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The pressure and volume dependence of the rate of wash-out in the bovine eye*

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ABSTRACT

The rate of increase of outflow facility (the wash-out rate) was measured in bovine eyes at 6 and 15 mm Hg. The time-rate-of-change of facility was less at 6 mm Hg (0.20: Δfacility/hour) than at 15 mm Hg (0.44). However, when the data was analyzed as a function of volume passing through the outflow system, the volume-rate-of-change of facility was the same at 6 (0.35: Δfacility/ml) and 15 mm Hg (0.34). This was consistent with the hypothesis of macromolecules "washing-out" of the aqueous outflow system, if these macromolecules were saturable in the perfusate.

When aqueous humor outflow facility is determined by perfusion methods in live (1) or enucleated eyes (non-human) (2), a steady increase in measured outflow facility with time is observed. This increase is known as the "wash-out effect". The cause of this flow-induced facility increase has not been established but has been postulated to result from a "washing-out" of extracellular material (2) (such as glycosaminoglycans (3) or proteins (4)) from the aqueous outflow pathway. Recent studies, however, have shown minimal evidence of "wash-out" of glycosaminoglycans (5). In this study, we have examined the wash-out effect at two different perfusion pressures (6 and 15 mm Hg), and have investigated the relationship between the wash-out rate and the volume of perfusate passing through the aqueous outflow pathway. Our results are consistent with the hypothesis of macromolecules "washing-out" of the aqueous outflow pathway.

Enucleated bovine eyes from two-week old calves were obtained from a local abattoir (Arenas & Sons, Hopkinton, MA) and transported in ice-saline. Perfusions were performed, beginning within eight hours postmortem, using Dulbecco's phosphate buffered saline (Life Science Technologies Inc., Chagrin Falls, OH) with 5.5 mM glucose added. A total of 21 eyes were perfused at either 6 or 15 mm Hg using a computer controlled syringe pump (Model 944, Harvard Apparatus Co., South Natick, MA). The eyes were placed into a gauze-filled beaker, wetted until the cornea was just covered with saline, and then placed into a constant temperature bath set to a temperature of approximately 33°C. Anterior chamber deepening was prevented by placing the perfusion needle in the posterior chamber or by placing an iridotomy in the iris.

The experiments were conducted such that the perfusions at 6 mm Hg were typically longer (4–8 hours) than those at 15 mm Hg (3-4 hours) in an attempt to perfuse the same total volume of fluid through the eyes. Typically 2-4 ml of perfusion fluid passed through the outflow pathway of the low pressure eyes while 3-5 ml passed through the high pressure eyes. Pressure and flow were monitored continuously throughout the experiment.

For data analysis, an average facility was determined for sequential time periods that varied between 2 and 5 minutes, depending on the total time of perfusion. The initial data during the equilibration period (30–120 minutes at 6 mm Hg; 10–45 minutes at 15 mm Hg) were rejected. The facility data were then fitted, either as a function of time (t) or volume that had passed through the perfusion system (V), as

$$c(t) = a_0 + a_1 t + a_2 t^2$$

or

$$c(V) = b_0 + b_1 V + b_2 V^2$$

with a_1 and b_1 the initial slopes representing the time or volume rates of wash-out, respectively. Correlation coefficients (r) were always greater than 0.97 and usually greater than 0.99.

Data from 3 eyes were rejected as too noisy to get a reliable slope, from one eye because of pump error, and from one eye (6 mm Hg) because its initial slopes (a_1 and b_1) were 4 standard deviations different from the mean value of that group (in fact, this eye had a wash-out rate that was greater even than any eye in the 15 mm Hg group).

Typical results are shown in Fig. 1 comparing a pair of eyes,

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