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DAILY CHANGES IN AMNIOTIC AND ALLANTOIC FLUID DURING THE LAST THREE MONTHS OF PREGNANCY IN CONSCIOUS, UNSTRESSED EWES, WITH CATHETERS IN THEIR FOETAL FLUID SACS

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SUMMARY

1. Catheters were inserted into the maternal and foetal vasculatures of ten ewes, 100–139 days pregnant, and daily samples of uterine and umbilical blood and maternal jugular vein blood were taken for periods of 5–27 days after operation.

2. Catheters were inserted into the fluid sacs of nineteen foetuses, 60–97 days post-conception, and daily samples were withdrawn for up to 90 days from amniotic sacs (eleven foetuses) and for up to 70 days from allantoic sacs (eight foetuses). Maternal jugular plasma was obtained 3 times weekly and an approximation from its composition to that of uterine and umbilical plasma was made using results from the ewes and foetuses with vascular catheters.

3. The pH, osmolality, $[Na^+]$, $[K^+]$, $[Cl^-]$, [urea] and [amino acid] of all samples were measured.

4. The nutritional status of all ewes was monitored throughout pregnancy. Most lambs were born naturally at ~ 147 days post-conception and their subsequent progress was observed.

5. Results at operation and from acute experiments were compared with those from conscious ewes and foetuses of the same gestational age to assess the nature and extent of the influence of the operative procedures on foetal fluid composition. The composition of amniotic fluid was influenced mainly by the anaesthetic and surgical procedures while that of allantoic fluid was affected largely by starvation of the ewe.

6. Changes during recovery from operation were followed and indicated that maternal and foetal plasma required about 3 days and the foetal fluids up to 7 days before stability of composition was achieved.

7. After recovery from operation, daily changes in the composition of each foetal fluid showed the same general pattern in all foetuses, but the absolute values of constituents sometimes showed large differences.

8. It is suggested that flow of foetal urine into the amniotic sac increased from 80 days gestational age, that urine flow into the allantoic sac decreased until about 100 days but did not cease thereafter, and that relative to foetal urine the influence of foetal pulmonary fluid on amniotic fluid composition was not great.

9. A relative impermeability of the amnion appeared to be a major factor influencing amniotic fluid composition, whereas pumping mechanisms in the chorioallantois seem to have been responsible largely for changes in the composition of allantoic fluid.

10. The quantity of solute relative to that of water within each sac appears to be a major determinant of changes in foetal fluid volumes.

11. Changes in the $[Na^+]$ and $[K^+]$ of allantoic fluid during the normal course of pregnancy were consistent with an increasing action of mineralocorticoids on pumping mechanisms in the chorioallantois. Similar but more rapid changes seemed to be associated with acute and chronic episodes of maternal hypoglycaemia. Under these circumstances foetal hypoglycaemia may effect a relative increase in the secretion of foetal corticosteroids having an action on the chorioallantois.

12. The results from this study demonstrate clearly the value of using chronically catheterized animals, and it is suggested that their use in physiological studies on the conceptus must eventually supersede that of acute, anaesthetized preparations.

INTRODUCTION

The composition of sheep foetal fluids has been examined (Malan, Malan & Curson, 1937; Cloete, 1939; McDougall, 1949), possible relationships between their composition and that of foetal urine and foetal and maternal plasma at different gestational ages have been suggested (Alexander, Nixon, Widdas & Wohlzogen, 1958*a*; Hervey & Slater, 1968), and possible associations between their ionic composition and the electrochemical gradients between the mother and the fluid compartments of the conceptus have been outlined (Mellor, 1970*a*). In all of this work, however, samples were taken during acute experiments or from pregnant ewes after slaughter. It is not known what artifacts were produced by the severe stresses associated with these procedures, or if the various conceptuses examined were normal or would have been so at birth. The development of a technique allowing daily withdrawal of amniotic and allantoic fluid samples from individual conceptuses of conscious unstressed ewes during the course of gestation (Mellor, 1970*b*) has obviated many of these problems, particularly as the lambs may be born naturally and their subsequent progress observed. This technique has been used in the present

study to investigate gestational variations in the composition of foetal fluids for periods of up to 90 days in pregnancies resulting in the birth of healthy viable lambs. Data from chronically catheterized animals have also been compared with those from acute, anaesthetized preparations to assess the nature and extent of the influence of these procedures on the conceptus.

METHODS

Animals. Thirty 5-year-old Scottish Black Face ewes (38–51 kg) of known mating date were used. They were housed in a well ventilated sheep house in individual pens from 40 days post-conception until their lambs were weaned at 7 weeks of age. The body of each ewe posterior to the last rib was completely shorn 4 days before operation. All ewes were weighed once weekly and their daily water and feed intakes were recorded. The quantity of pelleted feed (Ruminant B Diet, U.K. Compound Feeds Ltd., Cheshire) given to each ewe was individually regulated to give uniformity of lamb birth weights. The free fatty acid concentrations ([FFA]) were determined in maternal plasma samples taken once weekly from a jugular vein, and sufficient feed was given in an attempt to maintain the [FFA] below 0·6 m-equiv/l. following the procedure of Russell, Doney & Reid (1967). However, the ewes were partially shorn, unlike those of Russell *et al.* (1967), so that the maternal response to fluctuations in the ambient temperature caused the [FFA] to be too variable as an index. The [ketone body] proved to be more satisfactory and ewes carrying twins were fed to maintain their plasma [ketone body] below 10 mg/100 ml., and those with singletons below 6 mg/100 ml.

Ewes with foetal fluid sac catheters. A group of thirteen ewes was allowed to lamb naturally. During pregnancy they gained weight so that at term ($147 \pm 2{\cdot}4$ days) they weighed 13 ± 6 % more than their weight at 60 days post-conception (46 ± 4 kg). At birth single lambs weighed $4{\cdot}29 \pm 0{\cdot}24$ (eight) kg, and twins $3{\cdot}15 \pm 0{\cdot}48$ (ten) kg, and the mean growth rate for the eighteen lambs during the first 7 weeks post-partum was $1{\cdot}1 \pm 0{\cdot}3$ kg/week. The results from these ewes form the major part of this paper. The pregnancies of seven ewes (carrying eleven lambs) were terminated several weeks after operation to investigate the cause of sampling difficulties. Data from all twenty ewes were combined to determine the influence of the operative procedures on the composition of the foetal fluids, and the time required for recovery from operation.

Ewes with vascular catheters. The relative compositions of plasma samples withdrawn from a maternal jugular vein, uterine artery and vein, and umbilical artery and vein of ten conscious sheep were investigated. This enabled an approximation to the concentrations of solutes in uterine and umbilical plasma to be made from those in plasma from the maternal jugular vein. Thus, it was not essential to insert catheters into the maternal and foetal vasculatures of ewes with fluid sac catheters.

Preoperative procedure. All ewes were put in a heated room adjacent to the surgery 2–4 days before operation. Food but not water was withheld for 24–48 hr and animals received 60–75 mg progesterone, I.M. (Organon Laboratories Ltd, Surrey) 24 hr before the operation. Anaesthesia was induced and maintained with sodium pentobarbitone (Abbott Laboratories Ltd, Kent) given I.V.; an endotracheal tube was passed, and the animals breathed 100 % oxygen throughout the operation. Each animal was placed on its back and the whole of the ventral abdomen and a large area of the right flank was shaved and then washed and sterilized with the antiseptic solutions previously described (Mellor, 1970*b*). The animal was covered with sterile drapes leaving the operative area exposed, and this was sprayed with an antibiotic

powder containing neomycin sulphate, polymyxin B sulphate and zinc bacitracin (Framyspray, Fison's Laboratories Ltd). All instruments, syringes, saline and heparin-saline solutions, drapes and bandages were autoclaved, and catheters were sterilized by γ-irradiation (Ethicon Ltd).

Catheterization procedure. The uterus was exposed by an oblique incision parallel to the fibres of the rectus abdominus muscle, extending approximately 15 cm posteriomedially from the ventral border of the right subcutaneous muscle towards the mammary gland.

Fluid sac catheters and their method of insertion have been described by Mellor (1970*b*). In seven ewes, however, catheters with vinyl tubes attached were inserted into amniotic sacs of foetuses aged from 60 to 75 days. The extra length allowed the catheters to pass subcutaneously from a mid line incision to the flank of the ewe. This modification introduced sampling difficulties in five of the seven cases, and only three catheters did not block completely after 2–3 weeks. When blockages occurred the cause was investigated at *post-mortem* and was invariably due to the vinyl extension tube being compressed by the ewe when lying down. Exposing the uterus by an oblique ventrolateral incision (as above) and bringing the catheters out of its lateral end, obviated this difficulty and allowed the standard catheter (Mellor, 1970*b*) to be used at all ages. Systemic antibiotic was not given routinely at the end of each operation, but the operative site was sprayed with antibiotic powder and a sterile pad covering the wound was changed once each day for 3 days. These measures were generally effective since bacterial growth was detected in foetal fluids of only three of the twenty ewes within 4 days of operation. The three ewes received 750 mg procaine penicillin G with 750 mg dihydrostreptomycin, I.M. (Streptopen, Glaxo Laboratories) twice daily for 4 days; during this period samples were taken 1 hr after administration of antibiotic.

Vascular catheters were vinyl tubes (1·5 mm O.D.; Portex Ltd) with a 3-way stopcock (Baxter Ltd) fitted to one end. They were threaded into the umbilical vessels by the method of Meschia, Cotter, Breathnach & Barron (1965*b*) and, via small tributaries, into a uterine artery of the non-pregnant horn and the middle uterine vein of the pregnant horn, in ewes between 100 and 139 days gestational age. During insertion, catheters were filled with NaCl solution (0·9 %, w/v). Their final position was selected when blood samples could be withdrawn easily; they were then filled with heparin-saline solution (1·0 mg heparin/ml. in 0·9 %, w/v, NaCl) prepared from dry heparin (Pularin, Evans). During catheterization of the umbilical vessels this minimized the quantity of heparin introduced into the foetal circulation. All catheters were brought through the lateral end of the abdominal incision, were taken subcutaneously to the dorsal region of the flank and exteriorized. They were covered and protected by a padded bandage passed around the abdomen of the ewe. Each ewe was given systemic antibiotic, I.M. (Streptopen; dose rate as above) immediately after the operation and once daily for 3 days.

Post-operative procedure. Immediately following surgery the ewes were placed in a warm pen. For the first 18 hr drinking water contained NaCl (0·45 %, w/v). Pelleted feed and hay were available *ad lib.* for the first 3 days, after which the specific ration of pelleted feed was given. The ewes ate sparingly (rarely more than half one day's ration) during the first 18 hr, and almost not at all during the following 2 days. On the 3rd day feed and water were again consumed, and by the 4th or 5th day in most cases intake had returned to preoperative levels. All ewes were returned to the main sheep house 3–5 days after the operation.

Sampling procedure. Blood and foetal fluid samples were collected daily between 8 and 10 a.m. before ewes were fed. Sterile conditions were maintained and all samples were tested for bacteria (Mellor, 1970*b*). Before blood samples were with-

drawn the catheters were flushed with 0·5 ml. sterile heparin-saline solution (0·5 mg heparin/ml. in 0·9 %, w/v, NaCl), and afterwards refilled with 1·0 ml. of the same solution and sealed (capacity of catheter and tap 0·5 ml.). Each night between 5 and 6 p.m. all vascular catheters were flushed with 0·75 ml. sterile heparin-saline solution (1·0 mg heparin/ml. in 0·9 %, w/v, NaCl) and resealed. Samples were taken into 5 ml. syringes; those for blood contained dry heparin (0·25 mg). Air that occupied the dead space (0·2 ml.) was expelled and the syringe sealed immediately a blood (2·5 ml.) or foetal fluid (1·5 ml.) sample had been withdrawn (usually in less than 1 min), so that pH could be determined. This procedure was adopted since there was no significant difference between the pH of samples taken this way and that of samples not exposed to air. The pH was measured within 10 min of sampling. In addition, blood samples (5·0 ml.) were taken under vacuum into heparinized tubes from a maternal jugular vein by venepuncture, each day from the ten animals with vascular catheters, and every Monday, Wednesday and Friday from the twenty ewes with foetal fluid sac catheters. This blood sampling procedure does not stress the ewes (Slee & Halliday, 1968; Bassett & Hinks, 1969; D. J. Mellor & J. S. Slater, unpublished data). All samples were centrifuged for 30 min at 2000 *g*, and maternal and foetal plasma and amniotic and allantoic fluid were stored in sealed containers at −20° C until required.

Measurements. Osmolality was measured by freezing point depression (Precision Osmometer, Precision Systems, U.S.A.), and pH and P_{CO_2} by the standard methods using micro-electrodes (pH meter model 27; Radiometer, Copenhagen). The [Na^+] and [K^+] in samples suitably diluted with distilled water were determined with an atomic absorption spectrophotometer (model SP 90, Unicam Ltd), and the [Cl^-] and [urea], using an Autoanalyser (Technicon) by the methods given in the Technicon Handbook (files N-5b and N-1c). The [amino acid] were determined by the method of Slater & Dunnett (1970), [FFA] according to Patterson (1963), and [ketone body] as described by Reid (1960*a*). The osmolality, [Na^+], [K^+], [Cl^-], [urea] and [amino acid] of all samples were measured after lambs were born. When technique allowed, all plasma and foetal fluid samples from each animal were analysed as a group and in the order in which they were withdrawn from the animal. The concentrations of constituents have been expressed in m-equiv/l. (Na^+, K^+, Cl^-) or mM (urea, amino acid) to show relative contributions to osmolality.

Presentation of data. The following arbitrary criteria have been adopted.

1. *Maternal and foetal plasma values:* sequential changes in ten individual foetuses were followed for periods of 5–11 days (five; all alive at Caesarean section), 18–21 days (three; all born alive) and 23–27 days (two; both alive at Caesarean section), until samples were no longer obtainable. Data obtained at or on the 2 days following operation were considered unrepresentative of normal conditions and were excluded from calculations of mean values. Each foetus was not followed for exactly the same period and over the same stage of gestation, and not all catheters remained patent for the same time in any one animal. Fewer values were available from uterine artery and umbilical artery. Thus, different numbers of observations were available from each mother and foetus for comparison of the compositions of plasma from the different blood vessels.

Mean values were calculated for samples taken from a maternal jugular vein, and for the combined results from uterine artery and vein, and from umbilical artery and vein. The results from the two uterine, and from the two umbilical vessels were pooled because $A-V$ differences were relatively small and were not specifically relevant to the present study, and because this facilitated comparison of jugular, uterine and umbilical plasma values. When the means were calculated care was taken not to include disproportionately large numbers of results from any one animal, and to

include only those results that allowed a daily comparison of the constituents of jugular, uterine and umbilical plasma. The relationships have been expressed as ratios of these mean values (Table 2).

2. *Amniotic and allantoic fluid values:* sequential changes were followed for periods of 55–90 days in amniotic fluid from eleven foetuses, and for 55–70 days in allantoic fluid from eight foetuses (Table 1). Up to a week was required for recovery from the operation (see Results), so results from the first 5–7 days have been excluded from calculations of mean values. Between 130 and 136 days gestational age amniotic fluid became gelatinous, resulting in the complete blockage of some catheters; thus, fewer observations were available for the 2 weeks before birth. Allantoic fluid was obtainable until birth in all cases.

Mean values for 3-day intervals: the period of pregnancy from 69 to 149 days, inclusive, was divided into 27 intervals of 3 days, and the mean and standard deviation (S.D.) of all observations from each 3-day interval was calculated for each parameter. For foetal fluids each mean represents an average of 2·5–3·0 observations per foetus, and for maternal plasma 1·0–1·3 observations per ewe. Other relevant details are given in Tables and legends.

Anatomy of the conceptus. The relationship of the sheep foetus to the placenta and to the amniotic and allantoic sacs has been described in detail by Mellor (1969*b*).

TABLE 1. Details of no. of catheters implanted in single and twin foetuses. The no. of ewes is given in parentheses

Sac catheterized	Gestational age at operation (days)	Full pregnancy		Pregnancy terminated*	
		Singleton	Twin	Singleton	Twin
Amniotic sac	60–97	4 (4)	7 (4)	2 (2)	5 (4)
Allantoic sac	79–97	5§ (5)	3 (2)	—	2 (1)
Total no. of ewes		8†	5‡	2	5

* Samples obtained for at least 14 days.

† One foetus had a catheter in each fluid sac.

‡ One set of twins, one foetus with its amniotic sac and the other with its allantoic sac catheterized, and,

§ Two foetuses each with a catheter in both ends of the allantoic sac (in the end of each uterine horn).

RESULTS

Relation between mean solute concentrations in maternal jugular, uterine and umbilical plasma

The mean concentrations of solutes in all samples of jugular vein plasma taken during the period of withdrawal of amniotic fluid (70–148 days; see also Fig. 7) and allantoic fluid (88–148 days; see also Fig. 8) from ewes with foetal fluid sac catheters, and the ratios of the mean solute concentrations of jugular, uterine and umbilical plasma samples taken between 103 and 144 days gestational age from ewes with maternal and foetal vascular catheters, are given in Table 2. A uniformity of composition of jugular vein plasma from the two groups of ewes with fluid sac catheters

was found. Relative to maternal plasma the foetal plasma values were lower for osmolality (by 7 m-osmole/kg water), [Na^+] (by 6 m-equiv/l.) and [Cl^-] (by 2 m-equiv/l.). The higher [K^+] in jugular than in uterine plasma may have been due to haemolysis resulting from the technique of sampling; 1 % haemolysis would account for this elevation in the [K^+]. The umbilical plasma [K^+] was greater (by 0·6 m-equiv/l.) than that of uterine plasma. The [urea] and [amino acid] were greater in foetal plasma (by about 1·0 and 1·4 mM, respectively) than in maternal plasma. These differences (between 103 and 140 days) are comparable to those found in acute experiments on ewes 60–140 days pregnant (Alexander *et al.* 1958*a*; Meschia, Breathnach, Cotter, Hellegers & Barron, 1965*a*; Hervey & Slater, 1968; Mellor, 1970*a*). Therefore, in all discussion of the *mean* concentrations of solutes in maternal and foetal plasma relative to those in the foetal fluids, these differences (Table 2) have been assumed to be valid over the whole period of observation.

TABLE 2. The mean solute concentrations of jugular vein plasma from ewes with amniotic sac catheters ($\bar{x}_{Am}$) and allantoic sac catheters ($\bar{x}_{Al}$), and the ratios of the mean concentrations of the constituents of maternal jugular vein plasma ($\bar{x}_J$) to those of uterine plasma ($\bar{x}_{Ut}$) and umbilical plasma ($\bar{x}_{Umb}$) from ewes with maternal and foetal vascular catheters

					(mM)	
	(m-osmole/kg water)	(m-equiv/l.)				
	Osmolality	[Na^+]	[K^+]	[Cl^-]	[urea]	[amino acid]
$\bar{x}_{Am}$	293 ± 6	141 ± 8	5·8 ± 0·7	105 ± 3	4·55 ± 1·01	2·56 ± 0·28
	(219)*	(230)	(213)	(212)	(210)	(226)
$\bar{x}_{Al}$	293 ± 7	140 ± 8	5·7 ± 0·6	107 ± 4	4·28 ± 1·06	2·52 ± 0·30
	(177)	(159)	(165)	(184)	(165)	(161)
$\bar{x}_{Ut} - \bar{x}_J$	−2	−1	−0·6	+2	−0·14	−0·13
$\bar{x}_{Umb} - \bar{x}_J$	−7	−6	0·0	−2	+1·00	+1·36
$\bar{x}_{Ut}/\bar{x}_J$	0·993	0·994	0·887	1·018	0·947	0·950
	(61)*	(73)	(85)	(74)	(36)	(71)
$\bar{x}_{Umb}/\bar{x}_J$	0·976	0·962	1·00	0·982	1·225	1·540
	(61)	(73)	(85)	(74)	(36)	(71)

* No. of observations.

Post-operative changes in composition

Maternal and foetal plasma. Whether day-to-day variation was observed or not the relative concentrations of solutes in maternal and foetal plasma (Table 2) were usually maintained. There were no consistent differences between the values at operation for osmolality, [Na^+], [K^+], [Cl^-] and [amino acid] and those from samples taken during the following week, but a greater day-to-day variation was observed in each animal during

the first 2–3 days post-surgery. The [urea] took 3–5 days to decrease from the elevated values observed at and on the 1–2 days after operation (7–9 mM at about 60 days, and 10–18 mM at about 80 days; e.g. Fig. 10) to a stable post-operative level (4–6 mM). In acute experiments Alexander *et al.* (1958*a*) observed an increase in the maternal and foetal plasma [urea] as term approached. In the present study no such increase occurred in the ten ewes with vascular catheters (Table 2), or in maternal plasma of the fourteen ewes with catheters in their foetal fluid sacs (Figs. 7 and 8). The small variation in the [urea] after recovery from operation, and the uniformity of the whole group of ewes was due largely to the carefully regulated diet (J. S. Slater, unpublished data). With the exception of the [urea], therefore, there was remarkably little difference between the plasma values in conscious ewes and foetuses and those when ewes were anaesthetized. This appeared to be true also of whole blood pH, P_{CO_2} and P_{O_2} in the study of Comline & Silver (1970).

Amniotic and allantoic fluid. At operation the osmolality of both foetal fluids was consistently hypotonic (amniotic fluid by 20–50 m-osmole/kg water, and allantoic fluid by 20–30 m-osmole/kg water) to the values in conscious ewes of the same gestational age (Figs. 7 and 8). The day after operation the osmolality usually increased to be isotonic with, or hypertonic (by up to 20 m-osmole/kg water) to, maternal plasma, then showed smaller fluctuations until stable values were reached by 5 days post-surgery. Thus, the clear hypotonicity of both foetal fluids between 80 and 140 days in acute, anaesthetized preparations (Alexander *et al.* 1958*a*; Mellor, 1969*a*, 1970*a*) appears to have been an artifact, although the general trends with gestational age (Alexander *et al.* 1958*a*) approximated to those reported here (Figs. 7 and 8).

Post-surgery changes in the [urea] of both amniotic and allantoic fluid reflected similar changes in maternal plasma [urea] (e.g. Fig. 10), so that stable values were usually reached 3–5 days after operation (see above). In the majority of cases there were no significant differences between operative and immediately post-operative values for amniotic fluid [Na^+] and allantoic fluid [K^+], and any trends in these during the first week tended to be continuous from the day of operation.

In foetuses aged 60–80 days at operation there was a marked decrease in the amniotic fluid [K^+] to stable values 7 days post-surgery (Fig. 1). The magnitude of this post-operative decline decreased from 6 to 10 m-equiv/l. at 60 days to 0–6 m-equiv/l. at 80 days. Thus, values during surgery were greater than those in conscious animals at the same gestational age (Fig. 7). Most foetuses in this study were twins, but a comparison of the [K^+] at operation with those from single foetuses in acute experiments (Mellor, 1970*a*) shows a similar trend during the same stage of gestation (Fig. 1).

Post-surgery changes in amniotic fluid [Cl^-] followed the same pattern as the [K^+]. The magnitude of the decrease in the [Cl^-] in each animal was almost identical with that of the [K^+] (correlation coefficient $r = 0{\cdot}902$; $P < 0{\cdot}001$). During the same period the [Na^+] did not change significantly.

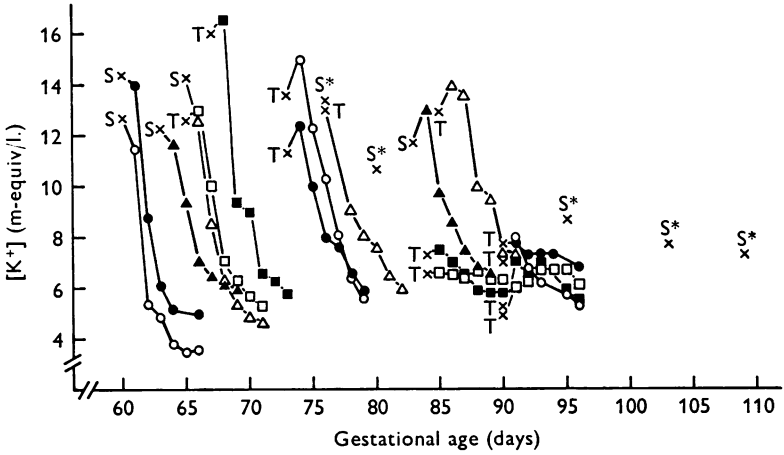

Fig. 1. Changes in the [K^+] of amniotic fluid during the first 6 days after operation in seventeen foetuses; S, singleton; T, twin; S*, singleton from Mellor (1970*a*); X, value at operation.

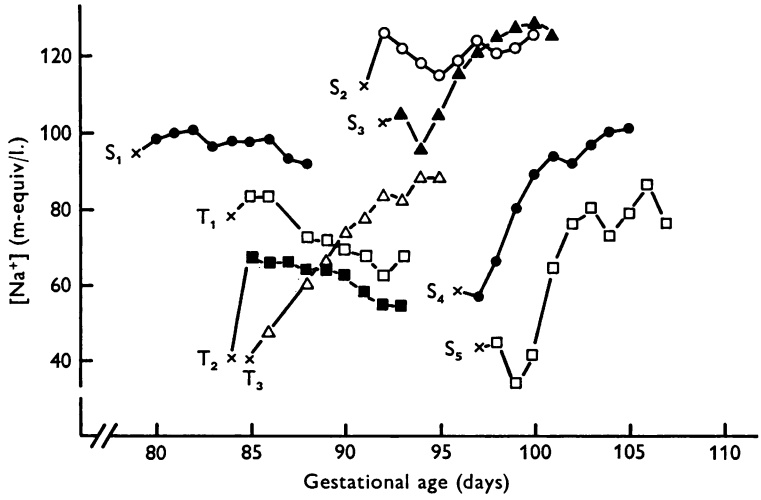

Fig. 2. Changes in the [Na^+] of allantoic fluid during the first 9 days after operation in eight foetuses; S, singleton, T, twin; X, value at operation. Ewes carrying foetuses S_{2-5} and T_{1-3} were starved for 48 hr before operation and the ewe with S_1 for 24 hr. All ewes returned to pre-operative levels of feed intake within 5 days, except the ewe with foetuses $T_{1 \text{ and } 2}$ which did not reach that level of intake until 10 days after operation.

Since Cl^- seems to be actively pumped into the amniotic fluid and K^+ appears to move passively (Mellor, 1970*a*), this suggests that under these conditions K^+ may enter the amniotic fluid in response to the additional anionic charge (in the form of Cl^-) being pumped into it. This elevation of the $[K^+]$ during surgery in foetuses 60–80 days old was also observed in animals after slaughter (Malan *et al.* 1937) and may account for the reported decline as term approached in the amniotic fluid $[K^+]$ in the acute experiments of Alexander *et al.* (1958*a*). In conscious animals the opposite trend was observed (Fig. 7). In each foetus the [amino acid] of amniotic fluid tended to follow the reverse pattern to that of the $[K^+]$ and $[Cl^-]$; the magnitude of the post-operative rise was 0·5–4·0 mM.

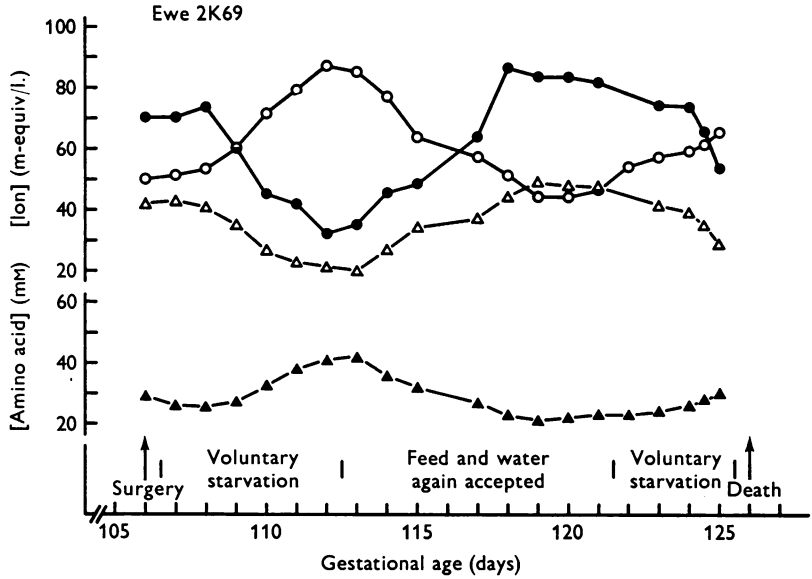

Fig. 3. The $[Na^+]$ (●), $[K^+]$ (○), $[Cl^-]$ (△) and [amino acid] (▲) of allantoic fluid between 105 and 125 days gestational age, showing the relationship between maternal feed intake and allantoic fluid composition.

Changes in the allantoic fluid $[Na^+]$ during the first 9 days after operation are shown in Fig. 2. In most cases the $[Na^+]$ started to increase within 3 days, and in all these animals feed intake had returned to pre-operative values by the 5th day after operation. In one case of a ewe carrying twins (T_1 and T_2; Fig. 2) daily feed intake only reached 80% of pre-operative levels 10 days after surgery, and the $[Na^+]$ decreased until then. All ewes were starved for 48 hr before operation, except one (carrying S_1; Fig. 2) which was starved for 24 hr. Its allantoic fluid $[Na^+]$ showed little variation after operation. These observations suggest that maternal fasting tended

to lower the allantoic fluid [Na^+]. This relationship was demonstrated clearly in a ewe (with a single foetus) that refused feed entirely for the first 6 days after operation, then accepted up to 60% of its daily pre-operative intake levels during the following 9 days, and finally refused feed until it died 4 days later showing clinical signs of pregnancy toxaemia (Fig. 3). During the periods of fasting the [Na^+] decreased, and when feed was consumed it increased. In acute experiments using the same breed (Mellor, 1970*a*) the relatively low [Na^+] at operation (Fig. 2) were not observed; in fact the [Na^+] followed an identical pattern to that found in conscious sheep (Fig. 8). However, feed and water were available *ad lib.* and the ewes were not starved before the acute experiments. The nutritional status of the ewe thus appears to have a greater influence on the allantoic fluid [Na^+] than anaesthesia or surgery, and this may account for the failure of Alexander *et al.* (1958*a*) to observe any definite trend in the [Na^+] as gestation advanced.

When a relatively large rise in the allantoic fluid [Na^+] occurred in the post-operative period ($S_{3,4,5}$, $T_{2,3}$; Fig. 2), during the rapid phase of the rise the [Cl^-] (range 5–45 m-equiv/l.) followed the same pattern, and thereafter tended to decrease (see 2N59; Fig. 9). If no large changes were observed ($S_{1,2}$; T_1; Fig. 2) the [Cl^-] varied independently of the [Na^+]. However, in all cases the [Cl^-] followed the reverse pattern to that of the [amino acid], which decreased (by 30–60 mM) in most animals during the first 5–7 days after operation. Thereafter they varied independently. The magnitudes of the changes in [Cl^-] were usually less than those of the [amino acid]. During the course of gestation these inter-relationships were not usually seen (Fig. 8) unless relatively rapid changes in the concentrations of allantoic fluid solutes occurred (e.g. Fig. 3).

Daily changes in foetal fluid composition

pH changes. The pH of amniotic fluid *in vivo* showed relatively little variation and tended to be more alkaline (range 7·00–7·50; e.g. Fig. 4*A*) than allantoic fluid (range 6·00–7·40; e.g. Fig. 4*B*, *C*). The pH and P_{CO_2} of three amniotic and three allantoic fluid samples exposed to air without agitation at 20° C were followed during equilibration with air, which was complete within 24 hr. As the P_{CO_2} decreased there was a greater increase in the pH of amniotic fluid than of allantoic fluid, suggesting that allantoic fluid has a greater buffering capacity than amniotic fluid. A comparison was made between the pH *in vivo* and the pH of all samples after more than 6 weeks storage. The general pattern of the daily changes was similar, but the correlation was more significant in allantoic fluid (Fig. 5*A*, *B*). For allantoic fluid, therefore, it is unlikely that changes in P_{CO_2} were responsible for the large daily fluctuations of pH *in vivo* (Fig. 4*B*, *C*). In all cases

there was a very close positive correlation between the [Cl^-] of allantoic fluid and its pH after equilibration with air (e.g. Fig. 6); there were no significant correlations between pH and [Na^+], [K^+], [urea] and [amino

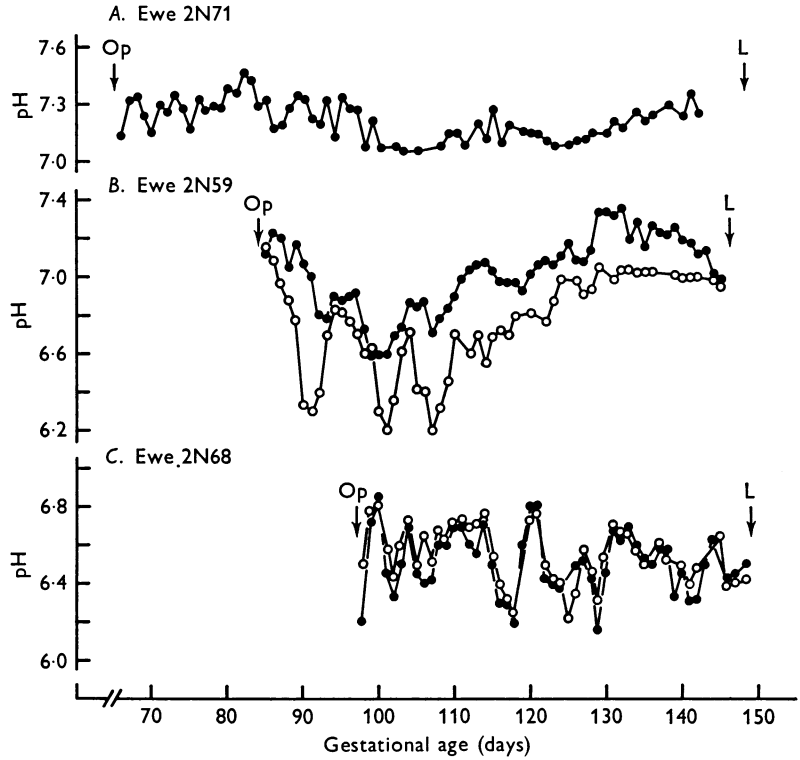

Fig. 4. Daily changes in the pH *in vivo* of amniotic fluid from a single foetus (*A*), of allantoic fluid from twin foetuses (*B*), and of fluid from both ends of the allantoic sac of a single foetus (*C*). Op, operation; L, lambing.

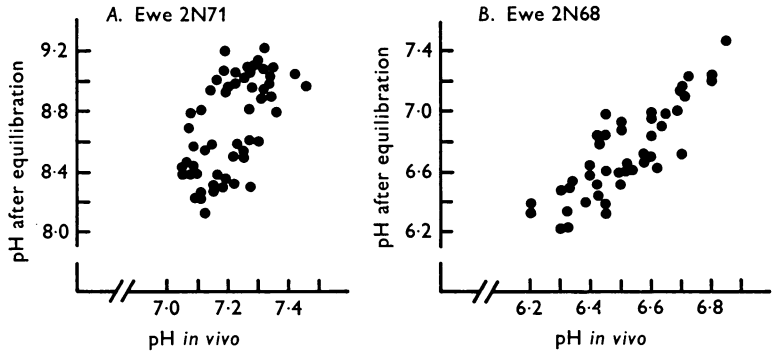

Fig. 5. The relationship between the pH *in vivo* and the pH of samples after equilibration with air in amniotic fluid (*A*) and in allantoic fluid (*B*).

acid]. Movement of Cl^- between allantoic fluid and maternal and foetal plasma seems to be passive and there is a deficiency of inorganic anion relative to cation in allantoic fluid (Mellor, 1970*a*). These observations, and the decrease in the [Cl^-] as the acidity of allantoic fluid increased (Fig. 6), suggest that Cl^- leaves the allantoic sac as organic acid (anion) enters it.

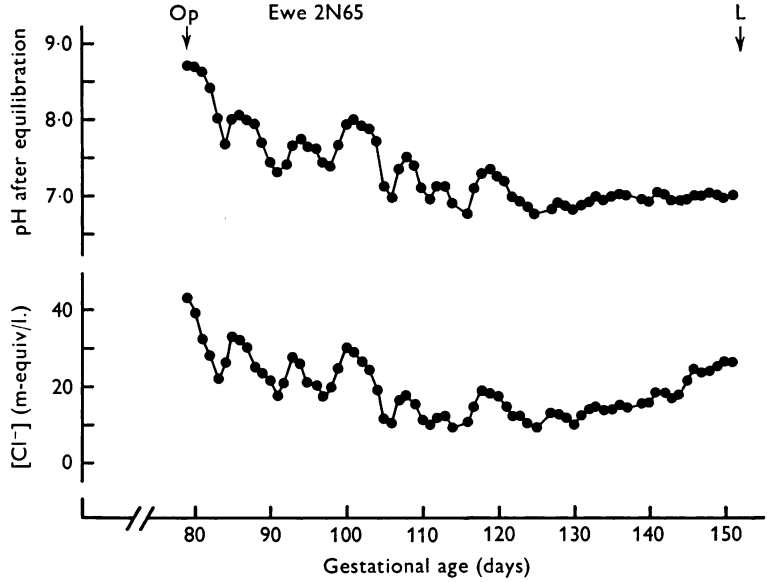

Fig. 6. Daily changes in the pH after equilibration with air and in the [Cl^-] of allantoic fluid from a single foetus between 79 and 151 days gestational age. Op, operation; L, lambing.

Amniotic fluid composition (*Fig. 7*). From 70 to 88 days the mean osmolality of amniotic fluid showed little variation (293–297 m-osmole/kg water) and tended to be slightly hypertonic to both maternal and foetal plasma. From 91 to 100 days it was slightly hypotonic to maternal plasma and its osmolality decreased to become approximately isotonic with foetal plasma (about 285 m-osmole/kg water) between 103 and 112 days gestational age. Thereafter it decreased steadily, reached its lowest value (262 m-osmole/kg water) at 136 days, and then increased (to 272 m-osmole/kg water).

After remaining fairly stable at 132–136 m-equiv/l. between 70 and 79 days, the mean [Na^+] decreased steadily to a plateau of about 95 m-equiv/l. which was maintained from about 136 days to term. In acute experiments a similar trend was observed by Malan *et al.* (1937) and Mellor (1970*a*) but not by Alexander *et al.* (1958*a*).

The mean [Cl^-] remained between 10 and 17 m-equiv/l. above the

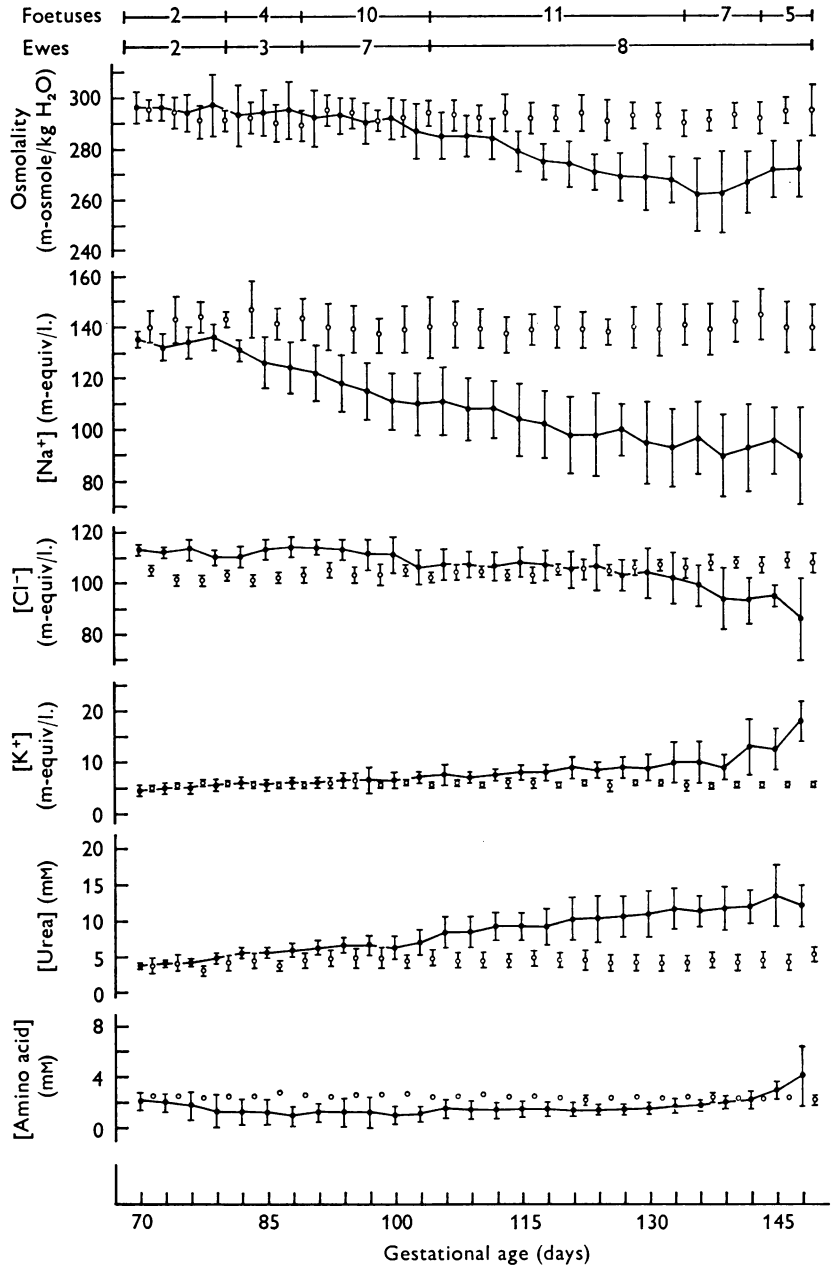

Fig. 7. The mean and S.D. over 3-day-intervals of osmolality, [Na^+], [Cl^-], [K^+], [urea] and [amino acid] of amniotic fluid (●) and maternal jugular vein plasma (○) from up to eleven foetuses and eight ewes between 70 and 148 days gestational age. Maternal values have been placed to the right of the corresponding amniotic fluid value for clarity of presentation. The mean and S.D. of all maternal plasma values for each parameter are given in Table 2.

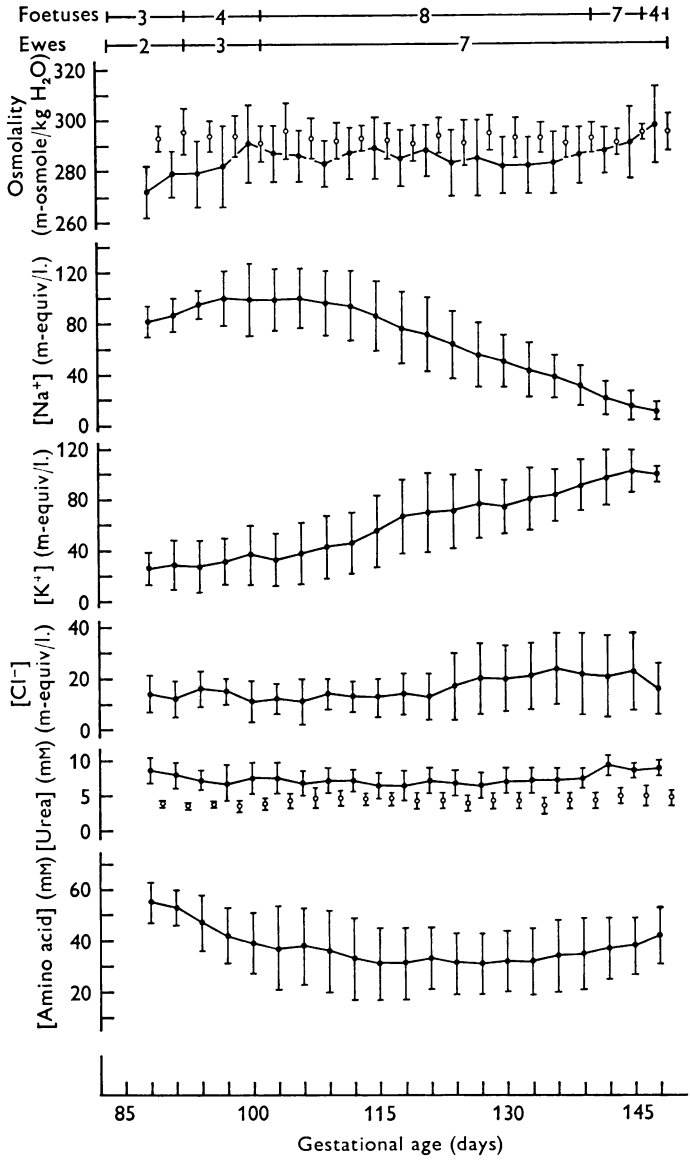

Fig. 8. The mean and S.D. over 3-day-intervals for osmolality, [Na^+], [K^+], [Cl^-], [urea] and [amino acid] of allantoic fluid (●) and maternal jugular vein plasma (○) from up to eight foetuses and seven ewes between 88 and 148 days gestational age. Maternal values have been placed to the right of the corresponding allantoic fluid value for clarity of presentation. The mean and S.D. of all maternal plasma values for each parameter are given in Table 2.

foetal plasma level (99–103 m-equiv/l.) until 100 days when it decreased to about 5 m-equiv/l. above the foetal plasma concentration where it remained until 115–118 days. There was a further decrease from the 124-day level, but the [Cl^-] did not descend below the foetal plasma level until after 133 days when it decreased more rapidly to a final value of 86 m-equiv/l. Similar changes were reported by Alexander *et al.* (1958*a*) and Mellor (1970*a*).

The mean [K^+] closely approximated to the foetal plasma concentration (about 5·8 m-equiv/l.) between 70 and 100 days, although it rose from 4·5 to 6·5 m-equiv/l. during that period. After 100 days the rate of increase was slightly greater and the [K^+] remained above the foetal plasma level reaching 10 m-equiv/l. at 136 days. During the following 12 days it increased more rapidly to 16 m-equiv/l. In each foetus a rise and fall in the [K^+] of 1·5–5·5 m-equiv/l. occurred over a 10–15 day interval during the period from 95 to 125 days. This was not related to any change in the [Na^+] or [Cl^-], and was not evident in Fig. 7 because it occurred at different times in each foetus.

Before 80 days the mean amniotic fluid [urea] approximated to maternal plasma values, between 80 and 100 days it remained slightly above foetal plasma levels, and thereafter it increased from 6·4 to 12·2 mM at term. In each foetus before 100 days parallel changes occurred in the maternal plasma and amniotic fluid [urea], similar to those observed in allantoic fluid (Fig. 10). After 100 days this relationship disappeared. Alexander *et al.* (1958*a*) report similar findings.

The mean [amino acid] of amniotic fluid remained below that of maternal plasma until 139 days gestational age, after which it increased until it was slightly greater than foetal plasma values (about 4·0 mM) at 148 days. In each foetus the [amino acid] tended to decrease before 90–100 days, after which it increased in all cases.

In general, the patterns of daily changes for each parameter were similar in all foetuses. In most foetuses there were small day-to-day fluctuations, but, in some, values varied as much as 1 S.D. about the mean in 1–2 days. Such large day-to-day variations were more often seen in osmolality [Na^+] and [Cl^-] than in the concentrations of the other constituents measured. For all parameters, differences between litter-mates (twins) were as great as those between singletons.

Allantoic fluid composition (*Fig.* 8). The mean osmolality increased from 272 m-osmole/kg water at 88 days to slightly above the foetal plasma level (285 m-osmole/kg water) at 100 days. From 100 to 136 days the allantoic fluid remained isotonic with or slightly hypotonic to foetal plasma, and then increased to become slightly hypertonic (298 m-osmole/kg water) to maternal plasma at term.

Between 88 and 100 days the mean [Na^+] increased from 82 to 100 m-equiv/l. where it remained until 106 days. It then decreased steadily to 12 m-equiv/l. at 148 days. The mean [K^+] increased from 23 to 36 m-equiv/l. between 88 and 100 days and after 106 days increased from 38 to 101 m-equiv/l. at 148 days. In each of the eight foetuses, the magnitudes of the fall in [Na^+] and the rise in [K^+] after 100 days approximated closely to each other ($r = 0{\cdot}792$; $0{\cdot}02 > P > 0{\cdot}01$). This inverse relationship has been observed in acute experiments (Malan *et al.* 1937; Mellor, 1970*a*). The precision of the relationship is demonstrated in Figs. 3 and 9, which show results from individual foetuses.

The gestational age at which the [Na^+] started to decrease and the [K^+] to increase could be related to the nutritional status of the ewe. The [ketone body] of maternal plasma was used as an index of this. Maternal plasma [ketone body] and [glucose] were estimated in thirty-three Scottish Black Face ewes maintained under the same conditions as those in the present study (A. R. Sykes & A. C. Field, unpublished data). After 100 days gestational age, plasma [ketone body] of less than 3·0 mg/100 ml., between 3·0 and 6·0 mg/100 ml., and between 6·0 and 12·0 mg/100 ml., were usually associated with [glucose] of greater than 40 mg/100 ml., between 28 and 36 mg/100 ml., and between 20 and 28 mg/100 ml., respectively. These findings agree with those of Reid & Hinks (1962*a*, *b*) who demonstrated that well nourished ewes had [ketone body] below 3 mg/100 ml., and that undernourished ewes had concentrations greater than 3 mg/100 ml., the degree of undernourishment being directly proportional to the plasma [ketone body].

Striking examples of the relationship of maternal nutrition to changes in the allantoic fluid [Na^+] and [K^+] are given in Fig. 9. The plasma [ketone body] of ewe 2N59, which was carrying twin foetuses, increased from 6·0 mg/100 ml., at 100 days to 14·0 mg/100 ml. at term, and that of ewe 2N68, which carried a single foetus, remained between 2·0 and 2·5 mg/100 ml. during the same period. In ewe 2N59, the allantoic fluid [K^+] of both foetuses increased rapidly and approached maximum values at 117 days. At this age the less rapid rise in the [K^+] of allantoic fluid from ewe 2N68 had just begun, and its maximum value was not reached until term. In the five other ewes, the increase in the [K^+] occurred at gestational ages approximately mid way between those at which the K^+ rise occurred in ewes 2N59 and 2N68, and maximum values were approached 10–15 days before term. In these animals the plasma [ketone body] increased from 2·5–3·5 mg/100 ml. at 100 days to 4·0–6·0 mg/100 ml. at term. In all ewes the [Na^+] followed the reverse trend to the [K^+]. It seems, therefore, that the greater the degree of maternal undernourishment the earlier the changes in allantoic fluid [Na^+] and [K^+] occurred. The low [Na^+] at operation

following a 48 hr fast (Fig. 2), and the decrease in the [Na^+] and increase in the [K^+] observed during fasting after operation (Fig. 3), may have resulted from a more extreme form of this phenomenon.

The mean [Cl^-] of allantoic fluid varied between 10 and 15 m-equiv/l. from 88 to 121 days. It then increased to a level of 20–25 m-equiv/l. which was maintained from 127 days until term. The lower value at 148 days resulted from a decrease, due to lambing, in the number of samples giving high [Cl^-]. In each of the eight foetuses the allantoic fluid [Cl^-] began to increase on the day that the [K^+] reached 70 m-equiv/l. (r = 0·996;

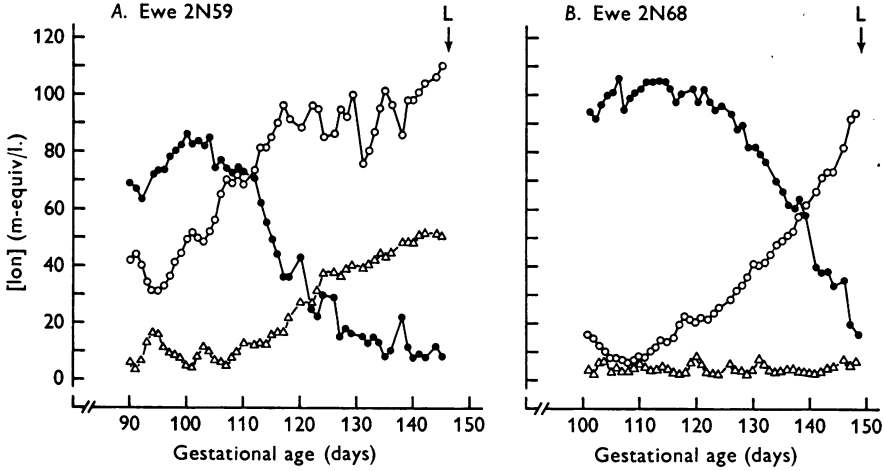

Fig. 9. Daily changes in [Na^+] (●), [K^+] (○) and [Cl^-] (△) of allantoic fluid from one of a set of twin foetuses (*A*) and a singleton (*B*) from the 7th day after operation until term (L). The maternal plasma [ketone body] of the ewe carrying twins (2N59) increased from 6·0 mg/100 ml. at 100 days to 14·0 mg/100 ml. at 142 days gestational age, and that of the singleton bearing ewe (2N68) remained between 2·0 and 2·5 mg/100 ml. throughout the whole period of observation.

P < 0·001), regardless of the [Na^+] (range, 34–102 m-equiv/l.) and gestational age (range, 97–142 days) at that point. The magnitude of the increase in [Cl^-] was greater the earlier the age at which it started (r = 0·749; 0·05 > P > 0·01). Fig. 9 gives results from two foetuses, one in which the allantoic fluid [K^+] reached 70 m-equiv/l. at 107 days, and the other at 142 days. The mean [K^+] and [Cl^-] showed the same relationship at 121 days (Fig. 8). In acute experiments the allantoic fluid [K^+] exceeded 70 m-equiv/l. between 121 days and term, and it was necessary to postulate active transport of K^+ into allantoic fluid to account for the high [K^+] during this period (Mellor, 1970*a*). Since Cl^- movement between allantoic

fluid and maternal and foetal plasma appears to be passive (Mellor, 1970*a*), the rise in its concentration after 121 days in these acute experiments (Mellor, 1969*a*) may have been due to a decrease in the negativity of the allantoic fluid relative to maternal and foetal blood (through electrical potential difference (p.d.) changes) or to a relative increase in the cationic charge in the allantoic fluid. The observation that between 95 and 121 days the allantoic fluid [Cl^-] approximated to that expected at electrochemical equilibrium, and the fact that after 121 days it was 2–3 times greater than the expected equilibrium concentration (Mellor, 1969*a*), together suggest the latter explanation. In all cases the relationship between [Cl^-] and pH was not as close after the [K^+] exceeded 70 m-equiv/l. (e.g. in ewe 2N65 after 130 days; Fig. 6); [K^+] above this level therefore seem to have a greater influence on [Cl^-] of allantoic fluid than that of organic anions.

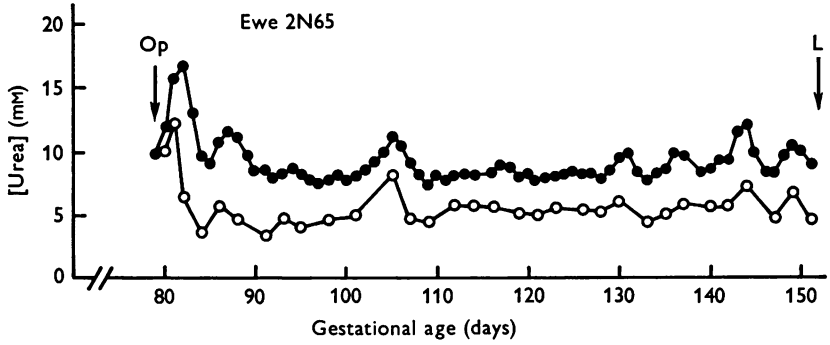

Fig. 10. Daily changes in the [urea] of allantoic fluid (●) and maternal, jugular vein plasma (○) between 79 and 151 days gestational age. Op, operation; L, lambing.

The mean [urea] decreased from 8·6 to about 7·0 mM between 88 and 100 days, then remained relatively steady until 139 days, and finally increased to 8·6–9·3 mM. Thus, the allantoic fluid [urea] remained above the foetal plasma level (about 5·25 mM) throughout, but day-to-day changes occurred in parallel with variations in maternal plasma [urea] (e.g. Fig. 10).

The mean [amino acid] decreased from 55 to 31 mM between 88 and 115 days, remained relatively unchanged until 133 days, and then increased to 42 mM at term. These changes did not seem to be related to variations in the mean concentrations of the other constituents measured (Fig. 8), and no obvious relationships were seen in individual foetuses during the normal course of pregnancy. However, when relatively rapid changes in solute concentrations occurred in two foetuses, the [amino acid] could be

correlated negatively with the [Na^+] and [Cl^-] and positively with the [K^+] (e.g. Fig. 3). These relationships may constitute evidence for the presence of Na^+ coupled amino acid transport mechanisms in the chorio-allantois; such mechanisms have been demonstrated in many tissues (Schultz & Curran, 1970). In each foetus the [amino acid] of allantoic fluid were rarely less than 5 times and were usually greater than 15–20 times maternal concentrations (2·5 mM). Therefore, they were also greater than foetal plasma levels (about 4·0 mM). These relative concentrations are in general agreement with the findings of Alexander *et al.* (1958*a*) and Hervey & Slater (1968). Within the usual pH range of allantoic fluid (6·00–7·40; Fig. 4*B*, *C*) most amino acids would be negatively charged. The large concentration differences could not have been maintained passively by the electrical p.d.s. between allantoic fluid and maternal and foetal plasma (Mellor, 1970*a*), since the negative polarity of allantoic fluid would act to drive anions out of the sac. This suggests that amino acid is actively transported into allantoic fluid. Whether transport occurs from the uterine lumen across the chorioallantois as a whole, or from foetal blood in the chorioallantois across vascular endothelium and allantoic membrane, or both, remains to be determined. The possibility that pumping into allantoic fluid occurs from the mother, and that amino acid reaches foetal plasma in the chorioallantois by diffusion down large concentration gradients, thereby forming a pathway in addition to the placenta, should not be neglected.

The electrolytes and nitrogenous compounds measured account for about 68% of allantoic fluid osmolality; Na^+, K^+ and Cl^- contributed 45–55%, and urea and amino acids 13–23% (Fig. 8). This is markedly different from amniotic fluid in which the electrolytes contributed 73–85% and nitrogenous compounds 2–6% of the osmotically active substances, i.e. 79–87% of the total amniotic fluid osmolality (Fig. 7).

Singletons. In two ewes carrying single foetuses (Table 1), fluid was withdrawn from both ends of the allantoic sac. Fluid from the non-pregnant horn in both cases was isotonic with or slightly hypertonic to maternal plasma, and was consistently hypertonic (by 5–10 m-osmole/kg water) to the fluid from the pregnant horn. This difference reflected consistently greater [Na^+], [K^+], [Cl^-] and [amino acid] in fluid from the non-pregnant horn; the [urea] was consistently lower. However, these differences were rarely greater than 15% so that gestational changes in composition of allantoic fluid from both uterine horns in the two animals were almost identical. A typical example is given in Fig. 4*C*.

Twins. Allantoic fluid was obtained from each foetus in two sets of twins, but the results from one set have not been included in Fig. 8 because the pregnancy was terminated at 124 days (Table 1). In both sets, differences

between the fluid from each foetus were rarely less than 15%. Gestational variations in each parameter followed the same general pattern, but were quite distinct. A typical example is given in Fig. 4*B*.

DISCUSSION

The technique for insertion of catheters into the maternal and foetal vasculatures of sheep has been outlined (Meschia *et al.* 1965*b*) and many of its attendant difficulties have been discussed in detail (Comline & Silver, 1970). In the present study it was frequently not possible to withdraw blood samples without first flushing the catheters. This forced the heparin-saline solution in the catheters into the blood stream. To minimize the quantity of heparin introduced into the foetal circulation in this way, the catheters were flushed twice daily with saline solution containing about one fifth the concentration of heparin used by these other workers. Heparin given to adult human subjects resulted in natriuresis arising from inhibition of aldosterone secretion (Bailey & Ford, 1969). Progesterone was given in one dose 24 hr before operation to ewes intended both for vascular or fluid sac catheterization, and not in a series of injections over several days. This reduced the possibility of disturbances to the foetal fluids, since exogenous progesterone has been shown to have an influence on allantoic fluid composition and volume (Alexander & Williams, 1968). The use of antibiotics was kept to an absolute minimum since marked changes in the composition and volume of allantoic fluid were observed after large doses of antibiotic had been given to ewes (Mellor, Slater & Cockburn, 1971). All animals were handled frequently for 3–8 weeks before operation to enable them to adapt more quickly to the daily sampling routine, which was carried out by the same personnel, at the same time, and using the same technique throughout the study.

Influence of the operative procedures

Feed was normally withheld for 48 hr before operation. During this period water was available *ad lib.* but the ewes rarely drank. While anaesthetized, all ewes lost saliva continuously, and in a few cases regurgitation of rumen fluid occurred. It is probable, therefore, that fluctuations in the osmolality of maternal and foetal plasma and amniotic and allantoic fluid during the first 2–4 days post-surgery were due to a readjustment of maternal and foetal fluid balance. It appears, however, that the hypotonicity of both foetal fluids at operation was due more to the anaesthetic and surgical procedures than to pre-operative feed and water intakes, since a similar degree of hypotonicity was found in the foetal fluids from anaesthetized ewes that had free access to both feed and water up to the

time of experiment (Mellor, 1969*a*, 1970*a*). The operative procedures also appear to have been the major cause of the elevation of the amniotic fluid [K^+], and perhaps [Cl^-], during surgery (Fig. 1). On the other hand, the pre-operative dietary restrictions and the return to normal intake levels after operation seem to have had a greater influence on the composition of allantoic fluid (Figs. 2 and 3). However, variations in the [urea] of maternal plasma during this period were reflected in similar changes in both foetal fluids. Thus, the pre-operative nutritional status of the ewe and the stress of the operative procedures produced artifacts during previous acute experiments (Malan *et al.* 1937; Alexander *et al.* 1958*a*; Mellor, 1970*a*). After operation in the present study most constituents of maternal and foetal plasma reached stable values within 3 days, but those of the foetal fluids required 5–7 days. In addition, during the first week after operation Bassett & Thorburn (1969) found a sixfold decrease in the corticosteriod concentration of maternal plasma, and in three of the four foetuses studied a 20–46% decrease in the corticosteroid concentration of foetal plasma. Thus 7 days are required for recovery from operation.

Daily changes in foetal fluid composition

Influence of foetal urine secretion. It has been shown in acute experiments (Alexander *et al.* 1958*a*; Hervey & Slater 1968; Mellor, 1969*a*, 1970*a*) that foetal urine has a lower osmolality and higher [urea] than both amniotic and allantoic fluid from 60 to 142 days gestational age; that its [Na^+] and [Cl^-] are lower than those of amniotic fluid during the same period; that the [K^+] is higher in foetal urine than in amniotic fluid between 100 and 142 days; and finally, that the foetal urine [Na^+] and [K^+] are lower than those of allantoic fluid between 80 and 110 days gestational age. In the present study, therefore, the first indication of the entry of foetal urine into the amniotic sac was a decrease in the amniotic fluid [Na^+] starting at 79 days (Fig. 7). However, the osmolality did not start to decrease until 88 days. This apparent discrepancy seems to have been due to a rise in the amniotic fluid [Cl^-] between 79 and 88 days, which was approximately equal in magnitude to the initial fall in the [Na^+]. Between 70 and 124 days gestational age the maintenance of the amniotic fluid [Cl^-] above maternal and foetal plasma values (Fig. 7) is consistent with active transport of Cl^- into amniotic fluid until about 130 days (Mellor, 1970*a*). The subsequent decrease in the [Cl^-] agrees with a previously suggested decrease in Cl^- pumping activity at this stage (Mellor, 1970*a*) and with the postulated entry of foetal urine into the amniotic sac. Between 88 and 100 days the allantoic fluid osmolality, [Na^+] and [K^+] increased and its [urea] decreased (Fig. 8). These changes suggest a decreasing contribution of foetal urine to allantoic fluid during this period. However, the

subsequent tendency of the allantoic fluid towards hypotonicity, and its greater [urea] than that of foetal plasma throughout the period of study (Fig. 8), suggest that foetal urine continues to enter the allantoic sac after 100 days.

It has been shown that the average rate of foetal urine flow varies between 8 and 14 ml./hr from 61 to 93 days, that after an initially rapid increase to 26–32 ml./hr at 104 days it remains between 28 and 39 ml./hr until 130 days, and then decreases to 7–14 ml./hr between 137 and 142 days (Alexander *et al.* 1958*b*). In the present study, the progressively greater hypotonicity of amniotic fluid between 100 and 136 days, the sudden decrease in its [Cl^-] and the more rapid increase in both the [K^+] and [urea] after 100 days (Fig. 7), are consistent with an increase in urine flow rate at this stage. A decrease in urine flow rate after 136 days is suggested by the terminal increase in the osmolality of both foetal fluids (Figs. 7 and 8), and the fact that between 136 and 148 days the [urea] of amniotic fluid remains relatively constant (Fig. 7) while that of foetal urine shows a five- to tenfold increase (D. J. Mellor & J. S. Slater, unpublished data). This rise in the [urea] of foetal urine may have been the cause of the increase in the allantoic fluid [urea] between 139 and 148 days gestational age (Fig. 8). The rapid increase in the [K^+] of amniotic fluid between 139 and 148 days (Fig. 7) and the terminal decrease in foetal urine flow rate (Alexander *et al.* 1958*b*), together suggest a marked and rapid increase in the foetal urine [K^+] during this period. Such a change would be consistent with an increase in K^+ secretion by the foetal kidney in response to the rapid pre-parturient rise in the foetal plasma concentrations of corticosteroids (Bassett & Thorburn, 1969; Comline, Nathanielsz, Paisey & Silver, 1970), which have both glucocorticoid and mineralocorticoid activity (Jones, Jarrett, Vinson & Potter, 1964).

It seems, therefore, that foetal urine starts to flow into amniotic fluid in quantities sufficient to alter its composition at about 80 days gestational age, 10 days earlier than Jacqué (1902) and Alexander *et al.* (1958*a*) suggested, and thereafter, that it makes significant contributions to both foetal fluids. However, it is not possible from these results to assess the relative flow rates of foetal urine into the two fluid sacs after 100 days gestational age.

Influence of foetal pulmonary fluid secretion and swallowing. By 56 days gestational age the production of pulmonary fluid has started (Berton, 1969), but flow rates or fluid composition at this early stage of pregnancy have not been measured. In two conscious ewes and foetuses between 118 and 127 days gestational age Merlet, Hoerter, Devilleneuve & Tchobroutsky (1971) found a mean flow rate of 8 ml./hr, and in acute experiments on foetuses near term (Adams, Desilets & Towers, 1967*a*) rates of

10–30 ml./hr have been observed. Between 115 days and term the osmolality and [Na^+] of pulmonary fluid approximate closely to foetal plasma values, but its [Cl^-] is 40–50 m-equiv/l. greater than that of foetal plasma (Adams *et al.* 1967*a*; Adamson, Boyd, Platt & Strang, 1969). It has been generally assumed that foetal pulmonary fluid makes significant contributions to amniotic fluid. The relative [Cl^-] of the two fluids suggest that pulmonary fluid may be a major source of the additional amniotic fluid Cl^- (Fig. 7). The foetal lung would then be a source of the electrical p.d. between foetal plasma and amniotic fluid, the magnitude of which seems to be related closely to the [Cl^-] of amniotic fluid (Mellor, 1970*a*). Moreover, this amniotic fluid p.d. would be expected to show fluctuations associated with opening and closing of the laryngeal sphincter (Adams *et al.* 1967*b*); in the rabbit the source of the amniotic fluid p.d. is the foetal stomach and variations in the p.d. appeared to be due to sphincter activity during swallowing (Mellor, 1969*c*). However, in the sheep the amniotic fluid p.d. showed little variation and was not altered when the head of the foetus was exteriorized through a small incision in the uterine wall and amnio-chorion (Mellor, 1970*a*). This observation, and the maintenance of a high amniotic fluid [Cl^-] while the osmolality and [Na^+] decreased (Fig. 7), are against the idea of foetal pulmonary fluid being a major source of amniotic fluid Cl^-. Indeed, pulmonary fluid would make a small contribution to amniotic fluid since most of it is swallowed (Adams *et al.* 1967*b*). There seem to be no determinations of the total volume of fluid swallowed by the foetus, but any further influence of swallowing on amniotic fluid composition would be largely indirect. Changes in amniotic fluid volume through swallowing would alter the degree of dilution of solutes, and the dilution effects of water, being added to amniotic fluid from all sources.

Influence of the amnion. It has been suggested that the amnion as a whole hinders considerably the passage of solute particles (Mellor, 1970*a*), and the observation in the present study of large concentration differences between solutes in amniotic fluid and those in allantoic fluid and maternal and foetal plasma (Figs. 7 and 8) tends to confirm this. The hypotonicity of amniotic fluid after 112 days also suggests a relative impermeability of the amnion to water. This relative impermeability of the membrane as a whole may be a major determinant of the tendency for ions (Mellor, 1969*a*) and urea (before 100 days; Fig. 7) in amniotic fluid to equilibrate with foetal plasma and not with maternal plasma. Thus, any influence of the ewe on amniotic fluid composition seems likely to be indirect via the placenta; that the influence of the foetus is greater is suggested by twin to twin differences in composition being no smaller than those between singletons. Whether the amnion or foetal skin is the site of Cl^- pumping remains to be determined.

Influence of the chorioallantois. On the basis of their relative permeabilities to Cl^-, Mellor (1970*a*) suggested that the chorioallantois was more permeable to solutes than the amnion, and the low [urea] of allantoic fluid relative to amniotic fluid (Figs. 7 and 8) agrees with this. However, the large differences in the $[Na^+]$, $[K^+]$ and [amino acid] between allantoic fluid and maternal and foetal plasma suggest that the chorioallantois is not freely permeable to these solutes. The concentrations of these substances and Cl^- seem to be regulated directly or indirectly by active transport mechanisms in the chorioallantois, balanced with the effects of foetal urine entering the sac via the urachus and with the movement of water and solutes by diffusion between allantoic fluid and maternal and foetal plasma.

The composition of fluid from both ends of the allantoic sac in singletons is almost identical (Fig. 4*C*) despite the fact that compression of the allantoic isthmus between the uterine wall and amnion would prevent mixing of fluid from each end of the sac (Mellor, 1969*b*), and the compositions of fluid from twin foetuses are distinct (Fig. 4*B*). This suggests that each foetus has a strong influence on the fluid in its own allantoic sac. The changes in the allantoic fluid $[Na^+]$ and $[K^+]$ during pregnancy (Fig. 8) are consistent with an increasing action of mineralocorticoids on pumping mechanisms in the chorioallantois. The concentrations of foetal plasma corticosteroids having both glucocorticoid and mineralocorticoid activity (Jones *et al.* 1964), slowly increase during pregnancy (Bassett & Thorburn, 1969) until the start of a rapid pre-parturient rise (Bassett & Thorburn, 1969; Comline *et al.* 1970), and the rates and directions of change in the allantoic fluid $[Na^+]$ after operation (Fig. 2) are consistent with post-operative changes in the concentrations of foetal plasma corticosteroids (Bassett & Thorburn, 1969). These hormones would have adequate opportunity to reach the chorioallantois since the intercotyledonary chorion receives approximately the same proportion of foetal cardiac output as the foetal brain, heart, and kidney (Makowski, Meschia, Droegemueller & Battaglia, 1968; Rudolph, 1969). It seems, therefore, that corticosteroids secreted by the foetal adrenal glands (Bassett & Thorburn, 1969; Comline *et al.* 1970) may be involved in regulating the ionic composition of allantoic fluid.

Influence of the ewe. Restricted maternal feed intake caused a marked decrease in the $[Na^+]$ and an increase in the $[K^+]$ of allantoic fluid, and these trends were reversed during the return to normal intake levels (Figs. 2 and 3). These changes during fasting and refeeding are consistent with an increase and a decrease, respectively, in the foetal plasma concentrations of corticosteroids. Therefore, the nature of possible maternal stimuli to foetal corticosteroid secretion will now be considered. It seems probable that the substance or substances effecting stimulation of the

foetal adrenal glands, directly or through the foetal pituitary-ACTH-axis (Liggins, 1969; Alexander, Britton, Forsling, Nixon & Ratcliffe, 1971*a*, *b*) must pass across the placenta. The placenta is relatively impermeable to both maternal and foetal corticosteroids (Bassett & Thorburn, 1969; Comline *et al.* 1970) and to other hormones (Alexander, Britton, Cohen, Nixon & Parker, 1968; Bassett, Thorburn & Wallace, 1970; Comline, Nathanielsz & Silver, 1970; Alexander *et al.* 1971*a*, *b*) which suggests that the stimulus is indirect through maternal metabolites. The normal response of pregnant and non pregnant ewes to fasting, particularly if the environment is changed during the fast, is the development of hypoglycaemia, hyperketonaemia, and an increased plasma [FFA] (Reid, 1960*b*; Reid & Hinks, 1962*b*; Saba, Burns, Cunningham, Hebert & Patterson, 1966; D. J. Mellor & J. S. Slater, unpublished data). Upon refeeding there is an almost immediate reversal of these effects. The placenta seems to be relatively impermeable to ketone bodies and to FFA, but it is permeable to glucose (Alexander, Britton, Cohen & Nixon, 1969). A highly significant positive correlation between glucose concentrations in maternal and foetal plasma, and a less significant but similar relationship between maternal plasma glucose and foetal plasma fructose, has been found in conscious ewes and foetuses (Comline & Silver, 1970). Thus it can be inferred that maternal hypoglycaemia during fasting would result in a decrease in foetal plasma glucose and fructose concentrations. A decrease in plasma glucose concentrations strongly stimulates the adrenal glands to secrete corticosteroids in ewes and 6-week-old lambs (Bassett & Hinks, 1969), and the adrenals of 88-day and older foetuses respond in a similar manner to ACTH infusion (Liggins, 1968). These facts indicate that during maternal hypoglycaemia the foetal adrenal glands may be stimulated to secrete additional corticosteroid by a reduction in foetal plasma glucose, and that a subsequent increase in maternal plasma glucose concentration would tend to reverse these effects. The elevated concentrations of corticosteroid in maternal plasma during the first 3–4 days after operation (Bassett & Thorburn, 1969) would act to increase maternal plasma glucose levels (Bassett, Mills & Reid, 1966; Saba *et al.* 1966). In pregnant ewes plasma glucose concentrations usually increased the day after operation (D. J. Mellor & J. S. Slater, unpublished data; Comline & Silver, 1970) which, according to the above hypothesis, should produce an immediate rise in the allantoic fluid [Na^+] even when feed intake levels remain below normal for several days. Such a response was seen in some cases, but a delay of 1–2 days before the [Na^+] increased was seen in others (Fig. 2). The delay may have resulted from an elevation, or maintenance of higher levels, of foetal plasma corticosteroids due to the stresses of the operative procedures acting on the foetus itself. The results of Bassett & Thorburn (1969) agree

with this. There was a 2-day delay in the fall of the allantoic fluid [Na^+] and the rise in the [K^+] in a ewe in which fasting continued after operation (Fig. 3). This may have been due to an influence on the foetus of the post-operative elevation in maternal plasma glucose concentrations.

During the course of pregnancy the glucose concentration of maternal plasma normally decreases (Reid & Hinks, 1962*a*). The decrease in foetal plasma fructose (Comline & Silver, 1970) and the slow increase in foetal plasma corticosteroid levels (Bassett & Thorburn, 1969) as pregnancy proceeds are consistent with this. During the last half of pregnancy, the plasma glucose levels of ewes carrying twins are lower than those of ewes with singletons, and the rate of fall of plasma glucose is greater in polytocous ewes (Reid & Hinks, 1962*a*). It follows that secretory activity of the foetal adrenal cortex may be greater in twin foetuses than in singletons of the same gestational age. If this is the case, and if the changes in the [Na^+] and [K^+] of allantoic fluid (Fig. 8) are due to corticosteroid secretion by the foetal adrenal glands, the allantoic fluid [Na^+] would be expected to decrease, and the [K^+] to increase, at an earlier gestational age in twin foetuses than in singletons. This appears to occur. In a twin bearing ewe (2N59; Fig. 9) with a significant degree of hypoglycaemia, as judged by the [ketone body] of maternal plasma (Reid & Hinks, 1962*a*, *b*), the [K^+] of allantoic fluid from each foetus increased rapidly from the values at operation (8 and 12 m-equiv/l.) at 84 days gestational age to approximate to maximum levels (96 and 118 m-equiv/l.) at 117 days. These levels were maintained until term. The [Na^+] decreased continuously after 100 days gestational age. On the other hand, in a ewe with a single foetus (2N68; Fig. 9), the allantoic fluid [K^+] started to increase at 110 days, and the [Na^+] to decrease at 115–120 days gestational age, reaching maximum and minimum values, respectively, at term. The plasma [ketone body] of this ewe indicated that its glucose level was in the non-pregnant range and would not have been lowered significantly between 100 days and term (Reid & Hinks, 1962*a*, *b*). In addition, in four moderately hyperketonaemic (hypoglycaemic) ewes with singletons, the allantoic fluid [K^+] started to increase at 95–101 days, the [Na^+] to decrease at 103–107 days, and maximum and minimum values, respectively, were approached 10–15 days before term. The results from the ewes with single foetuses, one ewe with relatively high plasma glucose concentrations (low [ketone body]) and four with moderate hypoglycaemia (hyperketonaemia), and those from the twin bearing ewe with relatively severe hypoglycaemia (hyperketonaemia), together suggest that stimulation of the foetal adrenal glands was effected by a lowering of maternal plasma glucose concentrations, and that the intensity of stimulation was directly proportional to the degree of maternal hypoglycaemia. However, in the absence of maternal hypo-

glycaemia the foetal plasma concentrations of corticosteroids still appear to increase as pregnancy proceeds (2N68; Fig. 9) and the rapid preparturient rise in their concentrations (Bassett & Thorburn, 1969; Comline *et al.* 1970) does not seem to depend on a terminal fall in the maternal plasma glucose concentration (2N68; Fig. 9). Therefore, maternal induced foetal hypoglycaemia is probably not the only stimulus effecting an increase in foetal corticosteroid output, but when present it seems to have a significant effect which is superimposed upon a background pattern determined by other factors. This appears to be worthy of further investigation.

It is possible that maternal hormones might act directly on the chorion after diffusion from blood in the endometrium, to produce the changes observed in allantoic fluid composition during fasting and refeeding and during the course of pregnancy. Data on plasma concentrations of aldosterone under these circumstances are not available. However, plasma cortisol concentrations during fasting and refeeding (Lindner, 1959; Reid, 1960*b*; Saba *et al.* 1966) and cortisol and corticosterone concentrations of maternal plasma between 110 days gestational age and term (Bassett & Thorburn, 1969) have been measured and do not change significantly. The effects of other hormones such as maternal and foetal progesterone and oestrogens (Harrison & Heap, 1968; Bassett, Oxborrow, Smith & Thorburn, 1969; Findlay & Cox, 1970; Challis, 1971) should also be studied since they have been shown to influence the ionic composition and the volume of allantoic fluid (Alexander & Williams, 1968).

Changes in foetal fluid volume

Foetal fluid volumes have been determined at different gestational ages in a total of fifty-three Merino ewes (Malan *et al.* 1937; Cloete, 1939). The mean volume of amniotic fluid increased from 170 to 550 ml. between 60 and 85 days, rose more slowly to 620 ml. at 115 days, and then decreased to about 360 ml. at 145 days. Assuming similar changes in volume, amniotic fluid was hypertonic to both maternal and foetal plasma while its volume increased rapidly (70–88 days; Fig. 7), it was slightly hypertonic to and then approximately isotonic with foetal plasma while the volume increased slowly (91–112 days; Fig. 7), and it was hypotonic to both maternal and foetal plasma during the terminal volume decrease (115–145 days; Fig. 7). The mean allantoic fluid volume was 170–180 ml. between 60 and 85 days, then increased continuously through 440 ml. at 115 days to 750 ml. at term. Before 90 days allantoic fluid was hypotonic (Alexander *et al.* 1958*a*; Fig. 8) and thereafter it approached isotonicity with foetal plasma (Fig. 8), so that its volume seems to have remained relatively unchanged while its osmolality was low. Thus, a low osmolality of either fluid,

apparently resulting from the entry of foetal urine (see above), seems to have been associated with no change (allantoic fluid before 85 days) or a decrease (amniotic fluid after 115 days) in fluid volume, whereas volumes appear to have increased when the foetal fluid osmolality approximated to that of maternal and foetal plasma (amniotic fluid before 115 days and allantoic fluid after 100 days).

The greatest expansion of fluid volume in the amniotic sac appears to occur before 85 days while urine flow rate is low (Alexander *et al.* 1958*b*) and before urine entry into the sac in large quantities is detectable (Fig. 7). The volume of allantoic fluid seems to increase rapidly after 100 days, which is apparently after the entry of foetal urine into this sac has decreased (Fig. 8). Therefore, although changes in fluid composition can be related closely to rates of foetal urine flow (see above), these flow rates (Alexander *et al.* 1958*b*) do not appear to be directly associated with fluid volume changes (Malan *et al.* 1937; Cloete, 1939).

During the rapid expansion of allantoic fluid volume between 85 days and term solutes appear to be pumped continuously into the allantoic sac (K^+ and amino acids; Fig. 8). However, during the same period in cases where pumping mechanisms in the chorioallantois were poisoned, or its permeability to solutes was increased, or both, loss of solute from within the sac was associated with a decrease in volume to less than 10 ml. (Mellor *et al.* 1971). This fact, and the apparent association between fluid volume changes and the relative osmolalities of foetal fluids and plasma, together suggest that the quantity of solute relative to that of water within each sac is a major determinant of fluid volume changes.

Acute and chronic preparations

Significant differences between properties of blood from anaesthetized ewes and foetuses and those from chronically catheterized animals have been described previously (Meschia *et al.* 1965*b*; Comline & Silver, 1970) but the present study is the first demonstration that pre-operative and operative procedures influence the composition of the foetal fluids. Two major causes of altered fluid composition are the sensitivity of the foetus to pre-operative starvation of the ewe and to the stresses of the operation itself. Fluid composition is also affected by the general nutritional status of the ewe. These influences are present in all acute experiments and inevitably make valid interpretation of results from individuals and from groups of animals difficult or impossible. However, in chronically catheterized ewes and foetuses observations need not start until these disturbances have passed. Also, the nutritional status of each ewe may be controlled in precisely the same manner as that of uncatheterized animals. The observations in the present study and those of Meschia *et al.* (1965*b*),

Bassett & Thorburn (1969), Comline & Silver (1970) and others, indicate that chronically catheterized preparations offer a wider scope for physiological studies on the conceptus, and their use can be expected to supersede that of acute, anaesthetized preparations.

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