Chang's conjunctiva and Henle's intestine 407, both derived from normal individuals, are indistinguishable cytologically from known tumor cell lines (*5*).

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## X-ray Microscopy of Veins of the Skull

The projection x-ray microscope at the University of Redlands is being successfully applied to the study of the distribution and communications of the veins of the diploë in the dog, an x-ray micrograph of which is shown in Fig. 1.

This type of x-ray microscope uses a point source of x-rays less than 1 μ in diameter to cast an enlarged shadow image on a photographic plate. The small source of x-rays is produced by focusing a beam of electrons with two magnetic lenses so that the point source of electrons strikes a window target in the vacuum wall of the x-ray tube. The specimen is kept at atmospheric pressure and yet can be placed within a few microns of the source of x-rays, so that high x-ray magnification can be obtained with the photographic plate or fluorescent screen only a few millimeters away.

The penetrating power of x-rays produces contrast in the image from internal detail of thick specimens, both from natural density differences and from injected radio-opaque material. In addition, the shadow-projection method of x-ray image formation produces an image of all planes of the specimen in focus at once. This makes possible stereoscopic views of the internal detail of a specimen, whereby the orientation within the object is shown in a three-dimensional relationship. A recent review article on x-ray microscopy compares all methods used and lists 102 references to the literature on the subject (*1*). Specific details of design and operation of x-ray microscopes can be found therein.

The veins of the diploë are difficult to expose and to visualize by the usual methods of serial sectioning and graphic reconstruction. Projection x-ray microscopy (*2*) that has been recently developed by Nixon and Cosslett (*3*) overcomes many of these difficulties.

To prepare the skull of each dog for study after the animals were sacrificed, the head was perfused through the carotid arteries with physiologic saline to flush the blood from all blood vessels. This was followed by perfusion with red vinyl plastic. To prevent confusion in observing arterioles and venuoles, blue vinyl plastic was injected into the veins by way of the external jugulars. The soft tissues were cut away from the skull, and the floor of the skull was opened to allow for removal of the brain. This procedure allowed the dura mater to remain intact and adherent to the brain case.

Fixation of the skull was achieved by placing it in 10-percent formalin. The skull was mapped into parts and cut, each part being about 1 in.[2] The skull parts were decalcified by using 5-percent nitric acid and were dehydrated by being passed through 15-percent, 40-percent, and 75-percent isopropyl alcohol solutions. The parts were kept in 75-percent isopropyl alcohol until they were x-ray photographed.

Figure 1 shows the plastic-filled veins of the diploë of the dog in longitudinal parallel formation as they lie between the inner and outer tables of the skull. The x-ray micrograph demonstrates the communications of the smaller diploic veins as branches of the large diploic vein at the top of the micrograph as they approach the center of the skull and connect with the superior sagittal sinus of the dura mater (not shown). In the background, trabeculae, which make up the porous structure of the diploë, appear as light lines, whereas spaces caused by osteoclastic activity are dark.

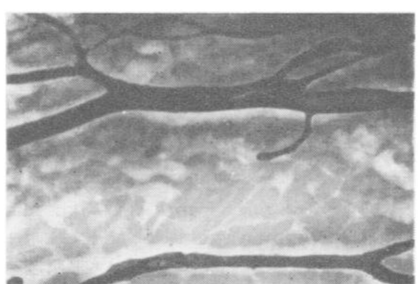

Fig. 1. X-ray micrograph showing diploic veins of the dog skull. Five-minute exposure on Eastman lantern plate contrast emulsion; 12-kv, 25-μa beam current; University of Redlands x-ray microscope (×3.5).

The normal methods of preparation for study and graphic representation of this tissue by light microscopy would have taken at least 25 hours, whereas the same information has been obtained with a single 5-minute x-ray micrograph. A detailed study of the entire calvarium of the dog by light microscopy would take several months, but the same information could be obtained in a few 5-minute exposures using the x-ray microscope.

Future studies are proposed, which will include x-ray micrographic representation of the veins of the diploë in skulls of man and monkey. From the observations made thus far, there appears to be a characteristic difference between the distribution of diploic veins in the skull of the dog and that of man.

Other applications of the x-ray microscope have been made with striking results. Engstrom, Bellman, and Engfeldt (*4*) have used contact microradiography on living tissues. Bohatirchuk (*5*) was successful in studying the aging of the vertebral column with this method of microradiography. X-ray micrographs have been made of the kidney by Nixon and studies of gallstones and kidney stones have been forecast.

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6. This work was done while W. C. Nixon was visiting research associate at the University of Redlands under a National Science Foundation grant.

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*We must never forget that metaphysics divides people, and science unites them.*
—Philipp Frank.