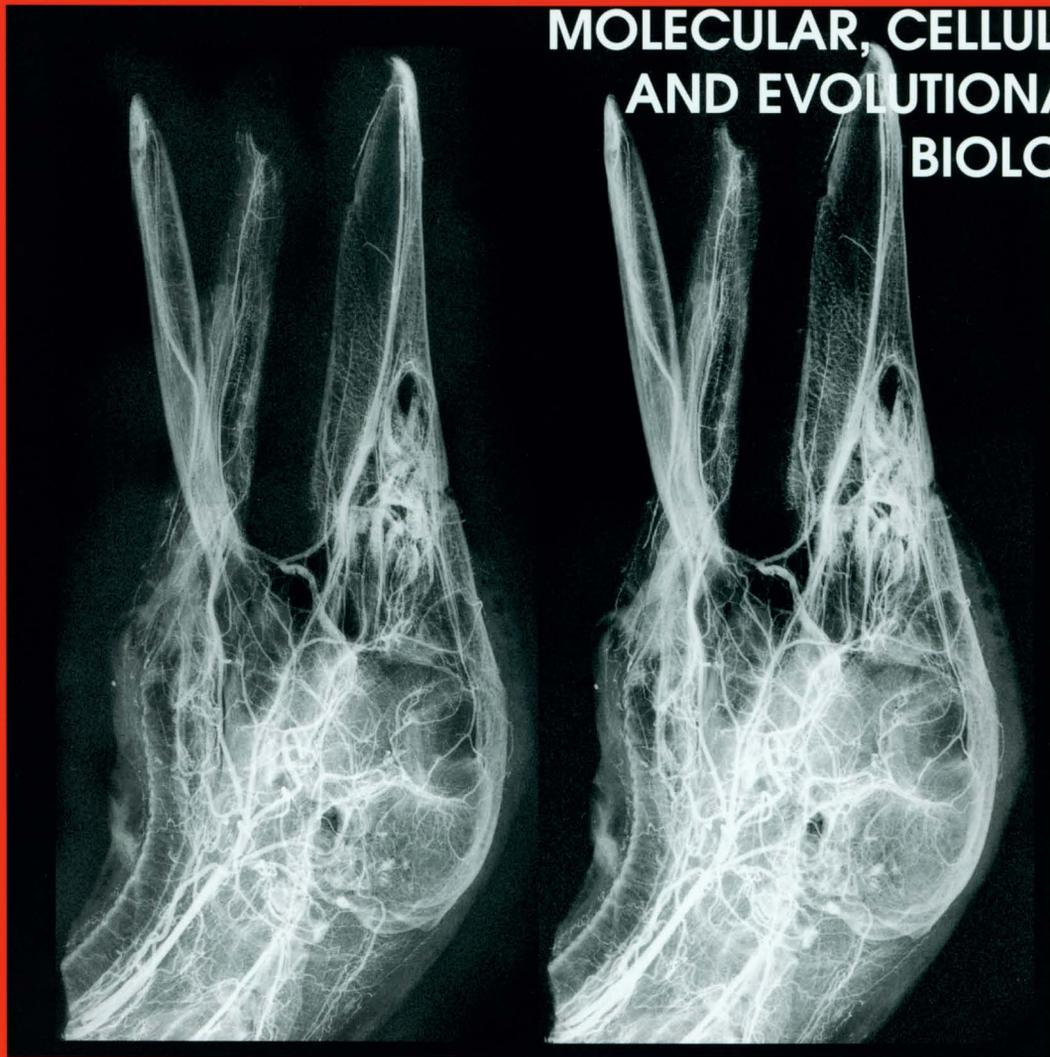


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# Rapid Technique for Imaging the Blood Vascular System Using Stereoangiography

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## ABSTRACT

Data on vascular anatomy traditionally have been derived from time-consuming gross dissection and histology, which has prevented the assembly of large sample sizes. Vascular injection of radiopaque medium (angiography) is a rapid technique, but, as typically performed, it has limitations (e.g., superimposition and poor subsequent dissectibility). We present a novel angiographic technique comprised of two elements: 1) a new, dissectible injection medium; and 2) stereoradiography. The injection medium consists of liquid barium (providing radiopacity) and latex (providing dissectibility). Domestic duck heads were the study system. The relative concentrations of barium and latex were varied, and the resulting radiographs were assayed for vessel radiopacity and the number of observable vessels. A wide range of barium percentages yielded excellent results, suggesting that preparation of the medium can be “eyeballed” for most applications, which enhances processing speed. The stereoradiographic element solved the superimposition problem, allowing stunning resolution of the spatial relationships of vessels to each other and to other tissues. Stereoangiography is a fast and easy technique that permits the acquisition of detailed anatomical data from many specimens, thereby enabling something rarely achieved: population-level anatomical studies. *Anat Rec* 267:330–336, 2002.

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**Key words:** imaging; stereoradiography; stereoangiography; vasculature; gross anatomy; duck

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Vasculature is a difficult aspect of vertebrate anatomy to study and document. Gross dissection and serial sectioning can be effective, providing precise three-dimensional anatomical relationships of vessels to each other and to other structures (O'Donoghue, 1921; Midtgard, 1984). These traditional methods, however, are destructive and time-consuming, and are feasible for only very small samples. Angiography, the radiography of specimens injected with radiopaque substances (i.e., contrast agents), does not destroy (consume) the specimen and is much faster, enabling preparation of larger samples. However, with traditional x-ray techniques, such as plain film radiography, angiography has the major limitation of superimposition. That is, all three-dimensional anatomy is compressed into two dimensions, such that in a lateral radiograph, for example, the left and right structures are superimposed. Thus, there can be great difficulty in determining three-dimensional position (e.g., is a vessel medial

or lateral, dorsal or ventral?) and the relationships of vessels to other structures (e.g., medial or lateral to a bone, through or external to musculature, supplying the brain or the scalp). As a result, the time efficiency of

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angiography comes at the expense of anatomical detail and clarity. Additionally, typical radiopaque injection media offer no advantages for subsequent dissection or sectioning in that they have such low viscosity, they essentially "bleed out" of the specimen during subsequent preparation.

We present here a new angiographic technique that meets the needs for 1) the elimination of superimpositioning problems; 2) subsequent dissectibility; 3) high vascular perfusion; 4) high radiopacity; and 5) a time- and cost-efficient means of generating a large sample size to assess variation.

There are at least four popular vascular injection media used in comparative anatomy: 1) Liquid latex is an excellent medium for dissection studies, offering high perfusion and excellent strength. However, latex is not radiopaque. 2) Unsaturated polyester resin (e.g., Batson's #17; Poly-science, Inc., Warrington, PA) is excellent for the production of vascular corrosion casts (e.g., Tompsett, 1970), but is not radiopaque, and its brittleness makes it unsuitable for dissection. 3) Microfil (Flow Tek, Inc., Boulder, CO), a low-viscosity silicone rubber, is a radiopaque substance and sets into a solid, making it both dissectible and well suited for radiography. However, Microfil is relatively very expensive, has only moderate radiopacity, and has a short shelf life, after which radiopacity declines. 4) Iodinated contrast agents are commonly used in clinical angiography, are safe, and have high perfusion. However, such media are expensive and do not allow for subsequent dissection. We have experimented with all four media.

Our technique consists of two novel elements: 1) a new vascular injection medium that combines the best attributes of the aforementioned media; and 2) the introduction of stereoradiography as the principle means of visualizing and analyzing angiographic data. The new vascular injection medium is a mixture of a barium sulfate suspension and latex. Latex is both a carrier and a stabilizer for the liquid barium, which otherwise is highly viscous and less likely to penetrate small caliber vessels, and will not "set up." Ganey et al. (1990) introduced the use of a barium enema solution to study vessels radiographically, but used gelatin as the carrier and stabilizer. We found this medium to be much less effective than the one proposed here in terms of subsequent dissectibility. Moreover, preparation of the medium has additional steps and hence is more time-consuming.

Stereoradiography and its variant discussed herein, stereoangiography, are radiographic applications of stereoscopic imaging, a well understood means of restoring three-dimensionality by viewing a pair of two-dimensional images taken from different angles with a stereoscope. Stereoradiography is an under-appreciated anatomical tool (Smith and Smith, 1967), and has seen only limited use in clinical and industrial settings (Kumazaki, 1991).

## MATERIALS AND METHODS

### Study Taxon

The sample was comprised of the heads of 30 domestic duck, *Anas platyrhynchos*, which were obtained from a commercial processor (Joe Jurgielewicz and Son, Ltd., Shartlesville, PA). Twenty heads were used in statistical analyses concerning the effectiveness of differing barium-to-latex ratios. Ten heads were used as replicates, and for more specialized techniques. All specimens and radiographs have been accessioned into the Ohio University

Vertebrate Collections (OUVC), and the data are available upon request from L.M.W.

### Injection Medium

The vascular injection medium tested in the present study consisted of a mixture of barium and latex. Again, barium is the radiologic contrast agent, and latex stabilizes the barium and allows subsequent dissection of the specimen. For the barium component, we used Liquid Polibar Plus barium sulfate suspension (BaSO<sub>4</sub>; E-Z-Em, Inc., Westbury, NY), which was developed for visualization of the gastrointestinal tract via rectal administration (barium enema). For the latex component, we used a laboratory grade latex (Ward's, Rochester, NY) specifically designed for vascular injection.

### Instrumentation

The injection apparatus consisted of 18-gauge Hamilton blunt-ended cannulae (Hamilton Co., Reno, NV) and B-D syringes with Luer-Lok tips (Becton Dickinson and Co., Franklin Lakes, NJ). Ethicon (Cincinnati, OH) 4-0 silk suture was sometimes used to ligate cannulated vessels. A Hewlett Packard (Corvallis, OR) Faxitron series 43805N cabinet x-ray system was the x-ray source. The x-rays were shot on Kodak (Rochester, NY) Industrex M professional x-ray film and processed with Kodak developer GBX and Kodak rapid fixer. Density readings of selected vascular structures were obtained by using a densitometer (X-Rite Co., Grandville, MI). Stereoscopes included a 4X pocket stereoscope (Alan Gordon, Hollywood, CA) for smaller images and a Geoscope mirror stereoscope (ASC Scientific, Carlsbad, CA) for larger images.

### Experimental Procedures

All injections were performed via both common carotid arteries, approximately midway along the neck. An opening was made by making a cut one-quarter to three-quarters across the artery. A blunt-ended cannula was inserted, and the needle was subsequently pushed as far cranially as possible. In some cases, suture material was used to hold the cannula in place, but with vessels of the size of duck common carotids, it was found that this could be accomplished more simply and cost effectively with hemostats.

Twenty different 60-ml solutions consisting of different barium-to-latex ratios were prepared, starting with a mixture of 95% barium and 5% latex, and decreasing in barium content in increments of 5% to end at a final solution of 100% latex. Large particles of latex that might have obstructed the injection apparatus were filtered out with fine-mesh cheesecloth. The filtered latex was then drawn into syringes and added to the barium, which previously had been measured into a beaker. The solution was then mixed for 3 min, after which it was drawn into syringes and completely injected into a duck head through the in-place cannulae. All injected heads were immediately frozen or refrigerated overnight to allow the medium to set.

In a previous study, Ganey et al. (1990) heated a barium sulfate and gelatin mixture to 57°C before injection. To test the effectiveness of a heated solution vs. an unheated solution, eight duck heads were injected with a barium/latex solution; in four the solution was heated to 57°C, and in four it was room temperature.

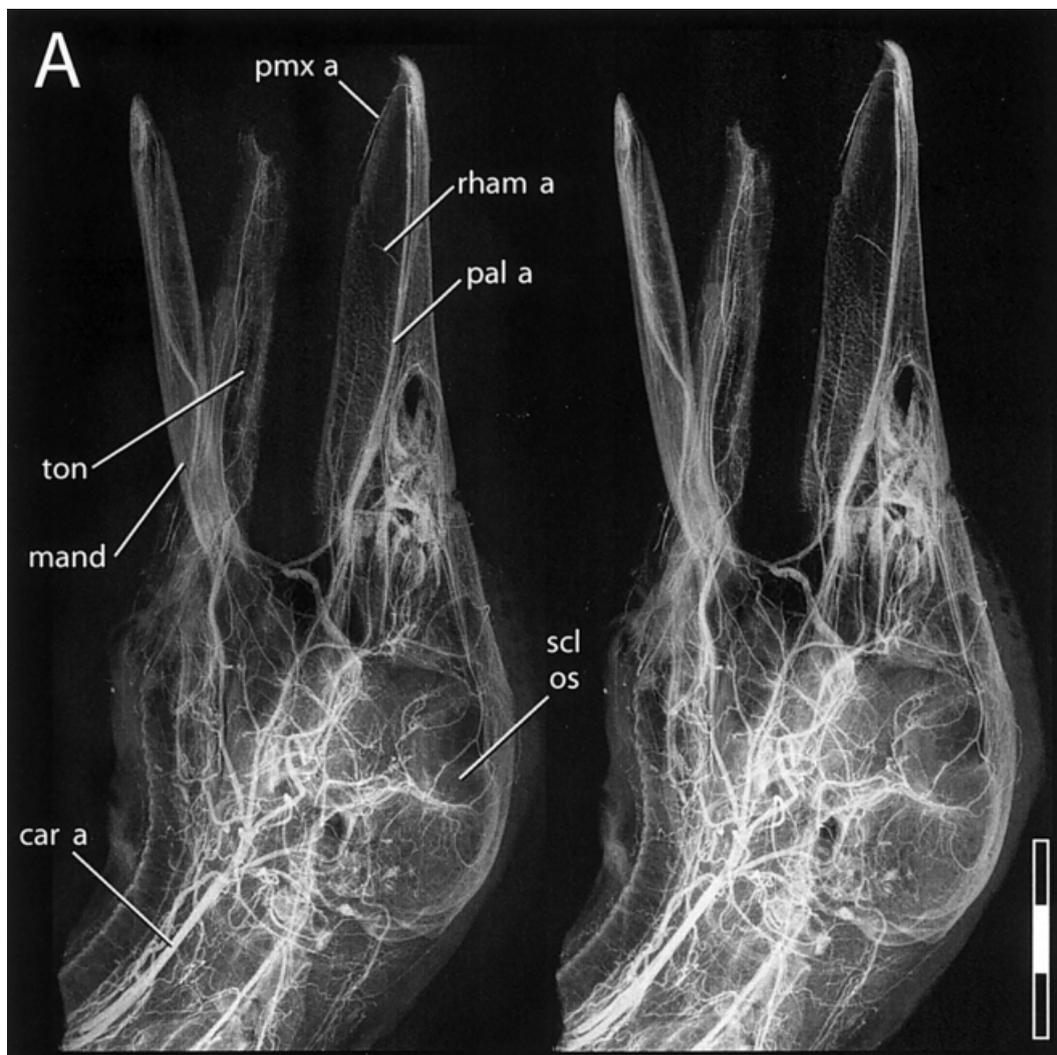


Fig. 1. Stereoangiographs of a parasagittally sectioned domestic duck head (*Anas platyrhynchos*; OUV 9441) injected with a medium consisting of 45% barium and 55% latex, demonstrating (A) a lateral perspective and (B) a medial perspective. In part B, the same stereoangiographs have been swapped (left for right), accounting for the reversed perspective. In part A, the eye and scleral ossicles (bony eye ring) stand out as superficial structures, and the tongue appears deep to the mandible. The common carotid artery is a large vessel coursing rostrally from the neck, dorsal to the trachea. The palatine artery is a median vessel running along the palatal vault; given that the head has been sectioned, the palatine artery appears as a deep structure in A and a superficial structure in B. The premaxillary artery originates from the rostral aspect

of the premaxilla, appearing superficial in A and deep in B. The ramphothecal artery is a small-caliber vessel that arises midway along the palatine artery, traveling laterally toward the observer in A and away from the observer in B. Note that despite the exact same information content, the different perspectives in A and B impart a tangibly different anatomical emphasis. Note that the 45% barium medium highlights physiologically important vascular devices, such as vascular plexuses and erectile tissues in the nasal conchae, along the pterygoid, and the ophthalmic rete caudoventral to the eye. Abbreviations: *car a*, common carotid artery; *mand*, mandible; *nas con*, nasal conchae; *pal a*, palatine artery; *pmx a*, premaxillary artery; *rham a*, ramphothecal artery; *scl os*, scleral ossicles; *ton*, tongue.

### Stereoradiography

Plain film x-rays of the duck heads were produced using standard techniques. All duck heads were exposed in lateral view, except for a few specifically prepared for dorsoventral radiographs. As mentioned above, stereoradiography requires that two slightly different x-rays be made of each subject. Two methods have been proposed to accomplish this (Zangerl and Schultze, 1989): the first involves moving the x-ray tube, and the second involves moving the specimen. The latter was employed in the present study.

Heads were placed on a thin plexiglas square, which was then set on a section of film, with the remaining film blocked off with lead sheets to prevent exposure. The plexiglas was then tilted by placing a 14-mm block just under its edge, taking care that the block did not overlap the specimen. The head was then exposed for 15 min at 30 kVp and 2.5 mA. The block was then carefully removed from beneath the plexiglas without moving the head, and the plexiglas was slid to an area of unexposed film. The block was then placed under the plexiglas edge directly

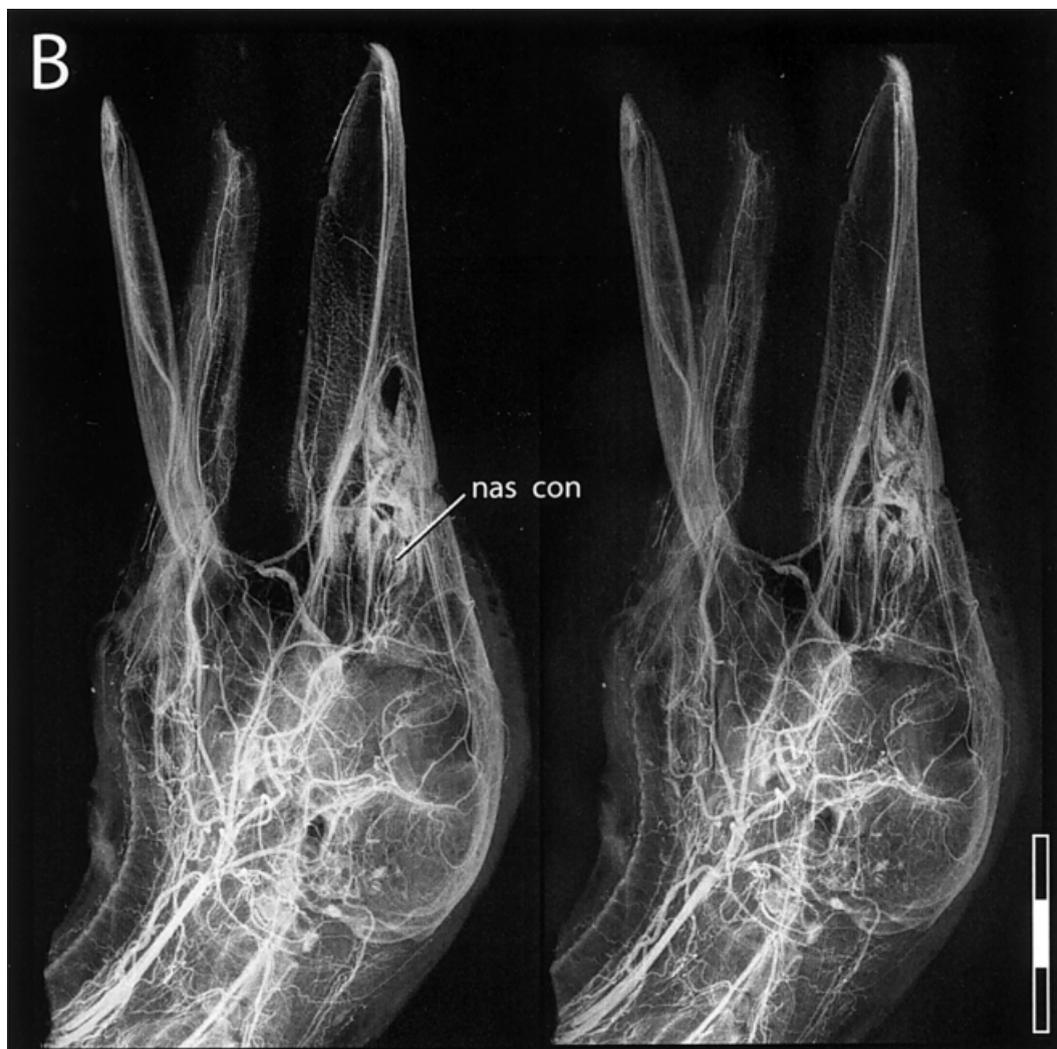


Fig. 1. Continued.

opposite its prior location, thus tilting in the opposite direction of the prior exposure. The head was then exposed as before.

After film development, the two images were cut out, placed on a light table beneath a stereoscope, and aligned to obtain the three-dimensional effect (Fig. 1). The opposite perspective of the subject can be gained by interchanging the two images. For instance, the original configuration of the two x-rays may give a lateral perspective of the head (Fig. 1A) so that lateral features (e.g., mandible) appear closer to the observer, and medial features (e.g., tongue) appear more distant. Swapping the x-rays left for right (Fig. 1B) reverses the perspective such that the observer now gains a medial view (e.g., with the tongue appearing closer than the mandible). Although the information content of the x-rays obviously has not changed, swapping the images and reversing the perspectives invariably leads to the perception of a "different" anatomical emphasis.

Finally, depth resolution can be artificially enhanced by increasing tilt, which can be useful for resolving anatomical components that are in nearly the same place.

#### Evaluation of Injection Media

To allow statistical analysis of the effects of injection media with different levels of barium, all specimens were x-rayed with precisely the same settings, and the films were processed simultaneously to eliminate any error resulting from the time-sensitive nature of the chemical processing. To assess the effect of barium percentage on the radiopacity of injected vessels, measurements were taken of the radiopacity of selected arteries using a densitometer for each of the 20 barium percentage levels. The four arteries selected for this analysis were (Fig. 1A): 1) the common carotid artery, which was the largest; 2) the palatine artery; 3) a rhamphothecal artery; and 4) the premaxillary artery, which is the most rostral and is one of the smallest identifiable vessels. For each vessel at each

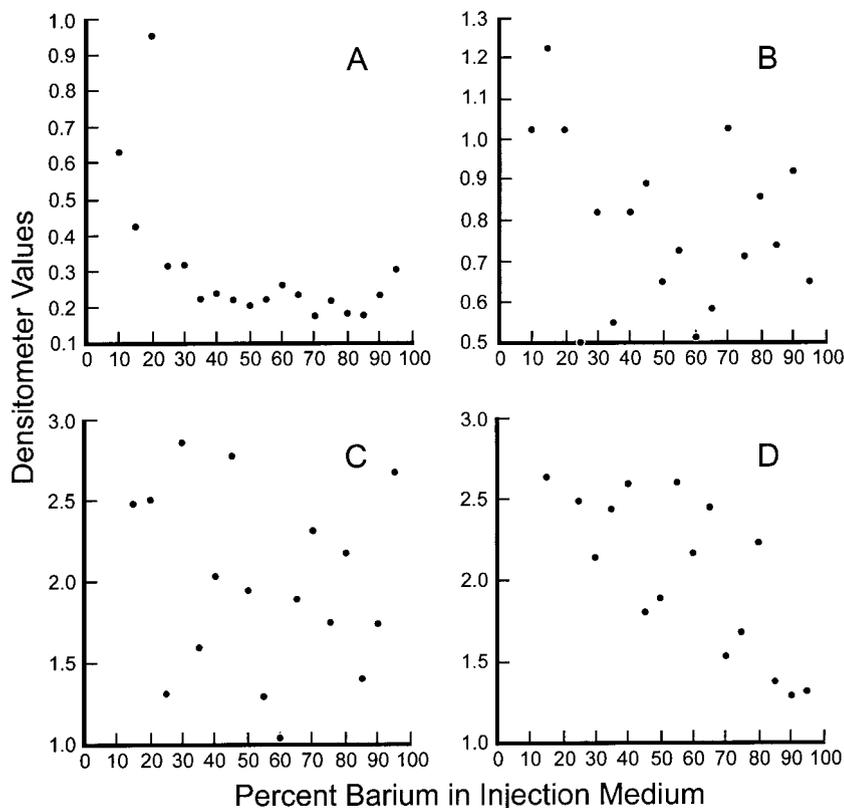


Fig. 2. Scatter plots of densitometer value vs. the percent of barium in the injection medium for the (A) common carotid, (B) palatine, (C) rhamphothecal, and (D) premaxillary arteries for 19 duck heads injected with a barium/latex solution varying in 5% increments. Densitometer values are unitless and are based on a scale of increasingly dark shaded bars numbered 1–21, with 1 being most radiodense (white: densitometer value of 0.0) and 21 most radiolucent (black: densitometer value of 3.24). Thus, the closer a reading is to 0.0, the more likely a vessel is to be visualized. The plots for the (A) common carotid and (D) premaxillary arteries illustrate an increase in vessel radiopacity with increasing barium content in the injection media; nonparametric Spearman rank correlation tests demonstrated a positively significant relationship between radiopacity and barium percentage (common carotid artery,  $Rho = 0.700$ ; premaxillary artery,  $Rho = 0.730$ ). The plots for the (B) palatine and (C) rhamphothecal arteries do not show statistically significant relationships (rhamphothecal artery,  $Rho = 0.18$ ; palatine artery,  $Rho = 0.200$ ).

barium percentage level, three readings were taken and then averaged. A nonparametric statistical test, the Spearman rank correlation, was used to test for a significant relationship between barium percentage and radiopacity. Additionally, for each of the tests, a corresponding bivariate scatter plot was generated to test for trends. In a second statistical study, the x-rays from the first analysis were scored for the presence or absence of 18 large- to intermediate-sized vessels. The total number of observed vessels was recorded for each of the 20 different solutions and the data were analyzed using the same statistical methods.

## RESULTS

### Injection Media

The scatter plot of the densitometer values recorded for the common carotid artery (Fig. 2A) indicates very little difference between treatments above a threshold at the 20% barium level, below which radiolucency steadily increases (i.e., densitometer values steadily increase) with each decrease in barium. The Spearman rank correlation test rejected the null hypothesis of no significant relationship between percent barium and the radiopacity of the common carotid artery ( $Rho = 0.700$ ).

Scatter plots for the rhamphothecal (Fig. 2B) and palatine arteries (Fig. 2C) show a more variable distribution of points than was seen with the common carotid artery. The rhamphothecal and palatine arteries could not be detected below 5% and 10% barium, respectively. Subsequent dissection revealed that these radiolucent vessels were in fact completely filled by the latex/barium solutions. The

Spearman rank correlation tests for both the rhamphothecal ( $Rho = 0.180$ ) and palatine ( $Rho = 0.200$ ) arteries accepted the null hypotheses that the radiopacity of these vessels was not dependent upon percentage of barium. These analyses indicate that there is no significant difference in the radiopacity of the rhamphothecal and palatine arteries for solutions of 15–95% barium. Nevertheless, inspection of the plots (Fig. 2B and C) reveals a threshold for visualizing these vessels at about 10% barium.

The scatter plot for the premaxillary artery (Fig. 2D) indicates a linear relationship between the percentage of barium in the injection medium and the radiopacity of the injected vessel. Radiolucency of the premaxillary artery increases with decreasing barium percentage. The premaxillary artery was not observable in x-rays from solutions with 5%, 10%, or 20% barium. The Spearman rank correlation test rejected the null hypothesis, and found a significant relationship between percent barium and radiopacity ( $Rho = 0.730$ ).

Analysis of the effect of barium percentage on the number of vessels observable in x-ray demonstrates a pattern similar to that found for the effect of barium percentage on radiopacity. The scatter plot (Fig. 3) indicates very little difference in the vessel counts for solutions with barium percentages of 15–95%. At both 15% and 95% barium, 17 out of 18 designated arteries could be identified in x-ray. A dramatic decrease in vessel count occurs at the level of 10% barium, with only four observable vessels out of 18 vessels, and at 5% barium with only three observable vessels. The Spearman rank correlation test for effect of barium percentage on the number of observable vessels

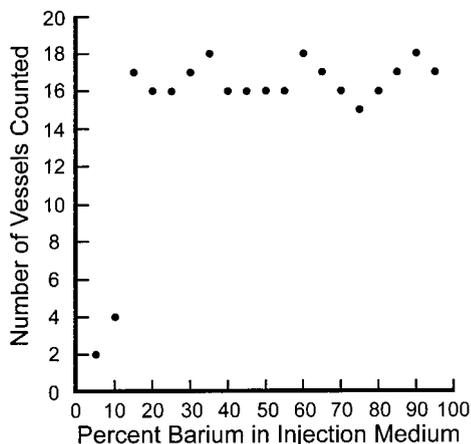


Fig. 3. Scatter plot illustrating the relationship between the number of vessels counted (with 20 total possible vessels) vs. percent barium for 19 different vascular injection solutions. The graph illustrates a fairly constant number of observable vessels (16–19 vessels) in solutions with 20–95% barium. However, a dramatic threshold occurs at 10% barium, where the number of observable vessels decreases (four vessels at 10% barium, two vessels at 5% barium). Between 40% and 55% barium, vessel number counts decrease slightly as the result of extreme vessel profusion, since some vessels are lost from view within a dense tangle of vessels (i.e., retia and erectile tissues). The Spearman rank correlation tests did not find a significant relationship ( $Rho = 0.400$ ).

for the 15–95% barium range accepted the null hypothesis ( $Rho = 0.400$ ).

The scatter plot nevertheless demonstrates a slight decrease in the number of vessels observable in the midrange levels (40–55% barium). Inspection of the x-rays from all of the solutions showed that the midrange barium percentages had filled a large number of small vessels associated with the conchal and pharyngeal arterial plexuses, such that large- and intermediate-sized arteries passing through or near these dense vascular structures were no longer identifiable as independent structures (Fig. 1B). Thus, the loss of vessel resolution with barium percentages of 40–55% can be attributed to an increase in barium in overfilled, small-caliber vessels (i.e., these plexuses were so bright they obscured other structures). Further analysis of the x-rays revealed a drastic decline in the number of intermediate- and small-caliber vessels beginning with the 20% barium solution. The conchal and pharyngeal plexuses were no longer detectable in the 20% and lower barium solutions. The same phenomenon was observed for the dense array of vessels associated with the jaw and cervical musculature.

Analyses of the number of observable vessels and their radiopacity demonstrate a threshold at 15–20% barium, below which resolution is severely compromised. Furthermore, we found no significant differences in vessel counts and radiopacity in solutions of 25–95% barium. At 20% barium, many large- and intermediate-sized vessels were visible but very faint, and resolution of small-caliber vessels, including important physiological structures such as the retia and plexuses, was nearly lost. Below 20% barium, only the largest vessels were observable, and they were so radiolucent they too were nearly undetectable.

The effects of using a heated injection medium (as done by Ganey et al., 1990) were compared to those of room-

temperature injections through a qualitative analysis of x-rays. No discernable differences in radiopacity or perfusion could be detected. Thus, this time-consuming step was dropped from our protocols.

Subsequent dissectibility of the injected heads represents a major advantage over conventional angiographic techniques. Even at moderate barium concentrations, the latex component of the injection medium ensured all of the dissection benefits of conventional latex injection (e.g., rubbery toughness of the injected vessels, and enhanced detectability due to coloration of the latex). However, there is a marked decrease in the dissectibility of specimens injected with high-barium (i.e., low-latex) solutions. Vessels injected with low-latex solutions are more delicate, easily torn, and difficult to see due to the lower levels of pigment. An unexpected (but welcome) result was that despite the “dilution” of latex with liquid barium, casts of vessels sometimes survived the skeletonization process (e.g., treatment with dermestid beetles), yielding vascular corrosion casts. Thus, the same injected specimen could yield useful data via angiography, dissection, and corrosion casting.

### Stereoradiography

As predicted, stereoscopic examination eliminated the problem of superimposition. Many vessels could only be resolved among the tangle of vessels when analyzed in stereo. The stereoangiographs provided information concerning vessel position in three-dimensional space, the precise origin of vessels, the relationships of the vessels to other vascular structures (e.g., the retia), and clarification of the tissues supplied by particular vessels. For example, the stereoangiographs demonstrated that particular vessels supply the brain, rather than run through the scalp. None of the above information was obtainable in normal two-dimensional analysis of the x-rays. In summary, the use of stereoradiographs dramatically increased the amount of meaningful information that could be recorded. In some ways, it duplicated information that was previously only obtainable through labor-intensive gross dissection; moreover, it revealed vessels and anastomoses that dissection would have missed.

### DISCUSSION

Our goal was to develop a technique that would go beyond the standard angiographic objectives of high perfusion and radiographic resolution. We also wanted to eliminate the problem of superimposition and to allow for subsequent gross dissection. Furthermore, our intent was to develop protocols that were fast, easy, and inexpensive, so that large samples could be surveyed. Thus, we developed an injection medium characterized by high vascular perfusion, high radiopacity, and high dissectibility, and analyzed the resulting angiographs stereoscopically, which solved the superimposition problem.

Any barium- and latex-based solution possessing 25–95% barium will produce high-resolution radiographs. Solutions with extremely high barium content (e.g., 75% or more) have highly radiopaque vessels, but suffer in two major areas: 1) diminished perfusion of small vessels because of the large barium particle size and hence increased viscosity; and 2) poor dissectibility because of the concomitant reduced latex content. Barium solutions with extremely low barium content (e.g., 25% or less) have

excellent perfusion and dissectibility (due to the high concentration of latex), but at the expense of radiopacity to the extent that very few (if any) vessels are observable in radiographs. Solutions approximating 50:50 barium : latex ratios are characterized by both high perfusion and radiopacity, and can illuminate important vascular structures. They have a low enough viscosity to allow the filling of small and far distal vessels, yet still contain enough barium particles to produce highly radiopaque vascular structures. As a result, these solutions provide important physiological insights by radiographically demonstrating vascular structures, such as the retia and arterial plexuses, that are involved in countercurrent heat and gas exchange. These solutions further provide indications of the relative blood supply to anatomical structures, such as jaw musculature used in feeding, or conchae (Fig. 1B) that are involved in protecting against heat and water loss during respiration. However, this increased filling can obscure vascular structures observable in higher or lower barium concentrations. If the extremes are avoided, a researcher can essentially "eyeball" the mixture and obtain excellent results. If the researcher is interested in gross dissection, we recommend lower barium concentrations (e.g., one-third barium, two-thirds latex). Obviously, each solution carries its own costs and benefits, and different solutions can be used to address different questions.

Stereoradiography remains a seldom used and, apparently, poorly known anatomical technique. The technique has been used and advocated for addressing paleontological questions (Zangerl, 1965) and assessing fish anatomy (Smith and Smith, 1967). Stereoangiography has been used only rarely in a clinical context (e.g., Lunsford et al., 1977; Kumazaki, 1991), and in those instances it was associated with high cost and (understandably) limited anatomical scope. To our knowledge, stereoangiography has never been used in comparative anatomical studies such as ours. Angiographs are notoriously difficult to decipher as a result of superimpositioning of vessels and other dense tissues such as bone. Stereoangiographs provide powerful resolution of the three-dimensional spatial position of individual vascular structures, and the positional relationships of vascular elements to other anatomical structures. The production of stereoradiographs is a simple technique that requires little additional expenditure of time or cost. Any radiographic study of vascular systems should use stereoradiography as an integral tool.

The technique advocated here is particularly advantageous for addressing variation in vasculature within and between populations, and in comparative studies of vasculature across groups of animals. For small- to medium-

sized organisms, several specimens can often be exposed and analyzed on a single film. Although documentation of the vascular system has in the past been a highly time-consuming occupation, often necessitating the recording of a system in great detail in a single specimen (or a small number of specimens), this technique can provide a new means of understanding the variation of vascular systems in natural populations.

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