J. Physiol. (1972), **227**, *pp.* 503–525
With 8 *text-figures*
Printed in Great Britain

DAILY CHANGES IN FOETAL URINE AND RELATIONSHIPS WITH AMNIOTIC AND ALLANTOIC FLUID AND MATERNAL PLASMA DURING THE LAST TWO MONTHS OF PREGNANCY IN CONSCIOUS, UNSTRESSED EWES WITH CHRONICALLY IMPLANTED CATHETERS

By D. J. MELLOR and J. S. SLATER
From the Moredun Research Institute, Gilmerton, Edinburgh EH17 7JH

(*Received 12 April 1972*)

SUMMARY

1. The fluid sacs and bladders of ten foetuses and the allantoic sacs of five foetuses were catheterized between 79 and 96 days gestational age and daily samples were withdrawn until lambs were born naturally at ~ 147 days. Maternal jugular plasma obtained daily allowed the nutritional status of each ewe to be regulated and monitored. All lambs were observed for 7 weeks, and at post-mortem no abnormalities were seen in those operated upon *in utero*.

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

3. Foetal surgery seemed to affect the actual concentrations of some solutes, but gestational trends in foetal fluid composition were unaltered.

4. Until about 7 days before birth the foetal urine osmolality, [Na^+], [Cl^-] and [fructose] decreased, its [urea], [amino acid] and pH remained relatively constant, and from about 120 days gestational age the [K^+] increased. During the last 7 days there was a marked increase in the osmolality and the concentrations of all these solutes, and a decrease in pH.

5. Entry of foetal urine into the fluid sacs tended to decrease the osmolality, [Na^+], [K^+], [Cl^-] and [glucose] of both foetal fluids and the [amino acid] of allantoic fluid, and tended to increase the [fructose] and [urea] of both fluids and the [amino acid] of amniotic fluid.

6. Changes in urine composition suggested large daily variations in the secretion of foetal antidiuretic hormone and also a rapid increase in its secretion during the last 7 days, and particularly the last 2–4 days before birth.

7. Changes in the [Na^+]/[K^+] ratios of foetal urine and allantoic fluid

were parallel during post-operative recovery, during the course of pregnancy and immediately before birth, and this was consistent with a simultaneous action of foetal plasma corticosteroids on the foetal kidneys and chorioallantois.

8. Variations in the [fructose] of foetal urine and allantoic fluid were parallel to changes in their $[Na^+]/[K^+]$ ratios and suggested an involvement of foetal corticosteroids in the regulation of the [fructose] of foetal plasma.

9. Further evidence has been presented supporting the hypothesis that maternal induced foetal hypoglycaemia effects a relative increase in the secretion of foetal corticosteroids having an action on the chorioallantois. Also, high concentrations of maternal plasma corticosteroids may decrease the permeability of the placenta to glucose.

INTRODUCTION

Suggested interrelationships between foetal and maternal plasma and the foetal fluids (Alexander, Nixon, Widdas & Wohlzogen, 1958*a*; Hervey & Slater, 1968; Mellor, 1970*b*; Mellor & Slater, 1971) involved speculation as to the role of foetal urine in foetal fluid formation, but all data on foetal urine were from acute experiments. Chronically catheterized preparations have been used to study sheep foetal fluids and have demonstrated that distinct differences between the composition of fluid samples from acute and chronic preparations were due largely to pre-operative and operative procedures (Mellor, 1970*a*; Mellor & Slater, 1971; Mellor, Slater & Cockburn, 1971). These findings, and those from sheep foetuses with chronically implanted vascular catheters (e.g. Meschia, Cotter, Breathnach & Barron, 1965; Bassett, Thorburn & Wallace, 1970; Comline & Silver, 1970; Mellor & Slater, 1971) emphasized the value of observations on chronically catheterized animals.

The present paper describes for the first time gestational changes in foetal urine composition in conscious, unstressed ewes, using the catheterization technique of Mellor, Williams & Matheson (1972). Natural parturition is not impeded by the technique and the subsequent progress of each lamb may be observed. It has been used here to examine daily changes in the composition of foetal urine for up to 10 weeks in pregnancies resulting in the birth of healthy viable lambs. In addition, its use in individual foetuses in conjunction with the method of daily sampling of amniotic and allantoic fluid (Mellor, 1970*a*) allowed interrelationships between simultaneous changes in composition of foetal urine, the foetal fluids and maternal plasma to be investigated in more detail than was hitherto possible. From distinct changes occurring during post-operative recovery, during the course of pregnancy and immediately before birth, it appears that the conceptus responds to maternal changes as an integrated and largely self-regulating system.

METHODS

Animals. Twenty 5-year-old Scottish Black Face ewes (35–58 kg) from a single flock, with known mating dates were used. The housing, husbandry and maintenance procedures were as before (Mellor & Slater, 1971), but the [glucose] of plasma sampled daily from a maternal jugular vein were determined in addition to weekly estimations of the [ketone body] of maternal plasma. This enabled the relative nutritional status of each ewe to be assessed (Russell, Doney & Reid, 1967; Mellor & Slater, 1971). Ewe weight gains during pregnancy, and lamb birth weights and growth rates to weaning were comparable to those reported by Mellor & Slater (1971).

Foetal bladder catheters. Foetal urine samples were obtained daily until term from nine ewes (carrying ten lambs; Table 1), and for shorter periods or irregularly from seven other ewes (carrying seven lambs).

Allantoic sac catheters were inserted into five conceptuses to investigate relationships between daily changes in maternal plasma and allantoic fluid composition.

Surgical and experimental procedures were as described by Mellor (1970*a*), Mellor & Slater (1971) and Mellor *et al.* (1972).

Measurements. Osmolality, pH, [Na^+], [K^+], [Cl^-], [urea], [amino acid] and [ketone body] were determined as before (Mellor & Slater, 1971). The [glucose] was determined according to Trinder (1969), the concentration of total reducing substances (TRS) using an Autoanalyser (Technicon; method file N-2b), and the [fructose] was estimated by difference. Creatinine contributed less than 10% to the [TRS] of amniotic fluid, allantoic fluid and foetal urine (J. S. Slater and E. J. Dunnett, unpublished data). Solute concentrations have been expressed in m-equiv/l. (Na^+, K^+, Cl^-) or mM (fructose, urea, amino acids) to show contributions to osmolality. Otherwise the [fructose] and [urea] have been given in mg/100 ml.

Presentation of data

Foetal urine. Daily changes in urine were followed (in nine ewes) for 60–68 days in nine foetuses, and 49 days in one foetus (Table 1). The results from the first 7 days were excluded from calculation of mean values to allow for recovery from operation; changes during recovery were followed in fourteen foetuses (Fig. 2).

Mean values for 3-day intervals. The period of pregnancy from 90 to 131 days, inclusive, was divided into fourteen intervals of 3 days, and the mean and standard deviation (S.D.) of all observations from each 3-day interval were calculated for each parameter (Figs. 4, 5). Each mean represents 2·8–3·0 observations per foetus.

Mean values for 14 days pre-partum. The mean and S.D. of all determinations on samples taken within 12 hr of birth (140–151 days gestational age), and on those taken on each successive day between 1 and 14 days before birth, were calculated for each parameter (Figs. 4, 5). No urine was obtained after birth. Each mean represents 0·9–1·0 observations per foetus.

This approach resulted in a gap of 2–6 days (nine foetuses) and an overlap of 5 days (one foetus) in the values represented by the 3-day interval mean at 130 days and the daily mean at 14 days pre-partum (Figs. 4, 5) but changes during this period were relatively small so the results may be regarded as continuous.

Foetal fluids and foetal urine. A daily comparison was made between the compositions of amniotic fluid and foetal urine from up to eleven foetuses, and of allantoic fluid and foetal urine from up to five foetuses, which enabled effects of foetal urine secretion on foetal fluid composition to be examined.

The anatomy of the sheep conceptus has been described by Mellor (1969).

TABLE 1. Details of number of catheters implanted in different foetal fluid spaces. The number of ewes is given in parentheses

Fluid space catheterized			Gestational age at operation (days)	No. of foetuses	
Amniotic sac	Allantoic sac	Foetal bladder		Full pregnancy	Part pregnancy*
+	+	+	82–91	4 (4)	4 (4)
+	−	+	79–84	6 (5)	3 (3)
−	+	−	82–96	5 (4)†	—

* Samples obtained for at least 30 days from one or more catheters.

† One foetus in a set of twins sham-operated for foetal bladder catheter insertion.

RESULTS

The patterns of change and interrelationships of all parameters measured in amniotic and allantoic fluid were the same in operated (bladder catheter) and unoperated foetuses and confirmed earlier data on unoperated foetuses (Mellor & Slater, 1971). However, the [Cl^-] of amniotic and allantoic fluid were higher (by 15–25 m-equiv/l.), the [amino acid] of allantoic fluid were lower (by 5–15 mM) and the pH of allantoic fluid was higher (by 0·2–0·7 units) in operated than in unoperated foetuses.

Post-operative changes in composition

Maternal plasma. In most ewes during the 3 days before operation there were no changes in the values for any parameters measured in maternal plasma, except the [glucose] which decreased while feed was withheld (e.g. Figs. 1, 8). The day after operation the [glucose] and [urea] increased and the [amino acid] decreased, all returning to pre-operative values during the following 4–6 days. There was no obvious association between maternal feed intake and plasma [glucose] in any ewe during the first 3–5 days after operation. This, and the changes in the plasma [urea] and [amino acid], may have been due to high maternal corticosteroid secretion during the recovery period (Bassett, Mills & Reid, 1966; Bassett, 1968; Bassett & Thorburn, 1969). Thereafter the correlation between feed intake and plasma [glucose] was again observed.

Allantoic fluid. Post-operative changes in the [K^+] of allantoic fluid from twin foetuses (*A* and *B*) are given in Fig. 1; foetus *B* was sham-operated for bladder catheterization and showed a relatively greater post-operative increase (between days 0 and 3) in its allantoic fluid [K^+]

which then decreased to approximately the same level as that of foetus *A* (by day 9). This pattern was observed in eight of the nine ewes with allantoic sac catheters which were also operated upon for bladder catheterization (Table 1); ewe 4V31 (Fig. 7) was the exception.

An inverse relationship between the [K^+] and [fructose] of allantoic fluid was observed in each foetus during the recovery period (e.g. Fig. 1) and the course of pregnancy. The [fructose] generally decreased between

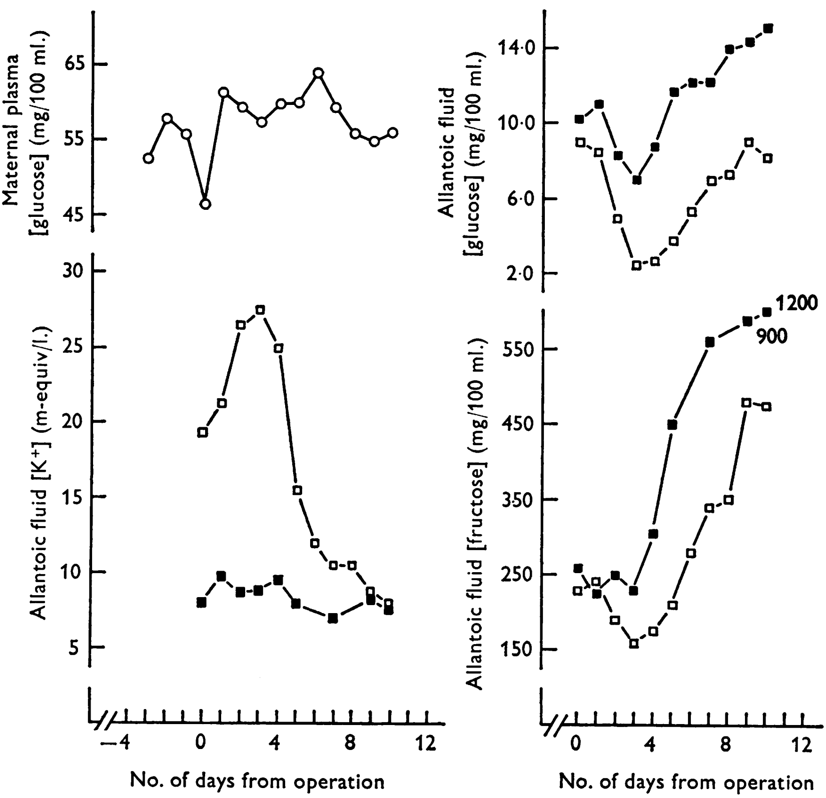

Fig. 1. Ewe 3V95; the [glucose] of maternal plasma (○) for 3 days before and 10 days after operation at 82 days gestational age, and the [K^+], [glucose] and [fructose] of allantoic fluid from the day of operation for 10 days in a set of twin foetuses (*A*, ■; *B*, □). A catheter was inserted into the allantoic sac of each foetus, and foetus *B* was sham-operated for bladder catheter insertion.

days 0 and 3, increased to a maximum on about day 8, and then declined progressively. In eight foetuses there was a significant negative correlation between the highest [fructose] of allantoic fluid (6–9 days after operation) and its corresponding [K^+] (correlation coefficient $r = 0{\cdot}774$; $0{\cdot}02 > P > 0{\cdot}01$).

Foetal urine. Major changes in foetal urine composition during recovery are shown in Fig. 2. The mean [K^+] decreased from 6·4 m-equiv/l. on day 2 to 1·0 m-equiv/l. on days 5 and 6 and then increased to 3·0 m-equiv/l. on day 14. These changes in the mean [K^+] were responsible largely for the changes in the mean [Na^+]/[K^+] ratio, which increased from 11 on day 2 to a plateau of 59–65 on days 4–7, and then decreased to 19 on day 14.

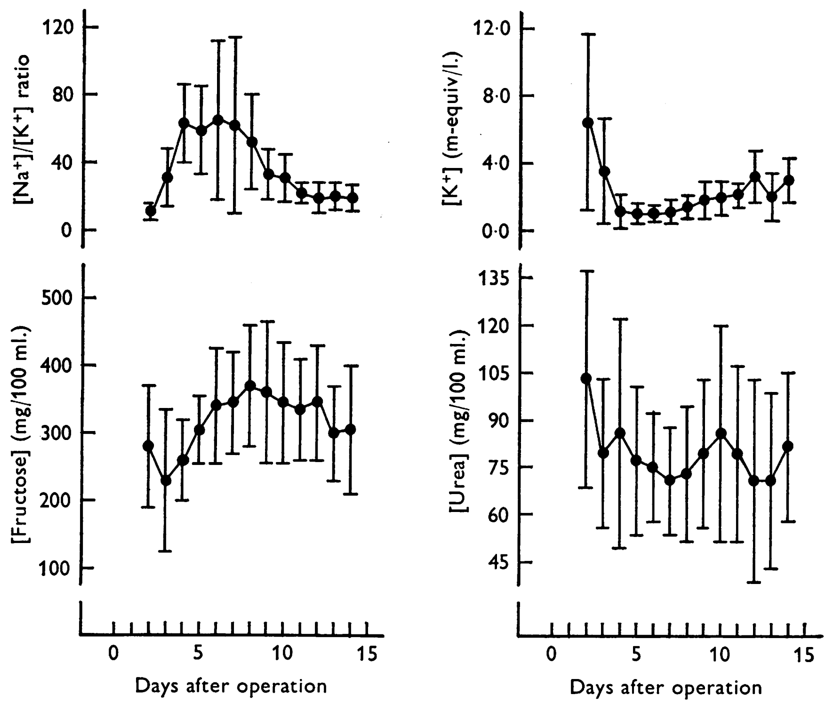

Fig. 2. Daily changes in the mean and S.D. for [Na^+]/[K^+] ratio, [K^+], [fructose] and [urea] of foetal urine from fourteen foetuses in thirteen ewes during the 2 weeks following operation. The operation was on day 0 and first foetal urine samples were usually obtained on day 2.

The mean [urea] decreased from 100 mg/100 ml. on day 2 to 70 mg/100 ml. on day 7, and the changes to the 4th day after surgery were approximately parallel to those of the maternal plasma [urea]. After an initial fall between days 2 and 3, the [fructose] rose to a maximum of 370 mg/100 ml. 8 days after surgery and changes in the [fructose] were parallel to, but occurred about 1 day later than changes in the [Na^+]/[K^+] ratio.

It has been shown in acute experiments that urea and fructose are excreted in a similar way by the sheep foetal kidney (Alexander *et al.* 1958*b*). In ten foetuses during post-operative recovery and the course of pregnancy there was a direct correlation between the [urea] and [fructose], which during the recovery period tended to be masked by the rapidly changing [fructose]. However, in one foetus the urine [fructose] rose to a relative plateau which was maintained between 90 and 115 days gestational age. The strikingly close relationship in this case (Fig. 3) and the correlations in the other foetuses agree with the previous findings (Alexander *et al.* 1958*b*).

The $[Na^+]/[K^+]$ *ratios of foetal urine and allantoic fluid.* In four foetuses with bladder and allantoic sac catheters the mean $[Na^+]/[K^+]$ ratios of foetal urine and allantoic fluid changed in parallel during the 14 days

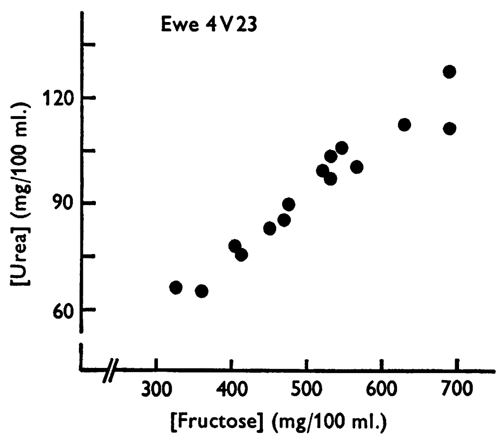

Fig. 3. The relationship between the [fructose] and [urea] of urine from a single foetus between 90 and 115 days gestational age, after the post-operative rise in the [fructose] had ceased.

after operation (Table 2). However, changes in the mean ratio of foetal urine preceded those of allantoic fluid by about 1 day.

The [fructose] of foetal urine and allantoic fluid. Changes in the [fructose] of foetal urine and allantoic fluid were parallel in four foetuses with bladder and allantoic sac catheters. Since the [fructose] of allantoic fluid remained relatively low or decreased during the first 3–4 days after operation (e.g. Fig. 1) the decrease in the mean [fructose] of foetal urine between days 2 and 3 may have been a continuation of a fall that started on the day of operation.

Daily changes from 90 days gestational age to term

Maternal plasma. Since no gestational trends were seen in maternal plasma for any parameter other than the [glucose], the mean values for the nine ewes with foetal bladder catheters are given in Table 3. The [fructose] were negligible. The mean [glucose] of maternal plasma from

TABLE 2. The daily mean and S.D. for the $[Na^+]/[K^+]$ ratios in allantoic fluid (R_{Al}) and foetal urine (R_{FU}) from four foetuses from the day of operation for 14 days

	Days from operation														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
R_{Al}	24	12	12	14	17	21	28	27	19	16	14	16	10	10	9
	± 12	± 10	± 10	± 8	± 4	± 8	± 21	± 17	± 4	± 4	± 4	± 4	± 2	± 3	± 3
R_{FU}	—	—	12	25	65	58	71	37	33	24	28	20	16	18	16
			± 4	± 12	± 31	± 20	± 43	± 13	± 21	± 12	± 17	± 9	± 11	± 10	± 11

TABLE 3. The mean and S.D. for osmolality, $[Na^+]$, $[K^+]$, $[Cl^-]$, [urea] and [amino acid] of maternal plasma from nine ewes with foetal bladder catheters

	m-equiv/l.			mM	
Osmolality (m-osmole/kg H_2O)	$[Na^+]$	$[K^+]$	$[Cl^-]$	[Urea]	[Amino acid]
289 ± 8 (501)	139 ± 9 (496)	4·1 ± 0·6 (497)	110 ± 4 (496)	4·48 ± 0·84 (502)	3·6 ± 0·5 (495)

these nine ewes decreased from 58·0 to 43·0 mg/100 ml. between 91 and 130 days gestational age, and thereafter remained relatively unchanged until 4–5 days before birth when it increased from 50·0 mg/100 ml. 1 day

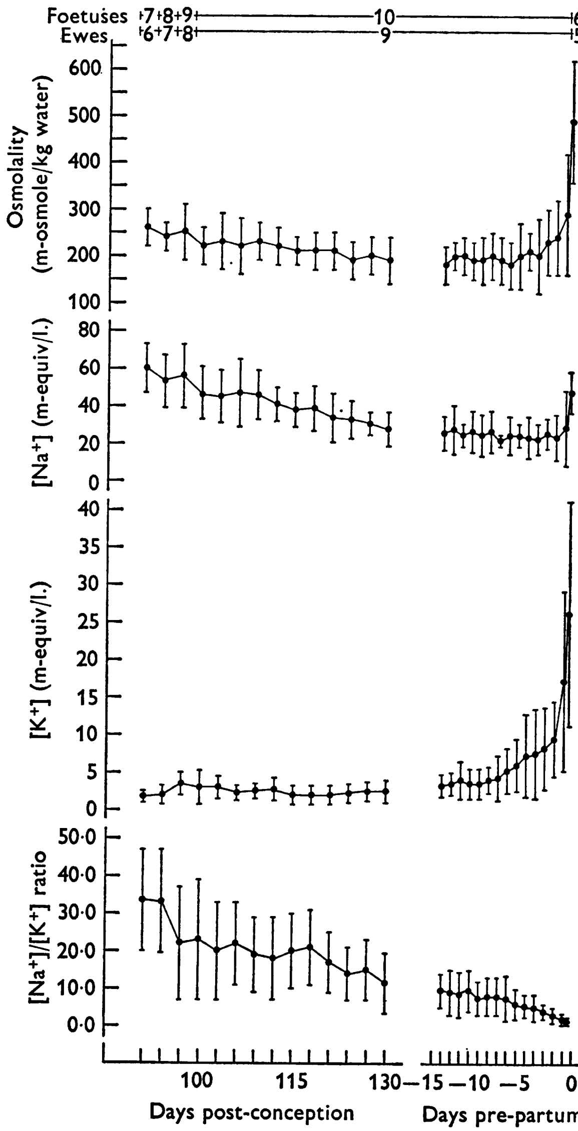

Fig. 4. The mean and S.D. over 3-day intervals for osmolality, $[Na^+]$, $[K^+]$ and $[Na^+]/[K^+]$ ratio of foetal urine from up to ten foetuses in nine ewes between 91 and 130 days gestational age, and their daily mean and S.D. during the last 14 days before birth.

before birth to 68·0 mg/100 ml. during labour. Four individual examples of this preparturient rise may be seen in Fig. 8.

Changes in foetal urine to 130 days (*Figs. 4, 5*). Between 91 and 130 days gestational age the mean osmolality decreased from 263 to 192 m-osmole/kg water, the mean [Na^+] from 60 to 28 m-equiv/l. and the mean [Cl^-] from 51 to 24 m-equiv/l. The mean [K^+] increased from 1·7 to 3·4 m-equiv/l.

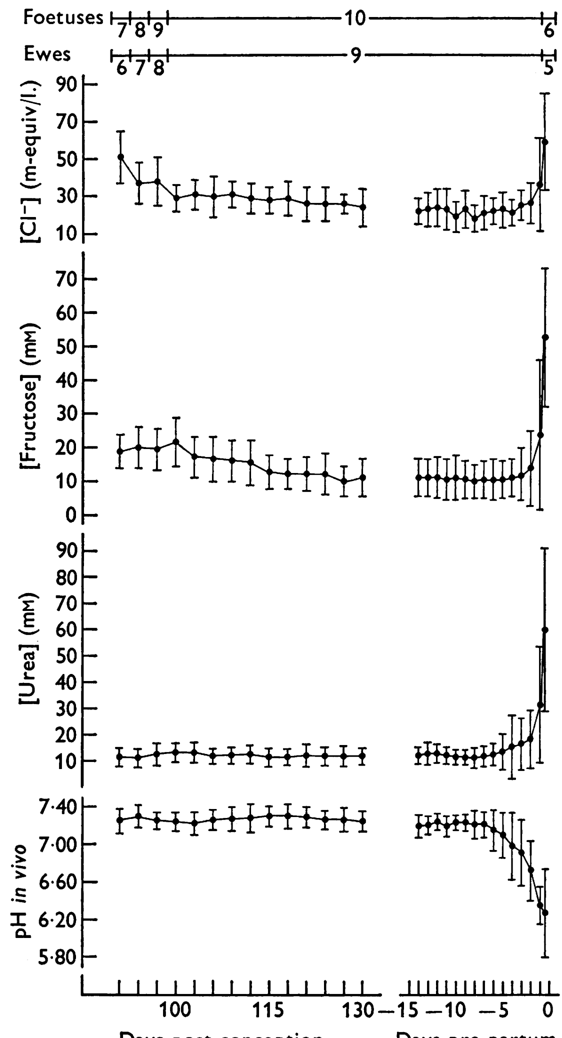

Fig. 5. The mean and S.D. over 3-day intervals for [Cl^-], [fructose], [urea] and pH of foetal urine from up to ten foetuses in nine ewes between 91 and 130 days gestational age, and their daily mean and S.D. during the last 14 days before birth.

between 91 and 97 days gestational age, decreased to 1·9 m-equiv/l. at 118 days, and then slowly increased to 2·5 m-equiv/l. at 130 days. Individual results are given in Figs. 6 and 7. The mean $[Na^+]/[K^+]$ ratio showed a general decrease throughout the period of observation. The mean [fructose] decreased from 19·0–20·1 mM at 91–94 days to 10·0–11·3 mM at 127–130 days gestational age. The mean [urea] and pH (Fig. 5), and the mean [amino acid] (not graphed) of foetal urine remained relatively constant (range: [urea], 10·5–13·4 mM; pH, 7·24–7·30; [amino acid], 2·58–3·01 mM).

Changes in foetal urine during the 14 days pre-partum (*Figs. 4, 5*). The mean osmolality of foetal urine fluctuated between 176 and 204 m-osmole/kg water from −14 to −8 days and then increased from 176 m-osmole/kg water 7 days before birth through 293 m-osmole/kg water 1 day before birth to 489 m-osmole/kg water during labour. The mean $[Na^+]$ continued its gestational decrease until 4 days before birth when it increased from 22·5 to 47·5 m-equiv/l. during labour. The rise in the mean $[K^+]$ which started at 118 days gestational age continued throughout the pre-partum period, increasing from 3·2 m-equiv/l. on day −14 to 26·2 m-equiv/l. during labour. The mean $[Cl^-]$ remained between 18·4 and 23·7 m-equiv/l. until 4 days before birth when it increased from 21·4 to 58·8 m-equiv/l. Changes in the mean $[Na^+]$ and $[Cl^-]$ were parallel and reflected a similar pattern in each foetus. The mean $[Na^+]/[K^+]$ ratio decreased from 9·6 on day −14 through 5·3 (on day −5) to 1·8 during labour, but the rate of decline between days −4 and $-\frac{1}{2}$ was about twice that of the previous 9 days. Although the mean [urea] started to increase 8 days before birth (from 8·5 to 47·1 mM), the mean [fructose] did not start to rise until 4 days later (11·1 mM rising to 52·7 mM), and the magnitude of its increase was 10 % less than that of the mean [urea]. The mean pH remained between 7·19 and 7·24 until 8 days before birth and then decreased to 6·27 during labour. Results from individual foetuses have been reported by Mellor & Slater (1972). The mean [amino acid] remained between 2·5 and 3·0 mM until 6 days before birth and then increased through 7·9 mM 1 day before birth to 14·1 mM during labour.

Interrelationships between the composition of maternal plasma, amniotic fluid, allantoic fluid and foetal urine

Osmolality and ion concentrations. Larger day-to-day variations (up to 90 m-osmole/kg water) were observed in the osmolality of foetal urine relative to those in maternal plasma and both foetal fluids. Between the day of operation and term, any general trend in urine osmolality during a period of 10 days or more was associated always with the same trend in the osmolalities of amniotic and allantoic fluid. However, allantoic fluid osmolality approximated to that of maternal plasma, while the osmolalities of amniotic fluid and foetal urine were more closely associated. Relative to the foetal fluids, foetal urine was hypotonic before 135–140 days gestational age. Its osmolality then increased until it was hypertonic to both fluids just before birth.

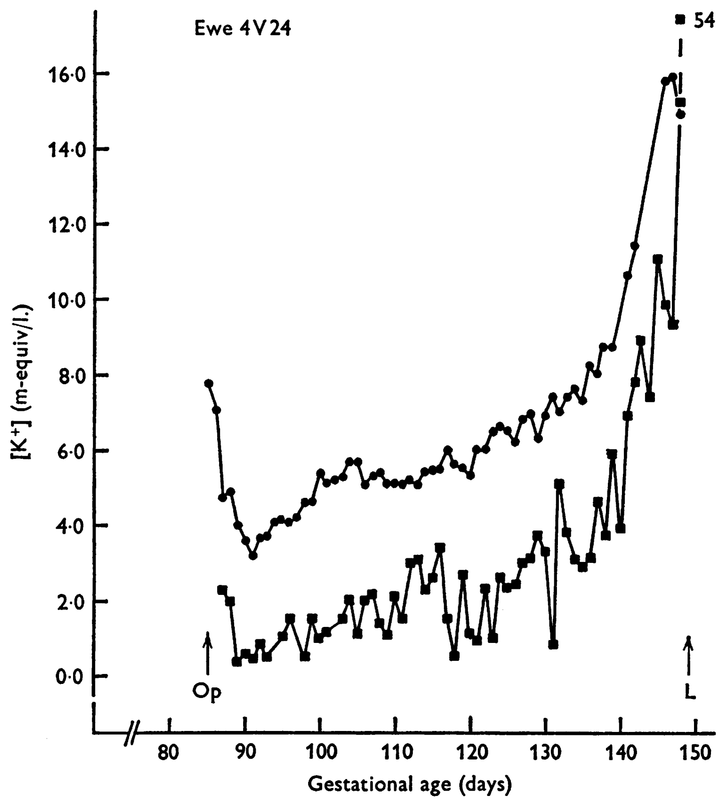

Fig. 6. Daily changes in the [K^+] of amniotic fluid (●) and foetal urine (■) from a single foetus from the day of operation (Op) to term (L).

Even after allowing for differences in osmolality, the [Na^+] and the [K^+] (except during the last 5–10 days of pregnancy) of foetal urine were significantly less than those of amniotic fluid. The [Cl^-] of amniotic fluid exceeded maternal plasma values until about 130 days gestational age, and during this period foetal urine [Cl^-] were less than 30% of amniotic fluid levels. In each foetus, trends in the [Na^+], [K^+] or [Cl^-] of foetal urine were reflected in similar changes in amniotic fluid (e.g. Fig. 6).

The close inverse relationship between the [Na^+] and [K^+] of allantoic fluid (Mellor & Slater, 1971) was again observed. There was no obvious parallel between changes in the [Na^+] of allantoic fluid and foetal urine. However, after recovery from operation the [Na^+] of both decreased, the magnitude and rate of the decrease being greater in allantoic fluid. There was a definite parallel between changes in the [K^+] of allantoic fluid and foetal urine in all foetuses. Fig. 7 shows a striking example. A close relationship between the [Na^+] and [K^+] of allantoic fluid and foetal urine was further suggested by parallel changes in their respective [Na^+]/[K^+] ratios (Table 2).

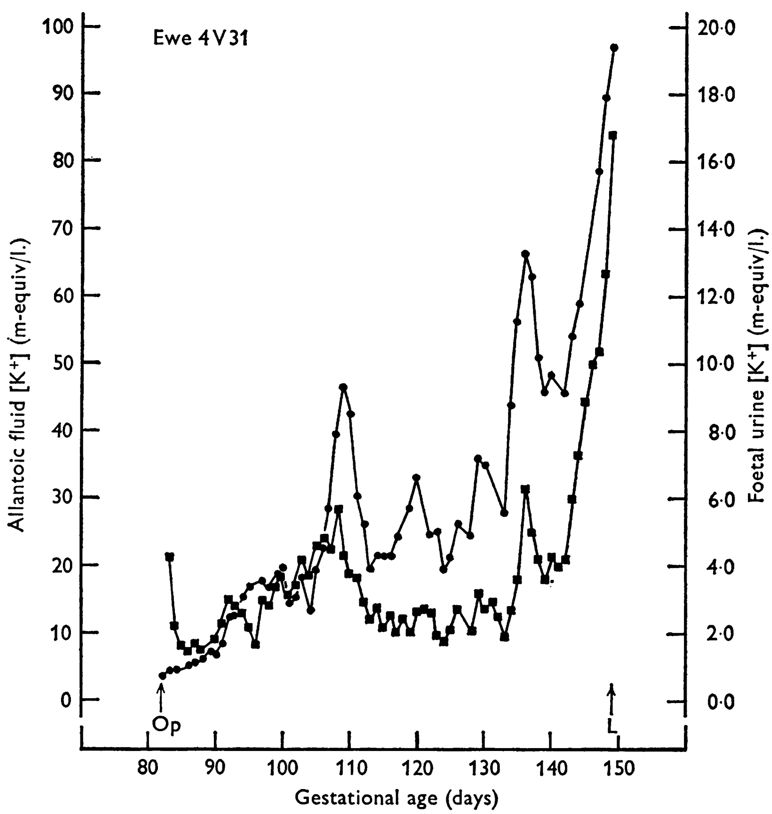

Fig. 7. Daily changes in the [K^+] of allantoic fluid (●) and foetal urine (■) from a single foetus from the day of operation (Op) to term (L). Note different scales.

The [Cl^-] of allantoic fluid and foetal urine varied independently. Their relative levels were difficult to assess because the [Cl^-] of allantoic fluid from operated (bladder catheter) foetuses were about 20–50 % higher than foetal urine values, and the foetal urine [Cl^-] were 50–150 % greater than those of allantoic fluid from unoperated foetuses.

The [glucose] and [fructose]. The [glucose] of amniotic fluid were comparable to those of allantoic fluid (e.g. Fig. 1), and after allowing for differences in osmolality the [fructose] of foetal urine were always greater

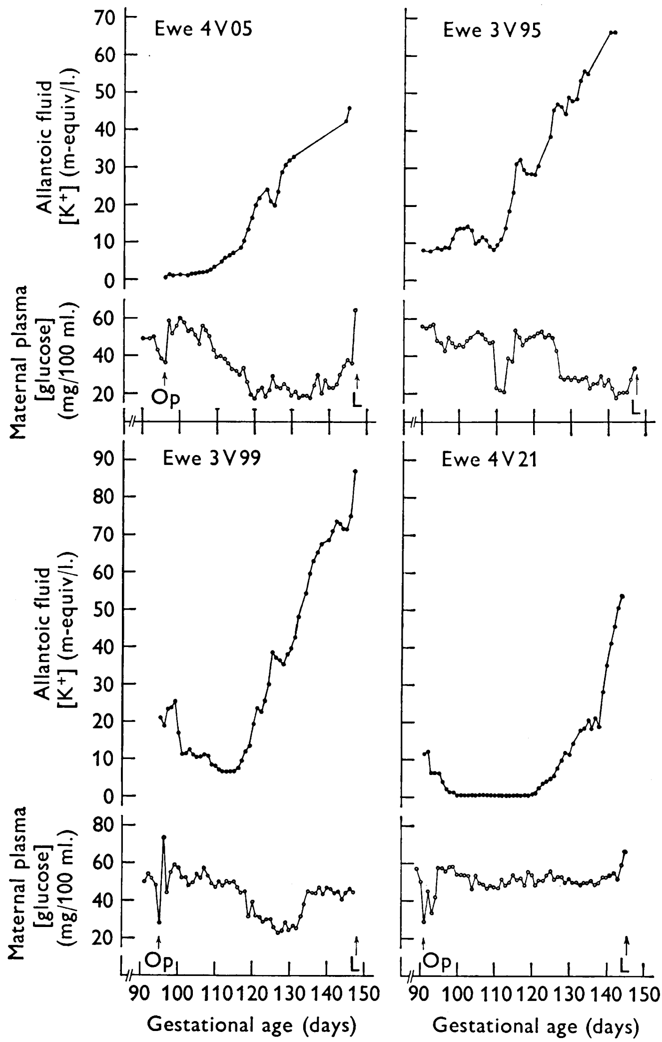

Fig. 8. Daily changes in the [K+] of allantoic fluid (●) and the [glucose] of maternal plasma (○) from three singleton bearing ewes (3V99; 4V05; 4V21) and from one foetus in a twin bearing ewe (3V95). Ewe 3V95 was operated upon at 82 days gestational age (Fig. 1) and after recovery changes in the [K+] of allantoic fluid from both foetuses were almost identical. Before 140 days gestational age, large changes in the [glucose] of maternal plasma resulted from variations in the quantity of the daily ration given the ewes. Op, operation; L, term.

than those of both foetal fluids. The parallel between the [fructose] of allantoic fluid and foetal urine during post-operative recovery was also observed throughout pregnancy.

The [*urea*] *and* [*amino acid*]. The [urea] of foetal urine generally exceeded those of both foetal fluids and a terminal rise was seen in all three. There was sufficient amino acid in foetal urine to account entirely for the concentrations in amniotic fluid and a terminal rise was observed in both. The urine [amino acid] were lower than allantoic fluid values which is consistent with the suggestion of Mellor & Slater (1971) that amino acid is actively transported into the allantoic sac.

Maternal plasma [*glucose*] *and allantoic fluid* [K^+]. All ewes were hypoglycaemic on the day of operation (Figs. 1, 8) as a direct result of the 48 hr pre-operative fast. There was a significant negative correlation between the [glucose] of maternal plasma sampled before operation and the [K^+] of allantoic fluid sampled at operation in seventeen foetuses (four not included in Table 1) in fourteen ewes ($r = 0{\cdot}662$; $0{\cdot}01 > P > 0{\cdot}001$). This relationship was also observed after recovery from operation in four ewes carrying five foetuses (Fig. 8). In these ewes the allantoic fluid [K^+] started a continuous rise at about 103 (4V05), 110 (3V95), 116 (3V99) and 120 (4V21) days gestational age, and in the first three cases this appeared to be associated with decreasing maternal plasma [glucose]. Before the rapid preparturient rise in the [K^+] of allantoic fluid (e.g. 4V21), the rate of increase in the [K^+] seemed to be influenced by such factors as the rate of development of hypoglycaemia, the actual degree of hypoglycaemia, and the gestational age at which hypoglycaemia was induced. In each case, as the [K^+] increased the [Na^+] decreased.

DISCUSSION

A major advantage of observations on chronically catheterized animals is the avoidance of artifacts produced by the abnormal conditions of acute experiments (Meschia *et al.* 1965; Comline & Silver, 1970; Mellor & Slater, 1971). Once such a technique has been devised it is clearly important to establish whether it introduces other artifacts. Such abnormalities appeared to be minimal in the work of Mellor & Slater (1971), so the precautions taken here were the same. After birth the lambs fed and behaved normally and at post-mortem (7–8 weeks post-partum) showed little evidence of the operation and were anatomically normal. In addition, operated (bladder catheter) and unoperated foetuses showed the same gestational trends in the compositions of their foetal fluids.

Influences of foetal urine on the foetal fluids

Amniotic fluid. Between 90 days gestational age and term (140–151 days) changes in the osmolality, the [Na^+], the [K^+], and the [Cl^-] of amniotic fluid were parallel to similar changes in foetal urine (e.g. Fig. 6). One of the major influences of foetal urine on amniotic fluid seems to be dilution, since these ion concentrations were lower in foetal urine (except for the [K^+] just before birth) even after allowing for differences in osmolality. Clearly foetal urine is not the only source of amniotic fluid Na^+, K^+ and Cl^-; these ions also appear to enter the amniotic sac by diffusion (Na^+, K^+) and active transport (Cl^-, until about 130 days gestational age) from foetal blood in the amnion or foetal skin, or both (Mellor, 1970*b*; Mellor & Slater, 1971). Since foetal urine is essentially devoid of glucose, urine entry into the amniotic sac will also tend to decrease the [glucose] of amniotic fluid. The amnion as a whole seems to be relatively impermeable to the passage of solutes (Mellor, 1970*b*; Mellor & Slater, 1971). This suggests that most amniotic fluid glucose originates from foetal plasma. Although there was sufficient fructose, urea and amino acid in foetal urine to account entirely for their concentrations in amniotic fluid, it is not suggested that this was their only source.

Allantoic fluid. Entry of foetal urine into the allantoic sac tends to decrease the osmolality, [Na^+], [K^+], [glucose] and [amino acid] and increase the [fructose] and [urea] of allantoic fluid. As stated above, the relative [Cl^-] are unknown. Chloride appears to be distributed according to electrochemical equilibrium between maternal plasma in the endometrium, foetal plasma in the chorioallantois and allantoic fluid (Mellor, 1970*b*), so passive diffusion in or out of the sac would compensate for any differences in [Cl^-].

It has been suggested that the chorioallantois contains pumping mechanisms operating between allantoic fluid and foetal blood which act to decrease the [Na^+] and increase simultaneously the [K^+] of allantoic fluid (Mellor, 1970*b*; Mellor & Slater, 1971). The results in the present study agree with this, since the rate of the gestational decrease in the [Na^+] of allantoic fluid was about twice that of foetal urine, and since the [K^+] of allantoic fluid were usually 4–5 times greater than those of foetal urine.

Preparturient changes in foetal urine

In individual foetuses large day-to-day variations were observed in the osmolality and solute concentrations of foetal urine (e.g. Figs. 3, 6), which may have been due to fluctuations in antidiuretic hormone (ADH) secretion by the foetus (Alexander, Britton, Forsling, Nixon & Ratcliffe, 1971*a*, *b*). Marked preparturient changes in foetal urine composition were also observed (Figs. 4, 5). The osmolality, [K^+], [urea] and [amino acid] of foetal urine increased 6–8 days before birth, and its [Na^+], [Cl^-] and [fructose] about 2–4 days before birth. The rate of rise before 2 days prepartum was relatively slow, but between days -2 and -1, and days -1 and $-\frac{1}{2}$, respectively, it increased at successively greater rates. It seems probable that these changes in urine composition were due largely to a progressively greater secretion of foetal ADH during the last 7 days, and particularly the last 2–4 days before birth. A preparturient decrease in urine flow rate of three sheep foetuses in acute experiments (Alexander *et al.* 1958*b*) agrees with this, but may have been due to an artificial elevation of ADH secretion through a greater sensitivity of older foetuses to stress (Alexander *et al.* 1971*a*, *b*). In these cases, however, the lower flow rates coincided with greater urine osmolalities (Alexander *et al.* 1958*b*), and administration of ADH to two other foetuses reduced urine flow rate (Alexander & Nixon, 1961).

Regulation of ion concentrations of allantoic fluid and foetal urine

Foetal plasma corticosteroids have both glucocorticoid and mineralocorticoid activity (Jones, Jarrett, Vinson & Potter, 1964). Between 80 and 143 days gestational age, foetal adrenocorticotrophin (ACTH) is secreted in response to haemorrhage (Alexander *et al.* 1971*a*, *b*), and the foetal adrenal glands appear able to respond to stimulation through the pituitary-ACTH-axis by secreting corticosteroids (Liggins, 1968, 1969*a*, *b*). It seems likely, therefore, that the relatively severe stress of bladder catheter insertion would increase foetal plasma concentrations of corticosteroids, which would be expected to decrease during recovery. The maintenance of relatively low [Na^+]/[K^+] ratios in allantoic fluid and foetal urine until the 3rd day after operation and the increase to the 6th or 7th day (Table 2), agree with this.

In all foetuses there was a close inverse relationship between the [Na^+] and [K^+] of allantoic fluid, such that after recovery its [Na^+]/[K^+] ratio decreased, the rate of decline being more rapid during the last 5–10 days before birth (Table 2; Figs. 7, 8). These changes were again parallel to those of the foetal urine [Na^+]/[K^+] ratio, which decreased slowly until 5 days before birth, and then decreased at twice the previous rate (Table 2; Fig. 4). The slow gestational increase in foetal plasma concentrations of corticosteroids (Bassett & Thorburn, 1969) and the rapid preparturient rise (Bassett & Thorburn, 1969; Comline, Nathanielsz, Paisey & Silver, 1970) are consistent with this.

It may be suggested therefore that the close parallel between the [Na^+]/

$[K^+]$ ratios of allantoic fluid and foetal urine during post-operative recovery and thereafter until birth reflect a simultaneous action of foetal plasma corticosteroids on the chorioallantois and foetal kidneys. The delay in the allantoic fluid response (Table 2) may have been due to the greater volume of allantoic fluid relative to that of foetal urine coupled with their different modes of formation. This also seems likely to be the explanation of the larger day-to-day variations in foetal urine composition relative to amniotic and allantoic fluid.

Regulation of fructose concentrations of allantoic fluid and foetal urine

In all foetuses, in allantoic fluid (Fig. 1) and in foetal urine (Fig. 2) there was an inverse relationship between the $[K^+]$ and [fructose], and a parallel relationship between the $[Na^+]/[K^+]$ ratios and the [fructose]. This suggests that the [fructose] of allantoic fluid and foetal urine were inversely related to the corticosteroid concentrations of foetal plasma. However, this may have been an indirect relationship acting through changes in the [fructose] of foetal plasma (see below), which produced parallel changes in those of foetal urine, and therefore allantoic fluid.

Fructose and urea appear to be excreted in a similar way by the foetal kidney (Alexander *et al.* 1958*b*; Fig. 3) and foetal plasma [urea] may be inferred from maternal plasma values (Alexander *et al.* 1958*a*; Mellor & Slater, 1971). Therefore, with a knowledge of the patterns of change in the [urea] of maternal plasma and of the [fructose] and [urea] of foetal urine, it should be possible to determine the directions of change in the [fructose] of foetal plasma. From the 4th day after operation until birth the [urea] of maternal and therefore foetal plasma remained relatively constant. It may be inferred, therefore, that the [fructose] of foetal plasma either decreased or remained relatively unchanged until 3–4 days after operation, that it then increased to relatively high values 6–8 days post-surgery (Fig. 2), and thereafter that it decreased slowly until the start of a more rapid preparturient decline (Fig. 5). This more rapid fall is suggested by the earlier and greater preparturient rise in the [urea] of foetal urine. Indeed, similar patterns have been found by direct observation of plasma [fructose] in foetuses with chronically implanted vascular catheters (Battaglia, 1969; Comline & Silver, 1970, 1972; Bassett & Thorburn, 1971; D. J. Mellor & J. S. Slater, unpublished data). Therefore the [fructose] of foetal plasma in addition to those of allantoic fluid and foetal urine appears to be inversely correlated with foetal plasma concentrations of corticosteroids (as inferred from changes in the $[Na^+]/[K^+]$ ratios of allantoic fluid and foetal urine). This may involve corticosteroid regulation of the utilization of glucose (Bassett *et al.* 1966) by fructose producing cells. If this is so, elevated concentrations of foetal plasma corticosteroids would be expected to decrease placental fructose synthesis and therefore the [fructose] of foetal plasma, with reduced corticosteroid concentrations having the reverse effect. The role of other foetal hormones in such a system would need to be investigated.

Maternal influences on allantoic fluid

Gestational changes in the [Na^+] and [K^+] of allantoic fluid seem to be regulated by foetal plasma corticosteroids. Mellor & Slater (1971) found that rapid changes appeared to occur during acute and chronic episodes of maternal hypoglycaemia. They suggested that maternal-induced foetal hypoglycaemia effects a relative increase in the secretion of foetal corticosteroids that would not be observed in normoglycaemic ewes. However, plasma [glucose] were not measured, the hypothesis being based on an assessment of relative [glucose] from the [ketone body]. In the present study, therefore, relationships between allantoic fluid composition and maternal plasma [glucose] have been investigated in detail (Fig. 8).

Hypoglycaemia induced in three ewes at 103 (4V05), 110 (3V95) and 116 (3V99) days gestational age was associated with an immediate rise in the [K^+] of allantoic fluid, which continued in two ewes (3V95; 3V99) after a return to normoglycaemia but at a reduced rate (Fig. 8). It appears that the effects of hypoglycaemic stimuli applied before 100–110 days gestational age were more easily reversible than in older foetuses, so that a greater degree of maternal hypoglycaemia could be tolerated at younger foetal ages without initiating an irreversible rise in the [K^+] of allantoic fluid (Figs. 1, 8). However, in a normoglycaemic ewe (4V21; Fig. 8) the allantoic fluid [K^+] started to increase slowly at 120 days and rapidly at 138 days gestational age, which supports the suggestion of Mellor & Slater (1971) that the background pattern of foetal corticosteroid secretion is determined by factors other than hypoglycaemia.

In the whole group of ewes on the day of operation there was a significant negative correlation between the [glucose] of maternal plasma and the [K^+] of allantoic fluid. This suggests that the inverse relationship found after recovery (Fig. 8) was also present before operation. However, during the recovery period it did not seem to hold (Figs. 1, 8). In general, after operation until the 4th day, higher allantoic fluid concentrations of K^+ were observed than would have been expected from the maternal plasma [glucose] if an inverse relationship existed. The elevated [K^+] of allantoic fluid in these ewes suggest that foetal plasma concentrations of corticosteroids were maintained at higher levels during the 4 days after operation. The major cause of this seems unlikely to have been an effect of surgical stress on the foetus since allantoic sac catheterization required only 1 hr of general anaesthesia and the foetus was not handled. In addition, although foetal bladder catheterization involved $2\frac{1}{2}$–$3\frac{1}{2}$ hr of general anaesthesia and exposed foetuses to greater direct stresses than insertion of allantoic sac catheters only (Fig. 1), comparable post-operative changes in allantoic fluid composition were found in some ewes (3V99; 4V21). Therefore, other factors seem to be implicated. Since foetal hypoglycaemia seems to be a major stimulus to foetal corticosteroid secretion, it appears that during the post-operative period glucose passage from mother to foetus may have been reduced. Corticosteroids administered to sheep produced a relative inhibition of glucose utilization (Bassett *et al.* 1966). It seems possible therefore that the elevated corticosteroid concentrations of maternal plasma during the first 2–4 days after operation (Bassett & Thorburn, 1969) decreased the permeability of the placenta to glucose. The results of Saba, Burns, Cunningham, Hebert & Patterson (1966) are consistent with this. They fasted three groups of pregnant (near term) ewes for 6 days. Plasma insulin activity of ewes from all three groups decreased throughout the fast. One group became moderately hypoglycaemic, the second severely hypoglycaemic, and the third, which received daily ACTH injections and had elevated plasma corticosteroid concentrations, remained normoglycaemic throughout. Lamb mortality for each group was zero, 39 and 66%, respectively. Since glucose is the major source of energy for the sheep foetus (Reid, 1968; Alexander, Britton, Cohen & Nixon, 1969) a decrease in its passage from mother to foetus may have been a primary cause of death. These data, and the results in the present study, suggest that maternal normoglycaemia or hyperglycaemia associated with high plasma corticosteroid concentrations is for the foetus equivalent to maternal hypoglycaemia.

A terminal increase in the [glucose] of maternal plasma coincided with the preparturient rise in foetal plasma concentrations of corticosteroids (e.g. 4V21; Fig. 8). This may have been due to foetal corticosteroids decreasing the passage of glucose from mother to foetus by decreasing the permeability of the placenta to glucose, or to the large preparturient changes in the progesterone (Bassett, Oxborrow, Smith & Thorburn, 1969) and oestrogen (Challis, 1971) concentrations of maternal plasma, or both. The relatively higher [glucose] of samples taken within 12 hr of birth (e.g. 4V05; Fig. 8) were probably due to the stress of labour (Comline & Silver, 1972).

Interrelationships

The following interrelationships seem to exist. A tendency towards foetal hypoglycaemia may be expected when the passage of glucose from mother to foetus decreases. This seems to occur during periods of maternal hypoglycaemia (induced by fasting or decreased feed intake) when the transplacental [glucose] gradient is reduced, or during maternal stress when there appears to be a relative decrease in the permeability of the placenta to glucose secondary to an increase in the concentrations of maternal plasma corticosteroids. Foetal hypoglycaemia, foetal stress and other factors (after about 120 days gestational age) seem to effect a relative increase in the secretion of foetal corticosteroids. These hormones, through their mineralocorticoid activity, appear to act on the foetal kidneys and chorioallantois to increase the [K^+] and decrease the [Na^+] of foetal urine and allantoic fluid. Simultaneously, through their glucocorticoid activity, foetal plasma corticosteroids in increased concentrations seem to decrease synthesis of fructose by placental cells, which causes the [fructose] of foetal plasma to decrease. This results in a parallel fall in the [fructose] of foetal urine and, indirectly, in those of amniotic and allantoic fluid. An increase in the [glucose] of maternal plasma in non-stressful circumstances tends to reverse these effects. These interrelationships, and the effects of other hormones, appear worthy of further investigation.

We are grateful particularly to Miss E. J. Dunnett, Mr I. C. Matheson, Mr D. C. Harkins and Mr A. Wilson for essential technical assistance throughout this study. Our thanks are also due to Dr F. Cockburn and Dr D. L. Mould for helpful discussion; to Mr C. C. Renwick and Mr K. W. Angus for mating arrangements and clinical attention to the sheep; and to Mr I. C. Butt, Mr R. D. Clark, Miss F. Maule Walker, Mr J. Redmond, Mr D. G. Watson and his staff, and many others for help in various aspects of the work.

REFERENCES

ALEXANDER, D. P., BRITTON, H. G., COHEN, N. M. & NIXON, D. A. (1969). Foetal metabolism. In *Foetal Autonomy*, Ciba Fdn Series, ed. WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 95–112. London: Churchill.

ALEXANDER, D. P., BRITTON, H. G., FORSLING, M. L., NIXON, D. A. & RATCLIFFE, J. G. (1971*a*). The release of corticotrophin and vasopressin in the foetal sheep in response to haemorrhage. *J. Physiol.* **213**, 31–32*P*.

ALEXANDER, D. P., BRITTON, H. G., FORSLING, M. L., NIXON, D. A. & RATCLIFFE, J. G. (1971*b*). The concentrations of adrenocorticotrophin, vasopressin and oxytocin in the foetal and maternal plasma of the sheep in the latter half of gestation. *J. Endocr.* **49**, 179–180.

ALEXANDER, D. P. & NIXON, D. A. (1961). The foetal kidney. *Br. med. Bull.* **17**, 112–117.

ALEXANDER, D. P., NIXON, D. A., WIDDAS, W. F. & WOHLZOGEN, F. X. (1958*a*). Gestational variations in the composition of the foetal fluids and foetal urine in sheep. *J. Physiol.* **140**, 1–13.

ALEXANDER, D. P., NIXON, D. A., WIDDAS, W. F. & WOHLZOGEN, F. X. (1958*b*). Renal function in the sheep foetus. *J. Physiol.* **140**, 14–22.

BASSETT, J. M. (1968). The relation of fat and protein catabolic actions of cortisol to glucose homeostasis in fasting sheep. *Metabolism* **17**, 644–652.

BASSETT, J. M., MILLS, S. C. & REID, R. L. (1966). The influence of cortisol on glucose utilization in sheep. *Metabolism* **15**, 922–932.

BASSETT, J. M., OXBORROW, T. J., SMITH, I. D. & THORBURN, G. D. (1969). The concentration of progesterone in the peripheral plasma of the pregnant ewe. *J. Endocr.* **45**, 449–457.

BASSETT, J. M. & THORBURN, G. D. (1969). Foetal plasma corticosteroids and the initiation of parturition in the sheep. *J. Endocr.* **44**, 285–286.

BASSETT, J. M. & THORBURN, G. D. (1971). The regulation of insulin secretion by the ovine foetus *in utero*. *J. Endocr.* **50**, 59–74.

BASSETT, J. M., THORBURN, G. D. & WALLACE, A. L. C. (1970). The plasma growth hormone concentrations of the foetal lamb. *J. Endocr.* **48**, 251–263.

BATTAGLIA, F. C. (1969). Placental exchange. In *Foetal Homeostasis*, N.Y. Acad. Sci. Series, ed. WYNN, R. M., pp. 35–46, New York: Meredith Corporation.

CHALLIS, J. R. G. (1971). Sharp increase in free circulating oestrogens immediately before parturition in sheep. *Nature, Lond.* **229**, 208.

COMLINE, R. S. & SILVER, M. (1970). Daily changes in foetal and maternal blood of conscious pregnant ewes, with catheters in umbilical and uterine vessels. *J. Physiol.* **209**, 567–586.

COMLINE, R. S. & SILVER, M. (1972). The composition of foetal and maternal blood during parturition in the ewe. *J. Physiol.* **222**, 233–256.

COMLINE, R. S., NATHANIELSZ, P. W., PAISEY, R. B. & SILVER, M. (1970). Cortisol turnover in the sheep foetus immediately prior to parturition. *J. Physiol.* **210**, 141–142 *P*.

HERVEY, E. J. & SLATER, J. S. (1968). The sources of sheep foetal fluids in the later stages of gestation. *J. Physiol.* **194**, 40–41 *P*.

JONES, I. C., JARRETT, I. G., VINSON, G. P. & POTTER, K. J. (1964). Adrencorticosteroid production of foetal sheep near term. *J. Endocr.* **29**, 211–212.

LIGGINS, G. C. (1968). Premature parturition after infusion of corticotrophin or cortisol into foetal lambs. *J. Endocr.* **42**, 323–329.

LIGGINS, G. C. (1969*a*). Premature delivery of foetal lambs infused with glucocorticoids. *J. Endocr.* **45**, 515–523.

LIGGINS, G. C. (1969*b*). The foetal role in the initiation of parturition in the ewe. In *Foetal Autonomy*, Ciba Fdn Series, ed. WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 218–231. London: Churchill.

MELLOR, D. J. (1969). Vascular anastomosis and fusion of foetal membranes in multiple pregnancy in the sheep. *Res. vet. Sci.* **10**, 361–367.

MELLOR, D. J. (1970*a*). A technique for chronic catheterization of the amniotic and allantoic sacs of sheep foetuses. *Res. vet. Sci.* **11**, 93–95.

MELLOR, D. J. (1970*b*). Distribution of ions and electrical potential differences between mother and foetus at different gestational ages in goats and sheep. *J. Physiol.* **207**, 133–150.

MELLOR, D. J. & SLATER, J. S. (1971). 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. *J. Physiol.* **217**, 573–604.

MELLOR, D. J. & SLATER, J. S. (1972). Preparturient changes in the pH of urine from chronically catheterized foetal sheep. *Res. vet. Sci.* **13**, 89–90.

MELLOR, D. J., SLATER, J. S. & COCKBURN, F. (1971). Effects of antibiotic treatment on the composition of sheep foetal fluids. *Res. vet. Sci.* **12**, 521–526.

MELLOR, D. J., WILLIAMS, J. T. & MATHESON, I. C. (1972). A technique for chronic catheterization of the bladder of the foetal sheep. *Res. vet. Sci.* **13**, 87–88.

MESCHIA, G., COTTER, J. R., BREATHNACH, C. S. & BARRON, D. H. (1965). The hemoglobin, oxygen, carbon dioxide and hydrogen ion concentrations in the umbilical bloods of sheep and goats as sampled via indwelling plastic catheters. *Q. Jl exp. Physiol.* **50**, 185–195.

REID, R. L. (1968). The physiopathology of undernourishment in pregnant sheep, with particular reference to pregnancy toxaemia. *Adv. vet. Sci.* **12**, 163–238.

RUSSELL, A. J. F., DONEY, J. M. & REID, R. L. (1967). The use of biochemical parameters in controlling nutritional state in pregnant ewes, and the effect of undernourishment during pregnancy on lamb birth weight. *J. agric. Sci., Camb.* **68**, 351–358.

SABA, N., BURNS, K. N., CUNNINGHAM, N. F., HEBERT, C. N. & PATTERSON, D. S. P. (1966). Some biochemical and hormonal aspects of experimental ovine pregnancy toxaemia. *J. agric. Sci., Camb.* **67**, 129–138.

TRINDER, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann. clin. Biochem.* **6**, 24–27.