
Dextran uptake into, and loss from, corneas stored in intermediate-term preservative

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This investigation demonstrates that dextran passes into rabbit corneas stored in M-K medium prior to corneal transplantation. An equilibrium between dextran in the cornea and in the M-K medium is reached at about 24 hours. Post-keratoplasty dextran efflux is rapid with about 70 to 75 percent being lost within one to three hours and 99 percent being lost in 12 hours.

A modified tissue culture medium (M-K medium) has been shown capable of preserving endothelial cell ultrastructure and function of corneas stored at 4° C. for periods longer than conventional eye bank techniques. Use of these corneas for penetrating keratoplasty in humans has demonstrated the clinical value of this technique.

The modified tissue culture medium consists of a mixture of Medium 199 (Microbiological Associates, Inc.) containing 5 percent dextran (Sigma Chemical Company, dextran 40). It was the purpose of this investigation to determine if the cornea is permeable to dextran under the usual conditions of corneal storage and to quantify the uptake and release of this solute.

Materials and methods. Albino rabbits (2 to 3 kilograms) were sacrificed with an overdose of sodium pentobarbital. The eyes were enucleated and the corneas isolated with a 3 mm. scleral rim. Each cornea was placed in a vial containing 20 ml. of M-K medium (Warner Lambert Laboratories) to which had been added 10 mg. of 14C dextran-molecular weight (M.W.) 40,000 (New England Nuclear Corporation, Boston, Mass.), specific activity, 1.125 mCi per gram, and stored at 4° C. The corneas were then subjected to one of five separate procedures.

Group I. Corneas were removed from the solution after storage at 4° C. for 3, 6, 12, 24, 48, and 72 hours. They were washed briefly in three separate aliquots of Ringer solution and a central corneal button was removed with an 8 mm. trephine. The corneal buttons were blotted dry on filter paper, weighed, and dissolved in 2 ml. of Protosol (New England Nuclear Corporation, Boston, Mass.). Aquafour (New England Nuclear Corporation, Boston, Mass.), 10 ml., was added and the samples were counted in a Packard Tricarb Liquid Scintillation Counter.

Group II. To determine whether dextran was adsorbed onto the epithelial and endothelial surfaces of the cornea, and to determine the stability of the 14C label, the following experiment was performed. Whole corneas, with scleral ring, were incubated for 72 hours in 14C-dextran-labeled M-K medium. After this time the epithelium and endothelium were scraped off the corneal surfaces and a central 5.5 mm. button trephined from the cornea. The central button was solubilized in Protosol as in Group I. The remaining peripheral ring of cornea was placed in a dialysis sac (pore size, 48 A) with 1 ml. of distilled water; this was immersed in 5 ml. of 0.9 percent NaCl solution and both solutions were stored for 24 hours at room temperature. Samples were taken of inside and outside dialysis solution and the peripheral corneal ring was digested in Protosol. All solutes were counted in the usual manner.

Group III. In order to determine if dextran was entering the cornea from the exposed lateral edge of the scleral rim, thereby creating a dextran gradient of decreasing magnitude from peripheral to central cornea, corneas were stored in the radioactive M-K medium for 6 and 24 hours at 4° C. After removal and three separate washings in Ringer solution a 5.5 mm. central corneal button was removed. Subsequently an 8 mm. trephine was applied and a ring of 8 mm. outer diameter and 5.5 mm. inner diameter was removed. Finally scissors were used to remove the scleral rim at the limbus giving two additional rings, one of cornea with an 8 mm. inner diameter and the limbus as its outer border and finally the scleral rim. The tissues were blotted, weighed, dissolved, and counted as in Group I.

Group IV. In order to further define the route of dextran penetration into the cornea, corneas were clamped between two chambers at 37° C. The lateral scleral rim was excluded as a possible route of entrance since the opposing faces of the
chambers occluded not only the scleral rim but also a small area of peripheral cornea. The endothelial and epithelial surfaces were bathed in radioactive M-K medium at 4°C for 48 hours. After removal from the chambers and washing in three aliquots of Ringer solution, an 8 mm. central corneal button was removed. Samples were then treated as Group I corneas.

Group V. In order to determine how rapidly dextran leaves the cornea after transplantation, rabbit corneas with their scleral rims were stored in radioactive M-K medium for 48 hours. Ten albino rabbits were anesthetized with intravenous urethane (25 per cent, in 0.9 per cent saline solution) and a 6 mm. penetrating keratoplasty performed in one eye using the corneas preserved in the radioactive M-K medium. Corneas were removed from the M-K medium, washed briefly in nonradioactive Ringer solution, and a 6 mm. corneal button sutured into the recipient eye. Rabbits were sacrificed at 1, 3, 6, 12, and 24 hours after keratoplasty, the transplanted corneal buttons (two at each time interval) were removed, washed, blotted, weighed, and counted as Group I corneas.

Results. Group I corneas demonstrated increasing uptake of dextran with time in the central 8 mm. button trephined from the stored cornea plus scleral rim (Fig. 1). A plateau was reached between 24 and 48 hours indicating an equilibration between influx and efflux rates (p > 0.2).

Group II denuded stroma contained 5.8 ± 0.3 μg dextran per milligram wet weight, a figure which is identical to that found for whole cornea for this incubation period (Group I, Fig. 1). Furthermore, 79 per cent of all retrieved counts are contained within the dialysis sac, either in the fluid or in the tissue. Since the pore size of the dialysis membrane is 48 A, dextran (M.W. 40,000, dimensions 100 x 700 A) will be retained but smaller breakdown products would escape from the sac. All reported dextran concentrations have been corrected to reflect this 79 per cent concentration of labeled dextran.

Group III corneas, in which samples were taken from the central, midperipheral, and peripheral cornea, demonstrated no statistically significant increased peripheral corneal concentrations of dextran within the six-hour group or within the 24-hour group when compared to more central samples, p > 0.2 (Table I). Concentrations in the scleral rim, however, were significantly higher than those found in the cornea (p < 0.01).

Group IV corneas with the peripheral scleral rim clamped demonstrated an uptake of 6.36 ± 0.81 μg dextran per milligram of corneal tissue (n = 4). Corneas stored in radioactive M-K medium 48 hours without the rim clamped demonstrated an uptake of 5.94 ± 0.58 μg dextran per milligram of cornea (n = 4). There is no statistical difference between these two values (p > 0.2).

Group V transplanted corneas showed a retention of approximately 25 to 30 per cent of dextran at one to three hours compared to untransplanted
controls (Table II). By 12 hours only 1 per cent of the original amount of dextran remained in the transplanted rabbit cornea.

**Discussion.** Corneas stored at 4° C. in Ringer's solution become edematous because the normal corneal swelling pressure is opposed by an endothelium and epithelium of low metabolic activity. Dextran acts as a colloidal osmotic agent preventing excessive corneal swelling, in a manner similar to that of other large molecules such as chondroitin sulfate and polyvinyl pyrrolidone. These compounds are assumed to be excluded from the corneal extracellular volume and thereby oppose the swelling pressure of the stroma due to their osmotic action. It has been assumed that the dextran molecule does not enter the corneal endothelium and stroma because of its large size (M.W. 40,000, dimensions 100 x 700 Å), although similar sized compounds do penetrate the endothelium into the stroma.

This investigation demonstrates that dextran enters corneas stored in M-K medium and that it enters from the epithelial-endothelial surfaces, that it is not adsorbed onto these surfaces, and that it does not enter from the exposed scleral edge. The corneal concentration of dextran increases rapidly with time until stabilization is reached between corneal water and bathing solution between 24 and 48 hours. The higher uptake of the scleral rim compared to cornea probably reflects a different effective extracellular space of the two tissues, or at least a difference in the nature of the available extracellular space.

It is of interest to calculate the amount of dextran in the cornea as a function of corneal water volume. At equilibrium the cornea contains 5.9 µg dextran per milligram of corneal wet weight; the average wet weight of the whole cornea is 80 mg, and the hydration of corneas stored in MK medium, for seven days, has been shown in our laboratory to be approximately 85 per cent. Thus the corneal water weight is 68 (or 80 x 0.85) mg (68 ml assuming a specific gravity of 1 for corneal water). The bathing solution contains 5 grams dextran per 100 ml. or 50 µg per microliter, thus the cornea should also contain 50 µg per microliter, but experimentally only 5.9 µg dextran per milligram of wet weight is found, which is (since hydration is 85 per cent) 5.9/0.85 or 6.9 µg dextran per milligram of corneal water. There is 13.8 per cent (6.9/50 x 100 per cent) of the corneal water, therefore, which contains dextran, indicating a large excluded volume effect (i.e., molecular exclusion due to the solute size relative to the geometrical space available within the tissue). Smaller molecules such as mannitol and inulin reach equilibrium with approximately 60 per cent (inulin) and 82 per cent (mannitol) of the corneal extracellular water. The equilibration of any compound with all free corneal water depends upon the size of the molecule relative to the available physical space. Obviously water has access to all regions of the stroma whereas large molecules, which offer physical limitations to penetration in this tissue, cannot insert themselves into all available space. The extracellular space of the cornea occupied by solutes, therefore, decreases with increasing molecular size in relation to the geometric network of collagen and glycosaminoglycan structure. The absence of dextran penetration into all corneal extracellular water has the practical implication that dextran still exerts a considerable osmotic pressure between the bathing solution and 85 per cent of corneal water.

Efflux of dextran from the transplanted rabbit cornea also occurs rapidly with about 70 to 75 per cent loss in one to three hours. The intraocular effects of the dextran efflux (e.g., osmotic cataract) are not known although none have yet been noted clinically. Assuming an efflux rate of about 1.5 µg dextran per minute per 15 mg. of corneal button (Group V, Table II),

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**Table I.** Dextran concentration of concentric segments of rabbit corneas stored in M-K medium

<table>
<thead>
<tr>
<th>Sample size (mm.)</th>
<th>µg Dextran/mg. cornea</th>
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<tbody>
<tr>
<td>5.5 mm.</td>
<td>1.88 ± 0.21*</td>
</tr>
<tr>
<td>5.5 to 8.0 mm.</td>
<td>2.06 ± 0.38</td>
</tr>
<tr>
<td>8.0 mm. to limbus</td>
<td>2.19 ± 0.19*</td>
</tr>
<tr>
<td>Scleral rim</td>
<td>11.3 ± 0.85</td>
</tr>
</tbody>
</table>

No statistically significant difference between the uptake of each corneal segment within each time period (p > 0.2). Scleral rim statistically different from corneal segments (p < 0.01).

N = 4 experiments except f (N = 3).

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**Table II.** Dextran concentration in rabbit corneas stored 48 hours in M-K medium after keratoplasty

<table>
<thead>
<tr>
<th>Time transplant in eye (hr.)</th>
<th>µg Dextran/mg. cornea*</th>
<th>Per cent of untransplanted control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.08 (0.85, 1.32)</td>
<td>62</td>
</tr>
<tr>
<td>3</td>
<td>1.30 (1.14, 1.43)</td>
<td>61</td>
</tr>
<tr>
<td>6</td>
<td>0.11 (0.05, 0.17)</td>
<td>50</td>
</tr>
<tr>
<td>12</td>
<td>0.02 (0.02, 0.03)</td>
<td>30</td>
</tr>
<tr>
<td>24</td>
<td>0.04 (0.01, 0.07)</td>
<td>26</td>
</tr>
</tbody>
</table>

* N = 2.
and an aqueous humor volume of 200 μl, an increase in the aqueous humor osmotic pressure of about $2 \times 10^{-3}$ milliosmoles would be expected over a 10-minute period (assuming no aqueous humor turnover) and this is totally negligible.

It is assumed that dextran passes through the trabeculum with relative rapidity.\(^5\)

The uptake of dextran by the corneas in M-K medium would be expected to cause an initial corneal swelling due to an imbibition of aqueous humor into the corneal stroma during the first few hours after keratoplasty, due to the slight increase in osmolarity of the stromal fluid. In fact an initial swelling prior to temperature reversal in the specular microscope has been noted by McCarey and Kaufman.\(^4\)

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Key words: cornea, rabbit, dextran, endothelium, tissue uptake, tissue loss, tissue preservation, corneal preservation.

REFERENCES


Rabbit corneal epithelial cells grown in vitro without serum. MARIAN I. ALLMAN, ROBERT A. HARPER, MYRON YANOFF, LESLIE J. CURFMAN, J. DOUGLAS CAMERON, AND B. ALLEN FLAXMAN.

Primary outgrowth cultures of normal rabbit corneal epithelium can be initiated and propagated in vitro up to 6 days in serum-free medium. By the eighth day the majority of cells have ceased to divide. Epithelial cells grown without serum show DNA synthetic activity at a level comparable to control cultures grown with added serum.

Numerous studies have shown that some continuous or transformed cell lines can be cultivated in vitro in serum-free media of defined composition.\(^1\) This property has been compared with the well-known serum requirements for normal diploid cells grown in vitro, and it has been suggested that there may be a relationship between reduced serum requirements and biological behavior. However, the real significance of this property is still unclear because there is much variation in serum requirements between different types of transformed cells. The present communication reports an instance of limited, but striking proliferation of normal cells in serum-free medium.

Materials and methods. Adult albino rabbits weighing approximately 2 kilograms were killed by fracture of the cervical spine. The eyes were enucleated and corneas excised according to methods previously described.\(^3\) - 4 Corneal tissue was placed in a 60 mm. Petri dish with Eagle's minimal essential medium, MEM (Grand Island Biological Co.), containing 100 U. per milliliter each of penicillin, streptomycin, and mycostatin, but without serum. After being placed epithelial side down on the bottom of the dish, small buttons were removed using a 2 mm. diameter trephine. The buttons were then placed in a 35 mm. diameter Petri dish (Falcon) containing MEM, where they were floated freely for 1 hour at room temperature in order to allow stromal swelling. The epithelial side of each button was then separated from the mass of underlying stroma by scissors and the final explants consisted of epithelium and minimal stroma. Three explants (each approximately 0.2 mm. thick) were placed epithelial side up on a glass coverslip lying in the bottom of a 35 mm. Petri dish. A drop of MEM was added and a second coverslip was placed on top of the first in order to hold the explants in place. Then 2.5 ml. of culture fluid (either MEM alone or MEM with 4 per cent fetal calf serum) was added and the cultures maintained at 37°C in a humidified 95 per cent