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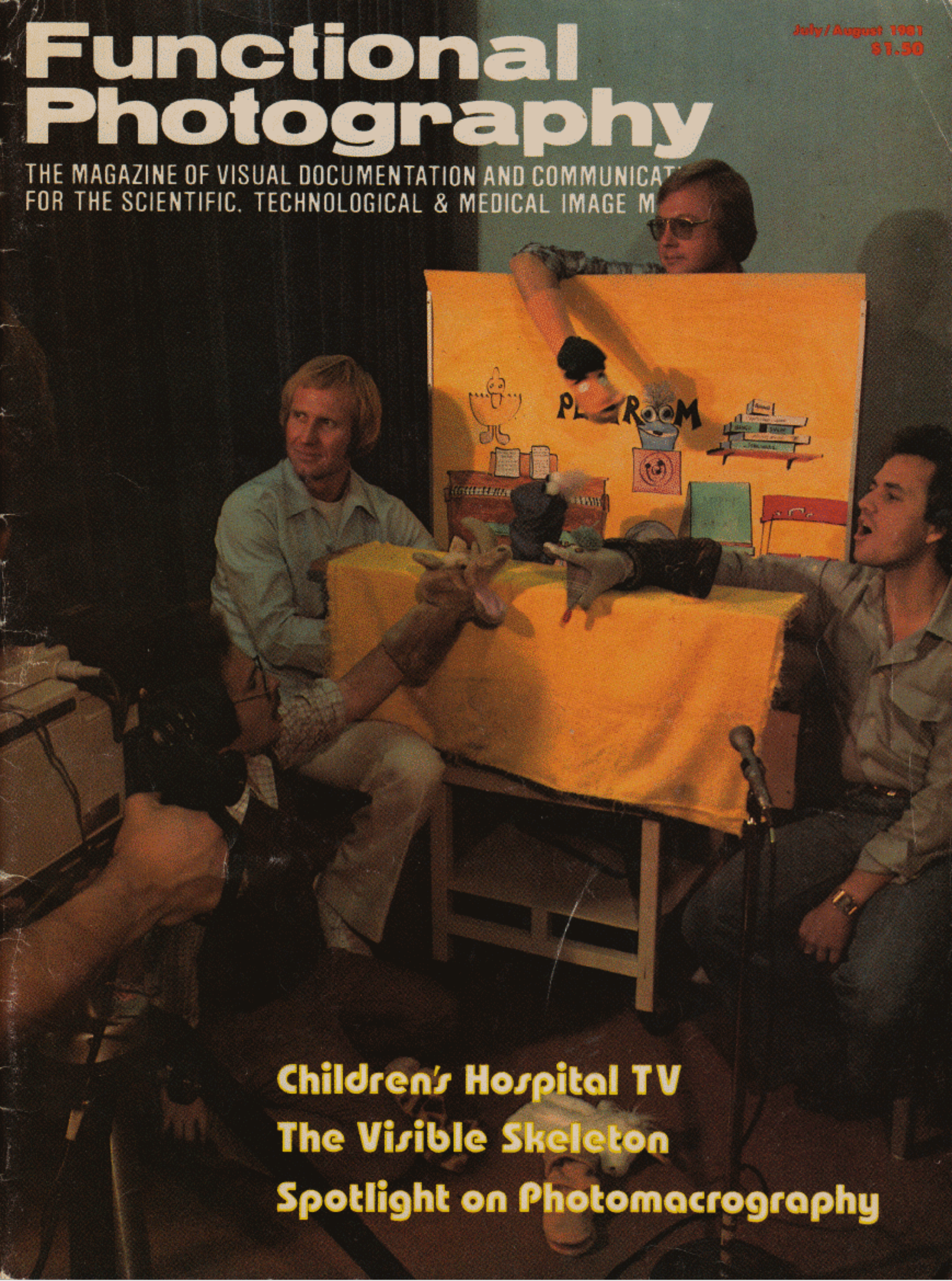

Children's Hospital TV
The Visible Skeleton
Spotlight on Photomacrography

Functional Photography

THE MAGAZINE OF VISUAL DOCUMENTATION AND COMMUNICATION FOR THE SCIENTIFIC, TECHNOLOGICAL & MEDICAL IMAGE MAKER

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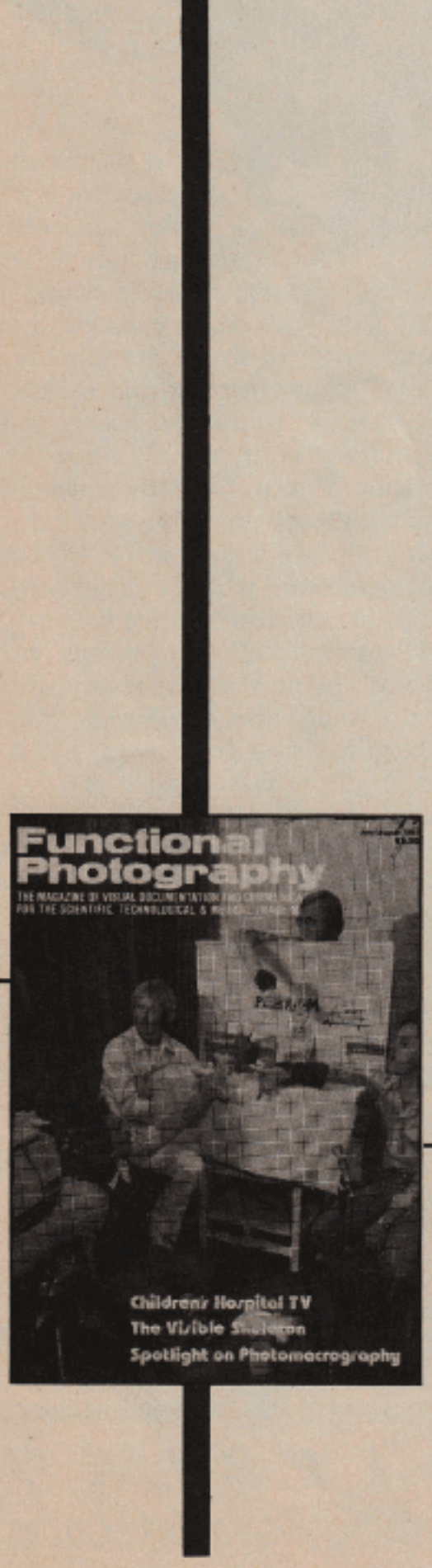

COVER: Medical television was founded eight years ago as an adjunct to the photography section at the sprawling Children's Hospital and Health Center of San Diego, CA but then became a separate entity. A small cadre of TV professionals produces a children's show as well as other functions to benefit the institution. See story on page 28.

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THE Visible Skeleton

A new double-stain technique reveals the nature of the "hard" tissues.

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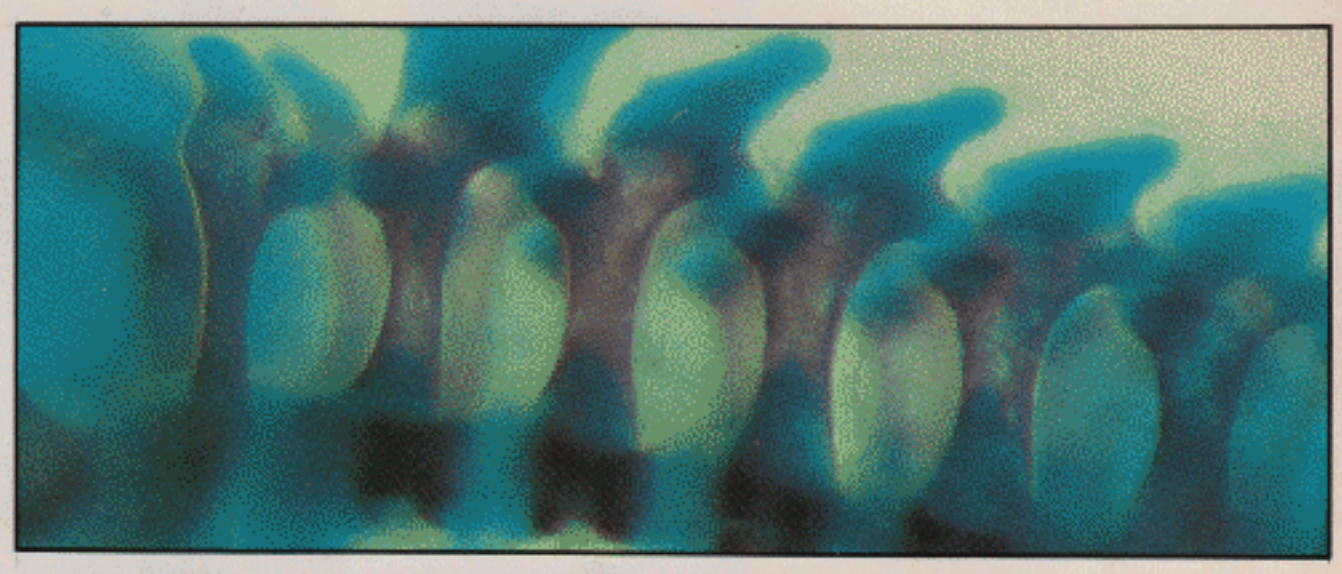
All of the photographs illustrate specimens which have been prepared by the double-stain procedure described in the text. In each case, cartilage is stained blue, bones and teeth (if present) are stained red. All of the photographs were taken on either a WILD M7S Zoom Stereomicroscope or Zeiss Tessovar dissecting microscope.



Lizard embryo. Lateral views illustrating developing skull and vertebral column (backbone). The cartilaginous skeleton is beginning to be replaced by bone.

Many scientific investigations in biology and medicine require detailed information on the skeletal system. Such studies can benefit greatly from specific stains that reveal the growth, location, and internal structure of the skeleton in whole, preserved specimens. Procedures for staining mineralized skeletal tissues (i.e., bones and teeth) in unsectioned specimens have been known for most of this century, but until only the last few years a reliable procedure for staining unmineralized cartilaginous structures (e.g., sensory capsules, epiphyses) in whole specimens has been lacking. A recently developed technique, however, now provides reliable and efficient staining of *both* bone and cartilage in whole, intact specimens.

Previously, the overall shape and details of cartilaginous skeletal elements could be obtained only by three-dimensional reconstruction from serial sections. Serial reconstruction, however, is both laborious and time consuming, and thus impractical in investigations in which many specimens are

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PHOTOGRAPHIC PUBLICATIONS AND PRIZES: First Prize (Photomicrography) *Natural History* Magazine contest, 1979; Honorable Mention (Natural World), *Natural History Magazine* contest, 1979; Honorable Mention, Nikon *Small World* Competition, 1979; publications in *Natural History* (including cover), *BioScience* (cover), field guides, biology texts, *Playboy*.

Dr. Richard Wassersug:
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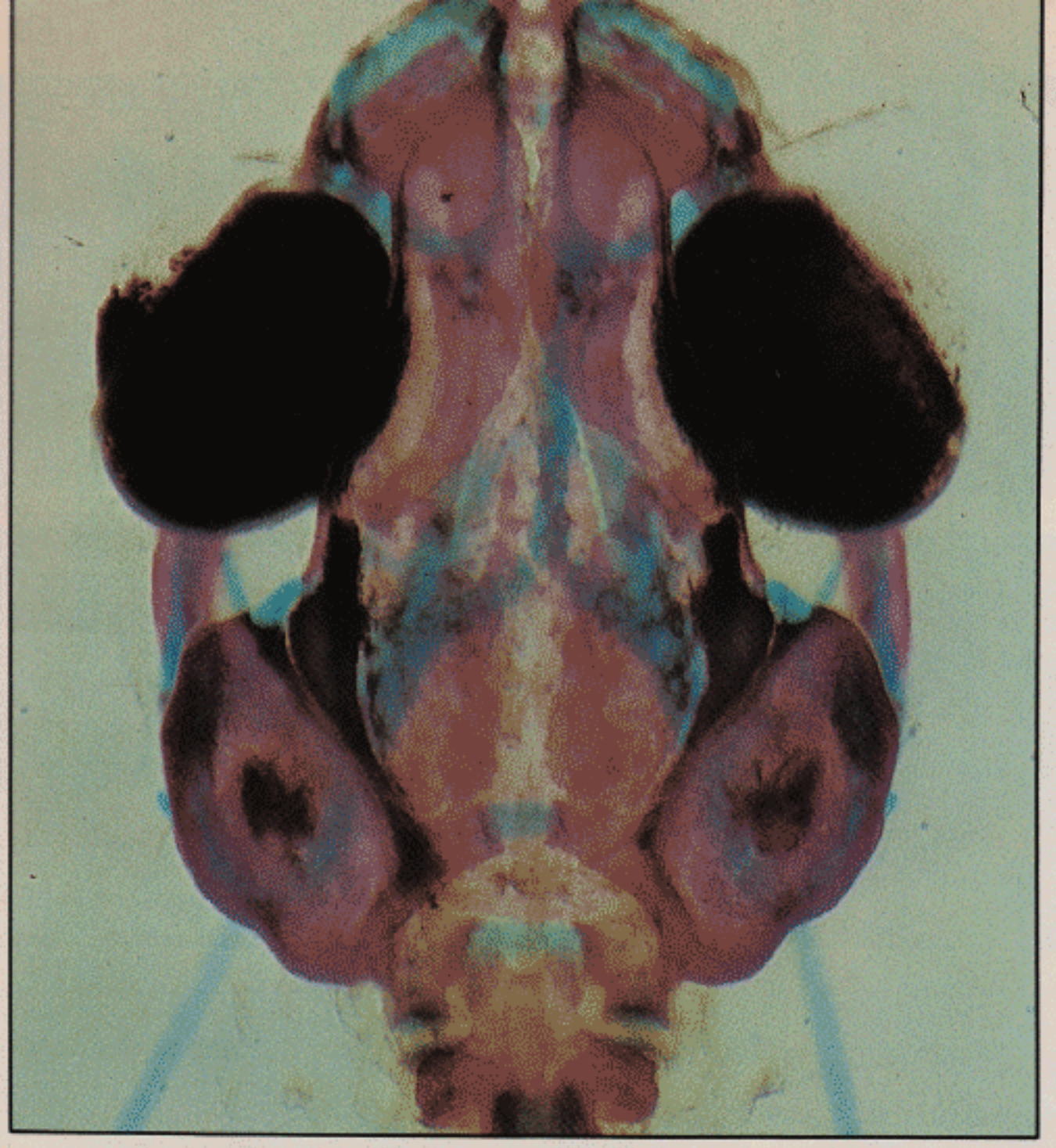
RESEARCH INTERESTS: Evolutionary and functional morphology of vertebrates, especially amphibians. Dr. Wassersug is one of the originators of the double-stain technique that we have described in our article.



Adult salamander. Dorsal (top) view of skull with pigmented eye distinguished from the cartilaginious or boney nasal capsules in front and the boney otic (ear) capsules at the rear of the skull.

needed; for example, in studies of successive stages of development, or estimates of population-wide variation within and between species.

The bone-cartilage double-stain technique reveals even slight amounts of cartilage and mineralized tissue, making it particularly valuable in studies of skeletal development in embryos, fetuses, and small adult vertebrates. It is quite general, in that with only slight modification it may be applied to all vertebrates regardless of size. We have successfully stained embryonic and adult amphibians, reptiles, fishes (including the strictly cartilaginous dogfish and boney fish), small mammals, and birds. Since the technique requires relatively little time on the part of the investigator, large numbers of specimens may be prepared in a fraction of the time required for serial reconstruction; we have processed hundreds of specimens simultaneously.

Varying Suggestions

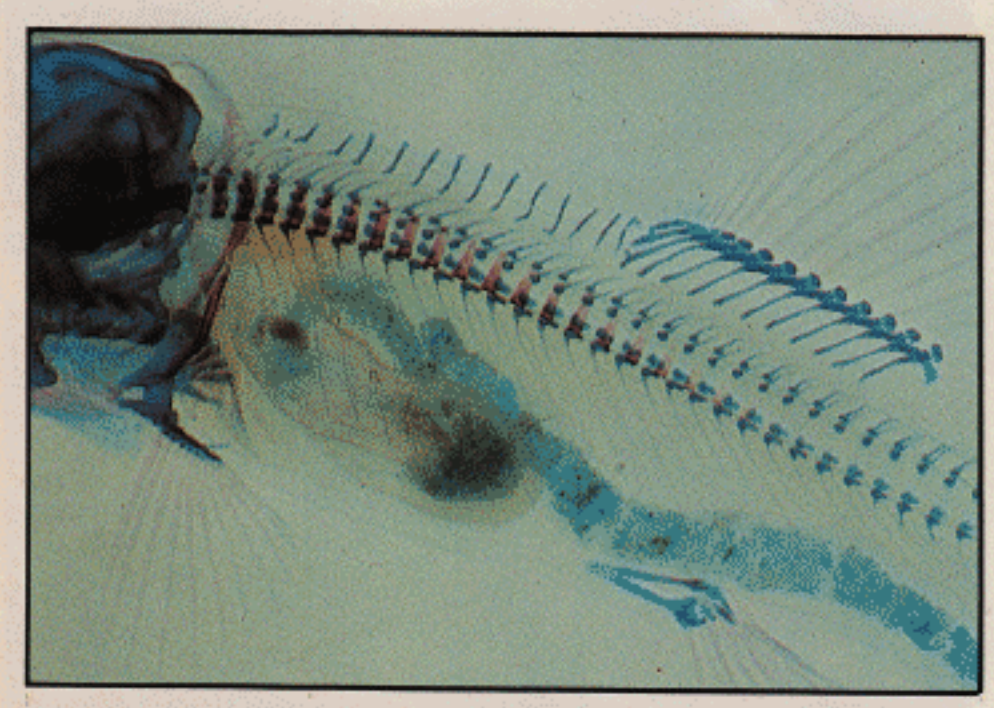
There are four basic steps in the technique: fixation, cartilage stain, maceration with bone staining, and clearing. In the last few years, numerous authors have proposed modifications of the technique which slightly alter one or another of these basic steps. Simons and Van Horn (1971) reviewed earlier procedures, most of which used methylene blue or toluidine blue as the cartilage stain, and suggested using alcian blue instead because of its greater permanence. Wassersug (1976) described a modification for use with formalin-fixed material; previous procedures had employed Bouin's fixative or ethyl alcohol-acetic acid exclusively. Dingerkus and Uhler (1977) suggested the use of trypsin as a clearing agent, instead of the traditional potassium hydroxide.

The following is a step-by-step schedule for the bone-cartilage double-stain technique. All chemicals used can be obtained from commercial biological supply houses or through a cooperative neighborhood pharmacist.

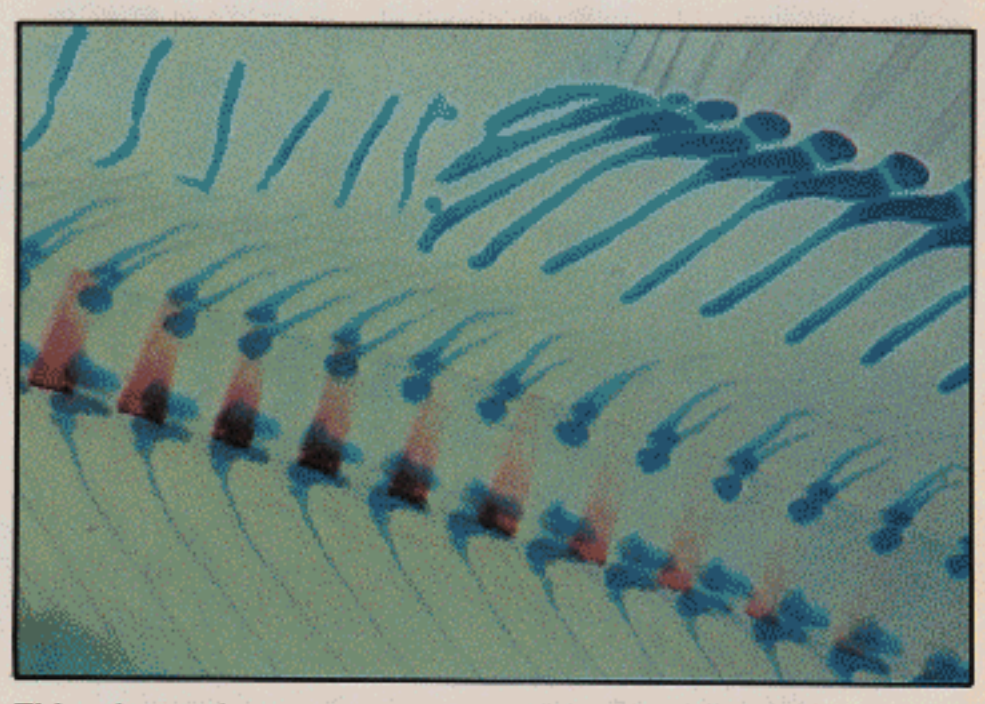
Fixation: Fix specimen in 10% neutral-buffered formalin for at least 24 hours.

Evisceration: Skin the specimen (may be omitted in the case of early embryos in which the skin has not formed). Remove eyes and viscera from body cavity.

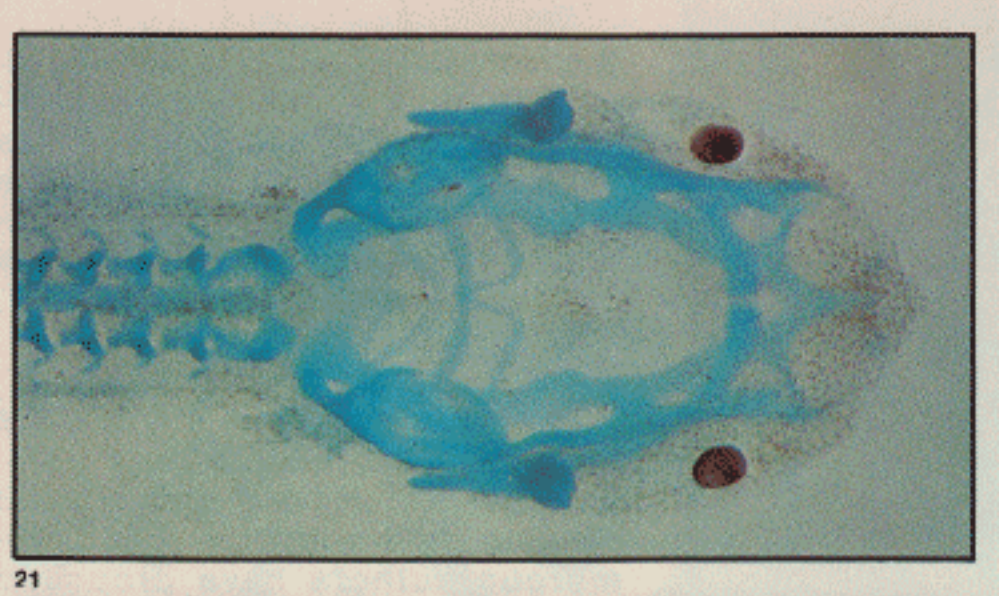
Washing: Wash specimen in sev-



Brook trout hatching is approximately one inch total length. Developing skull, fins, tail and vertical column (backbone) are all visible. In this specimen, the gut was not removed before staining. It is visible as the faint blue curving tube.



This photo shows the development of the vertebral column proceding as a ''wave'' of bone deposition beginning anteriorly near the head (left side of slide) and continuing towards the tail.



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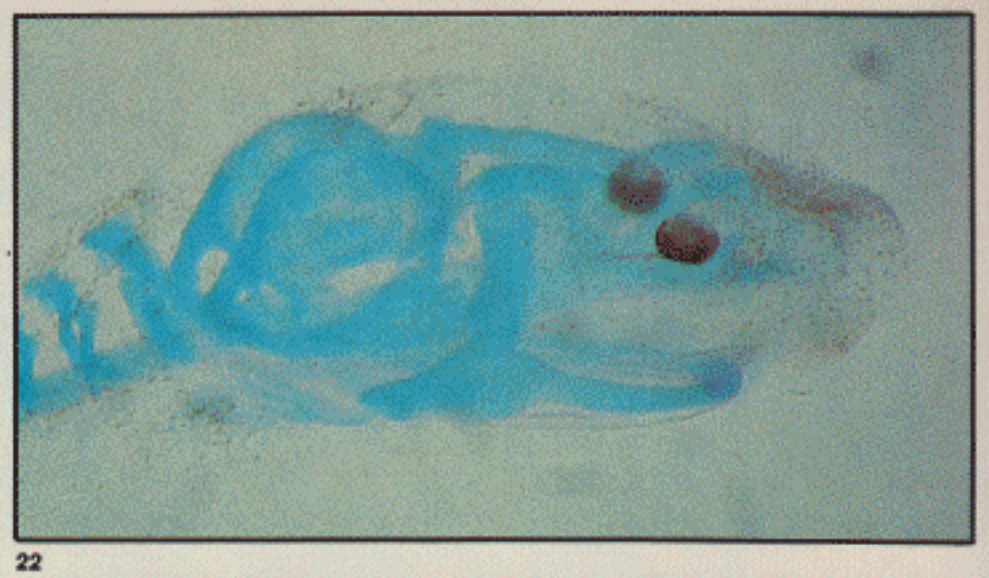

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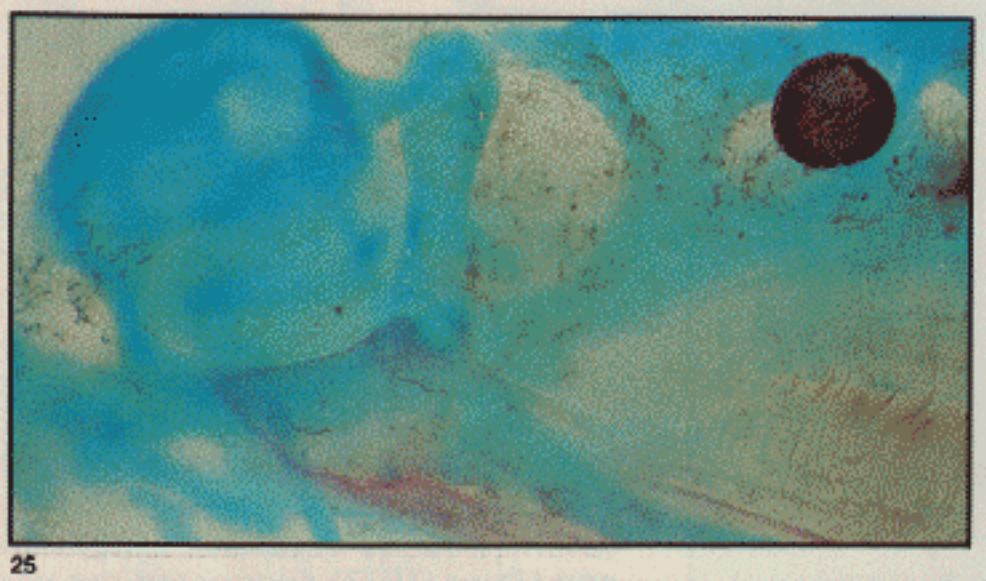

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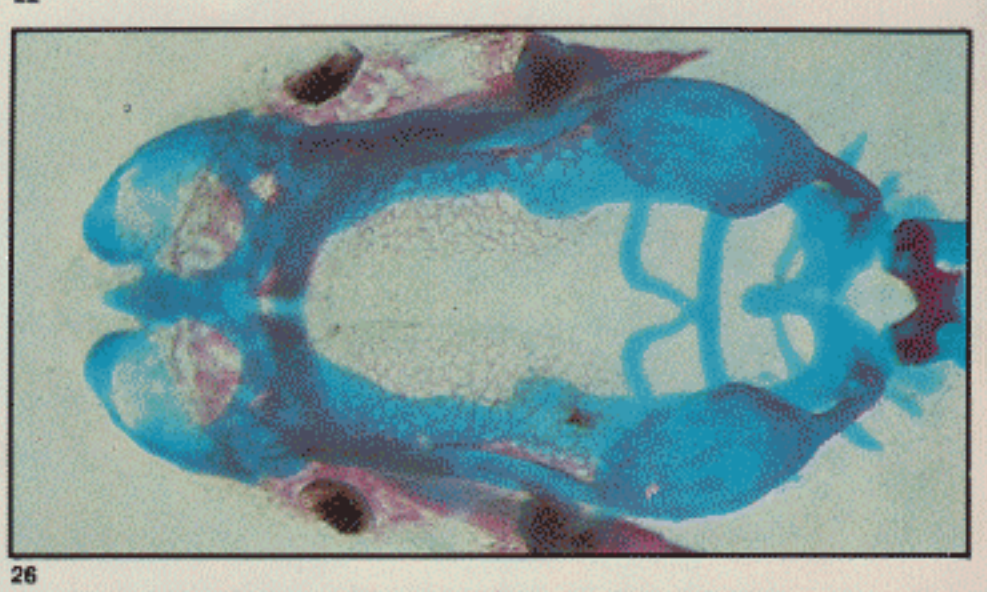

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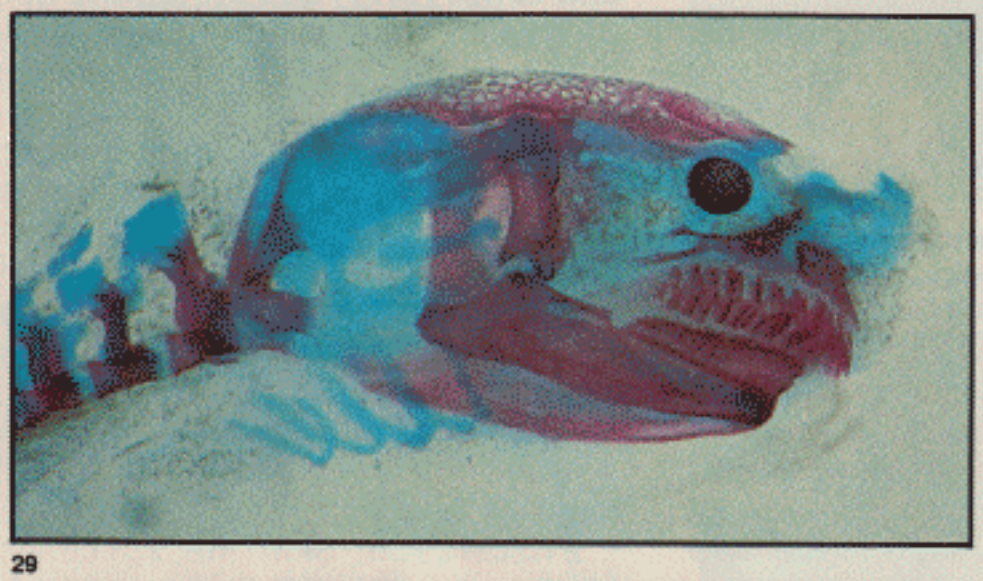

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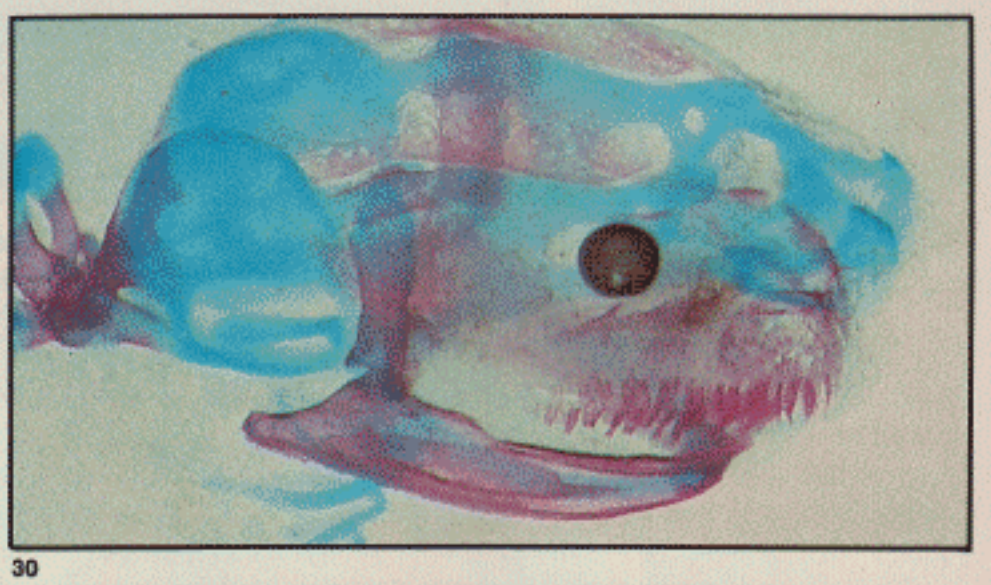

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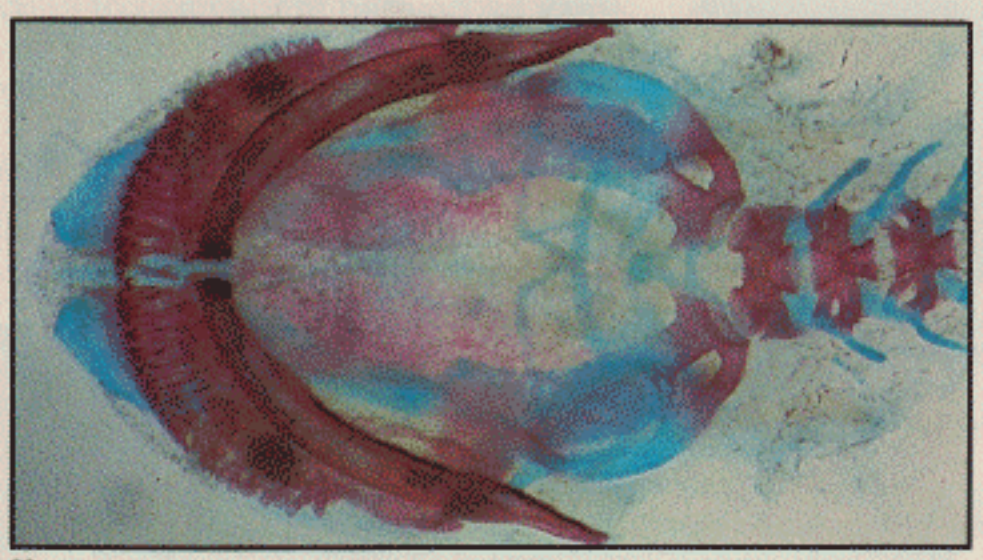

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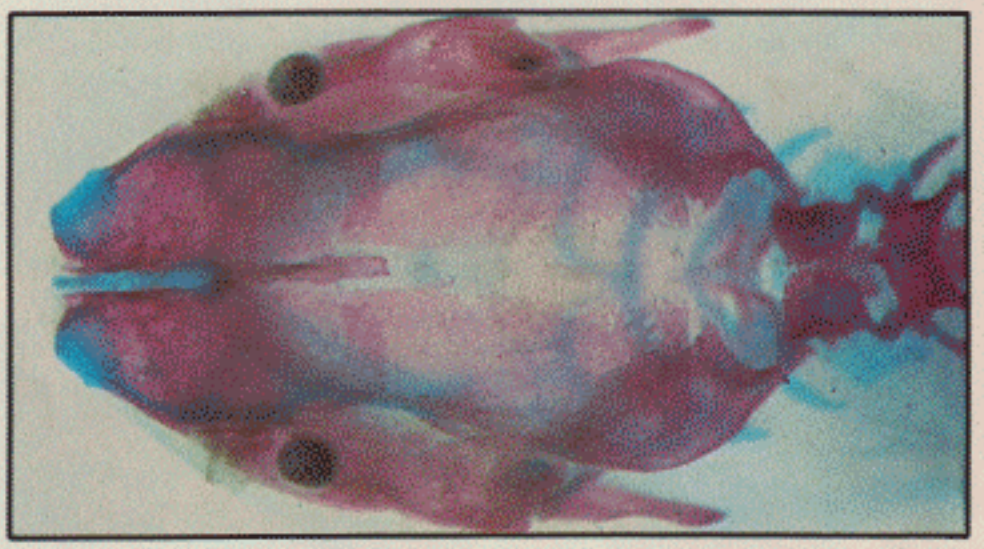

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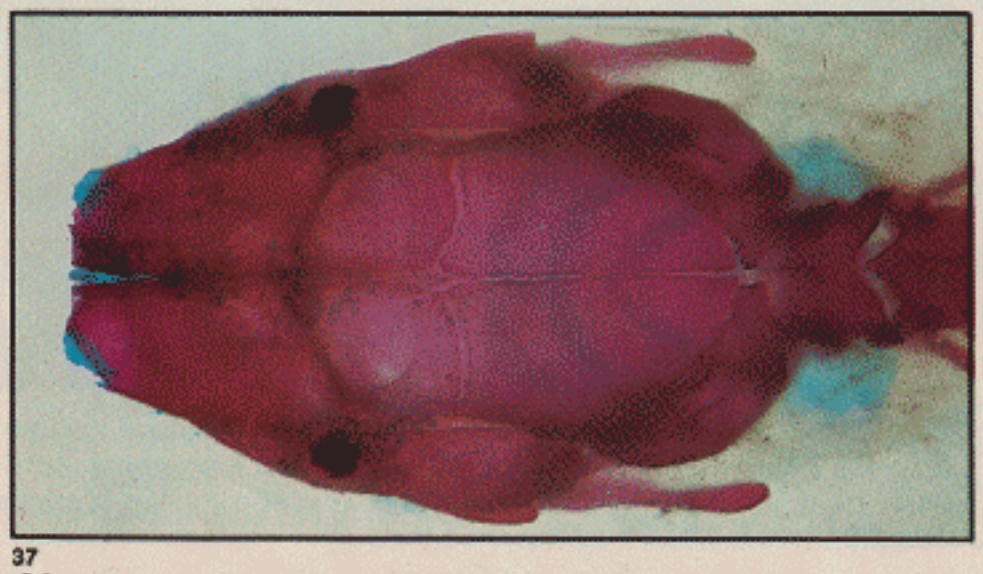

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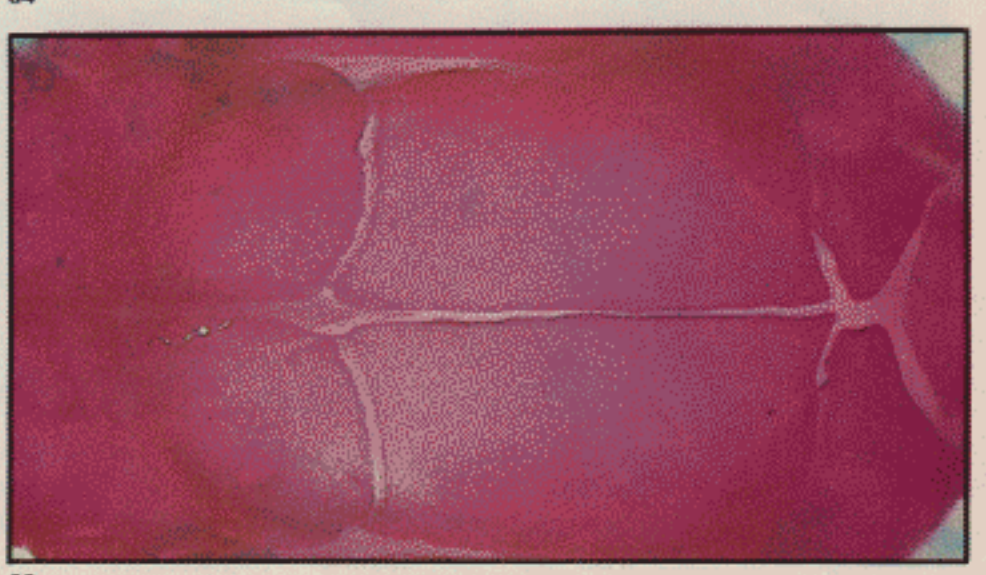

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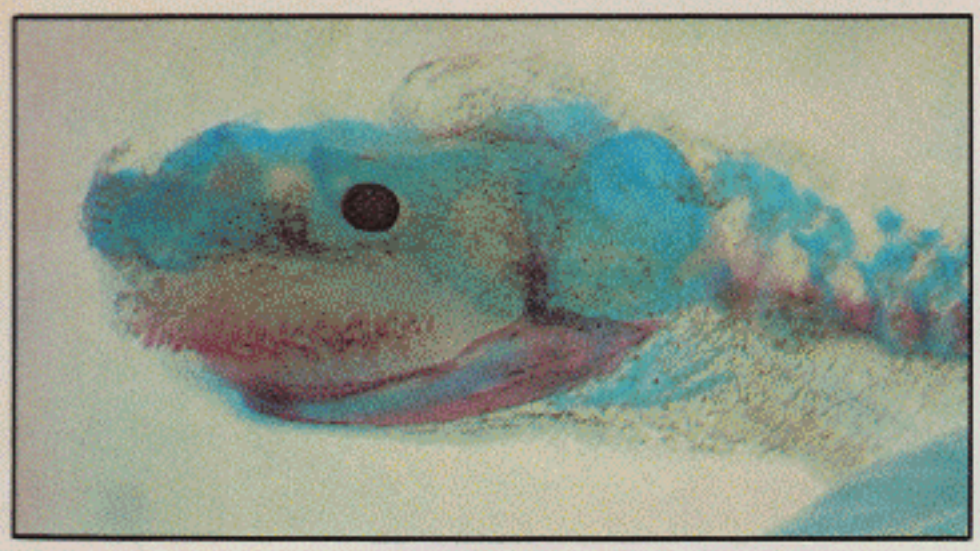

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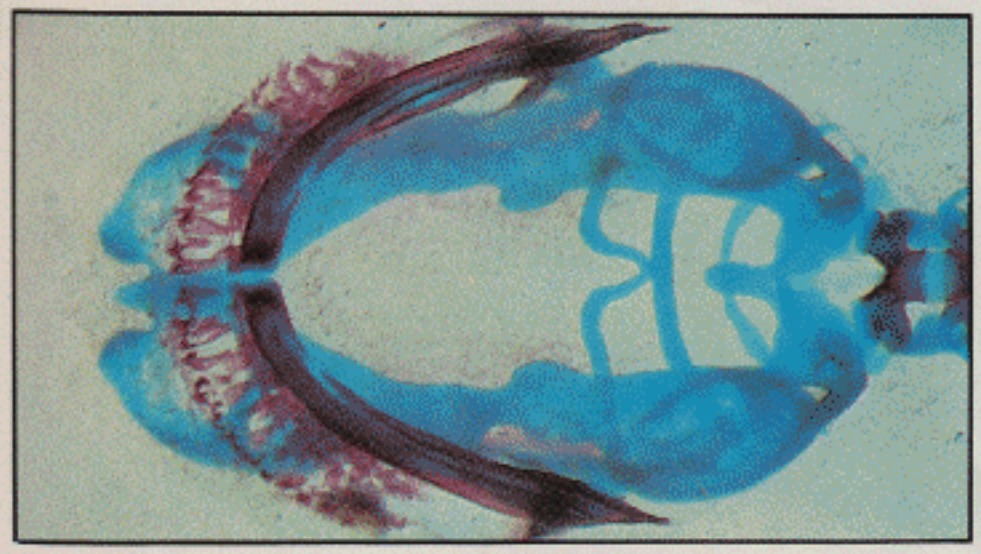

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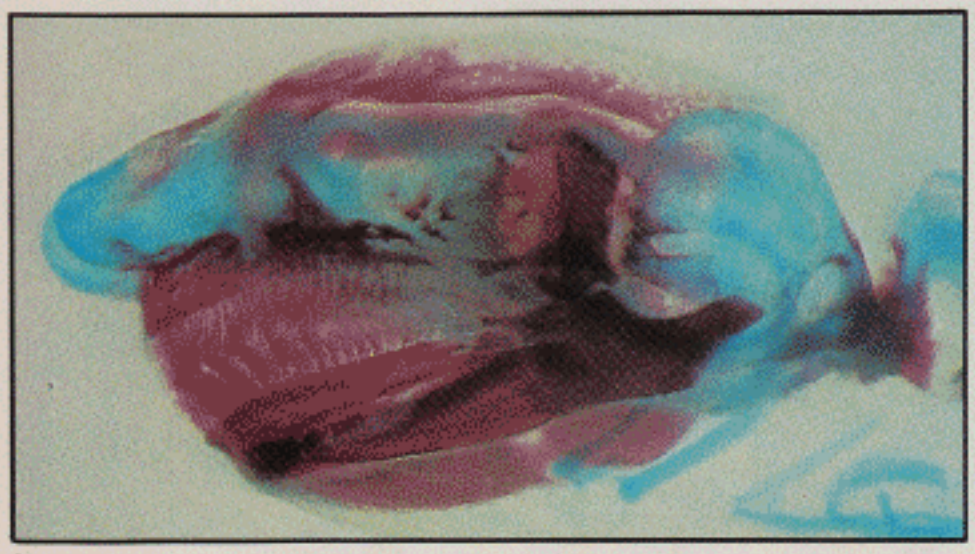

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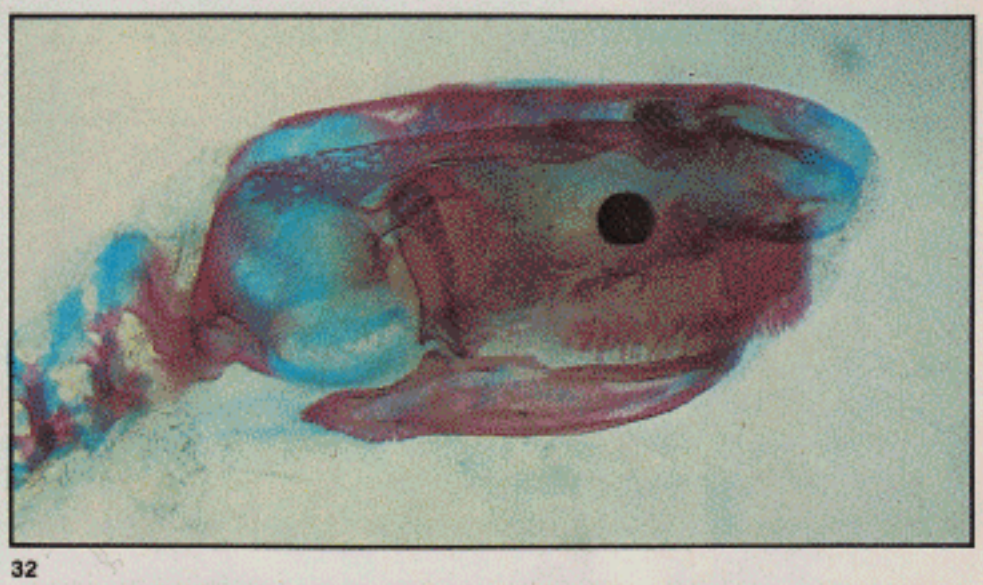

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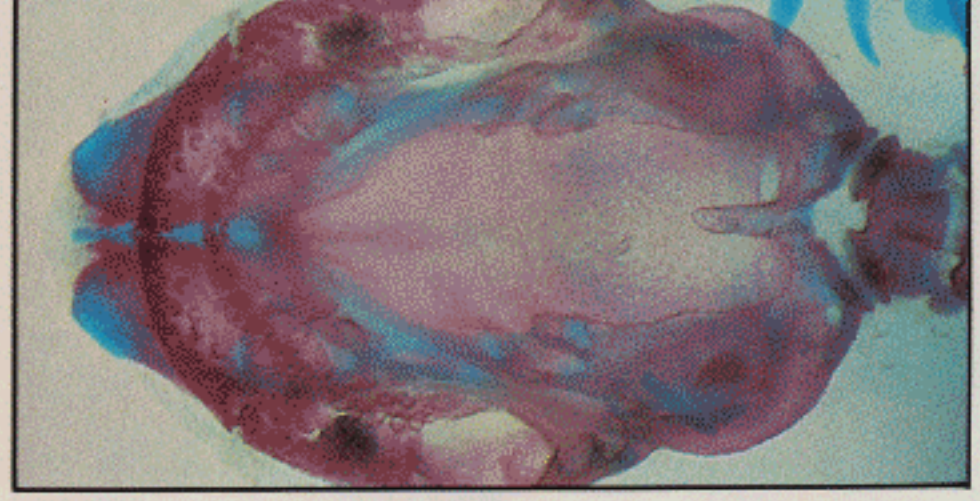

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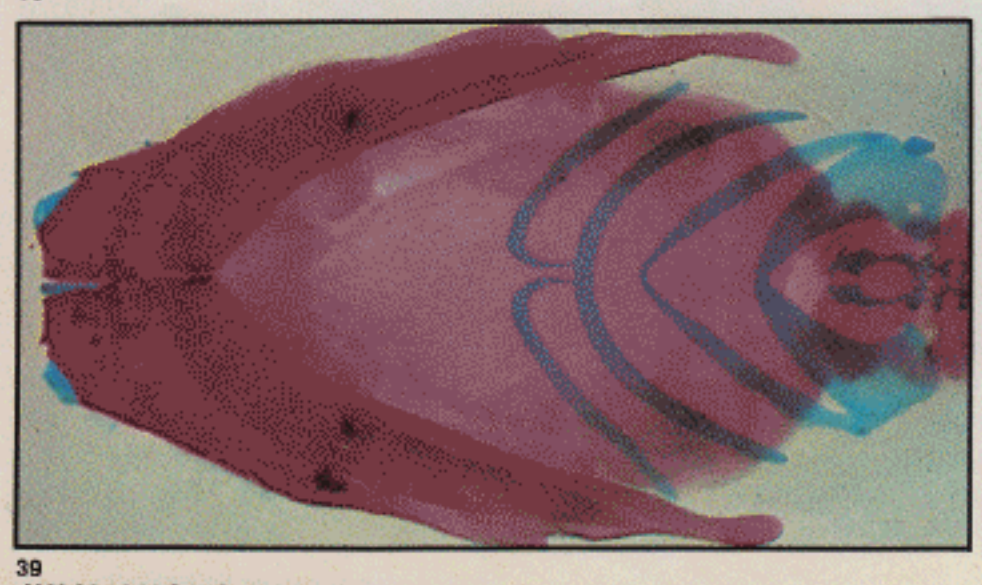

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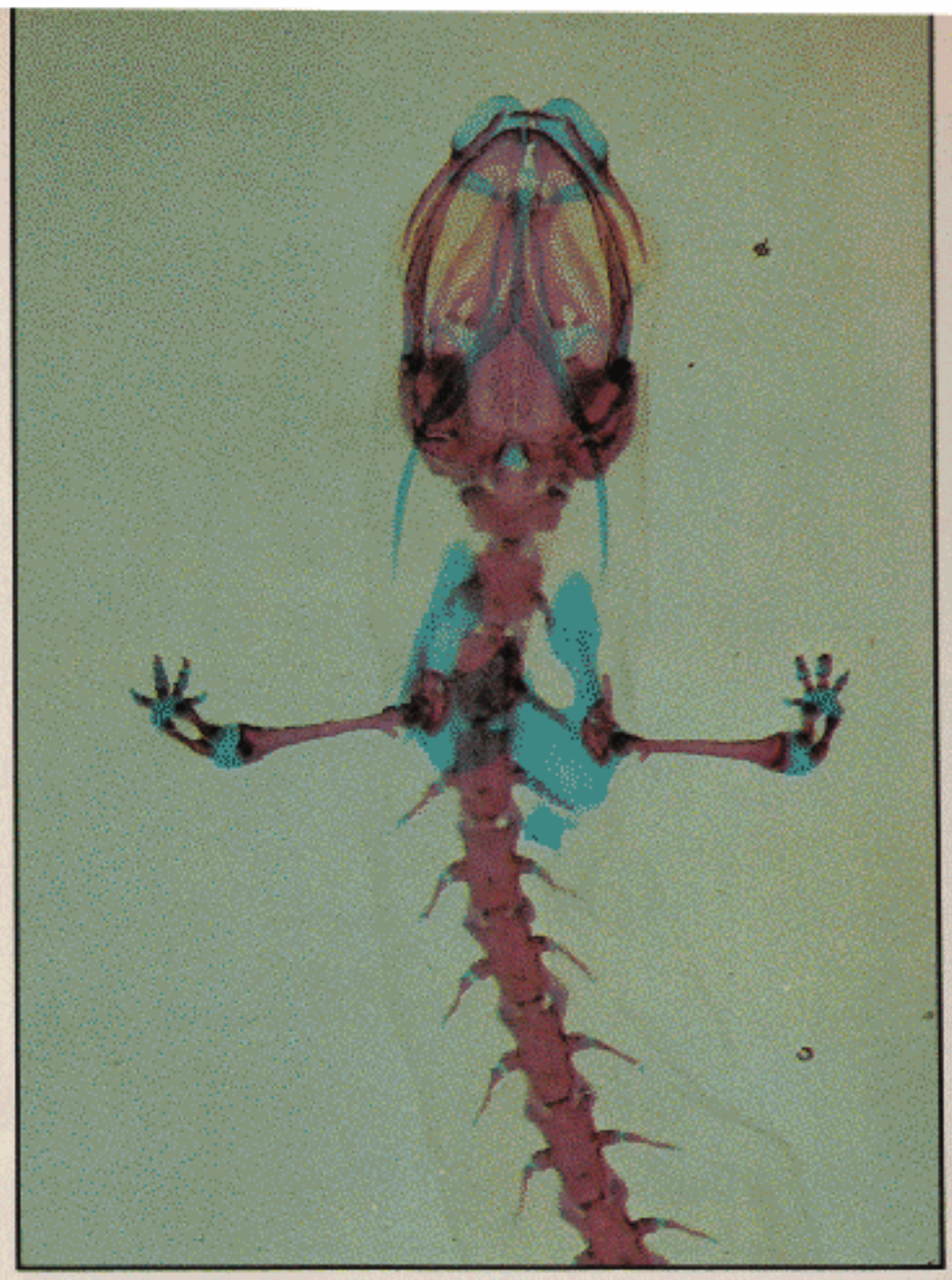
Caecilian (limbless amphibians) embryos. Photographs of a series of eight embryos and adults of increasing size that were used in a study of skull development performed by Dr. Marvalee H.Wake of the University of California at Berkeley and Dr. James Hanken. The eight specimens are represented by the following slides: 21-22; 23-25; 26-28; 29-30; 31; 32-33; 34-36; 37-39. The slides depict skull development beginning with the first structure to appear — the cartilaginous "chondrocranium" illustrated in #21-22 (in these specimens eyes are visible as black dots) — and continuing with increased ossification (bone development) until the nearly completely boney skull of the adult is reached (e.g., #37). However, the hyoid (tongue) skeleton is seen to remain cartilaginous throughout life, as is shown in slides 27, 28, 29, 36, and 39.

Adult salamander. Photo of a skull, including dorsal (top). The eyes (with lenses visible within), cartilaginous nasal capsules and hyoid skeleton, and bony skull are visible in most views.



The vertebral column, pectoral girdle, and forelimbs are shown here.

eral changes of distilled water for at least two days.

Cartilage staining: Place specimen in a solution of 10 mg alcian blue 8GX, 70 ml absolute ethanol, and 30 ml glacial acetic acid for 12-48 hours. Superficial cartilage will appear blue when specimen is stained. Do not leave the specimen in this solution longer than necessary as ossified tissues may begin to decalcify.

Dehydration: Place specimen in at least two successive baths of absolute alcohol, either ethanol or methanol. Leave in each bath for at least 24 hours. This dehydration will fix the alcian blue in cartilage and help de-stain surrounding soft tissues.

Maceration: Transfer through a graded, decreasing series of alcohol solutions (e.g., 75%, 50%, 25%) and then into two changes of distilled water, at least two hours in each step. Place in an enzyme solution of 1 g trypsin [e.g., Matheson Coleman, and Bell TX 1590 (1-100)], 30 ml saturated aqueous

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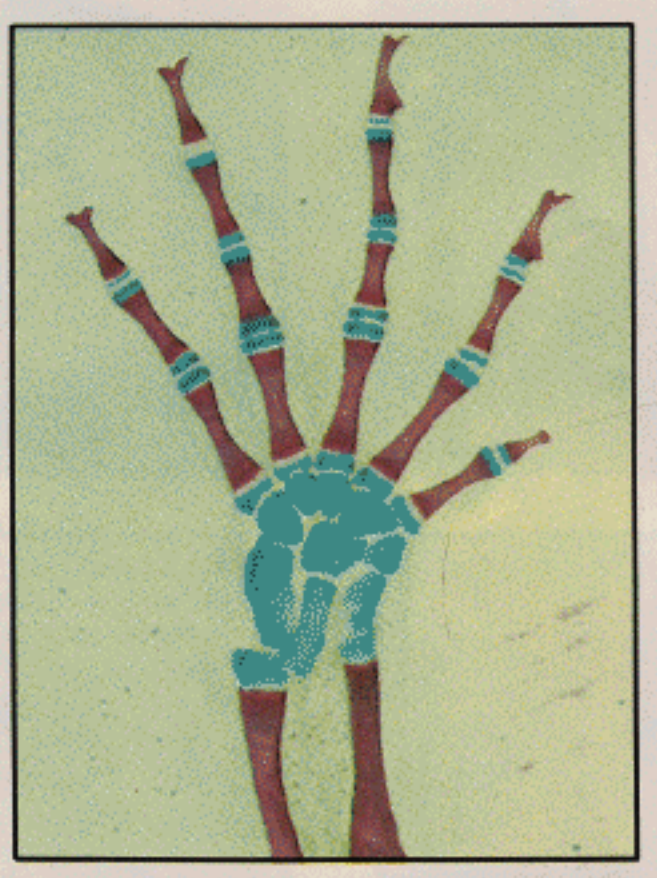
Aquatic salamander *(Siren intermedia).* Ventral view of underside of head showing the prominent hyoid (tongue) skeleton and the lower jaw with a cartilaginous core.



Adult salamander. Left rear foot. The cartilaginous ankle elements are clearly distinguishable from the bones of the lower leg (bottom) and the foot (top) which are mostly bone.

Adult salamander. Ventral view of hyoid (tongue) skeleton, and pectoral (shoulder) girdle at bottom of slide. Both structures contain boney and cartilaginous elements.

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sodium borate, and 70 ml distilled water. Leave here until soft tissues become transparent and fully destained, revealing underlying bone and stained cartilage. This step may require as little as a few hours to as many as several days, according to the size of the specimen. Alternatively, 0.5% potassium hydroxide (KOH) may be substituted for the enzyme solution.

Bone staining: Place specimen in a solution of 0.5% KOH to which enough alizarin red S stock solution (0.1% alizarin in water) has been added to turn the solution deep purple. Leave for 24 hours. When stained, bone will appear red to purple.

Clearing: Transfer to 100% glycerin via a graded series of glycerin-0.5% KOH solutions (e.g., 25%, 50%, 75%), 24 hours in each step. Pigmented internal fascia or unremoved skin may be bleached in the 25% solution by addition of a few drops of 3% hydrogen peroxide per 100 ml solution. Leave specimen in this solution until bleaching is complete.

Storage: Transfer specimen to fresh glycerin. A few crystals of phenol or thymol should be added to retard spoilage.

Modifications Possible

The above procedure should be used only as a guide, and can be modified according to need. We have found, however, that the sequence of steps is critical; for example, cartilage staining must precede bone staining. But the concentration of most solutions, and the time that a given specimen is left in each, may be varied to a large extent. While the times given for each step above may be approximately correct for a small frog or a mouse, larger specimens will require substantially more time. The concentration of the enzyme solution of KOH during maceration should be increased in such instances. The volume of each solution in the staining procedure should be at least ten times that of the specimen. Specimens should be drained and blotted when moved between solutions, but avoid excessive handling of specimens, especially in later stages when specimens are soft and fragile.

Photographing small specimens requires few additional procedures from those standard for low magnification photomicrography. First, for correct rendition of color the proper film-light source combination should be used. We prefer using Kodak Ektachrome 160 Tungsten film (ASA 160) with a tungsten lamp set at approximately 8 volts. Unfortunately, voltage dial settings on many microscope lamps are poorly calibrated; an initial test roll may be needed to determine the correct voltage setting for any given microscope and lamp.

One lamp will suffice to illuminate most specimens, although additional lamps may be used as is necessary. Arrange the lamp above the specimen and sufficiently distant from the optical axis between the specimen and microscope objective that the specimen's shadow falls out of the field of view (this may require elevating the specimen slightly above the microscope stage). The specimen should be fully immersed in glycerin to avoid glare and refractive effects, but use no more glycerin than is required to cover the specimen. Also, avoid warming the glycerin with the microscope lamp, as this will create convection currents in the viscous glycerin that will distort the specimen when viewed from above.

Differential staining of bone and cartilage in whole, intact specimens provides valuable information that may be used in a variety of disciplines that are concerned with both normal and abnormal skeletel development. These include comparative anatomy, experimental embryology, teratology, pharmacology, evolutionary biology, and taxonomy. We have used it to obtain estimates of skeletal variation in natural populations, to document the sequence and nature of skull development from embryonic cartilaginous chondrocranium to fully ossified adult skull, and in analyses of the evolution and functional morphology of feeding and respiratory structures of vertebrates. When combined with proper photographic techniques, it provides an effective means of illustrating the details of skeletal formation and morphology that has wide application in the fields of biological and medical education and research. □

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Abstracts

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quartz-controlled LCD/LED devices to record clock time and calendar date, but no other indications. To permit freer recording of any letter, symbol or number combinations, D. M. Harvey in the November 1980 issue of *Research Digest* proposes a device involving an electrical contact grid and an LED grid. The contact grid might have an array of say 10 x 20 (or more) contacts linked to a similar array of 10 x 20 LEDs. Tracing out figures or words on the contact grid causes the corresponding LEDs to light; that lighted image is projected into a recording area on the film through the back of the camera.

Information content would no doubt depend on the number of elements in the contact grid and LED grid. It should not however be difficult to extend the capacity to cover the amount of information likely to be required for recording in such situations.

From: BRITISH JOURNAL of PHOTOGRAPHY

The increasing publicity attention that the *Nimslo 3-D print* process is attracting has induced Charles W. Smith to research the patents by Nims and Lo. As listed in the January 30, 1981 issue of the *British Journal of Photography*, the Nimslo process is described in some 14 US patents between 1974 and 1979 and some eight British patents granted between 1976 and 1980. Most of these appear to cover the multi-image technique with movable cameras or lenses which has now been abandoned in favor of the Nimslo camera with four fixed lenses. □