

DISTRIBUTION OF IONS  
AND ELECTRICAL POTENTIAL DIFFERENCES BETWEEN  
MOTHER AND FOETUS AT DIFFERENT GESTATIONAL  
AGES IN GOATS AND SHEEP

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SUMMARY

1. Potential differences associated with the compartments of goat and sheep conceptuses have been measured *in vivo* and *in vitro* during the last half of gestation and the osmolarity, and the  $[\text{Na}^+]$ ,  $[\text{K}^+]$ , and  $[\text{Cl}^-]$  of maternal and foetal plasma and amniotic and allantoic fluid taken from these animals were determined.

2. The potential difference (p.d.) patterns of both goats and sheep were the same.

(a) The transplacental p.d. was about 71 mV (foetus negative) in the goat, and about 51 mV (foetus negative) in the sheep.

(b) The amniotic fluid p.d. (i.e. the p.d. measured between the maternal extracellular fluid and the amniotic fluid) decreased as gestation advanced (from 110 to 70 mV in the goat, and 90 to 50 mV in the sheep) and was equal to the sum of the transplacental p.d. and a p.d. between the foetal blood and the amniotic fluid. The amniotic fluid was negative relative to both maternal and foetal blood.

(c) An allantoic fluid p.d. (measured between the maternal extracellular fluid and the allantoic fluid) of about 107 mV in the goat, and about 96 mV in the sheep, was equal to the sum of the transplacental p.d. and a p.d. between the foetal blood and the allantoic fluid. The allantoic fluid was negative relative to both maternal and foetal blood.

(d) The results suggest that p.d.s of the fluid sacs arise from activity between the foetal fluids and the blood perfusing the foetal membranes, and not from activity across the full thickness of the foetal membranes.

3. The ionic concentrations were considered in relation to the electrochemical gradients found between the maternal and foetal fluid compartments to determine whether the ions were distributed according to electrochemical equilibrium.

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(a) It seems that ions in the amniotic fluid tend to equilibrate with foetal plasma, and not with maternal plasma or allantoic fluid, that changes in the  $[Na^+]$  and  $[K^+]$  of amniotic fluid can be accounted for largely in terms of passive factors, and that variations in the  $[Cl^-]$  are associated with activity of an electrogenic  $Cl^-$  pump directed from the foetal blood into the amniotic fluid.

(b) It appears that ions in the allantoic fluid can exchange with those of both maternal and foetal plasma, that an electrogenic pump effects absorption of  $Na^+$  from the allantoic fluid into the foetal blood, and that the  $[K^+]$  and  $[Cl^-]$  of allantoic fluid are maintained largely by passive exchange under the action of electrochemical gradients between maternal plasma and allantoic fluid, and between foetal plasma and allantoic fluid.

4. The results considered in the context of  $Na^+$  passage between mother and foetus call in question the general assumption that all  $Na^+$  reaches the foetus by passing across the placenta.

#### INTRODUCTION

Details of the ionic composition of goat foetal fluids do not seem to be available, but sheep foetal fluids have been examined (Malan, Malan & Curson, 1937; Cloete, 1939; McDougall, 1949), and possible relationships between their composition and that of foetal urine and foetal and maternal plasma have been suggested (Alexander, Nixon, Widdas & Wohlzogen, 1958*a*). When these observations were made details of electrochemical gradients between maternal and foetal plasma and amniotic and allantoic fluid were not available, and subsequent work in the goat (Meschia, Wolkoff & Barron, 1958) and sheep (Widdas, 1961) was relatively limited. The object of the present study was to investigate the potential difference (p.d.) patterns of goat and sheep conceptuses during the last half of gestation, and to assess their possible relevance to the distribution of ions between maternal extracellular fluid and the fluid compartments of the conceptus.

#### METHODS

Conceptuses from mixed strain goats, and Scottish Black Face sheep, aged from 81 to 141 days (term at about 150 days), and 67 to 140 days (term at about 145 days) gestational age, respectively, were used. Gestational ages were estimated from known mating dates and according to Huggett & Widdas (1951).

*Potential difference measurements.* All p.d.s were measured using a 'Vibron' electrometer (model 33B E.I.L.), and in some cases a Cambridge pH Meter (Cambridge Instruments Ltd.), two calomel electrodes, and salt bridges. The two meters were found to give almost identical readings under the same conditions. Corrections were made for asymmetry. The salt bridges consisted of vinyl tubing of a diameter

appropriate to the fluid compartment in which they were inserted (o.d. 1.0–2.5 mm.) and contained 154 mM-NaCl–2% agar.

**Terminology.** P.d.s between the fluid compartments of the conceptus and the maternal extracellular fluid (e.c.f.) will be referred to as (see Fig. 1) the amniotic fluid p.d., the allantoic fluid p.d., and the transplacental p.d. (measured between the foetal and maternal e.c.f.s.), and all signs will denote the polarity of the p.d.s relative to the maternal e.c.f. The term maternal e.c.f. is used because maternal plasma was shown to be electrically neutral relative to peritoneal fluid *in vivo*. Similarly, the term foetal e.c.f. is employed since foetal plasma in the placenta and in the foetus, foetal brain, and foetal peritoneal fluid were shown *in vivo* to be electrically neutral relative to each other. Thus, p.d.s measured between the fluid compartments of the

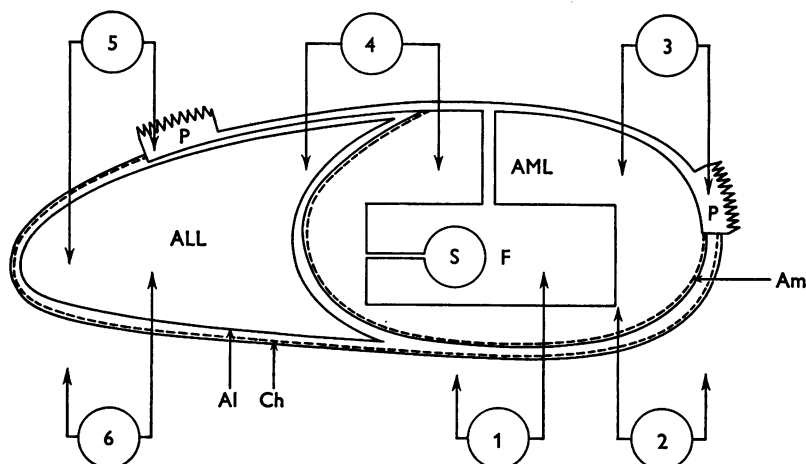


Fig. 1. A schematic representation of the goat and sheep conceptus showing the points between which potential differences were measured.

P.d. 1, the transplacental p.d.

P.d. 2, the amniotic fluid p.d.

P.d. 3, the foetal e.c.f. to amniotic fluid p.d.

P.d. 4, the amniotic fluid to allantoic fluid p.d.

P.d. 5, the foetal e.c.f. to allantoic fluid p.d.

P.d. 6, the allantoic fluid p.d.

F, the foetus; P, the placenta; AML, the amniotic fluid; ALL, the allantoic fluid; S, the foetal gastric lumen; Am, the amnion with its foetal vascular layer shown as a dashed line; Al, the allantois; and Ch, the chorion with its foetal vascular layer shown as a dashed line. The whole conceptus is surrounded by uterine tissue which is not shown in the diagram.

conceptus and the foetal e.c.f. will be referred to as (see Fig. 1) the foetal e.c.f. to amniotic fluid p.d., the foetal e.c.f. to allantoic fluid p.d., and the foetal e.c.f. to foetal stomach (abomasum) p.d. (not shown in Fig. 1), and all signs will show the polarity of the p.d.s relative to the foetal e.c.f. Any other p.d.s will be fully described in the text.

**Experimental procedure.** The procedure was the same for both goats and sheep. The anaesthetic was pentobarbitone given intravenously, and throughout the experiment the animals breathed 100% O<sub>2</sub>. The uterus was exposed by laparotomy.

*In vivo p.d. measurements.* One end of a salt bridge in the maternal peritoneal cavity acted as a reference electrode in the maternal e.c.f., and p.d.s were measured between this and the ends of salt bridges inserted in the foetal fluid compartments (Fig. 1). A small incision was made carefully in the uterine wall so that the underlying foetal membranes were exposed intact. The reference salt bridge in the foetus was inserted by either (a) one end of a salt bridge being passed towards the foetus in a foetal artery, or vein, associated with a cotyledon, and tied in place, or (b) in cases where the cotyledonary branches were too small to allow this an ear of the foetus was exposed through a small incision in the uterine wall and amniochorion, and one end of a salt bridge was passed into the foetal brain. Simultaneous measurements using these two methods in the same animal gave identical readings. Salt bridges were inserted through small punctures in the foetal membranes into the amniotic fluid, and the allantoic fluid, and were tied in place to prevent short-circuits. It was sometimes necessary to make a second incision in the uterine wall to allow a salt bridge to be inserted into one or other of these fluid compartments. Protruding foetal membranes were replaced in the uterine lumen and the incision was closed with artery forceps. P.d.s between the fluid compartments of the conceptus and the maternal e.c.f., and between these compartments and the foetal e.c.f., were then measured (Fig. 1).

*In vitro p.d. measurements.* P.d. measurements were performed according to the method of Ussing & Zerahn (1951). Measurements were made across the chorio-allantois, the amniochorion, and the amniocoel of both goats and sheep, and across the foetal skin of the sheep. Immediately after removal from the animal the membrane was sandwiched between two Perspex half chambers similar to those designed by Ussing & Zerahn (1951). The effective area of membrane in these chambers was 3.1 cm<sup>2</sup>. Fluids were placed in the half chambers so that chorion was bathed with maternal plasma, allantois with allantoic fluid, and amnion with amniotic fluid. The outside and inside of the foetal skin were bathed with amniotic fluid and maternal plasma, respectively. Fluids on both sides of the membranes were maintained at 37° C, and were stirred and oxygenated by the bubble lift in the apparatus. Frothing was prevented by adding several drops of octanol to the solutions, which were gassed with an 80% N<sub>2</sub>, 15% O<sub>2</sub>, 5% CO<sub>2</sub> mixture. In some cases Krebs bicarbonate Ringer solution of the following composition (mM): Na<sup>+</sup> 143.2, Cl<sup>-</sup> 128.0, K<sup>+</sup> 5.9, Ca<sup>2+</sup> 2.5, Mg<sup>2+</sup> 1.1, HCO<sub>3</sub><sup>-</sup> 24.9, H<sub>2</sub>PO<sub>4</sub><sup>-</sup> 1.1, SO<sub>4</sub><sup>2-</sup> 1.1, glucose 28.0; at pH 7.4, was used on both sides of the membrane, without the addition of octanol.

*Sampling and analysis.* Maternal blood was obtained from a jugular vein, and foetal blood from one of the placental vessels. The blood samples were taken into tubes containing dry heparin and plasma was obtained by centrifuging for 30 min at 2000 g. Amniotic and allantoic fluid samples were withdrawn by syringe before salt bridges were inserted into the fluid sacs. Osmolarity determinations were carried out using an Advanced Osmometer (Advanced Instruments Inc., U.S.A.). The [Na<sup>+</sup>], [K<sup>+</sup>], and [Cl<sup>-</sup>], of maternal and foetal plasma and amniotic and allantoic fluid were determined using the methods previously described (Mellor, 1969*a*).

*Estimated values* are expressed in the text as means and standard deviations with the number of observations in parentheses. The deep body temperature of both goats and sheep was found to be about 39° C, so that in all calculations involving the Nernst Equation the  $RT/zF$  value for monovalent ions was 62.8.

## RESULTS

*In vivo potential difference measurements*

In goats and sheep the pattern of the p.d.s observed was identical, but there were species differences in the magnitudes of individual p.d.s (Tables 1 and 2).

All p.d.s were negative whether relative to the maternal or the foetal e.c.f. Transplacental and amniotic fluid p.d.s were normally steady, and the allantoic fluid p.d. was sometimes steady but usually fluctuated. There was no gestational age variation in the magnitudes of the transplacental p.d., the allantoic fluid p.d., or the foetal e.c.f. to allantoic fluid p.d., but as gestation advanced there was a significant increase in the amniotic fluid p.d. and the foetal e.c.f. to amniotic fluid p.d.

As would be expected (see Fig. 1), the amniotic fluid p.d. was approximately equal to the sum of the transplacental p.d. and the foetal e.c.f. to amniotic fluid p.d. (e.g. in an 80-day goat conceptus,  $-110$  mV approx. =  $-(80 + 32)$  mV); the magnitude of the allantoic fluid p.d. approximated to the sum of the transplacental p.d. and the foetal e.c.f. to allantoic fluid p.d. (e.g. in a 130-day sheep conceptus,  $-85$  mV approx. =  $-(50 + 36)$  mV); and the p.d. between the amniotic fluid and the allantoic fluid was equal in magnitude to the difference between the allantoic fluid p.d. and the amniotic fluid p.d. (e.g. in a 124-day goat conceptus,  $-25$  mV approx. =  $-(106-80)$  mV).

The amniotic fluid p.d. was not altered after the head of the foetus had been exposed through an incision in the uterine wall and amniochorion. Foetal e.c.f. to foetal stomach p.d.s of  $-13$  mV at 81 days and  $-23$  mV at 124 days were observed in the goat, and of  $-19$  mV in a 103-day foetal sheep.

The results of transplacental p.d., amniotic fluid p.d., allantoic fluid p.d., foetal e.c.f. to amniotic fluid p.d., foetal e.c.f. to allantoic fluid p.d., and amniotic fluid to allantoic fluid p.d. measurements made on seven goat conceptuses are given in Table 1, and those made on sixteen sheep conceptuses are given in Table 2.

These results contradict the previously reported equality between the transplacental p.d. and the allantoic fluid p.d., and the reported rise of the transplacental p.d. towards 0 mV as gestation advances, in the goat (Meschia *et al.* 1958), and they are in general agreement with the p.d. pattern reported for the sheep (Widdas, 1961).

*In vitro potential difference measurements*

*In vitro* p.d. measurements were made on membranes from five goat and six sheep conceptuses ranging from 80 to 141 days gestational age. No

TABLE 1. Transplacental, amniotic fluid, allantoic fluid, foetal e.c.f. to amniotic fluid, foetal e.c.f. to allantoic fluid, and amniotic fluid to allantoic fluid p.d.s at different gestational ages in the goat

| Gestational age (days) | Transplacental p.d. (mV) | Amniotic fluid p.d. (mV) | Allantoic fluid p.d. (mV) | Foetal e.c.f. to amniotic fluid p.d. (mV) | Foetal e.c.f. to allantoic fluid p.d. (mV) | Amniotic fluid to allantoic fluid p.d.† (mV) |
|------------------------|--------------------------|--------------------------|---------------------------|---|--|--|
| 81 <i>a</i>            | -60                      | —                        | —                         | —   | —  | —  |
| 81 <i>b</i>            | -80                      | -110*                    | -115 to -120              | -32                                       | -34  | -7   |
| 94                     | -64                      | -89 to -94*              | -88 to -99                | -23 to -27                                | -30 to -34                                 | -7   |
| 110                    | -80                      | -90*                     | -130 to -136              | -11 to -13                                | -50  | -35  |
| 124                    | -74                      | -80*                     | -103 to -110              | —   | —  | -25  |
| 141 <i>a</i>           | -60 to -65               | -70 to -73*              | -95                       | -7 to -9                                  | -30  | -24  |
| 141 <i>b</i>           | -77                      | -82                      | -96                       | —   | —  | —  |
| Mean                   | -71                      | G.V.*                    | -107                      | G.V.**                                    | -33  |  |
| S.D.                   | ± 9                      |                          | ± 16                      |   | ± 10                                       |  |

*a* and *b* are twins.

\* Denotes potential differences which were unaltered after the head of the foetus had been exposed through an incision in the uterine wall and amniochorion.

† Polarity of the p.d. is relative to amniotic fluid.

G.V. significant gestational age variation (\* = 0.05 > *P* > 0.01; \*\* = 0.01 > *P* > 0.001).

TABLE 2. Transplacental, amniotic fluid, allantoic fluid, foetal e.c.f. to amniotic fluid, foetal e.c.f. to allantoic fluid, and amniotic fluid to allantoic fluid p.d.s at different gestational ages in the sheep

| Gestational age (days) | Transplacental p.d. (mV) | Amniotic fluid p.d. (mV) | Allantoic fluid p.d. (mV) | Foetal e.c.f. to amniotic fluid p.d. (mV) | Foetal e.c.f. to allantoic fluid p.d. (mV) | Amniotic fluid to allantoic fluid p.d.† (mV) |
|------------------------|--------------------------|--------------------------|---------------------------|---|--|--|
| 67                     | -45                      | -65 to -70               | —                         | -25 to -30                                | —  | —  |
| 76                     | -43 to -47               | -62 to -72               | -75 to -82                | -25 to -28                                | -30 to -36                                 | -6   |
| 80                     | -40                      | -90*                     | -70                       | -51                                       | -32  | +19  |
| 93a                    | -59                      | -76*                     | -98 to -104               | -12 to -17                                | -40 to -45                                 | —  |
| 93b                    | -69 to -72               | -85*                     | -100 to -110              | -13 to -17                                | -37 to -39                                 | -23  |
| 95                     | -60                      | -104*                    | -104 to -114              | -40 to -42                                | -44 to -50                                 | —  |
| 103                    | -53                      | -70 to -76               | -84 to -94                | —   | -35 to -44                                 | -18  |
| 109                    | -46 to -48               | -60 to -62               | -91 to -94                | -12 to -13                                | -38 to -43                                 | -28  |
| 113                    | -50                      | -64 to -66*              | -120 to -140              | -17 to -20                                | —  | —  |
| 121                    | -52                      | -69*                     | -123 to -131              | —   | -70 to -81                                 | -58  |
| 124a                   | -49                      | -51                      | -93 to -97                | -4  | -47 to -50                                 | -44  |
| 124b                   | -55 to -59               | -60 to -63               | -112 to -120              | —   | —  | -55  |
| 130                    | -55                      | -61 to -63               | -75 to -90                | -8 to -10                                 | -25 to -35                                 | -21  |
| 130                    | -50                      | -68                      | -82 to -88                | -13                                       | -32 to -40                                 | —  |
| 136                    | -46 to -48               | -55 to -57               | -70 to -79                | -7 to -10                                 | -20 to -34                                 | -19  |
| 140                    | -47                      | -50                      | -77 to -80                | 0 to -2                                   | -32  | -28  |
| Mean                   | -51                      | G.V.*                    | -96                       | G.V.***                                   | -44  | —  |
| S.D.                   | ±7                       |                          | ±18                       |   | ±16  |  |

a and b are twins.

\* Denotes potential differences which are unaltered after the head of the foetus had been exposed through an incision in the uterine wall and amniochorion.

† Polarity of the p.d. is relative to the amniotic fluid.

G.V. significant gestational age variation (\* = 0.05 > P > 0.01; \*\*\* = P < 0.001).

measurable p.d. was detected across the amniochorion, the amnioallantois, or the foetal skin (of sheep), whether the solutions bathing the membrane were plasma and the appropriate foetal fluid, or Krebs bicarbonate Ringer solution, at any of the ages studied. This was also true of the chorioallantois from two goat and two sheep conceptuses. However, p.d.s of 5–12 mV (allantois negative) were observed across chorioallantoic membranes from three goats and four sheep, but they became 0 mV when Krebs bicarbonate Ringer solution replaced the fluids on both sides of the membrane. Therefore, unlike the chorioallantois of the pig (Crawford & McCance, 1960), electrical activity could not be detected across these membranes as such *in vitro*.

#### *Ionic composition of foetal fluids*

The mean  $[Na^+]$ ,  $[K^+]$ , and  $[Cl^-]$  of maternal and foetal plasma in the goat and sheep are given in Table 3. The individual values, and the corresponding p.d.s (Tables 1 and 2), have been used to determine whether the distributions of the ions between maternal and foetal plasma and amniotic and allantoic fluid were according to electrochemical equilibrium.

*Amniotic fluid.* A comparison of the observed amniotic fluid  $[Na^+]$ ,  $[K^+]$ , and  $[Cl^-]$ , and those expected at electrochemical equilibrium between foetal plasma and amniotic fluid, is given in Table 4.

In both goats and sheep at all gestational ages the amniotic fluid  $[Na^+]$  was less than the expected equilibrium value. In the goat, the observed  $[K^+]$  was initially about one-third of the calculated value, but by 124 days electrochemical equilibrium appeared to have been reached. From 76 to 113 days in the sheep the observed  $[K^+]$  was less than the calculated value, and thereafter it was greater. The amniotic fluid  $[Cl^-]$  of the goat was greater than that expected at equilibrium over the whole age range, but the difference decreased from 90 m-equiv/l. at 81 days to about 30 m-equiv/l. at 141 days. In the sheep, before 124 days the observed amniotic fluid  $[Cl^-]$  was greater than that expected at equilibrium, but thereafter amniotic fluid and foetal plasma  $Cl^-$  was in electrochemical equilibrium since there was no significant difference between the observed and calculated  $[Cl^-]$  after 130 days.

*Allantoic fluid.* The  $[Na^+]$  of allantoic fluid ( $88 \pm 20$  (5) m-equiv/l. in the goat, and decreasing from 133 to 7 m-equiv/l. in the sheep; Fig. 2) was lower than the maternal and foetal plasma values at all ages studied in both species. Thus, both concentration and electrochemical gradients tended to favour diffusion of  $Na^+$  into the allantoic fluid.

In the goat the observed allantoic fluid  $[K^+]$  exceeded the maternal and foetal plasma values (cf. Tables 3 and 5), and was greater ( $0.01 > P > 0.001$ ; by about 14 m-equiv/l.) than that expected at electrochemical equilibrium



with foetal plasma only (Table 5). However, if the  $[K^+]$  is calculated using the mean of the allantoic fluid p.d. and the foetal e.c.f. to allantoic fluid p.d., the values obtained are an approximation to those expected at electrochemical equilibrium between allantoic fluid and maternal and foetal plasma. Since the values calculated in this way are 2 to 6 times greater

TABLE 3. The mean  $[Na^+]$ ,  $[K^+]$ , and  $[Cl^-]$  of maternal and foetal plasma of goats and sheep

| Species | Gestational age range (days) |   | Ion concentrations (m-equiv/l.) |                    |                  |
|---------|------------------------------|---|---------------------------------|--------------------|------------------|
|         |                              |   | $Na^+$                          | $K^+$              | $Cl^-$           |
| Goat    | 81-141                       | M | $168 \pm 6$ (5)                 | $5.2 \pm 0.3$ (5)  | $107 \pm 2$ (5)  |
|         |                              | F | $164 \pm 12$ (7)                | $5.2 \pm 1.0$ (7)  | $103 \pm 2$ (7)  |
| Sheep   | 76-140                       | M | $154 \pm 5$ (11)                | $5.5 \pm 0.7$ (12) | $106 \pm 4$ (11) |
|         |                              | F | $152 \pm 7$ (14)                | $5.7 \pm 1.6$ (15) | $105 \pm 5$ (13) |

M, maternal plasma; F, foetal plasma.

TABLE 4. The observed amniotic fluid  $[Na^+]$ ,  $[K^+]$ , and  $[Cl^-]$ , and those calculated assuming distribution of these ions according to electrochemical equilibrium between foetal plasma and amniotic fluid in the goat and sheep

| Gestational age (days) | Amniotic fluid [ion] (m-equiv/l.) |            |          |            |          |            |
|------------------------|-----------------------------------|------------|----------|------------|----------|------------|
|                        | $[Na^+]$                          |            | $[K^+]$  |            | $[Cl^-]$ |            |
|                        | Observed                          | Calculated | Observed | Calculated | Observed | Calculated |
| Goats                  |                                   |            |          |            |          |            |
| 81                     | 155                               | 555        | 5.4      | 14.6       | 123      | 32         |
| 94                     | 77                                | 445        | 5.2      | 13.0       | 110      | 41         |
| 110                    | 136                               | 252        | 7.9      | 10.0       | 123      | 67         |
| 124                    | 108                               | 182        | 7.3      | 6.5        | 102      | 82         |
| 141 $a$                | 136                               | 218        | 6.8      | 7.0        | 109      | 78         |
| 141 $b$                | 147                               | 180        | 5.4      | 6.5        | 108      | 85         |
| Sheep                  |                                   |            |          |            |          |            |
| 76                     | —                                 | —          | 13.3     | 19.5       | 119      | 38         |
| 80                     | 147                               | 1005       | 10.6     | 25.0       | 115      | 16         |
| 93 $a$                 | 132                               | 276        | 7.7      | 8.5        | 118      | 63         |
| 93 $b$                 | 139                               | 263        | 6.8      | 7.3        | 132      | 63         |
| 95                     | 136                               | 695        | 8.6      | 20.0       | 117      | 24         |
| 103                    | 136                               | 315        | 7.7      | 10.5       | 99       | 48         |
| 109                    | 138                               | 240        | 7.3      | 14.4       | 118      | 66         |
| 113                    | 107                               | 306        | 6.5      | 7.0        | 98       | 54         |
| 121                    | 93                                | 221        | 15.9     | 6.5        | 98       | 71         |
| 124 $a$                | 121                               | 163        | 10.1     | 6.6        | 119      | 94         |
| 124 $b$                | —                                 | —          | 8.6      | 6.1        | 108      | 97         |
| 130                    | 110                               | 204        | 13.4     | 10.4       | 92       | 69         |
| 130                    | 93                                | 239        | 16.5     | 7.4        | 90       | 93         |
| 136                    | 71                                | 189        | 31       | 9.4        | 76       | 94         |
| 140                    | 103                               | 154        | 11.6     | 6.1        | 90       | 102        |

$a$  and  $b$  are twins.

than the observed  $[K^+]$  (Table 5), it seems that diffusion in response to a joint action of the electrochemical gradients arising from the allantoic fluid p.d. and the foetal e.c.f. to allantoic fluid p.d. is sufficient to account for the relatively high allantoic fluid  $[K^+]$  in the goat. Thus it is not necessary to postulate an active transport mechanism in this species. In the

TABLE 5. The observed allantoic fluid  $[K^+]$  compared with that calculated assuming distribution of  $K^+$  according to electrochemical equilibrium between allantoic fluid and (a) maternal plasma, (b) maternal and foetal plasma, and (c) foetal plasma, in the goat and sheep

| Gestational<br>age<br>(days) | $[K^+]$ (m-equiv/l.) |       |     | Observed |
|------------------------------|----------------------|-------|-----|----------|
|                              | Calculated           |       |     |          |
|                              | (a)                  | (b)   | (c) |          |
|                              |                      | Goats |     |          |
| 94                           | 151                  | 86    | 21  | 32       |
| 110                          | 682                  | 357   | 33  | 57       |
| 124                          | 253                  | 137   | 20  | 26       |
| 141 <sub>a</sub>             | 186                  | 101   | 16  | 31       |
| 141 <sub>b</sub>             | 193                  | 103   | 12  | 27       |
|                              |                      | Sheep |     |          |
| 76                           | 103                  | 63    | 24  | 25       |
| 80                           | 65                   | 39    | 13  | 19       |
| 93 <sub>a</sub>              | 211                  | 118   | 25  | 23       |
| 93 <sub>b</sub>              | 244                  | 130   | 17  | 24       |
| 95                           | 537                  | 281   | 25  | 36       |
| 103                          | 157                  | 83    | 20  | 28       |
| 109                          | 191                  | 117   | 43  | 30       |
| 113                          | 470                  | 274   | 78  | 50       |
| 121                          | 685                  | 278   | 70  | 99       |
| 124 <sub>a</sub>             | 179                  | 105   | 32  | 70       |
| 124 <sub>b</sub>             | 386                  | 216   | 46  | 103      |
| 130                          | 115                  | 64    | 22  | 119      |
| 130                          | 118                  | 62    | 17  | 112      |
| 136                          | 89                   | 54    | 20  | 118      |
| 140                          | 86                   | 53    | 20  | 121      |

*a* and *b* are twins.

(a) was calculated using the allantoic fluid p.d., (b) the mean of the allantoic fluid p.d. and the foetal e.c.f. to allantoic fluid p.d., and (c) using the foetal e.c.f. to allantoic fluid p.d. (Tables 1 and 2).

sheep from 76 to 113 days the observed allantoic fluid  $[K^+]$  approximated to the values expected at electrochemical equilibrium with foetal plasma only (Table 5). Between 113 and 124 days the  $[K^+]$  doubled. Diffusion in response to a joint action of the two electrochemical gradients appears to have been sufficient to account for this rise. From 130 days until term, however, passive maintenance of the high allantoic fluid  $[K^+]$  by such a joint action does not seem to have been possible (Table 5).

In the sheep the  $[\text{Na}^+]$  of allantoic fluid decreased from 133 m-equiv/l. at 76 days to 7 m-equiv/l. at 140 days (the regression coefficient  $b = -2.18$  m-equiv/l. day;  $P < 0.001$ ), and the  $[\text{K}^+]$  increased from 25 to 121 m-equiv/l. during the same period ( $b = 1.88$  m-equiv/l. day;  $P < 0.001$ ), and there was a significant negative correlation ( $r = 0.931$ ;  $P < 0.001$ ) between the two ion concentrations (Fig. 2). No such correlation was seen in the goat.

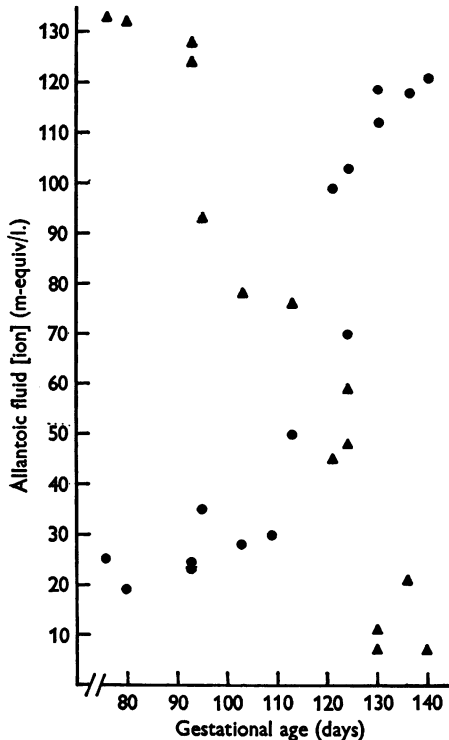


Fig. 2. The allantoic fluid  $[\text{Na}^+]$  (▲) and  $[\text{K}^+]$  (●) at different gestational ages in the sheep.

The observed allantoic fluid  $[\text{Cl}^-]$  of the goat was not significantly different ( $P > 0.1$ ) from that calculated using the mean of the allantoic fluid p.d. and the foetal e.c.f. to allantoic fluid p.d. (Table 6). This suggests that a joint action of these two electrochemical gradients determined the  $[\text{Cl}^-]$  of the allantoic fluid. In the sheep there was a significant difference ( $P < 0.001$ ) between the observed allantoic fluid  $[\text{Cl}^-]$  and that calculated using the mean of the two potential differences (Table 6), but the difference was less than that obtained from calculations involving the allantoic fluid p.d. or the foetal e.c.f. to allantoic fluid p.d., only. There was a significant

negative correlation ( $r = 0.745$ ;  $0.01 > P > 0.001$ ) between the individual allantoic fluid  $[Cl^-]$  and the individual means of these two potential differences. This suggests, as in the goat, that diffusion in response to a joint action of the two electrochemical gradients determined the  $[Cl^-]$  of sheep allantoic fluid.

TABLE 6. The observed mean allantoic fluid  $[Cl^-]$  compared with that calculated assuming distribution according to electrochemical equilibrium between maternal and foetal plasma and allantoic fluid, in the goat, and sheep. Individual calculations were made using the mean of the allantoic fluid p.d. and the foetal e.c.f. to allantoic fluid p.d. (Tables 1 and 2)

| Species | Gestational<br>age range<br>(days) | Allantoic fluid $[Cl^-]$ |                          |
|---------|------------------------------------|--------------------------|--------------------------|
|         |                                    | Calculated               | (m-equiv/l.)<br>Observed |
| Goat    | 94-141                             | $9 \pm 3$ (5)            | $12 \pm 3$ (5)           |
| Sheep   | 76-140                             | $9 \pm 4$ (15)           | $16 \pm 10$ (15)         |

TABLE 7. The mean osmolarity of maternal and foetal plasma and amniotic and allantoic fluid at different gestational ages in the goat and sheep

| Species | Gestational<br>age range<br>(days) | Osmolarity (m-osmole/kg water) |                  |                      |                     |
|---------|------------------------------------|--------------------------------|------------------|----------------------|---------------------|
|         |                                    | Maternal<br>plasma             | Foetal<br>plasma | Amniotic<br>fluid    | Allantoic<br>fluid  |
| Goat    | 81-141                             | $318 \pm 8$ (5)                | $307 \pm 28$ (7) | $278 \pm 26$ (7)**   | $294 \pm 7$ (5)**   |
| Sheep   | 80-140                             | $327 \pm 7$ (7)                | $316 \pm 5$ (10) | $271 \pm 27$ (10)*** | $294 \pm 8$ (10)*** |

Starred values are significantly different (\*\* =  $0.01 > P > 0.001$ ; \*\*\* =  $P < 0.001$ ) from the values for maternal and foetal plasma.

By comparing the observed allantoic fluid  $[Na^+]$ ,  $[K^+]$ , and  $[Cl^-]$ , with those calculated using the amniotic fluid to allantoic fluid p.d., it has been possible to determine whether these ions were in electrochemical equilibrium across the amnioallantois. Large differences in the ion concentrations in each animal strongly suggest that the distributions of these ions across the amnioallantois were not according to electrochemical equilibrium in either goats or sheep.

*Osmolarity of foetal fluids.* In both species the amniotic and allantoic fluids were significantly hypotonic relative to maternal and foetal plasma (Table 7), which agrees with the findings of Alexander *et al.* (1958a).

## DISCUSSION

*Sites of origin of potential differences*

In goats and sheep the overall pattern of the p.d.s observed was identical. They have therefore been discussed jointly and, unless otherwise stated, comments relating to one are considered to be equally applicable to the other.

The magnitude and sign of the transplacental p.d. were the same both before and after uterotomy. After uterotomy, temporary occlusion of the umbilical cord in the area between the tip of the foetal salt bridge and the placenta caused the negative transplacental p.d. to increase towards 0 mV; the p.d. returned to its previous value when the occlusion ceased. Similarly, when a salt bridge was passed into a blood vessel towards a single cotyledon, temporary occlusion of the vessel between the tip of the salt bridge and the cotyledon produced the same fluctuations in the negative (transplacental) p.d. being measured. Therefore, the transplacental p.d. appears to arise from activity within the cotyledons, but it is not possible from the results to establish the location and nature of the (active) mechanisms producing the transplacental p.d. with greater precision.

The site of origin of the amniotic fluid p.d. does not seem to be the amniochorion, the amnioallantois, or the foetal skin (of the sheep), since these membranes as such display no spontaneous electrical activity *in vitro*. This confirms previous *in vitro* observations on sheep amnion (Wright, 1964). The stomach of the foetal sheep has been shown to generate a negative p.d. (Wright & Nixon, 1961), which was confirmed in two goats and one sheep in the present study. Unlike the rabbit and guinea-pig (Mellor, 1969*a*), however, this activity of the foetal stomach does not seem to affect the amniotic fluid since the amniotic fluid, p.d. was not altered by exposing the head of the foetus through an incision in the uterine wall and amniochorion.

The amniotic fluid p.d. is equal to the sum of the transplacental p.d. and the foetal e.c.f. to amniotic fluid p.d. (Fig. 1). It seems, therefore, that a large proportion of the amniotic fluid p.d. arises indirectly from the transplacental p.d. The foetal e.c.f. to amniotic fluid p.d. provides the difference, and its changing magnitude seems to be responsible for the increase towards 0 mV of the amniotic fluid p.d. as gestation advances, since the transplacental p.d. shows no gestational age variation. The foetal e.c.f. to amniotic fluid p.d. appears to arise directly from activity between the amniotic fluid and foetal blood in the amnion or foetal skin vessels, and not from activity across the full thickness of membranes. The *in vitro* results are consistent with this hypothesis.

The allantoic fluid p.d. does not seem to arise from activity of the chorioallantois as such, as in the pig (Crawford & McCance, 1960), since no p.d. could be detected across this membrane *in vitro*. The allantoic fluid p.d. is equal to the sum of the transplacental p.d. and the foetal e.c.f. to allantoic fluid p.d. (Fig. 1), so that a large proportion of it arises indirectly from the transplacental p.d. The difference is provided by the foetal e.c.f. to allantoic fluid p.d., which seems to arise directly from activity between the allantoic fluid and foetal blood in the chorioallantois, or perhaps the amnion. The *in vitro* results agree with this. Results discussed in the next section suggest that the amnion severely hinders the passage of ions. Thus, the amnion does not seem likely to be involved, since ions would need to penetrate almost its entire thickness to reach the vascular layer (Fig. 1).

It appears, therefore, that the amniotic fluid p.d., and the allantoic fluid p.d., are each generated by activity at two different sites linked by foetal blood. Since no p.d. was generated across the full thickness of each of the foetal membranes (from *in vitro* findings), it must be assumed that any electrochemical gradients across them are maintained by relatively low permeabilities to ions.

#### *Electrochemical gradients and ion concentrations*

*Amniotic fluid.* The negative polarity of the amniotic fluid relative to both foetal and maternal blood would tend to promote diffusion of  $\text{Cl}^-$  out of the sac. Before 130 days, however, the amniotic fluid  $[\text{Cl}^-]$  was greater than that expected at electrochemical equilibrium with foetal plasma (Table 4); thereafter  $\text{Cl}^-$  was in equilibrium (in the sheep). This and the progressive rise of the negative foetal e.c.f. to amniotic fluid p.d. towards 0 mV (Tables 1 and 2) occurring concomitantly with the decline in the amniotic fluid  $[\text{Cl}^-]$  towards that expected at electrochemical equilibrium (Table 4), suggest the presence of an electrogenic  $\text{Cl}^-$  pump directed from the foetal blood towards the amniotic fluid. The changes observed with gestational age may have been due to a decrease in the activity of the  $\text{Cl}^-$  pump, or to a large increase in the ionic permeabilities which results in the electrogenic pump being short-circuited.

Before 113 days, the volume of the amniotic fluid increases (Malan *et al.* 1937; Cloete, 1939). In the goat, the amniotic fluid  $[\text{K}^+]$  was initially less than the value expected at electrochemical equilibrium with foetal plasma, but from 124 to 141 days electrochemical equilibrium had been reached (Table 4). In the sheep before 113 days the  $[\text{K}^+]$  was lower, and thereafter it was higher than the value expected at electrochemical equilibrium with foetal plasma (Table 4). In both species, therefore, it seems that the entry of  $\text{K}^+$  into the amniotic fluid was not sufficient to keep pace with the initial volume changes. After 113 days, in the goat  $\text{K}^+$  was in equilibrium,

but in the sheep the entry of  $K^+$  exceeded that required for the maintenance of electrochemical equilibrium values. This may be due to the secretion of foetal urine (Alexander, Nixon, Widdas & Wohlzogen, 1958*b*) which after 113 days has 2–3 times the  $[K^+]$  of the amniotic fluid (Mellor, 1969*b*).

The tendency of the amniotic fluid  $K^+$  and  $Cl^-$  to equilibrate with foetal plasma in the amnion or foetal skin vessels (Table 4), and not with maternal plasma (across the amniochorion) or allantoic fluid (across the amnio-allantois), suggests that the passage of solute particles across the amnion as a whole is severely hindered. The progressive rise in the urea concentration of the amniotic fluid relative to that of the maternal and foetal plasma and allantoic fluid in the sheep (Alexander *et al.* 1958*a*; Hervey & Slater, 1968), and the similar rise observed in goat amniotic fluid (D. J. Mellor, unpublished data), support this suggestion.

Although the electrochemical and concentration gradients from foetal plasma favour diffusion of  $Na^+$  into the amniotic fluid, the  $[Na^+]$  remains below that expected at electrochemical equilibrium. The increase towards 0 mV of the foetal e.c.f. to amniotic fluid p.d. (Tables 1 and 2) and the continued maintenance of a low amniotic fluid  $[Na^+]$  (Table 4) are against the idea of an electrogenic  $Na^+$  pump directed towards the foetal blood. It has been suggested above that the permeability of the amnion is low. Thus, diffusion across the amnioallantois into the allantoic fluid seems unlikely to occur at a sufficient rate to account for the difference. The low  $[Na^+]$  may merely reflect the low osmolarity of the amniotic fluid (Table 7).

*Allantoic fluid.* The  $[Cl^-]$  of allantoic fluid was less than that expected at electrochemical equilibrium with foetal plasma only and greater than that expected at equilibrium with maternal plasma only. However, the individual allantoic fluid  $[Cl^-]$  approximated closely to the values calculated assuming  $Cl^-$  moved passively in response to the electrochemical gradient between allantoic fluid and foetal plasma and between allantoic fluid and maternal plasma (Table 6). This suggests that in contrast to the amnion the chorioallantois as a whole is permeable to  $Cl^-$ , and that  $Cl^-$  exchange takes place between allantoic fluid and maternal plasma in the endometrium, and between allantoic fluid and foetal plasma in the chorioallantois.

It appears that large electrochemical and concentration gradients tended to favour diffusion of  $Na^+$  into the allantoic fluid from both foetal and maternal plasma. Thus, the maintenance of a low allantoic fluid  $[Na^+]$ , and the negative polarity of the fluid, together suggest the presence of an electrogenic  $Na^+$  pump directed out of the allantoic sac. No p.d. was generated across the full thickness of the chorioallantois *in vitro*, and the p.d. across it *in vivo* (allantoic fluid p.d.) seemed to arise from a p.d. generated in the cotyledons (transplacental p.d.) and a p.d. arising from

activity between the allantoic fluid and the foetal blood in the chorio-allantois (foetal e.c.f. to allantoic fluid p.d.). It therefore appears that  $\text{Na}^+$  is pumped electrogenically from the allantoic fluid into the foetal blood in the chorioallantois.

The high allantoic fluid  $[\text{K}^+]$  cannot be completely accounted for by diffusion in response to a joint action of the electrochemical gradients arising from the allantoic fluid p.d. and the foetal e.c.f. to allantoic fluid p.d. in the sheep after 130 days (Table 5). Thus it appears that  $\text{K}^+$  may be pumped into the allantoic fluid. If this is the case, the negative polarity of the allantoic fluid suggests that  $\text{Na}^+$  is being pumped out of the sac at a faster rate than  $\text{K}^+$  is pumped into it. Whether the  $\text{Na}^+$  and  $\text{K}^+$  pumps are independent or coupled remains to be determined. The highly significant correlation between the  $[\text{Na}^+]$  and  $[\text{K}^+]$  of allantoic fluid in the sheep (Fig. 2) could result from coupling of the pumps, or from the necessity to balance cationic and anionic charges. A conclusive demonstration of the presence of these pumps requires further work, and appears to be worthy of attention.

There appears to be a large difference between the concentration of allantoic fluid cations and the concentration of anions known to be present. The contribution of protein (Hervey & Slater, 1968), ionized amino acids (J. S. Slater, unpublished data), sulphate ion (Alexander *et al.* 1958*a*), inorganic phosphorus (D. J. Mellor, J. S. Slater & E. J. Hervey, unpublished data), bicarbonate (D. J. Mellor, I. W. Percy-Robb & F. Cockburn, unpublished data), and chloride (Table 6), are known to be small, so that the substance or substances that supply the remaining negative charge await identification.

#### *Sodium passage between mother and foetus*

For some time it has been accepted that the total flux of  $\text{Na}^+$  towards the foetus results almost entirely from passage across the placenta. The source of this idea appears to have been the report on  $^{24}\text{Na}^+$  uptake by foetuses of several species (Flexner & Gellhorn, 1942). Recently it has been suggested that in the rabbit, guinea-pig and man (Mellor, 1969*a*; Mellor, Cockburn, Lees & Blagden, 1969) a path involving the amniotic fluid may make a significant contribution. In goats and sheep, however, this seems unlikely since the amnion as a whole appears to be relatively impermeable to ions. There seems to be a dynamic cycle involving  $\text{Na}^+$  entry into the amniotic fluid in foetal urine (Alexander *et al.* 1958*a, b*) and in secretions of the nasopharyngeal and buccal cavities (Reynolds, 1953), its exchange between amniotic fluid and foetal blood in the amnion or foetal skin vessels, and its active absorption from the gastrointestinal tract of the foetus after being swallowed in amniotic fluid (Wright &



Nixon, 1961), but there appears to be little if any involvement of maternal plasma  $\text{Na}^+$ . When compared to the amnion, the chorioallantois of the goat and sheep seems to be relatively permeable to ions (cf. electrochemical equilibrium of  $\text{Cl}^-$  in amniotic fluid and in allantoic fluid). Thus, diffusion of  $\text{Na}^+$  from maternal plasma into the allantoic fluid down electrochemical and concentration gradients, followed by active uptake into the foetal blood in the chorioallantois appears to contribute to the total flux of  $\text{Na}^+$  towards the foetus as measured by Pohl, Flexner & Gellhorn (1941). Although the magnitude of this contribution remains to be determined, the possible importance of this path to the foetus may be assessed by considering net fluxes.

Net flux of  $\text{Na}^+$  towards the foetus, which of course equals the rate of foetal  $\text{Na}^+$  retention (about 0.3 g/day at 136 days; Field & Suttle, 1967), is less than the total flux towards the foetus (about 4 g/day at 136 days; Pohl *et al.* 1941). The foetal and maternal plasma  $[\text{Na}^+]$  are not significantly different (Table 3). This fact and the negativity of the foetal blood together indicate that the foetal plasma  $[\text{Na}^+]$  (about 150 m-equiv/l.) is less than that expected at electrochemical equilibrium (about 1000 m-equiv/l.). The maintenance of a low  $[\text{Na}^+]$  may be due to active  $\text{Na}^+$  transport across the placenta. If this were the case, and if the transport were electrogenic, it would account for the negative transplacental p.d. and would result in a net  $\text{Na}^+$  flux from foetal to maternal blood. Since foetal  $\text{Na}^+$  requirements are met, such a loss would need to be made good or exceeded by net  $\text{Na}^+$  flux towards the foetus along other transfer paths. Diffusion of  $\text{Na}^+$  from maternal blood in the endometrium into the allantoic fluid, followed by active uptake into the foetal blood in the chorioallantois would result in a net flux towards the foetus.

In the majority of ovine multiple pregnancies the chorionic vasculature of one foetus is located over regions of the allantoic sac of its neighbouring litter-mate (Mellor, 1969*c*). Considered in relation to the apparent involvement of the chorioallantois in foetal  $\text{Na}^+$  uptake, this suggests intrauterine competition for  $\text{Na}^+$  between litter-mates, or a relative increase in the surface area available for  $\text{Na}^+$  transfer to the individuals of multiple foetus pregnancies, or both.

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