BBA Report

BBA 71132

Membrane characteristics of human articular cartilage

ERIKA HANDLER-BERNICH [a,★], PAUL LOTKE [a,★★] and REVA RUBENSTEIN [b]

[a] *The Hospital for Special Surgery, affiliated with the New York Hospital-Cornell University Medical College, New York, N.Y. 10021, and* [b] *Cornell University-New York Hospital School of Nursing, New York, N.Y. 10021 (U.S.A.)*

(Received April 7th, 1972)

SUMMARY

The permeability, expressed as $A/\Delta x$, of human articular cartilage was determined using a two-chamber diffusion cell for a series of solutes and were found to decrease in the following order $H^3HO > Na^+ >$ urea $> Cl^- >$ proline $> SO_4^{2-} >$ glucose $>$ sucrose $>$ proteins. The pressure filtration coefficient, L_p, was measured as $3 \cdot 10^{-12}$ $cm^5 \cdot dyne^{-1} \cdot s^{-1}$. The apparent pore radius was determined as 17 Å.

Articular cartilage can be described as a tissue consisting of a relatively low density of cells (chondrocytes) embedded in a compact matrix consisting of collagen, protein polysaccharides, nutrients, cell metabolites and water (75% by weight). This network, often referred to as the ground substance, is produced and maintained by the chondrocytes. The tissue is completely non-vascular and has no limiting membrane. Its outer (articulating) surface is in contact with the synovial fluid[1]. Nourishment is provided to the chondrocytes by diffusion of nutrients across the cartilage matrix from the joint cavity which contains the synovial fluid. Since adult cartilage is non-vascular the movement of material through the ground substance can be treated like the transport of molecules through a classical physicochemical membrane. A membrane is defined in physicochemical terms as a "solid or liquid, film or layer with a thickness which is small compared to its surface; it includes any ion-exchange material irrespective of geometric form which can be used as a separation wall between two solutions"[2].

★Present address: 189 Ocean Avenue, Brooklyn, N.Y. 11225, U.S.A.
★★Present address: Department of Orthopedics, Hospital of University of Pennsylvania, Philadelphia, Pa. 19104, U.S.A.

A study of the membrane characteristics of cartilage is of interest since the integrity of this tissue is dependent on the diffusion of nutrients to the sustaining chondrocytes, whose biosynthesis in turn maintains the structural integrity of the cartilagenous matrix. In this note we will present data on three of the transport properties of cartilage, the apparent diffusion area per unit path length, $A/\Delta x$; the pressure filtration coefficient, L_p; and the apparent membrane pore size, r_p (ref. 3). It should be noted that $A/\Delta x$ is proportional to the permeability coefficient.

Human articular cartilage was obtained from the femoral condyles of fresh cadavers or amputation specimens. Only healthy adult cartilage was studied. All experiments were performed within 24 h after death or amputation. The membrane was a 200-μm section of articular cartilage cut tangent to the surface.

The permeability of solutes through articular cartilage, expressed in terms of the apparent diffusion area per unit path length, $A/\Delta x$, was obtained by evaluating Fick's first law of diffusion:

$$\frac{dn}{dt} = D\frac{A}{\Delta x}\Delta c \tag{1}$$

where dn/dt is the rate at which molecules cross the area A in the interface when a concentration gradient Δc is applied across the two sides of the interface or membrane. D is the diffusion coefficient of the solute in water. In practice Eqn 1 is integrated to give:

$$D\frac{A}{\Delta x} = \frac{V_1 V_2}{V_1 + V_2}\cdot\frac{2.3}{t}\cdot\log\left[\frac{c_0}{c_0 - c_2\left(1 + \frac{V_2}{V_1}\right)}\right] \tag{2}$$

where V_1 and V_2 are the volumes of the chambers on each side of the interface, t is the time in seconds, c_0 is the initial concentration of solute in V_1 and c_2 is the concentration of solute in V_2 at time t.

Here, as in many diffusion experiments, the electrical potential difference is zero. It is obtained by setting the concentration of solute equal in both chambers. Radioactive solutes were used to monitor the unidirectional flux[3]. The radioactivity was measured using a Nuclear Chicago liquid scintillation counter or a Packard 3002 spectrometer. Reagent grade tracers were obtained from New England Nuclear Corp., having 99% radiometric purity. The proteins were iodinated (^{125}I) by the chloramine T method[4]. All experiments were conducted in lactated Ringer's solution at a pH of 7.2 and 37 °C. The viscosity of this solution at 37 °C was measured in an Ostwald viscometer. The diffusion cell used for these measurements is illustrated in Fig. 1a. The solutions were stirred by magnetic stirring bars and the rates were estimated at 300 rev./min. In our experiments V_1 and V_2 were maintained equal, the initial volume was 15 ml. At three 1-h intervals 0.5 ml was withdrawn from each side, c_0 was between 10 000 and 15 000 cpm.

The pressure filtration coefficient L_p gives a measure of the rate of solvent flow

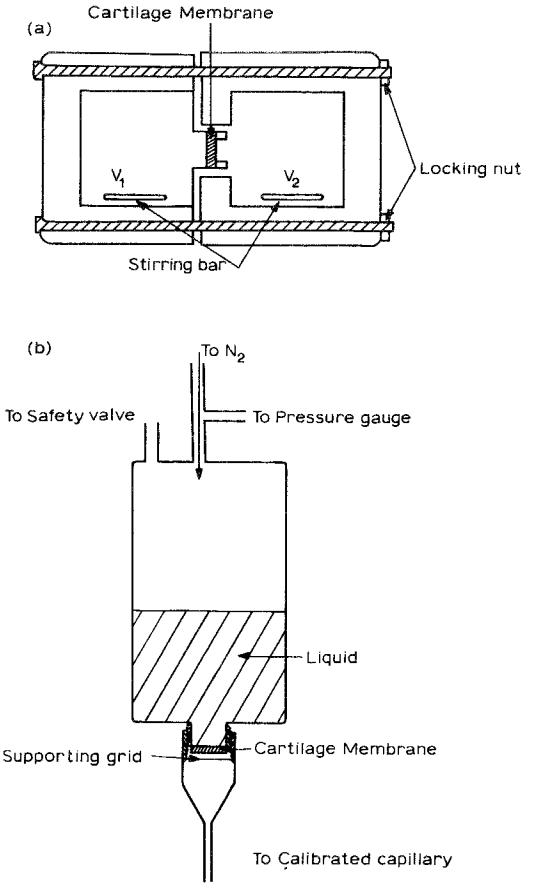

Fig. 1. Schematic diagrams of the apparatus. (a) Lucite diffusion cell. (b) Stainless steel high pressure cell.

through the membrane. It is evaluated by determining the rate of solvent volume flow under a pressure gradient ΔP and is defined as:

$$L_p = \frac{\Delta V/\Delta t}{\Delta P} \tag{3}$$

A stainless steel high pressure cell (see Fig. 1b) was built to measure the pressure filtration coefficient under an applied N_2 pressure of from 5 to 10 atm. The temperature was maintained at 37 °C by wrapping in heating tape.

Table I presents the values of $A/\Delta x$ for various solutes, through articular cartilage listed in order of increasing size. The pressure filtration coefficient, $L_p = 3 \cdot 10^{-12}$ cm^5 · dyne^{-1} · s^{-1}, was determined from the slope of the curve of applied pressure *versus* the

rate of volume flow. The data are shown in Fig. 2. A pore radius of 17 Å was calculated from the equation:

$$r_p = 2\left(\frac{2 L_p \eta}{A/\Delta x}\right)^{1/2} \quad (4)$$

where η is the bulk viscosity of the solvent and $A/\Delta x$ is the value for tritiated water H^3HO. Poiseuille flow and random orientation of the pore is assumed[5].

The permeabilities listed in Table I decrease in the following order: $H^3HO > Na^+ > Ca^{2+} >$ urea $> Cl^- >$ proline $> SO_4^{2-} >$ glucose $>$ sucrose $>$ proteins. These results are consistent with simple electrostatic and steric requirements for a highly hydrated negatively charged membrane like cartilage. In addition, such a membrane is known to exhibit ion-exchange characteristics[6]. This should lead to anomalous osmosis. The relatively low permeability observed for Na^+ and H^3HO confirms this expectation[2].

The value of the pore radius is comparable with others calculated for highly charged hydrated membranes[7]. Further, a value of 20 to 40 Å was reported for a pure collagen membrane[8]. This lends further support to a model of the cartilage skeleton as being structurally determined by the collagen network.

This work was supported by Training Grant AM 95414 of The National Institute of Arthritis and Metabolic Diseases and in part by The National Institute of General Medical Sciences Grant GM 10119.

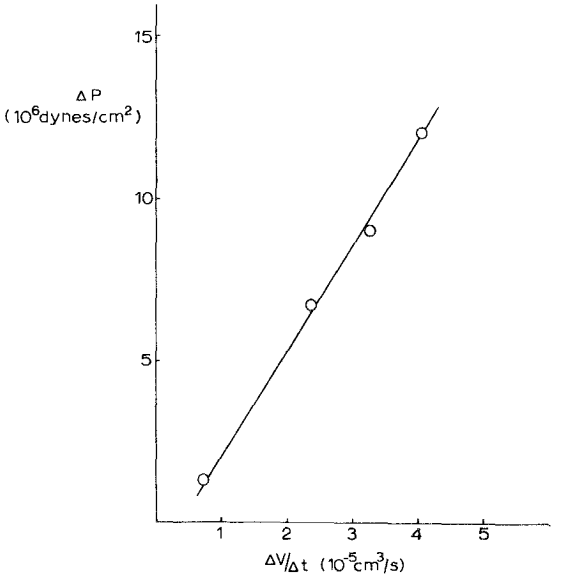

Fig. 2. Solvent flow through a cartilage membrane of 50 μm thickness, as a function of applied pressure.

TABLE I

PERMEABILITY DATA FOR HUMAN ADULT CARTILAGE

Solute	*Concentration (moles/l)*	*$(A/\Delta x)D$ (10^5 cm^3/s)*
Cl^-	0.109	8.69
Na^+	0.130	12.4
SO_4^{2-}	0.001	5.13
Ca^{2+}	0.003	10.6
H^3HO		14.0
Urea	0.001	9.50
Proline	0.001	7.65
Glucose	0.028	4.02
Sucrose	0.028	2.18
Lysozyme	$2 \cdot 10^{-7}$	0.812
RNAase	$2 \cdot 10^{-7}$	0.741
Bovine albumin	$3 \cdot 10^{-8}$	0.12
γ-Globulin	$2 \cdot 10^{-9}$	0.26

REFERENCES

1 A. Ham, *Histology*, J.B. Lippincott and Co., Philadelphia, Pa., 6th edn., 1966.
2 F. Helfferich, *Ion Exchange*, McGraw Hill, New York, 1962.
3 N. Lakshminarayanaiah, *Transport Phenomena in Membranes*, Academic Press, New York, 1969.
4 W.M. Hunter, F.C. Greenwood, *Nature*, 194 (1962) 495.
5 J.R. Pappenheimer, E.M. Renkin and L.M. Borrero, *Am. J. Physiol.*, 167 (1951) 13.
6 A. Maroudas, *Biophys. J.*, 8 (1968) 575.
7 E.M. Renkin, *J. Gen. Physiol.*, 38 (1954) 225.
8 A. Gliozzi, R. Marchio, A. Ciferri, *J. Phys. Chem;*, 73 (1969) 3063.