J. Pharm. Pharmac., 1975, **27**, 919–922 *Received June* 2, 1975

Cornea preparation for *in vitro* biopharmaceutical evaluation of ophthalmic dosage forms

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A system has been developed for a specific biopharmaceutical purpose: testing, for ophthalmic preparations, the influence of formulation on drug transport through the cornea. The apparatus is a lucite cell, divided in two compartments by a clamped rabbit cornea. Physiological conditions are ensured by a supply of oxygenated perfusion medium. They are monitored by electrical conductivity and corneal thickness measurements. Reliability of the system was tested in a set of experiments.

The intraocular transcorneal penetration of various drugs of ophthalmological interest appears fundamental in current therapeutics. Although the physiological behaviour of the cornea *in vitro* has been extensively investigated (Donn, Maurice & Mills, 1959; Ehlers & Ehlers, 1966; Green & Otori, 1970; McCarey, Edelhauser & Van Horn, 1973; Maurice, 1972), the basic biopharmaceutics of transcorneal drug transport is still not well understood. Our general purpose is to investigate the influence of physico-chemical factors. In this paper, we describe the system used for *in vitro* maintenance of the cornea and present experimental evidence of its reliability.

STRUCTURE AND FUNCTIONS OF THE SYSTEM

Cell and corneal clamp. The principal component of the system is a lucite cell composed of two chambers: the lower one (1·10 ± 0·05 ml) is bounded by the corneal endothelium and the bottom of the clamp. This clamp has a screwed ring, holding the cornea between two joints with lateral stop-pins. In this way, when the ring is screwed on the corresponding thread, a vertical pressure is exerted on the scleral edge of the corneal sample, preventing the tissue from slipping out of the clamp. The upper chamber (15 × 40 × 60 mm, volume 36 ml), where the dosage forms to be tested are introduced, is on the epithelial side. It is closed by a cover fixed with four screws. A hole bored into this cover allows the chamber to be filled with the test solution or suspension. An optical glass window faces the epithelial side of the perfused tissue and allows direct slit-lamp examinations and corneal thickness measurements. A magnetic stirrer ensures adequate stirring in the upper chamber, when preparations tending to sedimentation are tested. Two silver electrodes, one in the cover of the upper chamber and the other on the bottom of the lower chamber are used for monitoring transcorneal electrical conductivity. The whole cell is under adequate and accurate thermostatic control (Fig. 1).

Perfusion medium supply. The perfusion medium reservoir, a 30 ml double-walled beaker, is preheated by the return flow of the thermostatically controlled water system.

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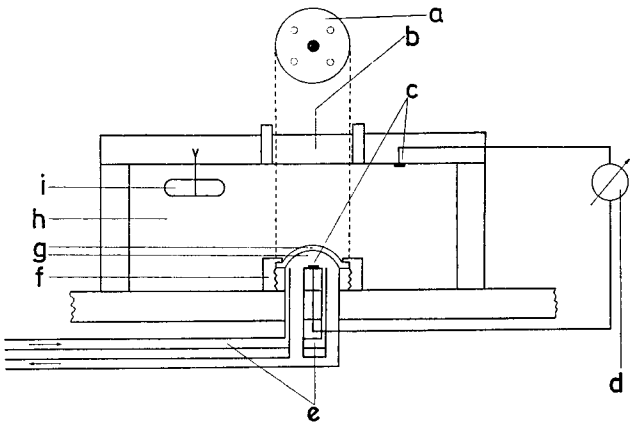

FIG. 1. General view of the system: a—bottom view of the lower chamber, b—glass-window for slit-lamp examinations, c—electrodes, d—conductivity meter, e—perfusion medium supply, f—corneal clamp, g—cornea lower chamber, h—upper chamber, i—magnetic stirrer.

The medium is pumped by a peristaltic pump (Ismatec mp-4) into a double silicone membrane oxygenator (Eschweiler) under pressure and flow control (0·55 $\pm$ 0·01 to 5·85 $\pm$ 0·02 ml min^{-1}). From the lower chamber, the medium can either be returned to the reservoir or collected in another flask (open or closed circuit). The pressure on the endothelial side is adjusted to 26 $\pm$ 0·5 cm H_2O by a screw-clamp on the outflow tube. If necessary for experimental purposes, extreme pressure conditions can be easily obtained. A bridge between inflow and outflow circuits allows pre-heating and pre-oxygenation of the medium and rapid recovery of correct pressure conditions immediately after corneal clamping (Fig. 2). Circulation of the media filling the upper chamber can also be arranged, but without oxygenation.

Experimental controls. Control of corneal transparency and thickness is performed by a Haag-Streit slit-lamp with special pachometer attachment. No optical correction appeared to be necessary under actual experimental conditions. Electrical conductivity measurements between the two silver electrodes mentioned above allow monitoring of physiological conditions and effectiveness of the seal made by the clamp. An output of 5 V, modulated by an oscillator of 1 kHz avoids any artificial

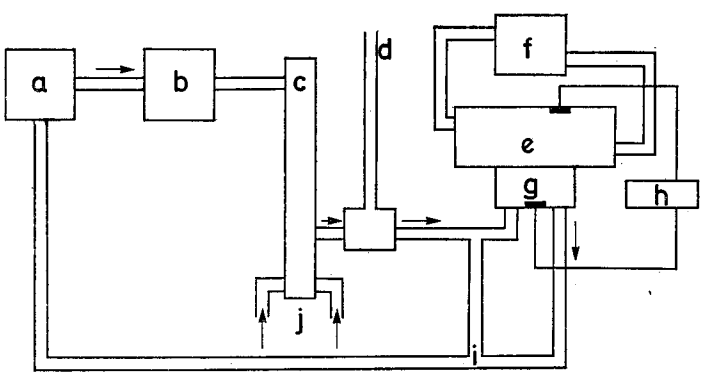

FIG. 2. Perfusion medium supply circuitry. a—Medium reservoir, b—pump (medium circulation), c—oxygenator, d—manometer, e—upper chamber, f—pump (circulation in upper chamber), g—lower chamber, h—conductivity meter, i—bridge with three-way taps for medium equilibration, j—gas inlet.

polarization of the perfused tissue. Drugs can be assayed on aliquots of the artificial aqueous humour by spectroscopic, colorimetric or liquid scintillation counting methods.

Procedure and preliminary experiments

Corneae, together with a 2 mm ring of sclera, were carefully removed from rabbit eyes, immediately after decapitation. The lens and iris were cut away avoiding as far as possible any damage to the tissues. The corneae were mounted between both joints and placed in the cell. The medium already circulating in the system, through the above mentioned bridge (Fig. 2) was allowed to irrigate the endothelial side of the tissue and pressure was adjusted to the required value. During these preparatory manipulations, the epithelium was frequently moistened with isotonic saline. The cover was placed on the upper chamber and fixed by its screws. The preparation to be tested was then added.

In order to test the reliability of the system a set of experiments was made with 10^{-2}M solutions of procaine hydrochloride at pH 7·38 in a Sørensen phosphate buffer rendered isotonic by addition of sodium chloride (Hind & Goyan, 1947).

Experimental conditions

The medium used in these experiments was the artificial aqueous humour described by Donn & others (1959), the pH of which was equilibrated at 7·38 $\pm$ 0·04 by saturation with a mixture of 5% CO_2 in oxygen delivered at 40 mm Hg. Perfusate temperature and flow were respectively 37 $\pm$ 0·5° and 1·93 $\pm$ 0·02 ml min^{-1}. The pressure on the endothelial side was 26 $\pm$ 0·5 cm H_2O.

Drug concentrations were assayed at 290 nm with a Perkin–Elmer UV-Visible spectrophotometer. The experiments were made over 2 h under these conditions. Two slightly differing procedures were compared, using the procaine hydrochloride solutions. In the first, the dosage form was applied immediately after the cornea was clamped; in the second the epithelium was at first bathed with the buffer solution alone, until corneal thickness had reached a steady state and the procaine hydrochloride was then applied.

RESULTS AND DISCUSSION

No significant change in electrical conductivity was observed during the experiments for both procedures, steady state and no steady state conditions (Fig. 3). Stabilization of the conductivity occurred regularly after about 40 min of perfusion. In the first type of experiment (without preliminary buffer application on the epithelium), the corneal thickness increased slightly, generally between 0·01 and 0·03 mm, over the time of perfusion. In the second procedure, involving preliminary conditioning of the tissue by the buffered vehicle of the drug, a stable corneal thickness was observed (average value: 0·40 $\pm$ 0·02 mm).

Fig. 4 illustrates the rate of passage of procaine through the whole corneal tissue, in both experimental conditions. Classical statistical analysis led to the conclusion that the increase of drug concentration in the artificial aqueous humour was well represented by a linear function of time, in both experimental conditions.

However, comparing the two plots, a difference was distinguished among the slopes and among the ordinates at origin. In the experiments where procaine was applied on the epithelium without waiting for the steady state to develop, a high rate of

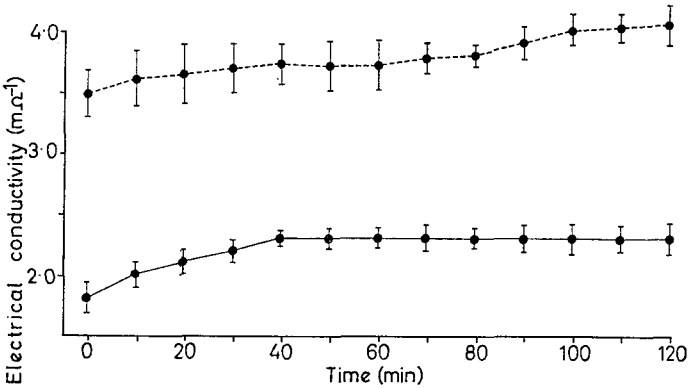

FIG. 3. Changes of transcorneal electrical conductivity during the experiments. Each point is the mean of 3 experiments for the control buffer solution and of 10 experiments for procaine hydrochloride. Vertical bars indicate the standard deviation of the mean. (Dotted line: control phosphate buffer solution, pH 7·38; solid line: 10^{-2} molar solution of procaine hydrochloride in the same buffer).

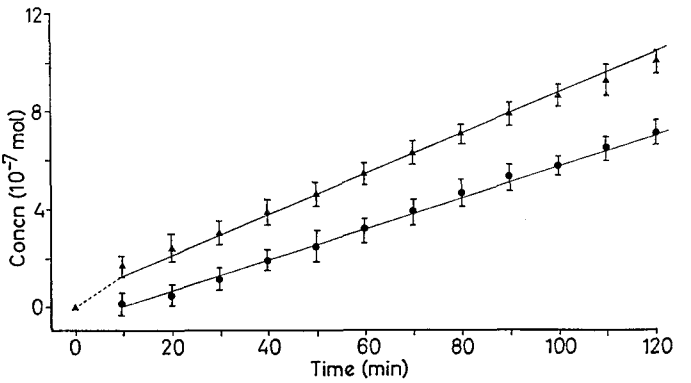

FIG. 4. Increase of procaine hydrochloride concentration in the artificial aqueous humour, plotted against time. Each point is the mean of 10 observations. Vertical bars indicate the standard deviation of the mean. (No steady state conditions: ▲; steady state conditions: ●).

passage was observed at the beginning of the sampling time. This fact could be explained by tissue metabolic changes due to surgery and preparatory manipulations. Consequently, it appeared that an adaptation time of about 40 min was necessary for a suitable study of drug transport on *in vitro* corneal preparations. After this time, there was confidence that the transcorneal passage process occurred through a normally functioning tissue.

The delay preceding the passage of procaine, when a steady state was established before the drug was applied could in the most part be explained by the pharmacological properties of the drug used as a tracer in these experiments.

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