Water and electrolyte acquisition across the placenta of the sheep

EDWARD E. CONRAD, JR., AND J. JOB FABER

Department of Physiology, School of Medicine, University of Oregon Health Sciences Center, Portland, Oregon 97201

Conrad, Edward E., Jr., and J. Job Faber. Water and electrolyte acquisition across the placenta of the sheep. Am. J. Physiol. 223(4): H475-H487, 1977. —Fetal plasma is known to be hyperosmotic with respect to maternal plasma. Although some solutes are actively transported across the placenta and occur in higher concentrations in fetal than in maternal plasma, the total concentration difference is reversed by an opposite difference in the concentrations of electrolytes. Calculations on the basis of the Kedem and Katchalsky equations for a homogeneous membrane demonstrate that active solute transfer and a physiologically plausible hydrostatic pressure difference account for the known rates at which water and electrolytes are accumulated in the fetus. Best estimates of the membrane parameters (per kilogram fetal weight) are \( P_{S_{na, cl}} = 0.23 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \), \( L_{a, S} = 5 \cdot 10^{-8} \text{ cm} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \cdot \text{dyne}^{-2} \), and \( \sigma_{NaCl} = 0.5 \). (where \( P_{S} \) is the permeability-surface area product, \( L_{a, S} \) is the filtration coefficient, and \( \sigma \) is the Staverman reflection coefficient). The driving forces for water and electrolyte transfer (active transfer and hydrostatic pressure) are not used as regulators. Rather, electrolyte permeability, which is the major constraint on fetal growth, and which continuously increases during gestation, makes possible the exponential increase in fetal weight during gestation.

Methods

Adaptation of Basic Equations

The equations with which Kedem and Katchalsky (24-26) described solute and water flow were presented in a variety of forms. Most useful for the present purpose are

\[
J_v = L_v (\Delta p - \sigma RT \Delta C), \text{ cm/min} \tag{1}
\]

\[
J_s = \omega RT \Delta C + (1 - \sigma) \bar{C} J_w, \text{ mmol/(cm}^2 \cdot \text{min)} \tag{2}
\]

Symbols are listed in Table 1. The first equation states that the volume flow \( J_v \) (in ml/min per cm² of membrane) is determined by a filtration coefficient \( L_v \) and the sum of the hydrostatic pressure difference (\( \Delta p \)) and...
### Table 1. List of symbols and meanings

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Molecular radius, Å = 10⁻⁸ cm</td>
</tr>
<tr>
<td>C</td>
<td>Concentration, mmol/g water</td>
</tr>
<tr>
<td>D</td>
<td>Coefficient of free diffusion, cm²·min⁻¹</td>
</tr>
<tr>
<td>Jc</td>
<td>Volume flow per cm² of membrane, cm/min</td>
</tr>
<tr>
<td>Jw</td>
<td>Water flow per cm² of membrane, cm/min</td>
</tr>
<tr>
<td>Jv</td>
<td>Solute flow per cm² of membrane, mmol·cm⁻²·min⁻¹</td>
</tr>
<tr>
<td>Lw</td>
<td>Filtration coefficient of 1 cm² of membrane, ml·min⁻¹·dyn⁻¹</td>
</tr>
<tr>
<td>N</td>
<td>Amount of solute, mmol/liter, Table 2</td>
</tr>
<tr>
<td>P</td>
<td>Diffusional permeability of 1 cm² of membrane, cm/min</td>
</tr>
<tr>
<td>Q</td>
<td>Placental blood flow, ml·min⁻¹·kg⁻¹</td>
</tr>
<tr>
<td>r</td>
<td>Pore radius, Å = 10⁻⁸ cm</td>
</tr>
<tr>
<td>R</td>
<td>Gas constant</td>
</tr>
<tr>
<td>S</td>
<td>Surface area of placenta per kg fetal wt, cm²·kg⁻¹</td>
</tr>
<tr>
<td>T</td>
<td>Absolute temperature, °K</td>
</tr>
<tr>
<td>V</td>
<td>Volume, ml</td>
</tr>
<tr>
<td>w</td>
<td>Thickness of placental barrier, cm</td>
</tr>
<tr>
<td>β</td>
<td>Concentration of inert solutes in ultrafiltrate of plasma arriving at fetal side of barrier, as required by fetal body composition, mmol/g water; defined by equation 13</td>
</tr>
<tr>
<td>ΔC</td>
<td>Concentration difference across placental barrier, mmol/g water</td>
</tr>
<tr>
<td>Δp</td>
<td>Hydrostatic pressure difference across placental barrier, dyn/cm²</td>
</tr>
<tr>
<td>σ</td>
<td>Reflection coefficient (dimensionless)</td>
</tr>
<tr>
<td>ω</td>
<td>P/RT, defined in equation 2 and in Ref. 24</td>
</tr>
</tbody>
</table>
that fraction (σ) of the Van't Hoff osmotic pressure difference (RTΔC) that is actually exerted across an imperfectly semipermeable membrane. The letter σ is the reflection coefficient (47) and ΔC is the solute concentration difference (mmol/g water). The second equation states that the solute flux J, across the membrane is the sum of a flux driven by the concentration difference ΔC and a flux carried across the membrane by the net volume flow Jv. The product ωRT is the diffusional permeability, P, measured under conditions of zero volume flow across the membrane (24), and C is the average solute concentration on the two sides of the membrane. Equations 1 and 2 apply to single-solute systems only.

It is proper to normalize all flows and the coefficients (Lp and ωRT) per kilogram fetal weight, because in the sheep the functional area of the placenta appears to be proportional to fetal weight in the last trimester of gestation. This is evident from measurements of the electrolyte permeabilities (3) and the urea permeability (15). It may and probably does arise elsewhere. The as-

The passive behavior of the placenta expressed by equations 1 and 2 may be rewritten in the form

\[ q_{tr} = L_p S \Delta p - \sigma L_p S \rho T \Delta C, \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \] (3a)

\[ q_s = P S \Delta C + (1 - \sigma) C \Delta p, \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \] (4a)

The pressure difference, Δp, and the solute concentration difference, ΔC, are defined positive when the pressure or the concentration in the maternal plasma exceeds the corresponding quantity in the fetal plasma. Since plasma is a polycomponent system, equation 3a must be modified to incorporate the sum of the osmotic pressures exerted by all the solutes, n

\[ q_{tr} = L_p S \Delta p - RT \Sigma (\sigma_n \Delta C_n), \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \] (3b)

Equation 4a applies to each of the n solutes separately; the total solute flux is therefore given by the sum of the individual fluxes

\[ \Sigma q_n = \Sigma (P_S \Delta C_n) \] (4b)

\[ + q_{tr} \Sigma (1 - \sigma_n C_n), \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \]

The passive behavior of the placenta expressed by equations 3b and 4b is determined by \( L_p S \) and the \( P_S \) and \( \sigma_n \) for each solute. Whereas \( L_p S \) is a function of the placental membrane and the solvent (water) only, \( P_S \) and \( \sigma_n \) are also functions of the solute under consideration. These coefficients must be determined from the available experimental data.

Membrane Quantity Determined with Osmotic Experiments

In the preceding paper (3) the calculated water flow through the membrane was obtained from the formula

\[ q_{tr} = Q^F - Q^M, \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \] (5)

The flow of the marker solutes \( q_m \) across the membrane is the difference between outflow of the marker solute in the umbilical vein and inflow in the umbilical artery:

\[ q_m = Q^V C^V - Q^A C^A, \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \] (6)

Rearrangement of equations 5-7 yields

\[ q_m = q_{tr} C^V - \bar{C}_m C^A, \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \] (7)

Combination of equation 8 with equation 4a applied to the marker solute (m) yields

\[ q_{tr} = (q_m C^V + P_m \Sigma C_n) / (C^V - \bar{C}_m + \bar{C}_n \sigma_n), \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \] (9)

The data reported in the preceding paper show that equation 9 may be simplified: \( C_m^V \) and \( \bar{C}_m \) are not grossly different after the infusion of hypertonic mannitol or sucrose, typical differences would be ±10%. It will be shown later that \( \sigma_m \) is of the order of 0.5-0.8. Hence, the denominator is approximately equal to \( C_m^V \sigma_m \). Figures 2 and 3 of the preceding paper (3) show that at a total concentration difference across the placenta of 40 mM, the calculated water flow is of the order of 3 ml/min·kg·kg, and the permeabilities (\( P_m \) and \( \sigma_m \)) of the marker solutes Na+ and Cl- were found to be of the order of 0.2-0.3 ml/min·kg·kg. Since \( C_m^V \) is also much greater than \( \Delta C_m \), equation 9 can be simplified to the form

\[ q_{tr} = q_m / \sigma_m, \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \] (10)

In order to apply equation 3b to the results of the osmotic experiments (3), it is rewritten

\[ q_{tr} = L_p S \left[ \Delta p - RT \sum \sigma_n \Delta C_n \right] - RT \sigma_n \Delta C_n, \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \] (3c)

1 An electrical potential between fetal lamb and ewe does not imply that there must be an electrical potential across the placental exchange area. It may and probably does arise elsewhere. The assumption of a transplacental membrane potential in the sheep would require a cascade of additional assumptions of such complexity that it must be rejected as implausible in the absence of compelling evidence.
where $x$ is the solute (mannitol or sucrose) that was used to make one of the two plasmas hypertonic. Differentiation of equation 3c yields

$$\frac{dq_i}{d\Delta C_x} = -a_x L_v S T R, \text{ mll}^2\cdot\text{min}^{-1}\cdot\text{kg}^{-1}\cdot\text{mmol}^{-1} \quad (11)$$

and combining equations 11 and 10 we find

$$\frac{dq_i}{d\Delta C_x} = \left(1 + a_i \frac{L_v S T P_i}{S} \right) \frac{dq}{d\Delta C}, \text{ ml}^2\cdot\text{min}^{-1}\cdot\text{kg}^{-1}\cdot\text{mmol}^{-1} \quad (12a)$$

where $dq_i/d\Delta C_x$ is the slope of the regression lines relating transplacental water flow to transplacental concentration gradient (Figs. 2 and 3, Ref. 3).

The preceding paper further showed that any of the marker solutes Na$^+$, K$^+$, and Cl$^-$ could be used to calculate transplacental water flow with no significant difference in the results. Equation 12a shows that this is only possible if there are no significant differences between the reflection coefficients $a_x$ of these solutes. Since the marker solutes belong to the class of "inert" solutes (defined below), we will write $\sigma_i$ for $a_x$. Appendix II demonstrates that the reflection coefficients for the solutes used to make the plasmas hypertonic (mannitol and sucrose) are essentially 1.0. We therefore rewrite equation 12a as

$$\frac{dq_i}{d\Delta C_x} = \left(1 + a_i \frac{L_v S T P_i}{S} \right) \frac{dq}{d\Delta C}, \text{ ml}^2\cdot\text{min}^{-1}\cdot\text{kg}^{-1}\cdot\text{mmol}^{-1} \quad (12b)$$

to describe the quantity (slopes of regression lines in figures 2 and 3, Ref. 3) that was measured in the osmotic experiments. The various simplifications made in the derivation of equation 12b could cause it to be in error by no more than 20%. This value may seem high, but the subsequent calculations will be shown to be quite insensitive to errors in equation 12b even if as large as 50% (Figs. 3 and 4 below).

Equations 3b and 4b in a Form Applicable to Placenta of Sheep

The preceding paper provides two experimental measurements for the evaluation of the membrane coefficients $L_v S, P T S$, and $a_i$; one to be used with equation 12b and the other the direct measurement of the diffusional permeability of the inert solutes Na$^+$ and Cl$^-$ ($P I S$). It is necessary, therefore, to derive a third independent expression to evaluate all three constants empirically.

Under conditions of normal fetal growth, the acquisition of certain solutes is independent of the pressure filtration and diffusion process. This may be so because of active transport or because the materials are derived from fetal metabolism (e.g., urea and bicarbonate). The transplacental concentration difference of the "active" solutes, represented by $\Delta C_a$, is relatively constant under normal conditions. All remaining solutes are defined as "inert" solutes. Normal fetal growth requires an influx of these inert solutes also, which is represented by $\Delta C_i$.

The molality of the transplacental ultrafiltrate of maternal plasma is defined by the variable $\beta$ as

$$\beta = \langle \Sigma q_i \rangle / q_i, \text{ mmol/g water.} \quad (13)$$

Although filtration may be assumed not to affect the transfer of active solutes, the transfer of these solutes by other processes still gives rise to concentration differences across the placenta that must be taken into account in the description of the filtration of water. Hence equation 3b becomes

$$\dot{q}_i = L_v S (\Delta p - R T \Sigma (\sigma_i \Delta C_i)) - R T \Sigma (\sigma_i \Delta C_a), \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \quad (14)$$

where the subscripts $a$ and $i$ identify the active and the inert solutes, whose osmotic forces are additive (23). The small difference in colloid osmotic pressures between maternal and fetal plasmas (15, 38) is legitimately incorporated into the hydrostatic pressure difference (24). Equation 4b is rewritten in terms of the inert solutes only and since $C_i = (C_i^M - 1/2 \Delta C_i)$, equation 4b becomes

$$\Sigma q_i = S \Sigma (P T S \Delta C_i)$$

$$+ \dot{q}_i \Sigma \left( (1 - \sigma_i) (C_i^M - 1/2 \Delta C_i) \right) \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \quad (15)$$

The variable $\Sigma q_i$ can be eliminated by combining equations 15 and 13.

The Na$^+$ and Cl$^-$ concentrations constitute 98% of the total concentrations of the inert solutes (Na$^+$, K$^+$, Mg$^{++}$, and Cl$^-$) in the plasmas of fetal and maternal sheep, and as explained above, there is good reason to believe that the reflection coefficients for the inert solutes Na$^+$ and Cl$^-$ are not very different. Equation 14 can therefore be simplified with little error by substitution of $\sigma_i \Delta C_i$ for $\Sigma (\sigma_i \Delta C_i)$, where $C_i$ is the total concentration of inert solute $\sigma_i$ and $\sigma_i$ is a weighted average inert solute reflection coefficient. Similarly, equation 15 can be simplified since $P Na^+S = P Cl^-S = P S$ (3). Hence, the system of the two equations 14 and 15 can be solved for $\Delta C_i$ and $q_i$. The result is a quadratic expression of the form

$$a(\Delta C_i)^2 + b(\Delta C_i) + c = 0 \quad (16)$$

where the variables $a$, $b$, and $c$ are defined by

$$a = -1/2 (1 - \sigma_i) \cdot (L_v S T R) \quad (16a)$$

$$b = \{- P S + 1/2 (1 - \sigma_i) L_v S \Delta p - (\beta - (1 - \sigma_i) C_i^M \cdot L_v S T R \} \quad (16b)$$

$$c = (\beta - (1 - \sigma_i) C_i^M \cdot (L_v S \Delta p - \sigma_i \Delta C_a S T R) \quad (16c)$$

and where $\sigma_i \Delta C_a$ stands for $\Sigma (\sigma_i \Delta C_a)$. The single physically possible solution of this equation can be shown to be

$$\Delta C_i = \left\{ \beta - \left( b^2 - 4ac \right)^{1/2} \right\} / 2a \quad (17)$$

With this solution in hand $\dot{q}_i$ can be solved from equation 14.

Since fetal water inflow ($\dot{q}_i$) is known from normal gestational growth data of the sheep fetus and its fluids, there are three equations (no. 12b, 14, and 17) with three unknowns ($L_v S, \sigma_i$, and $\Delta p$).

RESULTS

Estimation of Parameters $L_v S, P T S$, and $\sigma_i$ for Placenta of Sheep

The PS product for Na$^+$ was found to be 0.20 ml/ (min·kg) and for Cl$^-$ it was found to be 0.27 ml/
(min·kg) (3). We will assign the average value of 0.233 ml/(min·kg) to the salt NaCl. According to equation 12b, the value of $\sigma_L$, $SRT$ is 61 m$^2$·min$^{-1}$·kg$^{-1}$·mmol$^{-1}$ (3) and at 39°C, the value of the product $RT$ is 2.595·10$^9$ erg:mmol, hence $\sigma_L$ is equal to 2.35·10$^{-6}$ (cm$^2$·dyn$^{-1}$·min$^{-1}$·kg$^{-1}$). The most direct approach to solving all these variables would be the determination of $\sigma_L$ by the application of pore theory (10, 44, 46) as applied to the sheep placenta by Boyd and co-workers (6), but this is not valid for ions.

The theoretic relation between the ratio of pore diameter and solute diameter and the resulting steric and frictional hindrance to diffusion is visualized by a plot of the ratio of placental permeability and coefficient of free diffusion (PS/D) against molecular radius on logarithmic scales. The details of this application of Renkin's formula can be found elsewhere (16). Figure 1 shows such a plot for permeability data on the sheep placenta from a variety of sources, which are listed in APPENDIX II. Our new results (3) with Na$^+$ and Cl$^-$ are divergent to a degree that cannot be explained by errors of measurement. It would be hazardous, therefore, to use pore theory to predict reflection coefficients of electrolytes.

A more reliable method for estimating the reflection coefficient is the use of equation 17 and the normal rates of water and solute acquisition during gestation. According to the reasoning developed in METHODS, it is necessary to make estimates of: a) the osmotic pressure exerted by the concentration difference ($\Delta C_{pl}$) between maternal and fetal plasma of solutes that are transferred independently of water flux, and b) the ratio $\beta$ of inert solute and water flow across the placenta.

Ad. a: APPENDIX II lists those solutes whose influx into the fetal lamb does not significantly depend on the rate of water filtration for either of two reasons: they may be actively transported (e.g., glucose, lactate, fructose, amino acids, calcium, and inorganic phosphate), or they are produced by the fetus and their diffusional permeability is so high that the second term on the right-hand side of equation 4a is insignificant in comparison to the first, the diffusional term. Urea and bicarbonate (CO$_2$) fall in this latter category. APPENDIX II discusses the evidence for active transport and for predominantly diffusional transfer.

Ad. b: Table 2 identifies "inert" solutes (whose transfer is significantly affected by filtration). The quantities transferred to the fetus per unit time near the end of gestation were computed from fetal composition at various gestational ages (17) and fetal growth (4). Amniotic and allantoic fluid accumulation was also accounted for (37, 51). Total water required for fetal growth and the growth of the extrafetal fluid volumes was found to be 2.1·10$^{-2}$ ml/(min·kg), and total inert solute flux was found to be 5.054 µmol/(min·kg). Metabolic water accounted for 3.9·10$^{-3}$ ml/(min·kg), the ratio $\beta$ was therefore 0.295 mmol/ml.

The relation between $\dot{q}_r$, $\Delta C_T$ ($-\Sigma \Delta C_l + \Sigma \Delta C_f$, $\Delta p$, and $\sigma_L$ as specified by equations 14 and 17 is shown in Fig. 2. In the sheep, the normal gestational rate of water accumulation is 1.71·10$^{-2}$ ml/(min·kg), and there is a total concentration difference between maternal and fetal plasma of 7.2 mmol/kg water (the average of the osmolalities determined by freezing-point depression osmometry (3, 39) and vapor pressure osmometry (3) and the total difference in solute concentration (3) after correction for plasma water content). Figure 2 shows that the reflection coefficient $\sigma_L = 0.52$ and the hydrostatic pressure difference between maternal and fetal plasma in the placental capillaries $\Delta p$ is about 42 mmHg (not corrected for a difference in colloid osmotic pressures (15, 38) which is included in $\Delta p$). We conclude that these values are the normal ones for the placenta of the fetal sheep in the last trimester of gestation (Table 3).

The inert solutes exert an osmotic pressure of about −124 mmHg and the active solutes exert an osmotic pressure of about +84 mmHg. The hydrostatic pressure difference is 42 mmHg. Of these 42 mmHg, about 40 mmHg balance the osmotic pressure associated with the difference in inert and active solute concentrations. Thus, a net filtration pressure of only 2 mmHg suffices to supply the growing sheep fetus with water.

The concentration difference in inert solutes (major electrolytes) is generated by the sieving of these solutes when maternal plasma is filtered through the placental barrier. Fetal (major) electrolyte requirements are met in two ways. Part of it is met by electrolytes that diffuse across the barrier under the influence of the generated solute concentration difference (about 57%) and the remainder is the amount that is carried across the barrier with the filtrate (about 43%); these two contributions correspond to the first and the second terms, respectively, on the right-hand sides of equations 4 and 15.

Sensitivity of Derived Parameters $\sigma_L$ and $\Delta p$ to Experimental Error in Experimentally Determined Variables

Equations 14 and 17 can be solved for the value of the reflection coefficient $\sigma_L$ and the hydrostatic pressure

![Fig. 1. Restricted diffusion across sheep placenta. Ratio of permeability and diffusion coefficient in water PS/D declines with increasing size of diffusing particle, indicative of a geometric constraint. Line is the best fit of Renkin equation (44) for diffusion through cylindrical pores. The term $D/D_{av}$ is ratio of diffusion coefficient through porous membrane and coefficient of free diffusion in water, $\alpha$ is molecular radius and $r$, pore radius. Data from Table 4 in APPENDIX II. Best fit is obtained at $r = 4.55·10^{-8}$ cm. Note logarithmic scales.](image-url)
TABLE 2. Fluxes of water and inert solutes during last trimester of gestation in sheep

<table>
<thead>
<tr>
<th>Fetus</th>
<th>Amniotic Fluid</th>
<th>Allantoic Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water volumes, ml/g fetus</td>
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</tr>
<tr>
<td>Fetus</td>
<td>0.750</td>
<td>0.247</td>
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<tr>
<td></td>
<td>0.178</td>
<td>0.178</td>
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<tr>
<td></td>
<td>Solute concentrations, mmol/g water</td>
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<tr>
<td>Na⁺</td>
<td>0.143</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>0.086</td>
<td>0.066</td>
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<tr>
<td>Mg²⁺</td>
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<td>0.056</td>
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<tr>
<td>Cl⁻</td>
<td>0.077</td>
<td>0.095</td>
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<table>
<thead>
<tr>
<th>Fetus</th>
<th>Amniotic fluid</th>
<th>Allantoic fluid</th>
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<tbody>
<tr>
<td></td>
<td>Water flux, ml/min - kg</td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>1.2 × 10⁻²</td>
<td>+4.4 × 10⁻²</td>
</tr>
<tr>
<td>K⁺</td>
<td>+453</td>
<td>-104</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>+30</td>
<td>+230</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>+418</td>
<td>-126</td>
</tr>
</tbody>
</table>

(Average solute content is 5.050 mmol/1.17 × 10⁻² ml = 295 mmol/g H₂O = β.)

Total 5.050

1 From Refs. 8 and 54.  2 From Ref. 51.  3 From Ref. 17 (fetuses of 120 days' gestation or older only, data converted from per kilogram weight to per kilogram water).  4 From Ref. 37.  5 Based on assumption that 76% of fetal sodium content is extracellular (Ref. 54, p. 21), in a sodium-to-chloride ratio of 140/100.  6 Fetal growth estimated from Barcroft's data (4) at 1.62 × 10⁻² g/(kg min), suitably adjusted for water content and electrolyte contents which are constant from 120 days' gestational age onward (17).  7 If total content N is given by the product of volume and concentration, N = V · C, the time derivative (rate of inflow) is given by dN/dt = C(dV/dt) + V(dC/dt); data from Ref. 37.  8 Fetal oxygen consumption is approximately 6 ml/minute · kg⁻¹ (21). Since water production and carbon dioxide production are comparable for most metabolic fuels, we will assume a ratio of 0.8 mol of water produced for every molecule of oxygen used. This metabolic water production of 3.9 × 10⁻³ ml/(minute · kg) must be subtracted from the total fetal water requirements to obtain net transplacental water flow.

FIG. 2. Total transplacental solute concentration difference ΔC_T (= ΔC_A⁻ + Σ ΔC_i⁻) in mosmol/kg water, plotted against fetal water acquisition rate, q_w, in ml/(min · kg). A positive value of ΔC_T indicates maternal solute concentration exceeds fetal solute concentration. Total concentration difference and fetal water acquisition rate are a function of reflection coefficient of barrier for inert solutes, a_i, and hydrostatic pressure across barrier, Δp, (defined positive if maternal pressure exceeds fetal pressure in the placental capillaries). Conversely, if ΔC_T and q_w are known, values of reflection coefficient and hydrostatic pressure difference can be read off graph. Normal point is indicated (1). Values from Table 3 were used to solve equations 14 and 17.

The derived value for the average inert solute reflection coefficient σ_i (Fig. 3) is almost completely insensi-

σ_i, while all other variables were held constant at their best estimate (Table 3), except for one which was varied from 0.5 to 1.5 times its best estimate. Figure 3 and 4 show how σ_i and Δp vary when each of the other variables in turn deviates from its best estimate.
OSMOSIS AND PRESSURE FILTRATION IN PLACENTA

1.0 Value of $P_a$

FIG. 3. Inert solute reflection coefficient, $\sigma_i$, relative to its nominal value of 0.52, plotted as a function of each of placental permeability parameters as they are varied individually from 50% to 150% of their best estimates (Table 3). Slopes of these relations are measures of sensitivity of derived value $\sigma_i$ to errors in empirical values of placental permeability parameters. $\sigma_i\Delta C_i$ stands for $\Sigma (\sigma_i\Delta C_i)$.

1.0 Relative Value of Parameters

FIG. 4. Difference in hydrostatic pressure between maternal and fetal placental capillaries, $\Delta p$, relative to its nominal value of 42 mmHg, plotted as a function of each of placental permeability variables as they are varied individually from 50% to 150% of their best estimates (Table 3).

A Different Experiment to Estimate $\sigma_i$

Equations 14 and 15 show that $\sigma_i$ and $\Delta p$ can be solved if water flux $q_w$ and solute flux $\Sigma q_i$ are known. In addition to values derived from normal gestational growth, experiments in which transplacental fluxes were actually measured can be used also.

In an experiment on the renal clearances of fetal sheep (18), fetal urine was collected externally over long periods of time, causing a significant increase in transplacental water flow. One protocol was published in toto (Table 4 of Ref. 18). Over a period of 18 days, 9,250 ml of fetal urine was drained. It contained 281 mM of inert solutes (Na+, K+, Cl-). Fetal weight at birth was 4.9 kg, a value so high that one must assume that growth was not affected by the experiment. Water flow, $q_w$, therefore, was $7.3 \times 10^{-2}$ ml/(kg·min) plus the water flow necessary for normal growth, $1.7 \times 10^{-2}$ ml/(kg·min), a total of $9 \times 10^{-2}$ ml/(kg·min). Similarly, solute flow $q_i$ was $2.21 \times 10^{-3} + 5.054 \times 10^{-3} = 7.27 \times 10^{-3}$ mmol/(kg·min).

Although the results could have been presented in the form of a graph like Fig. 2, it is more instructive to plot the transplacental hydrostatic pressure and the transplacental total concentration difference $\Delta C_T$ as a function of the unknown inert solute reflection coefficient $\sigma_i$.

This has been done in Fig. 5. It is seen that the pressure difference $\Delta p$ is very sensitive to the assumed value of $\sigma_i$ and that only values of $\sigma_i$ in the range of about 0.76–0.78 are compatible with physiologically reasonable pressure differences. The center value of $\sigma_i$ of 0.77 agrees only moderately well with the value of 0.52 suggested by the analysis presented in Fig. 2 and presented again in Fig. 5. The drainage experiment analyzed in Fig. 5 is potentially capable of a much more precise estimation of $\sigma_i$ than the analysis of normal gestational values, but with only one experiment available, there is no basis for judging whether the difference in reflection coefficients is individual or systematic.

Constraints on Fetal Growth

Figure 2 shows that for a reflection coefficient of 0.52, the rate of fetal water acquisition is accelerated by an increase in the hydrostatic pressure difference between maternal and fetal capillaries in the placenta. It is evident, however, that this mechanism is a weak one. A large increase in pressure is required for a modest increase in transplacental water flow.

Nevertheless, it is known that fetal growth is continuously accelerating during gestation (4, 17, 51). Fetal water content being about 75% of fetal body weight (54), an accelerated growth rate directly reflects an acceler-
Inert Solute Reflection Coefficient, \( \sigma_i \), on the rates at which water and electrolytes can pass from mother to fetus. Figure 6 shows the effects of variations in the parameters of equations 14–17 on the rates of fetal water and electrolyte acquisition. Both \( q_r \), the water flow, and \( \Sigma q_i \), the electrolyte flow are insensitive to changes in the filtration coefficient, \( L,S \), and progressively more sensitive to changes in transplacental pressure difference, \( \Delta p \), the osmotic pressure exerted by the active solutes represented by the sum \( \Sigma (\sigma_i \Delta C_i) \) the electrolyte permeability \( P,S \), the required electrolyte concentration of the ultrafiltrate \( p \), and the reflection coefficient of the placental barrier for electrolytes, \( \sigma_i \).

**DISCUSSION**

**Applicability of Theory to Permeability of Sheep Placenta**

This study required some simplifications that are only approximately correct. The sheep placenta is not a homogeneous single-layer membrane as required by the theory in the form used here (26–28). Figure 7 shows the ultrastructure of this barrier in which the presence of several histological layers is evident. The ultrastructure of the sheep placenta differs significantly from those of the rabbit (49) or guinea pig (11) placentas. The dense extracellular material between maternal and fetal capillaries (arrows in Fig. 7) of the sheep placenta is absent in these other species. One may speculate, therefore, that the presence of this dense extracellular material in the sheep placenta is related to the very high diffusion resistance compared to the diffusion resistance found for the placenta of the rabbit and the guinea pig (13). It is known that, in the rabbit placenta, the diffusional resistance for small molecules is lower across the endothelial layer than across the center of the placental barrier (13). If this is also true for the sheep placenta, the behavior of the membrane as a diffusional barrier may be approximated with a single-layer model.

Although the osmotic pressures exerted by a variety of solutes are additive (23), it is not correct to assign a single reflection coefficient, \( \sigma_i \), to all electrolytes. However, the major electrolytes are Na\(^+\) and Cl\(^-\) whose diffusional permeabilities in the placenta of the sheep are at least comparable (3). The error was therefore accepted in the analysis.

It is clear that these approximations restrain the interpretation of the results. Quantitatively, the results are reliable for the estimation of the average inert solute reflection coefficient \( \sigma_i \), which is not unduly sensitive to errors in the empirically determined parameters. The hydrostatic pressure difference, \( \Delta p \), however, is so sensitive to errors of measurement in the empirical parameters that no certain value can be given for it. This uncertainty is compounded by the approximations that went into the analysis.

**Mechanism of Fetal Water and Electrolyte Acquisition**

The presence of a hydrostatic pressure difference across the placental barrier helps to explain that the maternal plasma osmolality exceeds the fetal plasma osmolality, in spite of a net inflow of water into the fetus during the entire gestational period. Although the quantitative value of this pressure difference is not yet known with acceptable accuracy, the values suggested by the present calculations are well within the range of physiologically reasonable pressure differences. Moll and Künzel (42) measured maternal arterial pressures in the smallest placental arteries that were accessible to direct puncture. In cotyledonary arteries of the sheep which were from 0.5 to 1 mm in outside diameter, they...
recorded a mean arterial pressure of 84 mmHg (42). This value was in sharp contrast to the corresponding values recorded in the guinea pig (12 mmHg), the rat (14 mmHg), and the rabbit (8 mmHg) (42). Fetal arterial blood pressure in the sheep is about 39 mmHg, umbilical vein pressure is about 7 mmHg, and uterine vein pressure is about 3 mmHg (48). The available evidence is compatible with hydrostatic pressure differences of 20 to 60 mmHg between the maternal and fetal capillaries in the placenta of the sheep. The existence of such a large pressure difference is also compatible with the finding that large increases in maternal placental vein pressure do not affect the pressures or the flow in the fetal placental circulation (48).

The mechanism of fetal water and electrolyte acquisition in the sheep appears to be a combination of hydrostatic pressure and the active transfer of some metabolically important substrates. The present best estimate of the hydrostatic pressure difference is of the order of 40 mmHg and the osmotic pressure exerted by the actively transported solutes is about 80 mmHg (Table 5 in Appendix III).

**Regulation of Fetal Water and Electrolyte Acquisition**

**Placental parameters.** Figure 6 shows that the magnitude of the hydraulic conductivity of the sheep placenta, \( L_p S \), is almost without influence on the rate of fetal water uptake. Placental hydraulic conductivity would be a significant factor in transplacental water flow only if the conductivity was less than \( 1/10 \)th of the observed value. This is because of the total hydrostatic pressure difference across the placenta of 42 mmHg. 40 mmHg are required to balance the osmotic disequilibrium due to the concentration differences across the barrier of inert and actively transported solutes. This finding means that the prime constraint on fetal growth is not water acquisition but electrolyte acquisition.

It follows that the magnitude of the diffusional...
permeability of the placenta for inert electrolytes ($P_S$) must have a major effect on the rate of water transfer. Figure 6 confirms this by showing that transplacental water flow and the diffusional permeability of the placenta are for practical purposes proportional to each other.

For the same reason, the magnitude of the inert solute reflection coefficient $\sigma_i$ must affect the rate of water transfer. Figure 6 shows that this reflection coefficient is indeed one of the most powerful controllers of the rate of fetal water acquisition. A decrease in the reflection coefficient decreases the counter osmotic pressure of the inert solute concentration difference generated by sieving. A greater fraction of the driving pressure difference remains available for pressure filtration.

The osmotic pressure difference exerted by the actively transported solutes is represented by the product $\sigma_a \Delta C_a$ in Fig. 6. Its control over fetal water acquisition is a relatively weak one.

Nonplacental parameters. The parameter $\beta$, the ratio of inert solute and water flows, has a significant influence on fetal water acquisition. This emphasizes again that the main constraint is on electrolyte uptake. This influence of $\beta$ explains why, in the experiments in which fetal urine was drained over a period of several weeks (18), transplacental water flow could be more than 5 times greater than the normal rate of fetal water acquisition. The value of $\beta$ in these experiments was only 87 mosmol/kg as opposed to 285 mosmol/kg during normal gestation. Under conditions of normal fetal growth, the value of $\beta$ is fixed by the compositions of the fetal body and the extrafetal fluids; therefore, $\beta$ cannot be considered a controlling variable.

The effect of the hydrostatic pressure difference, $\Delta \rho$, is much too small to explain the continuous increase in fetal water demands during the progression of gestation. It is too small even to account for the day to day regulation of fetal water content and cardiac output (14), in the sheep. Moreover, fetal arterial blood pressure rises with gestational age, which is hard to reconcile with an increase in $\Delta \rho$.

In conclusion, this analysis shows that the combination of a transplacental difference in hydrostatic pressure and the active transport of some metabolites accounts quantitatively for fetal water and electrolyte acquisition where neither one can do so alone. The analysis, however, does not shed light on the question: does the fetus control placental transfer or does the placenta control fetal growth?

If fetal control exists in the form of feedback steering, what is the most powerful possibility? Control of the placental electrolyte permeability ($P_S$) would be adequate to explain the ever increasing fetal water and electrolyte acquisition rates during the course of gestation. The experimental results (3) fully agree with the theoretic prediction that the permeability of inert solutes ($Na^+$, $Cl^-$, $K^+$, $Mg^{2+}$) must increase during the course of gestation. Urea permeability is known to increase also (29, 41), but fetal control over these increases has not been demonstrated.

Fetal control of the reflection coefficient $\sigma_i$, for instance by endocrine mechanisms, would powerfully control fetal water and electrolyte uptakes. Although such a mechanism need not account for the gestational increase in the acquisition rates, its existence would make available to the fetus an hour to hour control of extracellular water volume. We plan to investigate the possible role of antidiuretic hormone at a later time.

There is no evidence of fetal control of the concentration difference of actively transported solutes ($\Delta C_a$) and
there is no evidence of an increase of this difference during the course of gestation. The effect of $\Delta C_a$ on fetal water acquisition, moreover, is not a large one. The calculations do not give evidence for a major contribution of bicarbonate ion, which is only a fraction of the total concentration difference $\Delta C_a$, in the long term regulation of fetal water and electrolyte acquisition (32). Similarly, the hydrostatic pressure difference $\Delta p$ across the placental barrier has too little effect on the regulation of fetal water and electrolyte acquisition rates to be considered a regulator (14), at least in the sheep. These conclusions must be drawn notwithstanding the fact that the hydrostatic pressure difference and the difference in concentrations of actively transported solutes together constitute the driving force for water and electrolyte transfer. Somewhat contrary to our expectations, variation of the driving forces does not appear to be used for the regulation of water and solute flows. It seems, rather, that another variable, namely, the electrolyte permeability $\Psi S$ (3), is responsible for the long term regulation of these flows, in the face of comparatively constant driving forces.

It should be noted that these conclusions apply only to the placenta of the sheep, and perhaps to the very similar placenta of the goat. In the placenta of, for instance, the rabbit and the guinea pig, the diffusional permeabilities do not decline as rapidly with increasing molecular weight (13, 16). Theories of fetal water acquisition that are fallacious when applied to the placenta of the sheep (14) may well be valid in these species. The human placenta, judged histologically, is much more closely related to those of the rabbit and the guinea pig than to those of the sheep and goat. It is likely that the regulation of transplacental water and electrolyte fluxes is fundamentally different in these species.

APPENDIX I

Transplacental Difference in Temperature

Placental exchange can be shown to be essentially isothermal by comparing the diffusivities of oxygen and heat. The carbon monoxide diffusion capacity of the sheep placenta has been shown to be 0.54 ml/(min·mmHg·kg body wt), and the oxygen diffusion capacity has been estimated to be 1.2-2 times higher, ca. 0.86 ml/(min·mmHg·kg) (33). With an oxygen solubility of about 0.021 ml/(ml·760 mmHg) (2) the permeability-surface area product for oxygen is $P_O_2 S = 3.1 \times 10^4$ ml/(min·kg). Since oxygen may be assumed to have the entire placental area available for its diffusion, the permeability-surface area product is given by

$$D_{O_2}/S/w = P_{O_2}, S, \text{ml/(min·kg)} \tag{18}$$

where $w$ is the average thickness of the placental barrier and $D_{O_2}$ the coefficient of diffusion of oxygen in placental tissue. The value of $D_{O_2}$ is not exactly known, but estimates in plasma (55) and in other tissues (36) suggest a value about 1.2-10^{-8} m^2/s. Thus, equation 18 yields a value for $S/w$ of about 2.6 x 10^{-8} cm²/kg fetus.

The thermal conductivity of tissue is not much different from the thermal conductivity of water (53) at 40°C, for which 0.001499 cal·s^{-1}·cm^{-2}·°C·cm is given. If we approximate 1°C by 1 cal/ml, and convert seconds to minutes, the thermal conductivity is $(D_{th})$ about 9 x 10^{-2} cm²/min. The "thermal permeability" of the placenta is therefore given by

$$D_{th}/S/w = P_{th}, S, \text{ml/(min·kg)} \tag{19}$$
in analogy to equation 18. Substitution of the value of $S/w$ of 2.6 x 10^{-8} cm²/kg into this equation yields a thermal permeability of about 2.3 x 10^{-8} ml/(min·kg), or cm·min^{-1}·°C·cm^{-1}. The average temperature difference across the placental barrier is therefore about 30/(2.3 x 10^{-8} °C) = 1.3 x 10³ °C. Exchange is therefore isothermal to within a fraction of a millidegree Celsius.

APPENDIX II

Estimation of Molecular Radii and Reflection Coefficients

Molecular radii were calculated with the equation of Gierer and Wirtz, as described by Durbin (10). We used a water radius of 1.65 x 10^{-8} cm (5). Permeability-surface area products for the near-term sheep placenta were taken from the literature and, where necessary, corrected for fetal weight (Table 4). If the placental pathways are only slightly larger than the diffusing molecules, the ratios of the permeability-surface area products to the coefficients of free diffusion $(PS/DS)$ will decline with increasing molecular diameter. Such appears to be the case for inert nonelectrolytes in the placenta of the sheep (Fig. 1).

An equation derived by Renkin (44) describes the diminution in the ratio $PS/DS$ that is to be expected if the passages are cylindrical. The best fit between experimentally determined permeabilities of

<table>
<thead>
<tr>
<th>Substance</th>
<th>Molecular wt</th>
<th>$D_{th}$, Coef of Free Diffusion</th>
<th>$P_S, S$, Permeability</th>
<th>$\sigma_{mol}$, Molecular Radius, $\times 10^{-8}$ cm</th>
<th>$\sigma_{ref}$, Reflection Coeff</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>18</td>
<td>0.018</td>
<td>2.355 ± 0.5</td>
<td>(4)</td>
<td>1.69</td>
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<tr>
<td>Na⁺</td>
<td>23</td>
<td>0.206</td>
<td>2.021 ± 0.006</td>
<td>(5)</td>
<td>2.61</td>
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<tr>
<td>Na⁺ (goats)</td>
<td>23</td>
<td>0.267</td>
<td>2.056 ± 0.016</td>
<td>(6)</td>
<td>1.99</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>35</td>
<td>17.2</td>
<td>2.025 ± 0.006</td>
<td>(7)</td>
<td>2.85</td>
</tr>
<tr>
<td>K⁺</td>
<td>39</td>
<td>16.7</td>
<td>2.025 ± 0.006</td>
<td>(8)</td>
<td>2.85</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>40</td>
<td>6.67</td>
<td>2.025 ± 0.006</td>
<td>(9)</td>
<td>2.85</td>
</tr>
<tr>
<td>Urea</td>
<td>60</td>
<td>11.7</td>
<td>17.0 ± 1.8</td>
<td>(10)</td>
<td>2.55</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>84</td>
<td>10.0</td>
<td>1.99 ± 0.02</td>
<td>(11)</td>
<td>2.85</td>
</tr>
<tr>
<td>Ethylene</td>
<td>28</td>
<td>9.61</td>
<td>1.99 ± 0.02</td>
<td>(12)</td>
<td>2.85</td>
</tr>
<tr>
<td>Glycerol</td>
<td>92</td>
<td>7.81</td>
<td>1.99 ± 0.02</td>
<td>(13)</td>
<td>2.85</td>
</tr>
<tr>
<td>Erythritol</td>
<td>122</td>
<td>6.50</td>
<td>1.99 ± 0.02</td>
<td>(14)</td>
<td>2.85</td>
</tr>
<tr>
<td>n-Glucose</td>
<td>180</td>
<td>5.20</td>
<td>1.99 ± 0.02</td>
<td>(15)</td>
<td>2.85</td>
</tr>
<tr>
<td>L-Glucose</td>
<td>180</td>
<td>5.20</td>
<td>1.99 ± 0.02</td>
<td>(16)</td>
<td>2.85</td>
</tr>
<tr>
<td>Mannitol</td>
<td>182</td>
<td>5.41</td>
<td>1.99 ± 0.02</td>
<td>(17)</td>
<td>2.85</td>
</tr>
<tr>
<td>Sucrose</td>
<td>342</td>
<td>4.34</td>
<td>1.99 ± 0.02</td>
<td>(18)</td>
<td>2.85</td>
</tr>
</tbody>
</table>

$P_S$ values are means ± SE. $\sigma_{mol}$ Corrected to 39°C, where necessary, by adjustment of the factors for absolute temperature and the viscosity of water. Note units. $\sigma_{ref}$ Computed from the coefficients of free diffusion in water and the Gierer and Wirtz equation (10) with a water radius of 1.65 x 10^{-8} cm (5). Permeability-surface area products for the near-term sheep placenta were taken from the literature and, where necessary, corrected for fetal weight (Table 4).
inert non-electrolytes in the sheep placenta and the Renkin equation was obtained by assuming a pore radius of \(4.55 \times 10^{-8}\) cm (Fig. 1); also see Boyd et al. (6). The value of \(n\)-glucose (but not \(l\)-glucose) was anomalous, plausibly because of active transport of this sugar into the fetal circulation. The electrolytes \(Na^+\) and \(Cl^-\) also showed anomalous permeabilities; polarity of charge was not discriminated in inert nonelectrolytes in the sheep placenta and the Renkin equation was not determined, but the presence of charge was, suggesting a difference in dielectric constants in the membrane and in free solution (5).

Renkin (10, 44, 46) also derived an expression for the reflection coefficient as a function of the ratio of molecular and pore radii. The reflection coefficients calculated from this expression and an assumed pore diameter of \(4.55 \times 10^{-8}\) cm are listed in Table 4. Since diffusional permeabilities for electrolytes do not fit a model based only on geometric and frictional considerations (Fig. 1), it is not likely that their reflection coefficients can be accurately predicted from a similar model.

**APPENDIX III**

**Solute Whose Transfer is not Appreciably Affected by Filtration of Water**

Solute whose net transfer is from mother to fetus and which are present in fetal plasma in a higher concentration than in maternal plasma are presumably transferred by an energy-dependent molecular mechanism. Table 5 shows that the fetal plasma concentration exceeds the maternal plasma concentration for fructose, amino acids (20), and lactate (45) that are mostly actively transported into the fetal circulation, as well as calcium (17), and phosphate (17); the references given for each of these substances in the above line give evidence that net transfer is nevertheless in a direction from mother to fetus. Glucose is a special case since the maternal concentration of glucose in the sheep is greater than the fetal concentration. However, the permeability of \(n\)-glucose is more than 50 times greater than the permeability of \(l\)-glucose (Table 4 in APPENDIX II), a difference that is presumably due to the presence of a carrier mechanism or an active mechanism. In any case, \(n\)-glucose is so permeable that its rate of transfer is independent of water filtration. Lactate also is present in the maternal plasma in higher concentration than in fetal plasma. Recent evidence (7), however, shows that the lactate used by the fetus is produced in the placenta from glucose. The rate of fetal uptake of this material is therefore not dependent on the maternal plasma lactate concentration.

Bicarbonate and urea are products of fetal metabolism of materials that are mostly actively transported into the fetal circulation, glucose (60), amino acids (20), and lactate (45). Bicarbonate diffuses across to the maternal plasma in the form of molecular \(CO_2\) (31), a substance so permeable in the placental barrier that its rate of transfer is independent of water filtration. The permeability of urea also is so high (Table 4 in APPENDIX II) that its rate of transfer by filtration (second term on the right-hand side of equation 4a) can be calculated to be less than 1% of its rate of transfer by diffusion (first term on the right-hand side of equation 4a) from the data in Tables 2-4. Transfer of this solute also can be considered essentially independent of water filtration in the placenta of the sheep.

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Address requests for reprints to J. J. Faber.

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