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Extracellular volume and blood volume in chronically catheterized fetal sheep

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1. To determine the extracellular volume (ECV) in fetal sheep and its distribution between the plasma and interstitial spaces, ECV, blood volume (BV) and haematocrit were measured in ten chronically catheterized fetal sheep aged 121–133 days. Relationships with age, weight and other fetal variables, including glomerular filtration rate (GFR), were studied.

2. ECV was measured as the mean of the volumes of distribution of [^{3}H]inulin and [^{14}C]mannitol extrapolated to time zero. The time zero volume of distribution was 1506 ± 79 ml (means ± S.E.M.) for inulin and 1590 ± 80 ml for mannitol. The ECV was 1548 ± 79 ml (632 ± 18 ml (kg fetal wt)$^{-1}$). BV, measured using ^{51}Cr-labelled red cells, was 351 ± 27 ml (141 ± 6 ml kg^{-1}). Haematocrit, plasma volume and interstitial volume were 34 ± 1%, 229 ± 17 ml (92 ± 3 ml kg^{-1}) and 1319 ± 63 ml (540 ± 17 ml kg^{-1}), respectively.

3. Interstitial volume per kilogram fell with increasing fetal weight ($P = 0.026$). BV per kilogram did not change with weight or age.

4. The plasma : interstitial volume ratio was 0·17 ± 0·01. This ratio increased as fetal weight and age increased ($P = 0.026$ and $P = 0.044$), that is, the proportion of ECV that was contained outside the vascular compartment was lower in heavier or older fetuses.

5. Since GFR was 3·4 ± 0·4 ml min^{-1}, the entire fetal ECV was filtered by the fetal kidney only 3·1 ± 0·3 times per day.

During gestation in the human fetus, total body water decreases from 94 to 76%, extracellular volume (ECV) decreases from 62 to 43% and intracellular volume increases from 25 to 32% (Friis-Hansen, 1961). Little is known, however, about the distribution of the fetal ECV between the plasma and interstitial compartments.

In the late gestation sheep fetus, total body water is approximately 82% (Langlands & Sutherland, 1968; Rattray, Garrett, East & Hinman, 1974). Although a number of measurements of blood volume (BV) and plasma volume (PV) have been made in fetal sheep (Creasy, Drost, Green & Morris, 1970; Broughton Pipkin & Kirkpatrick, 1973; Brace, 1983; Kingsford & Lumbers, 1989), there are few measurements of ECV. Devaskar, Devaskar & Kleinman (1985), using [^{14}C]inulin, found that the ECV decreased in chronically catheterized fetal sheep from 59% of body weight at 120 days gestation to 34% at 145 days. However, [^{14}C]inulin was given as a bolus injection, whereas in non-nephrectomized animals a continuous infusion of inulin is required to compensate for urinary losses until equilibration is reached (Kruhoffer, 1946). As well, Devaskar *et al.* (1985) did not measure fetal weight but estimated it using data obtained from a different breed of sheep. Therefore, their estimates of ECV as a percentage of body weight are possibly inaccurate.

In a study that measured placental permeability, Thornburg, Binder & Faber (1979) determined the sodium and chloride spaces in chronically catheterized fetuses in the last third of gestation. Since these ions are mainly extracellular, these volumes (584 ± 24 ml kg^{-1} for sodium and 760 ± 51 ml kg^{-1} for chloride) could provide estimates of fetal ECV, although because these ions enter cells, they may be overestimates (Cheek, West & Golden, 1957).

More recently, Brace & Moore (1991) have estimated acute changes in ECV by following changes in plasma protein concentration. They used a value for the initial ECV of 320 ml kg^{-1}. This value was based on an assumed plasma : interstitial ratio of 1 : 3 (Brace, 1989), which was determined by kinetic analysis of the disappearance of atrial natriuretic peptide (ANF) after a bolus injection (Brace & Cheung, 1987). ANF is not a marker that is conventionally used to measure ECV, and it is possible that the ratio of 1 : 3 does not truly reflect the plasma : interstitial ratio in the fetus, because the value of 320 ml kg^{-1} for ECV is much lower than that measured in fetuses by others (Friis-Hansen, 1961; Thornburg *et al.* 1979; Devaskar *et al.* 1985). Moreover, despite the fact that this value includes the ECV of the extra-fetal tissues, it is lower than values measured after birth (Friis-Hansen, 1961; Longo, Allen, Niswonger, Pagel, Wieland & Gilbert,

1978; Wong, Sheng, Morkeberg, Kosanovich, Clarke & Klein, 1989).

In this study, both ECV and BV were measured in ten chronically catheterized fetal sheep aged 121–133 days. PV, interstitial volume and the plasma : interstitial volume ratio were also calculated, and relationships between ECV per kilogram and BV per kilogram and other fetal variables (weight, age, arterial pressure, blood gases, plasma electrolytes, lung liquid production and various renal parameters) were examined by simple linear regression.

METHODS

All procedures and protocols were approved by the Animal Care and Ethics Committee, University of New South Wales.

Surgical preparation

Cross-bred Merino ewes were anaesthetized with 1·5 g of sodium thiopentone (Pentothal; Abbott, Kurnell, NSW, Australia) I.V., intubated and maintained with 2–3 % halothane (Fluothane; ICI) in oxygen. As previously described, catheters were placed in a carotid artery and jugular vein of the ewe and into a femoral artery, both tarsal veins, the amniotic cavity and the bladder of the fetus (Lumbers & Stevens, 1983). The suprapubic bladder catheter ensured complete collection of fetal urine during experiments when it was drained under gravity, but enabled voiding into the amniotic and allantoic cavities at other times (Stevens, 1987). The trachea was cannulated with a T-piece cannula. This connected to an intrauterine latex bag into which lung liquid collected when it was not being drained to the exterior (Lumbers, Smith & Stevens, 1985). To prevent ingestion of amniotic fluid during ECV measurements the oesophagus was ligated. Although the fetus normally swallows up to 800 ml day^{-1}, oesophageal ligation in the sheep does not lead to polyhydramnios (Wintour *et al.* 1978), presumably because of increased absorption via the intramembranous pathway (Gilbert & Brace, 1989). Procaine penicillin (600 mg) and dihydrostreptomycin sulphate (750 mg) (Hydropen; Bomac Laboratories, Asquith, NSW, Australia) were given I.M. to the ewe and into the amniotic cavity at the end of surgery and into the amniotic cavity on the following 2 days.

Animal care

Ewes were housed in metabolic cages. They ate lucerne chaff and oats and had free access to water. Each day lung liquid was drained and discarded, and vascular catheters were flushed with heparinized saline (100 units ml^{-1}). At least 6 days were allowed for recovery from surgery prior to experimentation.

Experimental protocol

Urine and lung liquid were drained for at least 40 min before the experiment commenced, and samples of these fluids and fetal and maternal plasma were taken so that their background radioactivities could be measured. The ewe and the fetus were given I.V. lithium chloride (150 and 250 μmol kg^{-1}, respectively). At a known time ('time zero'), the fetus was given weighed loading doses of [^{3}H]inulin (3·6 μCi kg^{-1}; Amersham, UK) and [^{14}C]mannitol (1·8–3·6 μCi kg^{-1}; Amersham) mixed together in 0·15 M saline (4 ml), and flushed in with 5 ml of 0·15 M saline. A continuous infusion of [^{3}H]inulin (0·6 μCi kg^{-1} h^{-1}), [^{14}C]mannitol (0·3–0·6 μCi kg^{-1} h^{-1}) and lithium chloride (10 μmol kg^{-1} h^{-1}), contained in 0·15 M saline, was commenced within 2 min and continued for at least 3 h at 0·95 ml h^{-1}. Before this infusion commenced, the catheter dead space was filled with the same solution. The total time of infusion and the weight of solution that was infused were measured.

All urine and lung liquid were collected every 30 min for at least 3 h. Fetal (3·5–4 ml) and maternal (4 ml) blood samples were taken every 60 min and replaced with an equal volume of heparinized saline (100 u ml^{-1}). So that all radioactivity removed from the fetus could be accounted for, the blood that was removed from the catheter before taking a sample (in order to obtain pure blood) was re-injected. Maternal and fetal arterial pressures and heart rate were recorded continuously using pressure transducers (Bell & Howell, Pasadena, CA, USA) and a polygraph (Model 7 or 79D; Grass Instrument Co, Quincy, MA, USA). Fetal arterial pressure was corrected for amniotic pressure.

Measurement of ECV

ECV was measured by indicator dilution technique using [^{3}H]inulin and [^{14}C]mannitol. To determine the exact amounts of ^{3}H and ^{14}C injected into the fetus, weighed aliquots (approximately 1 ml) of both the loading dose and infusion (see above) were diluted to 100 ml in volumetric flasks. Ten samples of each diluted aliquot were treated and counted as described below. To determine the exact amounts of ^{3}H and ^{14}C removed from the fetus during the course of experiment, the amounts removed in urine, lung liquid and in fetal plasma samples were measured. To determine if any significant loss of indicator occurred as a result of placental transfer, maternal urine was collected for 48 h from the beginning of the experiment.

Before counting, samples of plasma, urine, lung liquid, diluted loading dose and diluted infusion were all treated identically to ensure equal quenching. Samples (600 μl) were mixed with 600 μl of 10% trichloroacetic acid to precipitate protein, and spun at 3000 r.p.m. for 10 min. Then 600 μl of supernatant was transferred to a glass scintillation vial, and 600 μl of 5% trichloroacetic acid and 9·5 ml of scintillant were added. Each vial was counted for 10 min on a Packard Tri-Carb Liquid Scintillation System (Model 1900TR; Packard, Meriden, CT, USA). The counts obtained were corrected to 1 ml of sample.

The volume of distribution (V_d) of each isotope at 1, 2 and 3 h was calculated using the following equations:

$$C_{\mathrm{inj},t} = C_{\mathrm{LD}} + C_{\mathrm{inf},t}, \qquad (1)$$

where $C_{\mathrm{inj},t}$ is the counts injected into the fetus by time t, C_{LD} is counts in the loading dose and $C_{\mathrm{inf},t}$ is counts infused by time t.

$$C_{\mathrm{lost},t} = C_{\mathrm{lostu},t} + C_{\mathrm{lostll},t} + C_{\mathrm{lostpl},t}, \qquad (2)$$

where $C_{\mathrm{lost},t}$ is the total counts removed from the fetus by time t, and $C_{\mathrm{lostu},t}$, $C_{\mathrm{lostll},t}$ and $C_{\mathrm{lostpl},t}$ are the counts removed in urine, lung liquid and previous fetal plasma samples by time t.

$$C_{\mathrm{rem},t} = C_{\mathrm{inj},t} - C_{\mathrm{lost},t}, \qquad (3)$$

where $C_{\mathrm{rem},t}$ is the counts remaining in the fetus at time t.

$$V_{\mathrm{d},t} = C_{\mathrm{rem},t}/\,C_{\mathrm{pl},t}, \qquad (4)$$

where $V_{\mathrm{d},t}$ is the V_d at time t (ml) and C is the counts in 1 ml of fetal plasma at time t.

Regression lines were computed for V_d against time to determine the V_d at 'time zero' (i.e. the y-intercept of the regression line). For convenience, this method of measuring the inulin and mannitol spaces will be referred to as the 'V_d extrapolation' method. ECV was calculated as the mean of the volume of distribution for inulin

($V_{d,in}$) and the volume of distribution of mannitol ($V_{d,man}$) at time zero. Interstitial volume was calculated as ECV − PV.

Measurement of inulin and mannitol spaces by the recovery method

To determine whether either inulin or mannitol were metabolized or penetrated the intracellular compartment, inulin and mannitol spaces were measured in a different group of six fetuses (aged 123–133 days) using both the V_d extrapolation method (above) and the recovery method (Gaudino, Schwartz & Levitt, 1948). In these experiments, [^{3}H]inulin and [^{14}C]mannitol were given I.V. to the fetus as loading doses followed by a combined continuous infusion for 5 h, in doses as described above. At the end of 5 h, the infusion was stopped and a fetal plasma sample was collected. To measure the total amounts of the indicators present in the fetus when the infusion was stopped, all fetal urine, lung liquid and maternal urine were collected for the following 2 days. In four fetuses, these fluids were collected for a further 5 days, but this additional collection period only increased the amount recovered by $3{\cdot}5 \pm 1{\cdot}0$% and $3{\cdot}3 \pm 0{\cdot}8$% of the injected doses of inulin and mannitol, respectively. Inulin and mannitol spaces were calculated by dividing the counts recovered during the 2 days following the infusion by the fetal plasma counts at the time the infusion was stopped. Using this recovery method, the calculated inulin and mannitol spaces are not falsely elevated by metabolism or intracellular penetration of the indicators, since indicator which has been metabolized or has entered cells will not be recovered (White & Rolf, 1957).

Measurement of BV

BV was measured by indicator dilution technique using ^{51}Cr-labelled red blood cells. For labelling, 4 ml of fetal blood was collected into 6 ml of citrate phosphate solution (trisodium citrate, 3·0 g; sodium dihydrogen phosphate, 0·015 g; dextrose, 0·2 g; water to 100 ml; Garby & Mollison, 1971), centrifuged and the supernatant discarded. The red cell pellet was incubated for 10 min at 37 °C with 20–50 μCi of ^{51}Cr-labelled sodium chromate (Amersham). To remove any unbound ^{51}Cr, the cells were washed 3 times with 0·15 M saline. They were then resuspended in 0·15 M saline and reinjected into the fetus. Using this procedure, counts in the supernatant of the third wash were $0{\cdot}3 \pm 0{\cdot}1$% ($n = 16$) of the counts injected. A value of < 1% is recommended (Brace, 1984).

Because ^{51}Cr interfered with the counting of [^{3}H]inulin and [^{14}C]mannitol, it was important that ^{51}Cr levels in all fetal fluids were constant during the course of the experiment. To achieve this, since elution of ^{51}Cr from the red cells declines exponentially (K. Gibson & E. Lumbers, unpublished observations), the cells were labelled and injected the night before the experiment. Absolute BV was obtained 1 h later. BV obtained at 1 h is similar to that obtained soon after injection (Brace, 1983; K. Gibson, unpublished observations). The dose of radioactivity injected was accurately determined as previously described by Kingsford & Lumbers (1989). ^{51}Cr was counted in a Packard Auto Gamma counter (Model 5650; Packard, Downers Grove, IL, USA).

PV was calculated as BV × (100 − haematocrit)/100. Red cell volume was calculated as BV × haematocrit/100.

Biochemical analysis

Arterial blood gases at 39·5 °C were determined using a Ciba-Corning blood gas system (Model 288; Medfield, MA, USA). Plasma bicarbonate concentration was calculated using a modified Henderson–Hasselbalch equation (Armentrout, Katz, Thornburg & Faber, 1977). Haematocrit was measured in duplicate using a haematocrit centrifuge and reader (Hettich, Tuttlingen, Germany). The remaining blood was centrifuged at 2500 r.p.m. for 10 min. Plasma, urine and lung liquid samples were stored at −20 °C.

Sodium and potassium levels were measured using a flame photometer (Model FLM 3; Radiometer, Copenhagen, Denmark). Chloride concentrations were measured using a chloride titrator (Model CMT 10, Radiometer). Osmolality was measured using a Fiske One-Ten osmometer (Needham Heights, MA, USA). Glomerular filtration rate (GFR) was measured as the clearance of [^{3}H]inulin. The fractional reabsorption of sodium by the proximal tubule was calculated from the formula: 1 − clearance of lithium/GFR. The fractional reabsorption of sodium by the distal tubule was calculated from the formula: clearance of lithium/GFR − clearance of sodium/GFR (Lumbers, Hill & Bennett, 1988). Lithium concentrations were measured using a Perkin-Elmer 272 atomic absorption spectrometer (Norwalk, CT, USA).

Analysis of data

At the time of the experiment, fetal body weight was estimated (BWE_{est}) from gestation age using a formula derived from postmortem weights and ages of eighty-four fetuses in this laboratory:

$$\text{Weight} = \text{age}^{2{\cdot}94182} / 530{\cdot}055,$$

where weight is in grams and age is in days. This formula was also used to determine the expected weight at the age of death (BWD_{est}). For analysis of data, the true body weight at the time of the experiment (BWE) was calculated by multiplying the measured fetal weight at death (BWD) by the ratio $BWE_{est}:BWD_{est}$ (Lumbers *et al.* 1985). To minimize error in BWE, a postmortem to obtain BWD was always carried out within 6 days of the experiment. For this, the ewe was killed with I.V. pentobarbitone sodium (6 g; Valobarb-Euthanasia Solution, Animal Health Australia Ltd, West Footscray, VIC, Australia). Also at postmortem, the placenta (cotyledons shelled from the caruncles) and membranes were weighed.

All results are expressed as means ± S.E.M. in ten fetuses. Calculated variables were derived using an IBM compatible personal computer and Statistical Package for the Social Sciences (SPSS/PC; SPSS Inc., Chicago, IL, USA). To examine the relationships between variables, simple linear regressions were computed by the method of least squares, and considered significant at $P < 0{\cdot}05$. Multivariate regressions were not performed.

RESULTS

The ten fetuses (six singletons and four twins) were aged 127 ± 1 days and weighed $2{\cdot}47 \pm 0{\cdot}16$ kg. Fetal weight was highly correlated with age ($r = 0{\cdot}904$, $P < 0{\cdot}001$). The placentae and membranes weighed 253 ± 26 g and 267 ± 31 g, respectively. Thus, total weight (fetus plus extrafetal tissues) was $2{\cdot}99 \pm 0{\cdot}17$ kg.

Fetal arterial blood gases (pH $7{\cdot}37 \pm 0{\cdot}01$; oxygen and carbon dioxide tensions, 22 ± 1 and 54 ± 1 mmHg, respectively), plasma electrolytes (mmol l^{-1}: sodium, $147{\cdot}7 \pm 0{\cdot}8$; potassium, $3{\cdot}65 \pm 0{\cdot}11$; chloride, 107 ± 1; and bicarbonate, $30{\cdot}0 \pm 0{\cdot}5$), plasma osmolality (282 ± 1 mosmol kg^{-1}), systolic and diastolic pressures (56 ± 2 and 36 ± 1 mmHg, respectively) and heart rate (183 ± 2 beats min^{-1}) were within the normal ranges for

this laboratory. Fetal urinary osmolality was 155 ± 18 mosmol kg^{-1}. Fetal plasma bicarbonate fell with increasing gestation age ($r = -0.682$; $P = 0.03$) and weight ($r = -0.821$; $P = 0.004$). Fetal heart rate fell with increasing gestation age ($r = -0.774$; $P = 0.009$).

In the 48 h after the experiment began, only 3.3 ± 0.8% of the dose of inulin given to the fetus and 4.1 ± 0.9% of the dose of mannitol were recovered in maternal urine.

Body fluid compartments

ECV

The time zero volumes of distribution of inulin ($V_{d,in}$) and mannitol ($V_{d,man}$) were 1506 ± 79 and 1590 ± 80 ml. There was a good correlation between $V_{d,in}$ and $V_{d,man}$ ($r = 0.981$; $P < 0.001$, Fig. 1). $V_{d,in}$ was always lower than $V_{d,man}$ (by 84 ± 16 ml; $P < 0.001$) and the ratio of $V_{d,in} : V_{d,man}$ was 0.95 ± 0.01. ECV (the mean of $V_{d,in}$ and $V_{d,man}$) was 1548 ± 79 ml.

ECV was highly correlated with fetal weight ($r = 0.904$; $P < 0.001$; Fig. 2) and age ($r = 0.854$; $P < 0.002$). Expressed relative to body weight, ECV was 632 ± 18 ml kg^{-1} (Fig. 3). Although it was not significant, there was a tendency for larger fetuses to have a lower ECV per kilogram ($r = -0.621$; $P = 0.055$).

BV

BV was 351 ± 27 ml or 141 ± 6 ml kg^{-1} (Fig. 3). Like ECV, BV was related to fetal weight ($r = 0.841$; $P < 0.003$) and age ($r = 0.752$; $P = 0.012$). Therefore it was not surprising that fetal BV and ECV were related ($r = 0.834$; $P < 0.003$). There were no relationships between BV per kilogram and weight, age or ECV per kilogram.

PV and red cell volume

Fetal haematocrit was 34 ± 1%; it did not correlate with fetal weight or age. Calculated PV was 229 ± 17 ml or 92 ± 3 ml kg^{-1} (Fig. 3) and red cell volume was 122 ± 13 ml or 49 ± 4 ml kg^{-1}.

PV correlated with fetal weight ($r = 0.912$; $P < 0.001$), age ($r = 0.866$; $P < 0.002$) and ECV ($r = 0.947$; $P < 0.001$), but there was no relationship between PV per kilogram and ECV per kilogram. Red cell volume tended to be higher in heavier fetuses but this was not significant ($r = 0.590$; $P = 0.072$). There was also no correlation between red cell volume and age or ECV. Neither PV per kilogram nor red cell volume per kilogram correlated with weight or age.

Interstitial volume

Calculated interstitial volume (ECV – PV) was 1319 ± 63 ml. Interstitial volume correlated with fetal weight ($r = 0.886$; $P < 0.001$), age ($r = 0.836$; $P < 0.003$) and ECV ($r = 0.996$; $P < 0.001$). Interstitial volume expressed per kilogram of body weight was 540 ± 17 ml kg^{-1} (Fig. 3). Interstitial volume per kilogram was closely correlated with ECV per kilogram ($r = 0.986$; $P < 0.001$). As well, interstitial volume per kilogram was lower in heavier fetuses ($r = -0.694$; $P = 0.026$). This, plus the fact that PV per kilogram was not related to weight, suggests that the tendency for ECV per kilogram to be lower in heavier fetuses ($P = 0.055$) was due to a reduction in interstitial volume per kilogram with increasing fetal weight.

The ratio of plasma to interstitial volume was 0.17 ± 0.01. This ratio correlated with fetal weight ($r = 0.695$; $P = 0.026$; Fig. 4) and age ($r = 0.646$; $P = 0.044$), i.e. the

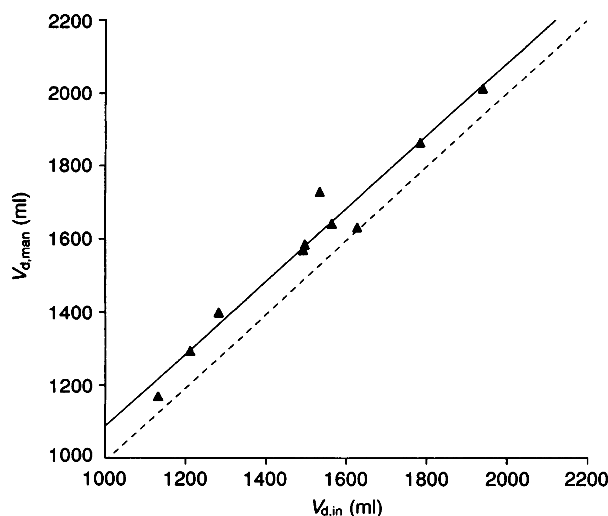

Figure 1. Comparison of the two indicators used to measure ECV

The volume of distribution at time zero of mannitol ($V_{d,man}$) against the simultaneously measured volume of distribution at time zero of inulin ($V_{d,in}$). Equation for the regression line: $y = 0.97x - 32.2$; $r = 0.981$; $P < 0.001$; $n = 10$. The dashed line is the line of identity.

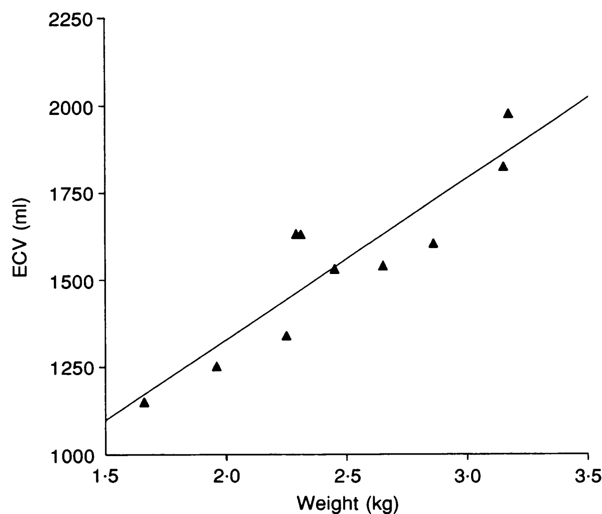

Figure 2. Extracellular volume (ECV) plotted against fetal body weight
Equation for the regression line: $y = 461x + 407$; $r = 0{\cdot}904$; $P < 0{\cdot}001$; $n = 10$.

proportion of ECV that was contained outside the vascular compartment was lower in heavier or older fetuses. Not surprisingly, the plasma to interstitial volume ratio also correlated with BV per kilogram ($r = 0{\cdot}662$; $P = 0{\cdot}037$), i.e. the proportion of the ECV in the vascular compartment was higher if the fetuses had a higher BV relative to their body weight. The ratio of plasma : interstitial volume was not related to fetal mean arterial pressure.

Volumes of body fluid compartments expressed relative to total weight

When corrected for total weight (i.e. fetus plus extra-fetal tissues), the volumes of the fluid compartments were

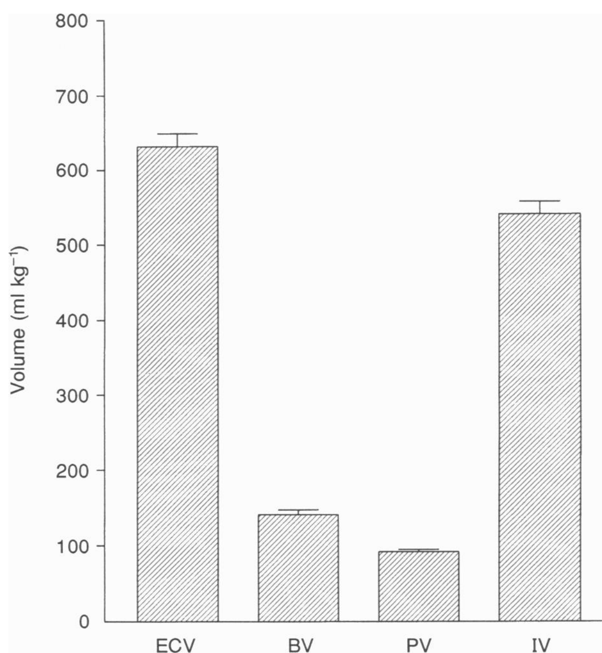

Figure 3. Fetal body fluid compartments
Extracellular volume (ECV), blood volume (BV), plasma volume (PV) and interstitial volume (IV) are all in ml kg^{-1}. Values are means ± S.E.M., $n = 10$.

(ml kg^{-1}): ECV, 519 ± 8; BV, 116 ± 6; PV, 76 ± 2; red cell volume, 41 ± 4; and interstitial volume, 444 ± 9.

Lung liquid function

Lung liquid flow rate was 0·19 ± 0·02 ml min^{-1}. It increased with increasing fetal weight ($r = 0·715$; $P = 0·020$) and age ($r = 0·854$; $P = 0·002$). Lung liquid flow per kilogram of fetal weight was 0·08 ± 0·01 ml min^{-1} kg^{-1}. Lung liquid sodium, potassium and chloride levels were 150·5 ± 0·7, 4·21 ± 0·13 and 151 ± 0·5 mmol l^{-1}, respectively, and osmolality was 295 ± 1 mosmol kg^{-1}. Lung liquid chloride levels fell with increasing fetal weight ($r = -0·755$; $P = 0·012$) and age ($r = -0·689$; $P = 0·028$).

Renal function

GFR was 3·4 ± 0·4 ml min^{-1}. It increased with increasing fetal weight ($r = 0·663$; $P = 0·037$) and age ($r = 0·692$; $P = 0·027$). Corrected for fetal body weight, there was no correlation between fetal GFR (1·4 ± 0·1 ml min^{-1} kg^{-1}), urine flow rate (0·18 ± 0·02 ml min^{-1} kg^{-1}), free water clearance (0·08 ± 0·01 ml min^{-1} kg^{-1}), and sodium, potassium and chloride excretion rates (7·7 ± 1·9, 1·1 ± 0·5 and 4·4 ± 1·5 μmol min^{-1} kg^{-1}) with weight and age. There was also no correlation between the fractional reabsorptions of sodium (96·4 ± 0·9%), potassium (77·8 ± 9·3%) and chloride (97·1 ± 1·0%), or the fractional reabsorptions of sodium by the proximal (59·8 ± 4·2%) and distal (36·6 ± 3·6%) tubules with weight and age.

Relationships between ECV per kilogram and BV per kilogram and other variables

There were no relationships between ECV per kilogram or BV per kilogram and fetal blood gases, arterial pressure or heart rate. However, BV per kilogram was inversely related to fetal plasma osmolality ($r = -0·631$; $P = 0·050$).

There were no relationships between either ECV per kilogram or BV per kilogram and lung liquid flow rate or composition, except that lung liquid chloride levels were higher in fetuses with a higher ECV per kilogram ($r = 0·673$; $P = 0·033$). This relationship was probably due to the dependence of both lung liquid chloride and ECV per kilogram on fetal weight (see above).

The fractional reabsorptions of sodium, potassium and chloride were inversely related to BV per kilogram ($r = -0·703$, $P = 0·023$; $r = -0·775$, $P = 0·009$; and $r = -0·654$, $P = 0·040$ – respectively) and potassium excretion per kilogram was directly related to BV per kilogram ($r = 0·687$; $P = 0·028$). No other relationships between ECV per kilogram or BV per kilogram and renal function were found.

When GFR was expressed on a daily basis (4·9 ± 0·6 l day^{-1}), it was possible to calculate that the entire ECV was filtered 3·1 ± 0·3 times per day; the entire PV was filtered 21·7 ± 1·9 times per day.

Inulin and mannitol spaces measured by the recovery method

Inulin spaces measured by the recovery method (1696 ± 160 ml, $n = 5$) were similar to those measured by V_d extrapolation (1563 ± 121 ml, $n = 5$, n.s.). Likewise, mannitol spaces measured by the two methods were similar (1807 ± 111 ml, $n = 6$ and 1813 ± 135 ml, $n = 6$, respectively).

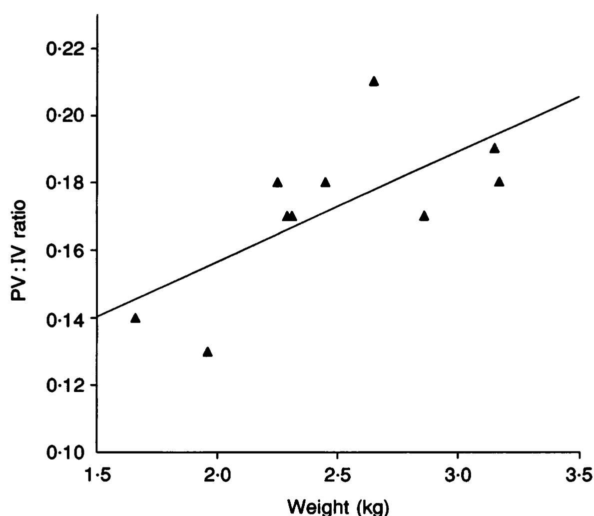

Figure 4. Changing distribution of the ECV

The ratio of plasma : interstitial volume (PV : IV) plotted against weight. Equation for the regression line: $y = 0·031x + 0·095$; $r = 0·695$; $P = 0·026$; $n = 10$.

DISCUSSION

To our knowledge this is the first study to measure both ECV and BV in chronically catheterized fetal sheep.

Fetal BV (351 ± 27 ml) was similar to the mean BV measured in twenty-seven fetuses by Brace (1984) using ^{51}Cr-labelled red cells (352 ± 16 ml). However, when both data were corrected for fetal body weight, our BV per kilogram was higher (141 ± 6 ml kg^{-1} compared with 111 ± 2 ml kg^{-1}; $P < 0{\cdot}001$). This difference may be related to the breed of sheep that was used. Broughton Pipkin & Kirkpatrick (1973) noted that although fetal BV was similar for the same gestation in different breeds, BV per kilogram varied between breeds, i.e. the smaller breeds tended to have higher BV per kilogram than larger breeds. The cross-bred Merino fetuses we studied were much smaller than the fetuses studied by Brace (1984) ($2{\cdot}47 \pm 0{\cdot}16$ kg compared with $3{\cdot}18 \pm 0{\cdot}14$ kg).

Alternatively, the difference may relate to animal husbandry. In Australia, intermittent droughts occur and consequently the quality and quantity of feed may vary considerably during early pregnancy before the sheep come into the laboratory to standardized conditions. We have found some variation in fetal BV per kilogram according to the year of study. In early 1993, BV per kilogram was 116 ± 4 ml kg^{-1} in eight fetuses aged 132–137 days and weighing $2{\cdot}86 \pm 0{\cdot}17$ kg (K. Gibson, unpublished observations). This value was much lower than in the current study (1990–1991), and similar to values obtained by Broughton Pipkin & Kirkpatrick (1973) and Brace (1984).

Within the group of fetuses, BV was positively correlated with weight and age, but the BV per kilogram was constant over the age range studied. This confirms previous findings (Creasy *et al.* 1970; Broughton Pipkin & Kirkpatrick, 1973; Brace, 1983).

To measure ECV, we used two markers, inulin and mannitol. We found that the volume of distribution of mannitol was consistently greater than that of inulin (by 84 ± 16 ml; $P < 0{\cdot}001$). This difference may have been partly due to mannitol penetrating liver intracellular water (Glasby, 1985). The liver weighs 107 ± 4 g ($n = 29$) in fetuses aged 124–137 days (N. Kingsford & E. Lumbers, unpublished observations). Assuming the liver has extracellular and total water contents similar to the fetus as a whole, about 20 ml, or 25%, of the difference between inulin and mannitol spaces is explained. The remaining 75% is probably accounted for by the smaller size of mannitol, allowing greater penetration of tissues (Law, 1982). As well, mannitol is small enough to cross the sheep placenta (Boyd, Haworth, Stacey & Ward, 1976; Basso, Fernandez, Althabe, Sabini, Piriz & Belitzky, 1977). However, since only small amounts of both inulin and mannitol were recovered in maternal urine in the 48 h after the experiment began, it is unlikely that placental transfer of mannitol contributed significantly to the higher mannitol volume of distribution during the experiment.

Our value for ECV per kilogram (632 ± 18 ml kg^{-1}), which was a mean of the inulin and mannitol spaces, was smaller than the fetal chloride space ($P < 0{\cdot}05$ by unpaired Student's t test) but was similar to the fetal sodium space measured by Thornburg *et al.* (1979). Although our value for inulin space (1506 ± 79 ml) was slightly higher than that obtained by Devaskar *et al.* (1985), this may be due to differences in breeds of sheep and animal husbandry or, as mentioned above, the lack of a continuous infusion in the study of Devaskar *et al.* (1985). Importantly, all three studies found the fetal ECV per kilogram to be larger than the 320 ml kg^{-1} assumed by Brace (1989) and used by Brace & Moore (1991). Presumably the high clearance of ANF (Brace & Cheung, 1987) prevents it distributing throughout the whole ECV after a single bolus injection (Kruhoffer, 1946). Hence the plasma : interstitial ratio obtained with ANF was 1 : 3 (Brace & Cheung, 1987; Brace, 1989), whereas it was 1 : 6 in the current study.

In all three studies in which conventional indicators for measuring ECV were used (this study; Thornburg *et al.* 1979; Devaskar *et al.* 1985) values obtained for fetal ECV per kilogram were higher than the ECV per kilogram in adult sheep (245 ± 9 ml kg^{-1}; Coghlan, Fan, Scoggins & Shulkes, 1977). Partly this is because all three fetal measurements include the ECV of placenta and membranes as well as the fetal body. Therefore, we divided ECV by total weight (i.e. the weight of the fetus, placenta and membranes). This value (519 ± 8 ml kg^{-1}) was still higher than the adult value, which is consistent with findings in humans (Friis-Hansen, 1961). We did not attempt to study the division of the ECV between the fetal body and extra-fetal tissues. However, since up to 30% of the BV can be contained within the extra-fetal tissues (Barcroft & Kennedy, 1939; Gibson & Lumbers, 1994), it is possible that these tissues may also have a relatively high ECV, i.e. although the mean ECV per kilogram is about 520 ml kg^{-1}, the ECV per kilogram of the fetal body may be lower than this, and the ECV per kilogram of the extra-fetal tissues correspondingly higher.

The agreement between ECV measured with inulin and mannitol in the current study and the sodium space of Thornburg *et al.* (1979) is perhaps surprising when one considers that in adult animals saccharide spaces are generally lower than the sodium space. This is because sodium is not completely excluded from cells (Cheek *et al.* 1957) and the saccharides penetrate dense connective tissue poorly (Law, 1982). However, Friis-Hansen (1961) noted that in the newborn there was good agreement between different extracellular indicators. Perhaps the ECV in the

fetus and newborn is more 'fluid' than in the adult, so that even slowly penetrating indicators, like inulin, can more readily penetrate all the interstitial water. In support of this, the ratio of the inulin space to the mannitol space in the fetus ($0{\cdot}95 \pm 0{\cdot}01$) was closer to unity than in nephrectomized adult dogs (0·74; Swan, Madisso & Pitts, 1954). As well, hyaluronidase increases the inulin space in some adult tissues (Law, 1982).

Another explanation for the good agreement between sodium and saccharide spaces in the fetus relates to the relative sizes of the ECV and intracellular compartments. The total water content of the ovine fetus and its extrafetal tissues is about 827 ml kg^{-1} (since the water content of the fetus and placenta/membranes are 82 and 86%, respectively; Langlands & Sutherland, 1968; Rattray *et al.* 1974). We found the ECV was 519 ml kg^{-1}. Therefore the intracellular compartment, at only 308 ml kg^{-1}, is relatively small compared with the ECV. Thus, the error introduced into ECV measurement by intracellular penetration of sodium must be much smaller than in the adult, where the ECV is relatively small and the intracellular compartment into which the ions can escape is relatively large.

Alternatively, the good agreement might be due to both inulin and mannitol penetrating fetal cells (in addition to mannitol penetrating liver cells, see above) or being metabolized. However, this is unlikely since inulin and mannitol spaces obtained by V_d extrapolation were similar to those obtained using the recovery method.

Like BV, ECV also increased with the weight and age of the fetus. However, unlike BV per kilogram, ECV per kilogram was not constant over the gestation range studied; rather, although not significant, it tended to decrease as fetal weight increased ($P = 0{\cdot}055$). Devaskar *et al.* (1985), studying a greater age range (120–145 days), found that the inulin space expressed per kilogram of body weight decreased with increasing gestation age. Thus, it seems that in the sheep fetus, like the human (Friis-Hansen, 1961), the ECV per kilogram decreases progressively in late gestation. Because it was possible for us to calculate PV and interstitial volume, we could determine that this decrease is mainly due to a reduction in interstitial volume per kilogram with increasing weight ($P = 0{\cdot}026$).

The mean plasma : interstitial volume ratio was 0·17. This value was slightly lower ($P < 0{\cdot}001$) than that obtained in adult sheep ($0{\cdot}212 \pm 0{\cdot}007$; Coghlan *et al.* 1977). Therefore, in the fetus, not only was the ECV per kilogram high compared with the adult, but a greater proportion of this large ECV was outside the vascular compartment. This difference probably occurred because fetuses have lower plasma colloid osmotic pressure than adults (Brace & Christian, 1981; Lumbers, Moore, Stevens & Gibson, 1991), although their lower capillary hydrostatic pressure would tend to offset this (Brace & Christian, 1981).

Interestingly, the plasma : interstitial volume ratio was higher in heavier fetuses (Fig. 4), i.e. the proportion of the ECV that was contained outside the vascular compartment was lower in heavier fetuses. A decrease in this interstitial proportion with growth is also seen by comparing the plasma : interstitial volume ratio of 0·12 in premature babies at 70% of gestation (Bauer, Bovermann, Roithmaier, Gotz, Prolss & Versmold, 1991), with the ratio of 0·17 in the current study (at 85% of gestation) and the ratio of 0·19 in lambs at 1–3 weeks of age (Longo *et al.* 1978). Similarly, in the first 2 years of childhood, ECV per kilogram decreases markedly but BV per kilogram does not change (Ely & Sutow, 1952). These findings suggest that the rise in plasma : interstitial volume ratio seen in Fig. 4 is part of a gradual trend that begins earlier in gestation and continues after birth until adult values are obtained.

During late gestation, the increase in the plasma : interstitial volume ratio might be due to the increase in plasma colloid osmotic pressure with age (Lumbers *et al.* 1991), which would tend to hold fluid in the vascular compartment, and the rise in lymphatic flow with age (Humphries, Normand, Reynolds & Strang, 1967), which would return escaped fluid to the vascular compartment. The effect of the rise in plasma colloid osmotic pressure may be magnified by a fall in capillary permeability to protein with age (Brace, 1989). By contrast, if capillary hydrostatic pressure rises during late gestation (Brace & Christian, 1981; Lumbers *et al.* 1991) this would tend to reduce the proportion of ECV in the vascular compartment.

A number of variables we measured were found to be dependent on fetal weight and/or age. Many of these relationships have been described previously, e.g. the fall in heart rate with age (Robillard, Matson, Sessions & Smith, 1979; Hill & Lumbers, 1988), the rise in lung liquid with weight and age (Mescher, Platzker, Ballard, Kitterman, Clements & Tooley, 1975) and the rise in GFR with weight and age (Robillard, Sessions, Kennedy, Hamel-Robillard & Smith, 1977; Kesby & Lumbers, 1986). However, the fall in fetal bicarbonate levels with weight and age was unexpected, especially considering that generation of bicarbonate by the fetal kidney increases with age (Kesby & Lumbers, 1986; Hill & Lumbers, 1988). The fall in lung liquid chloride concentration with weight and age was also unexpected because others have found either no change (Mescher *et al.* 1975) or, in acutely prepared animals, a rise (Olver, Schneeberger & Walters, 1981).

Several relationships were found between BV per kilogram and other variables. The inverse relationship between BV

per kilogram and fetal plasma osmolality ($P = 0{\cdot}05$) was probably due to variations in fetal hydration; a fetus that is well hydrated would have a higher BV per kilogram and a lower plasma osmolality (see also Stevens & Lumbers, 1990). The inverse relationships between the fractional reabsorptions of electrolytes and BV per kilogram (see also Stevens & Lumbers, 1990) were probably, in part, due to ANF. This hormone depresses tubular reabsorption of electrolytes (Castro, Ervin, Leake, Ross, Sherman & Fisher, 1991) and is released when fetal BV is expanded (Brace & Moore, 1991).

No relationships between ECV per kilogram and renal function were found. This was not unexpected because these relationships were only sought in a group of apparently normal fetuses in which there was no experimental manipulation of either ECV or renal function. Manoeuvres which would be expected to alter fetal ECV, like expansion with saline and contraction by dialysis, have been shown to affect fetal renal function (Hurley, Kirkpatrick, Pitlick, Friedman & Mendoza, 1977).

It is well established that the fetus has a low GFR relative to the adult when expressed per kilogram of body weight. Adult GFR per kilogram is approximately 1·5 times higher than fetal GFR per kilogram (Hill & Lumbers, 1988). However, it has been suggested that a better measure of kidney effectiveness is to relate GFR to ECV rather than to body weight (Brochner-Mortensen, 1980). If this is done, the fetal kidney is even less effective relative to the adult. An adult sheep has a GFR of 2·4 ml min^{-1} kg^{-1} (Hill & Lumbers, 1988) and an ECV of 245 ml kg^{-1} (Coghlan *et al.* 1977). Thus the adult sheep kidney filters the entire ECV about 14 times a day compared with 3 times a day in the fetus.

In summary, both ECV and BV have been measured in chronically catheterized fetal sheep. The fetus had a large ECV (632 ml kg^{-1} of fetal weight; 519 ml kg^{-1} of fetus, placenta and membranes) compared with the adult, and the proportion of the ECV contained outside the vascular compartment was higher than in the adult. With increasing gestation interstitial volume per kilogram fell, but PV per kilogram did not change. The effectiveness of the fetal kidney, as judged by the number of times the ECV was filtered each day, was only about 20% of the adult kidney.

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