Study of the retinal circulation

I. Effect of light

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Our knowledge of the retinal vascular development, structure, and response to various stimuli has increased greatly in recent years. Relatively little is known, however, of the relationship between retinal blood flow and a possible increase in metabolic activity induced by illumination.

We have found only a single published study on the effect of light on the retinal vasculature *in vivo*. By utilizing a scleral window technique, Friedman, Smith, and Kuwabara (1964) studied the retinal circulation under high magnification. They did not detect any change in vessel calibre in response to intraocular illumination by white, infrared, or ultraviolet light. Since the retinal microcirculation can be demonstrated *in vivo* only by means of a microscope with some form of illumination, the effect of retinal illumination may be masked by the viewing light of the microscope.

In this study we have employed a red blood cell staining technique to compare *in vitro* the effects of light-stimulation and dark adaptation on the quantity and distribution of these cells within the retinal vessels.

Material and Methods

GROUP I (CONTROL)

The eyes of eight adult Long-Evans rats (200–250 g.) were removed either immediately after decapitation or under pentobarbital sodium anaesthesia. In four rats one eye had been dilated with Mydriacyl 1 per cent. The enucleations were performed with minimal manipulation, and with the heads maintained in a level position. After several days' fixation in 10 per cent. formalin, the globes were opened, and the retinae were dissected free and stained for haemoglobin by Doherty's modification (Doherty, Suh, and Alexander, 1938) of Pickworth's benzidine-peroxide method (Pickworth, 1934). The retinae were then flat-mounted, either without further processing or after being dehydrated and cleared with alcohol and xylene. Clearing was done on the slide.

GROUP II

Eight rats, in five of which both eyes had been dilated with Mydriacyl 1 per cent. were anaesthetized with ether. A unilateral tarsorrhaphy was performed, and an opaque patch sewn over the closed lids. The animals were then immobilized in a nonconstricting holder, and upon awakening, they were dark-adapted for one hour; the uncovered eye was then exposed for 15 minutes to an ordinary unfocused high-intensity light source at a distance of 2 in. (4.07×10^3 cd/m.²). The rats were then decapitated and the eyes enucleated and processed as before. The covered eyes were first enucleated in half the rats, and the uncovered eyes in the remainder.

Received for publication August 27, 1969

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This work was supported by Grant No. NB-05-059-06, National Institute of Neurological Diseases and Blindness, National Institutes of Health, U.S.A.

GROUP III

Six rats were anaesthetized and unilateral tarsorrhaphy and patching were performed as in Group II. After dark adaptation they were exposed to room light (5.04×10^1 cd/m.²) for 30 min. and then decapitated. The eyes were enucleated and processed as above.

GROUP IV

Five rats, in which both eyes had been dilated with Mydriacyl 1 per cent. were anaesthetized and unilateral tarsorrhaphy and patching were performed as in Group II. After dark adaptation, they were subjected for 30 sec. to a strobe light at a distance of 6 in. (6.8×10^7 cd/m.²), flickering at the rate of twenty times per second. They were then decapitated and the eyes enucleated and further treated as in Group II.

GROUP V

Seven adult cats (2.5 to 4 kg.) were anaesthetized by parenterally administered pentobarbital sodium and their eyes were dilated with Mydriacyl 1 per cent. Unilateral tarsorrhaphies were performed and opaque patches placed over the closed lids. The animals were dark-adapted for 30 min., after which time they were placed prone, with their heads maintained in a level position. The uncovered eyes were exposed to an unfocused high-intensity light source at a distance of two in. (4.07×10^4 cd/m.²) for 15 min. The cats were killed by an intracardiac injection of pentobarbital sodium. The lids were dissected free, and the globes gently enucleated and processed in the same way as the rat eyes.

During our experiments, fundoscopy was performed when feasible. The mounted retinæ were examined microscopically, and equivalent areas of paired retinæ were photographed. The corresponding sets of photomicrographs were compared and evaluated using a dual-projector system. The benzidine-stained retinæ were also compared with India ink and Niagara blue dye-injected specimens.

Results

FUNDOSCOPY

The retinal vessels in the enucleated eyes of anaesthetized rats revealed only a slight narrowing as compared with their calibre before enucleation. After decapitation, however, fundus examination revealed marked narrowing of the arterioles, and in the veins, aggregates of red cells moving slowly toward the disc ("box-car" effect).

HISTOLOGICAL STUDIES

The vascular pattern of retinæ studied by the benzidine technique was similar to that seen in Indian ink preparations. In neither was the filling complete (Fig. 1). It is emphasized that with the benzidine method only the red cells, and not the vessel walls, accept the stain. Red cells were fairly uniformly distributed within the arterioles and venules in both cat and rat retinæ, but some of the larger and secondary vessels in the former appeared empty or only partially filled. In the benzidine-stained retina, the stained material is birefringent when subjected to polarized light, and outlines the retinal vessels (Fig. 2).

The superficial and deep capillary beds could be easily appreciated microscopically (Fig. 3). The superficial net was in the plane of the larger vessels. In cleared specimens the deeper bed was clearly seen, and its capillaries were more closely knit than the super-

FIG. 1 *Low-power view comparing Indian-ink-injected rat retina (A) with benzidine-peroxide stained retina (B). Note that in neither instance is vessel filling complete. The stain in (B) is specific for red cells. $\times 35$*

FIG. 2 *High-power view of benzidine-stained cat retina subjected to polarized light. The stained material is birefringent and outlines the retinal vessels. $\times 87$*

ficial net. Most capillaries in both layers contained red cells, not uniformly along the entire channel, but in aggregates of varying length. The channels of the deeper bed appeared more uniformly and more completely filled. However, some capillaries in the mesh appeared empty, especially toward the central areas. In the cat this was particularly apparent near the macular region. In most cases, the peripheral vessels appeared to be broader and better filled with blood (Figs 4 and 5). The distance separating the two layers diminished toward the periphery.

By projecting photographs of equivalent retinal areas side-by-side, the relative filling of the capillaries could be compared (Fig. 6). We could detect no significant difference in vessel filling patterns between the dark-adapted and light-stimulated retinae. Nor was any difference detected as a result of pupillary dilatation, tarsorrhaphy, mode of death, head position, or the order in which the eyes were enucleated.

FIG. 3 Comparison of superficial and deep capillary circulation in a benzidine-stained rat retina under low power (A and B) and under high power (C and D). The superficial bed is in the plane of the large vessels. The capillaries contain red cells in aggregates of varying length. The channels of the deeper bed are more uniformly and completely filled and the mesh is more closely knit. A and B $\times 82$; C and D $\times 158$

FIG. 4 Low-power view of benzidine-stained rat retina. The peripheral capillaries appear to be broader and better filled with blood. $\times 50$

FIG. 5 Higher-power view of benzidine-stained retina comparing central and peripheral retinal areas. A and B: central areas of two rat eyes. A' and B': Peripheral areas of the same two eyes. $\times 82$

Comment

At the outset of this study we had hypothesized that the vascular response of the light-stimulated retina might be distinguishable from that of the dark-adapted retina by a greater number of capillaries filled with red cells, by fewer spaces within the capillaries which did not contain red cells, or by the presence of more red cells within a widened lumen. In other words, we focused our attention on alterations in blood volume rather than in blood flow.

Several methods of stimulating the uncovered eye were used: exposure to room light for 30 minutes, exposure to an unfocused high-intensity light source for 15 minutes, and exposure to a strobe light flashing at twenty times per second for 30 seconds. The first type of exposure was considered the most physiologic, and the other two were employed to ascertain the possible long- and short-term effects of bright light on the circulation.

We were unable to demonstrate any significant differences in the vascular filling pattern in paired retinæ subjected to the conditions of the experiments. It is, of course, possible that subtle differences between the light-stimulated and the dark-adapted state could not be appreciated by visual comparison.

The possibility that such differences might be demonstrated was based on the following considerations:

The retina is essentially an outgrowth of the central nervous system and its vasculature may be anatomically and physiologically similar to the vasculature of brain substance.

FIG. 6 *Equivalent retinal areas of both eyes of a rat. A and C $\times 35$; B and D $\times 82$. A and B represent low and higher power views of the right eye, exposed to light-stimulation. C and D are equivalent areas of the left eye, which was dark-adapted.*

There is substantial indirect evidence for the concept that alterations in the metabolic requirements of brain cells in man have an effect on the vascular response (Cobb and Talbott, 1927; Dunning and Wolff, 1937; Craigie, 1938; Serota and Gerard, 1938; Landau, Freygang, Roland, Sokoloff, and Kety, 1935). In this regard, Jacobson and Basar (1956) and others have shown that any one of several vasodilators causes a marked increase in the amplitude of the scotopic *b*-wave in normal individuals. This suggests some relationship between the amplitude of the *b*-wave, which is thought to be generated by the bipolar cells (inner nuclear layer), and increased blood flow to the area. Further, Nichols, Jacobowitz, and Hottenstein (1967) demonstrated localized histochemical differences in the inner plexiform layer (an area supplied by the retinal circulation) in the light- and dark-adapted state.

It has been shown that the brain is continually active under normal circumstances. The dark-adapted retina also maintains a high level of "spontaneous" electrical activity as shown by Kuffler (1953) and others, using microelectrodes. The rate of retinal blood flow necessary to support this activity is about three times the rate for the brain as a whole, and most closely approaches the highest regional flow rate of 180 ml./100 g./min. in the

inferior colliculus (Hickam and Frayser, 1966). From the available evidence (Lennox, Gibbs, and Gibbs, 1938; Kety, 1960; Sokoloff, 1960), it is felt that the overall metabolic rate in the brain can be maintained independent of blood flow over a wide range; an increased flow, for example, would result only in a decreased arteriovenous difference for nutrients and metabolites. It would appear, then, that the total circulation in both brain and retina is fairly constant. This does not preclude the possibility that altered nutritional requirements can trigger localized vascular changes which would cause local variations in flow. Although we were unable to demonstrate these localized changes in the vasculature of paired retinæ, we made some interesting observations about the filling pattern of the retinal vessels as demonstrated by the benzidine-staining technique.

This technique has been previously used to study retinal vessels (Michaelson, 1954; Keeney and Barlow, 1956; Thuránszky, 1957). Michaelson (1954), in studying retinal vascular patterns, reinforced the natural blood content of the vessels by injecting a gelatin solution of sedimented red cells. It has been stated that one disadvantage of the benzidine technique is that many capillaries are either incompletely filled or become emptied of blood in the course of processing. We did not find the latter to be true, and the former is not a disadvantage when the purpose is to examine the distribution of red blood cells and not the vessels *per se*. In so doing, our findings were essentially in accord with the studies *in vivo* of Thuránszky (1957), and Keith, Cunha-Vaz, and Shakib (1967), who removed the anterior segment of the cat eye and observed the circulation using a microscope with a water objective. They found that most of the central capillaries appeared and disappeared, with red cells passing through these vessels in clumps. (Evidence that capillary closure does not account for the discontinuous flow of red blood cells was provided by Dollery, Henkind, Kohner, and Paterson (1968), whose fluorescein ciné-angiography studies in pigs indicated that at least plasma was always present in almost all capillaries.) Other larger capillaries, having a fairly direct route from arteriole to venule, had an almost constant flow of red cells. On the basis of the present study, we can make no statement on the preferential pathways described by these workers. However, we did find that some capillaries devoid of red cells are present at the posterior pole of the cat retina, and that in the cat and rat retinæ red cells grouped in discontinuous aggregates are present within most other capillaries. The peripheral vessels appear to be better filled with blood in most cases. Thuránszky (1957) felt that they were shunt vessels, and Keith and others (1967) noted their presence in Indian-ink-injected rat retinæ.

Michaelson (1954) had reported that the deep retinal capillary bed consists of a denser meshwork than the superficial bed, and that, at least in the rat, it is essentially venous in nature. In this regard, it is of interest that Cunha-Vaz (1966) found that, by inducing a temporary stasis in the retinal vessels of albino rats, permeability changes were produced within the deep capillary net, extending later to the larger venules. In addition to confirming the relatively greater density of the deeper net, we were able to show that the latter had consistently greater red cell filling than the superficial net. This might be expected if the deep bed were on the venous side of the circulation. However, it could also conceivably be the result of an increased regional metabolic requirement.

In our study, we were attempting to examine the circulatory pattern at isolated moments in time; the situation being analogous to looking at a single frame of a ciné film. One cannot assume that blood flow is constant, or that the total flow varies with the anatomical size of the bed. Blood flow itself may not necessarily be proportional to metabolic requirements since oxygen uptake may vary, and blood flowing through shunts may be poorly utilized. Dunning and Wolff (1937) have shown that the vascularity of the cerebral

cortex is about twice that of peripheral nerves, but oxygen consumption is about 30 : 1. Thus anatomical findings may be only roughly indicative of metabolic requirements.

Artefact must also be considered in the interpretation of our results, and this includes possible agonal or *post mortem* changes in red cell content or vessel calibre. Since the histological appearance of the benzidine-stained retina basically reflects the pattern *in vivo*, however, it is our feeling that, with careful handling of the material, the red cell distribution within the microcirculation is not greatly altered in processing. Because of this, and because of the simplicity of the benzidine technique, it may prove a useful adjunct in the study of retinal microcirculatory patterns and responses.

Summary

The effects of light-stimulation and dark adaptation on the quantity and distribution of red blood cells within the retinal vessels were studied. Using the benzidine-peroxide staining techniques, in which only red blood cells accept the stain, we were able to compare pairs of flat-mounted animal retinæ by photographing equivalent areas and projecting them side-by-side. Although no demonstrable difference in the vascular filling pattern was found between the two states, it was shown that the red cell distribution *in vitro* in the examined retinæ mirrored the findings of various techniques *in vivo*. It was felt that the benzidine-peroxide staining method may prove a useful adjunct in the study of patterns and responses of the retinal microcirculation.

We should like to thank Dr Harris Ripps and Dr Irwin Siegel for their assistance, Mr. Henrick Malpica for the photography, and Mrs. Doris Ortega for secretarial assistance.

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