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MEMBRANE TRANSPORT PROPERTIES OF BOVINE ARTICULAR CARTILAGE

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SUMMARY

The thermodynamic parameters which define transport of nonelectrolytes through bovine articular cartilage membranes were evaluated. H^3HO, glucose and sucrose were used as permeants. These solutes permeate more readily through the upper layers (near the articular surface) than through the denser deeper layers approaching bone. Cartilage is similar in many respects to a swollen cellulose gel. Viscous-flow contributes importantly to transport within cartilage thus greatly enhancing the movement of nutrients.

INTRODUCTION

Articular cartilage is the material which lines all healthy synovial joints in the body. Its structure is unusual since it is not directly connected to either the vascular or nervous systems. Its structural integrity and physiological properties are dependent on the passive transport of material to and from the adjacent joint cavity [1]. It has been shown that from this point of view articular cartilage behaves as though it were a simple ion-exchange membrane [2]. The present study was undertaken in order to investigate the thermodynamics of material transport.

The biochemistry and morphology of articular cartilage have been well documented [3]. It is composed of a matrix containing 75 % water, organic materials and a low density (10^5 per cc) of cells, the chondrocytes, which are located within lacunae in the matrix. The organic constituents are collagen, glycosamineglycans and other non-collagenous proteins. The "filler" material between the collagen fibers, which is also thought to account for the mechanical properties of cartilage, is the protein-glycan complex. The glycan units are long straight chains of either chondroitin-4, chondroitin-6 or keratin sulfates. These in turn are attached to a protein backbone. This proteo-glycan forms a very stable, viscous, hydrophilic, branched macromolecule of high molecular weight (in the range of 10^6 daltons). The water in articular cartilage is not tightly bound but forms a gel in combination with the muco-

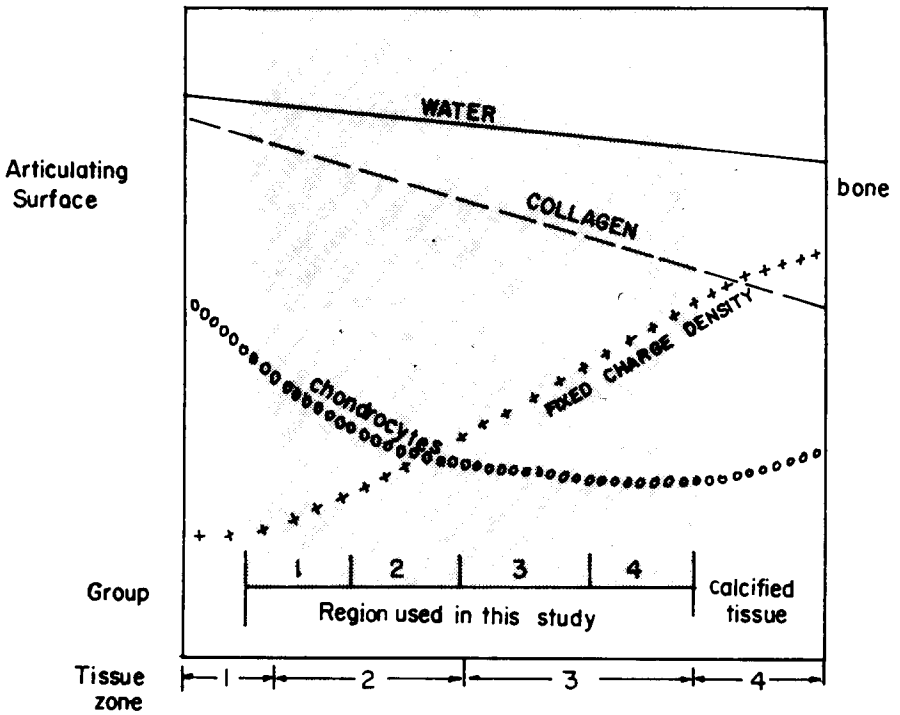

Fig. 1. Schematic representation of the composition of articular cartilage.

polysaccharides [4]. The matrix, also known as the ground substance, undergoes a continuous physiologic turnover, maintained by the chondrocytes.

The distribution of the major components of articular cartilage varies with distance from the articulating surface. The generalized relative composition is shown schematically in Fig. 1. The detailed composition of the tissue varies with species, age and health.

The permeability of a range of solute molecules dissolved in lactated Ringer's solution through human cartilage membranes 200 μm thick was investigated by Bernich et al. [5]. Using Fick's Law these investigators evaluated $A/\Delta x$, the apparent diffusion area per unit path length and L_p, the pressure filtration coefficient. From these two parameters the membrane's apparent pore radius was calculated.

Maroudas and coworkers [2, 6] reported on the permeability, diffusivity, electrical conductivity and streaming potential of thick sections (about 1000 μm) of human articular cartilage. These investigators treated the observed transport phenomena using a kinetic model. Their work, like that of Bernich et al. [5] was carried out in lactated Ringer's solution complicating the interpretation of the results. Moreover, diffusion and permeation are irreversible processes, and therefore require the application of irreversible thermodynamics. We wish to extend the early data of Maroudas [2, 6] and Bernich [5] using the thermodynamic treatment for passive transport across synthetic membranes described by Kedem and Katchalsky [7]. We report on the three parameters required to describe a membrane-solute-solvent system for non-electrolyte

solutes: the pressure filtration coefficient (L_p), the reflection coefficient (σ) and the solute permeability coefficient (ω). These parameters were used to calculate the frictional coefficients for the system. Values for the membrane pore radius and tortuosity were also calculated. It is our intention eventually to determine the value of these parameters for human adult articular cartilage in health and disease.

MATERIALS

Articular cartilage was obtained from the femoral condyles of freshly slaughtered cattle. This joint was chosen because it contains a large relatively flat area and is also a weight-bearing joint. The total thickness of the cartilage on this joint is between 1 and 1.5 mm. The joint was dissected, cut into cubes to fit the microtome, and stored moistened with lactated Ringer's solution at 4 °C for up to four days. The cartilage was sectioned using an American Optical rotary microtome. Serial sections were cut 60 ± 5 μm thick, and were separated into four groups covering about 250 μm each. Since chondrocytes are about 20–40 μm in diameter, sections thinner than 60 μm are likely to leak. Calcified tissue was never used. The four groups of sections thus correspond to the middle of zone I to the end of zone III (cf. Fig. 1). The sections were stored in lactated Ringer's at 4 °C. Prior to use they were equilibrated for several hours in the solution being studied.

Analytical grade sucrose and glucose were used as solutes. ^{14}C-labeled isotopes were obtained from Schwarz-Mann; tritiated water was obtained from New England Nuclear Company. All measurements were made at 25 °C.

METHODS AND CALCULATIONS

The equations describing the transport of solutes and solvent across a membrane as an irreversible thermodynamic process were developed by Kedem and Katchalsky [7] and evaluated for cellulose acetate membranes by Ginzberg and Katchalsky [8]. According to their treatment the following equations describe passive transport of non-electrolytes in such a system:

$$J_v = L_p \Delta P - \sigma L_p \Delta \pi \quad \text{(I)}$$

$$J_s = \bar{C}_s(1-\sigma)J_v + \omega \Delta \pi \quad \text{(II)}$$

The material fluxes J_v and J_s result from the driving forces ΔP and $\Delta \pi$.

$J_v = dv/dt$ is the rate of volume flow across unit area of the membrane in cm/s.

$J_s = dn/dt$ is the rate of solute flow across unit area of the membrane in mol/s/cm^2.

ΔP = hydrostatic pressure difference in dynes/cm^2.

$\Delta \pi$ = osmotic pressure difference (in dynes/cm^2) defined as $RT\Delta C_s$ (ΔC_s is the solute concentration difference).

$\bar{C}_s$ = mean of the solute concentrations on the two sides of the membrane.

The phenomenological coefficients σ, L_p and ω define the membrane characteristics:

(1) L_p, the pressure filtration coefficient, characterizes membrane-solvent interactions. It is determined by evaluating Eqn. I under conditions of zero concentration difference, so that $L_p = J_v/\Delta P$.

The measurement of the volume flow J_v caused by a pressure difference ΔP was performed with the cell illustrated in Fig. 2A, as described below.

(2) ω, the solute permeability coefficient, is a measure of solute membrane interactions. It is obtained from Eqn. II in the absence of a pressure difference and under conditions of zero volume flow, i.e., $J_v = 0$. Then $\omega = J_s/\Delta\pi = J_s/RT\Delta C_s$. J_s is measured as the rate of isotopically labeled solute flow across a membrane under conditions such that the total solute concentration is the same on both sides of the

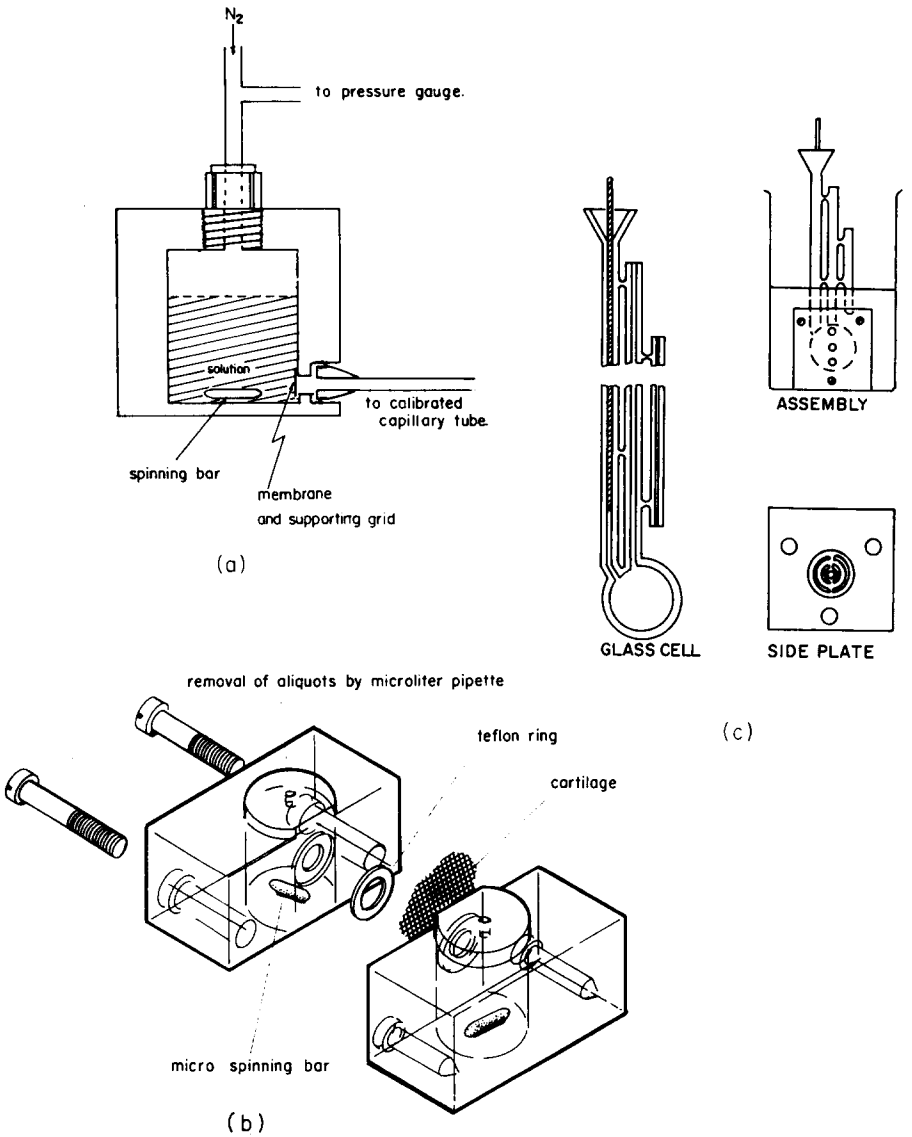

Fig. 2. Experimental apparatus. (a) Pressure filtration cell, (b) permeability cell, (c) osmometer.

membrane assuming, as is usual, no isotope interactions [2, 7]. The diffusion cell used in our measurements is illustrated in Fig. 2B.

(3) σ, the reflection or Staverman coefficient characterizes solute-solvent interactions. It is a measure of the selectivity of the membrane [9], $\sigma = 0$ signifies that the membrane is completely non-selective allowing free passage of solute. $\sigma = 1$ implies that the membrane is ideally semipermeable, permitting no solute transport. This parameter was evaluated using Eqn. I: J_v is measured under conditions where $\Delta P = 0$. Then $J_v = -L_p \sigma \Delta\pi$. The volume flux J_v is most conveniently observed in an osmometer as shown in Fig. 2C. $\Delta\pi$ is a result of the concentration difference in the osmometer. Thus $\sigma = (-J_v)_{\Delta P=0}/\Delta\pi L_p$, using the value of L_p determined in a pressure flltration experiment.

For the measurement of J_v (Fig. 2A) nitrogen was used to generate pressures up to 100 lb/inch^2. Magnetic stirring bars were used for stirring on the high-pressure side of the membrane. The initial volume of solution in the cell was 10.0 ml. the effluent volume flux (approx. 10^{-3} μl per s) was measured in a calibrated capillary tube.

The solute flux (J_s) was measured by following the diffusion of radioactive solute molecules across articular cartilage membranes (Fig. 2B). The initial volume on both sides of the membrane was 6.0 ml. Stirring was achieved with magnetic spinning bars placed on a shaft underneath each compartment of six cells. Twelve shafts were attached to one variable-speed motor by means of a timing belt. In this way the same stirring rate was simultaneously produced in six cells. At 3 to 5 min interval 0.1 ml samples were withdrawn, from both half cells, by micropipette for up to 1 h. These were placed in 4 ml of scintillation cocktail and counted in a Beckman 250 liquid scintillation spectrometer. The scintillation fluid was prepared by adding 5 g of PPO (2,5-diphenyl oxazole) to 100 g of naphthalene and diluting to one liter with dioxane; scintillation grade materials were used.

The volume flow resulting from a concentration difference was measured in a glass osmometer (Fig. 2C) built after the Zimm-Myerson design [10]. It was tested for leaks with a Teflon plug and calibrated using dialysis tubing as membrane and impermeable solutes of known molecular weights. The inside volume of the osmometer was approximately 2 ml, the outside volume 100 ml. The temperature was controlled at 25 ± 0.05 °C. The volume flux for articular cartilage membranes was of the order of 10^{-4} μl per s and was measured in a capillary tube using a cathetometer.

From the measured values of L_p, σ and ω one obtains the frictional coefficients f_{sw}, f_{sm} and f_{wm}:

$$f_{sw} = \frac{\varphi_w}{\omega \Delta x}\left[1 - \sigma - \frac{\omega \bar{V}_s}{L_p}\right]$$

$$f_{sm} = f_{sw}\left[\left(\sigma + \frac{\omega \bar{V}_s}{L_p}\right) \Big/ \left(1 - \sigma - \frac{\omega \bar{V}_s}{L_p}\right)\right]$$

$$f_{wm} = \frac{\varphi_w \bar{V}_w}{\Delta x}\left[\frac{1}{L_p} - (1-\sigma)\left(\sigma + \frac{\omega \bar{V}_s}{L_p}\right)\left(\frac{\bar{C}_s}{\omega}\right)\right]$$

φ_w is the water content (wet weight ratio) of the membrane; Δx is the membrane thickness and $\bar{V}_s$ and $\bar{V}_w$ are the partial molal volumes of solute and solvent respectively. These coefficients can be interpreted as hydraulic friction coefficients, f_{sw} expressing

the solute-solvent interaction, f_{sm}, the mutual drag of solute and membrane and f_{wm} the interaction of solvent and membrane [11].

The ratio of the frictional coefficient f_{sw} in the membrane to the frictional coefficient in bulk solution f_{sw}^0 is defined as the tortuosity (ν). The bulk frictional coefficient f_{sw}^0 is calculated from the Stokes-Einstein relation:

$$f_{sw}^0 = RT/D$$

where D = the self-diffusion coefficient. The tortuosity is related to the geometry of channels within the membrane and to the solute geometry.

The pore radius was estimated by:

$$r^2 = \left[\frac{L_p}{\omega \overline{V}_s} - 1\right] \frac{8\eta \overline{V}_s D}{RT}$$

where η is the bulk viscosity of the solution in centipoise.

A diffusion coefficient within the membrane was calculated from the relation between diffusion and viscosity [12].

$$\overline{D} = \frac{RT\Delta x}{\varphi_w \overline{V}_w}$$

The reproducibility of our measurements was calculated from replicate measurements on cartilage membranes obtained from up to ten different animals. Reproducibility values were as follows: $L_p \pm 9\,\%$, $\omega \pm 13\,\%$ and $\sigma \pm 19\,\%$.

RESULTS

The measurement of the transport properties of membranes is complicated by the possible development of a stagnant layer of molecules which can act as an additional barrier to permeation. Adequate stirring is necessary to minimize the formation of this Nernst layer. By measuring ω as a function of stirring speed, we found that, in our system, 1000 rev./min was sufficient to minimize interference from the Nernst layer when glucose or sucrose were the diffusing solutes. In pure water it was necessary to stir twice as fast. ω_{H^3HO} for sections 70 and 140 μm thick are related by a factor of 1.85, showing the unstirred layer to be constant and small [13]. Using the method outlined by Ginzburg and Katchalsky [8], we estimate the Nernst layer to be 20 μm thick in water, but negligibly small when glucose or sucrose are present. The values we report for ω have been corrected for the Nernst layer [8].

L_p was determined for water alone, and for solutions of glucose and sucrose varying in concentration from 0.01 to 1.0 M. Fig. 3 illustrates some representative data. The plot of J_v vs. ΔP deviates from linearity at pressures greater than 3 atmospheres, even in water. Hysteresis effects were also observed. Measurements were therefore always made from low to higher pressure. After being subjected to a pressure difference of approx. 6 atm, the membrane thickness was found to have decreased by about 25 % yet the amount of H^3HO extruded from the membrane was found to be independent of the applied pressure. Table I lists the values obtained for L_p measured in the linear, low-pressure region, i.e., $\Delta P < 3$ atm, as a function of solute concentration and section group number.

Fig. 4 illustrates some of the data obtained in the solute permeability measure-

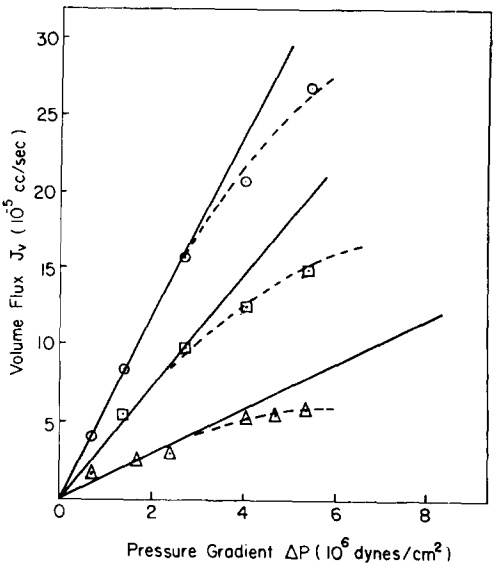

Fig. 3. Volume flux as a function of pressure gradient using H_2O (⊙), 0.1 M glucose (⊡), and 0.01 M sucrose (△). Sections from group 3.

TABLE I

PHENOMENOLOGICAL TRANSPORT COEFFICIENTS OF BOVINE ARTICULAR CARTILAGE

		O_p (10^{-11} cm^3/dyne-s)			
Section group No.:		1[a]	2	3	4
H_2O		16.7 (1.3)[b]	11.7 (1.5)	9.2 (1.0)	9.3 (0.4)
Glucose:	0.01 M	15.2 (0.8)	7.9 (0.8)	6.6 (0.3)	6.5 (0.5)
	0.10 M	9.3 (1.4)	6.5 (0.6)	4.8 (0.4)	4.9 (0.2)
	1.00 M	5.5 (0.3)	5.3 (0.5)	5.2 (0.6)	3.2 (0.2)
Sucrose:	0.01 M	10.4 (0.5)	10.5 (0.5)	6.3 (0.8)	4.2 (0.2)
	0.10 M	7.4 (0.8)	5.7 (0.8)	5.3 (0.6)	4.0 (0.5)
	1.00 M	3.5 (0.6)	3.0 (0.5)	1.7 (0.6)	1.0 (0.4)
Reflection coefficient (σ)					
Glucose:	0.01 M	0.035 (.008)	0.081 (.012)	0.101 (.020)	0.095 (.015)
	0.10 M	0.017 (.005)	0.028 (.005)	0.020 (.005)	0.032 (.005)
	1.00 M	0.029 (.005)	0.035 (.005)	0.029 (.005)	0.042 (.005)
Sucrose:	0.01 M	0.15 (.015)	0.099 (.015)	0.067 (.015)	0.10 (.015)
	0.10 M	0.060 (.010)	0.062 (.012)	0.027 (.014)	0.032 (.010)
	1.00 M	0.020 (.005)	0.097 (.020)	0.16 (.025)	0.082 (.032)
Permeability coefficient (ω) (10^{-15} mol/dyne-s)					
H_2O		117.0 (11.7)	86.0 (9.5)	66.0 (7.2)	43.0 (5.8)
Glucose:	0.01 M	10.2 (1.0)	9.5 (1.0)	7.1 (0.8)	6.9 (0.7)
	0.10 M	1.0 (0.5)	6.1 (0.4)	5.3 (0.4)	4.1 (0.4)
	1.00 M	5.3 (0.3)	4.9 (0.4)	3.9 (0.4)	4.1 (0.3)
Sucrose:	0.01 M	3.4 (0.5)	4.1 (0.5)	3.1 (0.5)	2.4 (0.5)
	0.10 M	2.8 (0.4)	1.8 (0.4)	1.5 (0.3)	1.7 (0.4)
	1.00 M	2.5 (0.4)	2.3 (0.4)	1.2 (0.3)	1.6 (0.3)

[a] 1 = closest to articular surface.

[b] Figures in parentheses = dispersion. Thus: 16.7 ± 1.3

ments. J_s and ω are proportional to the slopes of these lines. Table I lists ω as a function of solute concentration for sections obtained at different distances from the articular surface.

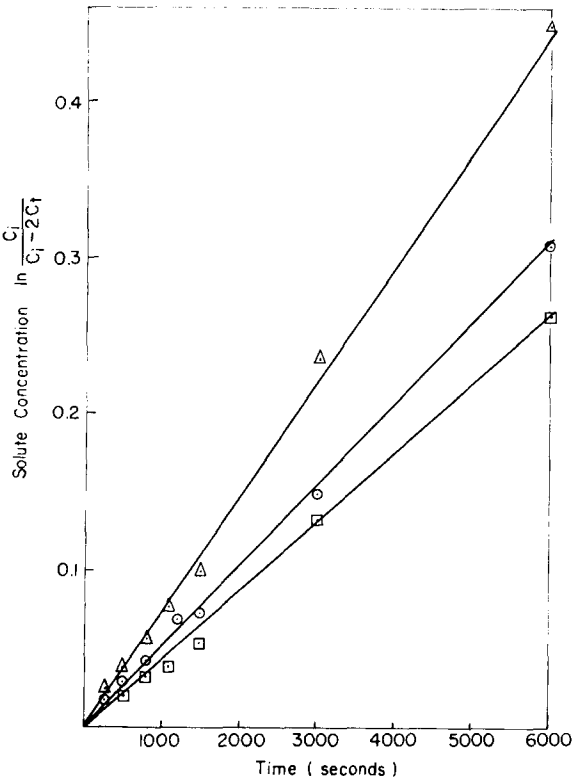

Fig. 4. Glucose permeation as a function of time using sections from group 4 and glucose as solute; 0.01 M (△), 0.1 M (○) and (⊡) 1.0 M, respectively.

TABLE II

TRANSPORT PROPERTIES OF BOVINE ARTICULAR CARTILAGE

Date from all section groups.

		Frictional coefficients (10^{15} × dyne-s/mol-cm)				Tortuosity	Diff. coeff. (10^{-5} cm²/s)	Pore radius (Å)
		f_{sw}	f_{sm}	f_{wm}	f_{sw^o} [a]	(f_{sw^o}/f_{sw})	$\bar{D}$	r_p
H^3HO		2.1			1.0	0.49	140	36
Glucose:	0.01 M	13	1.3	0.026	3.6	0.26	108	40
	0.10 M	24	0.88	0.034	3.8	0.19	76	42
	1.00 M	31	1.4	0.033	4.4	0.16	57	53
Sucrose:	0.01 M	36	3.1	0.030	4.8	0.13	94	56
	0.10 M	60	2.5	0.034	5.2	0.090	67	60
	1.00 M	59	4.5	0.067	5.5	0.10	28	62

[a] Calculated from data in Ginzberg and Katchalsky [8].

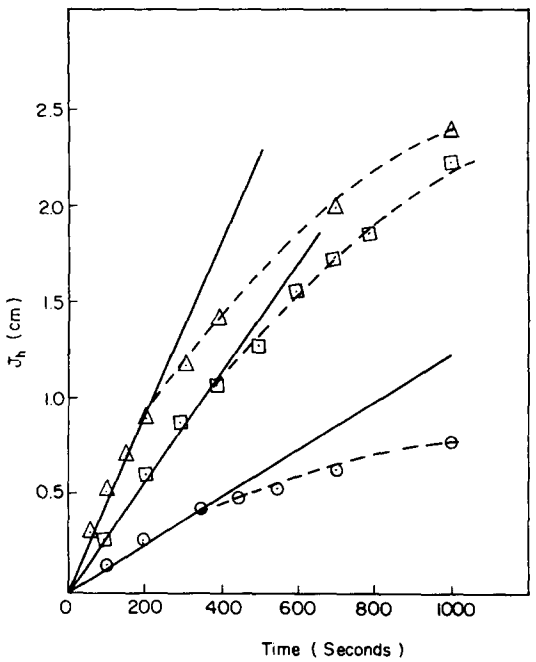

Fig. 5. Osmotic flow expressed as a function of capillary rise J_h for a 0.1 M sucrose solution for sections 1, (△), 2, (⊙) and 3 (⊡).

Fig. 5 illustrates the measurement of the volume flux obtained in the osmometer. The deviation from linearity with time shows that articular cartilage is permeable to both glucose and sucrose. Only initial flow rates can therefore be used to calculate σ. The calculated values are listed in Table I. From the measured values of L_p, ω and σ we calculated frictional coefficients, tortuosity, the apparent pore radius and the membrane diffusion coefficients, $\bar{D}$. These are listed in Table II.

DISCUSSION

The deviation of the relation between J_v and ΔP from linearity at high pressure gradients (Fig. 3) cannot be due to hyperfiltration (Eqn. I) since it occurs even in the absence of solute. The solutions on the high and low pressure side of the membrane do not differ in solute concentration. Since the membrane thickness decreases by about 25 % as a result of the application of the higher pressure gradient, while the amount of H^3HO expressed is independent of the applied pressure, increased "packing" of the membrane must be one result of the pressure gradient. This could decrease the membrane's tortuosity. In fact, the tortuosity for H^3HO at high pressure is 0.35, while a value of 0.49 was obtained at low pressure (Table I).

L_p decreases with increasing distance from the articular surface, and hence with density (Table I). The same effect is observed both with increasing solute concentration and size. Ginzburg and Katchalsky have shown for cellulose membranes that L_p does not vary significantly from the value obtained in water at non-electrolyte concen-

trations less than 0.1 M, while at higher solute concentrations L_p decreases due to increased viscosity [8]. For articular cartilage L_p decreases with sucrose and glucose concentration over the entire concentration range. One cannot ascribe this change to increasing viscosity alone.

The value obtained for L_p in bovine articular cartilage in water ($12 \cdot 10^{-11}$ cc/dyne-s) is similar to those obtained for wet gel ($9.7 \cdot 10^{-11}$ cc/dyne-s) and for a highly swollen cellulose acetate membrane ($6.5 \cdot 10^{-11}$ cc/dyne-s). Water transport in these three gel-like swollen systems thus seems similar in nature, although the supporting matrices are very different. Values of L_p generally decrease by about one fourth in the presence of electrolytes [14]. The L_p of human articular cartilage in Ringer's solution is about one fourth to one tenth smaller than that obtained for bovine articular cartilage in water [2, 5]. This difference can therefore only partly be attributed to differences in medium; species, age and membrane thickness also influence the results.

The L_p for water in red cell ghosts is an order of magnitude smaller than the values for bovine articular cartilage [15], again indicating the greater ease of water transport through the gel-like articular cartilage.

ω is a complex measure of the permeability of the membrane to the permeant. Our data show that it is less sensitive to solute concentrations than is L_p.

ω is greatest at the articular surface where the water content of the matrix is highest. This is consistent with observations reporting a correlation between ω and water content in synthetic membranes [8].

Since the rate of solute transport is independent of time (Fig. 4), Fick's Law applies [16]. We conclude that non-electrolyte transport through articular cartilage is not facilitated. It is apparent, however, that glucose permeates more readily than sucrose.

As in the case for L_p, our values for glucose permeability (ω) are approximately ten times larger than those obtained for human articular cartilage in physiological saline [5].

The low values of σ indicate that articular cartilage is a "coarse" or leaky membrane. This is true for glucose; it is somewhat more selective towards sucrose. Selectivity does not change appreciably with solute concentration or with depth from the articular surface. Most biological membranes, exhibiting active transport show σ values approaching unity [11]. In contrast for bovine articular cartilage $\sigma < 0.1$, a value similar to those reported for swollen artificial membranes [17].

The frictional coefficients, calculated from ω, L_p and σ, are listed in Table II. f_{sw}, the solute-solvent interaction, is by far the most important resistance to transport. Since f_{wm} is three orders of magnitude smaller, it can be assumed that the matrix is not significantly hindering transport of solvent. Eqn. III shows that f_{sw} will be small if $(1-\sigma)$ approaches $\omega\overline{V}_s/L_p$, an expected result if transport occurs through different paths for solvent and solute [7]. Since we observe that $(1-\sigma) \gg \omega\overline{V}_s/L_p$ the solute and solvent move together via the same pathways.

Transport through highly swollen membranes, such as cartilage may be viewed as the summation of viscous flow (molecules moving in groups), and diffusive flow (molecules moving singly by random walk). Ticknor [12] has shown that it is necessary to consider diffusion and bulk viscosity of the liquid in considering permeation. When the diameter of the permeant and the membrane's apparent pore size differ by

less than a factor of two the contribution of diffusion to the total flow increases greatly. We determined the apparent pore radius of bovine articular cartilage as 40–60 Å (Table II). The radii of glucose and sucrose are 4.4 and 5.3 Å respectively [11]. We therefore expect the bulk (viscous) flow to be significant. Our calculation for $\bar{D}$ (Table II) shows that $\bar{D}$ in the membrane is about 100-fold greater than D^0 (free diffusion). Hence the transport of nutrients through articular cartilage is greatly enhanced by viscous flow. Thus all our data show that bovine articular cartilage possesses unusual ability to channel nutrients to the chondrocytes which support its integrity.

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