Aqueous flow into the perivascular space of the rabbit ciliary body

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Through the use of the histochemical reagent, nitroblue tetrazolium chloride, to label anterior chamber aqueous in the eyes of living albino rabbits, we have been able to trace the pathway of aqueous leaving the anterior chamber angle. Although we could find no evidence that any of the marker had entered the trabecular veins (sometimes called Schlemm's canal) or the trabecular meshwork directly in front of the veins, there was evidence that the aqueous had moved through channels in the ciliary body posterior to the ciliary cleft. The pigmented epithelium of the ciliary body, but not the ciliary processes, was intensely stained with marker, particularly in the areas adjacent to the perivascular aqueous channels. From a detailed study of the distribution of the nitroblue formazan in serial sections of the ciliary body region of eyes injected in vivo with marker 10 minutes before excision and fixation, we have concluded that aqueous leaving the anterior chamber first enters the ciliary cleft from which it flows into the perivascular spaces and perivascular aqueous channels of the ciliary body. The aqueous channels lie adjacent to the pigmented epithelium in the posterior part of the ciliary body and some of them connect with the perivascular space of the suprachoroid. The uptake of stain by the pigmented epithelium of the ciliary body has been interpreted to mean that fluid from this channel system may be resecreted into the posterior chamber.

In 1943 Kiss reported some experiments which confirmed earlier reports describing the infiltration of colloidal pigments into the ciliary body of the rabbit, other mammals, and man following injection in vivo of India ink or other pigments into the anterior chamber. Kiss stated that if the anterior chamber was labeled with colloidal pigment without an increase of the intraocular pressure during the process, some hours later particles of pigment were to be found infiltrating the ciliary body but were not found in the structure we refer to as trabecular veins, nor were the particles concentrated especially in or about the trabecular meshwork in front of the trabecular veins. Presumably the particles which infiltrate the ciliary body were transported there by some sort of fluid flow from the anterior chamber although other possible modes of transport, i.e., diffusion, were not convincingly ruled out. Seidel, for example, in 1921 was very critical of earlier workers, who had injected pigments and soluble crystalloids into the anterior chambers of various living animals, on the grounds that when he repeated their experiments and examined the eyes microscopically a day or two after the injections, the colloidal pigment which was observed infiltrating the ciliary body was found mostly intracellularly in...
phagocytes. He thought the marker pigment which initially entered the outside layers of the tissue by diffusion was carried further into the ciliary body by the phagocytes. In addition, his experiments with injection of colloidal dyes under excess pressure into excised eyes was interpreted by him to mean that aqueous left the rabbit eye only via an episcleral venous system.

In a comprehensive description of the anatomy of the rabbit eye, Davis suggested (1929) that the aqueous may leave the anterior chamber of these animals by filtering into the perivascular lymph spaces in the root of the iris and also into similar spaces found "behind the lace-like uveal meshwork of the iris angle" as well as by Schlemm's canal. The structure which Davis labeled "Schlemm's canal" was called "trabecular veins" by Troncoso. In the rabbit this structure is located posterior to the iris-corneal junction about at the apex of the cilioscleral sinus, a structure called the ciliary cleft by Duke-Elder. In this report, we will call these structures trabecular veins and ciliary cleft, respectively, not out of preference or conviction but simply because a name for an identifiable structure is convenient. When we use these names we mean simply the specific structures as they are labeled in the figures of the references cited.

If aqueous leaves the anterior chamber by flowing into the ciliary cleft, as suggested by Davis, then if aqueous enters the intrascleral vascular plexus which includes the trabecular veins, as was suggested by Troncoso, this pathway should be marked by any label which had been injected into the anterior chamber a sufficient time before enucleation. Thus, microscopic sections from eyes which had been injected with a suitable label for aqueous, i.e., colloidal pigment or histochemical reagent, should show evidence of the label in the ciliary cleft and all other canals, ducts, or tissue through which the aqueous moves as it exits from the eye.

As part of a study of fluid movement in the eye, we have repeated some of the previous experiments and have extended the work to include experimental methods and controls which we believe take into account the question of passive diffusion into the ciliary body. To eliminate any possible role of phagocytes in the transport scheme and to minimize the effects of diffusion, we have used a very short time instead of the days or hours used by previous investigators. As a label and marker for fluid movement, we have again employed the water-soluble histochemical reagent, nitroblue tetrazolium chloride, which is reduced by all cellular tissues of the eye to insoluble nitroblue formazan.

### Materials and methods

The materials employed and the methods used were essentially the same as those reported in previous research where applicable. In the present experiments injections of 2 to 15 µl of nitroblue tetrazolium chloride (nitro BT) (10 mg. per milliliter) solution were made after insertion of a needle through the cornea of albino rabbits in such a way that the point of the needle was positioned in the filtration angle without the tip touching the iris or cornea.

At the expiration of the time of an experiment (which for all experiments reported was 10 minutes), the rabbit was beheaded and the experimental eye was enucleated, frozen in a dry ice-petroleum ether mixture, and transferred to Bouin's fixative which had been previously frozen to a mush. The bottles with the eyes and fixative were immediately placed in the freezing compartment of the refrigerator at about -10° C. for 24 hours, then were allowed to fix at room temperature another 24 hours or more before processing for sectioning.

After enucleation of the experimental eye, the head with the remaining eye was placed in a pan kept at 37° ± 1° C., and the second eye was injected in a manner as identical as possible to that used for the experimental eye. The second eye, which will be referred to as the control eye, was also enucleated after 10 minutes and was frozen, fixed, and prepared in the same way as the experimental eye.

After fixation and washing were completed, a coronal slice of each eye was prepared by cutting off the cornea and sectioning the eye just posterior to the ora serrata. After the zonules were cut the lens was removed.Microscopic sections of these preparations 8 µ thick were cut parallel to, or at a very acute angle to, the plane of the corona.
Fig. 1. Semischematic drawing of the filtration angle and ciliary body of the rabbit. A, the cornea; B, the iris; C, sclera; D, the pectinate ligament at the scleral end; E, anterior open spaces of the ciliary cleft; F, iris pillar; G, perivascular space around a large vein of the ciliary body just posterior to the apex of the ciliary cleft; H, the trabecular veins; I, perivascular aqueous channel; K, large vein posterior to the perivascular region, which lies above this level, showing absence of perivascular spaces; L, the distorted S-shaped twist of the large veins at the root of the iris.

ciliaris. Such sections, cut through the region of the nitroblue formazan stained portion of the eyes, were mounted serially on slides without additional staining. Occasional sections were stained with Harris's hematoxylin and eosin stain for identification of doubtful structures.

Pertinent anatomy of the rabbit ciliary body

The ciliary body of the rabbit is traversed by closely spaced, meridionally oriented, relatively large blood vessels. The blood vessels are veins which drain a capillary plexus of the iris. These veins also tend to have a distorted S-shaped course through the root of the iris. In meridional sections the ciliary cleft of the rabbit eye is shaped similar to an acute triangle with the base in the plane of the anterior surface of the iris. The outside of the ciliary cleft is defined by Descemet's membrane, an extension of the corneal endothelium, and the sclerocorneal trabeculum. The apex lies at the posterior terminus of the sclerocorneal trabeculum. The inside is bordered by stromal cells of the iris root and ciliary body except where this side is bordered by the walls of the large veins referred to previously as they begin their meridional course posteriorward. At its apex the ciliary cleft is crossed by numerous iris pillars which decrease markedly in number toward the base so that the anterior half of the ciliary cleft is crossed by very few iris pillars. The anterior surface of the ciliary cleft is closed with a membranous extension of the iris root, the pectinate ligament, which is attached to Descemet's membrane in such a way that a series of small openings is formed which connect the ciliary cleft with the anterior chamber.

The structures called the trabecular veins are found embedded in the scleral stroma immediately adjacent to the ciliary body. They are located just posterior to the terminus of Descemet's membrane and are covered on the ciliary body side with trabecular tissue. This tissue which Davis labeled sclerocorneal trabeculum extends anteriorly to where it inserts itself between Descemet's membrane and the sclerocorneal stroma and posteriorly to the apex of the ciliary cleft. These anatomical features can be recognized in Fig. 1.

Results

Regardless of how slowly the nitro BT solution was injected into the anterior chamber angle of the control eyes in these experiments, the nitro BT was carried throughout the anterior chamber by convective currents so that most of the anterior chamber contained some nitroblue formazan at the end of the experiments. In contrast, when nitro BT solution was injected into experimental eyes very slowly
Fig. 2. Photomicrograph of an unstained oblique section cut from a coronal ring, which includes the iris and corona ciliaris of an experimental eye of an albino rabbit. This photomicrograph shows part of the iris, the angle, and about one half of the length of the ciliary body region measured from the iris angle to pars plana. This eye was injected in vivo at the filtration angle with 10 μl of nitro BT solution 10 minutes before the eye was excised, frozen, and fixed. Dense deposits of nitroblue formazan are to be seen in the open spaces of the ciliary cleft, in the perivascular spaces around the large veins, and in the pigmented epithelium of the ciliary body. Formazan also stains certain structures in the iris and the pigmented epithelium of the iris. The five large veins seen in this view, each cut at a different distance posterior to the angle, illustrate the gradual conversion of the ciliary cleft into the perivascular spaces. (×280.)

(most of the nitro BT solution entered the uveal tissue immediately posterior to the site of injection and little or none escaped into the surrounding aqueous. Thus, only a small area of the filtration angle was stained with nitroblue formazan in these eyes.)

On gross examination of the coronal slices, which includes the corona ciliaris and iris and were prepared as described under materials and methods, it was found that the most obvious difference between the experimental and control eyes from the same albino rabbit was the amount of blue stain visible in the region of the corona ciliaris as viewed from the posterior side of the preparation. A blue-stained area was always found in the segment opposite the injection site but only in those preparations from experimental eyes. The size of the area correlated well with the amount of nitro BT solution injected—the more injected the larger the area. The stained area of the experimental eyes injected with the larger amounts of nitro BT solution often extended onto the iris, and the control eye from the same animal sometimes showed a little stain on the posterior of the iris. The stained area on the experimental eyes was confined to the tissue of the iris and ciliary body between the processes. The ciliary processes themselves showed no sign of formazan stain.

On examination of the serial sections made from experimental eyes, we found
Fig. 3. The trabecular vein (TV) region from a microscopic section of the same eye of Fig. 2. The portion of the trabecular meshwork (TM) directly in front of the trabecular vein did not contain formazan, although the ciliary cleft (CC) both anterior and posterior contained formazan in large amounts, and the sclera (S) both anterior and posterior to the trabecular vein contained formazan. The trabecular meshwork both anterior and posterior to the trabecular vein has appreciable stain. The layers of trabecular meshwork in front of the trabecular vein have some formazan stain but only those layers adjacent to the ciliary cleft. (x800.)

that the open spaces of the ciliary cleft contain large amounts of nitroblue formazan (Fig. 2). The same region in the control eyes has only barely detectable amounts of nitroblue formazan in the ciliary cleft, at the iris surface, inside the iris stroma (if more nitro BT than was usually used had been injected), and nowhere else except the anterior chamber. The experimental eyes which were injected slowly with 5 to 10 μl of nitro BT solution have dense deposits of granular formazan which extend posteriorly in the ciliary cleft all the way to the apex. In these eyes the trabecular meshwork directly in front of the trabecular veins was noted to be free of formazan even though the same structure anterior and posterior to the trabecular veins was stained. In some sections, particularly from eyes which had been injected with the larger amounts of nitro BT solution, the trabecular meshwork layers adjacent to the ciliary cleft were stained lightly in the region in front of the trabecular veins (Fig. 3). The trabecular veins and their lining were invariably free of the stain, except in the eye that had been injected with 15 μl of marker solution in less than 5 minutes. The distribution of formazan in the vicinity of the trabecular veins is particularly significant because in the same eyes appreciable quantities of formazan were invariably found in certain parts of the ciliary body posterior to the trabecular veins and in the sclera both anterior to and posterior to the veins.

When the pathway defined by the deposits of nitro BT formazan was traced from section to section it was found that the open spaces near the apex of the ciliary cleft are continuous with perivascular
spaces of the large, meridionally oriented veins. The well-defined perivascular spaces, such as those shown in Fig. 4, follow the vein posteriorly for a short distance, the perivascular region. At the anterior end of this perivascular region the vein has large open spaces on the scleral side only. The spaces gradually surround the vein until they have a typical perivascular appearance. Open spaces with no evidence of endothelial lining form continuous, tortuous channels which branch off from the apex area of the ciliary cleft and also from the perivascular region. These channels wind their way inside the ciliary body until they are adjacent to the pigmented epithelial layer. The channels can be identified on microscopic sections by the clumps of formazan seen in them and by the formazan stained cells adjacent to them. As seen in the usual microscopic section stained with hematoxylin and eosin or other stains, these spaces could easily be mistaken for artifacts since the continuity of the spaces is masked by their normal tortuous pathways which, when cut in a plane, results in a number of isolated spaces on any single section. In this respect they resemble the perivascular spaces which appear as continuous passageways or connected chambers only in reconstructions from serial sections.

There are a few similar channels in the stroma between the veins in the perivascular space region of the ciliary body. The channels are not numerous nor were they found to contain formazan in amounts equal to the formazan deposits in the channels in the perivascular region. The perivascular spaces of the perivascular region leave the large veins rather abruptly about
two thirds of the distance from the pectinate ligaments to the border between the ciliary body and pars plana. The large veins continue posteriorly from that level without perivascular spaces, but the spaces continue as channels both posteriorly and toward the inside with multiple branchings until the channels also are adjacent to the pigment epithelium of the ciliary body. In all respects they resemble the channels which branch off from the apex of the ciliary cleft and the perivascular spaces. Some of the channels proceed posteriorly in the tissue adjacent to the pigmented epithelium of the ciliary body until at the level of pars plana they become continuous with the perivascular spaces of the suprachoroid. We will refer to these channels in the remainder of this paper as perivascular aqueous channels.

Microscopic examination of sections from experimental eyes also reveals that the pigmented epithelium nearest a perivascular aqueous channel is usually more intensely stained than other portions of the pigmented epithelium in that area (Fig. 5). Cells were found in the ciliary epithelial layer adjacent to the most intensely stained pigmented epithelial cells which also contained recognizable amounts of nitroblue formazan.

While no quantitative measurements have yet been made, it appears from examination of our serial sections that the total cross-section area of the open spaces of the ciliary cleft, the perivascular spaces, and the perivascular aqueous channels decreases markedly from any given level to a more posterior level. It also appears that the total number of perivascular aqueous channels which reach the level of pars plana are only a fraction of the total number that branch off from the ciliary cleft and perivascular spaces.
Another observation which should be noted is that the walls of the veins in the perivascular region are usually unstained even though the adjacent perivascular space or ciliary cleft contains dense deposits of nitroblue formazan. Perhaps the stain that is occasionally found in the adventitia of these veins diffuses there during the time between beheading the animal and freezing the excised eye. Since the walls of these veins are not usually stained, it would appear that aqueous absorption into the veins in this region does not occur with great facility.

Fig. 1 is a fairly accurate schematic representation of the open spaces of the filtration angle and ciliary body of the rabbit. This illustration was drawn to show the open spaces as we were able to reconstruct the area from our serial sections. The photomicrograph (Fig. 2) of an oblique cut through the ciliary body with five of the large veins of the ciliary body visible, each cut at a different distance posterior to the iris, illustrates the typical changes in the ciliary cleft and perivascular space region of the large veins. It also shows the distribution we observe of nitroblue formazan in the open spaces of the ciliary cleft, perivascular spaces, perivascular aqueous channels, and in the ciliary body and pigmented epithelium of that structure. Fig. 5 shows the shadings in the intensity of formazan stain in the pigmented epithelium of the ciliary body at different distances from perivascular aqueous channels.

From examination of Fig. 2 it can be seen that the pigmented epithelium of the iris and the most anterior portion of the ciliary body are also deeply stained with formazan deposits. Certain areas of the iris are also visibly stained although with somewhat less intensity than the pigmented epithelium. Since our purpose was to report only the findings in the ciliary body, which involve the ciliary cleft and perivascular channels, and also because our researches on the uptake of nitro BT via the iris are not completed, discussion of these findings will be deferred to a subsequent paper. Suffice it to say at this time that the route from anterior chamber to the pigmented epithelium of the iris and the anterior part of the ciliary iris does not appear to include movement of reagent from the ciliary cleft.

Discussion

The earlier workers, including Kiss, who were concerned with the pathways of aqueous excretion apparently failed to appreciate the need to demonstrate a difference between the living and dead eye. Since we made injections into the dead eyes (control eyes) in situ about 5 minutes after the head was severed from the rabbit's body, it actually would be more accurate to describe them as eyes without a blood supply.

The question of the role played by the difference in the intraocular pressure of the experimental and control eyes, as a factor in the distribution patterns observed for the fluid movement tracer we employed, is difficult to answer with absolute certainty. It does not seem reasonable that the small reduction of intraocular pressure experienced by the control eyes could be the sole cause of the very marked difference in the distribution pattern of nitroblue formazan which was observed. It is a basic principle of fluid mechanics that a fluid in a closed vessel does not move in the absence of a specific driving force. The physical driving forces which have been so far discovered are mechanical, i.e., by a pump of some sort; electrical, of the endosmotic variety; or magnetic if the fluid has magnetic properties. Osmosis or processes associated with active transport can result in a net movement of water in one direction in certain biologic systems. If it is true that the intraocular pressure is supported by the sclera, cornea, and blood vessels, then it would be a gross violation of the basic principles of fluid mechanics to find fluid movement inside the eye which was primarily responsive to a change in intraocular pressure. At or
near a hole through the sclera, cornea, or blood vessel, flow, which was responsive to changes in the intraocular pressure, would occur, since flow within a fluid will always occur along a pressure gradient from any given region to a region of lower pressure, except along the gravitational-induced pressure gradient. At steady state the absolute pressure must be the same at all points inside the sclera and cornea if the infinitesimal corrections due to rigidity of the uveal tissue and also due to gravitational differences are ignored. The application of these principles of fluid mechanics to the present problem leads to the prediction that more flow should have been observed in the control eyes if the major exit of fluid from the eye was into the equivalent of a hole through the sclera or into a vein.

The 10 minute time period between the injection of tracer into the anterior chamber angle and freezing of the eye was so short, that the role of any possible transport mechanism except convective flow is quite minimal. If diffusion alone could account for the distribution patterns observed the control eyes should have shown the same distribution of formazan as was found in the experimental eyes. When pieces of tissue were incubated in nitro BT solution 10 minutes, then frozen, fixed, and cut perpendicular to the surface, formazan was found only in a 0.5 mm. thick layer adjacent to the surface. Within this layer the intensity of stain is greatest at the surface and decreases to none at the indistinct border. Fixation stops all reduction of nitro BT to formazan and since some of the formazan is chemically combined with protein as it is reduced it cannot move about appreciably during preparation of the tissues for sectioning although some of the unbound formazan is probably lost through solubilization. There was little evidence on any of the sections we examined that diffusion during fixation had occurred to any appreciable extent. Since large quantities of formazan were found in the eye tissue more than half a millimeter posterior to the anterior chamber, nitro BT must have been carried there by flow of a fluid.

The pigmented epithelial layer of the posterior portion of the ciliary body is more intensely stained than the adjacent structure on the anterior side. The epithelial layer adjacent to the posterior chamber was sometimes found to be stained very lightly but only adjacent to the most intensely stained pigmented epithelial cells. The reverse of this is observed if nitro BT solution is placed in the posterior chamber. This observation is a strong argument for the hypothesis that the reagent had moved from the anterior chamber by convective flow and was taken up by the pigmented epithelial cells because these cells were in the process of secreting at least some substances from the aqueous into the posterior chamber. The fact that a blood supply to the eye appears to be necessary before an appreciable flow will occur may indicate some sort of active role of the blood in this process. Two possibilities suggest themselves. The first, and most obvious, is the role of the blood as a supplier of oxygen to an active transport system such as the tissue layer made up of the pigmented epithelium and epithelial cells which would probably not operate efficiently without oxygen. A second possible role of the blood supply is suggested by the perivascular space region of the large veins which could serve to pump fluid through the perivascular spaces by utilizing rhythmic pulsations in the veins, the same mechanical principle which applies to certain laboratory pumps. Obviously both mechanisms may be operative. But, regardless of the role played by the blood supply in the transport of nitro BT to the pigmented epithelial layer of the posterior parts of the ciliary body, the pathway of aqueous from the anterior chamber through the ciliary cleft and into the perivascular aqueous channels is clearly evident from the distribution of formazan in the experimental eyes.
The continuity of aqueous-filled spaces from the anterior chamber through the full length of the ciliary body and into the suprachoroid has apparently not been described before, although Davis referred to perivascular lymph spaces around the large veins in the iris root "near the bases of the iris pillars." He does not refer to the perivascular spaces around these large veins posterior to the level of the trabecular veins, nor does he define very precisely the structure to which he refers when he uses the phrase "lace-like uveal meshwork of the iris angle" although he probably means the structure called the ciliary cleft by Sir Duke-Elder. Although Davis stated that the iris pillars are covered with an endothelial layer and the open spaces of the iris angle and ciliary body are lined with endothelium, this does not appear to be true in our preparations. It appears to us that the iris pillars are partially covered with endothelial cells but most of the other tissue surrounding the open spaces does not appear to have an endothelial layer. For this reason we would object to using the term "lymph spaces" because this term is usually applied to spaces which are lined with endothelium and do contain lymph. We prefer the term "perivascular aqueous channels," since they are perivascular for some portion of their course and contain fluid throughout their length which is continuous with anterior chamber aqueous.

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