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MEASUREMENT OF NET TRANSPLACENTAL TRANSFER OF FLUID TO THE FETAL SHEEP

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SUMMARY

1. If fetal drinking activity is prevented and it is assumed that in the latter third of gestation the fetus is capable of maintaining itself in fluid balance, then the net amount of fluid gained across the placenta by the fetus is equal to the amount of fluid lost from the fetus, by routes other than the placenta, plus fluid deposited in growing tissues minus the amount of water produced as a result of oxidative metabolism.

2. Net transplacental transfer of fluid to the fetus over a 3 h period was measured in eight chronically catheterized fetal sheep in which drinking activity was prevented by ligating the oesophagus. Urine and lung liquid flow rates were measured. In the latter third of gestation, these are the only significant sources of fluid loss from these fetuses during the 3 h experimental period. Water produced as a result of oxidative metabolism was calculated, as was the amount of fluid deposited in growing tissues during the course of the experiment.

3. The weight of the fetus at the beginning of the experiment and the change in weight that occurred during the experiment was calculated by measuring the weight of the fetus at death (within 30 h) and applying an equation which describes the body weight–gestation age relationship for merino sheep.

4. Net transplacental fluid transfer was $0{\cdot}40 \pm 0{\cdot}09$ ml min^{-1} kg^{-1} (range 0·30–0·54 ml min^{-1} kg^{-1}). Fetal urine flow rate averaged $0{\cdot}30 \pm 0{\cdot}11$ ml min^{-1} kg^{-1}. It was $72{\cdot}8 \pm 10{\cdot}0$ % of the volumes used to calculate net transplacental fluid transfer to the fetus. Lung liquid flow rate was $0{\cdot}079 \pm 0{\cdot}039$ ml min^{-1} kg^{-1}. It was $20{\cdot}2 \pm 9{\cdot}2$ % of the volumes used to calculate net fluid intake. The amount of fluid deposited as a result of tissue growth was $0{\cdot}023 \pm 0{\cdot}001$ ml min^{-1} kg^{-1}; it was $5{\cdot}94 \pm 1{\cdot}1$ % of the volumes used in the equation, while the production of water as a result of metabolism was $3{\cdot}9 \times 10^{-3}$ ml min^{-1} kg^{-1} (Conrad & Faber, 1977) and constituted $1{\cdot}01 \pm 0{\cdot}22$ % of the volumes used in the equation.

5. This method of measuring net transplacental fluid transfer to the fetus can be used to measure fetal fluid intake over relatively short periods of time. It also means that the effects of disturbances in maternal fluid and electrolyte balance on fluid transfer to the fetus can be studied and quantitated.

INTRODUCTION

By term, the fetal sheep of 3·5 kg body weight has accumulated about 2870 ml water (based on a water content of 82% body weight; D. J. Mellor, personal communication; Langlands & Sutherland, 1968; Rattray, Garret, East & Hinman, 1974). Furthermore, 1110 ml of fluid have accumulated within the amniotic and allantoic cavities (Barnes, 1976); i.e. over 148 days, 3980 ml of fluid have accumulated within the conceptus. The rate of accumulation of fluid within the conceptus is about 0·005 ml min^{-1} kg^{-1}; the rate of accumulation of fluid within the fetus is about 0·0038 ml min^{-1} kg^{-1}. This fluid is derived from the maternal compartment. Friedman, Gray, Hutchinson & Plentl (1959) estimated that in the monkey fetus more than 75% of this fluid was exchanged across the placenta.

These values are only an estimate of the average rate of water acquisition by the conceptus and the fetus. However, net transplacental fluid transfer to the fetus probably does not occur at a constant rate throughout gestation. For example, fetal growth is non-linear (Cloete, 1939; F. G. Smith, unpublished observations) and hence the rate of accumulation of fluid in growing tissues must also be non-linear. Furthermore, from time to time, net transplacental transfer is likely to vary if there is a change in the osmotic forces acting across the placenta or in the permeability of the placenta to electrolytes (Armentrout, Katz, Thornburg & Faber, 1977; Conrad & Faber, 1977). Studies have shown that net fluid transfer to the fetus is reduced when the maternal plasma osmolality is increased (Bruns, Linder, Drose & Battaglia, 1963; Bruns, Hellegers, Seeds, Behrman & Battaglia, 1964; Faber & Green, 1972). Faber & Green (1972) increased maternal osmolality by infusing hypertonic solutions of mannitol which does not cross the sheep placenta. They showed that the increased outflow of fluid from the fetus to the ewe, i.e. the reduction in transplacental fluid transfer to the fetus, caused increases in fetal plasma osmolality and haematocrit.

Since it is unlikely that net transplacental transfer of fluid to the fetus occurs at a constant rate throughout gestation and it is probable that it can be modified by alterations in maternal fluid and electrolyte balance, estimates of the average rate of water transfer are of limited use.

Net transplacental fluid transfer to the fetus can be measured if it is assumed that over a period of time the intake of fluid by the fetus is equal to its fluid output plus that volume of fluid deposited in growing tissues. The fetus in the latter third of gestation has three possible routes of fluid intake: drinking (Bradley & Mistretta, 1973), the placenta and that volume of water produced as a result of metabolism. Drinking activity can be prevented by ligating the oesophagus. The fetus has three routes by which fluid can be lost: the placenta, the lungs and the kidneys. Net fetal fluid intake is a measure of the difference between fluid intake across the placenta and fluid loss across the placenta. Urine flow rate and lung liquid flow rate can be measured, and water produced as a result of metabolism and water deposited in growing tissues can be calculated.

Net transplacental fluid transfer to the fetus was measured in chronically catheterized fetal sheep.

METHODS

Experiments were carried out in eight chronically catheterized fetal sheep aged 122 to 133 days.

Surgical preparation

Anaesthesia was induced with 1 g sodium thiopentone (Abbott Laboratories). The ewe was then intubated and anaesthesia was maintained with 3 % halothane in oxygen. Under aseptic conditions, the fetal hind limbs were exteriorized and polyvinyl catheters inserted into a femoral artery, a tarsal vein and the bladder (1·0 mm i.d.; 1·5 mm o.d.) (see also Lumbers & Stevens, 1983). The bladder cannula was inserted directly through the bladder wall. The fetus was returned to the uterus and the uterine incision closed. The fetal head was then delivered through a second uterine incision. The oesophagus was ligated to prevent the ingestion of amniotic fluid and the trachea was cannulated. The tracheal cannula was connected to a latex rubber bag by a T-piece joined to a polyvinyl catheter (1·5 mm i.d.; 2·7 mm o.d.) through which the contents of the bag and the lung could be aspirated (see also Mescher, Platzker, Ballard, Kitterman, Clements & Tooley, 1975).

An amniotic catheter was sutured to the fetal skin. Antibiotics were injected into the amniotic cavity (600 mg crystalline benzyl penicillin + 1 g streptomycin sulphate; Glaxo) and the uterus and abdomen were then closed. Polyvinyl catheters (1·5 mm i.d.; 2·7 mm o.d.) were inserted into a carotid artery and jugular vein of the ewe.

All ewes were housed in metabolic cages and fed a diet of chaff, oats and water. For 3 days post-operatively, the ewes were given an intramuscular injection of 3–5 ml procaine penicillin (250 mg ml^{-1}) and dihydrostreptomycin (250 mg ml^{-1}) (Glaxovet) as well as an intra-amniotic injection (see above).

Experiments were not begun before 5 days after surgery. During this period all fetal lung liquid that had collected in the intra-amniotic bag was drained once every 24 h. Since the urachus and urethra were still patent, it was not necessary to drain the bladder to the exterior except during experiments.

Experimental protocol

Ewes were allowed no access to food or water during the experimental period. For 45 min prior to the beginning of an experiment the lung liquid and bladder cannulae were drained. During the experiment, the end of the bladder cannula was about 10 cm below the level of the fetus. Thus urine flowed preferentially along the bladder catheter. Previous studies from this laboratory have shown that all urine passed during an experiment is collected via the bladder catheter (see Stevens & Lumbers, 1981). A loading dose of sodium [^{125}I]iothalamate (Amersham) (1·05 μCi kg^{-1}) was administered through the fetal vein catheter and infused continuously at 0·6 μCi kg^{-1} h^{-1}.

Fetal lung liquid and urine were then collected over 30 min periods for 3 h. The volumes were recorded and samples stored at −20 °C for analysis of sodium and potassium levels and osmolality using a FLM3 flame photometer (Radiometer), and an Advanced Digimatic Osmometer. Fetal intra-amniotic pressure and fetal and maternal blood pressures were measured using Statham pressure transducers and recorded on a Devices M19 eight-channel recorder. Maternal and fetal blood samples (4 ml) were collected 45 min and 165 min after the beginning of the experiment into airtight syringes. The samples were then spun at 4 °C and plasma osmolality was measured.

Ewes were killed within 30 h of the experiment and each fetus was removed and weighed. The presence of the oesophageal ligature and the integrity of the lung liquid collecting device were confirmed at post mortem.

Calculations

Fetal glomerular filtration rate was measured as the clearance of sodium [^{125}I]iothalamate. Free water clearance was calculated as the difference between urine flow rate (V) and osmolar clearance.

The fetal body weights at the time of the experiment and at the time of death were calculated from the equation:

$$\text{body weight (g)} = e^{(\ln \text{age (days)}) \, 5{\cdot}09 - 16{\cdot}88}$$

(F. G. Smith, unpublished observations) using data provided by Alexander (1978). The weight of the fetus at death (*BWD*) was measured and the weight at the time of the experiment (*BWE*) was then calculated from the equation:

$$BWE = \frac{\text{estimated } BWE \times BWD}{\text{estimated } BWD}.$$

From this equation the rate of growth of the fetus over the 3 h experimental period could be determined. The increase in fetal body water was calculated as 82% of the increase in fetal body weight (D. J. Mellor, personal communication; see also Langlands & Sutherland, 1968; Rattray *et al.* 1974). Metabolic water production was assumed to be $3{\cdot}9 \times 10^{-3}$ ml min^{-1} kg^{-1} (Conrad & Faber, 1977).

Net transplacental fluid transfer was calculated from the equation:

$$NFT = V + LV + FT - H_2O_m$$

(*NFT* = net transplacental transfer; *V* = urine flow rate; *LV* = lung liquid flow rate; *FT* = the amount of fluid deposited as a result of growth of new tissue and H_2O_m = water produced as a result of oxidative metabolism). This equation is based on the assumption that over a period of time the fetus can maintain the volume and composition of its body fluids constant. If this is the case then the net fluid gained by the fetus is equal to the net fluid lost plus water for growth. Since drinking activity was prevented by oesophageal ligation at the time of surgery, net fluid transfer to the fetus across the placenta must be equal to the sum of the volumes of fluid lost via the kidneys and lungs plus that volume of fluid deposited in newly formed tissues minus the volume of water generated from metabolism. *V* and *LV* were measured and *FT* and H_2O_m were calculated because they could not be measured directly.

Analysis of data

Data were expressed as mean ± standard deviation. Linear regression analysis was carried out using a Texas Instrument calculator (TI Programmable 58).

RESULTS

Urine flow rate

Urine flow rate (*V*) was measured in eight fetuses at 30 min intervals for 3 h (Fig. 1). Table 1 shows the average urine flow rate (ml min^{-1} kg^{-1}) for each fetus. In six fetuses, urine flow rates were constant, i.e the coefficient of variation was 8·0 ± 2·27% (mean ± S.D.) (Fig. 1 and Table 1). In two other fetuses urine flow rate was variable, i.e. the coefficient of variation was greater than 20% (Fig. 1 and Table 1). The mean urine flow rate was 0·30 ± 0·11 ml min^{-1} kg^{-1}; range 0·21–0·47 ml min^{-1} kg^{-1}; $n = 8$.

All fetuses excreted a urine that was hypo-osmotic with respect to fetal plasma, thus free water clearance was positive (range: 0·13–0·35 ml min^{-1} kg^{-1}; Table 2). The excretion rates of sodium and potassium and the osmolar excretion rates (for each fetus) are also shown in Table 2.

Glomerular filtration rate measured as the clearance of sodium iothalamate was 1·67 ± 0·25 ml min^{-1} kg^{-1} body weight and ranged from 1·15 to 1·97 ml min^{-1} kg^{-1}. Urine flow rate was not related to glomerular filtration rate. The percentage of the glomerular filtrate excreted as urine ranged from 10·5 to 28·2% (19·2 ± 5·96 (S.D.)%). The percentage of the filtered sodium load that was excreted ranged from 1·27 to 8·23% (4·00 ± 2·43 (S.D.)%, $n = 8$). The percentage of the filtered osmolar load that was excreted ranged from 4·11 to 11·3% (7·15 ± 2·41 (S.D.)%, $n = 8$).

Urine flow rate was related to both the sodium excretion rate ($r = 0{\cdot}84$; $P < 0{\cdot}001$; $n = 8$) and to the osmolar excretion rate ($r = 0{\cdot}75$; $P < 0{\cdot}01$; $n = 8$). There was no significant relationship between urine flow rate and potassium excretion. Urine flow

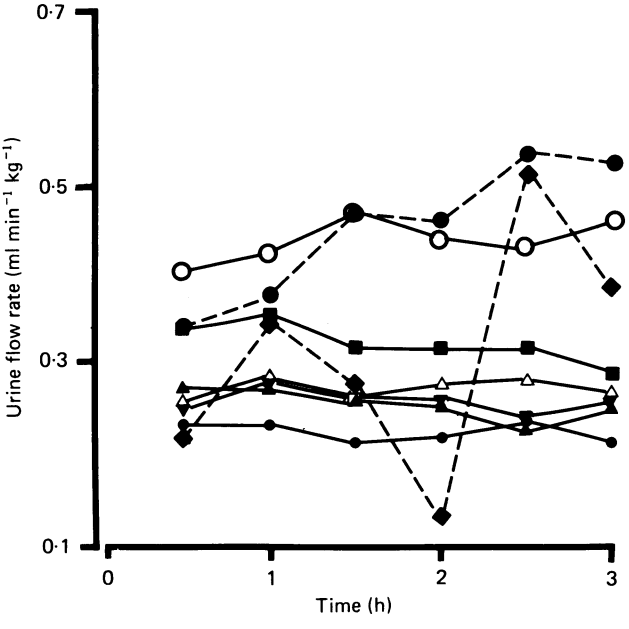

Fig. 1. The urine flow rates (ml min^{-1} kg^{-1} body weight) of eight fetuses over the 3 h of the experiment. Different fetuses are represented by different symbols. Continuous lines show those fetuses in which urine flow rate was constant (coefficient of variation $8{\cdot}0 \pm 2{\cdot}3$ %, $n = 6$). The dashed lines show the two fetuses in which urine flow rate was variable. See also Table 1.

TABLE 1. The mean (±standard deviation) urine flow rates and lung liquid flow rates for each fetus in ml min^{-1} kg^{-1} body weight. Also shown is the coefficient of variation around the mean. In six fetuses urine flow rates were constant (coefficient of variation = $8{\cdot}0 \pm 2{\cdot}3$ %). In two other fetuses urine flow rate was variable (coefficient of variation > 20 %); see Fig. 1. Lung liquid flow rates were more variable, especially in three fetuses (coefficient of variation 75 % or more); see Fig. 2

Urine flow rate (ml min^{-1} kg^{-1})	Coefficient of variation (%)	Lung liquid flow rate (ml min^{-1} kg^{-1})	Coefficient of variation (%)
$0{\cdot}21 \pm 0{\cdot}02$	9·5	$0{\cdot}068 \pm 0{\cdot}016$	23·5
$0{\cdot}24 \pm 0{\cdot}02$	8·3	$0{\cdot}065 \pm 0{\cdot}013$	20·0
$0{\cdot}28 \pm 0{\cdot}03$	10·7	$0{\cdot}053 \pm 0{\cdot}009$	17·0
$0{\cdot}47 \pm 0{\cdot}03$	6·4	$0{\cdot}053 \pm 0{\cdot}029$	54·7
$0{\cdot}23 \pm 0{\cdot}02$	8·7	$0{\cdot}090 \pm 0{\cdot}029$	32·2
$0{\cdot}46 \pm 0{\cdot}11$	23·9	$0{\cdot}037 \pm 0{\cdot}068$	184
$0{\cdot}29 \pm 0{\cdot}16$	55·2	$0{\cdot}16 \pm 0{\cdot}19$	119
$0{\cdot}23 \pm 0{\cdot}01$	4·4	$0{\cdot}102 \pm 0{\cdot}076$	74·5

rate was related to free water clearance ($r = 0{\cdot}98$; $P < 0{\cdot}001$; $n = 8$) and to free water clearance corrected for glomerular filtration rate ($r = 0{\cdot}94$; $P < 0{\cdot}001$; $n = 8$).

Lung liquid flow rate

Fig. 2 shows the lung liquid flow rates (LV) in ml min^{-1} kg^{-1} collected at 30 min intervals in each fetus. Table 1 shows the mean values. Lung liquid flow rate was variable in all fetuses (Fig. 2). In five fetuses, the coefficient of variation was

TABLE 2. The mean (±standard deviation) osmolar excretion rates and the excretion rates of sodium and of potassium are shown for each fetus. Also shown is free water clearance. Each value represents the mean±standard deviation of six samples except for fetus marked * ($n = 5$)

Osmolar excretion (μosmol min^{-1} kg^{-1})	Sodium excretion (μmol min^{-1} kg^{-1})	Potassium excretion (μmol min^{-1} kg^{-1})	Free water clearance (ml min^{-1} kg^{-1})
25·0±2·3	5·0±0·4	1·25±0·30	0·13±0·02
22·0±1·7	3·1±0·3	0·28±0·13	0·17±0·02
27·6±2·7	6·9±0·7	0·17±0·01	0·19±0·02
*64·8±27·8	*20·5±3·5	*1·31±0·29	0·29±0·02
28·5±2·4	8·1±0·8	0·22±0·05	0·13±0·01
30·4±8·0	11·3±3·9	0·17±0·07	0·35±0·08
36·6±17·7	12·0±5·9	2·62±1·51	0·17±0·11
26·3±1·6	5·1±0·4	2·85±0·41	0·14±0·01

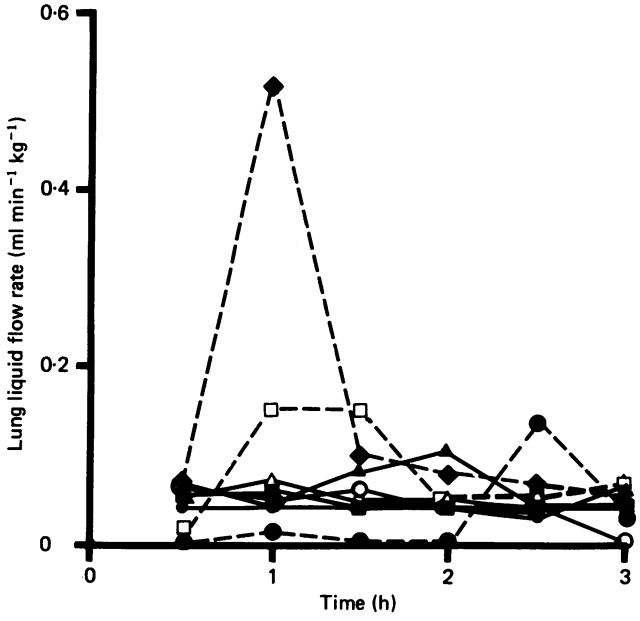

Fig. 2. The lung liquid flow rates of eight fetuses (ml min^{-1} kg^{-1} body weight) over the 3 h of the experiment. Different fetuses are represented by different symbols. In five fetuses, the lung liquid flow rates were constant (coefficient of variation of 29·5±15·2). In three other fetuses, the lung liquid flow rates were more variable (coefficient of variation 75% or more).

29·5±15·2 (S.D.)% while in the three other animals it was 75% or more (see Table 1). Mean lung liquid flow rate ranged from 0·037 to 0·16 ml min^{-1} kg^{-1} (0·079±0·039 (S.D.) ml min^{-1} kg^{-1}; $n = 8$).

Metabolic water production (H_2O_m) and fluid for tissue growth (FT)

Metabolic water production was $3{\cdot}9 \times 10^{-3}$ ml min^{-1} kg^{-1} (Conrad & Faber, 1977) while the change in total body water due to growth was 0·023±0·001 ml min^{-1} kg^{-1}.

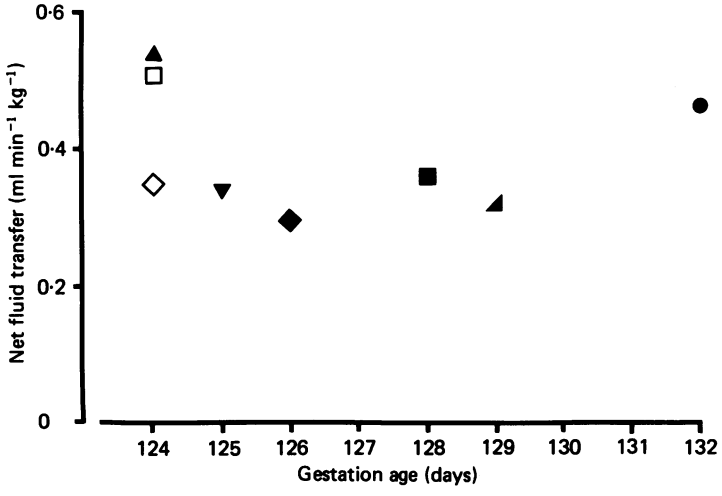

Fig. 3. The net transfer of fluid to the fetus (ml min^{-1} kg^{-1} body weight) at different gestation ages (days).

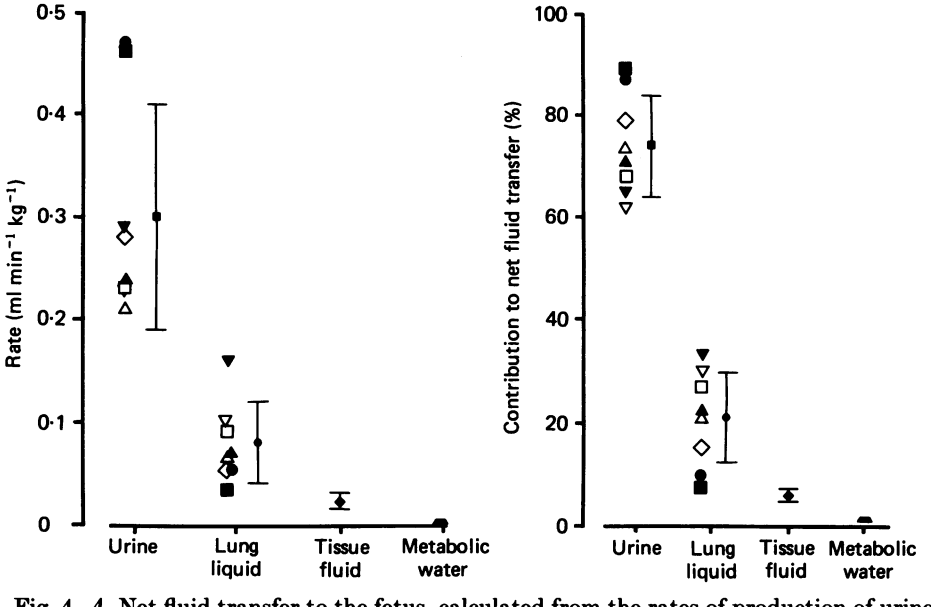

Fig. 4. *A*, Net fluid transfer to the fetus, calculated from the rates of production of urine, lung liquid, tissue fluid and metabolic water. Different symbols represent individual fetuses. The filled symbol and bar represents the mean ± S.D. *B*, the relative contribution (%) of each flow rate to the calculation of net fluid transfer. The major contributor to the equation $NFT = V + LV + FT - H_2O_m$, was urine flow rate (72·8 ± 10 %). Next was lung liquid flow rate (20·2 ± 9·2 %). These variables were measured. The rate of fluid deposition in growing tissues (FT) contributed 6·1 ± 4·1 % of the total volumes. The rate of production of metabolic water (H_2O_m) contributed even less (1·0 ± 0·2 %). These latter two variables were calculated.

Determination of net transplacental fluid transfer (NFT)

Net transplacental fluid transfer across the placenta to the fetus ranged from 0·30 to 0·54 ml min^{-1} kg^{-1} (Fig. 3). The mean rate of net transfer to the fetus of fluid was $0{\cdot}40 \pm 0{\cdot}09$ ml min^{-1} kg^{-1}.

The amount of fluid deposited as a result of growth of new tissue (*FT*) and the amount of water produced as a result of oxidative metabolism (H_2O_m) were only $5{\cdot}94 \pm 1{\cdot}1$ % (*FT*) and $1{\cdot}01 \pm 0{\cdot}22$ % (H_2O_m) of the total amounts used in the equation for calculating net fluid transfer. The greatest loss of fluid from the fetus was via the urine (see Fig. 4*A*). The contribution made by urinary fluid losses to the measurement of net fetal fluid intake across the placenta was greater than 60 % (range 62·3–88·5 %, Fig. 4*B*). Lung liquid flow rate accounted for 7·2–33·6 % of the volumes used to measure net fetal fluid intake by the fetus (Fig. 4*B*).

DISCUSSION

Normally there is no evacuation of the bowel contents by the fetus, and in fetuses aged 125–133 days there is insignificant movement of fluid across the thick integument (see Barnes, 1976). Thus the only possible sources of fluid loss from the fetus (apart from the placenta) are through the urinary and lung liquid excretions. Possible routes of fluid intake by the fetus are across the placenta, via drinking activity which can amount to 500 ml per day (Bradley & Mistretta, 1973) and by the formation of water during metabolism. Placental fluid losses from the fetus are incorporated in the equation since net transplacental fluid intake by the fetus is determined as the difference between fluid intake and output across the placenta. The oesophagus was ligated at surgery in all fetuses so that drinking activity was prevented. Water produced as the result of metabolism was calculated, and the equation, $NFT = V + LV + FT - H_2O_m$, includes a volume (*FT*) which is the amount of fluid that would be deposited as a result of tissue growth during the course of the experiment.

Fluid balance studies have not been used previously to determine fetal fluid intake, possibly because of the difficulty in accounting for fetal swallowing which can involve the ingestion of both lung liquid and amniotic fluid.

This method of determining net transplacental fluid intake is based on the assumption that over a period of time the fetus is capable of maintaining its volume and composition constant. It was difficult to decide on the length of the experimental period required to measure net fluid intake, since there must be a delay between changes in the rate of fluid intake and compensatory changes in the rate of fluid loss. To limit fluctuations in fetal fluid intake, the ewe was deprived of food and water from the time of the pre-collection period until the end of the experiment. It is known that feeding may affect fetal fluid intake (Mellor & Slater, 1973) and that maternal fluid intake can affect fetal fluid balance (Stevens & Lumbers, 1983). On the other hand, any lengthy period of restriction of maternal food and water intake might also disturb the fetus through effects on maternal osmolality (Lumbers & Stevens, 1983). Thus the period of maternal food and water deprivation was limited to 3·75 h. In six fetuses, it appeared that fluid intake across the placenta was reasonably constant

during the experimental period since fetal urine flow rate and free water clearance corrected for glomerular filtration rate remained constant, i.e. the coefficient of variation for 30 min collections was 6·4–10·7 % (Table 1). Fetal urine flow rate and the rate of absorption of water by the kidney are known to depend upon net fluid intake across the placenta (Lumbers & Stevens, 1983; Stevens & Lumbers, 1983).

Each fetus was weighed at the time of death (within 30 h of the experiment) and the change in body weight over the course of the experiment was calculated using a body weight–gestation age equation (F. G. Smith, unpublished observations). Thus both the increase in total body water and the fluid produced as the result of metabolism could be determined. This method of calculating the change in body weight and the weight of the fetus at the beginning of the experiment was the most accurate method available to us. Water for tissue growth was only 6 % of the total volumes used to calculate net fluid gain, and water produced as a result of metabolism was only 1 % of the total volumes used in determining net fluid intake (Fig. 4*B*).

Urine was the major route by which fluid was lost from the fetus, and lung liquid flow rate was next (Table 1; Fig. 4*A*). Lung liquid flow rate was variable (Fig. 2) possibly because of fetal activity, in particular respiratory efforts (Dawes, 1973), but the flow rates were similar to those measured by Egan, Olver & Strang (1975) and Mescher *et al.* (1975). The latter group used techniques similar to those described in this paper for the collection of lung liquid.

In the adult animal, fluid balance is maintained partly by the ability of the kidneys to vary the composition and volume of urine. The lack of a relationship between glomerular filtration rate and urine flow rate, and the variability in the percentage of the filtered load excreted as urine (10·5–28·2 %) suggests that in individual animals tubular mechanisms were the major determinants of fetal urine flow rate and its composition. This latter point is emphasized by the variability in the percentage of the filtered osmolar load that was excreted.

It has been shown that urine production by the fetus is determined by a number of renal mechanisms, the activities of some of which are influenced by the volume and composition of the fetal body fluids (see Lumbers, 1983). The volume and composition of the fetal body fluids is in turn influenced by the availability of fluid and electrolytes to the fetus from the maternal compartment. For example, Faber & Green (1972) showed that when maternal osmolality was increased by infusion to the maternal circulation of a solute which was unable to cross the placenta, the amount of water available to the fetus was reduced and fetal osmolality and haematocrit increased. Lumbers & Stevens (1983) applied the same experimental techniques to show that the fetal kidney could also respond to this reduction in net water intake across the placenta by increasing water reabsorption. Furthermore, there was an inverse correlation between maternal plasma osmolality and fetal free water clearance (Stevens & Lumbers, 1983). Since osmotic forces acting across the placenta have a major effect on net transplacental fluid transfer to the fetus (Faber & Green, 1972), the inverse correlation between maternal plasma osmolality and fetal free water clearance indicates that fetal renal function is influenced by the uptake of fluid across the placenta. Free water clearance ($0{\cdot}195 \pm 0{\cdot}082$ ml min^{-1} kg^{-1}) was much greater than the volume of water generated in the fetus as a result of oxidative

metabolism ($3{\cdot}9 \times 10^{-3}$ ml min^{-1} kg^{-1}). Conrad & Faber (1977) found that fetal plasma osmolality was 2 mosmol kg^{-1} less than maternal osmolality and suggested that the hydrostatic pressure difference between the two circulations was responsible for this hypo-osmotic state in the fetus. In addition, the movement of substrates such as glucose and amino acids and of ions such as sodium and chloride across the placenta would be accompanied by the movement of an isosmotic equivalent volume of water. As substrates such as glucose and amino acids are utilized, the water that they brought across must be liberated. In addition, metabolism of these substrates generates water (H_2O_m). Therefore, it was not surprising that urine flow rate was the major component in determining net fluid transfer.

The results from the present experiments indicate that the net movement of fluid across the placenta to the fetus is greater than that predicted by other workers, being of the order of 0·30–0·54 ml min^{-1} kg^{-1}. Other estimates based on the average rate of accumulation of fluid within the conceptus range from 0·005 to 0·017 ml min^{-1} kg^{-1} (Conrad & Faber, 1977; Wilbur, Power & Longo, 1978). Use of average rates of accumulation of fluid does not describe accurately fetal fluid intake at any particular gestation age because growth rate is not linear. In our experiments it was best described by the equation,

$$\text{body weight (g)} = e^{(\ln \text{age (days)})\ 5{\cdot}09-16{\cdot}88}$$

(F. G. Smith, unpublished data). Furthermore the average rate of gain of fluid by the conceptus cannot be equated with transplacental transfer, because it is possible that fluid can leave the conceptus by extraplacental routes.

The methods described in this study for measuring net transplacental transfer of fluid to the fetus suffer two limitations. First, the distinction between placenta and extraplacental membranes cannot be made; thus the term net transplacental transfer may incorporate some transfer across those membranes external to the placental cotyledons. Secondly, ligation of the oesophagus and continuous collection of lung liquid alters amniotic–fetal fluid exchange. Despite these limitations, the model can be used to study the effects of a variety of disturbances in maternal fluid balance on the availability of fluid to the fetus over short periods of time.

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