The effect of blood on ocular fundus reflectance and determination of some optical properties of retinal blood vessels. Robert W. Flower, D. Scott McLeod, and Sharon M. Pitts.

Light reflected from 50 μm diameter spots on the fundus of two adult rhesus monkeys was measured as a function of wavelength in 10 nm jumps from 400 to 900 nm. The areas measured were a retina artery and vein, areas of the disk, macula, and retina devoid of visible blood vessels, and the foveola. The eyes of the two monkeys were then exsanguinated, the blood vessels were filled with normal saline, and reflectances of the same spots on the fundus were again measured. The pairs of reflectance curves were compared and demonstrated that blood is not the major determinant of the characteristic shapes of the retinal tissue spectral reflectance curves. From the pre-exsanguination and postexsanguination data, the isolated retinal artery and vein wall reflectances were determined to be 0.020 and 0.009, respectively, their transmittances 0.987 and 0.967, and the fractions of light absorbed by them 0.143 and 0.014, respectively, in the visible spectral region below 500 nm.

Since blood accounts for a significant portion of the tissue volume comprising the retina and choroid, it would be expected to significantly influence the spectral characteristics of light reflected from the ocular fundus. Blood can be removed from the eye and replaced with saline without necessarily altering ocular geometry; therefore blood's effect on light reflected from various small fundus areas can be determined by measuring reflectance before and after exsanguination of the intact eye. Results of such measurements made on the eyes of two rhesus monkeys are reported here, and from these data approximations have been derived for the reflectances, transmittances, and the fractions of light energy absorbed by isolated retinal artery and vein walls. These data may ultimately prove useful in interpreting some of the phenomena resulting from the complex interactions between light entering the eye and the intraocular tissues, which occur during such procedures as fundus photography, fluorescence angiography, or even photo-coagulation.

Methods. The methods and equipment used in this study to measure light reflectance from small areas of the fundus of intact eyes were identical to those used in an earlier study. The subjects were the same two adult rhesus monkeys used in the earlier study to determine reflectance of light from small fundus areas of the living eye. Several days after completion of that study, each monkey was anesthetized with sodium nembutal, the common carotid arteries and jugular veins were exposed, 10,000 units of heparin were intravenously injected, and the exposed blood vessels were then cannulated. The carotid arteries were connected prograde to an elevated volume of heparinized normal saline, and the head was exsanguinated by flushing through approximately 8 L of saline at a pressure of 100 mm Hg. Each monkey's eye was then aligned in front of the fundus camera in the same position as in the earlier pre-exsanguination experiment, and the sequence of monochromatic fundus photographs made then at 10 nm intervals from 400 to 900 nm wavelength was repeated. During the photographic procedure, the cannulas in the jugular veins were occluded to allow only a constant slow leak, and a pressure of 100 mm Hg was maintained within the common carotid arteries. From the sequence of photographs obtained, reflectance of light was calculated by the same method used and for exactly the same fundus areas measured in the earlier pre-exsanguination experiments.

Results. Results of one of the monkey experi-
Fig. 1. Pre-exsanguination and postexsanguination reflectance as a function of wavelength for 50 μm diameter areas of the monkey fundus.

Fig. 2. Pre-exsanguination and postexsanguination reflectance as a function of wavelength for 50 μm areas of the monkey fundus. Reflectances of the fovea were specifically measured for a 0.17 degree area centered on the foveola.

ments are summarized in Figs. 1 and 2, which show the changes in reflectance of various 50 μm diameter fundus areas as a result of exsanguination. Identical relationships were found to exist in the second monkey in which this experiment was performed, although both pre-exsanguination and postexsanguination reflectances measured on the second monkey were about 0.5% higher than those measured on the first monkey.

Reflectances of disk, retinal, and macular tissue areas selected as being devoid of visible blood vessels were expected to be fairly insensitive to exsanguination, and results of both monkey experiments indicate this to be the case (Fig. 1). The highly reflective primate disk is normally only slightly pink, so that whatever pallor was produced by exsanguination did not significantly affect its reflectance, and the dense pigmentation of the macular and retinal areas measured apparently masked out any changes which might have resulted from removal of choroidal blood. However, the vein and artery, as shown in Fig. 2, evidenced significant shifts in reflectance of light when blood was removed from them, as did the very thin foveola when blood was removed from the underlying choroid. The gross similarity of all the reflectance curves derived is attributable to the fact that the eye behaves much like an integrating sphere; this phenomenon is discussed in detail elsewhere.2 The similarity between pre-exsanguination and postexsanguination curves demonstrates that blood is not the major determinant of the reflectivity curves’ characteristic shapes.

Calculation of retinal vessel wall optical properties. Reflectance measurements made of both 100 μm and 1 cm thick samples of whole, non-hemolyzed blood at different levels of oxygen saturation on a Beckman-DK reflectance spectrometer (Beckman Instruments, Inc., Palo Alto, Calif.) are shown in Fig. 3. Although no method currently exists to directly determine the oxygen saturation level of blood in a retinal artery or vein, for wavelengths at which the reflectance of blood is constant for all levels of oxygen saturation (e.g., 460 and 480 nm) enough information exists from pre-exsanguination and postexsanguination reflectance measurements to calculate the reflectances, transmittances, and fractions of light absorbed by isolated artery and vein walls.

In the equation representing the pre-exsanguination case (Fig. 3), light reflected from a blood vessel (S_l) consists of a component from the
PRE-EXSANGUINATION  POST-EXSANGUINATION

Fig. 3. Schematic representation of light components reflected from a retinal blood vessel before (left) and after (right) replacing the blood with saline.

In the equation representing the post-exsanguination case (Fig. 3), light reflected from a blood vessel ($S_2$) consists of a component reflected from the anterior vessel wall ($V$), a component reflected from the posterior vessel wall ($V_{k^2}$), and a component reflected from retinal tissue beneath the blood vessel ($R_{k^4}$), where $k$ is raised to the fourth power because the reflected incident light passes through vessel walls four times before it is detected. $R$ is the retinal tissue reflectance measured from a nonvascularized area similar in pigment density to those areas adjacent to the retinal artery and vein segments in question.

For a first approximation then, the pre-exsanguination and postexsanguination cases provide a pair of equations in two unknowns, $V$ and $k$:

$$S_1 = V$$
$$S_2 = V + V_{k^2} + R_{k^4}$$

The initial value calculated for $k$ is then used along with the measured reflectance of whole blood ($B$) in the pre-exsanguination case equation to determine a more precise value ($V_1$) for $V$ (this time the component reflected from blood is not ignored):

$$S_1 = V_1 + B_{k^2}$$

This more precise value $V_1$ is then used in the post-exsanguination case equation to determine a more precise value ($k_1$) for $k$:

$$S_2 = V_1 + V_{1k_1} + R_{k_1^4}$$

Repeating this process several times, $V$ and $k$ rapidly converge to values which satisfy both the pre-exsanguination and postexsanguination equations. When this method was applied to the retinal artery and vein for two wavelengths (460 and 480 nm), transmittance of the artery wall was found to be 0.837, and transmittance of the vein wall was found to be 0.977.

Once the transmittance ($k$) of the blood vessel walls has been determined, their reflectances may be determined by solving the pre-exsanguination case equation for $V$, the component reflected from the anterior vessel wall:

$$V = S_1 - B_{k^2}$$

Again, choosing a wavelength at which the reflectance of blood is constant, say 480 nm, the value of $S_1$ may be read from Fig. 2 (pre-exsanguination), and the value of $B$ may be read from Fig. 4. Thus the reflectance of the isolated artery wall is 0.020, and that of the vein wall is 0.009.

Having calculated the reflectances and transmittances for the artery and vein walls, the fraction of light apparently absorbed by each may be estimated from the relationship:

$$\text{Reflectance} + \text{Transmittance} + \text{Absorbed light} = 1$$

At 460 and 480 nm wavelengths, the fraction of light absorbed by the artery wall is 0.143, and that absorbed by the vein wall is 0.014.

**Discussion.** The pre-exsanguination reflectance data in Figs. 1 and 2 were previously shown to
compare favorably with the few other studies reporting similar data. Also, the unavoidable experimental shortcomings which prevented measurement of absolute reflectances were discussed, as was the fact that these shortcomings did not affect the relationship between measurements made on different tissue areas and certainly not the orders of magnitude of the values reported. Although there are no other studies reporting data to which the post-exsanguination reflectances can be compared, the accuracy of the values reported here and the level of confidence placed upon the relationships between reflectances of the different areas measured should be the same as for the pre-exsanguination data.

Of all the data reported, the fractions of light absorbed by vessel walls are obviously the least reliable, since (1) it is not clear that the fraction of incident light energy assumed lost by absorption is all converted to heat or whether some of it is lost by scatter within the vessel walls and (2) the fractions are very small and therefore susceptible to even small errors in determination of reflectance and transmittance. Unfortunately, there is no direct way to verify the values calculated; however, the data were consistent between two different experimental subjects, and the fractions of light absorbed by the artery and vein were found to vary by an order of magnitude. The vessels examined were a major retinal artery and vein, each approximately 1½ disk diameters away from the optic disk. Since the wall thickness of such an artery is from 1½ to 2 times thicker than that of such a vein, it seems reasonable to conclude that the absorbance of light energy by the retinal artery wall is much greater than by the vein wall.

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REFERENCES


Dexamethasone inhibition of experimental endothelial cell proliferation in retinal venules. JOHN T. HEFFERNAN, SIDNEY FUTTERMAN, AND ROBERT E. KALINA.

The observation that endothelial cell proliferation in retinal blood vessels is induced by ocular trauma in rats has been extended to mice. Indomethacin, 10 mg/kg/day, failed to block incorporation of tritiated thymidine into nuclei of venular endothelial cells in rat retinas observed 40 hr after puncturing the lens, but dexamethasone effectively suppressed tritiated thymidine incorporation, with 50% inhibition obtained with 0.2 mg/kg/day. The prostaglandin pathway does not appear essential to the activation of endothelial cell proliferation in this system.

Needle puncture of the lens in rats has been found to produce an increase of 30- to 70-fold in the frequency of tritiated thymidine labeling by retinal venular endothelial cells. Thymidine incorporation peaked 40 hr after ocular trauma. At the same time, an inflammatory process was observed, characterized by the presence of margined leukocytes within the lumina of retinal vessels.

Experimental ocular inflammatory processes, their relationship to prostaglandin biosynthesis and leukocytic infiltration, and their suppression by anti-inflammatory agents have been extensively studied. The present studies were carried out to determine whether the stimulation of endothelial cell proliferation is a presumptive early step in neovascularization of the retina, was unique to the rat and possibly prostaglandin dependent.

Methods. The retinas of 30 200 gm male Sprague-Dawley rats were exposed to tritiated thymidine, specific activity 20 Ci/mmol (New England Nuclear Corp. Boston, Mass.), by injecting 3 μCi of tritiated thymidine through the pars plana into the vitreous of each eye; subsequently the retinas were digested with trypsin, and the resulting vascular networks were exposed to autoradiographic emulsion, developed, and counterstained with hematoxylin so that labeled cell nuclei could be observed and counted. The techniques for intravitreal injection, trypsin digestion of the retina, and autoradiography have been described previously.

Labeling of the retinal vasculature was examined in a control group of eight 30 gm male CBA mice after exposure to tritiated thymidine, 10 μCi/gm administered intraperitoneally, and in an experimental group of eight mice previously subjected to ocular trauma.