Concentrations of Na^+ and Cl^- in transplacental ultrafiltrate in sheep

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1. Maternal blood was made transiently hypertonic by rapid I.V. infusion of a concentrated mannitol solution into pregnant ewes bearing lambs with an indwelling flow sensor and vascular catheters.

2. The transplacental flows of water and of Na^+ and Cl^- were calculated from the umbilical arteriovenous differences in the concentrations of ^{125}I-labelled albumin and electrolytes, and the fetal placental blood flow.

3. The reflection coefficients of Na^+ and Cl^- were calculated by means of the Patlak equation and found to be $0{\cdot}85 \pm 0{\cdot}04$ and $0{\cdot}68 \pm 0{\cdot}04$ (means ± S.E.M.). The filtration coefficient was $1{\cdot}02 \times 10^{-7} \pm 0{\cdot}12 \times 10^{-7}$ cm^5 dyne^{-1} s^{-1}.

4. The results fitted best to an equivalent pore radius in the placental barrier smaller than the currently accepted 0·44 nm but not less than 0·35 nm.

It is difficult to arrive at a satisfactory explanation for the flow of water from mother to fetus across the placenta of the sheep. Total maternal plasma osmolality is always higher than the fetal value, by several milliosmols per kilogram water (Meschia, Battaglia & Barron, 1957; Armentrout, Katz, Thornburg & Faber, 1977), and the equivalent pore radius of the barrier has been calculated to be about 0·44 nm (Boyd, Haworth, Stacey & Ward, 1976), which implies that all osmotically significant non-electrolytes in fetal and maternal plasmas exert osmotic pressures close to their van't Hoff theoretical value, i.e. have reflection coefficients close to 1·0 (Curry, 1984). However, there must be a net force driving water from mother to fetus.

It has been proposed (Conrad & Faber, 1977) that the net osmotic pressure difference can be in the fetal direction if the osmotic reflection coefficients of the major electrolytes, Na^+ and Cl^-, are less than their maximum value of 1·0. A computer simulation seemed to bear out that this assumption leads to a consistent explanation (Faber & Anderson, 1990) because the concentrations of the solutes with the lesser reflection coefficients (Na^+ and Cl^-) are higher in maternal than in fetal plasma while the opposite is true for most non-electrolytes, which have the higher reflection coefficients. The weakness of this explanation is that the reflection coefficients for Na^+ and Cl^- have not been measured directly. Thornburg, Binder & Faber (1979*a*) calculated reflection coefficients of 0·83 for Na^+ and 0·79 for Cl^-. However, their results depended on the assumption that the compositions and volumes of fetal soma and extrafetal fluids changed predictably over the several-days-long duration of the experiments. It was the purpose of the present experiments to obtain the reflection coefficients by direct measurement.

To this end, we forced an ultrafiltrate of fetal plasma through the placental barrier by osmotic means and measured the transplacental ultrafiltration rates of water and electrolytes. We computed the electrolyte reflection coefficients from the ratios of the concentrations of the electrolytes in the ultrafiltrate and in the donor plasma.

Chronically instrumented ewes and fetuses were used and the plasma of the ewe was temporarily made hyperosmotic by the infusion of a concentrated solution of mannitol while we took blood samples and recorded fetal placental blood flow. The transplacental flow of ultrafiltrate was calculated from the fetal umbilical blood flow and the measured arteriovenous change in the concentration of an impermeable marker solute injected into the fetal blood. The transplacental flow of electrolyte was calculated from the measured umbilical arteriovenous differences in marker solute and electrolyte concentrations. The Patlak modification of the Herzian equation (Patlak, Goldstein & Hoffman, 1963; Curry, 1984) was used in order to take into account both transplacental filtration and transplacental diffusion of the electrolytes. The assumptions that underlie the derivations of the necessary equations are presented in the Appendix.

METHODS

Bench experiments

Bench tests were performed to prove that the methods were adequate for the purpose of the experiments. Four pools of heparinized sheep blood were obtained with haematocrits from 17·5 to 33·0%. Some ^{125}I-labelled albumin was added to each pool as a plasma volume marker. Each pool was then divided into five or six aliquots during continuous mixing. Nothing was added to the first aliquot, which constituted mock arterial blood. To the second and following aliquots, we added either saline (to mimic ultrafiltrate that had passed through a barrier with a reflection coefficient, σ, of zero), or distilled water ($\sigma = 1{\cdot}0$), or a 50% dilution of saline with water ($\sigma = 0{\cdot}5$). In about half of the tests, the volumes added were 2·5% of the aliquot volume and in the remainder 5·0% of the aliquot volume. The aliquots were lightly spun before any fluid was added to prevent haemolysis. The aliquots were then mixed again. Part of each aliquot was used for determination of the freezing point osmolality and haematocrit. The remainder was centrifuged and the supernatant plasma taken for electrolyte analysis and gamma counting of the marker solute. Except for the first ('arterial') aliquot, the aliquots mimicked fetal venous blood emerging from the placenta after acquiring a small amount of transplacental ultrafiltrate of maternal plasma. Appendix equations (A7) and (A9) (in ratio form) were applied to the measurements. The ratios of the electrolyte and water fluxes so calculated constituted the Na^+ and Cl^- concentrations in the 'ultrafiltrate' and comparison of that concentration with the concentrations of Na^+ and Cl^- in the saline yielded the measured 'reflection coefficients'. The possible objection that these tests represented addition, rather than withdrawal, of ultrafiltrate can be met by reversing the appellations 'arterial' and 'venous'.

Surgical protocol

All surgical and experimental protocols were approved by the Institutional Animal Care and Use Committee. Four time-bred pregnant sheep were operated on at 119–126 days of gestation. Anaesthesia was induced by I.V. injection of 10 mg diazepam and 400 mg ketamine hydrochloride. After intubation, anaesthesia was continued with 0·8–1·2% halothane in 2 O_2 : 1 N_2O. Both the ewe and the fetus were fully anaesthetized.

All procedures were sterile. The uterus was exposed through a mid-line incision and entered. Indwelling polyvinyl catheters (i.d., 1·0 mm; o.d., 1·2 mm) were inserted into both fetal pedal arteries and veins and advanced about 20 cm. A left paravertebral incision was made on the fetus and the aorta was dissected free just above or below the external iliac arteries. An electromagnetic flow sensor (In Vivo Metric, Healdsburg, CA, USA) was placed around the aorta and an inflatable occluder (In Vivo Metric) was placed downstream from it. Finally, a medial incision was made in the fetal abdomen just cranial from the umbilicus and a polyvinyl catheter with a small external ring, 2 cm from its end, and multiple side holes near its end was inserted into the common umbilical vein and tied in place. All incisions were carefully repaired. A catheter for access to the amniotic fluid was sewn to the fetal skin and 1 million units of penicillin G were injected into the amniotic fluid at the end of surgery. No other antibiotics were used.

An arterial catheter was placed in a superficial branch of the maternal femoral artery and a large catheter (o.d., 4·0; i.d., 3·0 mm) was placed in a maternal jugular vein. All catheters were filled with heparinized saline and were kept in a pouch sewn to the flank of the ewe except for the maternal jugular vein catheter, which was taped to the neck.

Experimental protocols

Experiments were performed after 3–9 (mean, 5) days of recovery; the third animal was experimented on twice, at 3 and 7 days after surgery.

On the day of the experiment, the ewe was placed in a stanchion in the laboratory. The ewe had free access to food and water, except as noted. The ewe and the fetus were given I.V. doses of 7000 and 1000 units of heparin. This eliminated the need to control the degree with which blood samples were diluted with anticoagulant. After a control fetal blood sample had been taken for blood gases, the flow sensor was plugged into an electromagnetic flow meter (Gould Statham, Cleveland, OH, USA) and the mean blood flow in the distal aorta of the fetus was measured and averaged over a 15 min period of time. The blood flow was then reduced to 50% of its original value by partial inflation of the occluder. Thereafter, flow was held constant by means of control by a Hewlett-Packard computer, a Hewlett-Packard digital voltmeter and a modified Gilson peristaltic infusion pump connected to the inflatable occluder, as previously described by us (Anderson & Faber, 1984). Past experience had shown that when the maternal plasma is made hypertonic, fetal placental blood flow invariably falls (Armentrout *et al.* 1977). Clamping blood flow in the distal fetal aorta (where 77% of the flow is destined for the placenta; Anderson, Parks & Faber, 1986) in advance ensured that the placental blood flow would remain constant for the duration of the experiment. Fetal sheep tolerate substantial reductions in placental blood flow for periods of time much longer than 1 week, with little ill effect (Anderson & Faber, 1984; Anderson *et al.* 1986); the present experiments lasted less than 3 h.

The marker solute (10 μC ^{125}I-labelled albumin; Mallinckrodt, Chesterfield, MO, USA) was injected into a fetal pedal vein catheter and the fetus was given at least half an hour to stabilize. We then took simultaneous blood samples from a fetal femoral artery catheter and the umbilical vein catheter. A blood sample was taken from the femoral artery catheter of the ewe except in one case in which the arterial catheter would not draw and jugular vein blood was taken instead. Except for gamma counting of the plasma, these samples were immediately analysed, as described below.

A solution of about 360 g of mannitol, 40 g of NaCl and 30 g of glucose in a little under 1500 ml of water was then pumped as rapidly as possible into the jugular vein catheter of the ewe. Experience has shown that this composition of the hyperosmolal fluid minimized electrolyte changes in maternal plasma. During the infusion, the ewe was not allowed access to water.

Immediately at the completion of the maternal infusion, which required about 20 min, a second and final set of blood samples was taken. Thereafter, the occluder that regulated fetal blood flow was deflated and disconnected and the catheters and flow meter cable were also disconnected and the ewe returned to her pen with free access to water and food.

Analytical techniques

Blood gas measurements were performed on an Instrumentation Laboratory 1306 blood gas analyser (Lexington, MA, USA); all values were corrected to 39 °C and haematocrits were determined in duplicate.

Plasma concentrations of Na^+ and Cl^- were measured with a Beckman Lablyte System-810 (Fullerton, CA, USA) ion selective electrode system. The standard deviations were 0·28 and 0·24 mequiv l^{-1}, respectively. The order of sample analysis was two fetal arterial, two fetal venous and two maternal plasma measurements and this sequence was repeated five times for a total of ten determinations in each of the three plasma samples. This sequencing ensured that any cyclic change in machine sensitivity would be randomized. For the same reason, the machine was forced through its self-calibration routine before the analysis of the samples and no self calibration occurred again until the entire sequence had been completed. Since a S.E.M. = S.D./$n^{0.5}$, this procedure further improved the already considerable accuracy of the electrolyte determinations by a factor of about three. It was an advantage of the ion-selective electrode system that no pipetting operations with their attendant errors were required.

Two 1 ml samples of plasma were placed in weighed tubes and reweighed to reduce the normal pipetting error of about 1% to much less than 0·1%. The duplicate samples were counted at least four times in a Micrad germanium crystal multichannel gamma analyser system.

Statistical procedures

All data are presented as means ± S.E.M., unless the measure of dispersion is specified as a S.D. P values and 95% confidence limits were obtained by Student's t tests or paired t tests, where applicable (Winer, 1971).

RESULTS

Bench experiments

Ten samples were taken for electrolyte analysis from each of the twenty-two aliquots of pooled blood. The S.E.M. of the Na^+ concentration in each aliquot was, on average, 0·082 ± 0·028 (S.D.) mequiv l^{-1} and for Cl^- it was 0·080 ± 0·030 mequiv l^{-1}. Since the mean concentrations of Na^+ and Cl^- were 142·3 and 110·5 mequiv l^{-1}, the coefficients of variation were about 0·06 and 0·07%, respectively.

Several million counts, over a total counting time of 6 h (corrected for 'dead time'), were accumulated for each sample in the ^{125}I channel of the multichannel analyser; the accuracies of the ^{125}I-labelled albumin concentrations were, therefore, only limited by the accuracies of the weighing of the samples.

Figure 1 shows the reflection coefficients calculated from the analytical measurements on these aliquots as a function of the reflection coefficients of the mock ultrafiltrate that had been added. There is no systematic bias. The absolute values of the differences between the measured and the real reflection coefficients were 0·03 ± 0·03 for Na^+ and 0·04 ± 0·05 for Cl^- (means ± S.D.). We concluded that the proposed measurements and calculations were adequate to the task at hand.

Animal experiments

A–V differences associated with placental gas exchange

Control data. On the day of the experiment, fetal arterial P_{O_2} was 20 ± 1 mmHg, P_{CO_2} was 55 ± 2 mmHg, pH was 7·34 ± 0·01 and the haematocrit was 36 ± 3%. These were normal values for our sheep.

Control data after flow reduction. As expected, flow reduction caused a fall in the pressure in the fetal femoral artery roughly in proportion to the reduction in flow. Fetal venous pressure was unchanged. There was a fall of 5·8 mmHg in fetal arterial blood P_{O_2} and a rise of 6·5 mmHg in P_{CO_2} ($n = 4$). Table 1 lists the electrolyte and marker solute concentrations during the control period after reduction of fetal placental blood flow. For typographical

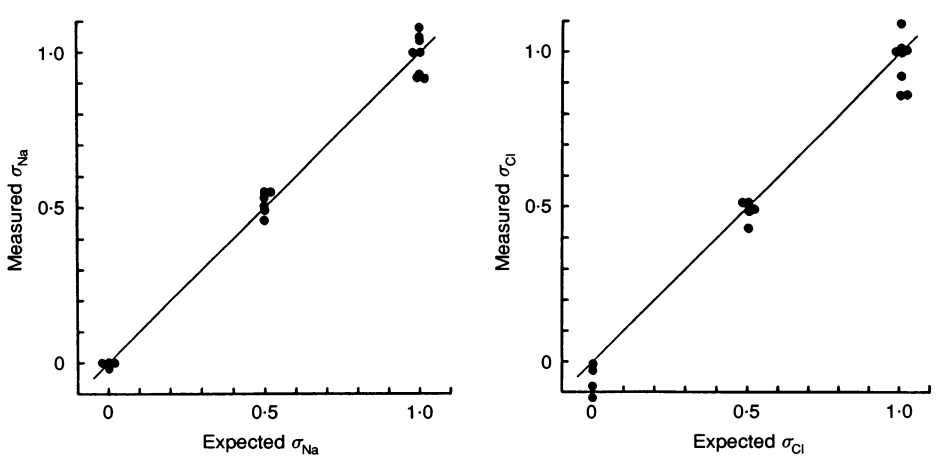

Figure 1. Agreement between expected and measured reflection coefficients in bench experiments on sheep blood

Overlapping points slightly displaced. Line is line of identity. Expected values were 1·0 when distilled water was added, 0·5 when diluted saline was added and 0 when saline was added.

Table 1. Experimental data

Fetus		[Na^+] (mequiv l^{-1})			[Cl^-] (mequiv l^{-1})			[Marker] (c.p.m./weight)		FPD (mosmol kg^{-1})	
		FA	FV	MA	FA	FV	MA	FA	FV	FA	MA
1	C	145·0	144·1	151·4	111·2	112·9	115·6	5038	4973	303	300
	E	159·9	165·4	132·4	120·5	126·5	106·4	4627	4790	344	377
2	C	140·9	140·4	148·0	109·3	110·1	112·6	1040	1023	297	301
	E	150·7	154·7	140·0	115·3	118·6	111·3	1134	1167	325	411
3A	C	143·0	142·3	150·2	107·3	108·5	115·9	1216	1198	300	304
	E	155·3	162·2	143·8	115·9	121·6	121·8	1302	1354	341	416
3B	C	143·2	142·2	148·5	108·9	110·2	115·6	1754	1748	302	304
	E	159·4	163·6	140·2	119·6	123·3	118·2	2061	2136	345	353
4	C	139·4	138·6	144·2	99·0	100·5	108·2	954	940	294	295
	E	150·3	156·3	137·8	106·7	112·9	108·5	1025	1068	325	365

FA, fetal arterial; FV, fetal umbilical vein; MA, maternal arterial; FPD, freezing point depression osmolality; C, control period 1 h after placental blood flow reduction; E, experimental period during elevated maternal blood osmolality. Two experiments, A and B, were done on the third animal. Number of significant digits reduced for typographical convenience. Placental blood flows ranged from 3·17 to 4·98 ml s^{-1}.

convenience no data in Table 1 are given to more than four digits, but all calculations were performed prior to rounding.

In each individual case, control umbilical venous [Na^+] was lower than fetal arterial [Na^+] and the same was true for the marker solute concentrations but umbilical venous [Cl^-] was always higher than fetal arterial [Cl^-]. The changes in [Na^+] and marker solute concentrations were considered to be due to the slight osmotic shrinkage of red cells as they lose osmotically active solute when giving up carbon dioxide in the placenta and the changes in [Cl^-] to the chloride shift (Meschia & Barron, 1956; Davenport, 1958). Thus, it was important to maintain fetal placental blood flow constant during the hypertonic phase of the experiment to ensure that any arteriovenous difference in concentrations that was not due to the transplacental flow of ultrafiltrate would remain unchanged.

A–V differences during the hypertonic phase

After the maternal blood had been made hypertonic, a second, experimental set of samples was taken. At that time, mean maternal blood osmolality had risen from 300·6 ± 1·6 to 393·0 ± 9·7 mosmol (kg water)$^{-1}$ ($P < 0·001$) and mean fetal arterial blood osmolality from 299·2 ± 1·7 to 334·5 ± 4·0 mosmol kg^{-1} ($P < 0·005$). Mean umbilical venous blood osmolality at 342·4 ± 4·3 mosmol kg^{-1} was significantly higher than fetal arterial blood osmolality ($P < 0·001$). All umbilical venous concentrations in Table 1 were higher than the corresponding fetal arterial concentrations, as was to be expected from the osmotic withdrawal of fetal blood water across the placental barrier. In order to correct the arteriovenous concentration differences in Table 1 for the differences due to respiratory gas exchange, we subtracted the relative arteriovenous difference during the control period from the relative

Table 2. Calculated quantities

Experiment	q_f (ml s^{-1})	q_f (% of Q)	σ_{Na}	σ_{Cl}	LpS (10^{-7} cm^5 $dyne^{-1}$ s^{-1})
1	0·125	3·1	0·90	0·78	1·11
2	0·129	3·3	0·72	0·58	0·57
3A	0·194	4·1	0·92	0·74	1·06
3B	0·151	3·0	0·88	0·58	1·12
4	0·124	3·9	0·83	0·76	1·23
Mean	0·145	3·5	0·85	0·68	1·02
S.E.M.	0·013	0·22	0·04	0·04	0·12
$P_1 <$	0·001	0·001	0·001	0·001	0·001
$P_2 <$			0·02	0·005	

P_1, probability that difference from zero is entirely due to random error; P_2, same for difference from 1·0. Abbreviations: q_f, transplacental ultrafiltration rate; Q, fetal placental blood flow; σ, reflection coefficient; LpS, filtration coefficient.

difference during the hypertonic phase. Thus, if umbilical venous [Na^+] was 0·6% lower than arterial [Na^+] during the control period and 4% higher during the hypertonic phase, we used an arteriovenous difference of 4·6% in the calculations. This correction eliminated any arteriovenous differences associated with placental metabolic activities as well as the very slight error due to radioactive decay of ^{125}I during the time the venous sample was waiting for the corresponding arterial sample to be counted.

Table 2 shows that the average transplacental water filtration rate was $0·145 \pm 0·013$ ml s^{-1}, or 3·5% of the fetal placental blood flow. Thus, the relative dilution (or concentration) of the placental blood during the hypertonic phase was about the same as the relative dilution of blood in the bench experiments.

The mean reflection coefficients for Na^+ and Cl^- were $0·85 \pm 0·04$ and $0·68 \pm 0·04$ and were significantly different from zero as well as from 1·0 (Table 2). The placental water filtration coefficient, LpS, was calculated by means of eqn (A12) in the Appendix. The mean LpS was $1·02 \times 10^{-7} \pm 0·12 \times 10^{-7}$ cm^5 $dyne^{-1}$ s^{-1}.

The experiment was repeated once on the third animal (experiments 3A and 3B in Table 2), with good agreement for σ_{Na} and LpS but only moderately good agreement between the two values for σ_{Cl}, although neither value for σ_{Cl} was outside the range observed in the other animals.

Effect of the experiment on the fetuses. The animal that was experimented on twice, with a 4 day interval, was killed after the second experiment. One of the other animals was killed 5 days after the experiment because its umbilical vein catheter would no longer function and a third fetus died *in utero* 2 days after the experiment (its twin still alive) for reasons that did not become clear at autopsy. The last ewe was killed while in the process of delivering a live lamb. These results confirm our previous findings that neither the hyperosmotic water withdrawal nor the reduction of fetal placental blood flow constitute insupportable stresses.

DISCUSSION

The main experimental challenge was accuracy. The bench experiments demonstrated, however, that useful accuracy could be achieved. Although the reduction in fetal placental blood flow was mandated by the need to keep blood flow the same during the control and hypertonic phases of the experiment, it fortuitously also accentuated the small arteriovenous differences that needed to be determined.

The values of the reflection coefficients obtained here can be compared to values that can be deduced from conventional pore theory. Curry (1984) gives the equation (his equation 5.79):

$$\sigma = 1 - 2(1-\alpha)^2 + (1-\alpha)^4, \quad (1)$$

where α is the ratio of the radius of the solute to the radius of the pore. The radius of the equivalent pore in the placental barrier of the sheep has been reported to be 0·44 nm (Boyd *et al.* 1976) and we calculated the atomic radii of Na^+ and Cl^- to be 0·261 and 0·199 nm by means of the procedure of Durbin (1960). These values substituted into eqn (1) yield $\sigma_{Na} = 0·70$ and $\sigma_{Cl} = 0·49$, which are smaller than the values of 0·85 and 0·68 in Table 2. However, the equivalent pore radius calculated by Boyd *et al.* (1976) may be somewhat too large. These authors presented evidence of partial degradation of the [^3H]mannitol tracer that was used in their series of molecular probes and an erroneously high apparent permeability of mannitol would have led them to overestimate the pore radius. Equation (1) gives the best agreement between the calculated and experimentally determined reflection coefficients when an equivalent pore radius of 0·35 nm is used, which gives $\sigma_{Na} = 0·87$ and $\sigma_{Cl} = 0·66$, close to the experimental values of 0·85 and 0·68 in Table 2. The agreement between the reflection coefficients calculated from the experimental results and calculated from pore theory is reassuring since the equations in the Appendix assume that there is no significant difference in electrical potential across the placental barrier. Although a large potential difference is unlikely (Thornburg, Binder & Faber, 1979*b*), a potential difference of the order of ±1 mV would be difficult to rule out. The effect of such a potential difference would have been to increase the value of one of the reflection coefficient, while decreasing that of the other.

Pore theory offers a second opportunity to verify the reality of the results against measurements made elsewhere because LpS can be calculated also, once the equivalent pore radius and the urea permeability of the placenta are known. Using eqn (5.32) from Curry (1984), we find that the permeability is given by:

$$PS = (N\pi r^2/l)D\mathrm{F}(\alpha), \quad (2)$$

where

$$\mathrm{F}(\alpha) = (1-\alpha)^2(1 - 2·104\alpha + 2·09\alpha^3 - 0·95\alpha^5).$$

The symbols (Table 3) are: N, number of pores; r, pore radius; l, length of the pore; D, coefficient of free diffusion ($D_{urea} = 1·9 \times 10^{-5}$ cm^2 s^{-1}; Faber & Thornburg, 1983); and α, ratio of the radius of the urea molecule (calculated to be 0·255 nm; Durbin, 1960) to the radius of the pore. We will use eqn (2) to calculate the quantity (N/l) from the urea permeability ($PS_{urea} = 1·020$ ml s^{-1}, Boyd *et al.* 1976)

Table 3. Glossary of symbols

D	Coefficient of free diffusion in water at 39 °C
E	Electrolyte flux (Na^+ or Cl^-) across the placental barrier
F(α)	Function of α, defined by eqn (2)
H	Fractional haematocrit ($0 < H < 1$)
K	Defined by eqn (A11)
LpS	Filtration coefficient of placental barrier
M	Marker solute that does not cross the placental barrier
N	Number of pores present
PS	Diffusion permeability of placental barrier
q	Water flow
Q	Fetal placental blood flow
r	Pore radius
R	Gas constant
S	Surface area
T	Absolute temperature
α	Ratio of radius of diffusing molecule and radius of pore
η	Viscosity of water at 39 °C
σ	Reflection coefficient
Π	Osmolality
Superscripts	
F	Fetal
FA	Fetal placental artery
FV	Fetal placental vein
M	Maternal
MA	Maternal placental artery
R	Ratio FV/FA
Subscripts	
f	Filtered through the placental barrier
pl	Plasma
rc	Red cell

and the data given in this paragraph and will substitute that quantity into eqn (3), below.

From Poiseuille's law it follows that:

$$LpS = \frac{(N/l)\pi r^4}{8\eta}, \quad (3)$$

where η is the viscosity of water at 39 °C ($6{\cdot}7 \times 10^{-3}$ dyne s cm^{-2}). With the pore radius of 0·44 nm (Boyd *et al.* 1976) one finds that $LpS = 8{\cdot}8 \times 10^{-8}$ cm^5 $dyne^{-1}$ s^{-1}, which is somewhat smaller than the experimentally determined value of $1{\cdot}02 \times 10^{-7}$ in Table 2. If a pore radius of 0·35 nm is assumed, $LpS = 2{\cdot}1 \times 10^{-7}$ cm^5 $dyne^{-1}$ s^{-1}, which is somewhat too high, indicating again that the true pore radius is less than the 0·44 nm calculated by Boyd *et al.* (1976) although not quite as small as the 0·35 nm calculated from our reflection coefficients. It is clear, however, that the published values of the urea permeability and pore radius (Boyd *et al.* 1976) and the experimentally determined filtration coefficient and electrolyte reflection coefficients are mutually consistent within the limits of the accuracy of the measurements and are consistent also with a single shared path for water and solutes across the placental barrier of the sheep, unlike the results obtained on some other tissues (Curry, Mason & Michel, 1976; Wolf & Watson, 1989), which require two parallel paths for a consistent explanation.

The experimentally determined filtration coefficient and reflection coefficients are well within the ranges previously shown (Faber & Anderson, 1990) to be required for the rates of transfer of electrolytes and water that are associated with the normal growth of the fetus and its extra fetal fluids. Thus, the present experimental results agree with the prediction that the electrolyte reflection coefficients must be less than 1·0 to ensure that there is no net osmotic force driving water from fetus to mother due to the despite maternofetal difference in total plasma osmolality.

APPENDIX

Calculation of transplacental flows of water, Na^+ and Cl^-

Table 3 is a glossary of symbols. Radio-iodinated plasma albumin was present in the fetal plasma as a 'marker solute', M, that cannot cross the placental barrier. The arterial inflow rate of M on the fetal side of the placenta is therefore equal to the venous outflow rate and by Fick's principle:

$$q_{pl}{}^{FA}[M]^{FA} = q_{pl}{}^{FV}[M]^{FV}, \tag{A1}$$

where q_{pl} is the fetal plasma flow and the superscripts FA and FV stand for fetal umbilical arterial and umbilical venous. It is convenient to define a concentration ratio $[M]^R = [M]^{FV}/[M]^{FA}$ so that the ratio of the arterial and venous fetal plasma flows is given by

$$q_{pl}{}^{FA}/q_{pl}{}^{FV} = [M]^R. \tag{A2}$$

The flow of water that filters through the placental barrier, q_f, taken to be positive when from mother to fetus, must be carried off (or be supplied) by either the fetal venous plasma water or the fetal venous red cell water. Hence:

$$q_f = (q_{pl}{}^{FV} - q_{pl}{}^{FA}) + (q_{rc}{}^{FV} - q_{rc}{}^{FA}), \tag{A3}$$

where q_{rc} represents the flow of water carried in the fetal placental red blood cells. The letter H represents the fetal fractional haematocrit ($0 \leqslant H \leqslant 1$). Since the red cell solids represent about 30% of the red cell volume (Czackes, Ullmann, Ullmann & Bar-Kochba, 1963), it follows that:

$$q_{pl} = Q(1 - H), \tag{A4}$$

$$q_{rc} = Q \times 0{\cdot}7H, \tag{A5}$$

where Q is the fetal placental blood flow.

The solute transfers between red cells and plasma that are associated with placental gas exchange were taken into account by subtracting arteriovenous differences observed during the control period (after reduction of fetal placental blood flow) from the arteriovenous differences during the hypertonic period before the latter were used in the calculations. It is assumed that no other exchanges of red cell contents with plasma took place and that the red cells were in osmotic equilibrium with the plasma at the time the experimental samples were centrifuged. Isotonicity and an absence of red cell to plasma solute exchange are formulated by

$$\Pi^{FA}/\Pi^{FV} = q_{rc}{}^{FV}/q_{rc}{}^{FA}, \tag{A6}$$

where Π is the osmolality of the blood.

Algebraic manipulation of the above expressions leads to an expression for the transplacental water filtration rate:

$$q_f = Q^{FA}\left\{\left(\frac{1}{[M]^R} - 1\right)(1 - H) + (0{\cdot}7H)\left(\frac{\Pi^{FA}}{\Pi^{FV}} - 1\right)\right\}, \tag{A7}$$

in which all variables on the right side are obtainable by experiment.

Let E_{Na} be the transplacental Na^+ flux (positive from mother to fetus). This sodium must be accounted for by application of Fick's principle to fetal plasma:

$$E_{Na} = ([Na^+]^{FV} q_{pl}{}^{FV}) - ([Na^+]^{FA} q_{pl}{}^{FA}), \tag{A8}$$

and it then follows from the above relations that:

$$E_{Na} = Q^{FA}(1 - H)\left(\frac{[Na^+]^{FV}}{[M]^R} - [Na^+]^{FA}\right), \tag{A9}$$

in which all quantities on the right side are measurable. The concentration of Na^+ in the transplacental ultrafiltrate is simply the ratio of the transplacental sodium and water fluxes. An equation analogous to A9 applies to chloride.

Calculation of the reflection coefficients of Na^+ and Cl^-

The plasma Na^+ and Cl^- concentrations were not precisely the same in maternal and fetal plasma during the osmotic experiments, although they were close. Thus, transplacental transfer of these ions not only occurred under the influence of ultrafiltration but possibly was affected also by diffusion. The transplacental electrical potential is known to be negligible (Thornburg, Binder & Faber, 1979*b*). Under these conditions the sodium and electrolyte fluxes are given by the Patlak equation (Bresler & Groome, 1981; Curry, 1984):

$$E_{Na} = \frac{q_f(1-\sigma)([Na^+]^F - e^{-K}[Na^+]^M)}{e^{-K}-1}, \quad \text{(A10)}$$

where the superscripts F and M stand for the average fetal and maternal placental capillary plasma concentrations. Since the arteriovenous differences were quite small on the fetal side and fetal placental blood flow was much smaller than maternal placental blood flow, the maternal arterial and venous concentrations were essentially the same. Thus, the fact that no measured maternal (uterine) venous concentrations were available was of little consequence and the mean maternal concentration was taken to be equal to the maternal arterial concentration; the mean fetal concentration was taken as the average of the fetal arterial and venous concentrations. The symbol K in eqn (A10) stands for:

$$K_{Na} = q_f(1-\sigma_{Na})/PS_{Na}, \quad \text{(A11)}$$

where PS_{Na} is the placental permeability surface area product for Na^+ and σ_{Na} is the placental reflection coefficient for Na^+. Equations analogous to (A10) and (A11) apply to Cl^-. The diffusion permeabilities of Na^+ and Cl^- have previously been measured and are $5{\cdot}2 \times 10^{-3}$ and $9{\cdot}8 \times 10^{-3}$ ml s^{-1} (kg fetal body weight)$^{-1}$ (Thornburg, Binder & Faber, 1979*a*).

Thus, the transplacental water and electrolyte fluxes were calculated from eqns (A7) and (A9), the latter being applied to both Na^+ and Cl^-. Once the electrolyte fluxes were known, the reflection coefficients were approximated by having a computer increment the values of σ_{Na} from 0 to 1 by increments of 0·01 until the sodium flux obtained from eqn (A10) was as close as possible to the sodium flux calculated from the experimental measurements by means of eqn (A9); the same was done for Cl^-. At the optimum value for the σ in eqn (A10), the largest discrepancy found in any experiment between the flux values obtained from eqns (A9) and (A10) was 0·26% of the true flux value for Na^+ and 0·13% for Cl^-. These discrepancies could have been reduced further by changing the increments of σ in the iterations from 0·01 to 0·001 but the results of the bench experiments showed that trying to obtain σ_{Na} or σ_{Cl} with greater accuracy than two significant digits was not justified.

Computation of the placental filtration coefficient

The placental filtration coefficient, LpS, was calculated from the observed water flux and a calculated osmotic pressure. The osmotic pressure was calculated with van't Hoff's law from the observed freezing point osmolalities (Π), after the contributions of Na^+ and Cl^- had been corrected for the fact that their reflection coefficients were less than 1·0. Thus:

$$LpS = \frac{q_f}{RT(\Pi^F - \Pi^M) - (1-\sigma_{Na})([Na^+]^F - [Na^+]^M) - (1-\sigma_{Cl})([Cl^-]^F - [Cl^-]^M)}. \quad \text{(A12)}$$

Anderson, D. F. & Faber, J. J. (1984). Regulation of fetal placental blood flow. *American Journal of Physiology* **247**, R567–574.

Anderson, D. F., Parks, C. M. & Faber, J. J. (1986). Fetal O_2 consumption during controlled long-term reductions in umbilical blood flow. *American Journal of Physiology* **250**, H1037–1042.

Armentrout, T., Katz, S., Thornburg, K. L. & Faber, J. J. (1977). Osmotic flow through the placental barrier of chronically prepared sheep. *American Journal of Physiology* **233**, H466–474.

Boyd, R. D. H., Haworth, C., Stacey, T. E. & Ward, R. H. T. (1976). Permeability of the sheep placenta to unmetabolized polar non-electrolytes. *Journal of Physiology* **256**, 617–734.

Bresler, E. H. & Groome, L. J. (1981). On equations on combined convective and diffusive transport of neutral solute across porous membranes. *American Journal of Physiology* **241**, F469–476.

Conrad, E. C. & Faber, J. J. (1977). Water and electrolyte acquisition across the placenta of the sheep. *American Journal of Physiology* **233**, H475–487.

Curry, F. E. (1984). Mechanics and thermodynamics of transcapillary exchange. In *Handbook of Physiology*, section 2, *The Cardiovascular System*, vol. IV, *Microcirculation*, part 1, ed. Renkin, E. M. & Michel, C. C., pp. 309–374. American Physiological Society, Bethesda, MD, USA.

CURRY, F. E., MASON, J. C. & MICHEL, C. C. (1976). Osmotic reflection coefficients of capillary walls to low molecular weight hydrophilic solutes measured in single perfused capillaries of the frog mesentery. *Journal of Physiology* **261**, 319–336.

CZACKES, J. W., ULLMANN, T. D., ULLMANN, L. & BAR-KOCHBA, Z. (1963). Determination of the red blood cell content of water, sodium, and potassium in normal subjects. *Journal of Laboratory and Clinical Medicine* **61**, 873–878.

DAVENPORT, H. W. (1958). *The ABC of Acid–Base Chemistry*, 4th edn, University of Chicago Press, Chicago.

DURBIN, R. P. (1960). Osmotic flow of water across permeable cellulose membranes. *Journal of General Physiology* **44**, 315–326.

FABER, J. J. & ANDERSON, D. F. (1990). Model study of placental water transfer and causes of fetal water disease in sheep. *American Journal of Physiology* **258**, R1257–1270.

FABER, J. J. & THORNBURG, K. L. (1983). *Placental Physiology*. Raven Press, New York.

MESCHIA, G. & BARRON, D. H. (1956). The effect of CO_2 and O_2 content of the blood on the freezing point of plasma. *Quarterly Journal of Experimental Physiology* **41**, 180–194.

MESCHIA, G., BATTAGLIA, F. C. & BARRON, D. H. (1957). A comparison of the freezing points of fetal and maternal plasmas of sheep and goat. *Quarterly Journal of Experimental Physiology* **42**, 163–170.

PATLAK, C. S., GOLDSTEIN, D. A & HOFFMAN, J. F. (1963). The flow of solute and solvent across a two membrane system. *Journal of Theoretical Biology* **5**, 426–442.

THORNBURG, K. L., BINDER, N. D. & FABER, J. J. (1979*a*). Diffusion permeability and ultrafiltration-reflection-coefficients of Na^+ and Cl^- in the near-term placenta of the sheep. *Journal of Developmental Physiology* **1**, 47–60.

THORNBURG, K. L., BINDER, N. D. & FABER, J. J. (1979*b*). Distribution of ionic sulfate, lithium and bromide across the sheep placenta. *American Journal of Physiology* **236**, C58–65.

WINER, B. J. (1971). *Statistical Principles in Experimental Design*. McGraw-Hill, New York.

WOLF, M. B. & WATSON, P. D. (1989). Measurement of osmotic reflection coefficient for small molecules in cat hind limbs. *American Journal of Physiology* **256**, H282–290.

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