Incident Dark-Field Illumination: a New Method for Microcirculatory Study

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INCIDENT DARK-FIELD ILLUMINATION: A NEW METHOD FOR MICROCIRCULATORY STUDY

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INTRODUCTION

Classically the microcirculation of semi-transparent tissues such as the mesentery and lung have been studied by transillumination with light from a water cooled quartz rod and a standard microscopic lens system.[1-4] In the past several years some use has been made of incident light directed at an oblique angle to the subject.[5] Both of these methods require careful alignment of the light source and the microscopic lens system.

During the past decade the growing fields of metallurgy and petrology have stimulated the development of special microscopes which do not require transillumination of the subject for its visualization. More recently these optics have been widely employed for quality control in the production of electronic microcircuitry.

In a casual conversation one of the authors (W.A.C.) was told of the use of these optics in plant biology. The possibility of their application to in vivo microcirculation of animal tissues suggested itself. The initial studies were directed at the semi-transparent lung and mesentery and subsequent studies included solid organs. We have now observed all major organs.

METHOD

Our basic microscope body is the Wild-Heerbrugge M-50 sub-stage metallurigcal microscope. This is fitted with a set of Wild Universal Epi-achromat objective lenses.* The system allows for delivery of light through the microscope body and the Epi-objective lens system. This incident light can be delivered as either bright-field or dark-field illumination by means of a simple push-rod attachment on the microscope body. We have found the incident dark-field illumination to be best for microcirculatory studies. (Figure 1) This light is delivered through a circular prismatic lens surrounding the objective lens which throws a halo of light around and beyond the objective focal point. (Figure 2) This illumination gives an unusual depth of field and a three-dimensional quality to the tissue observed. Most important it permits visualization of tissues beneath the surface of most organs. Our Epi-achromat objectives are 4×, 10×, and 20× magnification.

We have used two types of illumination. One is a low voltage tungsten lamp

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* Supplied by Eric Sobotka Co., Inc., Farmingdale, New York.

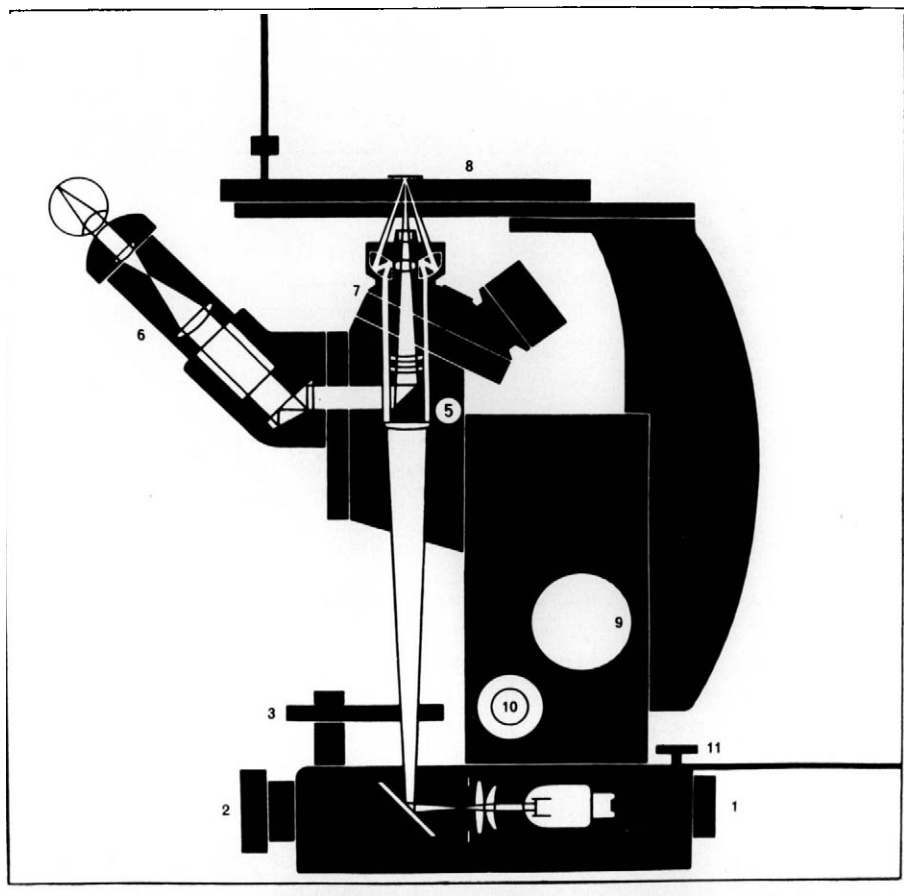


FIG. 1. The substage metallurgical microscope fitted with Epi-achromat objective lenses. Light path in dark-field mode is shown with tungsten filament lamp.

housed in the microscope base which is adequate for all routine observation. This lamp is shown in Figure 1. For high-speed color cinematographic studies we utilize a xenon, high-pressure burner which supplies light at 6000° Kelvin, and direct the light into the microscope body with a swing-out mirror. (Figure 3) The light is first passed through ultraviolet, and heat absorption filters, and a didymium contrast filter which is particularly good for blood.

As the image returns to the microscope body it is magnified an additional 1.5 times. Then 25% of the light goes to a binocular tube fitted with 10× wide-field eye pieces. The remaining 75% of the image light enters a phototube and passes through a photo eyepiece of either 6×, 10×, or 15× magnification. It then impinges on a prism which transmits either 5% or 50% of the light to a focusing telescope for field compositon and critical focusing. The remaining light passes through a special infinite focus lens into a Bolex 16mm cine camera body

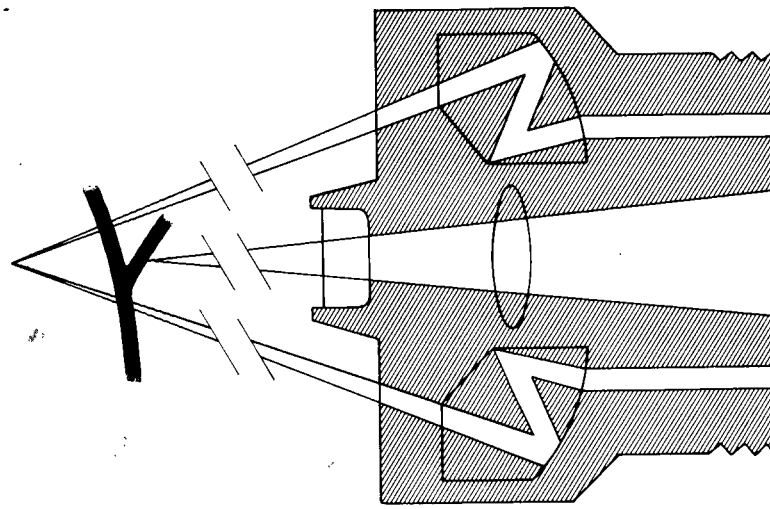


FIG. 2. A magnified view of light path through Epi-achromat lens showing the way light in the dark-field mode surrounds the vessel under study.

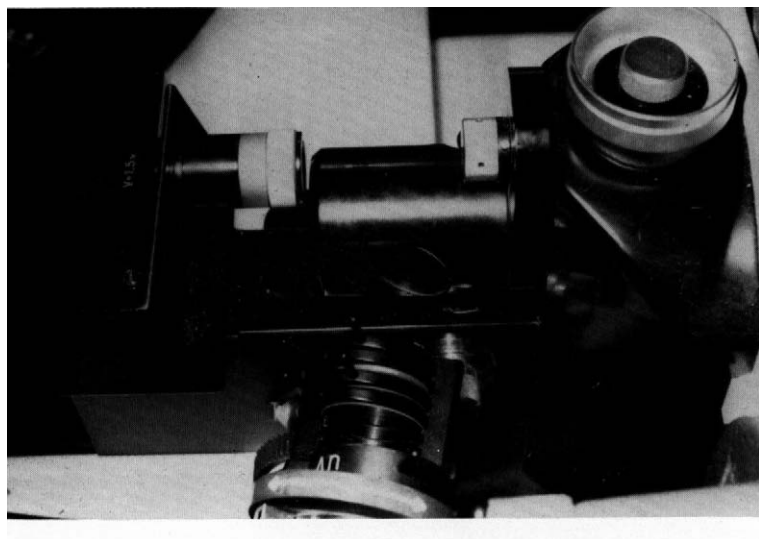


FIG. 3. This view shows the filters on the Xenon burner and the mirror which directs the light into the microscope body. This mirror swings away to allow light from the tungsten lamp to enter the microscope from its source in the microscope base.

equipped with battery powered motor film drive. (Figure 4) At the film plane our combinations of optics give us a magnification range of 36× to 450×. We usually work at 90× to 225× magnification. At this magnification we have adequate light to film at speeds up to 64 frames per second with Type A Kodachrome II.

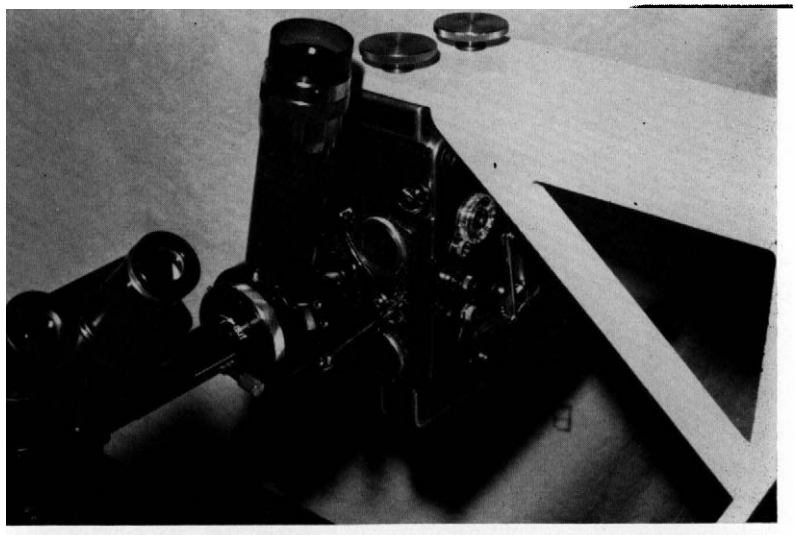


FIG. 4. Binocular eyepieces, photo tube, and cine camera assembly are shown. The phototube is used to obtain critical focus and composition of the field for photography. It also contains a light meter and a method of superimposing numbers on the field.

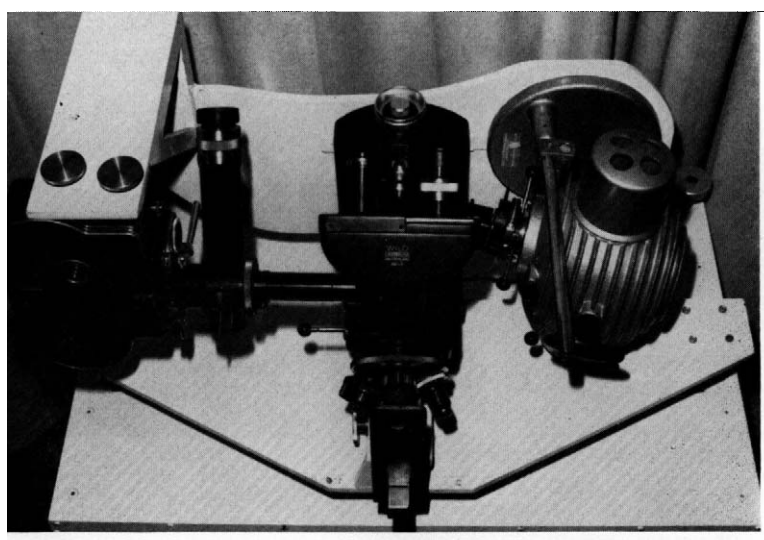
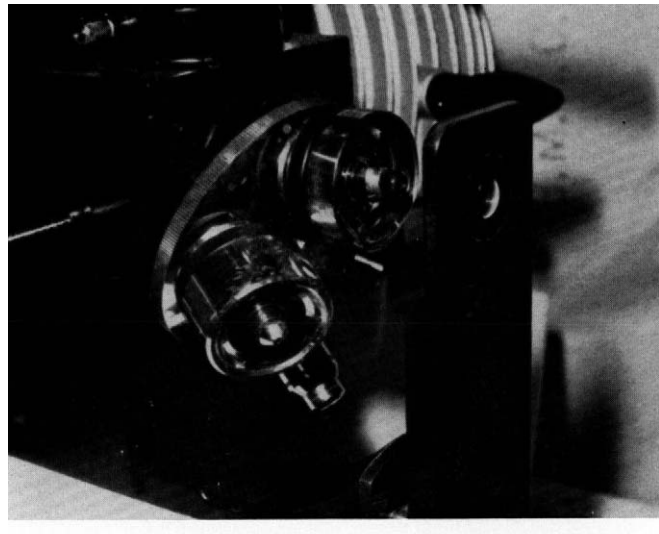


FIG. 5. A view from above of the entire assembly showing camera, phototube, microscope and light source mounted on special cart. Adjustment of the coarse or fine focus of the microscope moves the entire assembly horizontally. Vertical adjustment is made by a worm gear beneath the triangular base plate. The base of the cabinet contains transformers for the light sources and a galvanometric light meter.



Epi-achromat objectives are shown with the pylon containing a co

FIG. 6. The Epi-achromat objectives are shown with the pylon containing a cover slip to which the tissue is approximated. The pylon is fixed, the lenses movable by focus of the microscope.

This entire system is mounted on a specially constructed cart* in such a manner that the microscope observes the microcirculation from the horizontal aspect. It is also so arranged that height can be adjusted accurately with a worm gear and the entire optical system moves horizontally with the microscope when its coarse and fine adjustment are altered. (Figure 5)

A pylon containing a thin glass cover-slip is used to stabilize the organ being observed and to keep it plane to an axis perpendicular to the lens system. (Figure 6) All examinations were carried out in the cat which is easy to house and manage; and has a circulatory system similar to man.

RESULTS

Thus far we have visualized and photographed the circulation of the brain, lung, kidney, liver, mesentery and intestine successfully; and the spleen unsuccessfully. We attribute the failure of our spleen study to the thick, dense splenic capsule that prevented the light from penetrating the spleen's surface. Although some vessels can be seen beneath the capsule, focus on their plane is not possible and visualization of the flow is like watching water flowing beneath the ice of a frozen stream. It cannot be photographed. The remainder of the splanchnic vascular bed is easily visualized. Mesenteric flow has been under study for many years by standard methods. However, incident dark-field illumination is more satisfactory for the various portions of the gut. In the intestine, for

* Developed by the Ippolito Manufacturing Co., Bronx, New York.

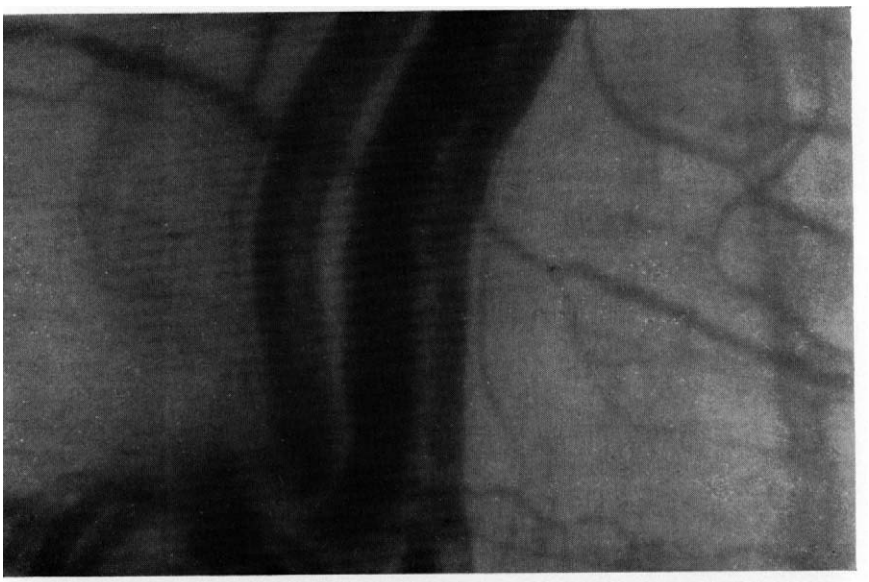


FIG. 7. Jejunum showing mesenteric artery and vein and capillaries

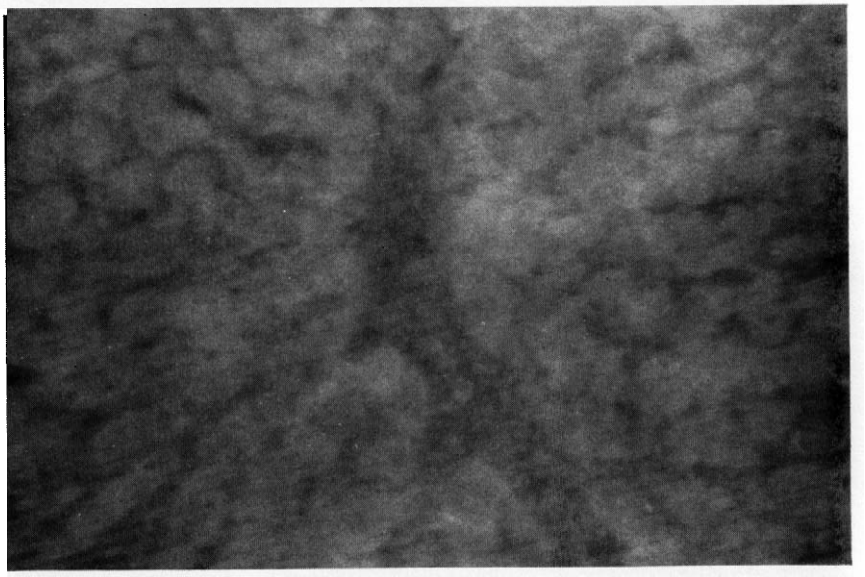


FIG. 8. Liver with arterioles emerging from the interior at darker points, dividing into capillaries around liver cords and draining into a central vein at center.

example, it is possible to study the circulation to the serosa and deeper layers simultaneously. (Figure 7) The surface circulation of the liver is well visualized and can best be characterized by reference to hepatic anatomy. An arteriole will be seen rising to the surface where it breaks up into a starburst of radiating

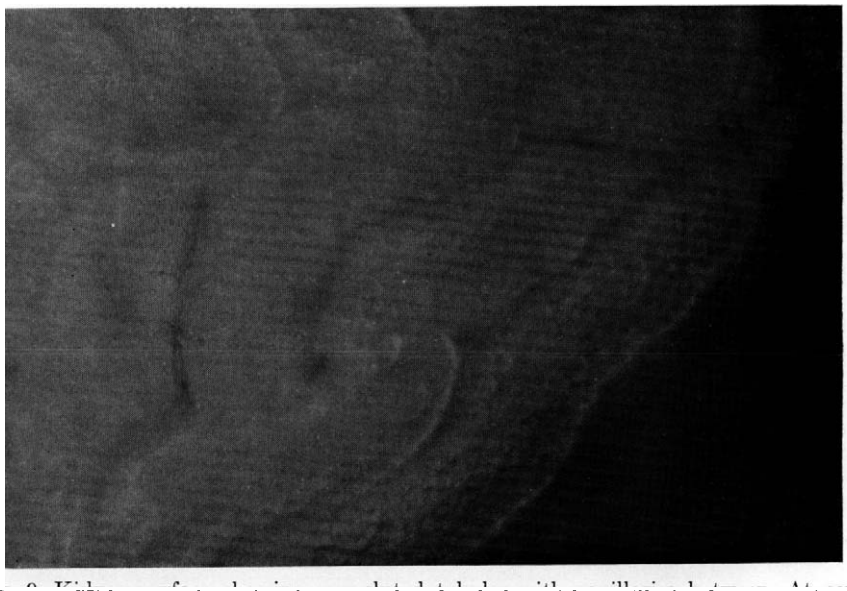


FIG. 9. Kidney surface showing convoluted tubules with capillaries between. At center an arteriole emerges from the cortex as a dark spot and after passing around the tubules empties into a large stellate vein at lower right.

vessels that surround liver cords, then congregate at a central vein and disappear back into the depths of the organ. (Figure 8)

Study of the kidney is more interesting because the renal tubules can be visualized as well as the vessels. This requires very careful removal of the capsule, however, and we are uncertain that this removal leaves the circulation in its normal state. In any case the arterioles can be seen emerging from the depths and breaking into capillaries which follow a tortuous course around the tubules as they lie convoluted upon the renal surface. Then the capillaries rejoin each other and are gathered together in large stellate veins which lie well exposed on the surface. (Figure 9) We have not as yet been successful in visualizing a glomerulus with its circulation. They may lie too deep in the renal parenchyma for the optics to penetrate.

The lung has provided by far the most dramatic visual and photographic studies with this method. Because of its transparency the three dimensional effect of the lighting is most pronounced, and several generations of vascular branching can be visualized. In addition, the expansion and contraction of the alveoli with ventilation is seen and the effects of these pressure changes on the blood flow is observable. Arteries give rise to pre-capillary arterioles which, in turn, divide into capillaries that cover the alveolar surface. The length of these capillaries is quite variable before they join together to form the pulmonary venules and veins. (Figure 10) In fact our observations have shown that some arterioles empty directly into venules with no real division into capillaries. Under conditions of reduced flow the longer capillaries which surround the

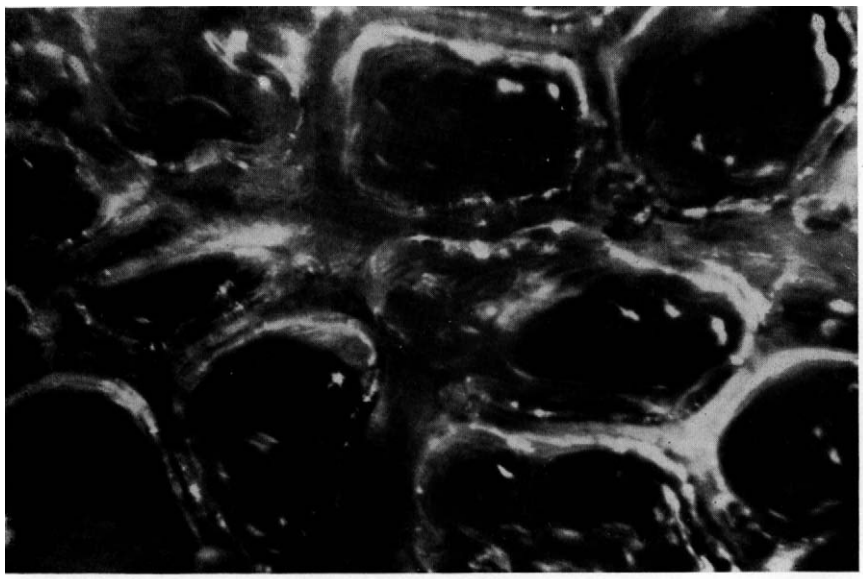


FIG. 10. Pulmonary alveoli surrounded by capillaries. At top center an arteriole enters and at the center bottom a venule collects capillary flow.

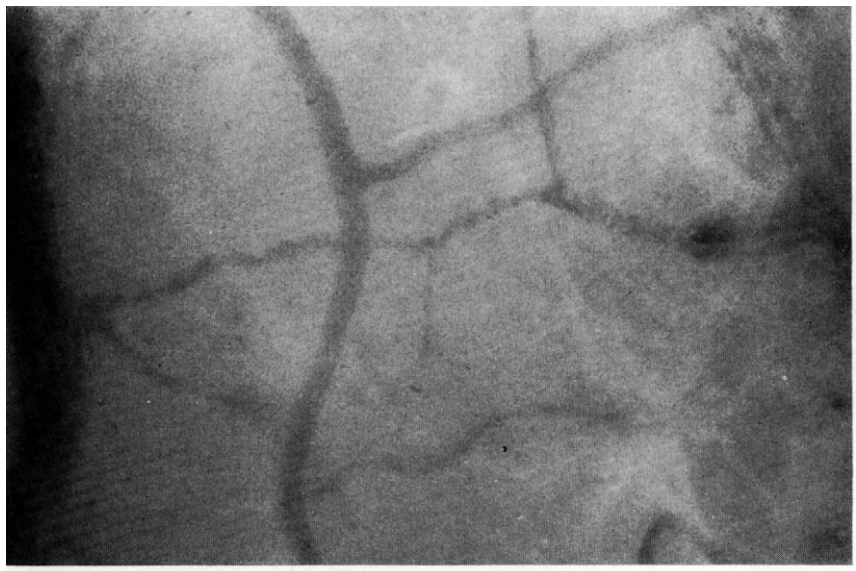


FIG. 11. Brain with arteriole on right, a capillary, with red cells, visible across the center and emptying into a venule at the left.

alveoli stop perfusing first while the short arteriole-venule pathways continue to flow rapidly. We suspect this is the basis for the apparent arterio-venous "shunt" effect found in various low-flow states by blood gas studies. Another interesting initial observation is that the flow through the smallest vessels of the lung seems to be oscillatory. This oscillation is related to heartbeat. It is distinct from an additional cyclic change in rate of flow which follows the expansion and contraction of the alveoli with ventilation.

Lastly, we have removed the calvarium and dura and exposed the vessels on the surface of the brain. The transparency of these tissues is also very suitable for microcirculatory studies by incident dark-field illumination. Small arterioles are seen on end as they rise to the surface. These divide, branch into capillaries, course over the gyri of the brain, and anastamose into veins of progressively larger size lying in the sulci. (Figure 11)

CONCLUSION

Incident dark-field illumination delivered through the optics of the observing lens provides a new method for study of the microcirculation. Due to the relative transparency of the covering of most organs it is possible to look into the organ and observe the arterial, capillary, and venous components of their circulation. This method has distinct advantages over the earlier methods of microcirculatory study which required transillumination and therefore made solid organ study difficult or unsatisfactory. Projection of the light to surround the observed vessels on all sides provides a three dimensional effect and eliminates the problem of alignment of the light source. This makes it possible to obtain better resolution and to study moving organs, such as the lung, to better advantage. We feel this tool should be helpful to those wishing to study the microcirculation of the living animal in greater detail, particularly the solid organs.

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