that the binding sites are saturable, characteristic of steroid receptor binding. The nuclear localization of \( ^3 \)H-dexamethasone is probably the result of binding to the cytoplasmic glucocorticoid receptor with subsequent translocation to the cell nucleus during incubation (30 C for 45 minutes), as is seen in virtually all steroid hormone target cells.\(^9,10\)

The lack of nuclear localization of \( ^3 \)H-dexamethasone in the scleral fibroblasts of the posterior segment of the eye, as opposed to those adjacent to the outflow pathway, suggests a functional difference between these two groups of scleral cells. Since this is a single observation, however, it requires confirmation with additional specimens.

The present study demonstrates the presence of glucocorticoid target cells in the outflow region of both glaucomatous and nonglaucomatous eyes. Additional studies will be required in order to determine whether or not there are disease-related differences in the distribution of glucocorticoid binding sites. It is clear, however, that this autoradiographic technique is applicable to small surgical specimens and will permit the testing of various competitors of glucocorticoid binding directly in the human tissue involved in the outflow of aqueous humor. Some of these competitors may be glucocorticoid antagonists of potential use in the clinical management of POAG.

Key words: glucocorticoids, target cells, glaucoma, autoradiography

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References


Coagulation Properties of Intraocular Humors and Cerebrospinal Fluid


Clinical observations indicated a prompt cessation of episcleral capillary bleeding after exposure of those tissues to aqueous humor during intraocular surgery. Based on this observation, both aqueous and vitreous humors were obtained from eyes provided by an eye bank and from human eyes prior to intraocular surgery. Samples of cerebrospinal fluid also were obtained from two patients, one with optic atrophy and another with nutritional amblyopia. A total of 49 specimens were studied. Aqueous humor shortened the average ear lobe puncture bleeding time from 4.74 ± 0.2 to 1.13 ± 0.1 minutes. Varying dilutions of aqueous humor, up to a dilution of 1:16, shortened prothrombin and partial thromboplastin
times when compared with normal control plasma without aqueous. Vitreous humor shortened partial thromboplastin time but had no effect on prothrombin time. The effect of cerebrospinal fluid on prothrombin and partial thromboplastin time but had no effect on prothrombin time. The effect of cerebrospinal fluid on prothrombin and partial thromboplastin times was similar to the aqueous, but to a lesser degree. Invest Ophthalmol Vis Sci 24:1616-1619, 1983.

The iris is a heavily vascularized tissue, yet when incised, it neither bleeds nor heals. The physiologic mechanism of these unusual properties of the iris is not known.

Recently, during the course of intraocular surgical procedures, we noted rapid cessation of episcleral capillary bleeding after exposure to aqueous humor. Irrigation of the same area with balanced saline salt solution prior to opening the anterior chamber did not change the course of bleeding. These clinical observations prompted us to evaluate the coagulative properties of aqueous and vitreous humors, as well as cerebrospinal fluid.

Materials and Methods. Both aqueous and vitreous humors were obtained from eye bank eyes (13 samples of each) and also aqueous humor from otherwise healthy human eyes prior to cataract surgery (21 specimens). In each case, care was taken not to traumatize the iris or the lens. We also obtained a sample of cerebrospinal fluid (CSF) from each of two patients, one with optic atrophy of unknown etiology and the other with nutritional amblyopia. Thus, a total of 49 specimens was obtained for evaluation of coagulation properties of these fluids. The following clinical and laboratory tests were performed.

(1) Bleeding time. This study was done with volunteer patients after we obtained informed consent according to the Helsinki Declaration for Human Subjects.

Using the Duke technique,1 bleeding time was measured for nine patients (five men and four women). This technique was used because it is a standardized technique in this institute for 30 years. Immediately after ear lobe puncture, two drops of the patient's own aqueous humor were applied to cover the area. The puncture site was blotted every 30 seconds with filter paper until bleeding had stopped. The time was recorded. Using balanced saline salt solution as the control, bleeding time was measured similarly on the patient's opposite ear lobe.

(2) Prothrombin and nonactivated partial thromboplastin time. To test the capacity of the aqueous humor, vitreous humor, and CSF to alter the coagulation time of blood, the prothrombin time (PT) and nonactivated partial thromboplastin time (PTT) were employed.1 Although similar results were obtained initially by either activated or nonactivated partial thromboplastin time, we preferred selecting nonacti-

Table 1. Effect of aqueous humor on bleeding time

<table>
<thead>
<tr>
<th>Patients and Sex</th>
<th>Number of</th>
<th>Right ear lobe</th>
<th>+2 gtt saline</th>
<th>+2 gtt aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right ear lobe</td>
<td>Left ear lobe</td>
<td>Right ear lobe</td>
<td>Left ear lobe</td>
</tr>
<tr>
<td></td>
<td>9 (5 M, 4 F)</td>
<td>4.74 ± 0.2</td>
<td>1.13 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

vated partial thromboplastin time. This selection was because activated PTT depending upon the activators employed (ellagic acid, diatomaceous earth, etc.) could actually fail to detect low levels of factors XII and XI. Using either of these techniques, the fluid to be tested was mixed with the reagents (including plasma) that are employed normally, in a 1:1 v/v ratio. The control employed a volume of 0.85% NaCl instead of the humor. The saline served as a control for the dilution effect of the fluid being tested. The endpoints of the time of clot formation using the saline control and the system containing the humor were compared. To identify the effectiveness of various concentrations of the clot-promoting activity in the various test fluids being studied, serial dilutions were examined, maintaining identical volumes in the test system and the controls.

(3) Coagulation factors. Fibrinogen quantification was performed by the method of Clauss2 and Ratnoff-Menzie.3 Fibrinogen antigen was described as previously by Karp and Bell.4 Fibrinogen-fibrin degradation products were determined by the tanned red cell hemagglutination immuno-inhibition assay.5 Kallikrein, prekallikrein, and high-low molecular weight kininogen were determined by the technique described by Alving et al.6 Employing known specifically deficient substrate human plasmas, coagulation factors, I, II, V, VII, VIII, and X were assayed in pooled samples of the aqueous humor employing a one-stage technique, according to the method described by Hardisty and MacPherson.7 Factor VIII:Ag in aqueous humor was measured employing a monospecific antibody to VIII:Ag in the Laurell technique.8 Platelet aggregation studies employing aqueous humor were performed in a platelet aggregation profiler (Bio Data Corp, Broomall, PA) according to the technique described by Triplett.9 In performing platelet aggregation studies, platelets that had been identified to aggregate normally to ADP, epinephrine, collagen, and ristocetin were employed. Using this normal platelet-rich plasma, increasing amounts of aqueous humor were added (0.1 ml–0.5 ml) to the 0.45 ml of platelet-rich plasma.

Statistical analyses were performed employing standard methodology described previously.10

Results. (1) Bleeding time. Aqueous humor shortened the average bleeding time from 4.74 ± 0.2 to 1.13 ± 0.1 minutes in the nine patients tested (Table
Table 2. Effect of aqueous, vitreous, and CSF on blood clotting time*

<table>
<thead>
<tr>
<th>Test (body fluid)</th>
<th>Control (saline)</th>
<th>1:1</th>
<th>1:16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous (34 specimens)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>15.4 ± 1.6</td>
<td>12.0 ± 0.3</td>
<td>13.8 ± 0.6</td>
</tr>
<tr>
<td>Partial thromboplastin time</td>
<td>78.7 ± 1.8</td>
<td>56.0 ± 1.4</td>
<td>70.7 ± 1.6</td>
</tr>
<tr>
<td>Vitreous (13 specimens)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>15.4 ± 1.6</td>
<td>17.3 ± 0.4</td>
<td>16.2 ± 0.5</td>
</tr>
<tr>
<td>Partial thromboplastin time</td>
<td>78.7 ± 1.8</td>
<td>64.4 ± 3.0</td>
<td>68.8 ± 5.0</td>
</tr>
<tr>
<td>CSF (2 specimens)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>15.4 ± 1.6</td>
<td>12.5 ± 0.5</td>
<td>15.8 ± 0.8</td>
</tr>
<tr>
<td>Partial thromboplastin time</td>
<td>78.7 ± 1.8</td>
<td>62.5 ± 1.5</td>
<td>79.6 ± 3.0</td>
</tr>
</tbody>
</table>

* Clotting time is given in seconds.

1). The effect was similar in both men and women. The degree of shortening was significant with \( P < 0.001 \). Fear of transmitting any disease process prevented us from using the aqueous humor of one patient to test another.

2) Prothrombin and nonactivated partial thromboplastin time. Aqueous humor and its varying dilutions, up to a dilution of 1:16, shortened both the prothrombin and nonactivated partial thromboplastin time (significant with \( P < 0.01 \)) when compared with normal control plasma without aqueous humor (Table 2, and Figs. 1 and 2). There was no difference between the shortening effect of aqueous humor obtained from eye bank eyes and those obtained from human eyes during surgery. Vitreous humor shortened partial thromboplastin time but did not affect prothrombin time (Table 2). The effect of CSF on prothrombin and thromboplastin times was similar to that of aqueous humor, but to a lesser degree.

None of these test fluids contained any measurable quantities of fibrinogen, fibrinogen antigen, fibrinogen-fibrin degradation products, factors I, II, V, VII, VIII:C, VIII:Ag, or X. Aqueous humor did not induce platelet aggregation even at the 0.5-ml concentration. Our studies revealed that the clot-promoting activities of aqueous humor were not from kallikrein, prekallikrein, or high-low molecular weight kininogen.

Discussion. Our clinical observations on the rapid cessation of episcleral capillary bleeding after exposure to aqueous humor were supported quantitatively by shortening of bleeding time, prothrombin time, and partial thromboplastin time by aqueous humor, as compared with control tests with normal saline.

The basic hemostasis mechanism consists normally of vascular spasm, platelet aggregation, and blood coagulation. Generally, these systems begin acting immediately after laceration of vascularized tissue, and are followed by growth of fibrous tissue within the clot to promote a permanent seal of the bleeding site.

Tests of bleeding time will measure the combined effect of the three main components of hemostasis. Aqueous humor applied topically at the puncture site markedly shortened the bleeding time, as compared with application of normal saline on an identical puncture of the patient’s contralateral ear lobe. Aqueous humor may affect any one of these components of hemostasis. Not knowing the effect of aqueous humor on vascular spasm at the present time, we cannot speculate if this component of hemostasis was altered by the aqueous humor in shortening the bleeding time. We have tested the effect of this humor on blood coagulation and platelet aggregation.

Our results on the effect of aqueous humor and two samples of CSF on prothrombin time and nonactivated
thromboplastin time have revealed a substantial hastening of blood coagulation, especially when comparing those times for aqueous versus normal saline.

Blood coagulation is the end product of a series of chemical interactions of coagulation-promoting factors (the "intrinsic" and "extrinsic" pathways). Prothrombin time will measure the activities of factors I, II, V, VII, and X. Partial thromboplastin time will determine the integrity of the entire coagulatory scheme (with the exception of factors VII and XIII), particularly factors VIII, IX, XI, and XII. Although none of these tests can determine the exact nature of the "site" of activity of aqueous humor or of CSF on clot promotion, our preliminary studies revealed that the effective factor was not factors I, II, V, VII, VIII:C, VIII:Ag, X, kallikrein, prekallikrein, or high-low molecular weight kininogen acting in the intrinsic pathway. The active agent may possibly be a tissue thromboplastin or thromboplastin-like substance. This also was suggested by Skelly and Binder to be present in vitreous body.11

We could not identify an effect of aqueous humor on platelet aggregation. The aqueous humor in humans does not contain any fibrinogen, which is in contrast to buphthalmic rabbits.12

The effect of vitreous humor or of CSF on platelet function or the vascular wall cannot be ascertained selectively with the tests performed. Prothrombin time was lengthened slightly in 1:1 ratio by the vitreous. This effect was no longer apparent in 1:16 dilution. The PTT was shortened in 1:1 ratio although less apparent, and still could be detected in 1:16 dilution. These findings suggest a mechanism other than a tissue thromboplastin as an effective agent, although we cannot exclude its presence. The number of samples of CSF was too small to arrive at a meaningful conclusion. About half of the samples shortened the PT similar to aqueous humor in 1:1 ratio. This effect was not apparent in 1:16 dilution. The PTT was shortened to a lesser degree than aqueous humor in 1:1 ratio. This effect was mild and disappeared in 1:16 dilution.

High concentrations of ascorbic acid in normal aqueous humor cannot explain its clot-promoting activity. If anything, ascorbic acid will decrease the pH of the mixture being tested, and reduction in pH exerts an inhibitory effect on coagulation of fibrinogen. Since the experiments were done in the presence of excess calcium concentration, this excludes the role of calcium in aqueous or vitreous humor, as suggested by Skelly and Binder.11

Lack of bleeding from an iris wound can play an important role in failure of iris wound healing. The fibrin mesh of clotted blood usually acts as a "ground substance" for the invasion by capillaries and fibroblasts necessary for wound repair. Absence of the fibrin mesh in an iris wound, plus the continuous flow of aqueous humor, frequent motility of iris tissue, and the direct inhibition by aqueous humor on cell proliferation,13,14 are the factors to be considered in explaining the absence of wound healing in iris tissue.

Further studies are required to identify the exact nature of clot-promoting activity of intraocular fluids and CSF. The effect of these fluids on vascular wall needs to be similarly investigated. The presence or absence of this activity in patients with various blood coagulation disorders, and in patients with various ocular disease processes that cause intraocular hemorrhage or neovascularization, clinically may be very important.

Key words: aqueous humor, vitreous humor, cerebrospinal fluid, blood coagulation, bleeding time, prothrombin time, partial thromboplastin time, iris wound repair

References