Biochimica et Biophysica Acta, 367 (1974) 349–360

BBA 76771

WATER AND ION PERMEABILITY OF A NATIVE COLLAGEN MEMBRANE

GÜNTHER WIEDNER[a] and GÜNTHER WILHELM[b]

With the technical assistance of G. JUREIT and A. ZIMMERSCHIED

[a] *Max-Planck-Institut für Biophysik, 6 Frankfurt/M 70, Kennedy-Allee 70 and* [b] *Zentrum für Kinderheilkunde der Universität Frankfurt am Main, Frankfurt/M (G.F.R.)*

(Received April 22nd, 1974)

SUMMARY

With the help of a ribonucleoprotein it is possible to precipitate collagen in a layer of fibres with a 700 Å period. As collagen is a constituent of many membrane systems in the body, it seemed interesting to investigate the permeability of ions and water through a native collagen membrane.

The experiments were carried out with the help of an acryl glass apparatus, where an osmotic pressure, a hydrostatic pressure difference or both can be maintained between the two bulk phases separated by the membrane. The diffusion coefficients for NaCl and KCl were found to be comparable with those in other biological membranes ($D_s = 9 \cdot 10^{-7}$ cm$^2 \cdot$ s^{-1}) whereas there is a difference of more than three orders of magnitude in the hydraulic permeability ($L_p = 6$ cm$^4 \cdot$ J$^{-1} \cdot$ s^{-1}).

Volume flow measurements caused by an osmotic gradient indicated that the reflection coefficient for NaCl and KCl is very small. In hydrostatic pressure experiments, the membrane shows a preferred direction for volume flows which seems to have something to do with the mode of preparation of the membrane.

INTRODUCTION

Collagen, which forms approximately one third of all mammalian proteins, is one of the most abundant proteins in the animal kingdom. The collagen content varies from organ to organ and changes with the growth of the body. The barrier between the blood and interstitial fluid, which includes the capillary wall, the intercellular substance and the cell membrane, has a high content of collagen as has the basement membrane. For transport across the capillary membranes and epithelial membranes, e.g. kidney tubules, intestines etc., the properties of collagen may be functually very important. Because of these reasons it was interesting to investigate the permeability of ions and water through a native collagen membrane.

A native collagen membrane was produced taking into account the fact that collagen, dissolved in an acid (e.g. acetic acid), can be changed into native collagen fibrils using a ribonucleoprotein group.

THEORETICAL SECTION

Consider an isothermal system in which a membrane separates two homogeneous solutions of the same solute. The left- and right-hand phase are characterized by the indices ′ and ″, respectively. The thermodynamics of irreversible processes gives an expression relating the entropy production within the membrane to the flux through the membrane and the conjugate driving force. In the stationary state this entropy production can be written in the form of Eqn. 1:

$$\frac{T}{A}\sigma = \phi_w \Delta\mu_w + \phi_s \Delta\mu_s \tag{1}$$

with

$$\phi_i = \frac{1}{A}\frac{dn_i''}{dt} = -\frac{1}{A}\frac{dn_i'}{dt}$$

σ = entropy production; T = absolute temperature (°K); A = membrane area (cm^2); ϕ_i = molar flow density of Component i into phase (″) ($mole \cdot cm^{-2} \cdot s^{-1}$); n_i = number of moles of Component i; index w = solvent; index s = salt; μ_i = chemical potential of Component i; Δ difference between (′) and (″) quantities ($\Delta \equiv ' - ''$).

In general, the flows ϕ_i will be functions of the driving forces $\Delta\mu_i$ as well as of an arbitrary reference state of the membrane system [1]. It is assumed that the reference state is a possible equilibrium state of the system.

From an experimental point of view it is more convenient to measure the volume flow ϕ_v and the salt flow ϕ_s^* relative to the volume flow instead of the flows ϕ_w and ϕ_s. The new flows ϕ_v and ϕ_s^* are introduced by the linear transformations:

$$\phi_v = V_w \phi_w + V_s \phi_s \tag{2}$$

$$\phi_s^* = \phi_s - \bar{c}_s \phi_v \tag{3}$$

$\bar{c}_s$ is the salt concentration of the bulk phases in the reference state of the membrane system. For systems near equilibrium, $\bar{c}_s$ can be identified with the arithmetic mean of the bulk concentrations ($\bar{c}_s = \frac{1}{2}(c'_s + c''_s)$). V_i is the partial molar volume of Component i. V_i is assumed to be independent of pressure and concentration. ϕ_v represents the volume change of the solution forming the bulk phases per unit area of the membrane. Taking into account the relation of homogeneity

$$\bar{c}_w V_w + \bar{c}_s V_s = 1 \tag{4}$$

and Eqns 2 and 3, the transformed entropy production is given by

$$\frac{T}{A}\sigma = \phi_v X_v + (\phi_s - \bar{c}_s \phi_v) X_s \tag{5}$$

with

$$X_v = \Delta P + \bar{c}_s (\Delta\mu_s)_P + \bar{c}_w (\Delta\mu_w)_P \tag{6}$$

$$X_s = (\Delta\mu_s)_P - \frac{V_s}{V_w}(\Delta\mu_w)_P \tag{7}$$

To obtain the driving forces X_v and X_s in this form, the pressure dependence and the concentration dependence of the chemical potential have been separated using

$$\Delta\mu_i = V_i \Delta P + (\Delta\mu_i)_P$$

For systems near equilibrium we postulate linear relations between the flows and the driving forces. It was shown by Kedem and Katchalsky [2] and more generally by Sauer [1] that it is possible to introduce the hydrostatic pressure difference across the membrane ΔP instead of the complicated volume force X_v without violating the symmetry relation of Onsager. The linear relations are obtained by developing the flows ϕ_v, ϕ_s^* in terms of the driving forces ΔP and X_s around the equilibrium state of the membrane system

$$\phi_v = L_{vv} \Delta P + L_{vs} X_s \tag{8}$$

$$\phi_s - \bar{c}_s \phi_v = \phi_s^* = L_{vs} \Delta P + L_{ss} X_s \tag{9}$$

In writing down Eqns 8 and 9, the Onsager reciprocity has been taken into account.

Introducing the reflection coefficient σ_s defined by Eqn 10 and the permeability p_s defined by Eqn 11

$$\sigma_s \equiv \frac{L_{vs}}{c_s L_{vv}} [1] \tag{10}$$

$$p_s \equiv \frac{1}{c_s} \left(L_{ss} - \frac{L_{vs}^2}{L_{vv}} \right) \left[\frac{\text{mole} \cdot \text{cm}}{\text{J} \cdot \text{s}} \right] \tag{11}$$

Eqns 8 and 9 then read

$$\phi_v = L_p(\Delta P - \sigma \Pi) \quad (L_{vv} \equiv L_p) \tag{12}$$

$$\phi_s = (1 - \sigma_s) \bar{c}_s \phi_v + p_s \Pi \tag{13}$$

The 'osmotic difference' [3] $\Pi = c_s X_s$ valid for dilute solutions was inserted.

METHODS

1. Production of acid-soluble collagen

300 g of tendon from the neck of a calf was dissected and cut into small pieces. After adding 3 l of 0.05 M acetic acid, the tendon was homogenized with an Ultra Turrax homogeniser. The homogenate was stirred at 4 °C for two days and then centrifuged 1 h at 3000 × *g* and 4°C. The supernatant was isolated and filtered through a glass filter (type G2). The filtered solution was brought up to a concentration of 7 % NaCl with the addition of a 30 % NaCl solution and centrifuged again for 40 min at 3000 × *g* and 4 °C. The sediment was washed with 400 ml of distilled water. Sedimentation and washing was repeated three times. The sediment was diluted in 300 ml of 0.05 M acetic acid and filtered again.

The protein content of the solution was estimated to be 7–8 % using the biuret reaction.

2. Isolation of ribonucleoprotein

The ribonucleoprotein was isolated from calf thymus and streptococci according to the method of Wilhelm [3].

Fig. 1. Electron micrograph showing the layer of collagen fibres on the edge of the membrane.

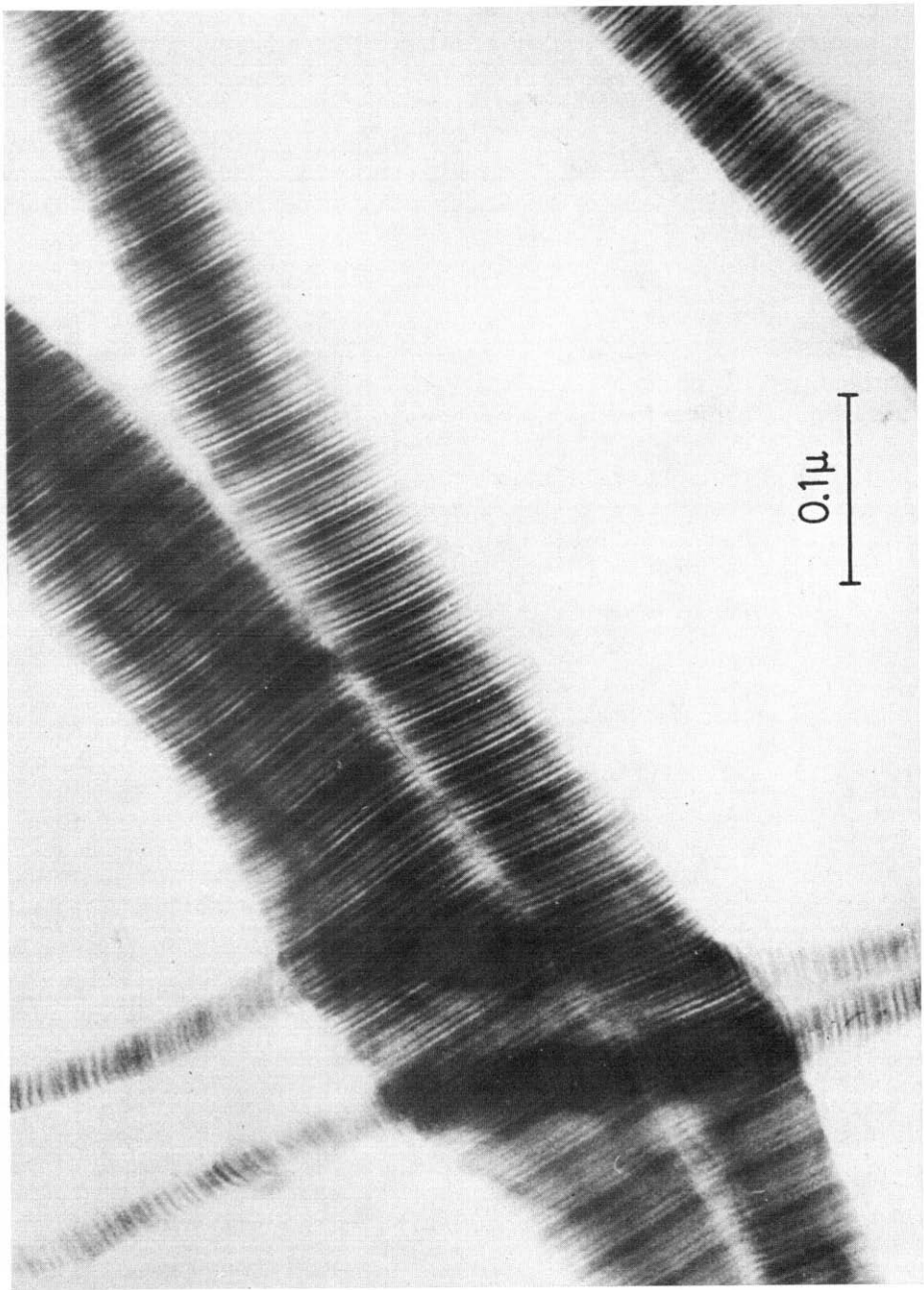

Fig. 2. Electron micrograph of a single collagen fibre.

3. Production of membranes

The membranes were formed by the action of ribonucleoprotein on the native collagen. A membrane filter (Sartorius Göttingen) was impregnated with ribonucleoprotein and, using a sledge, a 0.39-mm thick collagen layer was superimposed on this filter. The ribonucleoprotein precipitated the collagen in a layer of collagen fibres. (see Fig. 1). The filter was then floated off, leaving a collagen membrane 6 μm thick.

Electron microscopy showed that the collagen fibres were of the native type with a 700 Å period (see Fig. 2).

EXPERIMENTS

1. The chamber

Fig. 3 shows one half cell of the apparatus used to measure flows. The half cells are machined out of one piece of acryl glass and have a volume of 254 ml each. As these collagen membranes are extremely delicate to handle, they were held between two Monodur-Polyester nets (Vereinigte Seidenwebereien, Krefeld) with a mesh aperture of $4 \cdot 10^{-4}$ cm^2 fixed like an embroidery frame. The net was tightened on the membrane side with a ring of Parafilm and on the other side with a silicone ring. Vigorous stirring kept the two bulk phases homogeneous. The volume shift was observed in calibrated capillaries and the salt concentration of the bulk phases

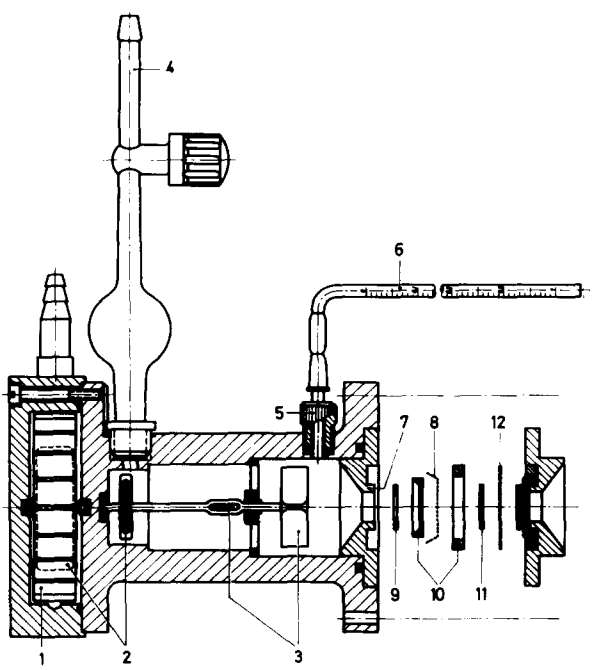

Fig. 3. One half cell of the chamber with the items: (1) stirring blade wheel; (2) magnetic coupling; (3) stirring blades; (4) filling cap; (5) screwed squeezing joint; (6) capillary for measuring volume flow; (7) membrane support; (8) polyester net; (9) silicone ring; (10) "embroidery frame"; (11) Parafilm ring; (12) membrane.

was measured conductrometrically with a Wayne Kerr Autobalance Universal Bridge (Brindi, Lörrach) and WTW (Wissenschaftlich-Technische Werkstätten, Weilheim) conductivity cells.

2. Pressure balance device

As the flows across the membrane showed an extremely high dependence on pressure, a pressure balance device was used in those experiments in which the flows were measured in the absence of pressure gradients. (see Fig. 4). A glass bulb was connected to each outer bulk phase. The bulbs communicated with each other via a three-way stop cock. When filling the two cell-compartments, the stop cock was opened to the bulbs and the atmosphere until the bulbs were about 1/3 filled with solution. The stop cock was then closed to the position shown in Fig. 4. If the hydrostatic pressure increased on one side of the membrane, the fluid level in the bulb on that side also increased. This caused liquid to be forced out of the other bulb until the pressure across the membrane was balanced. As the dead-(air) volume was very small, the hydrostatic pressure across the membranes was less than 2 mm of water.

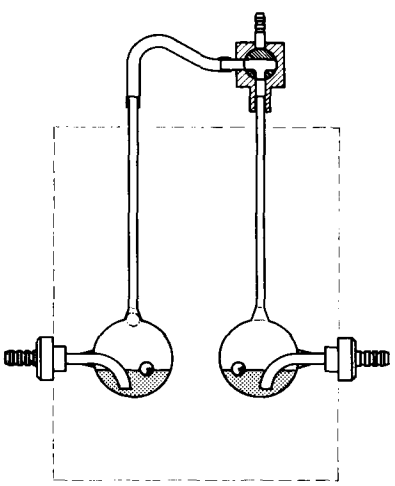

Fig. 4. Pressure balance device.

The chambers on each side of the membrane were filled simultaneously using two syringes. During the filling procedure, the small glass bulbs (marked by a dot-dash line in the left bulb of Fig. 4) acted as a valve to prevent mixing of the two solutions separated by the membrane.

3. Hydrostatic pressure

A glass bulb, containing water was connected to one side of the membrane by an air-filled tube. To increase the pressure, the bulb is lifted. The pressure was measured by an U-tube manometer. Pressures of less than 1 cm of water column (10^{-3} atm) were determined by an apparatus based on the principle of a water balance, where the level in both compartments is compared directly. The highest pressure applied was 15 cm of water.

The membrane area was 0.78 cm^2 but owing to the presence of the two support nets, the actual membrane area is undefined. For the purpose of calculation in the paper we used an effective area of 0.4 cm^2.

RESULTS

1. Flows caused by an osmotic difference

In these experiments the only driving force was the difference in osmotic pressure. The maximum concentration difference between phase ′ and phase ″ was 0.1 M, and the mean concentration was kept constant at 0.154 M.

(*a*) *Salt flow.* The two compartments, separated by the membrane, were filled with solutions of different salt concentrations. The concentration change in both outer bulk phases was observed by measuring the conductivity change. Fig. 5 shows the concentration change of a KCl solution with time. ○ and ● mark measurements starting with different concentration differences, but keeping the mean concentration constant. Knowing the concentration change with time and the volume, we could calculate the flow of solute ϕ_s and from Eqn 13 the permeability. Thereby the contribution of the volume flow was inserted from Section b. For KCl we obtained a permeability of $3 \cdot 10^{-7}$ mole $\cdot$ $cm^{-1} \cdot J^{-1} \cdot s^{-1}$. The diffusion coefficient is defined by the following relation:

$$\phi_s = -D_s \frac{dc}{dx} \tag{14}$$

With a membrane thickness of 6 μm and $\Pi = 2RT\Delta c$ (monovalent salt) one gets a $D_s = 9 \cdot 10^{-7}$ $cm^2 \cdot s^{-1}$, which is of the same order of magnitude as the tracer

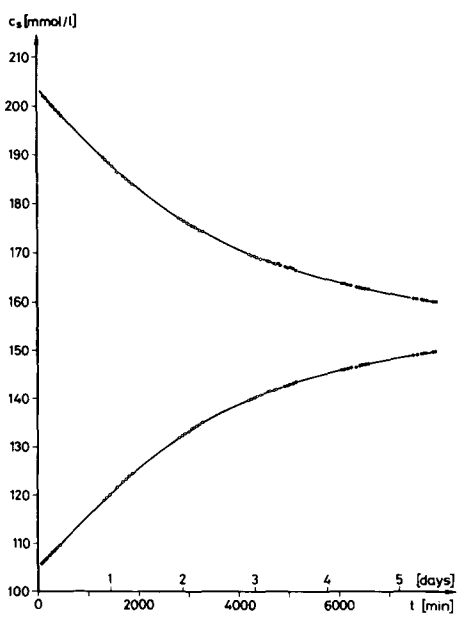

Fig. 5. Concentration change with time of a KCl solution.

permeabilities found on the proximal tubule of the rat kidney ($15–20 \cdot 10^{-7}$ cm$^{-2} \cdot$ s^{-1}) [4]. These results, reproducible with the same membrane for more than one month, showed only very small variations from membrane to membrane.

(*b*) *Volume flow.* The volume flow was observed in two calibrated capillaries, communicating with phase ′ and phase ″, respectively, and brought to the same level (there was of course no pressure balance device). Apart from small fluctuations at the beginning no volume change $> 2 \cdot 10^{-3}$ ml could be registered within 3.5 h.

2. Flows caused by a hydrostatic pressure difference

While there were only very small variations from membrane to membrane for osmotic effects, experiments with hydrostatic pressure difference showed a variation in magnitude up to 100 %. Qualitatively the same behaviour pattern was found.

(*a*) *Salt flow.* In hyperfiltration experiments no separation was measurable, the change in concentration for a pressure of 15 cm of H_2O was within the limit of error of 1 %. Together with the absence of a volume flow in osmotic experiments, this indicates that the reflection coefficient of KCl and of NaCl were negligibly small. So the concentration change in experiments with an applied hydrostatic pressure difference was practically determined by the volume flow.

(*b*) *Volume flow.* The volume was measured with a calibrated capillary connected to the side at atmospheric pressure. Fig. 6 shows the volume flow of a 0.154 M NaCl solution as a function of pressure. Notice that there is already an effect with pressures of a 1-mm water column (10^{-4} atm). The hydraulic permeability coefficient L_p is 6 cm$^4 \cdot$ J$^{-1} \cdot$ s^{-1}, which is more than three orders of magnitude higher than coefficients found with biological membranes [4, 5].

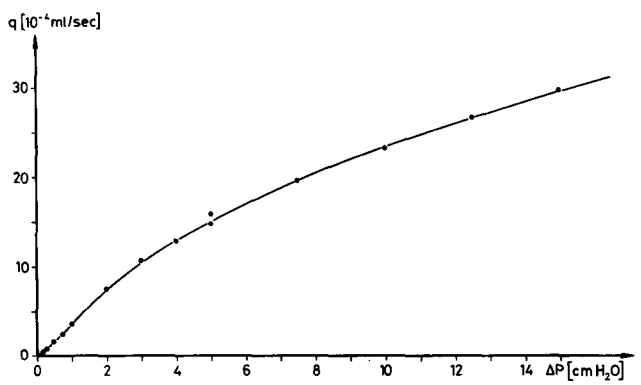

Fig. 6. Volume flow dependent on hydrostatic pressure.

3. Combination of osmotic difference and hydrostatic pressure

To demonstrate the striking difference in the behaviour of the membrane with respect to osmotic and hydrostatic pressure, we superimposed both driving forces. Fig. 7 shows, for a KCl solution, that a very small hydrostatic pressure caused a marked difference in concentration change per time as compared with the effect obtained with osmotic gradients.

With the same assumption that the reflection coefficient is negligibly small, Eqns 12 and 13 can be reduced to

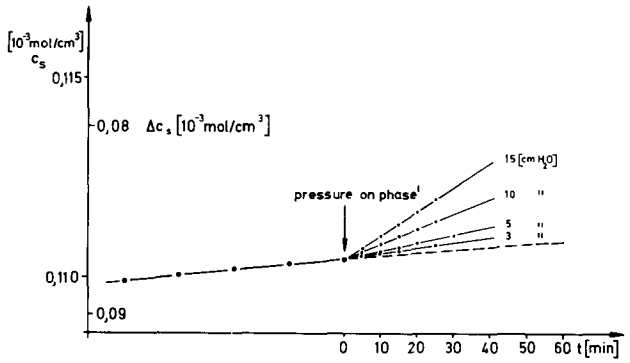

Fig. 7. Hydrostatic pressure superimposed on an osmotic difference.

$$\phi_s = L_p c_s \Delta P + P_s \Pi. \tag{15}$$

Fig. 8 shows the concentration change with time in cell compartments of 254 cm^3. The inner dotted (- - -) line represents the change caused by an osmotic difference only. The second, dot-dash (-·-·-) line is calculated with the known volume flow caused by a pressure of a 15-cm water column, assuming that the corresponding other phase is transported unchanged through the membrane. (The mean concentration is fixed at 0.154 M, so that the Δc gives the values of the concentrations of the bulk phases). The sum of both the osmotic and the hydrostatic pressure experiments is marked by a broken line (- - -). It agrees well with the values obtained in the experiments where the hydrostatic and osmotic pressures are combined (outer line). Of course, measurements in phase ′ and ″ are not made simultaneously (the membrane does not pick up solute).

Figs 7–9 show measurement made with the same membrane.

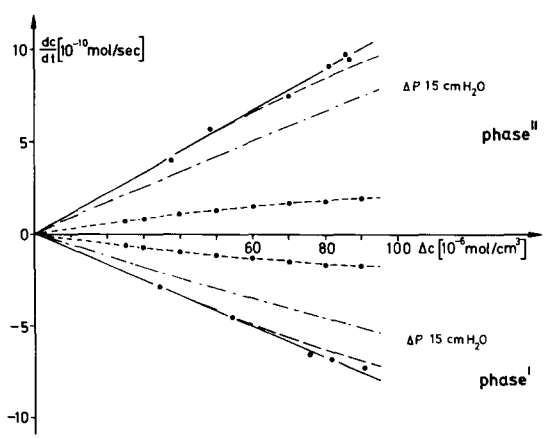

Fig. 8. Concentration change caused by either osmotic difference (inner line) or hydrostatic pressure (-·-). The outer line is the combination of both effects (the broken line gives the theoretic sum).

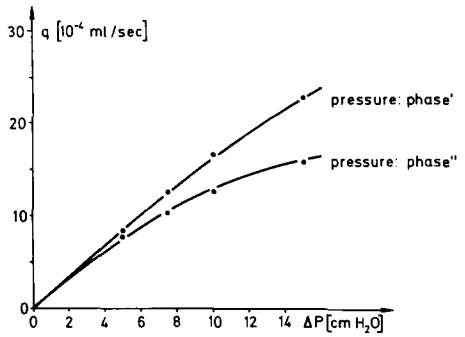

Fig. 9. Direction dependent volume flow. Pressure: phase ′ marks the privileged direction.

4. Direction-dependent effects

The asymmetry of flow shown in Fig. 8 prompted us to examine if there is rectification of the flow across the membrane. After eliminating the possible effects of cell geometry and stirring, a hydrostatic pressure was applied to phase ′ or phase ″, respectively. The volume flow shows a difference of up to 25 % depending on the direction (see Fig. 9). The direction of preferred flow is determined by the production of the membrane, it follows the direction in which the ribonucleoprotein diffuses into the collagen layer, precipitating the collagen in a layer of collagen fibres.

DISCUSSION

The experiments with a thin (6 μm) membrane made of native collagen, precipitated with ribonucleoprotein, show an extremely high sensitivity to hydrostatic pressure. The osmotic permeability is comparable with other biological membranes [4].

This behaviour indicates a change in membrane structure when a hydrostatic pressure difference is maintained. The discrepancy between our measurements and in vivo measurements on biological membranes (L_p is 10^3-fold higher with our membrane) may partly be due to the fact that during in vivo experiments a hydrostatic pressure difference is usually applied by non-permeating substances [4, 7].

Measurements of membrane potentials gave no evidence that the permeabilities of cations and anions are different.

We would like to point out that from our findings no direct conclusions about the mechanism of physiological functions can be made without knowing the other parameters participating and their mutual dependence. But as pressure regulation in living systems is very important, it seems reasonable to assume that membrane systems exist that are especially sensitive to pressure. It might be useful to show whether or not anomalous permeation behaviour is sometimes due to a hydrostatic pressure difference.

ACKNOWLEDGEMENTS

We thank Professor R. Schlögl for his encouragement to carry out this investigation, Mr Gröpl for experimental suggestions, and Mrs Pockrandt-Hemstedt for the electron micrographs.

REFERENCES

1 Sauer, F. (1973) Handbook of Physiology Sec. 8, Renal Physiology (Orloff, J. and Berliner, R. W., eds), pp. 399–414, The American Physiological Society, Washington D.C.
2 Kedem, O. and Katchalsky, A. (1958) Biochim. Biophys. Acta, 27, 229–246
3 Wilhelm, G. (1966) Z. Naturforsch. 21b Heft 8, 793–796
4 Ullrich, K. J. (1973) Handbook of Physiology Sec. 8, Renal Physiology (Orloff, J. and Berliner, R. W., eds), pp. 377–398, The American Physiological Society, Washington D.C.
5 Stein, W. D. (1967) The Movement of Molecules across Cell Membranes. Academic Press, New York
6 Weast, R. C. (1966/67) Handbook of Chemistry and Physics 47th edn.
7 Welling, L. W. and Grantham, J. J. (1972) J. Clin. Invest. 51, 1063–1075