AN EXPERIMENTAL DETERMINATION OF THE SHEEP PLACENTA'S HYDRAULIC CONDUCTIVITY

by

Thomas S. Armentrout

A THESIS

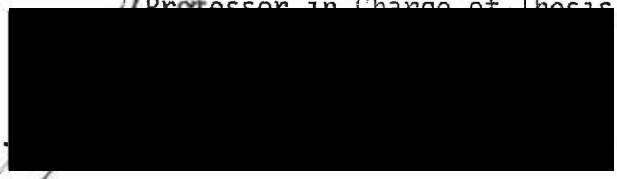
Presented to the Department of Physiology
and the Graduate Division of the University of Oregon Medical School
in partial fulfillment of
the requirements for the degree of

Master of Science
June 1978

APPROVED:

.....

(Professor in Charge of Thesis)



.....

(Chairman, Graduate Council)

ACKNOWLEDGEMENTS

The pronoun "we" has been used extensively in this thesis. It refers to Dr. J. Job Faber who invited me to participate in his on-going research concerning placental physiology. His teaching, advice and patience are gratefully acknowledged; to Dr. Kent Thornburg who participated in the research and was partly responsible for its inception; to Fred Arfmann, Tom Green and Carla Dow for their help but even more for their sense of humor which made the project bearable; and to Lydia Welden for her prompt secretarial assistance.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Statement of the problem: The experiments which are described here concern the factors which govern net fetal water aquisition during intra-uterine growth. Our experimental model was the pregnant sheep in its third trimester of gestation. In particular, we wanted to quantitate the conductance (reciprocal of resistance) afforded by the sheep placenta to transplacental water flow; that is, we wanted to quantitate the hydraulic conductivity of the sheep placenta. To analyze our data we have employed a mathematical model. Kedem and Katchalsky (1958) derived from thermodynamic laws an equation (equation 1, below) which describes steady-state water movement across homogenous, single-layered membranes.[1] One of the factors in this equation is the hydraulic conductivity. We derived a special form of Kedem and Katchalsky's equation which is valid under our experimental conditions and which can be solved for the hydraulic conductivity in terms of two experimentally determinable values. One of these values has been reported in the literature; the other is reported here.

Background: In 1947 Sir Joseph Barcroft reviewed the literature concerning the questions which are re-addressed in this thesis:

> ....Since the whole milieu of the placenta may be likened to layers of sponge saturated with water, there would seem to be little resistance to the passage of that fluid; yet for the moment there is a little mystery about the passage of water. The mystery is this: there is no explanation, in support of which experimental facts can be produced, of why the water on the whole passes from the mother to the foetus rather than from the foetus to the mother. The natural sequence would be that the growing cell requires water, that it draws water from the foetal blood, and that the foetal blood in turn

[1] See Conrad and Faber (1977) for a discussion of the applicability of this model to data from the sheep placenta.

> draws water from the maternal blood. But when we inquire more closely what we mean by the foetal blood drawing on the maternal blood we are at a loss. Two mechanisms suggest themselves...[2].

These are a hydrostatic pressure gradient, Δp, and/or an osmotic pressure gradient, $\Delta \Pi$, oriented across the placenta in such a way that transplacental water flow, $\dot{q}$, occurs from mother to fetus. Under conditions where it applies, Kedem and Katchalsky's equation relates these variables quantitatively: transmembrane water flow, $\dot{q}$, is directly proportional to the resultant of the transmembrane hydrostatic and osmotic pressure gradients:

$$\dot{q} = L_p S \ (\Delta p - \Delta \Pi). \qquad (1)$$

The proportionality constant ($L_p S$) is divided into two parts: the total surface area of the membrane, S[3], and the hydraulic conductivity per unit membrane area, L_p.

The lack of experimental facts to which Barcroft referred was this: there had not been by 1947 a direct measurement of the hydrostatic pressure gradient existing across that portion of the placenta where water transport takes place. Concerning the osmotic pressure gradient, Barcroft reported that the colloid osmotic pressure of maternal plasma was higher than that of fetal plasma; the effect of colloids, then, would be to draw water from the fetus to the mother.

[2] My underlining.

[3] When we apply equation 1 to water movement across the sheep's placenta, S is expressed in cm^2 per kg, the surface area of the placenta per kg fetal wt. This is done because, in the sheep, the functional area of the placenta appears to be proportional to fetal weight during the last trimester of gestation (Conrad and Faber, 1977). When S is expressed in the above units, $\dot{q}$ will have the dimensions ml / min. kg.

Since Barcroft's review, there still has not been a direct measurement of the transplacental hydrostatic pressure gradient. There have, however, been several published studies concerning the transplacental osmotic gradient.

Barcroft (1947) discussed the colloid osmotic pressure gradient. He was aware, of course, that colloids exert only a portion of the total osmotic pressure existing across the placenta; crystaloids exert the remaining, and major, portion. In 1957 Meschia, Battaglia and Barron reported their calculation of the total transplacental osmotic pressure gradient. They used van't Hoff's law which relates, for perfectly semi-permeable membranes, the transmembrane osmolality gradient, ΔC, to the resulting transmembrane osmotic pressure gradient:

$$\Delta \Pi = RT \ \Delta C, \qquad (2)$$

where R is the universal gas constant and T is the absolute temperature in degrees Kelvin. After measuring the total osmolalities of fetal and maternal plasma by freezing point depression osmometry, Meschia et al. calculated an average transplacental osmolality gradient. They reported that maternal plasma was hyperosmolar to fetal plasma. (Data reported in this thesis confirm their findings.) These results indicated that the total transplacental osmotic pressure gradient would tend, as did the colloid osmotic pressure gradient, to draw water from the fetus to the mother. Meschia et al. concluded that, to avoid postulating active transport of water across the placenta, one must postulate a transplacental hydrostatic pressure gradient with such

magnitude and direction that it would overwhelm the calculated trans-placenta osmotic pressure gradient.

The sheep placenta is not, however, a perfectly semi-permeable membrane; it exhibits measurable permeability to several plasma solutes (Armentrout, Katz, Thornburg and Faber, 1977). Any permeant solute, s, will exert only a fraction, σ_s, (the reflection coefficient), of the osmotic pressure predicted by van't Hoff's law:

$$\Delta \Pi_s = \sigma_s RT \Delta C_s. \qquad (3)$$

Since the osmotic pressures due to each of the n plasma solutes are additive, equation 1 can be rewritten:

$$\dot{q} = L_p S (\Delta_p - RT \Sigma \sigma_n \Delta C_n). \qquad (4)$$

Now, although the total osmolality of maternal plasma is greater than that of fetal plasma, several solutes have higher osmolalities in fetal plasma (see Table 2 of this thesis). Conrad and Faber (1977) point out that if these solutes have relatively high reflection coefficients, the total osmotic pressure gradient could be oriented in the direction opposite to that of the total osmolality gradient.

A further possible way in which osmotic pressure may contribute to fetal water acquisition - in spite of the measured hyperosmolality of maternal plasma - can be described as follows. Barcroft (1947) pointed out that an osmotic gradient tending to drive water to the fetus could arise in two ways: due to the accumulation in fetal plasma of by-products of fetal metabolism; or as the result of active transport

of solutes from mother to fetus. To incorporate these ideas into equation 4 we partitioned the transplacental osmolality gradient:

$$\dot{q} = L_p S \left[\Delta P - RT \left(\Delta C_{colloids}^{4.} + \Sigma\sigma_a \Delta C_a + \Sigma\sigma_b \Delta C_b + \Sigma\sigma_i \Delta C_i\right)\right] \quad (5)$$

where the subscript a refers to actively transported solutes (i.e., fructose, amino acids, Ca^{++}), b refers to by-products of fetal metabolism (i.e., bicarbonate, urea) and i refers to all remaining solutes (i.e., Na^+, Cl^-, K^+, Mg^{++}), termed "inert solutes" by Conrad and Faber (1977). Now, for any type of solute s, an average transplacental osmolality gradient can be calculated as:

$$\Delta C_s = 1/2 \left(C_s^{Ma} + C_s^{Mv}\right) - 1/2 \left(C_s^{Fa} + C_s^{Fv}\right). \quad (6)$$

In the case of actively transported solutes, however, this equation may yield a ΔC_a which leads to an important underestimate of the average osmotic pressure exerted by this class of solute: The concentration in fetal plasma of actively transported solutes can be expected to rise as fetal blood is exposed to that region of the placenta where active transport occurs. The resulting increase in fetal plasma osmolality would subsequently draw water 'fetusward', and this water would dilute the actively transported solutes in fetal plasma. Therefore, it is possible that the concentration in fetal plasma of actively transported solutes is higher in the fetal placental capillaries than in either fetal arterial plasma or umbilical venous plasma. Thus the average transplacental osmolality gradient due to actively transported solutes, ΔC_a, may be underestimated when calculated using

[4] The reflection coefficients for colloids, $\sigma_{colloids}$, are all very close to unity.

equation 6. Depending upon the magnitude of this effect, it is possible that the average osmolality of fetal plasma in that region of the placenta where water transport takes place is higher than the average osmolality of maternal plasma in the same region; this in spite of the fact that maternal plasma is of a slightly higher osmolality than fetal plasma in extraplacental regions.

To return to equation 5: The average rate of transplacental water flow, $\dot{q}$, has been calculated by Conrad and Faber (1977) for fetal sheep during their third trimester of gestation[5]. Conrad and Faber (1977) have also estimated for the sheep placenta the σ_a's, σ_b's and an average σ_i. $RT\,\Delta C_{colloids}$ has been reported for sheep by Meschia (1955). Measurements of ΔC_b and ΔC_i are presented in the results section of this thesis. Three terms remain unknown: L_pS, Δp and ΔC_a. It is the purpose of this thesis to determine from experimental data L_ps. Once this is achieved, the total pressure acting to drive water across the sheep placenta (the resultant of the bracketed terms in equation 5) can be calculated. That portion of the resultant pressure due to $\Delta p - RT\Sigma\sigma_a\Delta C_a$ can also be calculated, but how this quantity should be partitioned between the two terms Δp and $RT\Sigma\sigma_a\Delta C_a$ will be left as a further problem.

Theory: Our experimental determination of L_pS was based on the following reasoning: Equation 1 (rewritten here as equation 7),

$$\dot{q} = L_pS\,(\,\Delta p - \Delta\Pi\,), \qquad (7)$$

[5] $\dot{q}$ was calculated as the average rate of weight gain for the fetus times the average percent water of fetal tissue, plus the average rate of increase in amniotic and allantoic fluid volumes minus the amount of water produced within the fetus metabolically.

provides the formula for a straight line, the slope of which is

$$\frac{d\dot{q}}{d(\Delta p - \Delta \Pi)} = L_p S. \qquad (8)$$

In applying this equation to water movement across the sheep placenta we have so far been concerned with a particular solution of the equation: that point on the line whose co-ordinates are x, the pressure resultant $(\Delta p - \Delta \Pi)'$ which must exist across the placenta, as a time weighted average, in order to account for y, the known rate of fetal water acquisition during the third trimester of gestation, $\dot{q}'$. Other points on the line described by equation 7 could be generated by experimentally inducing various pressure resultants across the placenta while measuring the corresponding transplacental water flows. If equation 7 provides a good description of water flow across the sheep placenta, a straight line fit to such experimentally determined points would have a slope equal to $L_p S$.

In order to induce various pressure resultants across the placenta we injected hypertonic solutions of solute x (usually mannitol or sucrose in physiologic saline) into either the maternal or fetal veins. The effect of such injections on transplacental water flow is indicated by rewriting equation 5 with an additional term:

$$\dot{q} = L_p S\left[\Delta p - RT\left(\Delta C_{colloids} + \Sigma\sigma_a \Delta C_a + \Sigma\sigma_b \Delta C_b + \Sigma\sigma_i \Delta C_i + \sigma_x \Delta C_x\right)\right]. \qquad (9)$$

Although the primary effect of an injection of hypertonic solution will be to change the transplacental concentration of solute x, ΔC_x, some change will also be caused in the transplacental concentrations of the colloids and of solutes a, b and i; the transplacental pressure gradient

might also be affected. But if these secondary effects are small, most of the change occurring in transplacental water flow after an injection of hypertonic solution will be due to the change in ΔC_x. This approximation can be incorporated into the mathematical model provided by equation 9 by assuming that the partial derivatives with respect to ΔC_x of Δp, $\Delta \Pi_{\text{collids}}$, ΔC_a, ΔC_b and ΔC_i are zero. Then

$$- \frac{1}{RT\sigma_x} \left(\frac{\partial \dot{q}}{\partial \Delta C_x}\right) = L_p S. \qquad (10)$$

(Conrad and Faber (1977) have calculated reflection coefficients at the sheep placenta for mannitol and sucrose. They found that σ_x is very close to one.)

In order to construct a plot of $(\Delta p - \Delta \Pi)$ versus $\dot{q}$ we would have to know the $(\Delta p - \Delta \Pi)$'s which exist after hypertonic infusions. But since neither Δp nor ΔC_a can be measured, transplacental pressure resultants cannot be calculated from measurements of their component parts; nor is there any way to measure the pressure resultant directly. However, changes in the resultant can be estimated; according to the approximation made in the preceding paragraph

$$d (\Delta p - \Delta \Pi) \cong RT \, \partial \, \Delta C_x \qquad (11)$$

Fortunately, a plot of $\Delta(\Delta p - \Delta \Pi)$ versus $\dot{q}$ has the same slope as a plot of $(\Delta p - \Delta \Pi)$ versus $\dot{q}$ (see Appendix). Therefore, a plot of $RT \, \partial \, \Delta C_x$ versus $\dot{q}$ should have a slope of $L_p S$.

We measured changes in the transplacental osmolality gradient of solute x, $\partial \, \Delta C_x$, indirectly. Again according to the approximation

described in a preceding paragraph, most of the change in the total transplacental osmolality gradient will be due to a change in the transplacental osmolality gradient of solute x:

$$d\Delta C \cong \partial\Delta C_x \qquad (12)$$

Using equation 6 we calculated average transplacental osmolality gradients, ΔC, from direct measurements of maternal and fetal plasma osmolality. The changes which occurred in this gradient after hypertonic infusions, $\partial\Delta C$, provided the x co-ordinates for the points in Figure 2 (See Results Section, this thesis). Actually the x co-ordinates are plotted as the transplacental osmolality gradient, ΔC. But since ΔC under control conditions, $\Delta C'$, was much smaller than ΔC after hypertonic infusions $\partial\Delta C$ and ΔC provide approximately equivalent x - co-ordinates:

$$\partial\Delta C = \Delta C - \Delta C' \cong \Delta C \qquad (13)$$

The y co-ordinates for the points in Figure 2 are calculated transplacental water flows, $\dot{q}_c$. Water flows were calculated using Fick's principle. After hypertonic solution has been injected into the fetal circulation, water will be drawn transplacentally from the maternal blood at some rate $\dot{q}$. Therefore

$$\dot{q} = \dot{Q}^{Ma} - \dot{Q}^{Mv}, \qquad (14)$$

where $\dot{Q}^{Ma}$ is arterial blood flow to the maternal side of the placenta and $\dot{Q}^{Mv}$ is venous drainage from the maternal side of the placenta[6]. If the fractions A and V are known, where

$$A = \frac{\text{liters blood-water}}{\text{liter arterial blood}} \qquad (15)$$

[6] Fetal and maternal placental blood flows appear constant during the last trimester of gestation when expressed per kilogram fetal weight (Conrad and Faber, 1977). Therefore we have expressed each of the flows in equation 14 with dimensions ml / min • kg.

and $$V = \frac{\text{liters blood-water}}{\text{liter venous blood}} \qquad (16)$$

then equation 14 can be rewritten in terms of arterial and venous blood-water flows:

$$\dot{q} = A\dot{Q}^{Ma} - V\dot{Q}^{Mv} \qquad (17)$$

Now, if there is some solute m ("m" for marker) in maternal blood to which the placenta is impermeable, then the flux of that solute (moles per minute) in venous blood-water leaving the maternal side of the placenta will equal its flux in arterial blood-water entering the maternal side of the placenta:

$$C_m^{Mv}\ V\dot{Q}^{Mv} = C_m^{Ma}\ A\dot{Q}^{Ma} \qquad (18)$$

where C_m is the concentration of solute m in moles per liter of blood-water. Solving equation 17 for $V\dot{Q}^{Mv}$ and substituting this expression in equation 18 yields

$$(\dot{q} - A\dot{Q}^{Ma})\ C_m^{Mv} = A\dot{Q}^{Ma}\ C_m^{Ma} \qquad (19)$$

which can be solved for $\dot{q}$:

$$\dot{q}_c = A\dot{Q}^{Ma}\left(\frac{C_m^{Ma} - C_m^{Mv}}{C_m^{Mv}}\right)^{7,8}. \qquad (20)$$

The subscript c is introduced in writing equation 20 to indicate that the transplacental water flow calculated using this equation, $\dot{q}_c$, will

[7] An analogous equation can be derived for the calculation of $\dot{q}_c$ when the maternal plasma has been made hypertonic:

$$\dot{q}_c = A\dot{Q}^{Fa}\ (C_m^{Fa} - C_m^{Fv})\ /\ C_m^{Fv}\ .$$

[8] Although the value of A will fluctuate somewhat due to our experimental infusions, we have used a value of 0.82 (liters blood-water per liter arterial blood) in all calculations. Also, for convenience, we have used marker solute concentrations in units of moles per liter plasma instead of in units of moles per liter blood-water.

equal the actual transplacental water flow, $\dot{q}$, only when the placenta is completely impermeable to the marker solute m. We have used Na^+, K^+ and Cl^- as marker solutes; the sheep placenta is measurably permeable to all three of these ions. Fortunately, Conrad and Faber (1977) have shown that, under the conditions of our experiments,

$$\dot{q} \cong \dot{q}_c / \sigma_m \qquad (21)$$

where σ_m is the reflection coefficient at the sheep placenta for the marker solute used to calculate $\dot{q}_c$. Furthermore, Conrad and Faber (1977) have estimated the reflection coefficients at the sheep placenta for Na^+, K^+ and Cl^-; they argue that the three coefficients are not very different from each other and are in the range from 0.5 to 0.8. Therefore, for the purpose of calculating L_pS, we have used the approximate relation

$$\dot{q} \cong \dot{q}_c / 0.65 \qquad (22)$$

Summary: Our intent has been to experimentally determine the hydraulic conductivity-surface area product, L_pS, of the sheep placenta:

$$\frac{d\dot{q}}{d(\Delta p - \Delta \Pi)} = L_pS \ . \qquad (23)$$

Under the conditions of our experiments we suggest that

$$d\ (\Delta p - \Delta \Pi) \cong - \partial (RT\ \sigma_x \Delta C_x) \qquad (24)$$

and therefore that

$$- \frac{1}{RT\sigma_x} \left(\frac{\partial \dot{q}}{\partial \Delta C_x} \right) \cong L_pS \ . \qquad (25)$$

Now, $\sigma_x = 1$; and under the conditions of our experiments $\partial \Delta C_x \cong d\ \Delta C$. Since $\dot{q} = \dot{q}_c / 0.65$,

$$- \frac{1}{RT(0.65)} \left(\frac{d\dot{q}_c}{d\ \Delta C} \right) \cong L_pS \ . \qquad (26)$$

Therefore, to determine L_pS we measured the rate of change of transplacental water flow as the transplacental osmolality gradient was varied experimentally.

METHODS

Plasma osmolality was measured by vapor-pressure osmometry (VPO) in the first four animals. A Hewlett Packard model 302B vapor-pressure osmometer was calibrated at 39°C (the normal body temperature of sheep) with NaCl solutions of 280, 300, and 360 mOsmol/liter. Osmolalities of plasma samples were reproducible to a standard deviation of ± 1 mOsmol/liter. However, because VPO exposes droplets of sample to air, an undetermined amount of CO_2, and hence osmotically active bicarbonate, is lost. For this reason VPO underestimates plasma osmolalities.

In order to correct for bicarbonate loss, plasma osmolalities in the remaining eight animals were determined by freezing-point depression osmometry (FPD). In this technique a large plasma sample is used (2 ml as opposed to a drop for VPO), so both pH and PCO_2 can be determined from the same sample before and after osmometry. From the changes occuring in pH and PCO_2 we calculated with the Henderson-Hasselbalch equation the minimum number of mOsmol/liter bicarbonate lost during osmometry. The osmolality determined by FPD was corrected for this loss. The average bicarbonate loss was about 1 mOsmol/liter. An Advanced FPD osmometer was calibrated with the same standard NaCl solutions used for VPO calibration. Determinations were reproducible to a standard deviation of ± 0.5 mOsmol/liter. Plasma pH and PCO_2 (also PO_2) were measured with a Radiometer Model 27 blood gas analyzer at 39°C. A published pK for human plasma at 39°C (Severinghaus, 1971) was used in the Henderson-Hasselbalch equation.

Fetal placental blood flow was not measured directly; rather, an electromagnetic flowmeter measured flow through the fetal distal aorta

and this flow was multiplied by the fraction of distal aortic flow going to the placenta.[9] The fraction was determined by injecting microspheres labeled with a gamma emitting isotope into the fetal venous circulation. Since about 85% of fetal venous return bypasses the lungs, most of the microspheres find their way into the arterial system. Those passing through the flow sensor will be distributed to dependent tissues in proportion to tissue blood flow. After we finished collecting data and killed the sheep and fetus, we recovered the fetus and placenta. The fetus was divided at the level of the flow sensor in a plane perpendicular to its spine; the posterior portion so obtained approximated all extra-placental tissues supplied by flow through the sensor. This posterior portion was incinerated in an electric oven and aliquots of the ash were counted in a gamma scintillation spectrometer. The fetal placenta was also incinerated and counted. The ratio of placental counts per minute (cpm) to placental plus posterior portion cpm yielded the placental fraction of flow passing through the distal aortic flow sensor.

We used an Omnicraft Mediflow Electromagnetic Flowmeter. The internal diameters of the flow sensor heads ranged from 5 to 8 mm. Flowmeter output was recorded on a Leeds and Northrup Speedomax recorder. To calibrate the flow sensors we placed them on excised sheep arteries and monitored their outputs as sheep blood was pumped through at known flow rates. Calibration was repeated until calibration factors were known with a standard error of less than 2% of their mean values. When flow through the excised arteries was stopped flowmeter output was always less than 2% of full-scale deflection. Later,

[9] All fetal placental flow arrives by way of the distal aorta.

when experiments were completed and the fetus was killed, flowmeter outputs coincided with meter zeros within 2% of full-scale deflection.

Microspheres 15 μm in diameter labeled with either Strontium-85 or Cerium-146 were obtained from the 3M Co. They were suspended in physiologic saline for injection and each injection contained about 250,000 microspheres. Gamma activity was determined in a Packard Tri-Carb gamma scintillation spectrometer.

To calculate maternal placental blood flow, we began by injecting tritiated water into the fetal circulation. In quasi-steady state we may neglect the rate of tritiated water up-take by placental tissue; then the rate at which tritiated water leaves the fetal placental circulation equals the rate at which it appears in the maternal placental circulation:

$$\dot{Q}^F (C^{Fa}-C^{Fv}) = \dot{Q}^M (C^{Mv}-C^{Ma}) \qquad (27)$$

where $\dot{Q}$ is blood flow through the fetal (F) or maternal (M) placenta, and C is tritiated water concentration in the fetal femoral artery (Fa), umbilical vein (Fv), uterine vein (Mv) or maternal carotid artery (Ma). Plasma samples drawn from these vessels were precipitated with 5%, final concentration, trichloroacetic acid and their tritiated water concentrations determined in a Packard Tri-Carb liquid scintillation spectrometer. With these tritiated water concentrations and the fetal placental blood flow (derived as described in the preceeding paragraph), equation 9 was solved for the maternal placental blood flow.

The electrolytes sodium, potassium and chloride served as marker solutes for the calculation of transplacental water flow (equation 20). Their concentrations in plasma samples were determined by

a Technicol SMAC AutoAnalyzer System. This system was also used to quantitate other plasma constituents which contribute to plasma osmolality (magnesium, calcium, inorganic phosphate, blood urea nitrogen and glucose) or affect plasma osmolality (plasma protein, by virtue of its excluded volume).

Blood pressures were measured with Statham pressure transducers (Models Gb and Db) and recorded on a Beckman Offner RB polygraph. The pressure transducers were calibrated against a water manometer before each experiment.

Preparation of animals: Pregnant ewes of a variety of western breeds were surgically prepared (see Fig. 1) at least three days before beginning data collection. A ewe was selected that was in her third trimester of gestation judging from roentgenograms showing fetal skeletal development. She was shorn and deprived of food the night before surgery. Anesthesia was induced with 4% halothane in a gas mixture of one-third oxygen and two-thirds nitrous oxide administered by face mask. After the ewe lost consciousness, she was intubated and tied on a surgical table in the supine position. The concentration of halothane was reduced to 1-2% for the remainder of the operation. The entire abdomen was shaven, scrubbed with iodine containing soap and rinsed with alcohol. The ewe was wheeled into the surgery and draped. Sterility during surgery was carefully maintained as the fetus of the sheep is agammaglobulinemic until it suckles colostrum after birth. Its resistance to infection in utero is therefore very much inferior to that of rabbit or guinea pig fetuses who obtain antibodies in utero.

Surgery began with a midline abdominal incision extending from the umbilicus to the udder. Splitting the peritoneum exposed the uterus which was palpated to ascertain fetal orientation within. The uterine region overlying the fetal pelvis was ringed with a purse-string suture of no. 3 silk and incised; when possible the purse string was sewn into a region of myometrium under which there were no placental cotyledons. The exposed fetal membranes were broken and the fetal hindquarters and pelvic region pulled out through the purse string. Broken edges of fetal membranes were also pulled out through the purse string, tightening of which then prevented further spilling of amniotic fluid.

A skin incision on the fetal left flank was extended by blunt dissection through the retroperitoneal space to the dorsal aorta just proximal to its iliac bifurcation. This region of aorta was then mobilized; occasionally a small dorsal branch had to be tied and cut. The head of an electromagnetic flow sensor was placed around the aorta and secured in place. The cable of this flow sensor was anchored with sutures to paraventebral musculature and to the fetal skin in a manner designed to prevent later torsion of the sensor head. The subcutaneous tissue and skin were closed with 00 silk.

Next, a fetal hindlimb was grasped and extended to expose the groin. A skin incision there allowed mobilization of the femoral vein and artery which were cannulated with polyvinyl catheters. The arterial catheter was not advanced so far as to interfere with the electromagnetic flow sensor. These catheters, and those whose placement is described below, were filled with heparinized saline (200U/ml) and their free ends closed by knotting. The groin incision was closed with 00

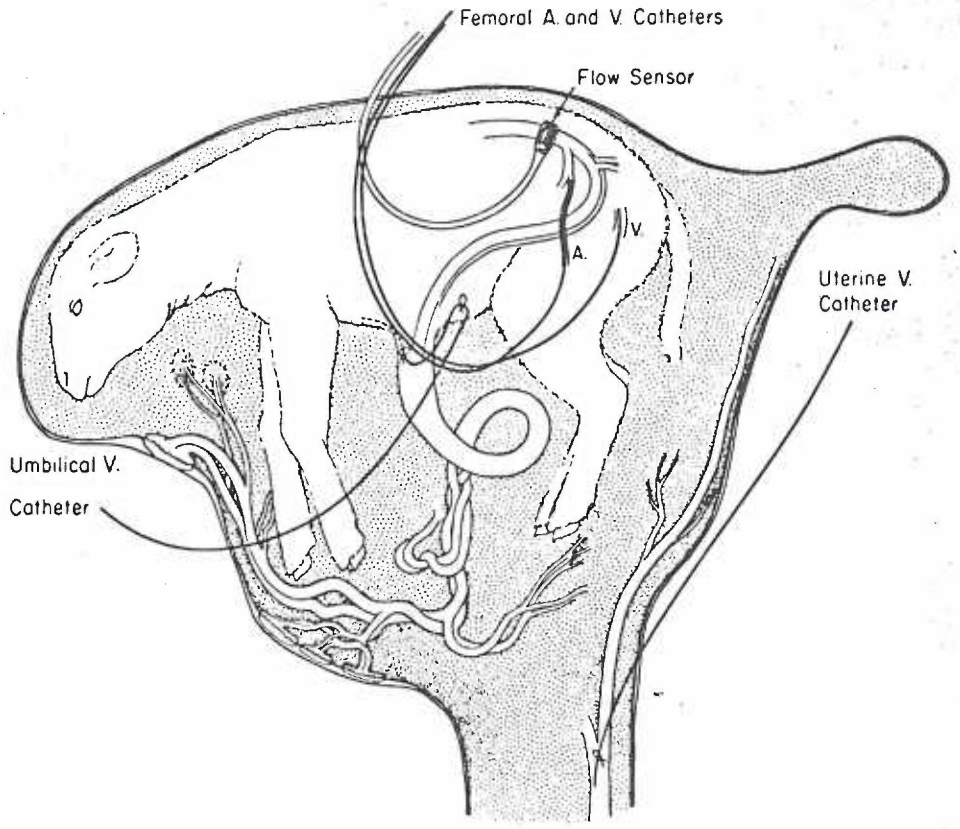


Fig. 1. Fetal lamb surgically prepared for transplacental water flux experiments. In addition to catheters shown in this figure, a catheter was placed opening into the amniotic cavity and a maternal carotid artery and jugular vein were cannulated.

silk. The final stitch was used to anchor the end of a silastic catheter; this catheter would open into the amniotic cavity once the caudal portion of the fetus was returned there.

Next the purse-string about the fetal membranes and trunk was loosened so that the fetal umbilical region could be exposed. As the umbilical cord now passed through the purse-string, care had to be taken to avoid impeding placental venous return. A 14 gauge hypodermic needle was stuck into one of the umbilical veins 4 or 5 centimeters from where the vein entered the fetus. A catheter was fed through the needle lumen into the vein and advanced until its tip was estimated to be in the common umbilical vein. The needle was then pulled out of the vessel and off the catheter. Cord and vein wall tissue immediately surrounding the perforation was bunched around the catheter so that a noose of 00 silk could be used to seal and secure the catheter. A second anchor for this catheter was made with suture in the fetal abdominal skin. Occasionally the needle puncture in the umbilical vein would cause the vein to go into spasm preventing insertion of the catheter. In that case, it was necessary to expose the common umbilical vein by incising abdominal skin just cephalad to the umbilicus. The common umbilical vein proved much less reactive to needle puncture than the umbilical veins in the cord, and catheter placements through needle punctures were always successful here. The fetal abdomen was closed with a few stitches of 00 silk.

The exteriorized portion of the fetus was returned to the amniotic cavity: We grasped the cut edges of the uterus and fetal membranes and lifted and shook, allowing the fetus to "fall back" into place. This

method proved far superior to "stuffing". The broken edges of fetal membrane were then gathered around the emerging catheters and flow sensor cable and sealed with a noose of 00 silk. Before tightening the noose, at least 20 centimeters of catheter and cable lengths were pushed into the amniotic cavity as slack for fetal movement. Pulling the free ends of the purse-string closed the uterine incision around the catheters and cable; the cut edges of uterus were folded inward as they were drawn together thereby apposing uterine serosa to uterine serosa. Finally, the purse-string was tied. At this time the only antibiotic treatment used during our experiment was given: one million units of penicillin G were injected into the amniotic cavity through the silastic catheter tied to the fetal groin.

Before closure of the ewe's abdomen, a uterine vein was catheterized using the same technique as employed for placement of the umbilical vein catheter. We chose a vein for catheterization which drained the uterine horn containing the operated fetus. The catheter was anchored to the vessel wall and to myometrium.

The knotted ends of the five implanted catheters and the flow sensor cable were lead from the ewe's abdominal cavity through a hole we punctured in her peritoneum where it lay exposed adjacent to the peritoneal incision. The peritoneal incision was closed with matress sutures of no. 3 silk. The catheters and cable were then threaded through a subcutaneous tunnel leading to the ewe's side where they emerged through a small skin incision; the tunnel began in subcutaneous tissue exposed along the abdominal incision and was extended by pushing a steel rod into the subcutaneous tissue, which separated

easily. The emerging catheters and cable were coiled and stored in a pouch pinned to the ewe's flank. The abdominal incision was closed with wound clips.

Finally, catheters filled with heparinized saline were placed in a maternal jugular vein and carotid artery. Their free ends were knotted and they were coiled and taped to the ewe's neck for storage.

Anesthesia was discontinued. The ewe regained consciousness after about 5 minutes at which time the endotracheal tube was withdrawn. She was then moved back to the sheep pen, propped in a kneeling position and encouraged to stand as soon as she was able. Catheters were flushed with heparinized saline at least once a day. Ewes were always kept in the company of other sheep.

Experimental Protocol:

After a minimum of three days (mean 3.8 days) recovery from surgery the ewe was restrained in a standing position against the sheep pen wall. Food and water were placed within her reach. The catheters and flow sensor cable, stored since surgery in the purch on the ewe's flank, were lead through a hole in the wall separating the sheep pen and laboratory. The flow sensor cable was connected to its flow meter and control values for distal aortic flow were obtained; the frequency of flow pulsations was recorded as fetal heart rate in beats per minute. Next, the knotted ends of the seven surgically placed catheters were cleaned and disinfected with tincture of iodine, hemostats were placed on the sheep side of the knots and the knots snipped off. Care was taken to maintain sterility of the open catheter ends, particularly those leading to the fetus. A few milliliters of sterile heparinized saline (30 U/ml) was used to flush all catheters. Control values for fetal and maternal blood pressures were obtained by connecting five of the catheters to Statham pressure transducers; the five catheters were those in the fetal femoral artery and umbilical vein, the maternal carotid artery and uterine vein and the amniotic cavity. Blood pressures were recorded as differences from amniotic fluid pressure in mmHg.

About 0.2 mCi of tritiated water was then injected into the fetal circulation through the catheter in the fetal femoral vein. About 5 minutes after this injection catheter dead spaces were cleared by withdrawing more than 3 dead space volumes of fluid from each catheter. Three ml samples were then drawn from the fetal femoral artery and

umbilical vein and the maternal carotid artery and uterine vein. Four people were required to draw these samples simultaneously--to the nearest one or two seconds. Samples were drawn into syringes the dead spaces of which had been filled with heparin solution (1000 U/ml). Care was taken to keep blood samples iso-aerobic: After drawing the sample, the sampling syringe was closed with a Luer-Lock cap containing a droplet of mercury; the droplet of mercury from the cap served to mix blood and heparin. Immediately after drawing the first set of four samples a second set of four was drawn, this set with a sample size of 10 ml.

The first set of samples was placed on ice until it could be analyzed for PO_2, PCO_2 and pH; analysis was begun immediately and completed within half an hour. The second set, still in the sampling syringes, was centrifuged at 20,000 RPM for 10 minutes. After centrifugation the Luer-Lock cap was replaced with a hypodermic needle fitted into a length of polyethylene tubing. Three aliquants of supernatant plasma were pushed from each sampling syringe through the tubing into three vials:

(1) ½ ml samples were prepared for liquid scintillation spectrometry in order to determine tritiated water concentrations.

(2) 3 ml samples of plasma were pushed into vials, capped, frozen and later sent to the Department of Clinical Pathology for chemical analyses.

(3) 2 ml samples were pushed into osmometer vials. To reduce loss of plasma CO_2 during filling of these vials the stream of plasma leaving the polyethylene tubing was directed along the

vial wall to minimize turbulence and exposure to air. Immediately after filling, the vials were transferred to the freezing point depression osmometer for the determination of plasma osmolalities. Next, the partially frozen plasma samples in the osmometer were allowed to thaw and then immediately were drawn into syringes with mercury filled dead-spaces; these syringes were capped until plasma pCO_2 and pH could be re-analyzed. Thus the loss of bicarbonate during osmometry could be calculated.

As soon as the blood samples had been drawn from the ewe and fetus microspheres were injected through the fetal femoral vein catheter in order to determine the fraction of fetal distal aortic flow going to the placenta.

The above procedures provided control data. When these procedures were completed, food and water were moved out of the ewe's reach and then either the maternal of fetal plasma was made hypertonic by infusion of a hypertonic solution. Hypertonic solutions were 1 mol of mannitol per liter physiologic saline, 1 mol of sucrose per liter physiologic saline, or concentrated ($\sim$2M) Ringer's solution. These solutions were sterilized by boiling or autoclaving.

Maternal infusions were made through the ewe's jugular vein catheter. A Sigmamotor T4 finger pump moved two liters of hypertonic solution into the ewe in about 10 minutes. A consistent finding during maternal infusions was a fall in fetal distal aortic flow; if this flow dropped to 60% of its control value infusion was immediately

terminated in order to save the fetus. In all cases but one, infusions did not appear to distress the ewes; i.e. we noticed no change in the ewes' behavior during or after infusion, although there was a brisk diuresis. The exception occurred the first and only time concentrated Ringer's solution was infused: Towards the end of the infusion into the maternal jugular vein the ewe began to tremble violently, exhibited nystagmus and fell against her restraints. The infusion was immediately terminated, the ewe recovered within a few minutes with no apparent residual effects.

Fetal infusions were made through the catheter in the fetal femoral vein. Depending on the size of the fetus estimated at the time of surgery, between 200 and 300 ml of solution were infused. An IV bottle connected to the femoral vein catheter was suspended about two meters above the fetal level; gravity was sufficient to drive fetal infusions.

Immediately following infusions, the data gathering procedures performed under control conditions were repeated: fetal heart rate and distal aortic flow were monitored; fetal and maternal blood pressures were recorded; a second injection of tritiated water was followed by the drawing of simultaneous samples for liquid scintillation spectrometry, blood gas and pH determinations, and plasma chemistries and osmometry. Finally, radioactive microspheres were again injected into the fetal circulation; however the microspheres for this injection carried a different isotope: Strontium ^{85} or Cerium^{146} were used as labels for control or experimental determinations in random order.

These isotopes were separately counted by the channels-ratio method of gamma scintillation spectrometry.

Following this second microsphere injection the ewe was again offered food and water. Ewes that received hypertonic infusions drank, with evident enthusiasm, several buckets of water within a few minutes. Catheters were re-closed by knotting, coiled and returned along with the flow sensor cable to the pouch on the ewe's side. If the ewe and fetus remained in good health experiments were repeated, but no sooner than two days hence. If the fetus died, if the catheters became clogged or if a ewe went into labor both the fetus and mother were given lethal doses of barbituate via venous catheters. An autopsy was performed to confirm the locations of flow probe and catheters.

RESULTS

Tables 1 through 4 present control data recorded immediately before hypertonic infusions were made.

Table 1 contains data pertaining to the well-being of our chronically prepared ewes and fetuses. For comparison, values previously reported in the literature for chronically prepared sheep are also listed.

Table 2 lists the data from which we calculated a transplacental molality gradient. Concentrations for the first nine constituents listed were measured in samples drawn from our sheep; for the five remaining constituents concentrations were taken from the literature. As defined in equation 6, a positive concentration gradient indicates a higher concentration in maternal plasma than in fetal plasma. If it is assumed that all plasma constituents which occur in high enough concentrations to contribute measurably to plasma molality have been included in Table 2, then the total molality gradient acting across the placenta can be calculated as the algebraic sum of the gradients due to individual plasma constituents.

The concentration gradients in Table 2 are, however, expressed as mols per liter plasma. Since the osmotic pressure acting across the placenta is proportional to transplacental molality gradients, concentration gradients should be expressed as mols per liters H_2O. We converted liters plasma to liters H_2O by subtracting from the former that volume from which water is excluded by plasma proteins. The

amounts of protein we found in maternal and fetal blood are reported in Table 3. Assuming that the excluded volumes of ewe and fetal plasma proteins are similar to the excluded volume of adult human plasma proteins (0.8 ml H_2O/g protein) the total gradient of + 3.26 mmol/liter plasma (from Table 2) becomes about +11 mmol/liter H_2O.

Table 4 lists the results of vapor pressure osmometry and freezing point depression osmometry. Again, a positive osmolality gradient (ΔC^{M-F}) indicates a higher osmolality in maternal than in fetal plasma. Although the average transplacental gradients found by both osmometric methods were significantly different from zero (Table 4) they were not significantly different from each other ($t=1.28$, $p> 0.1$).

For each measurement of the transplacental osmolality gradient we calculated the concomitant transplacental water flow using Fick's law (equation 20). In fact, we calculated three water flows for each osmolality gradient using arterial-venous concentration differences (from Table 2) of the three marker solutes Na^+, K^+ and Cl^-. In Figure 2 each measured transplacental osmolality gradient is plotted against the three corresponding estimates of transplacental water flow. Nearly all of the points representing control conditions are clustered between ±5 mOsmols/kg H_2O. Although the transplacental osmolality gradients of about a third of the points graphed in Figure 2 are based on VPO determinations and the rest on FPD determinations, all points appeared to belong to a single population. They are therefore plotted together; this is true for the control points and for the post-infusion points now to be described.

The points on the left in Figure 2 represent transplacental osmolality gradients and water flows after the fetuses had received intravenous infusions of hypertonic mannitol (four experiments) or sucrose (two experiments). Fetal plasma osmolality rose to an average of 367.1 mOsmol/kg H_2O with a range of 324.4 to 417.0 mOsmol/kg H_2O. Maternal plasma osmolality changes were negligible. The average change in the transplacental gradient was -66.3 mOsmol/kg H_2O. Changes in fetal hemodynamics accompanying infusions are listed in Table 5.

The points on the right in Figure 2 represent transplacental osmolality gradients and water flows after the ewes had received intravenous infusions of hypertonic mannitol (11 experiments) sucrose (one experiment) or Ringer's solution (one experiment). Maternal plasma osmolality rose to an average of 368.1 $\pm$ 13.7 (SD) mOsmol/kg H_2O; fetal plasma osmolality also rose, to an average of 326.1 $\pm$ 5.9 (SD). The average change in the transplacental gradient was +41.9 mOsmol/kg H_2O. Changes in fetal hemodynamics are listed in Table 5. Changes in maternal hemodynamics (placental blood flow, arterial blood pressure and uterine vein pressure) were not significant after infusions into either fetus or ewe.

Of the 129 points graphed in Figure 2, 45 were obtained after mannitol infusions, 9 after sucrose infusions and three after Ringer's infusion. The remainder represent control conditions. In spite of using three types of hypertonic solution, the data appear to follow a single trend and so all 129 points were fitted with a single

regression equation by the method of least squares:

$$\dot{q}_c = 0.23 - 0.0635\ (osmol^M - osmol^F),\ ml \cdot kg^{-1} \cdot min^{-1} \quad (28)$$

This equation is graphed as the middle dotted line in Figure 2. As an estimator of the deviation of the data points about this regression line we calculated the standard error of the estimate: SEE = 3.94 ml/(min • kg). This established a range about the regression line of ± 3.94 ml/(min • kg) within which about 68% of the data points will fall provided that the variations of water flow values above and below the regression line are uniform over the range of osmolalities represented; and provided that this variation follows approximately a normal distribution. For the slope of the regression line we calculated 95% confidence limits of 0.046 and 0.082 ml/(min•kg•Osmol); this slope is very significantly different from a slope of zero: $p \ll 0.001$. The correlation coefficient (r) for the regression is -0.525. In Figure 2, curving dotted lines have been graphed above and below the regression line. These established the 95% confidence limits for predictions by the regression equation of mean transplacental waterflow given any particular transplacental osmotic gradient. For example, it can be seen that zero water flow falls within these limits when the osmotic gradient is zero. Said another way, transplacental water flow as measured by our methods was not significantly different from zero when the transplacental osmotic gradient was zero.

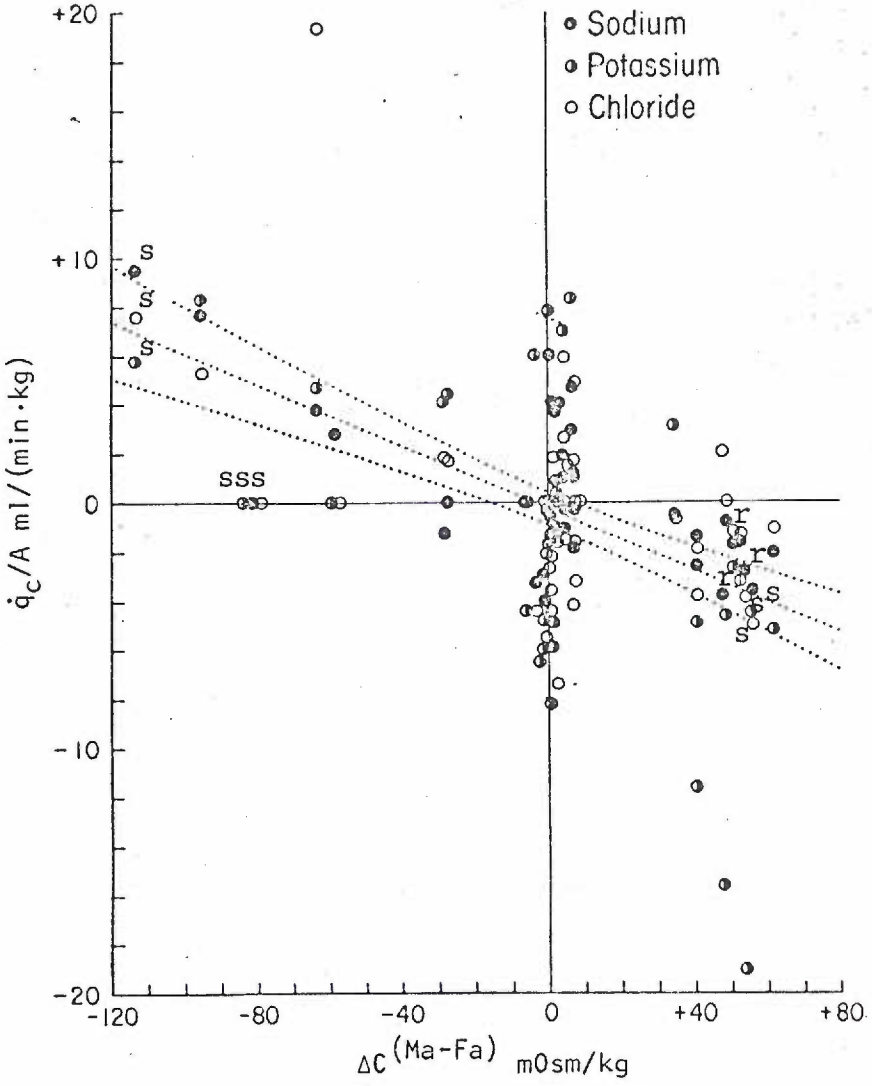


FIG 2. Transplacental water flow as a function of the transplacental osmotic gradient. The species of marker solute (Na^+, K^+ or Cl^-) used to calculate each water flow is indicated. Osmotic gradients measured after infusion of Ringer's or sucrose solution are labeled R and S; the other gradients were measured after mannitol infusions. On the scale used for the x-axis, osmolality gradients calculated using equation 6 were indistinguishable from gradients calculated simply as the maternal-fetal arterial difference (C^{Ma-Fa}); this simplification has been used to plot the above figure and to calculate its regression equation. The factor A, part of the y co-ordinate, is liters blood-water per liter arterial blood (See equation 15). Individual regressions for transplacental flow calculated using Na^+, or K^+, or Cl^- concentrations were not statistically different from each other.

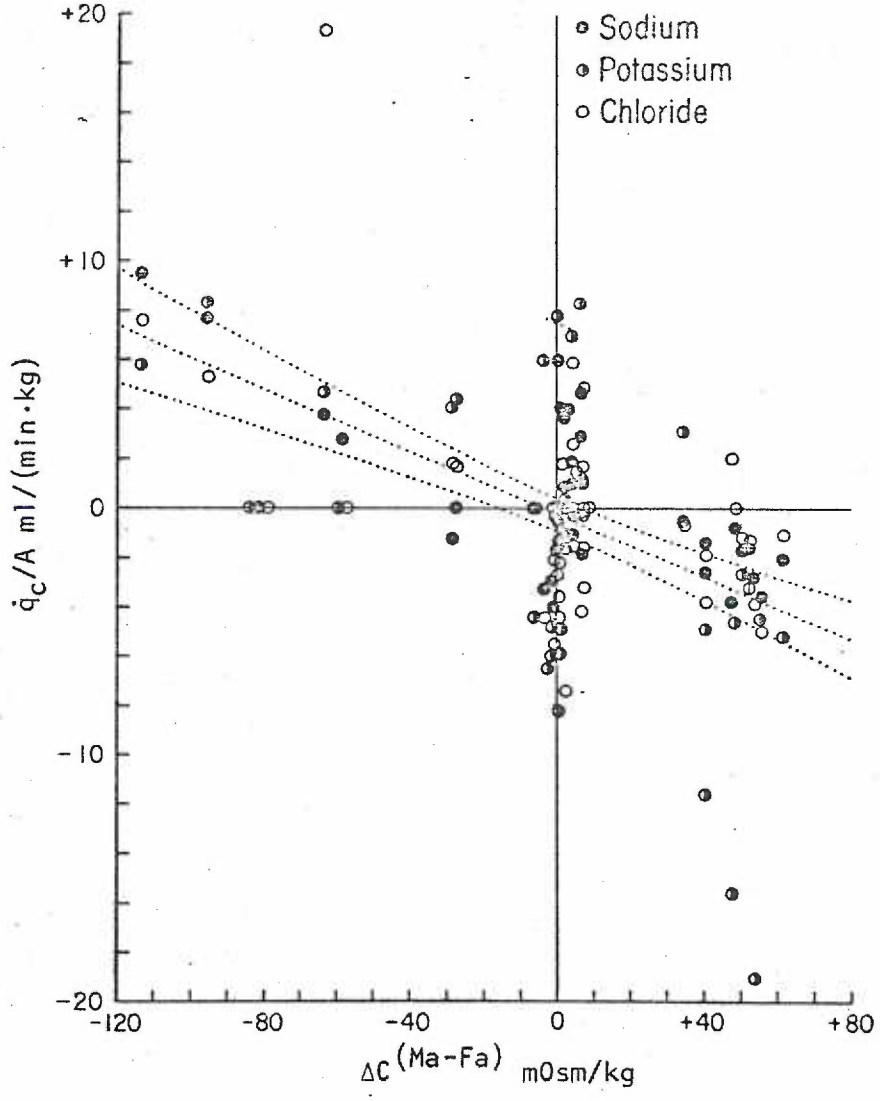


FIG 2. Transplacental water flow as a function of the transplacental osmotic gradient. The species of marker solute (Na^+, K^+ or Cl^-) used to calculate each water flow is indicated. Osmotic gradients measured after infusion of Ringer's or sucrose solution are labeled R and S; the other gradients were measured after mannitol infusions. On the scale used for the x-axis, osmolality gradients calculated using equation 6 were indistinguishable from gradients calculated simply as the maternal-fetal arterial difference (C^{Ma-Fa}); this simplification has been used to plot the above figure and to calculate its regression equation. The factor A, part of the y co-ordinate, is liters blood-water per liter arterial blood (See equation 15). Individual regressions for transplacental flow calculated using Na^+, or K^+, or Cl^- concentrations were not statistically different from each other.

DISCUSSION

Reliability of Methods: The control hemodynamic data we recorded from our fetal sheep immediately before hypertonic infusions were made are compatible with those commonly recorded from chronically prepared sheep fetuses in good condition (See Table 1).

Calculation of the hydraulic conductivity-surface area product, L_pS, for the sheep placenta: The slope of the regression equation (equation 28) fit to the data points in figure 2 was found to be

$$\frac{1}{A}\frac{d\dot{q}_c}{d\Delta c} = -0.0635 \left(\frac{ml}{min \cdot kg} \text{ per } \frac{mOsmol}{liter} \right). \qquad (29)$$

When this slope is multiplied by an average value for the factor A of 0.82 (liters blood-water per liter arterial blood) and substituted in equation 26 (rewritten here as equation 30),

$$- \frac{1}{RT\ (0.65)} \quad \frac{d\dot{q}_c}{d\Delta c} \cong L_pS \qquad (30)$$

where the product RT at 39°C equals 19.5 (mmHg-liter per mmole), the value calculated for L_pS is 4.09×10^{-3} ml per min.kg.mmHg.

Calculation of an average transplacental pressure resultant which would account for the known rate of fetal water aquisition: Equation 1 can be solved for the transplacental pressure resultant:

$$(\Delta p - \Delta \Pi) = \dot{q}/L_pS. \qquad (31)$$

Substitution into this equation of the average rate of transplacental water flow occuring during the third trimester of gestation, $\dot{q}_c$, and the value of L_pS just calculated, yields an average transplacental pressure resultant of 4.15 mmHg.

General discussion: The main finding of this study is that the placenta of the sheep offers a rather low resistance to the passage of

water; that is, it appears that a transplacental pressure resultant of less than 5 mmHg will sustain the growing fetus' water needs. Perhaps Barcroft (1947) was anticipating this result when he wrote,

> ...The idea...of the dependency of the fetus for its water supply upon the balance between the 'foetuswards' hydrostatic gradient, and the 'motherwards' osmotic gradient, is not a very pleasant one; it leaves the foetus so much at the mercy of its mother's blood pressure...

Thus it might be argued from our results that an average increase of 4.15 mmHg hydrostatic pressure in the maternal placental capillaries would double the rate of water flow to the fetus.

However, Conrad and Faber's (1977) calculations indicate that such an increase in maternal hydrostatic pressure would cause about a 3%, not a 100%, increase in transplacental water flow. The reason for their results is that, when a transplacental pressure resultant acts to force plasma through the placental barrier, plasma water encounters much less resistance than plasma solutes. The result is sieving of plasma solutes: when water is forced through the placenta at an increased rate, plasma solutes accumulate in the maternal placental capillaries, also at an increased rate. Therefore, any increase in the transplacental hydrostatic pressure gradient will be countered immediately by an increase in the transplacental osmotic pressure gradient. Conrad and Faber's calculations indicate that this effect is so powerful that fetal water volume will be only slightly effected by even fairly large transcients in maternal blood pressure.

The effectiveness of solute sieving in protecting fetal water volume from transients in the transplacental hydrostatic pressure gradient is dependent upon two parameters: (1) How effectively plasma

solutes are sieved by the placental barrier; this will vary for each species s of plasma solute in proportion to $(1-\sigma_s)$, where σ_s is the reflection coefficient at the placenta of the solute species in question (Kedem and Katchalsky, 1958) and; (2) How rapidly the osmolality gradient created by solute sieving is dissipated by diffusion of solutes across the placenta; this will vary for each species s of plasma solute in proportion to P_sS, the diffusional permeability - placental surface area product for the solute species in question. Increases in the factors P_sS or decreases in the factors σ_s will, by decreasing the effectiveness of solute sieving, translate into an increased effectiveness for any transplacental pressure resultant, $(\Delta P - \Delta \Pi)$, in driving transplacental water flow. Conrad and Faber (1977) have calculated that a given percent change in either σ_s or P_sS is several times as effective as the same percent change in ΔP in controlling the rate of transplacental water flow. They have also noted that, because the surface area of the placental barrier, S, increases in proportion to fetal weight during the last trimester of gestation, the factors P_sS will tend to increase; and this will allow a given pressure resultant $(\Delta P - \Delta \Pi)$ to drive water transplacentally at an increasing rate as gestation progresses. Such a mechanism could sustain the ever increasing water needs of the fetus as fetal growth accelerates during the third trimester of gestation.[10]

[10] Of course, as the P_sS increases during gestation, solute sieving will become less effective in creating counter osmotic pressure gradients; that is, transcients in ΔP will become more effective in altering the rate of transplacental water flow. However, fetal water volume also increases as gestation progresses, and transcients in ΔP will then have to be of greater magnitude and/or of longer duration to effect a given percent change in fetal water volume.

At the beginning of the background section of this thesis Barcroft (1947) was quoted as suggesting that, for fetal water aquisition, "... The natural sequence would be that the growing cell requires water, that it draws water from the foetal blood, and that the foetal blood in turn draws water from the maternal blood..." Also quoted from Barcroft (1947), in the general discussion section of this thesis, was the following remark:

> ...The idea...of the dependency of the fetus for its water supply upon the balance between the 'foetuswards' hydrostatic gradient and the 'motherwards' osmotic gradient, is not a very pleasant one; it leaves the foetus so much at the mercy of its mother's blood pressure...

Perhaps Barcroft was suggesting that the "natural sequence" would have the parameters controlling fetal water acquisition under the control of the parameters determing fetal water needs; that is, that the rate of fetal water acquisition would be linked by feedback control to fetal water needs. Conrad and Faber (1977) have suggested that one means whereby feedback control could be exerted would consist of a fetal endocrine mechanism capable of altering the reflection coefficients, σ_s. As indicated in the preceeding paragraph, these reflection coefficients strongly influence the rate of transplacental water flow. Conrad and Faber state: "...Although such a mechanism need not account for the gestational increase in the acquisition rates (of water and solutes - these would be accounted for by the increase in placental surface area, S), its existence would make available to the fetus an hour to hour control of extracellular water volume..."

A further mechanism whereby the rate of fetal water acquisition might be linked to fetal water needs would involve control of the rate

at which solutes are actively transported across the placenta: As was postulated in the background section of this thesis, active transport of solutes across the placenta could create an osmotic gradient which would draw water to the fetus. Such water flow would result in solute sieving, and thus the establishment of a counter osmotic gradient. Taking these opposing osmotic gradients into account, Conrad and Faber (1977) have calculated that a 15% decrease in ΔC_a [11] would result in a 10% increase in transplacental water flow (see figure 6 of their paper). Assuming that the average concentration gradient ΔC_a existing across the placenta during the third trimester of gestation is about -2.2 mmoles per liter[12], such a 10% increase in transplacental water flow would be effected by an increase in ΔC_a of about (15% x 2.2 mmoles per liter =) 0.33 mmoles per liter. This calculation indicates that control of the rate at which solutes are actively transported across the placenta offers a potentially powerful mechanism for controlling the rate of fetal water acquisition.

[11] i.e., a 15% increase in the concentration of actively transported solutes in fetal plasma over the concentration of these same solutes in maternal plasma,

[12] This gradient was calculated from data in table 2 of this thesis, assuming that fructose, lactate, amino acids, calcium, inorganic phosphate and D-glucose are the major solutes actively transported across the placenta. The concentration gradients listed in table 2 were calculated using equation 6 and may therefore be underestimates (see discussion following equation 6). An underestimate in ΔC_a would lead to a proportionate underestimate of the change in ΔC_a needed to effect a given change in transplacental water flow.

If the rate of transplacental active transport is linked by feedback control to the rate of fetal growth, and if, as postulated above, the rate of active transport affects the rate of transplacental water flow, then fetal water needs, which are proportional to the rate of fetal growth, would be linked to the rate of fetal water acquisition. The suggestion that changes in the transplacental concentration gradient of less than 1 mmole per liter could effect significant change in the rate of fetal water acquisiton is dependent upon our finding that the placenta offers a low resistance to transplacental water flow.

SUMMARY AND CONCLUSION

Our experimental determinations on unanesthetized, chronically prepared sheep indicate that the hydraulic conductivity-surface area product per kilogram fetal weight of the sheep's placenta is about 4×10^{-3} ml per min•mmHg. Using this result it can be calculated that an average transplacental pressure resultant, the resultant of hydrostatic and osmotic pressures acting across the placenta, of about 4 mmHg will drive sufficient water across the sheep's placenta to meet the growing fetus' water needs.

APPENDIX:

The transplacental pressure resultant existing under control conditions, $(\Delta_P - \Delta\Pi)'$, drives a certain amount of transplacental water flow:

$$\dot{q}' = L_pS \quad (\Delta_P - \Delta\Pi)'. \qquad \text{(A-1)}$$

A change in the transplacental pressure resultant causes a proportionate change in transplacental water flow:

$$d\dot{q} = L_pS \quad d\ (\Delta_P - \Delta\Pi). \qquad \text{(A-2)}$$

The new water flow, $\dot{q}$, will equal flow under control conditions plus the change in flow caused by changing the pressure resultant:

$$\dot{q} = \dot{q}' + dq. \qquad \text{(A-3)}$$

Substituting equation A-1 and A-2 in equation A-3,

$$\dot{q} = L_pS\ (\Delta_P - \Delta\Pi)' + L_pS\ d\ (\Delta_P - \Delta\Pi). \qquad \text{(A-4)}$$

Thus, $\dot{q}$ is a linear function of $d\ (\Delta_P - \Delta\Pi)$ with a $\underline{y}$-intercept of $L_pS\ (\Delta_P - \Delta\Pi)'$ and a slope of L_pS. QED.

REFERENCES:

1. Armentrout, T., Katz, S., Thornburg, K., and Faber, J. J. "Osmotic Flow Through the Placental Barrier of Chronically Prepared Sheep". Am. J. Physiol., 1977. 233 (4), H466-H474.

2. Barcroft, J. Researches on Pre-Natal Life. Springfield: Thomas, 1947. (pages 14-17).

3. Comline, R. S., Silver, M. "Daily Changes in Foetal and Maternal Blood of Conscious Pregnant Ewes, With Catheters in Umbilical and Uterine Vessels". J. Physiol., London, 1970. 209, 567-586.

4. Conrad, E. E., and Faber, J. J. "Water and Electrolyte Acquisition Across the Placenta of the Sheep". Am. J. Physiol., 1977. 233 (4), H475-H487.

5. Diamond, J. M. and Tormey, J. M., "Studies on the Structural Basis of Water Transport Across Epithelial Membranes". Fed. Proc. 1966. 25, 1458-1463.

6. Faber, J. J., Green, T. J. "Foetal Placental Blood Flow in the Lamb". J. Physiol., London. 1972. 223, 375-393.

7. Hopkins, L., McFadyen, I. R., Young, M. "The Free Amino Acid Concentrations in Maternal and Foetal Plasmas in the Pregnant Ewe." J. Physiol., London, 1971. 215, 9-10P.

8. James, E., Meschia, G., Battaglia, F. C., "A-V Differences of Free Fatty Acids and Glycerol in the Ovine Umbilical Circulation." Proc. Soc. Exptl. Biol. Med., 1971. 138, 823-826.

9. Kaiser, I. H., Cummings, J. N. "Plasma Electrolytes of the Pregnant Ewe and Fetal Lamb". Am. J. Physiol., 1958. 193, 627-633.

10. Kedem. O. and Katchalsky, A. "Thermodynamic Analysis of the Permeability of Biological Membranes to Non-Electrolytes". Biochem. Biophys. Ada., 1958. 27, 229-246.

11. Meschia, G. "Colloidal Osmotic Pressure of Fetal and Maternal Plasmas of Sheep and Goats." Am. J. Physiol., 1955. 181, 1-8.

12. Meschia, G., Battaglia, F. C. and Barron, D. H. "A Comparison of the Freezing Points of Fetal and Maternal Plasmas of Sheep and Goat." Quant. J. Exp. Physiol., 1957. 42, 163-170.

13. Severinghaus, J. W. "Carbon Dioxide Solubility and First Dissociation Constant (pK') of Carbonic Acid in Plasma and Cerebrospinal Fluid: Man". In P. L. Altman and D. S. Dittmer (Eds.) Respiration and Circulation. Bethesda, Md.: Fed. Am. Soc. Exptl. Biol., 1971. pp. 218-219.

14. Thornburg, K. L., Bissonnette, J. M., Faber, J. J. "Absence of Fetal Placental Waterfall Phenomenon in Chronically Prepared Fetal Lambs." Am. J. Physiol., 1976. 230 (4), 886-892.

15. Tsoulos, N. G., Colwill, J. R., Battaglia, F. C., Makowski, E. L., Meschia, G. "Comparison of Glucose, Fructose, and Oxygen Uptakes by Fetuses of Fed and Starved Ewes." Am. J. Physiol., 1971. 221, 234-237.

TABLE 1: Control data recorded 3 to 8 days after surgery (mean 3.8 days)

	Control data	n*	Values from lit.	n	Source
Pressures with respect to intrauterine pressure (mm Hg)					
Maternal carotid artery	96 ± 3*	7			
Maternal Uterine vein	2.9 ± 0.5	11			
Fetal femoral artery	44.4 ± 1.8	12	39 ± 3	11	Thornburg et al, 1976
			40 ± 1.5	15	Faber & Green, 1971
Umbilical vein	6.3 ± 0.6	11	7.4 ± 1.0	12	Thornburg et al, 1976
			2.0 ± 0.3	15	Faber & Green, 1971
Fetal femoral arterial blood					
pH	7.367 ± 0.001	12	7.37 ± 0.9	9	Thornburg et al, 1976
pO_2 (mm Hg)	21.1 ± 1.5	12	19 ± 3	6	Thornburg et al, 1976
pCO_2 (mm Hg)	49.3 ± 2.0	9	45.7 ± 1.9	7	Comline & Silver, 1969
Blood flow (ml/min•kg)					
Umbilical	188 ± 12	12	199 ± 20	15	Faber & Green, 1971
Uterine	244 ± 34	9			

* Mean ± S.E. *n is the number of ewes or fetuses in which the measurement was made.

TABLE 2: Concentrations (mmol/liter plasma) of plasma constituents and their transplacental concentration gradients (control)

Constituent	Maternal Artery	Uterine Vein	Fetal Artery	Umbilical Vein	ΔC	P	Source of data
Na^+	145.3 ± 1.2 (9)@	146.4 ± 1.5 (8)	143.7 ± 1.9 (9)	143.3 ± 1.4 (9)	+2.29 ± 0.64*	<0.01	Present study
K^+	4.49 ± 0.10 (9)	4.46 ± 0.11 (8)	3.91 ± 0.14 (9)	3.91 ± 0.14 (9)	+0.55 ± 0.14	<0.01	
Mg^{++}	0.85 ± 0.08 (7)	0.81 ± 0.06 (6)	0.81 ± 0.05 (7)	0.79 ± 0.05 (7)	+0.04 ± 0.05	N.S.	
Ca^{++}	2.12 ± 0.07 (9)	2.16 ± 0.08 (8)	2.90 ± 0.05 (9)	2.97 ± 0.04 (9)	-0.79 ± 0.06	<0.01	
Cl^-	107.7 ± 1.7 (9)	107.5 ± 1.9 (8)	101.3 ± 2.4 (9)	103.0 ± 2.2 (9)	+5.47 ± 1.31	<0.01	
HCO_3^-	24.5 ± 1.1 (9)	26.9 ± 1.4 (7)	27.4 ± 1.3 (9)	27.5 ± 1.4 (8)	-2.11 ± 0.57	<0.01	
P_i‡	0.48 ± 0.03 (6)	0.50 ± 0.02 (5)	0.73 ± 0.05 (6)	0.75 ± 0.06 (6)	-0.27 ± 0.03	<0.01	
BUN§	6.2 ± 0.7 (8)	6.3 ± 0.8 (7)	7.1 ± 0.8 (8)	7.0 ± 0.7 (8)	-0.98 ± 0.07	<0.01	
Gluc.	3.4 ± 0.3	2.8 ± 0.2	1.0 ± 0.2	1.5 ± 0.5	+2.12 ± 0.12	<0.01	
Fructose					-1.70 NA‡		Barcroft, 1947; Tsoulos et al, 1971
Lactate					+1.25 NA		Kaiser et al, 1958
Glycerol					0.00 NA		James et al, 1971
Amino Acids					-2.90 NA		Hopkins et al, 1971
Free Fatty Acids					+0.29 NA		James et al, 1971
TOTAL TRANSPLACENTAL CONCENTRATION GRADIENT					+ 3.26		

* Mean ± S.E. @ number of animals in which measurements were made.

‡ Inorganic phosphate. § Blood urea nitrogen (converted to urea).

TABLE 3: Plasma proteins; maternal and fetal concentrations and transplacental gradient (control)

	Maternal Artery	Uterine Vein	Fetal Artery	Umbilical Vein	Average Transplacental Gradient	P
Total plasma protein g/100 ml	6.60 ± 0.25*	6.70 ± 0.28	3.43 ± 0.22	3.40 ± 0.20	+3.32 ± 0.25	<0.01
Number of animals in which measurement was made	6	5	6	6		

* Means ± SE

TABLE 4: Plasma Osmolalities and Transplacental Osmolality Gradients (Control)

	Maternal Artery	Uterine Vein	Fetal Artery	Umbilical Vein	$\Delta\pi^{(M-F)}$	P
Vapor Pressure Osmometry	294.1 ± 1.6 (4)	293.8 ± 2.3 (4)	287.0 ± 1.9 (4)	289.3 ± 2.4 (4)	+5.8 ± 1.4	<0.01
Freezing Point Depression Osmometry	301.1 ± 2.9 (8)	302.0 ± 3.3 (7)	300.0 ± 2.3 (8)	299.4 ± 2.8 (8)	+2.2 ± 0.7	<0.01

Values are means ± SE. Numbers in parentheses are number of animals in which measurement was made.

TABLE 5: Changes in fetal placental hemodynamics after infusions of hypertonic solutions.

	Fetal placental flow ml/min • kg	Fetal femoral artery pressure mm Hg	Umbilical vein pressure mm Hg
Control values before infusions	188 ± 12	44.4 ± 1.8	6.3 ± 0.6
Change from control values after infusion into fetal plasma	- 70.1		+4.3
Significance of change	$P < 0.01$	NS	$P < 0.02$
Change from control values after infusion into maternal plasma	- 61 ± 11	+ 7.8 ± 1.9	+ 1.9 ± 0.7
Significance of change	$P < 0.01$	$P < 0.01$	$P < 0.03$

Values are means ± SE. Significance of change evaluated by paired "t" testing.