J. Membrane Biol. 18, 49–60 (1974)

Water Transport 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

Received 29 January 1974; revised 5 April 1974

Summary. The hydrodynamic permeability of normal term human amnion is measured using pressure-driven bulk flows. The permeability coefficient is found to vary widely, variations between tissues taken from different subjects being significantly greater than those from samples taken from one subject. No correlation is observed between this coefficient and either tissue thickness or the diffusional permeability coefficient measured using tritiated water; it is, however, found to be very sensitive to epithelial damage.

The results indicate that the bulk transport of water through amnion is largely controlled by the amniotic epithelium alone. This contrasts with water diffusion which is a function of total membrane thickness. The two permeability coefficients cannot therefore be employed to formulate an equivalent pore model of the whole tissue. An equivalent pore model of the epithelial layer only is considered and the results assessed in the light of other evidence bearing on the structure of amnion. It is concluded that the epithelial layer is intersected by a large number of pores with radius 10 to 30 Å, and a smaller number of much broader pores.

This paper will examine the relationship between the structure of normal term human amnion and its properties with respect to tritiated water diffusion, and to pressure-driven bulk flows.

The amnion is a composite membrane consisting of a single layer of cuboidal epithelial cells resting on a basement membrane, this in turn being supported by a layer of connective tissue. For the present purposes it will be convenient to term the region of the epithelial cells and basement membrane as the "epithelial layer," and the remainder of the membrane as the "connective tissue layer." There is strong histological evidence for the presence of a well-developed network of intercellular canals in the epithelial layer (Bourne, 1962; Wynn & French, 1968).

Despite the histological complexity of this membrane, Barton and Baker (1967) have attempted to represent it by an equivalent pore structure. By taking the ratio of the hydrodynamic permeability coefficient to the dif-

fusional (or exchange) permeability coefficient of water, these workers obtain an equivalent pore radius of 85 Å for the membrane. These two permeability coefficients can only be compared, however, if they both refer to the same rate-controlling step within the membrane (Solomon, 1968). As demonstrated by Hays and Franki (1970) in their studies of toad bladder, this requirement may not be met when the membrane structure is complex.

The present paper will show that the treatment by Barton and Baker (1967) is not valid owing to their neglect, both of membrane inhomogeneity, and of unstirred layer effects in the solutions immediately adjacent to the membrane. Modifications of the equivalent pore model to allow for the first of these factors will be considered. The physiological significance of these findings will then be discussed.

Materials and Methods

Specimens of amnion were obtained from the amnio-chorion of normal term placentae, the tissue being kept moist at all times with Gey's solution (Gey & Gey, 1936). Specimens were subjected to study with the minimum of delay, normally within 15 min of delivery.

Methods were generally as described in Page, Abramovich and Smith (1974) except for the measurement of the hydrodynamic permeability coefficient L_p defined by Eq. (1):

$$L_p = J_v/\Delta P. \tag{1}$$

Here J_v is the volume (or bulk flow) of water (in cm/sec) induced by a hydrostatic pressure differential ΔP (in dynes/cm^2) applied across the membrane. Two types of apparatus were employed to measure this quantity.

The first apparatus, termed the small aperture apparatus, was employed in cases where the tissue had already been studied using a diffusion cell. It was based on the apparatus described by Robbins and Mauro (1960). The tissue was supported on a circular glass sinter (porosity 2) of 5-mm radius, and clamped at its periphery by a 1-mm broad perspex joint. The high pressure side of the membrane consisted of a glass compartment with a filling port and a horizontal capillary tube (Veridia tubing, 0.6-mm diameter). This compartment was filled with Gey's solution. The capillary tube was connected by a T-joint to one side of a mercury manometer, the mercury making direct contact with the Gey's solution in the capillary. The low pressure compartment was open, being filled with gassed Gey's solution. The whole apparatus was thermostatted to 22 ± 0.1 °C.

The applied pressure was obtained by using a cathetometer to measure the height of the mercury in the manometer. The volume flow was measured by following the movement of the mercury interface in the capillary using an etched mirror scale and a stop watch. A plot of the volume flow as a function of the pressure yielded L_p.

The second apparatus, termed the large aperture apparatus, was similar to that employed by Ginzburg and Katchalsky (1963). In this the tissue was supported by a circular glass sinter (porosity 2) of 15-mm radius, and clamped at its periphery by a 1-mm broad perspex joint. Both the low and high pressure compartments were filled with Gey's solution. The solutions were neither gassed or stirred. The absolute pressure differential was measured using a mercury filled U-tube connected between the compartments. Pressure was applied by a mercury column connected via a U-tube to the cell

interior. Volume flows were obtained by observing the movement of the Gey's solution-air interface in a 1-mm diameter Veridia capillary tube connected to the low pressure compartment. A horizontally mounted cathetometer was used to observe the position of the interface. The apparatus was thermostatted to 22 ± 0.1 °C. As in the previous apparatus the hydrodynamic permeability coefficient was calculated from the ratio of the volume flow to the pressure differential.

Results

Experimental Errors

The errors for thickness and diffusion measurements were the same as reported by Page *et al.* (1974), these being ± 1.8 and $\pm 15.5\%$, respectively. The error in measuring the hydrodynamic permeability coefficient using the broad aperture apparatus was determined using Visking dialysis tubing, the error expressed as a percentage standard deviation of the mean being $\pm 2.3\%$. This method could not be used in the narrow aperture apparatus. Repeated measurements made on the same piece of amnion indicated the error of this latter method was approximately $\pm 10\%$.

Variations in Hydrodynamic Permeability Coefficient

Fig. 1, obtained using the narrow aperture apparatus, illustrates five separate measurements of the volume flow J_v using different applied pres-

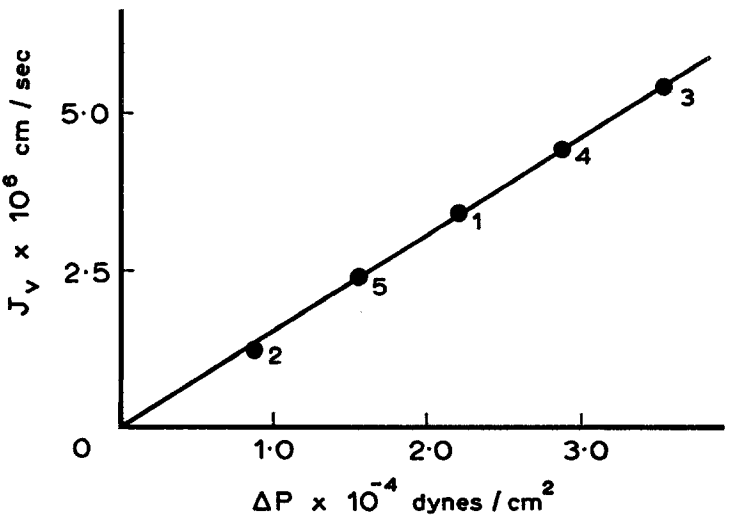

Fig. 1. The determination of hydrodynamic permeability L_p. Values of the volume flow J_v plotted as a function of the hydrostatic pressure differential ΔP. The numbers by the points give the order in which measurements were made. The slope gives L_p direct. Data from experiment number 5

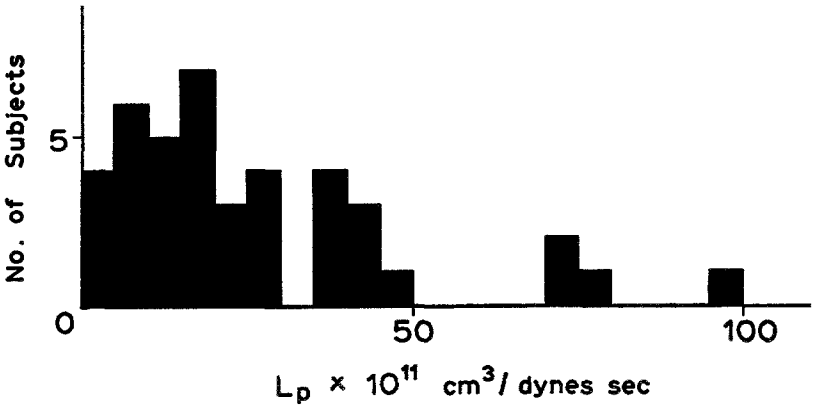

Fig. 2. Distribution of hydrodynamic permeability coefficients for 41 different tissue samples taken from 26 subjects

Table 1. Determination of hydrodynamic permeability coefficients L_p from pairs of areas each from the same placenta

Experiment	$L_{p1} \times 10^{11}$ cm^3/dynes sec	$L_{p2} \times 10^{11}$ cm^3/dynes sec
16	74.1	77.4
17	17.2	9.92
20	19.5	2.19
21	39.9	19.6
22	19.3	40.6
25	37.4	46.8
31	36.0	38.0
32	20.0	14.0
34	9.0	4.7
33	7.7	8.0
35	98.0	71.0
37	21.7	22.7
40	26.8	26.7
41	2.7	6.4

sures ΔP. It shows that the coefficient L_p remained constant over a pressure range of 0 to 3×10^4 dynes/cm^2 (approximately 0 to 3 cm Hg). Such experiments indicated also that L_p remained constant for 6 hr after delivery. Beyond this time there was evidence that L_p began to fall and the tissue swell. No volume flows were observed when the pressure differential was set to zero.

The distribution of L_p obtained from 41 intact samples of tissue taken from 26 subjects is indicated in Fig. 2. It shows that there is a quite broad variation in L_p. The wide aperture apparatus was used to study the values

of L_p from two pieces of amnion taken from the same subject. In most cases a sample was taken 2 cm from the placental edge, L_{p1}, and a second 8 cm from the placental edge, L_{p2}. The variance ratios of the logarithmically transformed coefficients indicated a highly significant difference between subjects ($P < 0.001$) despite the variations within a subject. The logarithmic mean coefficient from this set of data was 19.7×10^{-11} cm^3/dyne sec (*see* Table 1).

Relationship between Hydrodynamic and Diffusional Permeability Coefficients

In 13 experiments the diffusional permeability of the tissue was first determined, and then the tissue transferred with the minimum delay from the diffusion cell to the small aperture apparatus. The hydrodynamic permeability coefficient was then determined. In all cases the measurements were complete within 6 hr of delivery. The membrane diffusional permeability coefficients P are plotted against the corresponding values of L_p in Fig. 3. As shown by this Figure variations in L_p do not correlate with variations in P.

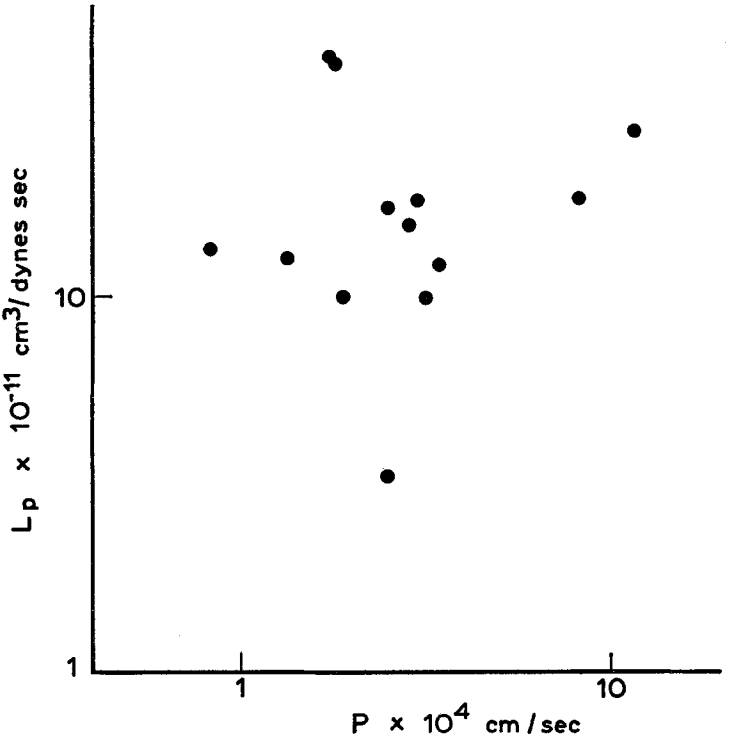

Fig. 3. Paired determinations of the hydrodynamic permeability coefficient L_p and the membrane permeability coefficient to tritiated water P. The plot illustrates the absence of any correlation between these two quantities

Relationship between Hydrodynamic Permeability and Membrane Thickness

The hydrodynamic permeability coefficients and mean membrane thicknesses of 14 samples of tissue obtained from eight different subjects was determined. The results are shown in Fig. 4 which plots the reciprocal hydrodynamic permeability coefficient against the tissue thickness *d*. If the membrane were homogeneous a linear relationship should exist between these variables, the line passing through the origin. No correlation of this type is observed.

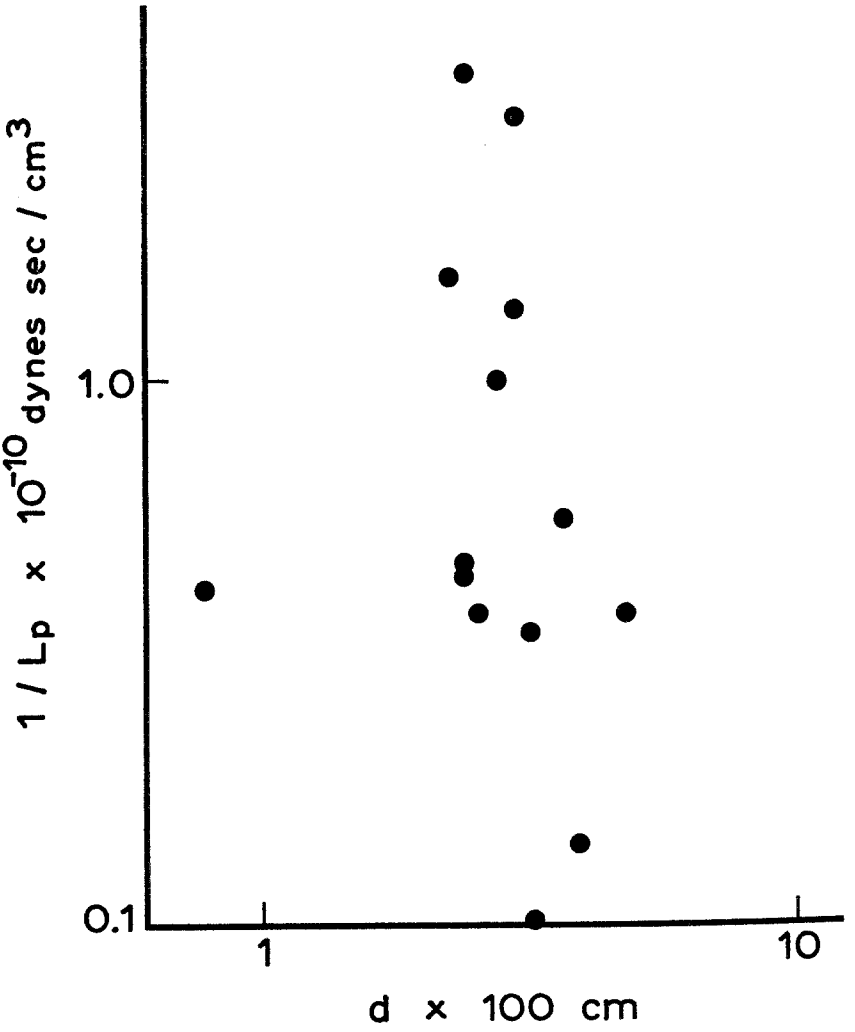

Fig. 4. Reciprocal hydrodynamic permeability coefficient $1/L_p$ plotted against membrane thickness *d*. The plot illustrates the absence of a physically meaningful correlation between these quantities

Effect of Epithelial Damage

In the course of the experiments just described tissues with epithelial damage were encountered and these were found to have markedly high values of L_p. In these cases the connective tissue layer was intact, but small patches of epithelium were observed to be torn and hanging free from the connective tissue. Although results from these experiments were rejected for the purposes of the investigations described above, it was decided to examine this phenomena further. Occasionally, tissues were encountered that had separate regions of damaged and undamaged epithelia. In three such cases measurements of L_p were made on samples taken from both regions. In six more cases intact tissues were obtained and divided into half. The L_p of one half was determined directly, while small patches of epithelia were scraped off the remaining half using a scalpel, care being taken to preserve the integrity of the underlying tissue. The hydrodynamic permeability of the damaged half was then determined. In two cases the last

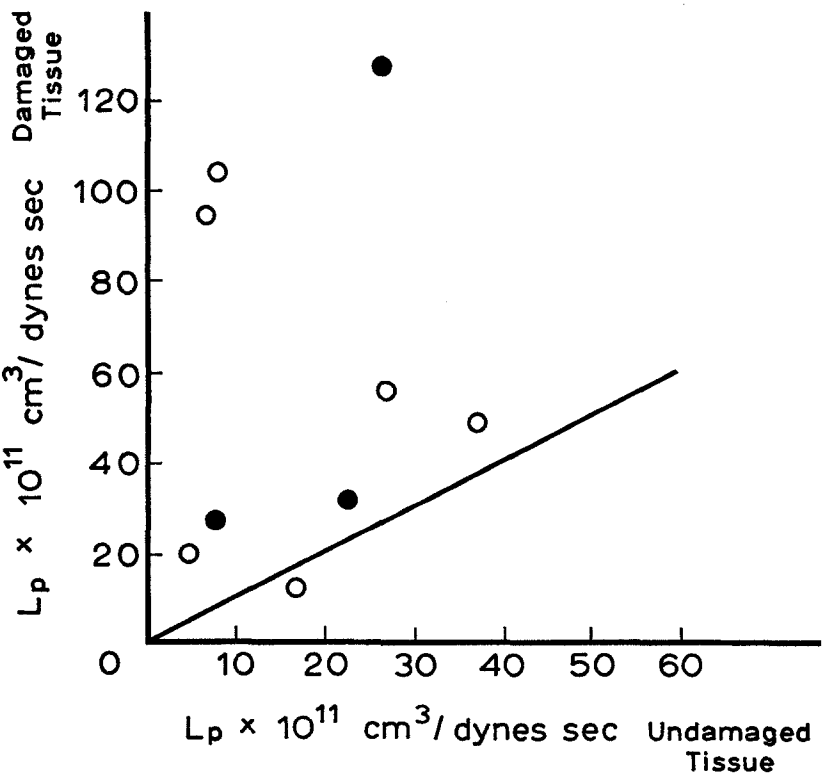

Fig. 5. Effects of epithelial damage. The hydrodynamic permeability coefficient of damaged tissue plotted against that of undamaged tissue from the same placenta. Open circles indicate artificially damaged and closed circles naturally damaged tissues. Coefficients of equal magnitude on each scale will fall on the line shown

experiment was repeated except that the diffusional permeability was determined instead. In all the cases considered the area of damage amounted to between 5 and 10% of the total membrane surface. Fig. 5 plots the L_p of the damaged tissue as a function of the L_p of the intact tissue. In all but two cases the L_p of the damaged tissue was significantly higher than the undamaged tissue. In the case of the two diffusion experiments, the products of the thickness and diffusional permeability, $d \times P$, between damaged and undamaged tissues did not differ significantly.

Discussion

The consistency of L_p and d for periods of up to 6 hr indicates there is no significant tissue degradation within this time period. The linearity of J_v with ΔP for pressures up to 3.6×10^4 dynes/cm^2 (approximately 3 cm Hg) also suggests there are no important changes in tissue structure arising from pressures of this magnitude.

All measurements of L_p were conducted in the absence of stirring. The osmotic measurements of Seeds (1970) indicate, however, that none of the solutes present in Gey's solution are likely to have reflection coefficients greater than 0.06. In contrast to the measurement of the diffusional permeability therefore, errors in L_p through lack of stirring are considered negligible. Preliminary work has also failed to detect significant streaming potentials with bulk flows of the present magnitude. Errors through electrokinetic effects are therefore considered negligible.

Page *et al.* (1974) have found that the reciprocal of the diffusional permeability coefficient P correlates linearly with the tissue thickness d. This behavior contrasts strongly with the present results concerning L_p, and is consistent with the finding that variations in L_p do not correlate with variations in P. These factors, taken together with the measurements on damaged tissues, strongly indicate that the rate-controlling step for bulk flows lies within the epithelial layer of amnion. The ratio L_p/P cannot therefore be used to calculate a meaningful equivalent pore radius as attempted by Barton and Baker (1967). In point of fact, these latter workers also neglected to allow for the presence of unstirred layers in the solutions adjacent to the amnion when measuring P. As indicated by Page *et al.* (1974) owing to the very high values of P (in order of 10^{-4} cm/sec), it is essential to investigate the effects of these layers if a true membrane quantity is desired.

Although a precise treatment of the structure of the amnion cannot be attempted on the basis of the present data, some information may be obtained if it is assumed that the observed L_p derives totally from the epithelial

Table 2. Frictional coefficient ratios f_{ww}/f_{wm} and equivalent pore radii r for amniotic epithelia calculated for different values of the hydrodynamic permeability coefficient L_p and for an epithelial thickness of 20 μm

$L_p \times 10^{11}$ cm³/dyne sec	f_{ww}/f_{wm}	r Å
3	2.5	9.3
10	10.5	19.1
15	16.2	23.7
20	21.9	27.6
70	79.8	52.6

layer. Page *et al.* (1974) find that the diffusion coefficient D^* of tritiated water for amnion is 7.68×10^{-6} cm²/sec. Danforth and Hull (1958) report that the thickness of the epithelial layer of term human amnion d_1 is approximately 2×10^{-3} cm. If water-solute interactions are neglected, then from the treatment of Thau, Block and Kedem (1966) we may write for the epithelial layer alone

$$\frac{L_p}{D^*} d_1 \frac{RT}{V_w} = 1 + \frac{f_{ww}}{f_{wm}} \tag{2}$$

where R is the gas constant, T the absolute temperature, and V_w is the partial molar volume of water; f_{ww} represents a frictional coefficient relating to water-water interactions, and f_{wm} a similar coefficient relating to water-membrane interactions. If further we assume an equivalent pore structure for the epithelial layer then

$$\frac{f_{ww}}{f_{wm}} = \frac{RT}{V_w} \frac{r^2}{8\eta D_w} \tag{3}$$

where η is the coefficient of viscosity for water, D_w the self diffusion coefficient for tritiated water and r is the equivalent pore radius. Table 2 lists values of f_{ww}/f_{wm} and r for various values of L_p. It will be seen that even with the smallest value of L_p the value of f_{ww}/f_{wm} is much larger than unity indicating a predominance of water-water interactions over water-membrane interactions. This is consistent with the presence of large water-filled spaces within the epithelium. The equivalent pore radii range from 9 to 53 Å, with typical values in the range of 20 to 30 Å. Seeds (1970) reports that term amnion is impermeable to Dextran 10, a molecule with a Stokes-Einstein radius of about 30 Å. The same author also reports a reflection coefficient of 0.07 for sucrose whose Stokes-Einstein radius is 4.6 Å.

Although the equivalent pore radii given here would therefore appear to be a good deal more realistic than the value of 85 Å given by Barton and Baker (1967), too much confidence should not be placed on them. They at best represent an average, whereas variations of actual pore sizes may be of more physiological significance. As discussed by Garby (1957) the presence of a few large pores will dramatically alter the value of L_p. If a pore is treated as a cylinder, L_p will depend upon the fourth power of the pore radius, but only the first power of its length. This factor probably explains the marked variations in L_p observed between different tissues combined with the absence of any simple correlation between L_p and thickness. There is strong histological evidence for variations in the epithelial layer which would fit this line of reasoning. Both Wynn and French (1968) and Bourne (1962) have observed patches of dead epithelium in normal term tissue. Other epithelial abnormalities have also been reported in otherwise normal tissue, in particular that of pseudostratification. According to Bourne (1962) this latter defect can cause gaps in the epithelium as large as 10 μm in diameter.

The magnitudes of L_p reported here are in general considerably larger than those reported by Seeds (1970) which were made using an osmotic method of measurement. The reason for this probably lies in the method of observation. In osmotic measurements made on membranes of many small pores and a few large pores, the volume flows will be dominated by the small pores, as the solute reflection coefficients will be highest in these. Bulk flows generated by hydrostatic pressure differentials will on the other hand be dominated by the large pores owing to the radius effect discussed above. It is noteworthy that the lower values of L_p in this work fall within the range reported by Seeds.

The results of the present work therefore indicate that in normal term human amnion volume (or bulk) flows are largely controlled by the epithelial layer alone. There is strong evidence that this layer is intersected by a large number of pores of equivalent radii from 10 to 30 Å, and by a small number of very much broader pores. These conclusions are in broad agreement with those of Garby (1957) made on the basis of diffusion measurements using solutes of different molecular sizes. Although there is evidence that there are different pore distributions between individuals the significance of this has yet to be determined. Variations in pore distribution also occur within tissues obtained from the same placenta, but as shown in Table 1 there is no evidence that any given distribution may be localized to a particular region of the amnio-chorion.

The amnion appears to be a tissue well adapted to the passage of large volume flows, and hence could play a role in the control of bulk flows in and out of the amniotic cavity. This adaptation could, however, also represent the metabolic requirements of an avascular tissue. As discussed by Bourne (1962) there is strong evidence that the amnion gains most of the nutrients necessary for its maintenence from the amniotic fluid. Therefore, although the present work cannot provide evidence as to the particular physiological significance of the adaptation, it does provide a number of criteria which may ultimately permit such an assessment. It would in particular be of interest to examine if the pattern of water flow found here is the same in tissue taken from an earlier state of gestation, and in tissues taken from subjects suffering from such pathological abnormalities as hydramnios.

We would like to thank Mrs. Margaret Still for her technical help. M.R.S. was supported by the Scottish Home and Health Department. We would like to thank the Labour Ward and Theatre Staff, Aberdeen Maternity Hospital for their cooperation, and Professor Kerridge of the Department of Statistics, University of Aberdeen for help in data analysis.

References

Barton, T. C., Baker, C. 1967. Permeability of human amnion and chorion membrane. *Amer. J. Obstet. Gynec.* **98**:562

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

Danforth, D. N., Hull, R. W. 1958. The microscopic anatomy of the fetal membranes with particular reference to the detailed structure of the amnion. *Amer. J. Obstet. Gynec.* **75**:536

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

Hays, R. M., Franki, N. 1970. The role of water diffusion in the action of vasopressin. *J. Membrane Biol.* **2**:263

Page, K. R., Abramovich, D. R., Smith, M. R. 1974. The diffusion of tritiated water across isolated term human amnion. *J. Membrane Biol.* **18**:39

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

Seeds, A. E. 1970. Osmosis across term human placental membranes. *Amer. J. Physiol.* **219**:551

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

Thau, G., Bloch, R., Kedem, O. 1966. Water transport in porous and non-porous membranes. *Desalination* **1**:129

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