Protein permeability of the ciliary epithelium of enucleated arterially perfused cat eyes was studied. Tracer concentrations of 125I-albumin, in an arterial perfusate solution consisting of a balanced salt solution (BSS) without added colloid, was found in the anterior chamber fluid (AH) in a percentage similar to the AH/plasma ratio found for protein in the living cat eye. Stimulation of AH formation by acetylcholine plus eserine increased protein permeability markedly and by a similar amount in eyes perfused either with BSS or with 100% serum. These data indicate that arterial perfusion of the enucleated eye with BSS alone induces no gross abnormality of the blood-aqueous barrier. (INVEST OPHTHALMOL VIS SCI 22:478-481, 1982.)

Key words: aqueous humor, protein, permeability, cat, eye, enucleated, perfused

We have published a number of reports on the physiology and pharmacology of aqueous humor (AH) production with data obtained from enucleated arterially perfused cat eyes. From these data a model of mechanisms affecting AH production has been proposed. In this model system, concepts have been advanced that AH is probably formed by a process of ultrafiltration. Additionally, drugs and physiologic mechanisms have been described that increase or decrease the rate of AH formation by stimulation or blockade of two distinct intraocular neuronal pathways.

The data obtained from these isolated eye experiments may be subject to at least two forms of criticism: (1) the eyes were enucleated and were therefore no longer under neural or cardiovascular control, and (2) the vascular perfusion of the eyes was accomplished with an isotonic balanced salt solution (BSS) that contained no colloid (protein) to duplicate osmotic or viscosity effects present in vivo.

It was because of the first criticism that we undertook the development and use of this enucleated eye preparation. It was intended to study the eye free from all possible modifications introduced by either the central nervous system or by an intact cardiovascular system. The use of an arterial perfusate, without colloid, may indeed be injurious to the eye by altering the permeability of the ciliary body or by abnormally influencing the neuronal receptors. Because of the increasing interest in this preparation and the conclusions resulting from its use, we believed it important to determine its physiologic status during the arterial perfusion procedure with BSS.

Initial studies were made to ascertain whether the permeability of the blood/AH barrier had been affected. This was done by comparing results obtained during perfusion...
Table I. Protein permeability of the ciliary body

<table>
<thead>
<tr>
<th>No. of eyes</th>
<th>Distribution ratio (AH/plasma)</th>
<th>p value</th>
<th>Group vs. control</th>
<th>Between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact living eye</td>
<td>18</td>
<td>0.037 ± 0.009</td>
<td>&gt;0.3</td>
<td></td>
</tr>
<tr>
<td>Enucleated eye</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>0.022 ± 0.004</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>AChB + Es</td>
<td>6</td>
<td>0.477 ± 0.091</td>
<td>&lt;0.001</td>
<td>}</td>
</tr>
<tr>
<td>AChC + Es (100% Serum)</td>
<td>4</td>
<td>0.301 ± 0.055</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean ± S.E.; all enucleated eyes were arterially perfused with BSS, except as noted.
ACh 0.01 ng/ml. All other drug concentrations were 10.0 µg/ml.
ACh 10.0 µg/ml.

Materials and methods

Cats of either sex were anesthetized with pentobarbital sodium (30 mg/kg, intraperitoneally) and were subsequently given an intracardiac injection of 1000 units of sodium heparin. In experiments dealing with isolated cat eyes, the animals were given a subsequent lethal dose of the anesthetic agent. The eyes were immediately enucleated deep within the orbit and placed in oxygenated perfusate solution. The procedure for the arterial perfusion of the whole eye has been reported. No ligatures were placed around the insertion of the optic nerve to prevent blood flow to the retina and choroid. Briefly, the eyes were perfused with warmed (37° C) BSS (Eagle's Basal Medium) or 100% serum (fetal calf or cat) through the ophthalmic artery. Both perfusate media were oxygenated and maintained under an atmosphere of 95% oxygen and 5% carbon dioxide, pH of 7.4.

The rate of AH turnover (Kout) was measured by following the decay of intracamerally injected (one bolus) of 125I-labeled human serum albumin with a gamma probe. Calculations of AH formation were made by multiplying the Kout/min of the radioactive albumin by a presumed anterior chamber volume of 900 µl.

The procedure for enucleated eye experiments in which the distribution ratio (AH/plasma) was calculated were as follows: no needles were placed in the eye so that trauma would be held to a minimum. The eyes were arterially perfused with BSS for a 30 min stabilizing period, and the perfuse flow was altered to a bottle containing the selected perfusate with or without trace amounts (20 µCi/100 ml) of 125I-labeled human serum albumin.

Exactly 120 min later the perfusate flow was stopped and the anterior chamber fluid was aspirated with a 1 ml syringe with a 25-gauge needle. AH and perfusate solutions were sampled and all counts for radioactivity were made in triplicate. Scintillation counting was done with a Nuclear Chicago (Model 6850) Unilux counter. The specific activity of the AH samples were divided by the specific activity of the arterial perfusate samples to give the AH/plasma distribution ratio.

Cats used for in vivo protein determinations were given a lethal dose of anesthetic and AH was immediately aspirated. The protein concentration was determined by the Folin phenol method.

Concentrations of drugs are expressed as the salts.

Statistical analyses were performed with the Student's t test for paired or unpaired data. Results were considered significant at p < 0.05.

Results

Protein distribution ratios (AH/plasma)
Intact cat. In 18 eyes of nine cats the protein concentration of previously unmedicated and untouched eyes was found to be 274.44 mg/100 ml ± 68.47 (S.E.M.), with a range between 39 to 987 mg/100 ml. The average AH/plasma ratio at steady state for the normal intact cat eye was calculated to be 0.037 ± 0.009 (S.E.M.) (Table I).

Enucleated eyes
ARTERIAL PERFUSION WITH BSS

(1) Control experiments. The AH/plasma protein distribution ratio of these eyes was determined by the use of tracer amounts of 125I-labeled human serum albumin (RISA). Preliminary experiments in which eyes had been perfused for 1, 2, 5, and 6 hr indicated...
that protein concentration in the anterior chamber was at steady state by 2 hr. A group of eight eyes was therefore perfused 2 hr with a BSS arterial perfusate containing RISA. A mean AH/plasma distribution ratio of 0.022 ± 0.004 (S.E.M.) was obtained (Table I). This value is not significantly different (p > 0.3) from the 0.037 value calculated for the intact living eye.

2. Effects of acetylcholine (ACh) plus eserine (Es). ACh (0.01 ng/ml) + Es (10 μg/ml) induced an AH/plasma RISA ratio increase to 0.48, approximately 20-fold higher than control values (Table I).

**ARTERIAL PERFUSION WITH 100% FRESH FETAL Calf Serum**

1. Effects of ACh + Es. An ACh concentration of 10 μg/ml, together with Es (10 μg/ml), was necessary to produce a consistent increase in AH production. After a 2 hr perfusion period with these drugs the protein concentrations in the anterior chamber was determined by the Lowry procedure. A mean AH/plasma ratio of 0.30 ± 0.055 (S.E.M.) was obtained in four eyes. This value is 40% less than the 0.48 value found when BSS alone was used, but the difference is not statistically significant (p > 0.2) (Table I).

**Discussion**

The pharmacology and physiology of AH production have been studied for almost 20 years using enucleated arterially perfused cat eyes. Early experiments indicated that vascular responses of the anterior segment of the eye were not perceptibly influenced by the presence of 5% to 10% serum in the BSS perfusate medium. Therefore most of the data subsequently reported for the whole eye have been obtained from eyes arterially perfused with BSS containing no serum, or in one report with BSS containing 4% dextran. The question remains as to whether the eye, in an environment without protein, is in a normal physiologic state or whether observed responses are modified by an induced intraocular membrane abnormality.

Protein concentration in the AH of the normal cat eye is much higher than that of the human, monkey, or rabbit. In the experiments reported here, a normal mean concentration of 274 mg/100 ml was found, which is in agreement with the approximate value of 400 mg/100 ml reported by Oppelt. Blood plasma of the anesthetized cat contains 7500 mg/100 ml protein; therefore a mean AH/plasma distribution ratio calculated for normal in situ eyes centers around 0.037. Perfusion of the enucleated eye with BSS that contained only trace amounts of RISA produced a steady-state albumin, AH/plasma distribution ratio of 0.022. These in vivo and in vitro data are quite similar and the difference is not statistically significant (p > 0.3).

Stimulation of the AH inflow system by ACh2 produced, in the eyes perfused with BSS alone, a RISA AH/plasma distribution ratio of 0.48. This value is 20 times greater than prestimulus values and is qualitatively similar to results obtained by N III stimulation in the intact rabbit. The comparison of the BSS-perfused eye, stressed with ACh, was next compared with eyes perfused with 100% serum under identical conditions. The AH/plasma distribution ratio for protein in these experiments was found to be 0.30, which was not statistically different from the value of 0.48 obtained with the BSS-perfused eyes.

These data would indicate no gross abnormality in the permeability characteristics of the ciliary body resulting from the enucleation of the cat eye and its arterial perfusion with BSS.

We thank both Dr. Gerald Chader and Mr. Theodore Fletcher for having performed the protein analyses for us.

**REFERENCES**

4. Lowry OL, Rosebrough NJ, Farr AL, and Randall RJ:


