DISTRIBUTION AND DIFFUSION OF SOLUTES IN ARTICULAR CARTILAGE

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ABSTRACT An experimental study was made on the distribution of solutes between articular cartilage and external solution, and on their diffusivity in cartilage. The solutes were classed as small ions, small uncharged molecules, and uncharged molecules of increasing size ranging from glucose to hemoglobin. The distribution of sodium and chloride ions obeys the Donnan equilibrium when cartilage is equilibrated in physiological saline solution. However, in cartilage immersed in dilute solution the concentration of chloride ions is higher than predicted. This is probably due to the presence in cartilage of some microscopic regions depleted of mucopolysaccharide in which the Donnan exclusion does not operate. The molal distribution coefficients of small uncharged molecules like urea are close to unity, which indicates that all water in cartilage seems to behave as solvent water. For larger molecules the distribution as well as the diffusion coefficients decrease with increase in molecular weight and are very sensitive to variations in fixed charge density. The results have been interpreted on the basis of the "steric exclusion" principle. The largest molecules which can penetrate into cartilage are of the size of the hemoglobin molecule.

INTRODUCTION

The application of ion exchange theory to cartilage and its use to correlate experimental results on fluid flow, electrical conductivity, and the diffusion of small ionic solutes has been described (Maroudas, 1968). This work is now extended to study the distribution, between cartilage and external solution, of solutes of different type and size. These range from small ionic and nonionic solutes to dextrans comparable in size to chondroitin sulphate. The diffusion of larger molecules through cartilage is also investigated. Wherever possible the experimental results are correlated on the basis of ion exchange theory. The applicability of Ogston's theory of solute exclusion by macromolecules to the present results is also discussed.

MATERIALS AND METHODS

Human postmortem cartilage taken from the femoral condyles was used in all experiments. Cartilage slices 200–600 μ in thickness were cut on a sledge microtome at various depths, from the articular surface to the deep zone. Depth series of slices were obtained from several subjects in the age range 20–70 yr.

The solutes used in the investigation were: sodium and chloride ions, urea, glucose, sucrose, inulin carboxylic acid, hemoglobin, and dextrans 10 and 40. The dextrans were supplied by Pharmacia Ltd., Uppsala, Sweden.

Fig. 1 gives the molecular weight distribution curves for dextrans 10 and 40 as supplied by the manufacturers.

Determination of Distribution Coefficients

The following procedure was adopted to determine the distribution coefficients of the various solutes between cartilage and Ringer's solution.

Each cartilage slice was mopped dry with absorbent paper and weighed. It was then immersed in Ringer's solution (or, as in the case of Na^+ and Cl^-, in Ringer's solution diluted 1:10) which contained a suitable amount of the solute under consideration. The concentration of the solute was about 1% for the dextrans, inulin, and hemoglobin but was much lower for urea in order to avoid any risk of denaturing the cartilage proteins. Experiments with glucose were carried out at two concentration levels: one at 0.1% (i.e. approximately the physiological level) and the other at a trace level (of the order of 0.0001%). Within this range the concentration was found to have no effect on the distribution coefficient.

The liquid containing the cartilage slice and the given solute was stirred for a suitable period of time. In the case of the small solutes, one-half hour at room temperature was found in

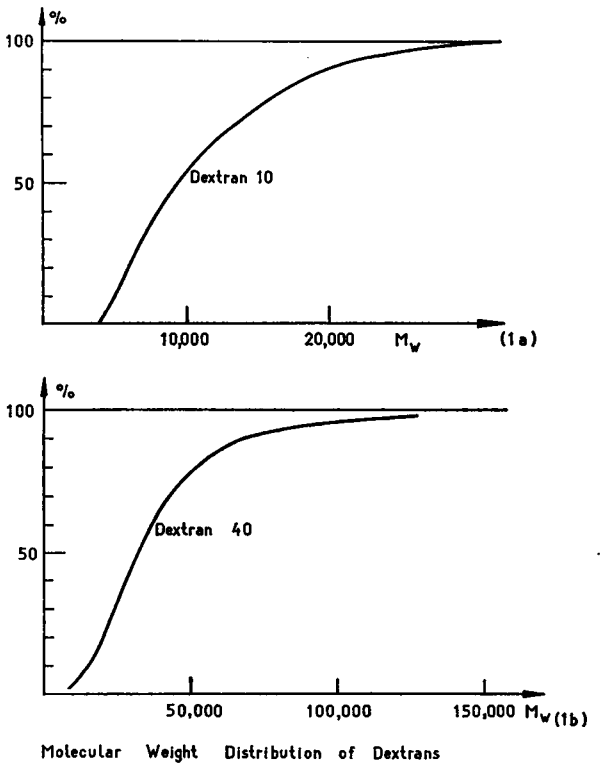

FIGURE 1 Molecular weight distribution of dextrans 10 and 40.

preliminary experiments to be amply sufficient to ensure complete equilibrium. However, when dextrans, hemoglobin, and inulin were used, the cartilage was kept in the rich solution for approximately 12 hr. In order to prevent enzymatic degradation of cartilage during this period, the solutions were kept at 4°C. Where the solute consisted of macromolecules, vigorous stirring was found to cause breaking up of these molecules; accordingly, the solutions were occasionally swirled by hand only.

Since the volume of the cartilage slice was very small compared with that of the solution in which it was equilibrated (3 cm^3 of solution to about 0.04 cm^3 of cartilage), the change in the concentration of the solution due to the uptake of solute by cartilage was negligible. In most cases the concentration of the solute in cartilage could not be determined directly because of the interference by the constituents of cartilage. Accordingly, the simplest procedure was to desorb the solute into fresh Ringer's solution by leaving the cartilage specimen in contact with the solution until equilibrium. Since the volume of the slice was again only about 1% that of the solution, the proportion of solute remaining in the cartilage in equilibrium with the solution was negligible. This method has a further advantage, viz. that the solute irreversibly taken up by cartilage is not desorbed and that the results, therefore, give the reversible equilibrium distribution.

The amount of solute desorbed was assessed either by scintillation counting, in those cases where a solute labeled with a radioactive isotope had been used (sodium and chloride ions, urea, glucose, sucrose, and inulin carboxylic acid), or by colorimetry (dextrans and hemoglobin).

Where γ-radiation was emitted (^{22}Na), a solid potassium bromide crystal was used as scintillator, while for β-radiation a water mixible scintillator suitable for solutions containing a high salt concentration was employed (Turner, 1968).

Dextran concentration was determined by Wallenius' modification of the anthrone method (Wallenius, 1954). A Unicam Colorimeter SP 1300 (Unicam Instruments Ltd., Cambridge, England) was used with a 620–750 μ filter. By this method it was possible to determine accurately concentrations down to 20 ppm, corresponding to a dilution of 1:1000 of the rich dextran solution. Since the anthrone reagent is extremely sensitive to the presence of traces of sugar, blanks were carried out on the cartilage slices by themselves and, where necessary, corrections were applied to the readings.

The hemoglobin determinations were also carried out colorimetrically. The optical density of the solutions was determined using a 370–515 μ filter.

Determination of Solute Permeability Coefficients

The diffusion experiments were carried out in a diffusion cell as previously described (Maroudas, 1968). Both compartments of the cell contained Ringer's solution and the solute whose rate of diffusion was to be investigated was introduced on one side of the cartilage membrane at the same concentration as was used in the equilibrium experiments. The solute gradually diffused across the membrane into the second compartment, the contents of which were withdrawn for analysis at approximately ½-hr intervals in the case of small solutes, and 24-hr intervals in the case of dextrans and hemoglobin. As in the case of the equilibrium experiments, diffusion runs involving the higher molecular weight solutes was carried out at 4°C without stirring.

The same methods of analysis were employed as in the equilibrium studies.

It was found that the rate of diffusion did not alter with time after the initial unsteady-state period, which showed that the cartilage matrix was not undergoing significant degradation in the course of the experiments. Degradation of the matrix leads to a considerable increase in

the rate of diffusion of large molecules, as was indeed observed when some preliminary experiments with dextrans were carried out at room temperature and not at 4°C.

Fixed Charge Density Determination

Fixed charge density was determined by the streaming potential method (Maroudas, 1968; Maroudas and Muir, 1969) on each slice which had been used in the distribution and the diffusion studies.

Dry Weight Determination

The water content of each slice was determined at the end of the experiments by the same method as has previously been described (Maroudas and Muir, 1969).

RESULTS AND DISCUSSION

Distribution of Various Solutes between Cartilage and Ringer's Solution

There are many ways of expressing the distribution of a solute between two phases, depending on the choice of units for the concentration.

In the present work the distribution coefficients have been expressed in three ways:

(*a*) in the way they were obtained experimentally, i.e. as the ratio of solute concentration per unit weight of cartilage to solute concentration per unit volume of solution (which, in the case of dilute solutions, is practically the same as per unit weight of solution), $\bar{w}/w$,

(*b*) on a molar basis, i.e. as the ratio of solute concentration per unit volume of cartilage to solute concentration per unit volume of solution, $\bar{C}/C$,

(*c*) on a molal basis, i.e. as the ratio of solute concentration per unit weight of water in cartilage to solute concentration per unit weight of water in solution (which, in the case of dilute solutions is practically the same as the weight or the volume of solution), $\bar{m}/m$.

The experimentally measured distribution coefficient $\bar{w}/w$ was converted to the molar coefficient $\bar{C}/C$ by multiplying by the average density of cartilage, i.e. by a factor of 1.08 (Maroudas and Muir, 1969), and to the molal coefficient $\bar{m}/m$ by dividing by the fractional water content of the given cartilage specimen.

Distribution Coefficients of Na^+ and Cl^-. Table I shows the distribution coefficients $\bar{w}/w$ and $\bar{m}/m$ for Na^+ and Cl^- between cartilage and normal Ringer's solution.

If the distribution of ions between cartilage and solution can be described in terms of the Donnan equilibrium the following relationship should hold between the molal distribution coefficients of a monovalent cation such as Na^+ and a monovalent anion such as Cl^- (Helfferich, 1962):

$$\left(\frac{\bar{\gamma}_{\pm}}{\gamma_{\pm}}\right)^2 \frac{\bar{m}_{Na^+}}{m_{Na^+}} = \frac{1}{\dfrac{\bar{m}_{Cl^-}}{m_{Cl^-}}}, \qquad (1)$$

TABLE I

DISTRIBUTION OF Na^+ AND Cl^- BETWEEN CARTILAGE AND RINGER'S SOLUTION

Age and code of specimen	Slice No.	% H_2O content	$\left(\frac{\bar{w}_{Na^+}}{w_{Na^+}}\right)_R$	$\left(\frac{\bar{w}_{Cl^-}}{w_{Cl^-}}\right)_R$	$\left(\frac{\bar{m}_{Na^+}}{m_{Na^+}}\right)_R$	$\left(\frac{\bar{m}_{Cl^-}}{m_{Cl^-}}\right)_R$	$\left(\frac{1}{(\bar{m}_{Cl^-}/m_{Cl^-})}\right)_R$
42M	3 and 4	76.0	1.51	0.376	1.98	0.495	2.02
42L	3 and 4	77.1	1.30	0.475	1.68	0.62	1.62
21A	1 and 2	77.2	1.16	0.624	1.50	0.81	1.24
"	3 and 4	73.0	1.20	0.450	1.65	0.615	1.62
"	6 and 7	71.9	1.20	0.430	1.67	0.60	1.66
71	1 and 2	77.5	0.97	0.575	1.25	0.74	1.35
"	3 and 4	72.2	1.21	0.436	1.68	0.605	1.66
"	5	73.1	1.30	0.430	1.77	0.596	1.70

where $\bar{\gamma}_\pm$ is the mean electrolyte activity in cartilage and $\gamma_\pm$ is the mean electrolyte activity in solution.

It can be seen from Table I that there is a very close agreement between the experimentally determined ratios $(\bar{m}_{Na^+}/m_{Na^+})_R$ and $1/([\bar{m}_{Cl^-}/m_{Cl^-}])_R$. It can hence be concluded that Donnan equilibrium is obeyed in this case and that the mean electrolyte activity coefficients in cartilage and in Ringer's solution are approximately equal so that $(\bar{\gamma}_\pm/\gamma_\pm) = 1$.

It is also to be noted that in a given subject $(\bar{m}_{Na^+}/m_{Na^+})_R$ increases with distance from the articular surface. This is in accordance with the finding that the mucopolysaccharide concentration increases with depth (Stockwell and Scott, 1967; Maroudas and Muir, 1969).

Table II shows the ratios $\bar{w}/w$ and $\bar{m}/m$ for Na^+ and Cl^- for cartilage equilibrated in 1:10 Ringer's solution. In this case the relationship $(\bar{m}_{Na^+}/m_{Na^+}) = 1/(\bar{m}_{Cl^-}/m_{Cl^-})$ no longer appears to hold since $\bar{m}_{Na^+}/m_{Na^+}$ is 2–3 times as high as $\bar{m}_{Cl^-}/m_{Cl^-}$.

This means that the concentration of the chloride ion in cartilage is much higher than predicted.

Anomalously high co-ion concentrations in synthetic cation exchange materials immersed in dilute solution have been reported in the literature. Teorell (1953) and Overbeek (1956) were the first to draw attention to this effect. Teorell attributed it to a reduction in the degree of dissociation of the fixed groups of the ion exchanger at low electrolyte concentrations whilst Overbeek explained it by a microscopic inhomogeneity in the distribution of fixed negatively charged groups in the material, resulting in the existence of some regions of very low fixed charge density where pools of the electrolyte could gather without the co-ion being subject to Donnan exclusion.

There have also been suggestions of an electrostatic interaction between collagen and glycosaminoglycans (Mathews, 1965) which would be expected to increase at low salt concentration, leading to an effective decrease in fixed negative charge density.

TABLE II

DISTRIBUTION OF Na^+ AND Cl^- BETWEEN CARTILAGE AND 1:10 RINGER'S SOLUTION

Age and code of specimen	Slice No.	% H_2O	$\left(\frac{\bar{w}_{Na^+}}{w_{Na^+}}\right)_{1:10R}$	$\left(\frac{\bar{w}_{Cl^-}}{w_{Cl^-}}\right)_{1:10R}$	$\left(\frac{\bar{m}_{Na^+}}{m_{Na^+}}\right)_{1:10R}$	$\left(\frac{\bar{m}_{Cl^-}}{m_{Cl^-}}\right)_{1:10R}$	$\left(\frac{1}{(\bar{m}_{Cl^-}/m_{Cl^-})}\right)_{1:10R}$
42M	3 and 4	76.0	6.2	0.21	8.2	0.28	3.6
42L	3 and 4	77.1	7.0	0.22	9.1	0.28	3.6
21A	1 and 2	77.2	4.9	0.34	6.4	0.44	2.3
"	3 and 4	73.0	6.2	0.20	8.6	0.28	3.6
"	6 and 7	71.9	6.5	0.20	9.1	0.28	3.6

Of the above three possibilities we have been able to eliminate two, by measuring the concentration of counterions in cartilage equilibrated in dilute solution. We have found that the counterions present balance the concentration of fixed negatively charged groups, as determined both by chemical analysis and by the streaming potential method (Maroudas, in preparation). This implies that there is no decrease in the degree of dissociation of these groups at low electrolyte concentration. Furthermore, we can also conclude that there are no significant electrostatic interactions between the negative groups on the glycosaminoglycans and the amino groups on collagen.

Thus, the high co-ion concentration observed in cartilage at low electrolyte concentration is most probably due to a microscopically inhomogeneous fixed charge density distribution; it would mean that there are some regions of the matrix which are depleted of acid glycosaminoglycans while in other regions the glycosaminoglycan content might be higher than the average. This interpretation would also be supported by our observation that the increase of Cl^- concentration in dilute solution over and and above that predicted by the ideal Donnan equilibrium becomes very considerable indeed at low values of fixed charge density.

Distribution of Small Nonionic Solutes between Cartilage and Ringer's Solution. In the absence of interactions of any kind, it should be expected that the molal concentrations of a small nonelectrolyte in cartilage and in external solution would be equal. However, in practice it is difficult to find small organic solutes which do not interact with any of the cartilage constituents. Of the small solutes so far examined, only urea did not exhibit irreversible behaviour; acetone, methyl alcohol, and ethyl alcohol all proved to be to a greater or lesser extent irreversibly taken up by cartilage.

The distribution coefficients for urea are shown in Table III. The molal distribution coefficients are on the whole close to unity, although in a few cases they appear to be somewhat higher.

Although ethyl alcohol is in part absorbed by cartilage irreversibly, it was possible to determine the distribution coefficients for the reversibly absorbed fraction alone and these also turn out to be around unity (see Table III).

TABLE III

DISTRIBUTION COEFFICIENTS OF UREA AND ETHANOL

Age and code of specimen	Slice No.	% H_2O	$\frac{\bar{w}}{w}$	$\frac{\bar{m}}{m}$
			Urea	Urea
21B	1 and 2	78	0.79	1.0
"	3 and 4	76	0.80	1.05
"	5	71.5	0.75	1.05
71	1 and 2	77.5	0.79	1.01
"	3 and 4	72.2	0.74	1.00
"	5	73	0.73	1.00
27A	1	81.5	0.81	0.99
"	2	79.2	0.78	0.98
"	4	76.2	0.75	0.98
27B	1	82	0.80	0.98
"	2	79	0.79	1.00
"	3	75	0.745	0.995
37A	1 to 4	75	0.8	1.07
37B	1 to 4	76	0.77	1.01
22A	1 to 5	75	0.92	1.22
			Ethanol	Ethanol
37A	1 to 4	75	0.80	1.06
37B	1 to 4	76	0.80	1.05
22A	1 to 5	75	0.72	0.96

These results, together with the close adherence to the Donnan equilibrium of the molal distribution coefficients of Na^+ and Cl^-, seem to indicate that all the water in cartilage behaves as solvent water. However, it could also be argued that the presence of bound water is masked by a "salting in" of the solutes. More work is needed to discriminate between the two possible explanations, and also to study the apparently irreversible absorption of acetone and alcohol.

Distribution Coefficients of Higher Molecular Solutes between Cartilage and External Solution. Towards small ions and molecules, all water in cartilage appears to behave as solvent water. However, where higher molecular weight solutes are concerned, some of the water becomes inaccessible because of steric exclusion effects exerted by the mucopolysaccharide molecules.

In Fig. 2 are plotted values of the molal distribution coefficient versus fixed charge density obtained for solutes of different size, ranging from glucose (mol wt 180) to dextran 40 (mol wt 40,000). The curve for a small solute, viz. urea, is also included for comparison.

The curves for sucrose, inulin, and hemoglobin were each obtained on cartilage from one individual at different depths from the articular surface. The curves for urea, glucose, and dextrans 10 and 40 were plotted from pooled results obtained for cartilage specimens of different individuals and corresponding to different depths from the articular surface.

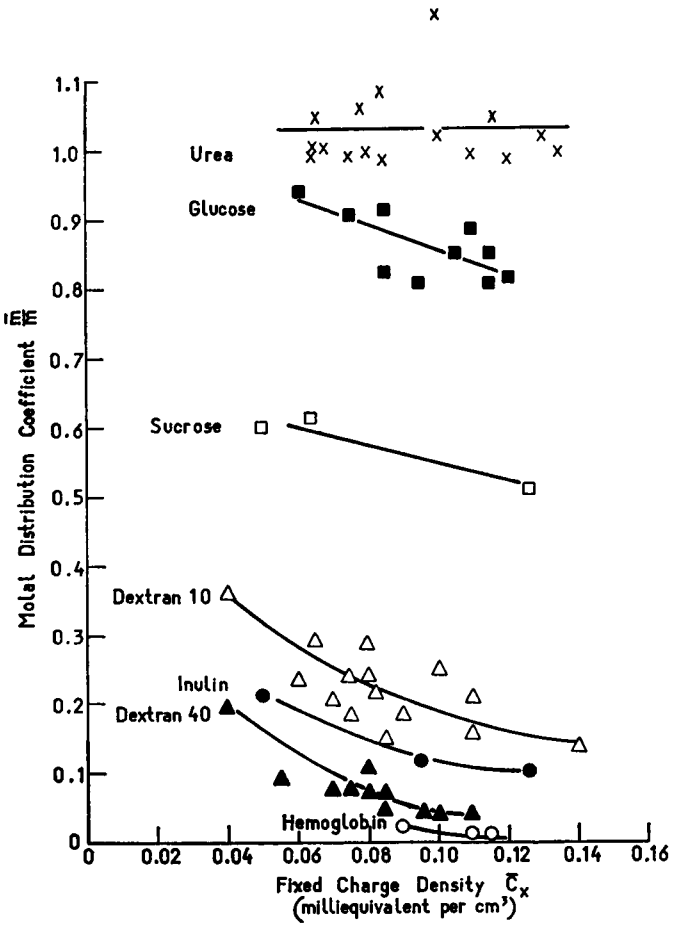

FIGURE 2 Variation of the distribution coefficient with fixed charge density for solutes of different size.

The following general effects emerge.

(*a*) The distribution coefficient decreases as the molecular weight of the solute increases, ranging from approximately 0.85 for glucose down to less than 0.1 for dextran 40 and hemoglobin.

(*b*) For any given solute there is a decrease in the molal distribution coefficient with increase in fixed charge density of the cartilage. This decrease is small in the case of glucose but becomes more and more significant as the molecular weight of the solute increases.

The molal distribution coefficients for each solute will now be considered in some detail.

(*a*) For urea, $\bar{m}/m$ is independent of fixed charge density and approximately equal to unity.

(*b*) In the case of glucose, the distribution coefficients, though high, are definitely less than unity, and show a small decrease with increase in fixed charge density. Thus, the glucose molecule which has the dimensions (5 × 7 × 9) A appears to be large enough to be excluded from a fraction of the solvent volume in cartilage.

(*c*) The distribution coefficient of dextran 10 is higher than that of inulin carboxylic acid, although the molecular weight of the latter is 5,000 while that of the former is 10,000. Two factors may be responsible for this apparent anomaly. First, dextran has only an average molecular weight of 10,000. As can be seen from the molecular weight distribution curve, 10% of dextran 10 has a molecular weight of less than 5,000 and since it is the smaller molecules which are preferentially taken up by cartilage, the mean molecular weight of the fraction of molecules actually in cartilage is certainly lower than 10,000 and in most cases lower than 5,000. Second, inulin carboxylic acid is an anion and, apart from steric exclusion effects, the Donnan exclusion would also be expected to operate.

(*d*) Hemoglobin has the lowest distribution coefficient of the substances so far tested.

In the case of the larger solutes, the effect of fixed charge density on the distribution coefficient is striking. Thus, for instance, in the case of dextran 10 an increase in $\bar{C}_x$ from 0.04 to 0.11 leads to a reduction in $\bar{m}/m$ from 0.36 to 0.18. In the case of dextran 40, this effect is even greater: for the same increase in $\bar{C}_x$, $\bar{m}/m$ drops from 0.2 down to 0.04.

It is of interest to consider to what extent the above results can be accounted for by the theory developed by Ogston (1958) for the treatment of the exclusion of globular solutes by linear macromolecules in solution.

Ogston derived the following equation for the volume available to a spherical particle (e.g. a protein) in a system of randomly distributed long rodlike molecules.

$$k_{av} = e^{-\pi L(r_r + r_s)^2}, \tag{2}$$

where L = concentration of rods expressed in cm/cm^3, r_s = radius of sphere, r_r = radius of rod, k_{av} = fractional volume available to the spherical particles. The parameter k_{av} is effectively equal to the distribution coefficient.

It can be readily seen from equation 2 that (*a*) the volume available to any given solute should decrease exponentially as L increases, i.e. as the concentration of macromolecules increases, and (*b*) for solute molecules such that $r_s \geqslant r_r$, the available volume should be extremely sensitive to changes in the size of the solute. The results shown in Fig. 2 are in full qualitative agreement with the above predictions.

Laurent (1964) plotted the distribution of various solutes between a hyaluronic acid gel and buffer versus solute size, and obtained very good agreement between his experimental curve and the theoretical curve calculated from Ogston's equation.

A similar experimental curve for the distribution of solutes between cartilage and Ringer's solution is given in Fig. 3. (Values of $\bar{m}/m$ corresponding to $C_x = 0.11$ were taken from curves in Fig. 2) The theoretical curve is also shown. It was obtained as-

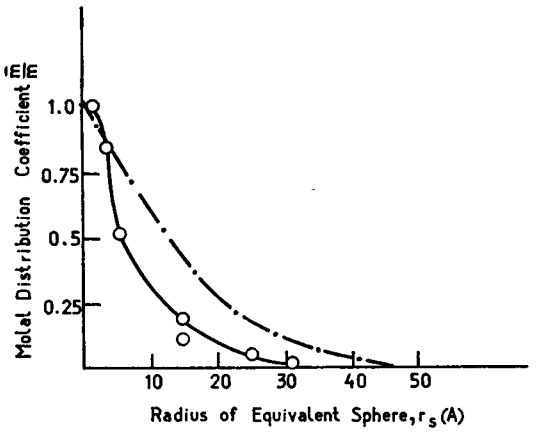

FIGURE 3 Variation of the distribution coefficient with solute size.

suming a 6% by volume concentration of mucopolysaccharide protein complex. To simplify the calculation, the whole of the mucopolysaccharide protein complex was assumed to consist of chondroitin sulphate chains. The weight of the repeating disaccharide unit is 503. The disaccharide length was taken to be 10×10^{-8} cm and $r_r = 3.5 \times 10^{-8}$ cm, in analogy with the figures used by Laurent for the hyaluronate molecule.

The mean molecular weight of the dextran 10 fraction actually penetrating into cartilage was assumed to be 5000 (corresponding to the mean molecular weight of the lowest 20% as given by the distribution curve in Fig. 1). The corresponding radius of equivalent sphere r_s was taken as 15 A (Wallenius, 1954). Similarly, the mean molecular weight of the dextran 40 fraction able to penetrate into cartilage was taken as 12,000 and the corresponding r_s was found to be equal to 25 A.

The experimental points lie below the theoretical curve, showing an exclusion volume 20–50% greater than predicted.

In view of the many assumptions made in the calculation of the theoretical curve, this discrepancy is not surprising. Thus, for instance, too low a value for r_r may have been assumed. Schubert (1966) who determined experimentally the distribution coefficients of albumin and chondroitin sulphate between solutions of PP-L and buffer, used for r_r a figure of 10 A to make his experimental results fit the theory.

Diffusion of Solutes through Cartilage

The diffusion of small ionic solutes through cartilage has been discussed by the author in a previous publication (Maroudas, 1968). In the present work the diffusion of nonionic solutes of different size was investigated, in parallel with the study of their distribution coefficients.

The rate of movement of solutes from external solution into cartilage and vice versa is governed by two factors: (*a*) the distribution coefficient of the solute between

cartilage and external solution; (*b*) the effective diffusion coefficient of the solute in cartilage. Thus, the solute permeability coefficient $\bar{P}$ is defined by the relation

$$\bar{P} = \bar{D}\frac{\bar{C}}{C}, \tag{3}$$

where $\bar{P}$ = effective solute flux per unit cross-sectional area of cartilage per unit concentration gradient across it, $\bar{D}$ = effective diffusion coefficient of solute in cartilage, and $\bar{C}/C$ = molar distribution coefficient of solute between cartilage and external solution.

In the case of molecules which are small in comparison with the cross-sectional area of the diffusion channel, the movement of the solute in the membrane is governed by its free diffusion in the water-filled pores. Thus, equation 3 can be written as:

$$\bar{P} = \left(\frac{A_d'}{\lambda}\right)\left(\frac{\bar{m}}{m}\right)D, \tag{4}$$

where A_d' = total cross-sectional area of diffusion channels per unit cross-sectional area of cartilage, λ = tortuosity factor = actual length of diffusion path per unit thickness of cartilage slice, and D = diffusion coefficient of solute in free solution.

Equation 4 is equivalent to equation 5 of Page and Bernstein (1964) which they use to describe steady-state diffusion through a sheet of cat's heart muscle.

Now the cross-sectional area of the diffusion channels can be expressed in terms of the total porosity of the membrane and the actual length of the flow channels:

$$A_d' = \frac{\epsilon}{\lambda}. \tag{5}$$

The porosity ϵ is equivalent to the fractional water content of the cartilage membrane.

Equation 4 can thus be rewritten as:

$$\bar{P} = \left(\frac{\epsilon}{\lambda^2}\right)\left(\frac{\bar{m}}{m}\right)D. \tag{6}$$

But by definition

$$\epsilon\left(\frac{\bar{m}}{m}\right) = \left(\frac{\bar{C}}{C}\right). \tag{7}$$

Hence

$$\bar{P} = \left(\frac{\bar{C}}{C}\right)\left(\frac{D}{\lambda^2}\right). \tag{8}$$

Comparison of equations 3 and 8 gives

$$\frac{\bar{D}}{D} = \frac{1}{\lambda^2}. \tag{9}$$

Thus, for a small solute such as urea, the ratio of its effective diffusion coefficient in cartilage ($\bar{D}$) to its diffusion coefficient in free solution depends only on the tortuosity of the diffusion path in cartilage and the latter can readily be obtained from equation 9.

For molecules whose dimensions are not negligible in relation to channel size, the permeability depends not only on the friction between the solute and the solvent but also on the friction between the solute molecules and the matrix. Using an approach similar to that of Kedem and Katchalsky (1961), though a somewhat different nomenclature, it can be shown that

$$\bar{P} = \left(\frac{\bar{C}}{C}\right)\left(\frac{1}{\lambda^2}\right) D \left(\frac{1}{1 + DF_{sm}}\right), \tag{10}$$

where F_{sm} = solute-membrane friction coefficient. Hence, in this more general case

$$\frac{\bar{D}}{D} = \frac{1}{\lambda^2}\left(\frac{1}{1 + DF_{sm}}\right). \tag{11}$$

When friction between the solute molecules and the pore walls becomes much higher than the friction between the solute molecules and the solvent, then the term $DF_{sm} \gg 1$ and equation 10 reduces to

$$\bar{P} = \left(\frac{\bar{C}}{C}\right)\left(\frac{1}{\lambda^2}\right)\left(\frac{1}{F_{sm}}\right), \tag{12}$$

or

$$\bar{D} = \left(\frac{1}{\lambda^2}\right)\left(\frac{1}{F_{sm}}\right). \tag{13}$$

Table IV gives typical diffusion data for a number of solutes in cartilage. Examination of these data lead to the following observations.

(*a*) Using the ratio $\bar{D}/D = 0.55$ obtained for urea it is possible to estimate the tortuosity of cartilage on the basis of equation 9. The value of λ thus obtained is equal to 1.35.

(*b*) The diffusion coefficient of glucose is considerably lower than that of urea, being equal to only about one-third of its own value in water.

(*c*) In general, it appears that as the molecular weight of the solute increases, so the ratio $\bar{D}/D$ decreases. This is entirely consistent with equation 11, since the larger the solute molecule, the higher the solute-membrane friction coefficient F_{sm}. In the case of dextrans 10 and 40, D is negligible compared with $\bar{D}$ and equation 13 applies; the effective diffusion coefficient is dependent only on the tortuosity of the diffusion path and the frictional interactions between the solute molecules and the solid matrix. It is not possible to say from the present results whether the tortuosity of the diffusion path increases or decreases as the size of the solute increases.

TABLE IV

DIFFUSION OF SOLUTES THROUGH CARTILAGE

Age and code of specimen	Slice No.	$\bar{C}_x$	Solute	$\bar{C}/C$	$\bar{P}$	$\bar{D}$	D_{water}	$\frac{D_{cartilage}}{D_{water}}$
					$cm^2\ sec^{-1}$	$cm^2\ sec^{-1}$	$cm^2\ sec^{-1}$	
27A	1	0.085	Urea	0.81	4.85×10^{-6}	6×10^{-6}	1.1×10^{-5}	0.545
	2	0.095	"	0.79	4.95×10^{-6}	6.15×10^{-6}	"	0.555
	4	0.110	"	0.75	4.45×10^{-6}	5.9×10^{-6}	"	0.535
27A	1	0.085	Glucose	0.69	1.42×10^{-6}	2.1×10^{-6}	6×10^{-6}	0.35
	2	0.095	"	0.64	1.46×10^{-6}	2.3×10^{-6}	"	0.38
	4	0.110	"	0.60	1.35×10^{-6}	2.25×10^{-6}	"	0.375
55	3	0.090	Sucrose	0.485	6.3×10^{-7}	1.3×10^{-6}	4.8×10^{-6}	0.27
55	3	0.090	Inulin	0.09	2.3×10^{-8}	2.5×10^{-7}	1.8×10^{-6}	0.14
41L	2	0.060	Dextran 10	0.17	2.5×10^{-8}	1.47×10^{-7}	Approx. 1.8×10^{-6}	0.08
	3	0.070	"	0.155	2.3×10^{-8}	1.47×10^{-7}	"	"
	6	0.14	"	0.10	0.6×10^{-8}	0.6×10^{-7}	"	0.04
41M	1	0.055	Dextran 40	0.07	1.4×10^{-9}	2×10^{-8}	Approx. 7×10^{-7}	0.027
	2	0.085	"	0.055	0.84×10^{-9}	1.53×10^{-8}	"	0.022
	3	0.081	"	0.055	0.63×10^{-9}	1.14×10^{-8}	"	0.016
	4	0.096	"	0.031	0.46×10^{-9}	1.5×10^{-8}	"	0.022
	5	0.110	"	0.030	0.31×10^{-9}	1.03×10^{-8}	"	0.014
56	3	0.085	"	0.056	1.57×10^{-9}	2.8×10^{-8}	"	0.040
	5	0.098	"	0.040	0.68×10^{-9}	1.7×10^{-8}	"	0.024
	7	0.11	"	0.030	0.48×10^{-9}	1.6×10^{-8}	"	0.023
56	3	0.085	Hemoglobin	0.03	2×10^{-9}	1.6×10^{-7}	6×10^{-7}	0.28
	5	0.098	"	0.01	1.27×10^{-9}	1.27×10^{-7}	"	0.21
	7	0.11	"	0.007	1.03×10^{-9}	1.15×10^{-7}	"	0.16

(*d*) It is interesting to note that hemoglobin does not quite seem to fit in with the other solutes: although its molecular weight is higher than that of the two dextrans used and of inulin, its $\bar{D}/D$ ratio is also higher. This may be due to the comparatively more compact shape of the hemoglobin molecule which would mean less contact and hence less friction with the surrounding macromolecules than in the case of the more branched dextrans.

(*e*) Although neither urea nor glucose show any significant variation in their diffusion coefficients with fixed charge density, in the case of the larger solutes D appears to decrease with increase in $\bar{C}_x$. As has been shown in an earlier section, these solutes have a considerable excluded volume which increases sharply as $\bar{C}_x$ increases. An increase in exclusion volume results, in turn, in a reduction in the ratio of cross-sectional pore area to the size of the solute molecules and hence in an increase in the solute-membrane friction. This results in a lower effective diffusion coefficient.

It is interesting to note the similarity in their dependence on fixed charge density between the diffusional permeability coefficient $\bar{P}$ of a large solute and the hydraulic permeability coefficients, K. As can be seen from equation 3, $\bar{P}$ depends on $\bar{C}/C$ and $\bar{D}$. Since in the case of large solutes both $\bar{C}/C$ and $\bar{D}$ decrease as $\bar{C}_x$ increases, $\bar{P}$ will decrease very sharply as fixed charge density increases (see results for dextrans 10 and 40 and hemoglobin in Table IV).

The hydraulic permeability K in a porous network is dependent on the diameter of the flow channels as well as on their tortuosity. It is reasonable to assume that the

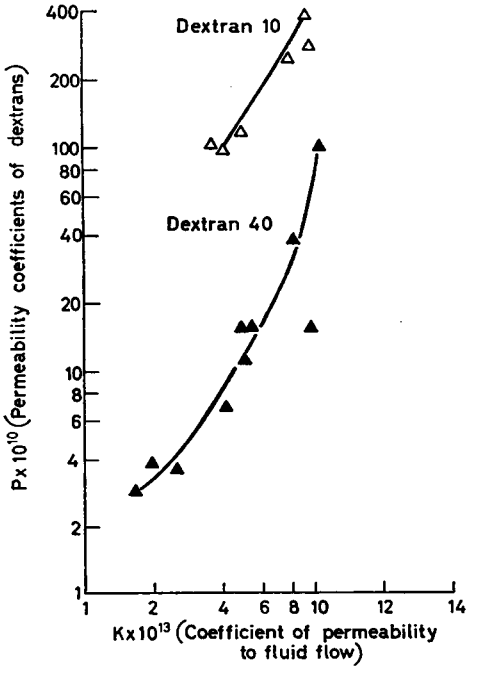

FIGURE 4 Relation between permeability of cartilage to dextrans and permeability to fluid flow.

former will decrease while the latter will increase when the concentration of mucopolysaccharide in the matrix of cartilage increases. Thus, K also will decrease sharply as fixed charged density increases. This has been shown to happen experimentally (Maroudas, 1968). There thus exists a similarity between the diffusion of larger solutes and fluid flow through cartilage since both these processes depend on friction inside the pores as well as on the tortuosity of the paths.[1]

Since, from an experimental point of view, fluid permeability is one of the easiest parameters to measure, it may be of interest to know that it can also be used as a guide to estimate the diffusibility of large solutes through a given specimen of cartilage.

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[1] Typical curves of $\bar{P}$ versus $\bar{K}$ for dextrans 10 and 40 are shown in Fig. 4.