizing a less invasive vascular access technique.

MATERIALS AND METHODS

Six Sprague-Dawley rats (350–450 g) were used in this assessment of our technique. General anesthesia was achieved utilizing intramuscular injection of a mixture of ketamine (Ketaset, Aveco, Ft. Dodge, IA), 43 mg/ml; xylazine (Gemini, Rugby, Rockville Center, NY) 8.6 mg/ml; and acepromazine (Promace, Aveco, NY) 1.4 mg/ml; at a

dose of 0.7 ml per kilogram body weight. National Institute of Health guidelines for use and care of experimental animals were followed throughout this study.

Vascular Access

Vascular access is obtained at the femoral artery opposite the vessel under investigation (Fig. 1). A contralateral groin incision is performed, and the femoral artery is dissected free. Any branches proximal to the inferior epigastric artery are tied off. A single clamp is applied to the proximal femoral artery as it emerges from under the inguinal ligament. A 6-0 silk tie is then placed loosely around the vessel just distal to the clamp. A second clamp is placed just proximal to the inferior epigastric artery, thereby cutting all blood flow to the central portion of the artery. An arteriotomy of 50–60% of the vessel diameter is performed. A 22 gauge intravenous catheter (Intracath, Deseret Medical, Sandy, UT), cut to a length of 45–50 mm, is inserted into the vessel. After advancing the catheter tip past the proximal silk tie, the tie is gently snugged down over the catheter. The proximal clamp is released and the catheter advanced the desired length. A second 6-0 silk tie is then gently snugged down over the catheter in the vicinity of the first proximal tie to prevent bleeding and catheter movement. A tuberculin syringe containing 0.75 ml heparinized saline (10 U/cc) is attached to the catheter hub, and the catheter is tested for patency and then flushed.

An insertion length of 36–40 mm will place the catheter tip in the distal aorta and allow for excellent visualization of the contralateral femoral vessels. Further catheter advancement can provide for angiograms of the renal, splenic, mesenteric, or other intraabdominal vessels.

Radiological Procedure

In order to optimize x-ray resolution, mammographic equipment (Phillips Diagnost U-M, Phillips Medical, Pur-

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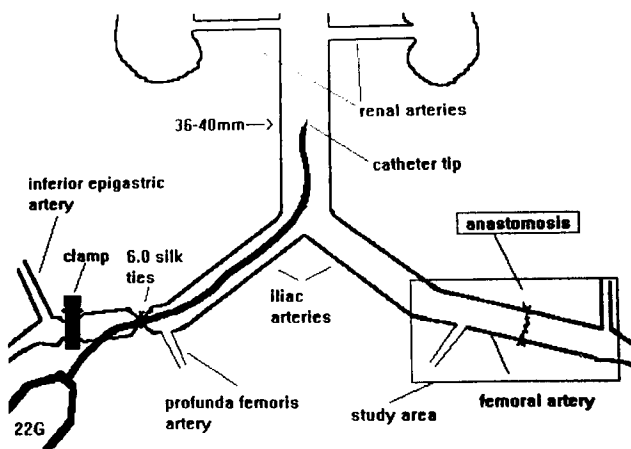


Figure 1. Schematic of catheter positioning.

chase, NY) and processing (Agfa Compact Mammoray, Agfa-Gevaert, Teterboro, NJ) are used. Anesthetized rodents are restrained on a standard operating cork board and placed on the mammography unit. Dupont Cronex Microvision film (E.I. du Pont de Nemours, Wilmington, DE), 18 × 24 cm or 24 × 30 cm, and Agfa "MR Detail" cassettes (Agfa-Gevaert) are employed throughout this study. All exposures, whether mediolateral or anteroposterior (AP), are taken at 30 kV and 100 mA manual settings. A phototimer is not used. Intravenous contrast injection consists of 0.5–0.6 cc of Reno-76 (E.R. Squibb, Princeton, NJ), hand injected over 1–1.5 sec, with X-ray exposure commencing towards the end of the bolus injection.

Mediolateral limb views are taken directly against a mammoray screen with the rodent in a supine position and the X-ray centered over the mid thigh (Fig. 2). Mediolateral X-rays are not magnified. In order to separate the femoral artery from the femur, a 1.5–2.5 cm skin incision is made over the course of the femoral artery and a skin hook is placed in the superior femoral musculature. Gentle cranial traction is then applied. This action displaces the femoral vessels off the femur and allows proper visualization. The exposure time for mediolateral X-rays is 0.6 seconds.

AP limb views are performed with the mammography unit rotated to 90 degrees so that the X-ray beam is parallel to the floor. The supine rodent is placed on a supporting table between the tube and film. AP views are magnified by employing a subject-image distance of 21 cm. The ipsilateral thigh and pelvis are elevated using a 2–3 cm wad of gauze, thereby clearing the image area from the cork board (Fig. 3). The study leg is abducted to 90° and simultaneous traction is applied to the tail, pulling it away from the midline and toward the contralateral leg (Figs. 3,4). This maneuver displaces the pelvis and adjacent soft tissue from the study field. The X-ray is centered on the posterior midthigh



Figure 2. Rodent positioning for mediolateral x-ray. Skin incision has been performed and skin hook is in place.

and the beam aimed perpendicular to the path of the femoral artery. Exposure time for the AP X-ray is 1.6 sec.

In Vivo Evaluation

Six rats were examined in this study. Two rats (A1, A2) were control animals and were used to verify catheter placement and technique. Two (B1, B2) underwent partial occlusion of their left femoral artery using a 9-0 nylon noose. The occluded areas were flanked with ties of thin (9-0–10-0) copper wire (individual strand of braided electronic wire No. 3006-023-29-2-2/5, Calmont Engineering, Santa Ana, CA) to eliminate any error in interpretation. High frequency pulsed Doppler ultrasound (HFPDU; Crystal Biotech, Hopkinton, MA) was used to quantify the degree of stenosis prior to radiography. The final two rodents (C1, C2) underwent exposure of their left femoral artery with subsequent division and reanastomosis. Postoperative hemodynamics were assessed with HFPDU. A 25-gauge needle was used to mark the anastomosis prior to X-ray.

Following arteriography, rats B1 and B2 were sacrificed and corrosion casts (Polysciences, Warrington, PA) were made of the noose occluded regions to verify and further quantify stenosis. Magnification photographs of the original X-rays were taken using a Polaroid MP-4 camera (Polaroid, Cambridge, MA) with Kodak Ectapan film (Kodak, Rochester, NY)

RESULTS

Four examples of our microangiographic technique are presented. Rat A1 (Fig. 5), demonstrates the intravascular



Figure 3. Rodent positioning for AP X-ray. Photo taken in direction of X-ray beam (as marked by arrowhead).



Figure 4. Position for AP X-ray as seen from above. Traction on tail pulls the pelvis out of the study field. X-ray path marked by arrow.

course of the catheter (arrows), with its tip lying just above the rat's pelvic brim (arrowhead). An angiogram after the catheter was advanced to the 45–50 mm region was taken (Fig. 6) and clearly displays the kidneys, right adrenal gland, and mesenteric vasculature. Figures 7 and 8 show the difference in outcome in the mediolateral plane when gentle skin hook traction on the femoral musculature is applied.

An AP image of rat B1 is shown (Fig. 9). The noose occlusion is clearly seen lying between the marking copper wires (arrows). In the mediolateral limb view of the same rat (Fig. 10), the contrast column distal to the occlusion is attenuated, but the marking wires (arrows) clearly denote the area of stenosis lying between them. HFPDU study of the occluded area revealed a stenosis of roughly 70% of the diameter (90% of the cross-sectional area). Also note the skin hook displacing the vessels cranially for proper exposure. A corrosion cast of the occluded area of rat B1 directly demonstrates the degree of noose occlusion (Fig. 11). AP arteriography of rat C1 (Fig. 12) 4 hours after standard end-to-end anastomosis reveals a mild stenosis (arrows and radio-opaque pointer), which measured roughly 30% diameter reduction by HFPDU.

DISCUSSION

The femoral artery is involved in many microvascular study models, yet previously described microangiographic techniques have been directed more toward aortic than femoral study.^{1,4} This trend is likely a function of catheter and radiological technology, since earlier equipment was better suited for the study of the larger caliber aorta. However, with today's ubiquitous mammographic equipment, all medical centers have the ability to perform high resolution biplanar microarteriography. Additionally, since there is no

need for the specially manufactured catheters utilized in other reports,³ intravascular access is easy and inexpensive.

The potential morbidity to the test rat is lessened using these techniques. Previous attempts at aortofemoral angiography have used transthoracic left ventricular puncture² or laparotomy with aortic puncture under direct vision.^{1,4} These approaches are clearly more invasive and carry a higher morbidity than a contralateral femoral cutdown. Furthermore, with careful technique, our method allows the catheter to be removed and the femoral artery to be repaired using standard microsurgical methods. This ability permits a repeat angiogram at a later date without ever having to violate the thoracic or abdominal cavities. These latter approaches could then be used for further follow up if needed.

Proper positioning of the rat prior to injection is crucial. Elevation of the study leg along with traction on the rodent's tail to remove the pelvis and excess soft tissue from the image field are important maneuvers in the AP x-ray. In the mediolateral plane, the use of a skin incision and gentle traction to isolate the study vessel is vital as it provides excellent visualization without altering vessel anatomy. Repeated angiograms in the AP and mediolateral planes of our test rats revealed that gentle traction on the femoral musculature did not change angiographic characteristics in any way.

We have found that the elective nature of outpatient mammography allowed ample time to work with the mammographic equipment without interfering with patient care. The use of mammographic equipment and standard intravascular catheters, therefore, allows any medical center to perform high-quality microarteriography in two planes. We find these techniques to be extremely useful in the follow up of microvascular experiments.

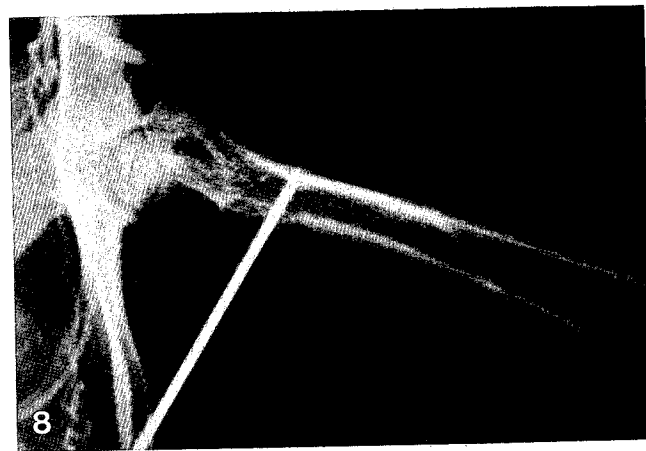
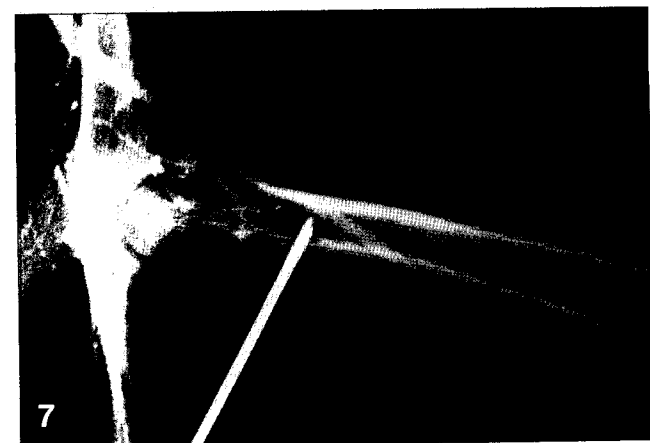
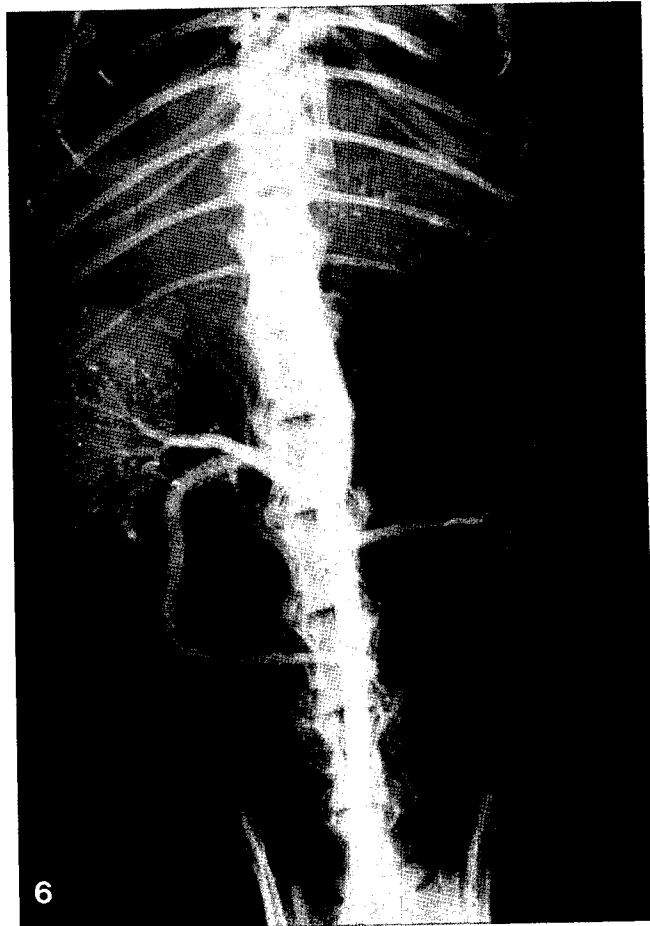
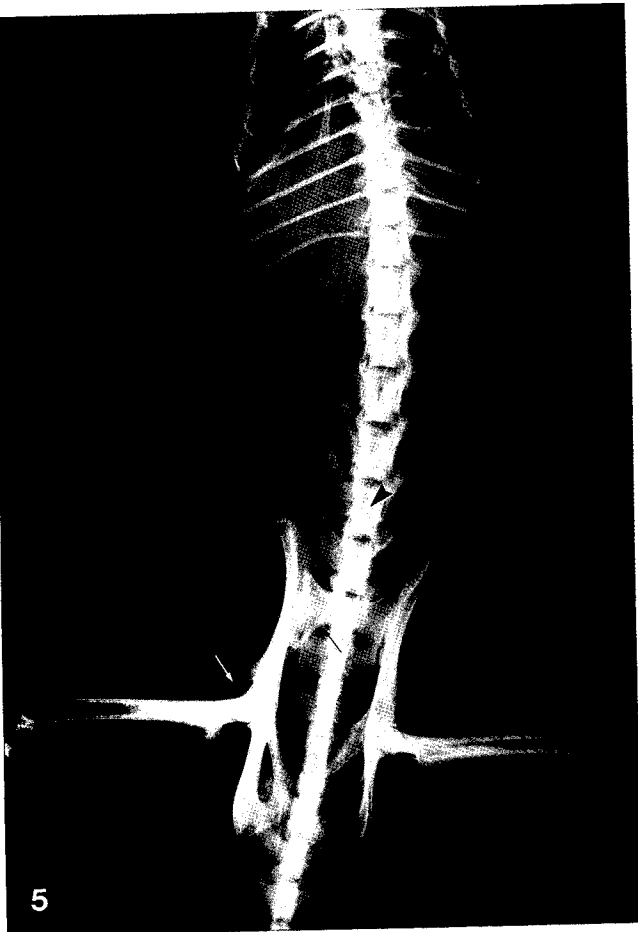


Figure 5. Intravascular course of catheter (arrows) and catheter tip (arrowhead).

Figure 6. Angiogram taken after catheter advanced to 45–50 mm. Renal and mesenteric vessels, kidneys, and right adrenal gland are well delineated.

Figure 7. Mediolateral femoral arteriogram without skin hook traction (rat A1). Needle points to usual microvascular study area at midfemoral artery. Study area is obscured by femur.

Figure 8. Rat A1 with skin hook traction applied to musculature. Femoral artery is now displaced from the femur without vessel distortion. Pointer delineates study area.

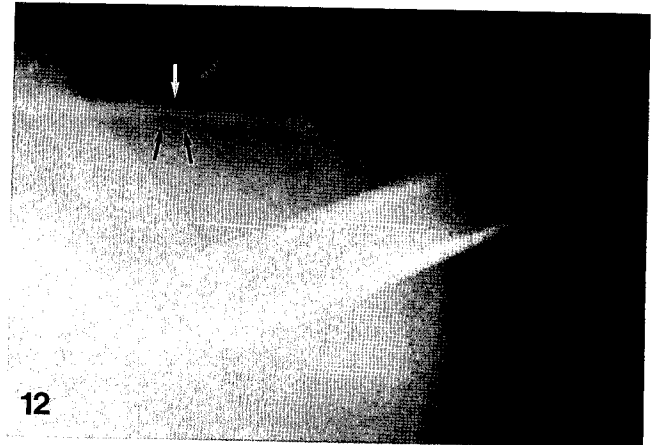
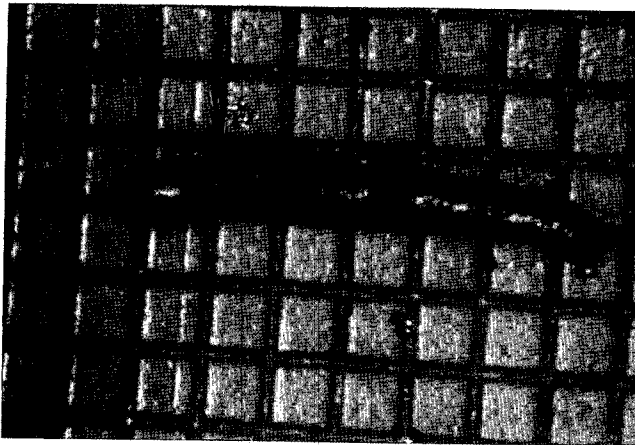
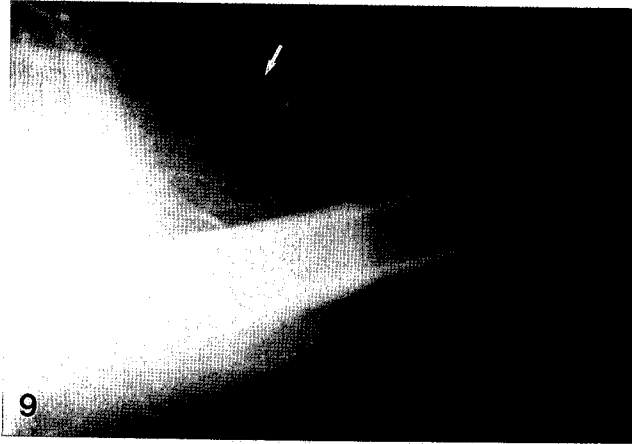


Figure 9. AP angiogram (rat B1) of noose occlusion (70% diameter reduction). Arrows mark copper wires with occlusion between them.

Figure 10. Mediolateral arteriogram (rat B1). Arrows mark copper wires. Occlusion lies between markers.

Figure 11. Corrosion cast of rat B1. Noose suture (arrow) still in place. Stenosis well displayed (1 grid box = 1 mm).

Figure 12. AP arteriogram 4 h after standard end-to-end anastomosis. Arrows and radioopaque pointer mark anastomosis with stenotic region.

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