Meridional flow from the corona ciliaris through the pararetinal zone of the rabbit vitreous

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From the data we have obtained we are led to the conclusion that in the in vivo rabbit eye there is a stream of fluid in the pararetinal vitreous flowing posteriorly from the corona ciliaris. This flow occurs in a well-ordered pattern best described as a meridional flow pattern. Fluid moves from this stream through the retina apparently in extracellular spaces but faster and with a different pattern than is observed under conditions which presumably favor diffusion into the retina. The difference of formazan distribution in the retinal epithelium compared to the formazan staining pattern in the overlying tissue leads to the hypothesis that the retinal epithelial cells may be secreting fluid into the choroidal blood. In addition there appears to be a well-ordered unidirectional diffusion field of fluid from the corona ciliaris to all points of the retina. This field is well ordered in the sense that it is approximately parallel to the sides of a cone with a base in the plane of the equator of the lens and an apex on the optic axis near the retina. Within such a cone the movement most often observed was that which one would expect from diffusion of the tracer unaltered, by flow or unidirectional diffusion of the solvent. Both the meridional flow and the diffusion field require the integrity of the blood supply to the eye since appreciable posteriorly oriented movement of the injected tracer was not observed in eyes injected in situ in the isolated head.

Sir Stewart Duke-Elder in his monograph on "The Nature of the Vitreous Body" summarized the data then available on the origin and exit of vitreal fluid. He stated that vitreal fluid was thought to originate from the capillaries of the ciliary body or pars plana, that it "traveled backwards through the substance of the vitreous in a slow stream," and that it had an exit "by way of the optic nerve." In further discussion of this clear-cut picture he presented some data and possible arguments for the hypothesis that the choroid was about equally involved as a source of the fluid in the vitreous body and that the "slow stream" was probably "simple diffusion." He took great care to point out that "the demonstration of a free

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drainage at the optic nerve has been demonstrated almost entirely in animals, especially in rabbits." He concluded: "It thus appears that the fluid dialyses from all the vascularized tissues of the eye, more especially from the ciliary body, that it spreads through the vitreous body by diffusion, and that it finds exit by a reciprocal process of dialysation in the manner as does the aqueous humor." Except for the concept of dialysis of the aqueous humor this view of the origin of vitreal fluid has remained as the most widely accepted opinion of eye physiologists to the present time. 3 although the fact that fluid does exit via the optic nerve, at least in the rabbit, has apparently been forgotten in the intervening years.

This problem has been re-examined recently by the author. Fluid movement in the rabbit vitreous was mapped by use of tracer amounts of the histochemical reagent nitro blue tetrazolium chloride (nitro BT)4 and India ink.

**Biologic materials and reagents**

Rabbits, both pigmented and albino, from commercial sources weighing 3 to 6 pounds were used. For most experiments the rabbits were anesthetized systemically with Nembutal given intravenously, and topically with Pontocaine applied to the cornea.

Solutions of nitro BT* for injection were made by dissolving 10 mg. of the reagent per milliliter of distilled water or 0.9 per cent sodium chloride solution. India ink used for tracing the flow pattern was obtained by dissolving commercial product with 4 volumes of distilled water. The injection site was marked in some experiments with 0.2 ml of colored oil included in the nitro BT injected volume or with carbon particles.

**Methods**

Injections into the vitreous were made from a microburet through a 15 cm. polyethylene catheter which was attached to a 1 cm. section of No. 30 hypodermic needle tubing. For injections made through the cornea and lens a 2 cm. section of No. 27 needle tubing was used. To facilitate entry into the eye the bevel of the needle was sharpened to a conical point.

After anesthesia the rabbits were disturbed as little as possible. The eye was gently rotated into position and the needle inserted with slight pressure and rotation. When the needle was in position the restraints to the eye movement were removed. A delay of 30 seconds or more from the time of insertion of the needle to the beginning of the injection was regularly allowed. In these experiments 2 to 10 pl of tracer solution was used, most often the smaller volumes. The maximum rate of injection was 1 pl per 10 seconds. After the injection the needle was left in place until just before the animal was decapitated. The in vivo eye was then enucleated and the head was placed in a pan with a tight-fitting lid. The pan and contents were kept at 37° ± 2° C. by immersion in a bucket of water. After 2 to 5 minutes the second eye was injected in a manner as identical to the in vivo eye as possible, and at the end of the specified time period it also was enucleated. Following enucleation two different methods were used for subsequent examination. The enucleated eyes from some nitro BT experiments were dropped immediately into Bouin’s fixative. For the second procedure the enucleated eyes were frozen immediately in a mixture of dry ice and petroleum ether and subsequently sectioned while still frozen.

The fixed eyes were opened and examined under the stereomicroscope for observation of the distribution pattern of nitro blue formazan deposited inside the eye. Sections were prepared of some fixed eyes by a paraffin procedure modified so that the tissue was in contact with baths containing more than 50 per cent alcohol or chloroform for minimum times. Slightly shorter embedding times were also used. Preliminary alcohol treatment to remove picric acid was also eliminated. These eyes were usually sectioned through or parallel to the optic axis and, if possible, through the site of injection. In order to examine in more detail the distribution of formazan in the retina and optic nerve, serial 8 μ thick sections of appropriate eyes were cut parallel to a plane tangent to the posterior segment of the eye near the optic nerve.

Eyes which had been immediately frozen were mounted while still frozen in the cavity of a simple microtome designed especially for cutting sections of frozen eyes. The eyes were mounted so that the plane of cutting would be parallel to the optic axis. The mounting medium was 1 per cent gelatin, which was added in small increments to the frozen eye positioned in the cavity of the dry ice-cooled cutter. Photographs were taken of some of these frozen preparations when
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Fig. 1. A, Second eye from Dutch rabbit into which 2 μl of nitro BT solution (10 mg. per milliliter) was injected 5 minutes after the animal was decapitated. The eye was excised 15 minutes after the injection and was frozen and sectioned immediately. The site of injection is clearly evident from the location of the oil droplet 2.5 mm. posterior to the line joining the equator of the lens and the posterior edge of the corona ciliaris, and 2.5 mm. from the choroid in a radial direction. The mean diameter of the stained area is estimated at 3.5 mm. and appears quite spherically symmetrical. Since it takes approximately 5 minutes to develop sufficient color in the vitreous to be seen, this represents the extent of diffusion in 10 minutes in this region of the vitreous.

B, In vivo eye from the same animal, injected 2.7 mm. behind the line joining the equator of the lens and the posterior edge of the corona ciliaris, and 1.5 mm. from the choroid in a radial direction. The point of injection is the slightly darker spot at the anterior end on the choroidal side of the dark area in the vitreous. This dark area is 3.5 mm. long and 2.2 mm. wide, and fits the shape to be expected from diffusion of nitro BT in a diffusion field of the solvent. Note that the edge of this stained area facing the choroid is sharply defined by the pararetinal fluid stream in that region.

the blue formazan cloud or colloidal carbon was uncovered.

The principal parameters investigated were, first, the relationship between location of the injection site within the vitreous and the differences in distribution pattern of nitro blue formazan observed in the in vivo eyes compared to the second eye. Second, the time course of development of some of the distribution patterns observed was investigated. To study the first parameter the injections were made by entering the eyes through pars plana near the insertion of the superior rectus muscle on the temporal side, near the inferior rectus muscle, and the nasal side. In addition other injections were similarly made at intermediate locations in the plane of pars plana. In addition a series of injections at various radial distances from the pars plana were made by entering the eye near the superior rectus muscle and also through the temporal side. Also the effect on the distribution pattern of nitro blue formazan resulting from injections made by entering the eye through the cornea and lens was investigated. The effect of making the injection...
Fig. 2. A, Second eye, injected under conditions the same as in Fig. 1 but in the retrolental zone. Although the pattern is distorted due to a movement of the needle during the injection it is clearly evident that otherwise diffusion was the principal force distributing the nitro BT.

B, In vivo eye from the same animal. The pattern again is that to be expected for diffusion, except for the thin line of stain posterior to the main body, which was caused by a movement of the needle when the animal jumped shortly after the injection was completed. Movement in the fluid diffusion field of Zone II (Fig. 4) from the small injection at the end of this line is clearly evident.

Results

All tissues of the eye including the vitreous body reduce nitro BT to an insoluble blue formazan which binds to protein at the site of deposition and which also survives the fixing and sectioning procedures. The formalin in the fixative or freezing effectively stops the deposition of formazan. Thus nitro BT is an effective tracer since the path from origin to most distant site is clearly marked with formazan deposition.

In all experiments in which nitro BT was injected into the vitreous outside a central retrolental cone-shaped zone, there was obviously greater deposition of formazan posterior to the point of injection in the in vivo eye than in the second eye. The amount of apparent posterior movement was dependent upon the radial distance from the sclera. When nitro BT was injected 2 mm. or closer to the retina or pars plana, it was swept posteriorly in a meridional flow pattern. Blue formazan was found to stain that retina immediately posterior to an injection at or near the
corona ciliaris and as far back as the posterior pole and in as little as 15 minutes after injection. Outside the central retrolental zone but in the region between it and a 1.5 to 2 mm. wide pararetinal zone at the periphery of the vitreous, the reagent moved as though in a diffusion field of solvent moving by the shortest pathways from the corona ciliaris to all points of the retina (Fig. 1). In all experiments in which nitro BT was injected outside the central retrolental zone of the vitreous, the apparent posterior movement in the in vivo eyes was greater than the reagent could have diffused in the time allotted. But in the second eyes, regardless of the site of injection, the distribution pattern found was essentially that which one would expect from diffusion alone. In the central retrolental zone of the vitreous for experimental times shorter than ¾ hour the distribution of formazan in both the in vivo and second eyes was essentially identical and in the pattern one would expect to

Fig. 3. A, Posterior chamber injection. The oil droplet marking the site of injection was found resting on the tertiary vitreous—posterior chamber interface inside the posterior chamber about midway between the sclera and the lens. This is the in vivo eye of an albino rabbit 15 minutes after injection of the nitro BT solution (3 µl, 10 mg. per milliliter). No stain is visible on the iris anterior to the corona ciliaris nor was any found on this structure in the microscopic sections prepared from the eye. Although the vitreous in the pararetinal zone is clearly stained 3.8 mm. in a meridional direction posterior to the tertiary vitreous in the 1 mm. wide pararetinal zone, it is stained only 1.2 mm. posteriorly near the lens. On examination of the microscopic sections formazan-stained particles were found in the retina 9 mm. posterior to the tertiary vitreous along a meridional line.

B, Second eye from same animal with point of injection into posterior chamber posterior to midpoint of corona ciliaris. More movement anteriorly is evident and no stained particles were found in the retina posterior to the equator.
result from diffusion alone (Fig. 2). For experimental times longer than one hour after injection into the central retrolental zone of the vitreous, formazan was found to stain the retina in the posteriormost region of the eye but only in the in vivo eyes.

In no experiment was formazan found anterior to the corona ciliaris if the injection was made posterior to the median plane of this structure (Fig. 3). However, if injections were made about at the mid-point of the corona ciliaris, formazan was found anterior and posterior to the point of injection. More nitro BT seemed to move posteriorly in this situation. A schematic summary of these results is given in Fig. 4.

The patterns obtained were found to be independent of the amount injected (within the range of volumes used), the circumferential location (at the equator) of the injection, the position of the rabbit at time of injection, or movement of the eye subsequent to injection. The patterns were also essentially the same if the injection was made by entering the vitreous through the cornea and lens or through the sclera at the pars plana or more posteriorly.

In the serial sections made of some of the formazan-stained retinas it was possible to find formazan-stained particles at all levels of the retina out to and including the retinal epithelium. It was noted that in most layers of the retina the stained particles were usually found at the places where three or more cells joined and were just inside the cell membranes of all cells at such junctures. The outer segments of the rods and cones were quite free of stain, but occasionally some isolated stain was found in this layer. In contrast to the stain of the inner layers of the retina the retinal epithelial cells had stained particles in all parts of the cell or none at all. It was also noted that areas of the retina with minimum formazan, such as at the leading edge of the staining pattern, were stained in such a way that formazan found deep in the retina was contiguous with the partially stained overlying cells even though there were unstained cells lying between stained cells in a lateral direction. Fifteen to twenty minutes contact with nitro BT solution in vivo was sufficient time for all cells to stain quite uniformly in a given layer even though all layers did not stain the same. From our observations it thus appears that the nitro BT solution moves through the retina in somewhat discrete passageways apparently in extracellular spaces. It is also noteworthy that the amount of formazan found in the retinas of the in vivo eyes was invariably greater than in the second eye at comparable locations. Formazan was found in the retinal epithelium with minimal formazan deposition in overlying structures only in the in vivo eyes. In the second eyes or in isolated eye pieces
the retinal epithelium was found to be stained only if all the overlying tissues were also quite uniformly stained.

The possibility of rotation or streaming of the vitreous as a mechanism for the observed distribution can be discounted on the following grounds: First, there was no difference in distribution between otherwise identically treated eyes if one was deliberately rotated while the other was carefully not rotated. Second, the oil or carbon marker was invariably found at the expected site of injection.

In all experiments in which nitro BT was injected into the segment of the eye-ball which contains the optic nerve, blue formazan was found in the perivascular spaces of the optic nerve. It should perhaps be noted that the walls of the blood vessels in the optic nerve were never found to be stained nor was formazan found posterior to the place where the retinal artery leaves the optic nerve. This finding is consistent with all older reports on the movement of fluid from the vitreous via the optic nerve. However, it did not appear that the optic nerve provided a major exit route.

In addition it was noted that nitro BT injected near the lens but off the optic axis was moved into the lens through the posterior suture line and anteriorly inside the lens along the cortical fibers.

Discussion

Some authors have been puzzled by the fact that the retina, with a high metabolic rate especially when light adapted, has such a meager blood supply. In the rabbit there are few if any blood vessels within the retina itself. It has also been noted that if metabolites diffuse into the retina from choroideal blood and if the metabolic products diffuse from the retina into the choroideal blood, the distance each must diffuse is inconsistent with the high metabolic activity of the retina. The findings reported here that a vitreal fluid moves readily through the retina then apparently into the choroideal blood provides an explanation of this enigma at least for the rabbit. This finding also provides sufficient explanation for the observation that the concentration of hyaluronic acid in the vitreous is larger nearer the retina, since a radial component of flow near the retinal surface of the vitreous could result in a pile up of hyaluronic acid molecules there. Similarly the observation that vitreal collagen fibrils in the pararetinal region are oriented parallel to the surface of the vitreous while the collagen fibrils near the center of the vitreous are randomly oriented could be due to fibril orienting forces in a meridional flow pattern.

The absence of anatomical structures penetrating the pigmented epithelium which suggest outflow channels would require a secretory function for this cell layer. Such a function has been suggested previously by Bernstein on the basis of his study of electron micrographs of the retinal epithelium. It was not clear from his discussion if secretion would necessarily occur in the direction he assumed. From the studies reported here, it would of necessity be in the vitreal to choroideal direction.

Historical notes

During the years between 1863 and 1925 at least 30 papers appeared which were concerned with the problem of the movement of fluid in the vitreous. These papers are about equally divided between the reports of those who experimented on various animals and man in vivo and those who used excised eyes for their experiments. It is interesting to note that except for one paper all those who experimented on eyes in vivo concluded that there was a posterior movement of fluid through the vitreous. The one paper which states the opposite conclusion deserves special comment, which will be given later. It is equally interesting and highly significant that the conclusion of no flow in the vitreous was reached by all those who experimented with excised eyes and that the most often cited reviews were also written by those who experimented with excised eyes.
It is also unfortunate that most of these earlier workers who concluded that there was posterior movement of fluid were concerned with lymph flow and that even when all the data did not support it, they erroneously concluded that the flow was down Cloquet's canal. This conclusion arose apparently because these earlier workers generally examined the eyes 1 to 2 days after injections were made into the vitreous.

A great deal of weight has been given to the experiments of Ovio, who injected strychnine into the vitreous of some animals and the aqueous of others and observed that after injection into the anterior chamber the rabbit showed immediate tetany while there was about an hour's delay between the injection and tetany in animals with strychnine injected into the vitreous. It seems pertinent to comment that even these experiments would support the author's data if, as is highly probable, Ovio made the injections retrolentally near the center of the vitreous. Also because the anionic vitreous gel would be expected to retard the diffusion of the cationic alkaloid, a point which seems to have been entirely overlooked, the wonder is that the vitreally injected animals ever developed tetany. One would suspect that such animals that did develop tetany did so because the injected material leaked out of the vitreous along the needle path.

Ulrich in some experiments in which he injected diluted India ink into the vitreous found that some of the pigment was in the perivascular spaces of the optic nerve when he killed the animals 24 to 48 hours later. He also reported that some of the pigment was found in the retina although he noted that the vitreous near the retina had been cleared of carbon in the hours after the injection. Ulrich also used his observation that India ink was found staining the zonules and some of it was moved posteriorly into the vitreous along the retina following injection into the posterior chamber as support for his theory that the iris was the source of part of the aqueous and vitreal fluids.

As part of a study of the movement of radioactive sodium into the eye, von Sallmann in 1949 reported that in all experiments in which this tracer was injected into the vitreous of living rabbits' eyes the tracer moved posteriorly from the point of injection more rapidly than it could by diffusion alone. In excised eyes this posterior movement was not observed.

Maurice in 1957 reported that Na-24 injected into the "center" of the vitreous appeared in the anterior chamber of the injected eye in higher concentration than in the contralateral eye. He concluded that this occurred because the labeled sodium diffused through the vitreous into the posterior chamber although he offered no experimental proof of this hypothesis nor did he exclude with experimental evidence all other possible pathways from vitreous to anterior chamber.

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