Studies on the effects of osmotically active substances on the circulation and structure of the retina. Part I. Observations in vivo

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The retinal circulation in the cat was studied by intravital microscopy, and experiments confirmed that the passage of red cells in the capillaries is intermittent, although plasma may be circulating continuously. Preferential pathways, in which blood flows almost continuously, have been found in the superficial capillary network, forming a direct communication between arterioles and venules. They form an alternative pathway for the blood which would otherwise pass down to the deep plexus of capillaries. When distilled water is injected into the vitreous, the retina becomes edematous, usually causing narrowing of the arteries and veins and capillary closure. This closure may be due partially to the arterial constriction, but it was thought that tissue tension and swelling of the capillary wall played a part in the effect. Hypertonic saline usually caused dilatation of the retinal vessels, but the effect was much more marked when it was used to reverse a previous closure induced by distilled water. Therefore, it seems possible that both tissue tension and swelling of the capillary wall (pericytes and endothelial cells) may be concerned with the autoregulation of the microcirculation in the eye. Furthermore, this mechanism of vessel closure might be involved in the development of some of the pathological lesions that characterize the retinopathies of diabetes mellitus, hypertension, and the collagenoses.

The circulation of blood in the retina is believed to be controlled by both general and local factors. Many experiments have been undertaken to demonstrate the effects of altering the blood pressure, of stimulating the autonomic nerve supply to the eye, and of applying various chemical and pharmacological agents (either systemically or directly) to the retinal vessels. Thurnanszky† carried out extensive research into this problem in the cat, and concluded that, under normal circumstances, the capillary circulation was unaffected by the above factors; but that the pattern of blood flow in the retina was dictated by the metabolic requirements of the cells, and varied from moment to moment. The mechanism by which metabolic needs alter the blood flow is not known, but it has been suggested that metabolic changes in the surrounding retinal cells may stimulate the swelling of endothelial cells, or the contractile properties of intramural pericytes to shut off or open the capillaries.

Another mechanism for the autoregulation of the retinal circulation has been postulated by Ashton,§ who suggested that localized swelling of the retina (either intracellular or extracellular edema) could, by putting pressure on the capillaries, cause profound changes in the circulation, which
might be responsible for some of the pathological lesions occurring in the retina in such diseases as hypertension, diabetes, or the collagen disorders. In order to further investigate these possibilities, we have experimentally induced swelling and shrinkage of the retina by altering the tonicity of its environment through the application of anisotonic solutions, and have then attempted to relate the observed effects in the retinal circulation to the structural changes found by electron microscopy.

This part of our report deals solely with the appearances of the normal circulation as seen by intravital microscopy, and the changes which follow the intravitreal injection of distilled water and hypertonic saline.

**Methods**

The experiments were carried out in two parts: the normal circulation in the cat retina was observed and photographed; distilled water or hypertonic saline was then injected into the vitreous, and the circulation was observed and photographed. Following this, the retina was removed and processed for electron microscopy.

The retinal circulation was observed by the method described by Thuranszky. Mature tabby cats were used when available (in immature cats and black cats the posterior sclera is often too thin, and tends to collapse after the vitreous has been removed). The animal was anesthetized with intraperitoneal pentobarbitone 40 milligrams per kilogram of body weight. The nictitating membrane and eyelids were excised, and sometimes part of the bony rim of the orbit was removed in order to give adequate exposure. The bleeding usually stopped spontaneously, and then the cornea was excised at the limbus. The sphincter pupillae was cut away 2 mm. from the pupil margin, usually without hemorrhage; the anterior lens capsule was excised, and sometimes part of the bony rim of the orbit was removed in order to give adequate exposure. The bleeding usually stopped spontaneously, and then the cornea was excised at the limbus. The sphincter pupillae was cut away 2 mm. from the pupil margin, usually without hemorrhage; the anterior lens capsule was excised, and the lens was delivered in one piece with forceps or a needle. The posterior lens capsule sometimes had to be incised if it was very dense. A Leitz incident light microscope (Fig. 1) was used with a 5x objective lens and a dipping cone, in order to avoid the light reflexes that occur when passing from one refractive medium to another. A 10x eyepiece was used for observation and a 3x one for photography, as the magnification was increased by the length of the photographic attachment. The illumination was provided by a Phillips 50 W, 12 v, lamp with heat filters and condensing lenses. Ilford R20 or HPS plates were used for photography. For cinematographic studies of the circulation, a Bolex H12 camera with Kodachrome II film and a Kern Paillard ½” lens was used. The dipping cone was passed down into the eye until the retinal vessels were clearly in focus, and a suitable part of the vasculature was chosen for photography or cinematography. The field of view was changed by carefully moving the whole cat so that the eye rotated around the dipping cone.

The area chosen was photographed, and the objective lens removed from the eye. Distilled water or hypertonic saline was injected deeply into the vitreous, often expelling the latter. The dipping cone was reinserted into the eye, the circulation observed, and serial photographs taken over a period of time. In other experiments, 2.7 per cent hypertonic saline was injected into the vitreous after distilled water, and the changes observed and recorded; more distilled water was then added, and further photographs taken. In later experiments, local application of Priscol and Papaverine to the retina was used in an attempt to avoid arteriolar spasm. When sufficient observations had been made, the eye was irrigated with chilled 1 per cent osmium tetroxide, and removed for electron microscopy. In most experiments, the blood pressure was measured in the tail by means of a transistorized plethysmograph.
Findings and discussion

Normal circulation (Fig. 2). Using the method described by Thuranszky we have been able to confirm many of his findings in the retinal circulation of the cat. The flow of red blood cells in the central capillaries is discontinuous, and these vessels are visible only when red cells are flowing in them. The red cells often appear to pass in clumps along the smaller vessels, and their speed indicates the rate of flow in the capillaries. No flow can be seen in the larger vessels where the blood column is continuous, unless the column is broken up by reducing the speed of circulation so that clumps of red cells can be seen. This phenomenon is called granular flow.

While most of the central capillaries are appearing and disappearing continually, others seem to have an almost constant flow of red cells, even when observed over several hours; they are larger than the other capillaries, and have a fairly direct pathway from arteriole to venule, although they often give off branches to the deeper capillary plexus. We consider them to be preferential pathways in the capillary network, and they may play an important role in the local distribution of blood in the retina. The photographic recording of these pathways has proved very difficult, both with plates and cinematography; but they become very dilated in artificially induced retinal edema, and then can be easily demonstrated. They are also demonstrable in flat retinal mounts of India ink-injected specimens. It was not possible to verify Thuranszky's concept of the retinal circulation—that blood is brought to the eye in excess of its metabolic requirements, most of it passing via peripheral shunts from artery to vein. These peripheral shunts cannot be seen in vivo, as they are too peripheral and have no tapetal background. While study of India ink-injected specimens confirmed his findings of short wide capillaries (which could act as shunts) connecting the terminal arterioles and venules near the ora serrata, no functional conclusions can be reached by means of purely anatomical studies. Thuranszky thought that the excess of blood coming to the retina acted as a regulating mechanism to protect the eye against fluctuations in blood pressure; but not all the retinal vessels can participate in this mechanism. Michaelson described several arteries which arise at the optic disc, supply only the macular area, and do not reach the periphery; so that all the blood in these vessels passes through the central capillary network.

Among workers who have studied the retinal circulation, there seems some difference of opinion as to whether the capillaries are constantly fully engorged with blood, or whether a proportion of them may contain only plasma. According to Thuranszky, who injected dyes intravenously and then immediately arrested the circulation by freezing the retina, there is a continuous flow of plasma without red cells in some of the capillaries, even when they cannot be seen biomicroscopically. Unfortunately, this method has been unsuccessful in our hands, but injection of dilute India ink into the circulation certainly increases the number of visible capillaries. It does not follow, however, that all capillaries unseen by intravital microscopy con-
tain only plasma, for they may be invisible for purely technical reasons. The small vessels can be seen only against the tapetal background. Elsewhere, the dark choroid absorbs the incident light, and even then, the deeper capillaries may not be seen due to obscuration by the inner retinal layers, or inadequate illumination by the light reflected from the tapetum.

Similarly, the contrary evidence that the capillaries are constantly full of blood is somewhat inconclusive. Although Dollery found that the capillary bed appeared fully engorged in fluorescein angiography, this does not resolve the problem; for fluorescein is only carried in the plasma. Although Friedman and co-workers found that the circulation of red cells was continuous in all the capillaries that they observed with the scleral window technique, it was our impression—from seeing their film—that the vessels studied contained several red cells which passed down the lumen at the same time, and therefore were larger than true capillaries. In all our electron micrographs of capillaries, one red cell usually filled the whole diameter of the vessel.

Finally, it must be admitted that the methods used by Thuranszky, Friedman, and ourselves involve considerable disturbance to the eye, and cannot provide unequivocal evidence of the normal behavior of the retinal vessels.

Circulation after the instillation of anisotonic solution. With the injection of distilled water into the vitreous, the appearance of the fundus changed due to a rapidly developing retinal edema which caused the retina to become so opaque that the tapetum and choroidal stars were no longer visible (Fig. 3). In the majority of experiments most of the capillaries disappeared, apart from some of the preferential pathways which became dilated. The arterioles and venules in the affected area were often markedly narrowed, and the granular flow of blood in them indicated a slowing of the circulation. The blood flow in the preferential channels was also sluggish.

The disappearance of the capillaries is thought to be due to their closure and not to obscuration by retinal edema, because the circulation would sometimes spontaneously return after a variable period, without any apparent change in the degree of edema. The capillaries may be closed by a direct action on their wall, or closure may be secondary to the arteriolar constriction; but it was thought that the extent of it was

![Fig. 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/933258/)

**Fig. 3.** The cat retina after the application of distilled water, which shows edema and the disappearance of most of the capillaries.

![Fig. 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/933258/)

**Fig. 4.** The same specimen as seen in Fig. 3 after the addition of hypertonic saline, which has caused the reopening of the capillary network.

![Fig. 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/933258/)

**Fig. 5.** The same specimen as seen in Fig. 4, but after the reapplication of distilled water.
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more than could be accounted for by the latter, and there was still some circulation present via the preferential pathways. Why these should become dilated (Fig. 6) is not known, but they do lie on the surface of the retina, which makes them less subject to tissue tension. If the latter is an important mechanism in controlling the pattern of capillary blood flow, then the phenomena observed here may be an exaggeration of the normal process. When retinal edema is produced, there is almost certainly a rise in tissue tension which could cause closure of the deep capillaries; but similarly, edema of the pericytes and endothelial cells composing the capillary wall could obstruct the lumen. Possibly both factors are involved. There are also several possible causes for the observed arteriolar constriction: it could be due to a direct stimulating effect of distilled water on the muscle wall; it might be due to edema of the wall causing a reduction in the size of the lumen; or it could be secondary to obliteration of the capillary bed. The venular narrowing could be due to either of the latter two factors. Attempts were made to eliminate arterial contraction by the instillation of Papaverine drops before the introduction of distilled water, but this caused white flocculi to form in the vitreous, and prevented any further observations. Priscol drops did not seem to alter the vascular reactions, but the results were not conclusive. The systemic blood pressure remained approximately constant throughout the experiments, so it seems that only local factors were responsible for the phenomena observed. Some experiments were unsuccessful, as the retina became detached after the introduction of distilled water. In others, retinal edema did not produce any capillary closure; but, on the contrary, engorgement of the superficial capillaries occurred. This could be due to extensive disruption of the retina with loss of the internal limiting membrane; then the tissue tension would fall and allow the blood vessels to dilate. The secondary dilatation of the blood vessels, after an initial closure, may be caused

Fig. 6. Dilatation of the preferential pathways in the capillary network. One connection between arteriole and venule is arrowed.

Fig. 7. Normal retina. This is a poor photograph due to technical reasons.

Fig. 8. The same specimen as seen in Fig. 7, but ten minutes after the application of hypertonic saline. The retina is semiopaque and the vessels are dilated.
by the passage of fluid from the tissues into the bloodstream by means of osmosis; and, if the process were allowed to continue, the normal tonicity of the retina might be re-established. The conflicting results and the complexity of the many possible factors affecting the circulation and structure of the retina make any conclusions difficult, but our electron microscopic studies helped to elucidate some of the problems.

When hypertonic saline was injected into the vitreous, most of the retinal vessels became dilated within a few minutes (Figs. 7 and 8). This seemed to affect particularly the preferential pathways in the capillary network, but the fine capillaries were sometimes dilated as well. The retina became semiopaque, and this was thought to be due to disruption of the optically homogeneous structures.

Hypertonic solutions will cause shrinkage of all cells, and this will result in a fall in tissue tension, which tends to allow the blood vessels to become dilated. Shrinkage of the endothelial cells lining the blood vessels will cause widening of this lumen, and allow a freer flow of blood; but shrinkage of the outer coats of the blood vessels (muscle in the case of the arterioles, and pericytes in the capillaries and veins) might well cause constriction of the blood vessels. So the rather equivocal results obtained are not surprising, even on a theoretical basis. The changes were much more marked when the retina was first treated with distilled water which caused capillary closure, and then treated with hypertonic saline. The saline caused a very marked dilatation of all the retinal blood vessels (Fig. 4), but this could be completely reversed by adding more distilled water when all the small vessels were closed again (Fig. 5). These experiments indicate that the microcirculation can be affected by changes in osmotic pressure, and there is some suggestion that the capillary circulation is not entirely and passively dependent on the arterioles supplying it, but that there is some degree of independent closure and opening.

Both tissue tension and capillary wall swelling may be involved in controlling the capillary size, but the former factor may prove to play a bigger role in the intact eye where retinal swelling will cause a definite rise in tissue tension; while, in the experimental eye with the cornea removed, the intraocular pressure is reduced to atmospheric level, and the retina can swell freely with less increase in tissue tension. Johnson has shown that the autoregulation of the circulation in an organ surrounded by a rigid capsule may depend entirely on tissue tension without any metabolic factors or arteriolar contraction being involved. The criteria he has established may well be applicable to the eye; but it seems more probable that all three mechanisms are involved.

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REFERENCES