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Diffusion-Permeability of Labeled Compounds of Various Transparent Synthetic Polymers in Relation to the Rabbit Cornea*

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INTRODUCTION

In our work at the Eye Research Division of the National Institute for Scientific Research, properties of certain polymeric materials in the transmission of water, and electrolytes had to be evaluated.

Three-dimensional networks of hydrophilic polymers with varying degrees of cross-linking in the form of hydrophilic gels have been suggested for medical uses where hydrophilicity is important. Of particular interest to ophthalmology are the hydrogels of methacrylates because of their chemical stability and transparency. A great deal of publicity has been given to hydrogel contact lenses fabricated in Czechoslovakia [11]. The hydrogels of these lenses were included in this study.

The diffusion-permeability characteristics of hydrogels (namely, of hydroxyethyl-methacrylate with varying degrees of cross-linking) in relation to water, to labeled compounds, and to oxygen were examined and compared with the diffusion-permeability of intact rabbit corneas *in vitro*.

MATERIALS AND METHODS

The labeled materials used were: (a) tritiated water, (b) sodium-22, and (c) iodine-125.

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All hydrogels were prepared as thin films by casting between two 4×4 in. sheets of Pyrex glass. Silicone rubber was used between the glass plates as a sealing gasket. The thickness of the compressed gasket determined the thickness of the cast hydrogel film.

A redox system of ammonium persulfate, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, and sodium metabisulfate, $\text{Na}_2\text{S}_2\text{O}_5$, was used to polymerize the hydrogels. After mixing, the mixture was poured into the glass-plate mold. Polymerization was completed after maintaining the mold at 55°C for 90 min.

After polymerization, the glass plates were separated, and the film was removed. The thickness of the film was then measured and recorded. Each film was soaked in distilled water for approximately 1 week, during which time several changes of distilled water were made. After this soaking, the thickness of the film was measured and recorded. Those films which changed substantially in thickness were discarded. Those that remained the same thickness were considered isovolumetric and suitable for permeability testing. Some hydrogels were expected to change in thickness as the distilled water removed the solvents from the films [1].

The free water content was determined for each film. Each film was equilibrated in distilled, deionized water, its weight being noted. The film was dried at 90°C for 1 hr. After cooling, its weight was again noted. The weight difference was attributed to the loss of all free water.

The hydrogel films were cut into disks with a trephine. The diameter of the trephine was selected so that the sample disks would fit loosely in the testing apparatus. The sample was then equilibrated in the labeled solution.

Intact rabbit corneas were trephined immediately after death and equilibrated in the labeled solution in the same manner as described above.

The testing apparatus is shown in Fig. 1. The sample membrane was clamped in place between two silicone rubber gaskets and a precisely measured volume of labeled solution then added to the vessel above the membrane. The upper vessel was equipped with a freely moving plunger made of T.F.E. fluorocarbon. A calibrated weight was placed on the plunger to maintain a constant pressure on the labeled solution. A precisely measured volume of distilled, deionized water was placed in the lower vessel. Distilled, deionized water was used to minimize ionic interactions with the labeled solution. A magnetic mixing bar was placed in the lower vessel to keep the solution agitated during the test.

Just prior to each test, a small, precisely measured sample volume of the labeled solution was withdrawn from the upper vessel. This sample was inserted in a Packard scintillation counter, using a specific Packard scintillation liquid. The number of counts per unit time of the sample was recorded and used as a standard for the radioactivity of the labeled solution.

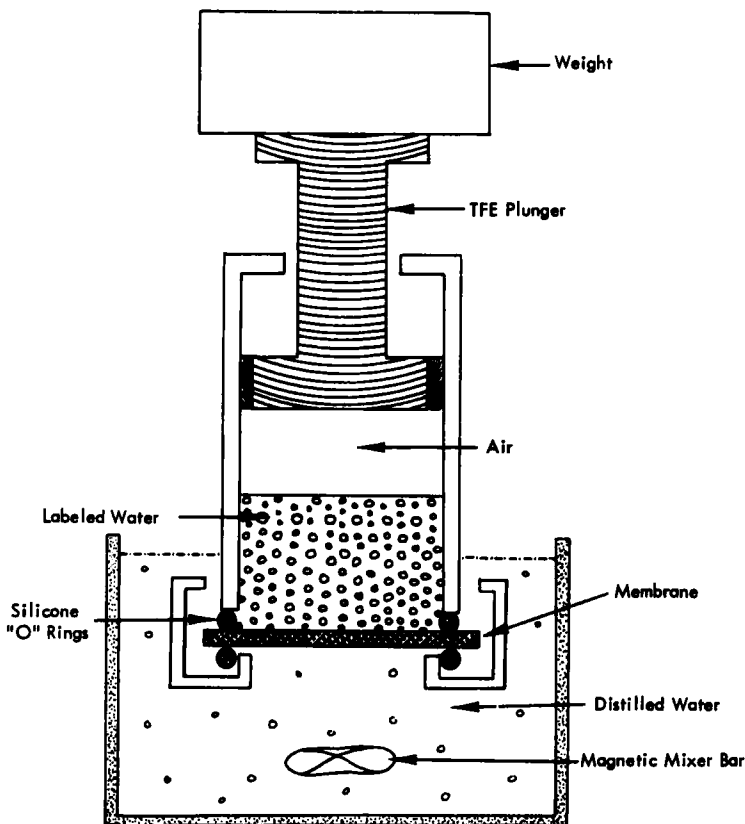


Fig. 1. A simple apparatus was designed to hold a membrane between two silicone "O" rings. The chamber at one side of the membrane contained labeled water and the chamber on the other side of the membrane contained distilled, deionized water.

The test commenced when the upper vessel was lowered into the distilled, deionized, agitated water. At fixed time intervals, small, precisely measured sample volumes were withdrawn from the lower vessel and introduced into the scintillation counter. The number of counts per unit time, for each sample, was recorded. When tritiated water was used, samples were withdrawn every hour for 24 hr. Samples were taken only at the end of 24 hr when sodium-22 and iodine-125 were used. Fick's law was used to calculate the coefficient of diffusion-permeability for tritiated water. The resulting units for this coefficient are expressed as square centimeters per second. Sodium and iodine permeabilities are expressed in terms of

relative permeability, i.e., the ratio of the scintillation count rates of the lower vessel to the upper vessel, in per cent, after a 24-hr period.

Oxygen permeability tests were detailed as to methods and materials in earlier work [2].

RESULTS

Oxygen

The results of gas-to-gas and water-to-water oxygen permeability tests of polymer membranes are given in Table 1. As these data indicate, polymers differ considerably in their oxygen permeability, when tested by the two methods. It was therefore concluded that gas-to-gas oxygen permeability could not be used as a measure of polymer water permeability. However, the earlier results with gas-to-gas oxygen permeability of hydrogels, shown in Table 2, show a pronounced dependence on the water content of the hydrogel. However, the permeability is much greater in translucent and opaque gels than in transparent gels of similar hydration. This increase in permeability in a translucent or opaque gel suggests that these gels, while

Table 1. Comparison of Gas-to-Gas and Water-to-Water Oxygen Permeability (P) of Polymers [2]

Polymer	P(G/G) ^a	P(W/W) ^a
Poly(dimethylsiloxane) ^b	$665. \times 10^{-10}$	4000×10^{-10}
Polyethylene (low density)	2.34×10^{-10}	50×10^{-10}
Poly(fluoroethylenepropylene) ^c	3.86×10^{-10}	105×10^{-10}
Poly(tetra fluoroethylene) ^d	23.7×10^{-10}	91×10^{-10}
HEMA hydrogel ^e	—	180×10^{-10}
Polyelectrolyte complex ^f	—	270×10^{-10}

^aP expressed in units of cc(STP)—cm/cm²-sec-cm Hg.

^bDow Corning "Medical Silastic" with SiO₂ fillers.

^cDow Teflon FEP.

^dDow Teflon TFE.

^eCross-linked poly(hydroxyethyl-methacrylate); water content, 38%.

^fPoly(vinyl trimethylammonium)—poly(styrene sulfonate); water content, 35%. Courtesy of Amicon, Inc., Cambridge, Mass.

Table 2. Oxygen Permeability (P) of Hydroxy-Ethylmethacrylate Hydrogels [2]

Gel	pa	% H ₂ O content	Ethylene glycol solvent		
			% solvent	% H ₂ O	% E.G.
Transparent	1.6×10^{-8}	35.5	10	60	40
Transparent	1.7×10^{-8}	36.7	30	40	60
Transparent	1.9×10^{-8}	38.2	40	60	40
Translucent	7.6×10^{-8}	49.9	70	80	20
Opaque	27.0×10^{-8}	58.7	80	80	20

^aP expressed in units of cc (STP)-cm/cm²-sec-cm Hg.

being optically heterogeneous, may be composed of microscopic aggregates of microgels which, nevertheless, are invisible with light microscopy.

Tritiated Water

Figure 2 shows a comparison of our tests of diffusion-permeability using tritiated water, with that of the *in vivo* rabbit cornea (fluorescein) [7], the contact lens hydrogel [6, 8], and the cellular layers of the cornea [4]. A quantitative comparison is not practical because of the various experimental methods and expressions of the permeability constant.

Sodium-22 and Iodine-125

In addition to the materials tested with tritiated water, polymethylmethacrylate and a cross-linked terpolymer hydrogel of vinylpyrrolidone were tested with sodium-22 and iodine-125. The results are shown in Fig. 3 and 4.

As expected, polymethylmethacrylate had a low relative permeability. However, the terpolymer hydrogel containing 84.3% free water was roughly twice as permeable to sodium and iodine as the same hydrogel containing 40% free water. The relative permeability to sodium and iodine can be used as a rough approximation of permeability selectivity [12].

Permeability tests with rabbit corneas were not made using sodium-22 and iodine-125 as there are data available from the literature [4, 9, 10].

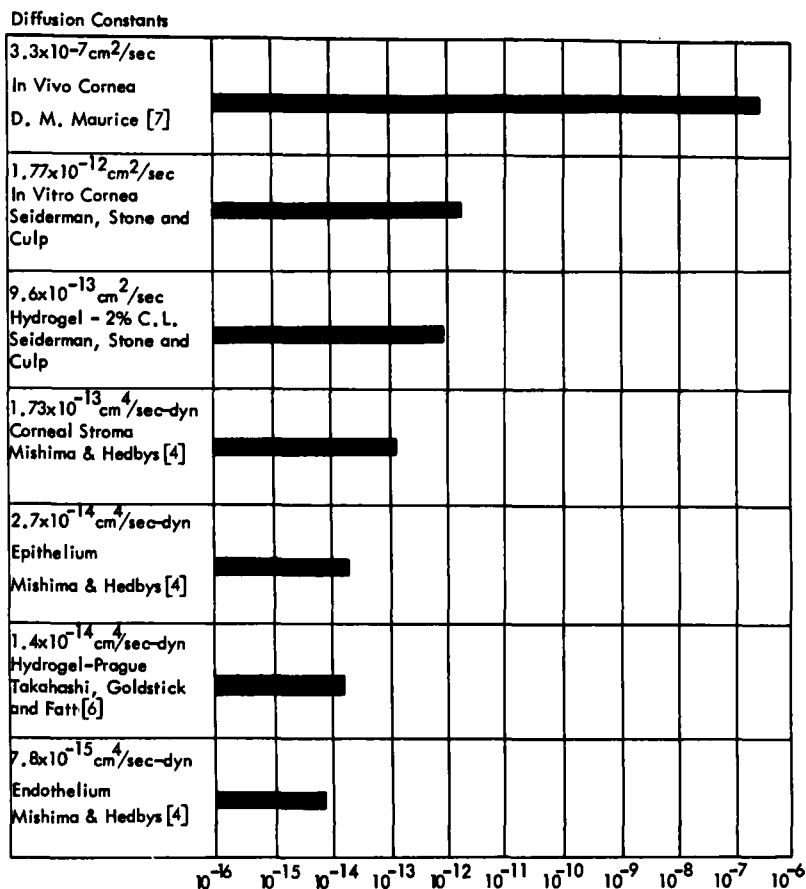


Fig. 2. Fluid transport across hydrogel membranes and rabbit corneas, using tritiated water, and as reported in the literature by various investigators, using different methods and different expressions for permeability.

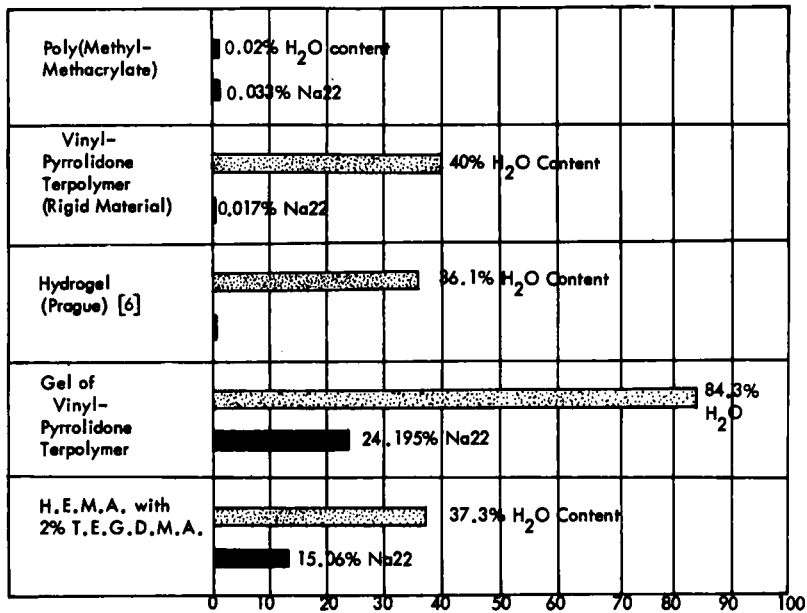


Fig. 3. Effects of water content on relative permeability of polymers to sodium-22.

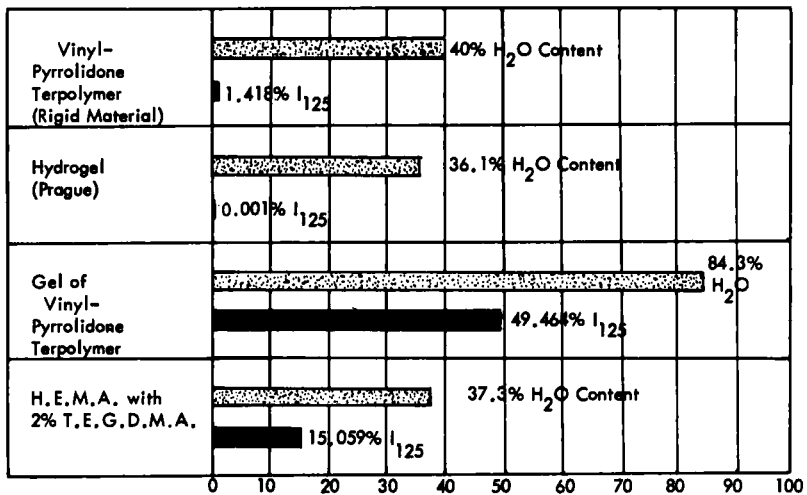


Fig. 4. Effects of water content on relative permeability of polymers to iodine-125.

DISCUSSION

Oxygen Diffusion-Permeability

The results obtained with oxygen were inconclusive because of the poor pressure-volume relation between the gas-to-gas and water-to-water testing methods [3]. Therefore, the oxygen diffusion-permeabilities of the polymer membranes will not be compared with those using tritiated water, sodium, and iodine.

Tritiated Water

Previously given were the results of the tests of hydrogels and *in vitro* rabbit corneas for diffusion-permeability coefficient using tritiated water. The term "diffusion-permeability coefficient" is used to identify the transport of the labeled water across the membranes, which is actually a parallel transport resulting from permeability by capillary flow, and from diffusion, *i.e.*, square centimeters per second because of the small pressure differential used in the experiments (approximately 20 mm hg).

Our experimental results, and our attempt to compare them with what is believed to be the fluid transport through the living cornea as reported in the literature, strongly suggest that all future measurements on synthetic materials, as potential cornea substitutes, be made with standardized methods, apparatus, and quantitative nomenclature.

The cornea is a complex material in which the various layers of cells are responsible for maintenance of fluid and ionic balance. Fluid transport across these layers is a sum of (a) permeability by capillary flow; (b) permeability by diffusion, *i.e.*, thermal energy of the fluid and the corneal tissues; and (c) permeability by ionic interaction, *i.e.*, ionic concentration gradients. Before a synthetic cornea can be successfully fabricated, experiments on live, *in vitro* corneas must be standardized. The same experimental conditions must prevail for the artificial corneas so that meaningful comparisons can be made. In this manner, the artificial cornea can be made to give the same performance as the natural cornea, insofar as the fluid and ionic balance of the anterior chamber and the exterior surface of the eye will be preserved. This is a fundamental requirement for the eye, even though many of the necessary fluid and ionic balances may not be necessary for the performance of the artificial cornea as an optical element.

Standardization of transport measurement apparatus will require that the temperature, pressure, and humidity be controlled, such that the body environment is duplicated as closely as possible for living cornea testing. The artificial corneas should then be tested under the same conditions.

Standardization of the nomenclature will result in a mathematical relation involving the three modes of fluid transport. This relation will be indeterminate, i.e., a single datum cannot conveniently be separated into its fundamental fluid transfer modes. However, experimental technique can perform this separation. For example, the permeability by capillary and microvoid flow can be isolated by varying the pressure differential across the cornea, then extrapolating the fluid transport to zero pressure gradient. This leaves the transport due to diffusion and ionic concentration gradients. A further separation is effected by varying the ionic concentration and again extrapolating to zero gradient, but keeping in mind that although no net ionic transport is observed there is an equal and opposite transport by random ionic interaction. Admittedly, the procedure is no more simple than the cornea itself.

From the biological standpoint, the cornea is a complex structure with barrier properties and built-in selectivity in the cellular membranes. About 90% of the thickness of a rabbit cornea is stroma. Corneal stroma in the living eye is constructed of collagen fibrils uniformly distributed and uniformly spaced. The cellular membranes, epithelium and endothelium, play an important role in controlling the steady-state hydration of this composite living tissue, and by selective permeability, keep the structure of the stroma transparently patent [9]. However, experiments with the *in vivo* cornea have shown that its permeability increases with increased water content, resulting in swelling, and that control of the swelling is achieved by bulk fluid flow [5, 10]. Thus, a comparison of the diffusion-permeability coefficient of a homogeneous polymer membrane, to the bulk fluid transport of the *in vivo* cornea, must be one of gross evaluation only.

The bulk flow in the cornea or across the cornea depends entirely on the experimental procedure employed because of the control of flow by the cellular membranes. The comparison of constants, for an intact cornea and one denuded of its cellular membranes, is shown in Table 3 [13].

The epithelium and the endothelium have a much higher selectivity to solute permeation than the stroma of the cornea. Movements

Table 3. Tritiated Water Diffusion Constant (cm^2/sec)

	Aqueous to tear	Tear to aqueous
Intact cornea	0.78×10^{-5}	0.73×10^{-5}
Without epithelium	1.52×10^{-5}	1.18×10^{-5}
Without endothelium	1.23×10^{-5}	1.04×10^{-5}

of water and electrolyte solute are different because they interact. A change in solute concentration occurs in the membrane because the permeability of water is higher than that of the solute.

Comparison of corneal stroma to synthetic polymer hydrogel makes clear that corneal stroma depends to a large extent on its heterogeneous structure, and strongly indicates the necessity for testing heterogeneous composite materials for a more mathematically agreeable comparison in developing films that may be used in corneal surgery.

In all previous literature, only a comparison between individual layers of the cornea, or an entire cornea, *in vitro*, and the equations derived from tests and measurements were used in selecting polymers for corneal surgery. For example, the diffusion constants are, as shown in Fig. 2:

$$\begin{aligned} \text{Epithelium} &= 0.27 \times 10^{-13} \text{ cm}^4/\text{sec-dyne} \\ \text{Stroma} &= 1.73 \times 10^{-13} \text{ cm}^4/\text{sec-dyne} \\ \text{Endothelium} &= 0.078 \times 10^{-13} \text{ cm}^4/\text{sec-dyne} \end{aligned}$$

The ideal polymer should have the properties of the above figures [4].

Sodium and Iodine

The results with sodium-22 and iodine-125 were given as percentages for lack of a basis of meaningful comparison. The data indicate that the diffusion-permeability of the hydrogels is a function of water content, and there is some selectivity of permeability, probably due to differences in ionic activity and atomic size.

CONCLUSIONS

The results of our work were consistent in themselves. However, insufficient basis for quantitative comparison is available for proper evaluation of polymeric hydrogels and living corneas. The work indicates that homogeneous, transparent, polymer gels thus far fabricated are several orders of magnitude too impermeable for corneal replacement. It is clear that the permeability of the hydrogels increases as their water content increases, and as the degree of opacity increases. Also shown is that the hydrogels are somewhat selective in their permeability to different ionic species, at least to the extent that the selectivity can be regulated by altering the composition and hydration during the formulation of the hydrogels, within the constraints of acceptable physical properties such as mechanical strength, resistance to biodegradation, and transparency.

Because of the relatively high permeability of the corneal stroma, and the control of its degree of hydration by the cellular membranes, it may be necessary to construct an artificial cornea in layers, each layer to approximate as closely as possible the fluid and ionic transport properties of their analogous layers in the living cornea.

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