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The Diffusion of Tritiated Water Across Isolated Term Human Amnion

K. R. Page, D. R. Abramovich, and M. R. Smith

Biophysical Chemistry Unit, Department of Chemistry and the Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen, United Kingdom

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Summary. The diffusional permeability of isolated human amnion to tritiated water is studied in relation to its histological structure. It is shown that the high permeability of this tissue requires an evaluation of the effects on diffusion of unstirred layers of solution adjacent to the membrane. After correction for such effects the magnitude of the permeability coefficient is found to be both larger and more variable than previously reported. The principal reason for this is found to lie with variations in tissue thickness. The results show that for the purposes of water diffusion the amnion may be treated as a homogeneous membrane in the axial co-ordinate of flow. This finding is discussed in relation to other evidence concerning membrane structure.

Information concerning the structure and function of a biological membrane may be obtained by studying its permeability to tritiated water. This information becomes especially valuable when it can be linked with the osmotic properties of the same membrane (Solomon, 1968). As with other types of study involving diffusion, however, it is necessary to ensure that the properties examined derive wholly from the membrane, and are not influenced by the boundary conditions at the membrane surface. This is a particularly important point when diffusional permeabilities are high (Ginzburg & Katchalsky, 1963).

The present study arises from an interest in factors controlling water movements in the pregnant human uterus. One of the pathways that may be of importance concerns water transport through the amnion. As with most biological tissues the amnion is a composite membrane. It consists of a single layer of cuboidal epithelial cells supported on a basement membrane, this in turn resting on a thick layer of connective tissue. This epithelium is somewhat unusual in that large spaces occur between the cells, and

these spaces appear to be joined by a network of narrow channels (Bourne, 1962). There is strong histological evidence that the narrow channels and the much larger intercellular spaces permit direct communication between the amniotic fluid and the basal region of the epithelia (Wynn & French, 1968). The tissue, therefore, appears well adapted to the rapid transit of water.

The diffusional permeability of amnion to labeled water has been studied by a variety of workers, in particular Garby (1957), and Seeds, Schruefer, Reinhardt and Garlid (1973). Despite the high permeabilities observed, however, none of these workers has attempted to assess the effects of unstirred layers in the solutions immediately adjacent to the membrane boundaries. As will be shown, neglect of these layers can lead to considerable errors in the calculation of the diffusional permeability coefficient.

The present work will examine the relationship between the structure of term human amnion and its diffusional permeability as measured using tritiated water. In a later communication the information gained here will be related to the permeability of amnion to bulk flows of water.

Materials and Methods

Tissues were obtained within a few minutes of delivery from placentas of normal uncomplicated term pregnancies. The placenta was immersed in gassed Gey's solution (Gey & Gey, 1936) and strips of amnion dissected out from the placenta and from various parts of the amnio-chorion. The strips were kept immersed in Gey's solution at all times and normally subjected to study within ten minutes of delivery.

Diffusional permeabilities were measured using an apparatus similar to that described by Ussing and Zerahn (1951). The cell employed was constructed from perspex and exposed a circular area of amnion, 2 cm in diameter. The tissue was clamped by a peripheral joint 3 mm in width, and supported on each side by a broad mesh cotton gauze.

Each cell compartment was filled with 25 ml of Gey's solution. Solutions were gassed and stirred by air lift pumps supplied by a mixture of 95% oxygen and 5% dioxide, this maintaining the pH at 7.8 ± 0.1.

Membrane potentials were measured using a Vibron electrometer (EIL model 33B-2) connected to saturated calomel electrodes, these making contact with the cells via salt bridges consisting of 4 % w/v agar in Gey's solution. All experiments were conducted at 22 ± 1 °C.

The permeability of amnion to water in the absence of bulk flows was determined by adding a 10-μC sample of tritiated water (Amersham 5 mC/ml) to one compartment of the cell, and withdrawing samples at specified time intervals from the opposite cell compartment. All samples taken from the cell were replaced by equal volumes of unlabeled Gey's solution. Samples were also withdrawn from the high activity compartment at the beginning and at the end of each experiment. The activities of the samples were determined using a Nuclear-Chicago Isocap/300 scintillation counter.

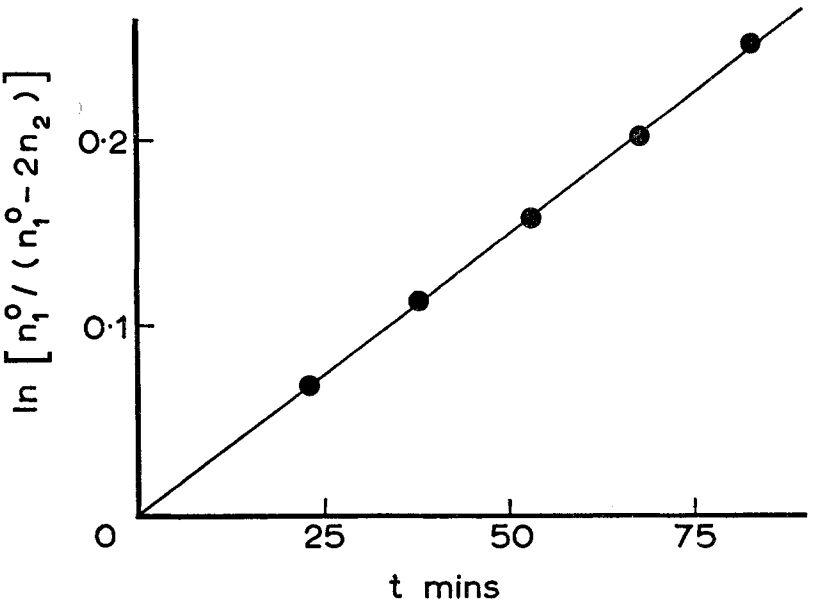

Fig. 1. The measurement of the observed permeability P_0 for experiment No. 8 (the functions explained in the text). The slope of this plot multiplied by the volume of a cell compartment and divided by twice the membrane area yields P_0

In work to be published later it was found that in the absence of pressure gradients, no bulk flow occurs through amnion when it is bathed on either side by solutions of the same composition. Under the conditions of the present experiments it is therefore permissible to define a permeability coefficient P_0 by

$$J = P_0 C_w. \tag{1}$$

Here J is the diffusional flux in moles $cm^{-2} sec^{-1}$ and C_w the water concentration in moles cm^{-3}. P_0 was calculated from the activity-time measurements using a modified Northrop-Anson equation (Robbins & Mauro, 1960). This involved plotting the time t as a function of $\ln[n_1^0/(n_1^0 - 2n_2)]$, where n_1^0 represents the initial activity of the high activity compartment, and n_2 the activity of the low activity compartment at any time after withdrawing the initial high activity sample. Plots were found to be linear. Lines were best fitted to the points by the method of least squares and values of P_0 obtained from their slopes. Fig. 1 illustrates a typical result.

The effects of unstirred layers of solution adjacent to the membrane were determined using the method first derived by Nernst. Each region of poor stirring is represented as an equivalent homogeneous layer of stagnant solution with a defined thickness δ. This thickness is wholly a function of the rate of stirring, cell geometry, and the reciprocal cube root of the solute diffusion coefficient, and is independent of the nature of the membrane. If the thickness δ is the same on either side of the membrane, then P_0 is related to the membrane permeability coefficient P by

$$\frac{1}{P_0} = \frac{1}{P} + \frac{2\delta}{D_w} \tag{2}$$

where D_w is the self diffusion coefficient of water.

The thickness of the unstirred layer in each cell compartment was measured by the method of Scattergood and Lightfoot (1968). The amnion was replaced by a sheet of silver, and a silver gauze of large surface area was inserted into the main body of the compartment. The compartment was filled with a solution of 5×10^{-4} M silver nitrate and 1 M sodium nitrate, this being stirred in the normal way. The current-voltage relation of the compartment was then determined by passing a known current between the electrodes (the silver sheet acting as the cathode) and measuring the potential between the electrodes. Each current-voltage plot contained a plateau whose height corresponded to the limiting current density. The quantity δ was obtained from this current density, and a knowledge of the concentration and self diffusion coefficient of the silver ion.

The thickness of amnion was determined using an apparatus similar to Polishuk, Kohane and Peranio (1962). This consisted of a micrometer gauge fitted with a metal base electrically insulated from the main body of the gauge. The resistance between the base and the anvil of the gauge was monitored using a multimeter (Avometer 8MK III). The anvil had a cross-section of 0.1 cm^2. A sample of tissue, soaked in Gey's solution was placed on the base and smoothed out. The anvil was then gently lowered until a fall in resistance was registered by the meter. The device permitted measurement of thickness correct to 10 μm with the minimum of tissue distortion.

Results

Experimental Errors

The error in measuring membrane potentials was estimated as being ± 0.2 mV. Errors in measuring diffusional permeability coefficients were determined by making eight separate measurements of the permeability of Visking dialysis tubing to tritiated water. The error, expressed as the percentage standard deviation of the mean, was found to be $\pm 15.5\%$. Repeated determinations of the thickness of Visking dialysis tubing showed that the error in thickness determinations, expressed as the percentage standard deviation of the mean, was $\pm 1.8\%$.

The Flux Ratio

Electrical measurements confirmed the findings of Garby (1957) and Lind, Kendall and Hytten (1972) that no significant membrane potential exists across human amnion when it is bathed on either side by solutions of the same composition.

In five separate experiments the permeability of amnion to oppositely directed diffusional fluxes was measured. In each experiment the flux was first measured directed from the epithelial to the connective tissue sides of the amnion, the cell and tissue washed, and the flux re-determined with the direction of isotope flow reversed. Each experiment had a duration of about 6 hr. In every case the pairs of fluxes so determined were the same within the experimental error.

The Effects of Unstirred Layers

Four sets of Ussing cells were employed in the diffusion experiments, all of identical dimensions and stirring arrangements. The thicknesses of the unstirred layers in all cell compartments were found to be the same within 5%. With no stirring, the thickness in each chamber was 395 μm. This quantity decreased sharply with the commencement of pumping but then fell off more slowly as the pumping strength increased. All diffusion experiments were conducted at medium to high rates of pumping. The value of δ for this strength of stirring was found to be 160 ± 8 μm. The large magnitude of this quantity probably reflected the presence of the cotton gauze meshes placed either side of the membrane.

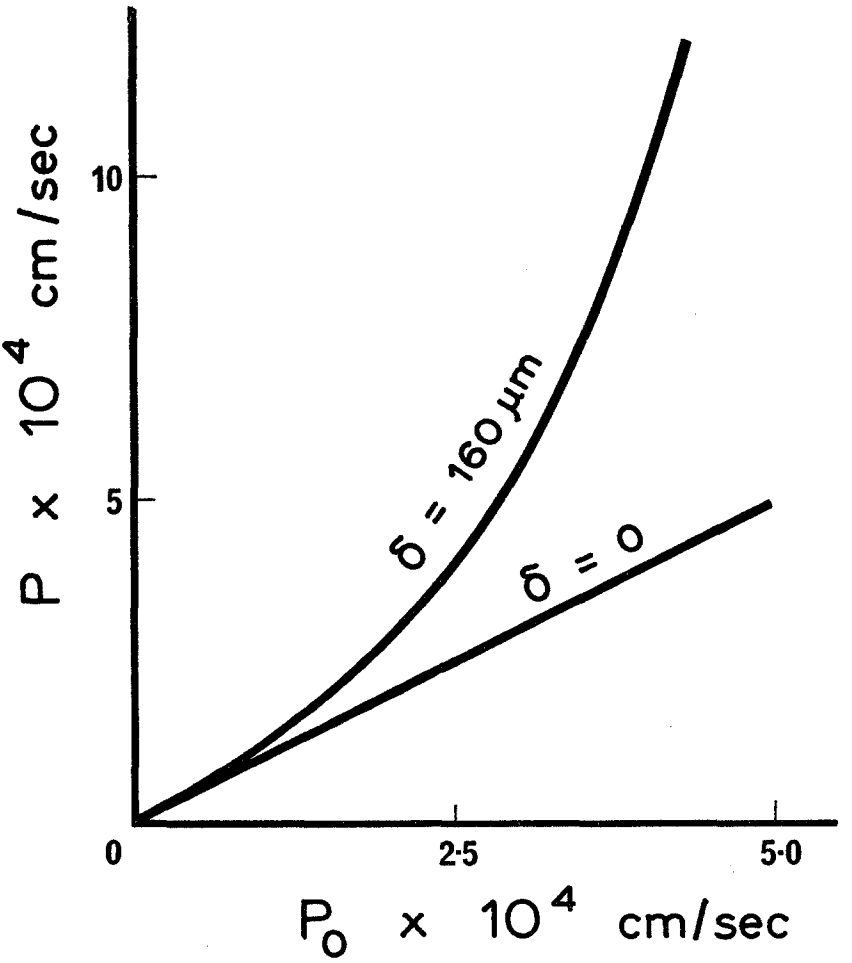

Fig. 2. The effect of unstirred layers on permeability. The membrane permeability P is plotted as a function of the observed permeability P_0 for two different values of δ, the thickness of the unstirred layer of solution adjacent to each membrane surface

The diffusional permeabilities P_0 were found to range from about 5 to 40×10^{-5} cm/sec with the majority having values close to 20×10^{-5} cm/sec. The effect of unstirred layers for permeabilities of this magnitude are illustrated in Fig. 2.

In this Figure, Eq. (2) has been used to plot the membrane permeability P as a function of P_0, the observed permeability.

By comparing the curves for $\delta = 0$ and $\delta = 160$ μm it will be seen that the membrane permeability is from 10 to 150% greater than the observed permeability when determined using the present apparatus. Unless otherwise stated, all permeabilities quoted in the remainder of this paper will be membrane permeabilities and not observed permeabilities.

Diffusional Permeability and Membrane Structure

Fig. 3 illustrates the distribution of amnion permeability coefficients. The coefficients are taken from 38 separate samples of tissue obtained from 20 different subjects.

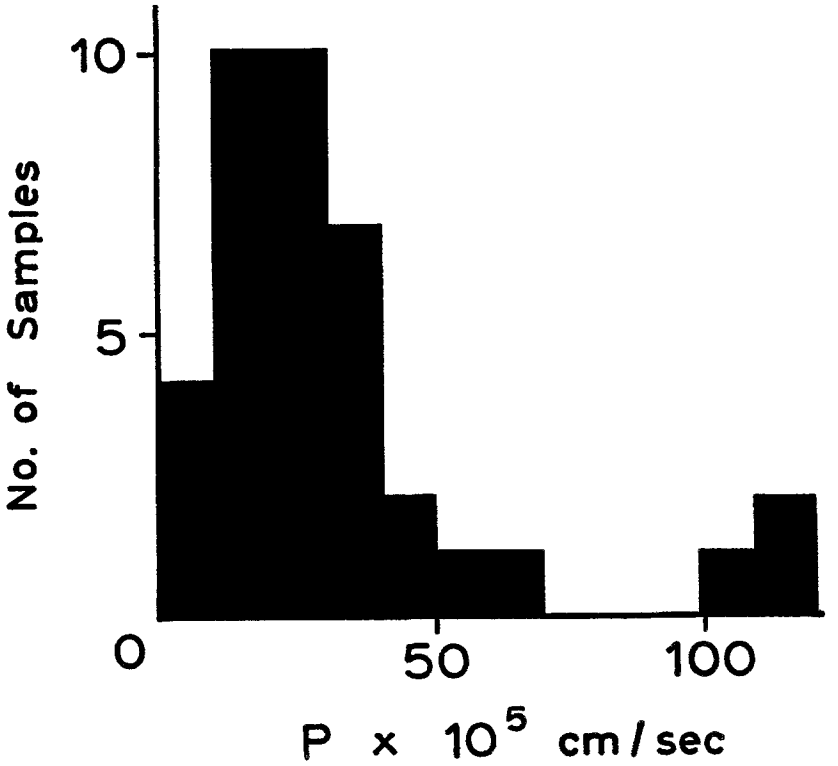

Fig. 3. Distribution of the membrane permeability for 38 different tissue samples taken from 20 subjects

Table 1. Permeabilities obtained from different sites of a single placenta

Subject	$P \times 10^5$ cm/sec			
	X_A	X_B	Y_A	Y_B
16	6.35	–	18.2	20.0
17	25.0	19.0	34.4	17.1
19	32.6	44.5	21.9	25.0
26	–	31.0	25.8	13.6
27	19.9	21.2	10.1	7.95
29	63.7	24.3	32.1	54.3

$X_A = 2$ cm from point of rupture; $X_B = 8$ cm from placental plate; $Y_A = 2$ cm from placental plate; Y_B = placental amnion.

To gain some idea of the nature of this distribution, parallel determinations of the properties of amnion taken from four different sites in the same subject were made. The results are shown in Table 1.

The permeability coefficients varied considerably within samples taken from the same subject. The variance ratio of the logged coefficients showed, however, that variations between subjects was significantly greater than those within the same subject ($P < 0.01$). These results also demonstrated that there were no systematic variations between amnion taken from different sites.

The relation between diffusional permeability and membrane thickness was examined. The permeability of the tissue was first determined, and then ten separate measurements of the tissue thickness made. These last were taken from different regions of the tissue so that an average membrane thickness could be determined for the total area exposed to transport. Blanks were also run in order to check if thickness variations occurred during the course of each experiment. No such variations were detected.

Fig. 4 plots the reciprocal permeability against the tissue thickness *d*. It shows that there is a strong correlation between thickness and permeability. Points were obtained for 19 separate samples of amnion taken from five subjects. The results provide evidence for a linear correlation. The continuous line shown in the figure has been fitted by the method of least squares on the points alone. The intercept does not significantly differ from one passing through the origin.

The distribution of membrane thicknesses was also investigated. The thicknesses of five samples of amnion taken from different regions in the same subject were measured, four from the amnio-chorion and one from the placenta. Sixteen different subjects were examined in this manner.

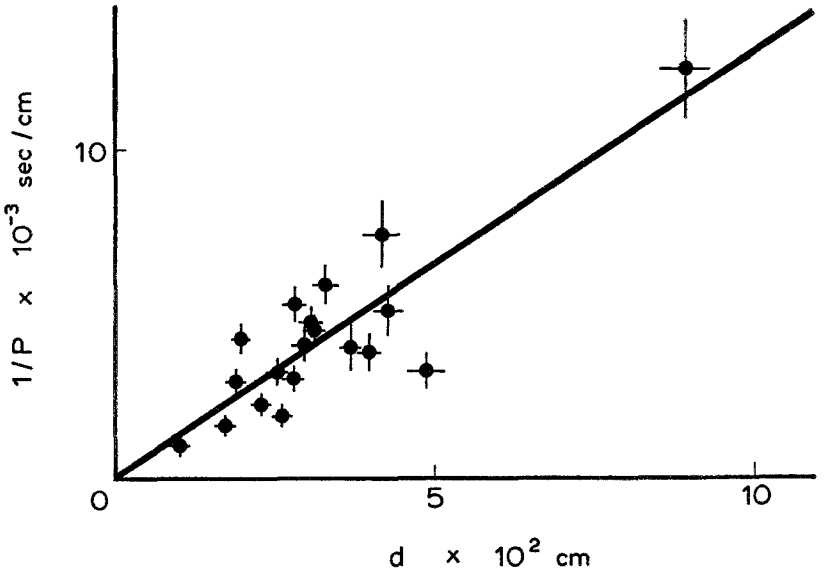

Fig. 4. The correlation between reciprocal membrane permeability $1/P$ and the membrane thickness d

Statistical analysis showed that despite variations within one subject, significant variations occurred between subjects ($P < 0.01$). The analysis also showed there were no significant differences between the thickness of placental amnion and amnion taken from the amnio-chorion.

The pooled thickness determinations gave a distribution similar to that reported by Polishuk, Kohane and Paranio (1962). The thicknesses ranged from 30 to 900 μm although values larger than 500 μm were exceptionally rare. Typically, measurements fell within the range 100 to 400 μm with a mean of about 200 μm.

Discussion

The results demonstrate the importance of allowing for the presence of unstirred layers of solution when determining the diffusional permeability of amnion. The data taken in respect to the flux ratio also shows there is no metabolic influence on diffusional water transport. The consistency of data indicates that there are no significant changes in the properties of amnion over a period of about 6 hr.

There appears to be a greater variability in the diffusional permeability than has been previously reported (Lloyd, Garlid, Reba & Seeds, 1969). As demonstrated by Fig. 4, the principal cause for this lies in variations in

tissue thickness. Histological evidence points to the outermost part of the connective tissue layer as the seat of these variations (Bourne, 1962). On the basis of the present evidence it is not possible to say whether there are variations in the absolute thickness or variations in the plane of scission between amnion and chorion.

Despite the scatter of points in Fig. 4 there is no strong evidence for a correlation other than a straight line between the reciprocal permeability and tissue thickness. This provides a strong indication that for the purposes of water diffusion, amnion is homogeneous in the axial co-ordinate of flow. In this case it is possible to define a diffusion coefficient D^* by

$$P=\frac{D^*}{d}. \tag{3}$$

The mean value of D^* determined from the slope of the line shown in Fig. 2 was found to be $(7.68 \pm 1.08) \times 10^{-6}$ cm^2 sec^{-1}. This value is substantially higher than the value of 3×10^{-6} cm^2 sec^{-1} calculated for 22 °C from the data of Seeds *et al.* (1973). A direct comparison is not possible however as Seeds did not evaluate the effect of unstirred layers on his data. The stirring arrangements in Seeds' cell differed considerably from those used in this work, his membranes being supported on one side by a fiber glass filter disc perforated by 6-mm diameter holes. In the present work, the apparent value of D^* would be 5.7×10^{-6} cm^2 sec^{-1} if no allowance were made for unstirred layers.

Although thickness is the predominant cause for variations in P, the scatter of the points in Fig. 4 show that it is not the only cause. The homogeneity of amnion to diffusion along the axial coordinate of flow does not necessarily imply a corresponding homogeneity of membrane structure. Garby (1957) has reported that the water content of amnion ranges from 85 to 95% with a mean of 90%. This indicates that the volume fraction V_p of the tissue is approximately 0.1. Mackie and Meares (1955) have shown that for a homogeneous membrane

$$\frac{D^*}{D_w}=\left(\frac{1-V_p}{1+V_p}\right)^2. \tag{4}$$

Eq. (4) predicts that the ratio D^*/D_w should be 0.67 ± 0.15 if the amnion is homogeneous. The observed ratio however is significantly smaller being only 0.36 ± 0.05. This finding may be explained if some of the membrane water does not participate significantly in the diffusion process, thereby

lowering the effective water content. Water contained within the epithelial cells may well be of this nature.

These observations when compared with the structure of amnion revealed by histological methods suggest that water diffusion occurs predominantly in the intercellular spaces of the epithelium.

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References

Bourne, G. L. 1962. The Human Amnion and Chorion. Lloyd-Luke, London

Garby, L. 1957. Studies on transfer of matter across membranes with special reference to the isolated human amniotic membrane and the exchange of amniotic fluid. *Acta Physiol. Scand.* **40**, Suppl. 137:1

Gey, G. O., Gey, M. K. 1936. The maintenance of human normal cells and tumor cells in continuous culture. *Amer. J. Cancer* **25**:45

Ginzburg, B. Z., Katchalsky, A. 1963. The frictional coefficients of the flows of non-electrolytes through artificial membranes. *J. Gen. Physiol.* **47**:403

Lind, T., Kendall, A., Hytten, F. E. 1972. The role of the fetus in the formation of amniotic fluid. *J. Obstet. Gynaec., Brit. Cwlth.* **79**:289

Lloyd, S. J., Garlid, K. D., Reba, R. C., Seeds, A. E. 1969. Permeability of different layers of the human placenta to isotopic water. *J. Appl. Physiol.* **26**:274

Mackie, J. S., Meares, P. 1955. The diffusion of electrolytes in a cation-exchange resin membrane. *Proc. Roy. Soc. A* **232**:498

Polishuk, W. Z., Kohane, S., Peranio, A. 1962. The physical properties of fetal membranes. *Obstet. and Gynec.* **20**:204

Robbins, E., Mauro, A. 1960. Experimental study of the independence of diffusion and hydrodynamic permeability coefficients in colloidion membranes. *J. Gen. Physiol.* **43**:523

Scattergood, E. M., Lightfoot, E. N. 1968. Diffusional interaction in an ion-exchange membrane. *Trans. Faraday Soc.* **64**:1135

Seeds, A. E., Schruefer, J. J., Reinhardt, J. A., Garlid, K. D. 1973. Diffusion mechanisms across human placental tissue. *Gynec. Invest.* **4**:31

Solomon, A. K. 1968. Characterization of biological membranes by equivalent pores. *J. Gen. Physiol.* **51**:335s

Ussing, H. H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short circuited isolated frog skin. *Acta Physiol. Scand.* **23**:110

Wynn, R. M., French, G. L. 1968. Comparative ultrastructure of the mammalian amnion. *Obstet. and Gynec.* **31**:759