THE PASSAGE OF MATERIALS

BETWEEN

MOTHER AND FOETUS

by

David J. Mellor

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SUMMARY

1. Potential differences associated with the compartments of the conceptus have been measured in vivo and in vitro in species with Haemochorial placentae (rat, rabbit, guinea-pig, and human), and with Syndesmochorial placentae (goat and sheep).

2. In the Haemochorial group the $[\mathrm{Na}^+]$ and $[\mathrm{Cl}^-]$ in maternal plasma and amniotic fluid were determined. In the Syndesmochorial group the $[\mathrm{Na}^+]$, $[\mathrm{K}^+]$, and $[\mathrm{Cl}^-]$ of maternal and foetal plasma, and of amniotic and allantoic fluid, together with the $[\mathrm{K}^+]$ of foetal urine were determined.

3. In the Haemochorial group:

(a) Transplacental potential differences of about 15 mV (foetus positive) were found in the rat, of approximately zero mV in the rabbit and human, and of about 18 mV (foetus negative) in the guinea-pig.

(b) In the rat a potential difference (p.d.) measured between the maternal extracellular fluid and the amniotic fluid (the amniotic fluid p.d.) was identical with the transplacental p.d., and thus appeared to arise indirectly from it. In the rabbit, a fluctuating amniotic fluid p.d. with a maximum value of 22 mV (amniotic fluid negative) seemed to arise from activity of the foetal gastric mucosa. There was no amniotic fluid p.d. in the human at term. In the guinea-pig, an amniotic fluid p.d. of about 50 mV (amniotic fluid negative) appeared to arise from the foetal gastric mucosa and indirectly from the placenta.

(c) The results are discussed in the context of Na^+ transfer to the foetus, and on this basis tend to question the general assumption that almost all Na^+ reaching the foetus passes across the placenta. This assumption might be justified in the rat, but only if Na^+ passage along other paths is relatively small. Passage of Na^+ across the placenta seems to account for only part of the total flux towards the foetus in the rabbit, and possibly also in the human and guinea-pig. Net flux of Na^+ across the placenta may be directed towards the foetus in the rat, could be in either direction in the rabbit and human, and may be towards the mother in the guinea-pig.

4. In the Syndesmochorial group:

(a) The overall pattern of the potential differences in the goat and sheep was similar, but differed in magnitude.

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

(c) The amniotic fluid p.d. 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. The variation in the amniotic fluid p.d. seemed to result from changes in the magnitude of the p.d. between the foetal blood and the amniotic fluid.

(d) A p.d. between the maternal extracellular fluid and the allantoic fluid (the allantoic fluid p.d.) 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.

(e) The results suggest that the p.d. between foetal blood and amniotic fluid arises from an electrogenic Cl^- pump directed towards the amniotic fluid, and that the p.d. between the foetal blood and the allantoic fluid is generated by an electrogenic Na^+ pump directed towards the foetal blood.

(f) The results are discussed in the context of Na^+ transfer to the foetus, and again suggest that all Na^+ reaching the foetus does not pass across the placenta. It is possible that Na^+ diffusion from maternal blood into the allantoic fluid followed by active transport into the foetal blood may constitute an additional pathway. In the species in this group, Na^+ diffusion from maternal plasma into the amniotic fluid does not seem likely to be involved.

5. It appears that grouping conceptuses from different species according to their morphological characteristics does not necessarily indicate similarity of physiology within any one group; indeed, the results emphasise the necessity to demonstrate such a similarity before it is assumed.

6. Futher investigation is required on such topics as: the precise location and nature of the mechanisms producing the potential differences observed, the p.d. patterns in conceptuses of other species, the origin of the foetal fluids and the regulation of their composition and volume, and the interactions of neighbouring conceptuses in multiparous pregnancies in goats and sheep.

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INTRODUCTION

DURING THE LATER stages of gestation the rapidly growing foetus requires large quantities of minerals. Investigations of factors associated with this phenomenon have usually so emphasized the placenta that other possible transfer paths have been completely neglected. This is particularly true of the work initiated by the observations of Flexner & Gellhorn (1942a) who discussed the injection of radioactive ^{24}Na into dams of several species and assumed that almost the entire quantity reaching the foetus did so via the placenta. Such an assumption is probably only justified in cases where uterine and umbilical arterial and venous bloods are sampled, or when all other possible transfer paths, or exchange surfaces, have been demonstrated to be of no significance. Two possible transfer paths involve the outer membrane of the conceptus. Since this membrane is perfused by foetal blood and is closely apposed to the uterine mucosa, it is theoretically possible for minerals to enter the foetal blood from secretions of the uterine decidua, or from the foetal fluids after the minerals have passed across the membrane from the maternal side. Passage of radioactive Na^+ into the amniotic fluid of the rat (Quigley, Phillips & McKay, 1965), the guinea-pig (Flexner & Gellhorn, 1942b), and the human (Vosburgh, Flexner, Cowie, Hellman, Proctor & Wilde, 1948), after injection into the mother, has been shown to occur. In addition, it is possible that minerals entering the gastrointestinal tract in swallowed amniotic fluid could be absorbed into the foetal blood. Absorption of Na^+ from the gastrointestinal tract of the foetal sheep (Wright & Nixon, 1961), and from the gastric lumen of the foetal rabbit (Wright, 1962), has been demonstrated.

Since exchange of minerals between foetal blood, foetal fluids, and maternal blood can only occur across multicellular membranes consisting of one or more layers of cells, a knowledge of the membrane transport mechanisms involved is essential if the paths of transfer are to be identified. The properties of mechanisms of transport across membranes, varying from simple homogeneous cell membranes to more complex multicellular types, have been outlined by Shanes (1958), Fuhrman (1959), Edelman (1961), Whittam (1964), Csaky (1965), and Caldwell (1968). Free diffusion (or passive diffusion) occurs without expenditure of metabolic energy, the driving force being a concentration or an electrochemical gradient. Transfer can occur against concentration gradients passively when the driving force is an electrochemical gradient. The driving force of facilitated diffusion is a concentration difference, but combination with a carrier in the membrane allows faster transfer than would be possible by free diffusion alone. Exchange diffusion is another carrier-mediated transport mechanism, but in this case transfer occurs in both directions without net gain or loss of solute on either side of the membrane. Active transport is carrier-mediated translocation which may proceed even against concentration and electrochemical gradients, and requires metabolic energy expenditure. Its forms are said to include a coupled exchange of two different ions with the same charge (neutral pump), and an uncoupled exchange resulting in an ultimate separation of charge (electrogenic pump).

The minerals, Na, K, and Cl, are present in the body fluids in the ionic state. Since electrical potential differences are generally associated with the active transport of an ion across a membrane, simultaneous observation of the ion concentrations and potential differences associated with the fluid compartments of the conceptus could yield valuable information. However, in order to interpret the data in a meaningful manner it is necessary to consider the different circumstances in which potential differences can be generated.

Homogeneous cell membranes will first be considered. An uneven distribution of a particular ion species on each side of such a membrane will give rise to a potential difference (p.d.) across it, but unless there is absolute impermeability or a mechanism maintaining the differential ion concentrations the p.d. will eventually disappear. If an ion is distributed according to electrochemical equilibrium the Nernst equation applies:

$$E_m = \frac{RT}{zF} \log_e \frac{{}^{a}C_x}{{}^{b}C_x}$$

where E_m = the p.d. across the membrane,
R = the gas constant,
T = the absolute temperature,
z = the valency of the ion species,
F = the Faraday,

and ${}^{y}C_{x}$ = the concentration of the ion x (y = a or b, the solutions on either side of the membrane).

Whenever a membrane is known to be permeable to an ion which is not distributed according to electrochemical equilibrium, there is a priori evidence for an active transport mechanism.

A homogeneous cell membrane separating solutions of identical ionic composition will not have a p.d. across it if there is only a neutral pump present, but an electrogenic pump in such a system would generate a p.d. across the membrane. A p.d. across a homogeneous cell membrane containing a neutral pump only, will tend to remain at about the same value for a relatively long period after inactivation of the pump by a metabolic poison, whereas inactivation of an electrogenic pump will result in an almost immediate decline in the p.d. it generates.

Investigations of factors associated with the generation of the p.d. across frog skin in vitro (Johnsen, Levi & Ussing, 1952; Johnsen & Ussing, 1958; MacRobbie & Ussing, 1961; Ussing & Windhanger, 1964; Ussing, 1965) have revealed a relatively complex mechanism, which has been discussed by Heinz (1967). A model, treating the frog skin simply as consisting of two parallel membranes in series, the outer one acting as a Na^+ electrode and the inner one as a K^+ electrode, with an electroneutral $Na^+ - K^+$ exchange pump in the inner membrane, was postulated. This appears to have been an oversimplification since there is evidence (cited by Heinz, 1967) suggesting the involvement of intercellular bridges, shunt pathways through intercellular spaces, transcellular pathways, and electrogenic ion pumps not located in the basement membrane. The precise details of p.d. generation across the frog skin, therefore, remain to be elucidated, but one phenomenon associated with this process appears to be a net flux of Na^+ from the outside to the inside across the skin.

The p.d. across the chorioallantoic membrane of the pig has been investigated in vitro (Crawford & McCance, 1960) and in many ways appears to be similar to the frog skin. It seems that although the allantois alone is electrically neutral it is essential for p.d. generation, as potential differences were only found across the full thickness of the chorioallantoic membrane. This may result from a special (unknown) metabolic association. The p.d. across the membrane as a whole appears to be associated with net flux of Na^+ from the allantoic to the chorionic side, and active Na^+ transport appears to require the presence of K^+ in the solution bathing the chorionic side. A large proportion of the p.d. possibly arises from an electrogenic pump, since poisoning the membrane with dinitrophenol caused a rapid decline in the p.d. Support for the idea that this pump may be an electrogenic Na^+ pump, is that the 'short-circuit current' is equivalent to the net flux of Na^+ across the membrane (see Ginzburg & Hogg, 1967).

It appears, therefore, that potential differences across multilayered multicellular membranes may arise from a combination of the factors responsible for p.d. generation across relatively simple membranes. However, the complex inter-relationships of these factors are exceedingly difficult to clarify.

Terminology

For ease of expression it is convenient to introduce a section on terminology at this stage.

Potential differences measured between the fluid compartments of the conceptus and the maternal extracellular fluid (e.c.f.) will be referred to as, the amniotic fluid p.d., the allantoic fluid p.d., the foetal stomach 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 potential differences relative to the maternal e.c.f. Potential differences measured between the fluid compartments of the conceptus and the foetal e.c.f. will be referred to as, 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 p.d., and all signs will show the polarity of the potential differences relative to the foetal e.c.f. Any other potential differences will be fully described in the text.

Potential differences of the conceptus

In the goat, transplacental potential differences have been shown to increase from a mid-pregnancy value of -130 mV to about -25 mV at term (Meschia, Wolkoff & Barron, 1958). The allantoic fluid p.d. was measured in a small number of the animals observed and was found to have the same magnitude and sign as the transplacental p.d. No amniotic fluid p.d. measurements were made. In 3 sheep, transplacental potential differences of about -30 mV, and allantoic and amniotic fluid potential differences ranging from -50 to -80 mV have been observed (Widdas, 1961). Amniotic fluid potential differences of -30 to -40 mV in the cat (Widdas, 1961) and of -30 mV (maximum) in the rabbit (Wright, 1963) have been found also.

Detailed investigations of the site or sites of origin of these potential differences have not been carried out in any one species, but the electrical activity of tissues of conceptuses from different species have been studied. The isolated amnion of the human (Garby, 1957; Wright, 1964), of the rabbit (Wright, 1963), and of the sheep (Wright, 1964) seem to be electrically neutral, while the pig chorioallantoic membrane (Crawford & McCance, 1960) and possibly the goat chorion (Meschia et al, 1958) generate potential differences with the foetal side negative. In the rabbit, fluid continuity along the foetal oesophagus enables a p.d. generated by the foetal gastric mucosa (Wright, 1962) to be a source of the negative amniotic fluid p.d., but it is not known if the inverted yolk sac splanchnopleur, or uterine wall, contribute to the amniotic fluid p.d.

Of the few reports of potential differences associated with the fluid compartments of the conceptus that have appeared, the majority seem to be of incomplete or restricted studies. The work outlined in this thesis comprises an extensive comparative study of such potential differences measured during the later stages of gestation in the rat, rabbit, guinea-pig, human, goat, and sheep. These potential differences have been related to simultaneously measured ion concentrations, to *in vitro* observations, and to published data on Na^+ transfer between mother and foetus. In this way it has been possible to clarify, to a very large extent, the situation with regard to mechanisms and paths involved in the transfer of Na^+ between mother and foetus in the above mentioned species.

It is important to note, however, that the approach to this problem has necessarily been qualitative because of the multicompartmental nature of the conceptus and because no ion fluxes were measured. In order to establish between which compartments ion exchange is occurring it is necessary to approach the individual components of the system separately by *in vitro* and *in situ* techniques. Although these techniques can give rise to valuable information relating to directions of net flux between compartments, the rates of ion flux determined can only be applied to the conceptus as a whole with great caution. Similarly, quantitative results obtained from *in vivo* use of radioactive isotopes are only of limited value because techniques that enable continuous simultaneous sampling of all important fluid compartments have yet to be developed.

METHODS

Potential Difference Measurements

All potential differences 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. A *terminology* which facilitates description of the potential differences measured has been outlined in the Introduction.

Rat, Rabbit, and Guinea-pig Measurements

Conceptuses from Sprague-Dawley rats, New Zealand White rabbits, and mixed strain guinea-pigs, aged from 15 to 22 days, 20 to 30 days, and 35 to 65 days gestational age, respectively, were used. Gestational ages were estimated from known mating dates and according to Huggett & Widdas (1951).

Salt bridges consisted of polythene tubing (o.d. 1.0 mm) containing saturated KCl-2% agar.

Experimental procedure. Rats were anaesthetized with ether, and rabbits and guinea-pigs with 30 mg/kg body weight of pentobarbitone given intravenously and intraperitoneally, respectively, and ether. The uterus was exposed by laparotomy.

In vivo potential difference measurements. One end of a salt bridge placed in the maternal peritoneal cavity acted as a reference electrode in the maternal e.c.f., and potential differences were measured between this and one end of a probe salt bridge (Fig. 1.). Before uterotomy one end of the probe salt bridge was passed through a small puncture in the uterine wall and foetal membranes into the amniotic fluid, then down the oesophagus into the foetal stomach, and finally into the foetal peritoneal cavity by rupturing the stomach wall. This allowed amniotic fluid, foetal stomach, and transplacental p.d. measurements, respectively, to be made. Potential differences were also measured after uterotomy in exposed foetuses with ruptured membranes lying on non-conducting material so that the only contact between mother and foetus was at the placenta. Under these circumstances in the rabbit and guinea-pig, the transplacental p.d. had the same magnitude and sign whether the probe salt bridge was placed in the foetal peritoneal cavity, or in the umbilical vein, thus indicating that the p.d. as measured was unaffected by rupturing the stomach wall. The rat umbilical vessels were too small to allow this test to be made, but there was no difference in the magnitude or sign of the transplacental p.d. whether the probe salt bridge entered the foetal peritoneal cavity by rupturing the stomach wall, or entered through a small puncture in the abdominal wall. It was not technically possible to use the umbilical vein for transplacental p.d. measurements before uterotomy. In some experiments, usually of longer duration, a terminal decline in the magnitude of the potential differences was observed. In such cases, the potential differences reported refer to those measured before the condition

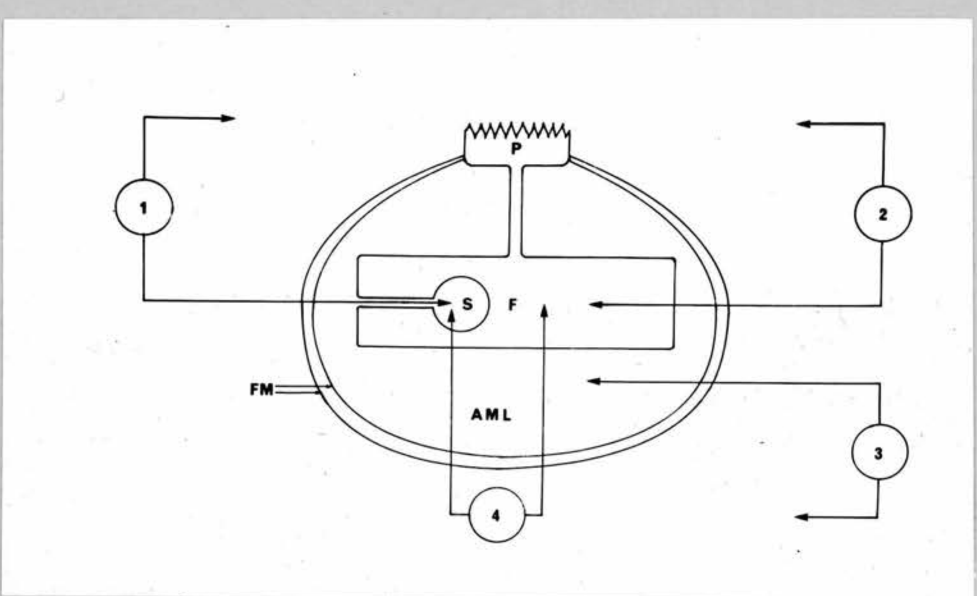

Fig. 1. A schematic representation of the rat, rabbit, guinea-pig, and human, conceptus, showing the points between which potential differences were measured.

P.d. 1 - the foetal stomach p.d.
P.d. 2 - the transplacental p.d.
P.d. 3 - the amniotic fluid p.d.
P.d. 4 - the foetal e.c.f. to foetal stomach p.d.

The diagram shows the foetus (F) bathed in amniotic fluid (AML) which is contained in a sac formed by the placenta (P) and the foetal membranes (FM). The foetal gastric lumen (S) communicates with the amniotic sac via the oesophagus. The whole conceptus is surrounded by uterine tissue which is not shown in the diagram.

of the animal deteriorated. The foetuses were weighed within 4 minutes of being removed from the uterus.

In vitro potential difference measurements. Potential difference measurements were performed according to the method of Ussing & Zerahn (1951). Measurements were made across the uterine wall and amnion of both rabbits and guinea-pigs, and across the inverted yolk sac splanchnopleur of the rabbit, and the yolk sac splanchnopleur of the guinea-pig. No in vitro measurements were made on rat membranes. Intact conceptuses, and pieces of uterine wall overlying them, were removed immediately after uterotomy and placed in Krebs bicarbonate Ringer solution at 4°C. Washed membranes were sandwiched between 2 Perspex half chambers similar to those designed by Ussing & Zerahn (1951). The effective area of membrane in these chambers was 3.1 cm^2. Solutions on both sides of the membrane were maintained at 30°C, and were stirred and oxygenated by the bubble lift incorporated in the apparatus. All solutions were Krebs bicarbonate Ringer solution, were continuously gassed with a 95% O_2, 5% CO_2 mixture, and were at pH 7.4. The Krebs bicarbonate Ringer solution had the following composition (mM): Na^+ 143.2, Cl^- 128.0, K^+ 5.9, Ca^{++} 2.5, Mg^{++} 1.1, HCO_3^- 24.9, $H_2PO_4^-$ 1.1, SO_4^{--} 1.1, glucose 28.0.

Human Measurements

Potential differences were measured during standard elective lower segment Caesarian Sections, carried out on normal patients at term (see acknowledgements).

Salt bridges consisted of vinyl tubing (o.d. 4.0 mm) containing 154 mM NaCl-2% agar, and were sterilized by gamma irradiation (Ethicon Ltd.). Probe salt bridges were of two types; one was fitted with an 18 gauge syringe needle, and the other with a graduated infant stomach tube (o.d. 1.5 mm).

Experimental procedure. Patients were given atropine prior to surgery. Anaesthesia was induced with Thiopentone and maintained with $N_2O/30\% O_2$. There was no intravenous fluid therapy prior to, or during surgery. The uterus was exposed by laparotomy.

In vivo potential difference measurements. One end of a salt bridge in the maternal peritoneal cavity acted as a reference electrode in the maternal e.c.f., and potential differences were measured between this and one end of a probe salt bridge (Fig. 1). The amniotic fluid p.d. was measured before uterotomy by passing the end of a probe salt bridge fitted with a syringe needle through the uterine wall and foetal membranes into the amniotic fluid. After uterotomy, when the foetus had been carefully delivered onto the mother's abdomen so that the umbilical cord and placenta were retained intact, the syringe needle end of a probe salt bridge was inserted into the umbilical artery, or vein, and the stomach tube end of another salt bridge was passed down the oesophagus into the foetal stomach. This allowed transplacental p.d. and foetal stomach p.d. measurements, respectively, to be made. Foetal e.c.f. to foetal stomach potential differences were measured using these two probe salt bridges.

Goat and Sheep Measurements

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

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.

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_2. The uterus was exposed by laparotomy.

In vivo potential difference measurements. One end of a salt bridge in the maternal peritoneal cavity acted as a reference electrode in the maternal e.c.f., and potential differences were measured between this and the ends of salt bridges inserted in the foetal fluid compartments (Fig. 2). A small incision was carefully made in the uterine wall so that the underlying foetal membranes were exposed intact. The insertion of a salt bridge into the foetal e.c.f. was achieved by either (a) one end of a salt bridge being inserted towards the foetus in a foetal artery, or vein, associated with a cotyledon, and tied in place, or (b), in cases where the cotyledonary

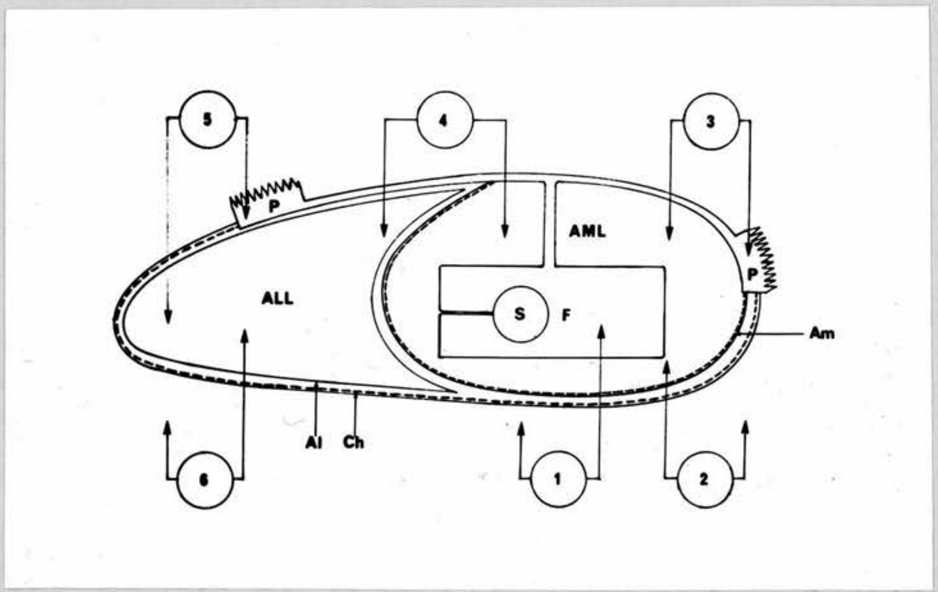

Fig. 2. A schematic representation of the goat and sheep conceptus showing the points between which potential differences were measured.

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 vascular layer shown as a broken line; Al - the allantois; and Ch - the chorion with its vascular layer shown as a broken line. The whole conceptus is surrounded by uterine tissue which is not shown in the diagram.

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 2 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. Potential differences 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. 2).

<u>In vitro potential difference measurements</u>. Potential difference measurements were performed according to the method of Ussing & Zerahn (1951). Measurements were made across the chorioallantois, the amniochorion, and the amnioallantois, of both goats and sheep, and across the foetal skin of the sheep. Immediately after removal from the animal the membrane was sandwiched between the 2 Perspex half chambers previously described (page 11). 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_2, 15% O_2, 5% CO_2 mixture. In some cases Krebs bicarbonate Ringer solution of the composition previously described (page 11) was used on both sides of the membranes.

Mineral Determinations

In the rat, rabbit, guinea-pig, and human, determinations of the $[Na^+]$, and $[Cl^-]$, were carried out on maternal plasma and amniotic fluid. In the goat and sheep, the $[Na^+]$, $[K^+]$, and $[Cl^-]$, were determined in maternal and foetal plasma, and in amniotic and allantoic fluid. The $[K^+]$ of goat and sheep foetal urine was also determined. The $[Na^+]$ and $[K^+]$ in samples suitably diluted with distilled water, were determined with an atomic absorption spectrophotometer (model SP 90, Unicam Ltd.), and the $[Cl^-]$ was determined with a Technicon AutoAnalyser using the standard mercuric thiocyanate, ferric nitrate reaction described by Technicon (method file N-5b). Na^+ was measured to ± 3 m-equiv/l, K^+ less than 20 m-equiv/l was to ± 0.5 m-equiv/l, and greater than 20 m-equiv/l was to ± 3 m-equiv/l, and Cl^- less than 50 m-equiv/l was to ± 0.5 m-equiv/l, and greater than 50 m-equiv/l to ± 2 m-equiv/l.

Osmolarity Determinations

The osmolarity of maternal and foetal plasma, and amniotic and allantoic fluid, of both goats and sheep was determined with an Advanced Osmometer (Advanced Instruments Inc., Mass., U.S.A.) to ± 4 m-osmole/kg water.

Estimated values have been expressed in the text as means and standard deviations, with the number of observations in parenthesis.

Nernst equation calculations. Rats, rabbits, guinea-pigs, and humans, were assumed to have a deep body temperature of 37°C, and goats and sheep 39°C, so that the corresponding $\frac{RT}{zF}$ values for monovalent ions were 62.5, and 62.8, respectively.

RESULTS

PLACENTAE HAEMOCHORIALIS

Potential Difference Measurements

Rats. Individual transplacental potential differences, both before and after uterotomy, were steady, but within each litter values were spread over ranges of up to ± 5 mV. At each gestational age there was no association between foetal weight and this p.d. Over the age range of 15 to 21 days the mean transplacental p.d. from each litter showed no gestational age correlation, and the overall mean was +15 ± 3 mV(63). On the 22nd day of gestation, however, the transplacental p.d. decreased towards zero mV.

Within individual conceptuses a steady positive amniotic fluid p.d. with a magnitude not significantly different from that of the transplacental p.d. was found at all gestational ages studied. Between 15 and 21 days gestational age its overall mean was +15 ± 4 mV(63), and on the 22nd day it decreased towards zero mV. The p.d. was the same whether measured in the amniotic fluid, or the foetal buccal cavity.

The results of transplacental p.d. and amniotic fluid p.d. measurements made on 73 conceptuses from 9 litters ranging from 15 to 22 days gestational age are to be found in Table I.

Rabbits. At all ages studied, both before and after uterotomy, transplacental potential differences of approximately zero mV were seen.

Table I. Transplacental and amniotic fluid potential differences in the rat at different gestational ages.

Gestational age (days)	Transplacental p.d. (mV)	Amniotic fluid p.d. (mV)
15	+17 ± 3 (7)	+17 ± 2 (7)
16	+16 ± 4 (9)	+14 ± 4 (9)
17	+15 ± 4 (11)	+15 ± 4 (11)
18	+14 ± 2 (9)	+13 ± 3 (9)
19	+13 ± 4 (8)	+13 ± 3 (8)
20	+15 ± 3 (10)	+14 ± 3 (10)
21	+17 ± 3 (9)	+18 ± 3 (9)
22	+ 1 ± 2 (5)	+ 2 ± 3 (5)
22	0 ± 2 (5)	- 1 ± 3 (5)

One litter was examined at each age. The results are expressed as means and standard deviations with the number of foetuses in parenthesis. There was no significant difference ($P > 0.1$) between individual transplacental and amniotic fluid potential differences.

Fluctuations between +2 and -2 mV were frequently observed in individual conceptuses.

A fluctuating negative amniotic fluid p.d. was seen, and within individual litters maximum values were spread over ranges of up to ± 5 mV. Within each litter there was no association between the maximum value of this p.d. and foetal weight. The mean maximum amniotic fluid p.d. from each litter showed no gestational age correlation, and the overall mean was -22 ± 5 mV(120). The amniotic fluid p.d. was sometimes steady, but usually fluctuated between -5 to -7 mV and the maximum. It was never seen to increase to zero mV.

Within individual conceptuses a steady negative foetal stomach p.d. was observed to be usually 5 to 10 mV more negative than the maximum amniotic fluid. This difference may have been due to an artifact arising from the saturated KCl in the salt bridge producing a very high [KCl] in the small volume of the foetal stomach. The magnitude of the p.d. was not associated with foetal weight at each gestational age, nor was there any correlation between the mean value for each litter and gestational age. The overall mean was -27 ± 5 mV(67). The magnitude and sign of the foetal stomach p.d. measured before uterotomy were the same as those of the foetal e.c.f. to foetal stomach p.d. measured after uterotomy.

The results of transplacental p.d., amniotic fluid p.d., and foetal stomach p.d. measurements made on 120 conceptuses from 14 litters ranging from 20 to 30 days gestational age are to be found in Table II.

Table II. Transplacental, amniotic fluid, and foetal stomach potential differences in the rabbit at different gestational ages.

Gestational age (days)	Transplacental p.d. (mV)	Maximum amniotic fluid p.d. (mV)	Foetal stomach p.d. (mV)
20	-1 ± 2 (12)	-20 ± 5 (12)	—
21	0 ± 1 (9)	-19 ± 3 (9)	—
21	+1 ± 1 (10)	-30 ± 3 (10)	—
22	0 ± 2 (8)	-14 ± 4 (8)	—
23	0 ± 1 (7)	-29 ± 4 (7)	-34 ± 2 (5)
24	0 ± 2 (10)	-20 ± 2 (10)	-24 ± 3 (7)
25	+1 ± 2 (7)	-27 ± 2 (7)	-30 ± 4 (6)
26	-1 ± 1 (12)	-17 ± 5 (12)	-24 ± 4 (10)
26	+1 ± 2 (7)	-26 ± 2 (7)	-29 ± 3 (5)
27	0 ± 1 (9)	-22 ± 2 (9)	-25 ± 3 (8)
28	-1 ± 2 (8)	-21 ± 2 (8)	-27 ± 2 (8)
29	0 ± 2 (7)	-27 ± 3 (7)	-31 ± 1 (5)
30	0 ± 2 (7)	-24 ± 2 (7)	-26 ± 4 (6)
30	0 ± 1 (7)	-20 ± 2 (7)	-21 ± 2 (7)

One litter was examined at each age. The results are expressed as means and standard deviations, with the number of foetuses in parenthesis.

In vitro no measurable p.d. was detected across the uterine wall, the inverted yolk sac splanchnopleur, or the amnion, either individually or combined, on each successive day between 20 and 30 days gestational age inclusive. Two preparations of each tissue, or combination of tissues, were examined at each age.

Guinea-pigs. Negative transplacental potential differences which were steady were observed in every conceptus. The mean transplacental p.d. from each litter showed no gestational age correlation, and the overall mean was -18 ± 4 mV(35).

Within individual conceptuses a steady negative amniotic fluid p.d. was seen. This p.d. increased abruptly from a mean value of -50 ± 11 mV(23) before the 60th day of gestation, to a mean of -23 ± 4 mV(12) subsequently. At all gestational ages a steady negative foetal stomach p.d. was found to be usually between 2 and 7 mV more negative than the amniotic fluid p.d., but its magnitude (mean before the 60th day = -53 ± 9 mV(23); mean after the 60th day = -28 ± 4 mV(12)) was not significantly different from that of the amniotic fluid p.d.

The results of transplacental p.d., amniotic fluid p.d., and foetal stomach p.d. measurements made on 35 conceptuses from 10 litters ranging from 35 to 65 days gestational age are to be found in Table III.

In vitro no measurable p.d. was detected across the uterine wall, the yolk sac splanchnopleur, or the amnion, either individually or

Table III. Transplacental, amniotic fluid, and foetal stomach potential differences in the guinea-pig at different gestational ages.

Gestational age (days)	Number of foetuses	Transplacental p.d. (mV)	Amniotic fluid p.d. (mV)	Foetal stomach p.d. (mV)
35	4	-16 ± 2	-46 ± 7	-48 ± 8
45	3	-16 ± 3	-46 ± 18	-50 ± 18
47	4	-16 ± 3	-58 ± 11	-63 ± 11
50	3	-18 ± 4	-45 ± 6	-48 ± 7
55	3	-25 ± 1	-60 ± 17	-61 ± 17
57	3	-19 ± 7	-51 ± 5	-53 ± 4
59	3	-22 ± 4	-44 ± 7	-51 ± 8
61	4	-16 ± 1	-21 ± 2	-23 ± 1
63	3	-20 ± 1	-19 ± 2	-23 ± 2
65	5	-17 ± 3	-27 ± 2	-30 ± 3

One litter was examined at each gestational age. The results are expressed as means and standard deviations. There was no significant difference ($P > 0.1$) between the magnitudes of the amniotic fluid and foetal stomach potential differences.

combined, at each of 30, 40, 50, 55, 60, and 65 days gestational age. Two preparations of each tissue or combination of tissues were examined at each age.

Humans. The transplacental and amniotic fluid potential differences of the group as a whole were not significantly different from each other, and had mean values of 0 ± 3 mV(6) and +2 ± 4 mV(9), respectively. Values ranging between -13 and -37 mV were obtained for the foetal stomach p.d., and for the foetal e.c.f. to foetal stomach p.d., and as would be expected, within individual conceptuses these two potential differences differed by approximately the magnitude of the transplacental p.d.

The results of transplacental p.d., amniotic fluid p.d., foetal stomach p.d., and foetal e.c.f. to foetal stomach p.d. measurements made on 9 conceptuses ranging from 36 to 40 weeks gestational age are given in Table IV. This work was carried out in collaboration with Dr. Forrester Cockburn and Dr. Martin Lees.

Sodium and Chloride Determinations

In all cases the $[Na^+]$ and $[Cl^-]$ of maternal plasma and amniotic fluid were determined, and the results are given in Table V. In the rat, rabbit, and guinea-pig, amniotic fluid was pooled to give a representative sample from each litter. In the human values are from individual uniparous pregnancies.

Table IV. Transplacental, amniotic fluid, foetal stomach, and foetal e.c.f. to foetal stomach potential differences in the human at term.

Gestational Age (weeks)	Reason for section	Transplacental p.d. (mV)	Amniotic fluid p.d. (mV)	Foetal stomach p.d. (mV)	Foetal e.c.f. to foetal stomach p.d. (mV)
36	diabetes melitus	-	+2 to +5 (+3)	-	-
38	essential hypertension	+2 to +4 (+3)	0 to -3 (-2)	-	-
38	essential hypertension	-2 to +3 (+1)	-3 (-3)	-30 to -34	-24 to -30
39	contracted pelvis	-	+2 to +5 (+4)	-	-
39	contracted pelvis	-	+3 to +7 (+5)	-	-
39	contracted pelvis	+2 to +4 (+3)	+1 to +2 (+1)	-	-
39	contracted pelvis	-3 to -8 (-5)	-1 to -3 (-2)	-15 to -18	-13 to -15
40	contracted pelvis	-2 to -3 (-3)	+9 (+9)	-	-37
40	contracted pelvis	-1 to -2 (-1)	+2 to +4 (+3)	-29 to -30	-28 to -29

The values in parenthesis are those used for the statistical calculations. There was no significant difference ($P > 0.1$) between the magnitudes of the transplacental and amniotic fluid potential differences.

Table V The $[\mathrm{Na}^+]$ and $[\mathrm{Cl}^-]$ of maternal plasma and amniotic fluid from rats, rabbits, guinea-pigs, and humans.

Species	Gestational age range (d = days; w = weeks)	Maternal plasma $[\mathrm{Na}^+]$ m-equiv/l	Maternal plasma $[\mathrm{Cl}^-]$ m-equiv/l	Amniotic fluid $[\mathrm{Na}^+]$ m-equiv/l	Amniotic fluid $[\mathrm{Cl}^-]$ m-equiv/l
Rat (5)	16 - 20d	152 ± 5	103 ± 2	$133 \pm 5^{***}$	105 ± 4
Rabbit (11)	20 - 30d	155 ± 5	103 ± 5	$138 \pm 12^{***}$	107 ± 5
Guinea-pig (6)	30 - 65d	145 ± 4	97 ± 2	$134 \pm 8^{**}$	$140 \pm 15^{***}$
Human (7)	36 - 40w	138 ± 2	107 ± 2	$123 \pm 10^{**}$	103 ± 17

The results are expressed as means and standard deviations, with the number of animals in parenthesis. Starred values are significantly different ($^{***} = P < 0.001$; $^{**} = 0.01 > P > 0.001$) from those of the maternal plasma.

The $[\mathrm{Na}^+]$ and $[\mathrm{Cl}^-]$ of maternal plasma and amniotic fluid in the 4 species did not change with gestational age. However, the $[\mathrm{Na}^+]$ of amniotic fluid was significantly less than that of maternal plasma in all 4 species; in the rat and rabbit the difference was about 20 m-equiv/l, and in the guinea-pig and human about 12 m-equiv/l. In the guinea-pig the amniotic fluid $[\mathrm{Cl}^-]$ was about 40 m-equiv/l greater than that of maternal plasma.

A comparison of the observed amniotic fluid $[\mathrm{Na}^+]$, and $[\mathrm{Cl}^-]$ and those calculated on the assumption that these ions were distributed according to electrochemical equilibrium between maternal plasma and amniotic fluid is presented in Table VI.

In the rat, rabbit, and guinea-pig, it appears that the distribution of Na^+ between maternal plasma and amniotic fluid was not according to electrochemical equilibrium. In the rat the observed amniotic fluid $[\mathrm{Na}^+]$ was about 46 m-equiv/l greater than the calculated value, and in the rabbit and guinea-pig the observed concentrations were about 80 and 770 m-equiv/l, respectively, less than the calculated values. In the rat, therefore, it seems that Na^+ being driven by the electrochemical gradient would tend to diffuse out of the amniotic fluid against its concentration gradient. In the rabbit and guinea-pig it appears that both the concentration and the electrochemical gradient would tend to favour diffusion of Na^+ from maternal plasma into amniotic fluid. Na^+ appears to be distributed according to electrochemical equilibrium in

Table VI. The observed amniotic fluid $[Na^+]$, and $[Cl^-]$, and those calculated assuming that distribution of these ions between maternal plasma and amniotic fluid was according to electrochemical equilibrium.

Species	Amniotic fluid [ion] (m-equiv/l)			
	$[Na^+]$		$[Cl^-]$	
	Observed	Calculated	Observed	Calculated
Rat	133	87	105	180
Rabbit	138	218	107	73
Guinea-pig	134	906	140	16
Human	123	124	103	118

The observed concentrations are the means from Table V. The calculated concentrations are derived from the relevant mean maternal plasma concentration (Table V) and the observed mean amniotic fluid potential differences; in the rat + 15 mV, in the rabbit - 5 mV and -22 mV, in the guinea-pig - 50 mV, and in the human + 2 mV. The calculated concentration for each ion in the rabbit is the mean of the concentration obtained using -5 mV and that obtained using -22 mV as the amniotic fluid p.d.

the human.

The observed amniotic fluid $[Cl^-]$ was less in the rat and human, and greater in the rabbit and guinea-pig, than the values calculated on the assumption that Cl^- was distributed according to electrochemical equilibrium.

PLACENTAE SYNDESMOCHORIALIS

Potential Difference Measurements

Goats. All potential differences were negative whether relative to the maternal or the foetal e.c.f. Transplacental and amniotic fluid potential differences were normally steady, and the allantoic fluid p.d. was sometimes steady but usually fluctuated. There was no gestational age variation in the magnitude of the transplacental p.d., the allantoic fluid p.d., or the foetal e.c.f. to allantoic fluid p.d., and their mean values were -71 ± 9 mV(7), -107 ± 16 mV(6), and -33 ± 10 mV(6), respectively. As gestation advanced there was a significant increase in the amniotic fluid p.d. ($b = 0.49$ mV/day; $0.05 > P > 0.01$) from -110 to -70 mV, and in the foetal e.c.f. to amniotic fluid p.d. ($b = 0.42$ mV/day; $0.01 > P > 0.001$) from -32 to -6 mV.

As would be expected (see Fig. 2), 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 conceptus; -110 mV app. = -(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 141-day conceptus, -95 mV app. = -(63 + 30)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

Table VII. Transplacental, amniotic fluid, allantoic fluid, foetal e.c.f. to amniotic fluid, and foetal e.c.f. to allantoic fluid potential differences 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)
81a	-60	—	—	—	—
b	-80	-110*	-115 to -120	-32	-34
94	-64	- 89 to -94*	- 88 to - 99	-23 to -27	-30 to -34
110	-80	- 90*	-130 to -136	-11 to -13	-50
124	-74	- 80*	-103 to -110	—	—
141a	-60 to -65	- 70 to -73*	- 95	- 7 to - 9	-30
b	-77	- 82	- 96	—	—

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.

p.d. (e.g. in a 124-day conceptus, -25 mV app. = -(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 (abomasum) potential differences of -13 mV at 81 days, and -23 mV at 124 days were observed.

The results of transplacental p.d., amniotic fluid p.d., allantoic fluid p.d., foetal e.c.f. to amniotic fluid p.d., and foetal e.c.f. to allantoic fluid p.d. measurements made on 7 conceptuses from 5 animals ranging from 81 to 141 days gestational age (term at about 150 days) are outlined in Table VII.

<u>In vitro</u> p.d. measurements were made on membranes from conceptuses at each of 81, 94, 110, 124, and 141 days gestational age. No measurable p.d. was detected across the amniochorion or the amnioallantois at any of these ages. The chorioallantois was electrically neutral at 81 and 141 days, and the potential differences of 5 to 10 mV (allantoic fluid negative) across it at 94, 110, and 124 days, became zero mV when Krebs bicarbonate Ringer solution replaced the fluids on both sides of the membrane.

<u>Sheep</u>. All potential differences were negative whether relative to the maternal or the foetal e.c.f. Transplacental and amniotic fluid potential differences 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., and their mean values were

Table VIII. Transplacental, amniotic fluid, allantoic fluid, foetal e.c.f. to amniotic fluid, and foetal e.c.f. to allantoic fluid potential differences 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)
67	-45	-65 to -70	—	-25 to -30	—
76	-43 to -47	-62 to -72	- 75 to - 82	-25 to -28	-30 to -36
80	-40	-90*	- 70	-51	-32
93a	-59	-76*	- 98 to -104	-12 to -17	-40 to -45
b	-69 to -72	-85*	-100 to -110	-13 to -17	-37 to -39
95	-60	-104*	-104 to -114	-40 to -42	-44 to -50
103	-53	-70 to -76	- 84 to - 94	—	-35 to -42
109	-46 to -48	-60 to -62	- 91 to - 94	-12 to -13	-38 to -43
113	-50	-64 to -66*	-120 to -140	-17 to -20	-83
121	-52	-69*	-123 to -131	—	-70 to -81
124a	-49	-51	- 93 to - 97	- 4	-47 to -50
b	-55 to -59	-60 to -63	-112 to -120	—	—
130	-55	-61 to -63	- 75 to - 90	- 8 to -10	-25 to -35
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
140	-47	-50	- 77 to - 80	0 to - 2	-32

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.

-51 ± 7 mV(16), -96 ± 18 mV(15), and -44 ± 16 mV(15), respectively. As gestation advanced there was a significant increase in the amniotic fluid p.d. ($b = 0.38$ mV/day; $0.05 > P > 0.01$) from -90 to -50 mV, and in the foetal e.c.f. to amniotic fluid p.d. ($b = 0.53$ mV/day; $P < 0.001$) from -51 to zero mV.

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 a 93-day conceptus, -76 mV app. = -(59 + 15) 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 conceptus, -85 mV app. = -(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 93-day conceptus, -28 mV app. = -(101 - 76) 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. A foetal e.c.f. to foetal stomach (abomasum) p.d. of -19 mV was observed in a 103-day foetus.

The results of transplacental p.d., amniotic fluid p.d., allantoic fluid p.d., foetal e.c.f. to amniotic fluid p.d., and foetal e.c.f. to allantoic fluid p.d. measurements made on 16 conceptuses from 14 animals ranging from 67 to 140 days gestational age (term at about 145 days) are given in Table VIII.

Table IX. The $[Na^+]$, $[K^+]$, and $[Cl^-]$, of maternal and foetal plasma, and amniotic and allantoic fluid, of the goat.

ION CONCENTRATIONS

Gestational age (days)	Maternal plasma			Foetal plasma			Amniotic fluid			Allantoic fluid		
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
81a	174	5.2	109	171	3.4	107	116	4.8	—	—	—	—
b				176	4.5	103	155	5.4	123	—	—	—
94	171	5.0	106	178	6.4	101	77	5.2	110	81	32	11
110	163	5.2	108	163	5.2	103	136	7.9	123	61	57	14
124	159	5.0	105	147	6.1	101	108	7.3	102	84	26	8
141a	171	5.7	106	163	5.4	104	136	6.8	109	108	31	16
b				151	5.4	104	147	5.4	110	108	27	11
Mean	168	5.2	107	164	5.2	103	125	6.1	113	88	35	12
S.D.	± 6	± 0.3	± 2	± 12	± 1.0	± 2	± 26	± 1.2	± 8	± 20	± 13	± 3
n	5	5	5	7	7	7	7	7	6	5	5	5

a and b are twins

All concentrations are given in m-equiv/l.

In vitro p.d. measurements were made on membranes from conceptuses at each of 80, 95, 110, 120, 131, and 141 days gestational age. No measurable p.d. was detected across the amniochorion, the amnioallantois, or the foetal skin at any of these ages. The chorioallantois was electrically neutral at 95 and 131 days, and the potential differences of 5 to 12 mV (allantoic fluid negative) across it at 80, 110, 120, and 141 days gestational age, became zero mV when Krebs bicarbonate Ringer solution replaced the fluids on both sides of the membrane.

Sodium Potassium and Chloride Determinations

Goats

There was no correlation between gestational age and the $[\mathrm{Na}^+]$, $[\mathrm{K}^+]$, or $[\mathrm{Cl}^-]$, in maternal and foetal plasma, or in amniotic and allantoic fluid. There was no significant difference between the maternal and foetal plasma $[\mathrm{Na}^+]$ and $[\mathrm{K}^+]$, but the foetal plasma $[\mathrm{Cl}^-]$ was significantly lower ($0.05 > P > 0.01$; by about 4 m-equiv/l) than that of maternal plasma. Concentrations that were lower than those of foetal plasma were the amniotic fluid $[\mathrm{Na}^+]$ ($0.01 > P > 0.001$; about 40 m-equiv/l lower), the allantoic fluid $[\mathrm{Na}^+]$ ($P < 0.001$; about 75 m-equiv/l lower), and the allantoic fluid $[\mathrm{Cl}^-]$ ($P < 0.001$; about 90 m-equiv/l lower). Concentrations higher than those of foetal plasma were the amniotic fluid $[\mathrm{K}^+]$ ($0.01 > P > 0.001$; about 1 m-equiv/l higher), the allantoic fluid $[\mathrm{K}^+]$ ($P < 0.001$; about 30 m-equiv/l higher), and the amniotic fluid

Table X. The observed amniotic fluid $[\mathrm{Na}^+]$, $[\mathrm{K}^+]$, and $[\mathrm{Cl}^-]$, and those calculated assuming distribution of these ions according to electrochemical equilibrium between foetal plasma and amniotic fluid, in the goat.

Gestational age (days)	Amniotic fluid [ion] (m-equiv/l) $[\mathrm{Na}^+]$		$[\mathrm{K}^+]$		$[\mathrm{Cl}^-]$	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
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
141a	136	218	6.8	7.0	109	78
b	147	180	5.4	6.5	108	85

a and b are twins

The calculated concentrations were obtained using the foetal e.c.f. to amniotic fluid potential differences given in Table VII.

Table XI. 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.

Gestational age (days)	$[K^+]$ (m-equiv/l) Calculated			Observed
	(a)	(b)	(c)	
94	151	86	21	32
110	682	357	33	57
124	253	137	20	26
141a	186	101	16	31
b	193	103	12	27

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. (Table VII).

$[Cl^-]$ ($0.05 > P > 0.01$; about 10 m-equiv/l higher).

The $[Na^+]$, $[K^+]$, and $[Cl^-]$, of maternal and foetal plasma, and amniotic and allantoic fluid measured at different gestational ages are given in Table IX.

<u>Amniotic fluid</u>. 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 X.

At all gestational ages the amniotic fluid $[Na^+]$ was less than the expected equilibrium value, but the difference decreased from 400 m-equiv/l at 81 days to about 60 m-equiv/l at 141 days. Initially the observed $[K^+]$ was about one third of the calculated value, but the difference decreased to the extent that electrochemical equilibrium appeared to have been reached by 124 days. At all ages the amniotic fluid $[Cl^-]$ was greater than that expected at equilibrium, but the difference decreased from 90 m-equiv/l at 81 days to about 30 m-equiv/l at 141 days.

<u>Allantoic fluid</u>. The $[Na^+]$ of allantoic fluid was about 75 m-equiv/l lower than the maternal and foetal plasma values, and the allantoic fluid was also negative relative to the maternal and foetal plasma. It appears, therefore, that both concentration and electrochemical gradients tended to favour diffusion of Na^+ into the allantoic fluid. This suggests the presence of a pump maintaining the $[Na^+]$ below the electrochemical equilibrium value.

Table XII. The observed allantoic fluid $[Cl^-]$ compared with that calculated assuming distribution according to electrochemical equilibrium between maternal and foetal plasma and allantoic fluid, in the goat. The calculation was made using the mean of the allantoic fluid p.d. and the foetal e.c.f. to allantoic fluid p.d.

Gestational age (days)	Mean p.d. (mV)	$[Cl^-]$ (m-equiv/l)	
		Calculated	Observed
94	-63	10	11
110	-91	4	14
124	-69	8	8
141a	-62	11	16
b	-58	12	11

a and b are twins.

Table XIII. The amniotic fluid to allantoic fluid p.d. at different gestational ages, and the difference between the observed allantoic fluid $[\mathrm{Na}^+]$, $[\mathrm{K}^+]$, and $[\mathrm{Cl}^-]$, and those calculated assuming these ions were in electrochemical equilibrium across the amnioallantois, of the goat.

Gestational age (days)	Amniotic fluid to allantoic fluid p.d. (mV)*	Concentration differences (m-equiv/l)		
		$[\mathrm{Na}^+]$	$[\mathrm{K}^+]$	$[\mathrm{Cl}^-]$
94	- 7	- 19	25	-96
110	-35	-429	29	-73
124	-25	-186	8	-44
141a	-24	-219	15	-70
b	-14^x	-138	18	-92

a and b are twins

* The polarity of the p.d. is relative to the amniotic fluid.

^{x} Values obtained by subtraction.

Negative signs indicate observed values are less than calculated values, and vice versa.

The observed allantoic fluid $[K^+]$ was greater ($0.01 > P > 0.001$; about 14 m-equiv/l higher) than that expected at electrochemical equilibrium with foetal plasma only (Table XI). 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 2 to 6 times greater than the observed concentrations (Table XI). It seems, therefore, that 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 $[K^+]$.

There was no significant difference ($P > 0.1$) between the observed allantoic fluid $[Cl^-]$, and that calculated using the mean of the allantoic fluid p.d. and the foetal e.c.f. to allantoic fluid p.d. (Table XII). This suggests that a joint action of these two electrochemical gradients determined the $[Cl^-]$ of allantoic fluid.

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 (Table XIII). Large discrepancies in concentration strongly suggest that none of these ions was in electrochemical equilibrium across the amnioallantois.

Table XIV. The $[\mathrm{Na}^+]$, $[\mathrm{K}^+]$, and $[\mathrm{Cl}^-]$, in maternal and foetal plasma, and amniotic and allantoic fluid of the sheep.

ION CONCENTRATIONS

Gestational age (days)	Maternal plasma			Foetal plasma			Amniotic fluid			Allantoic fluid		
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
76	150	5.9	103	170	7.1	—	—	13.3	119	133	25	24
80	163	5.0	101	155	3.9	104	147	10.6	115	132	19	30
93a	155	5.2	108	159	5.0	109	132	7.7	118	124	23	6
b				151	4.3	110	139	6.8	132	128	24	13
95	171	5.7	108	155	4.5	106	136	8.6	117	93	36	16
103	163	6.0	102	152	5.0	99	136	7.7	99	78	28	11
109	149	6.3	103	155	9.6	—	138	7.3	118	—	30	5
113	151	4.0	107	158	3.7	104	107	6.5	98	76	50	3
121	156	6.5	—	148	4.3	105	93	15.9	98	45	99	3
124a	148	5.5	114	141	5.5	108	121	10.1	119	48	70	15
b				—	5.5	108	—	8.6	108	59	103	13
130	—	—	—	147	7.4	95	110	13.4	92	11	119	14
130	—	5.2	104	148	4.6	108	93	16.5	90	7	112	33
136	157	5.7	102	148	7.2	100	71	31	76	21	118	30
140	151	4.9	112	148	6.1	105	103	11.6	90	7	121	24
Mean	154	5.5	106	152	5.7	105	GV	11.9	GV	GV	GV	16
S.D.	± 5	± 0.7	± 4	± 7	± 1.6	± 5	—	± 6.4	—	—	—	± 10
n	11	12	11	14	15	13	—	15	—	—	—	15

a and b are twins
GV denotes gestational age variation
All concentrations are given in m-equiv/l.

Sheep

There was no correlation between gestational age and the $[\mathrm{Na}^+]$, $[\mathrm{K}^+]$, or $[\mathrm{Cl}^-]$, of maternal and foetal plasma, and there was no significant difference between the individual ion concentrations of these plasmas. The amniotic fluid $[\mathrm{K}^+]$, and the allantoic fluid $[\mathrm{Cl}^-]$, did not vary over the period studied, but the amniotic fluid $[\mathrm{Na}^+]$ and $[\mathrm{Cl}^-]$, and the allantoic fluid $[\mathrm{Na}^+]$ and $[\mathrm{K}^+]$ showed significant gestational age variations (Table XIV). Concentrations that were lower than those of foetal plasma were the amniotic fluid $[\mathrm{Na}^+]$ ($P < 0.001$; between 8 and 76 m-equiv/l lower), the allantoic fluid $[\mathrm{Na}^+]$ ($P < 0.001$; between 37 and 141 m-equiv/l lower), and the allantoic fluid $[\mathrm{Cl}^-]$ ($P < 0.001$; about 90 m-equiv/l lower). Concentrations higher than those of foetal plasma were the amniotic fluid $[\mathrm{K}^+]$ ($0.01 > P > 0.001$; about 6 m-equiv/l higher), the allantoic fluid $[\mathrm{K}^+]$ ($P < 0.001$; between 18 and 115 m-equiv/l higher), and the amniotic fluid $[\mathrm{Cl}^-]$ ($0.005 > P > 0.001$; between 6 and 13 m-equiv/l higher) before 109 days gestational age. After 109 days there was no significant difference between the amniotic fluid and foetal plasma $[\mathrm{Cl}^-]$.

The $[\mathrm{Na}^+]$ of allantoic fluid decreased from 133 m-equiv/l at 76 days to 7 m-equiv/l at 140 days ($b = -2.18$ m-equiv/l/day; $P < 0.001$), and the $[\mathrm{K}^+]$ increased from 25 m-equiv/l at 76 days to 121 m-equiv/l at 140 days ($b = 1.88$ m-equiv/l/day; $P < 0.001$), and there was a significant negative correlation ($r = 0.931$; $P < 0.001$) between the 2 ion concentrations (Fig. 3).

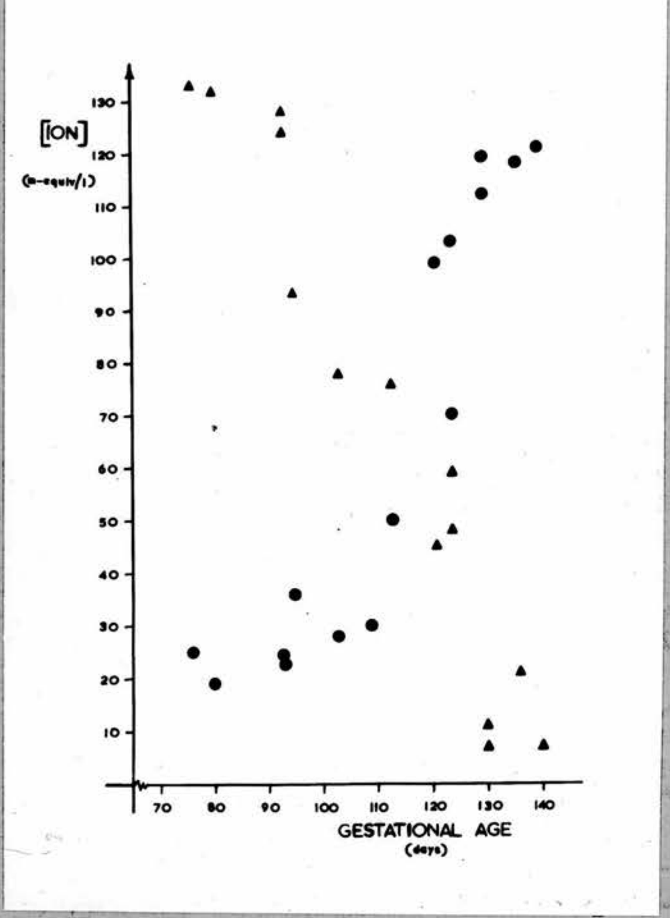

Fig. 3. The allantoic fluid $[Na^+]$ (▲) and $[K^+]$ (●) at different gestational ages in the sheep

Table XV. The observed amniotic fluid $[\mathrm{Na}^+]$, $[\mathrm{K}^+]$, and $[\mathrm{Cl}^-]$, and those calculated on the assumption that these ions are distributed according to electrochemical equilibrium between foetal plasma and amniotic fluid, in the sheep.

Gestational age (days)	Amniotic fluid [ion] (m-equiv/l) $[\mathrm{Na}^+]$ Observed	 $[\mathrm{Na}^+]$ Calculated	 $[\mathrm{K}^+]$ Observed	 $[\mathrm{K}^+]$ Calculated	 $[\mathrm{Cl}^-]$ Observed	 $[\mathrm{Cl}^-]$ Calculated
76	---	---	13.3	19.5	119	38
80	147	1005	10.6	25.0	115	16
93a	132	276	7.7	8.5	118	63
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
124a	121	163	10.1	6.6	119	94
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	31.0	9.4	76	94
140	103	154	11.6	6.1	90	102

a and b are twins.

The calculated concentrations were obtained using the foetal e.c.f. to amniotic fluid potential differences given in Table VIII.

Table XVI. 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 sheep.

Gestational age (days)	$[K^+]$ (m-equiv/l) Calculated (a)	(b)	(c)	Observed
76	103	63	24	25
80	65	39	13	19
93a	211	118	25	23
b	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
124a	179	105	32	70
b	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. (Table VIII).

The $[\mathrm{Na}^+]$, $[\mathrm{K}^+]$, and $[\mathrm{Cl}^-]$, of maternal and foetal plasma, and amniotic and allantoic fluid, at different gestational ages are given in Table XIV.

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

At all gestational ages the amniotic fluid $[\mathrm{Na}^+]$ was less than the expected equilibrium value. From 76 to 113 days the observed $[\mathrm{K}^+]$ was less than the calculated value, and after 113 days the observed $[\mathrm{K}^+]$ was greater than the calculated value. Before 124 days the observed amniotic fluid $[\mathrm{Cl}^-]$ was greater than that expected at equilibrium, but thereafter it appeared that amniotic fluid and foetal plasma Cl^- was in electrochemical equilibrium. This is clearly demonstrated by a decrease ($b = -0.005$/day; $P < 0.001$) in the observed amniotic fluid/foetal plasma $[\mathrm{Cl}^-]$ ratio occurring concomitantly with an increase in the calculated ratio, to the extent that there was no significant difference between them after 130 days (Fig. 4).

Allantoic fluid. The $[\mathrm{Na}^+]$ of allantoic fluid was between 20 and 140 m-equiv/l lower than the maternal and foetal plasma values, and the allantoic fluid was negative relative to the maternal and foetal plasma. It appears, therefore, that both concentration and electrochemical gradients tended to favour Na^+ diffusion into the allantoic fluid. This suggests the presence

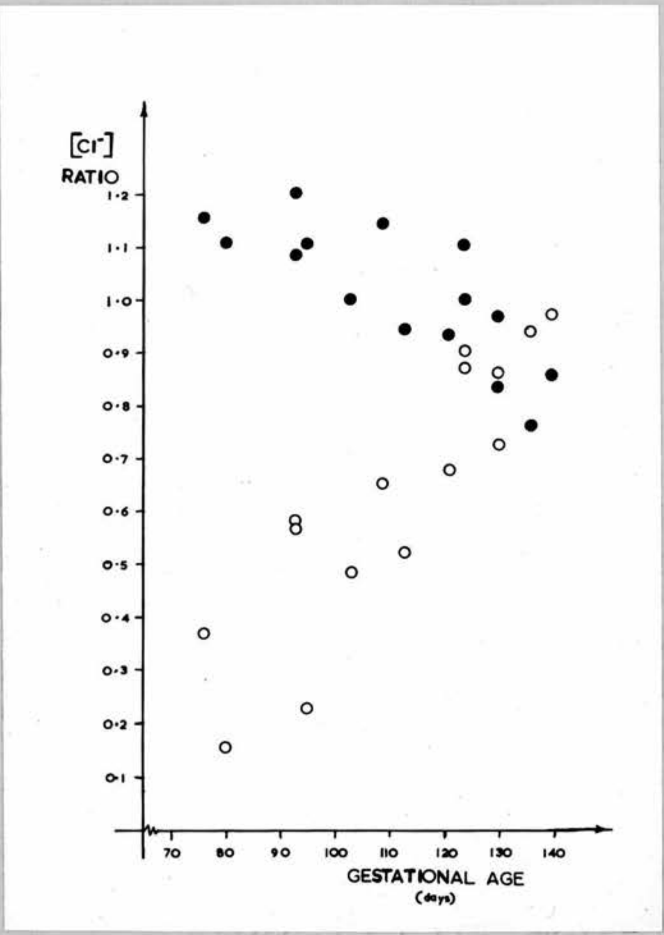

Fig. 4. The Amniotic fluid/Foetal plasma $[Cl^-]$ ratio at different gestational ages in the sheep. The observed ratio (●) is compared with that calculated assuming distribution of Cl^- according to electrochemical equilibrium (o).

Table XVII. The observed allantoic fluid $[Cl^-]$ compared with that calculated assuming distribution according to electrochemical equilibrium between maternal and foetal plasma and allantoic fluid in the sheep. The calculation was made using the mean of the allantoic fluid p.d. and the foetal e.c.f. to allantoic fluid p.d.

Gestational age (days)	Mean p.d. (mV)	$[Cl^-]$ (m-equiv/l) Calculated	Observed
76	-56	13	24
80	-51	16	30
93a	-72	8	6
b	-71	8	13
95	-78	6	16
103	-64	9	11
109	-67	9	5
113	-107	2	3
121	-101	2	3
124a	-71	8	15
b	-87	4	13
130	-57	11	14
130	-60	12	33
136	-51	15	30
140	-55	14	24

a and b are twins.

Table XVIII. The amniotic fluid to allantoic fluid p.d. at different gestational ages, and the difference between the observed allantoic fluid $[\mathrm{Na}^+]$, $[\mathrm{K}^+]$, and $[\mathrm{Cl}^-]$, and those calculated assuming these ions were in electrochemical equilibrium across the amnioallantois, of the sheep.

Gestational age (days)	Amniotic fluid to allantoic fluid p.d. (mV)*	Concentration differences (m-equiv/l)		
		$[\mathrm{Na}^+]$	$[\mathrm{K}^+]$	$[\mathrm{Cl}^-]$
76	- 6	—	8	- 89
80	19	59	14	-100
93a	-28[x]	-242	2	-101
b	-23	-195	8	-103
95	- 6[x]	- 76	25	- 97
103	-18	-185	13	- 78
109	-28	—	10	-104
113	-64[x]	-1042	18	- 67
121	-58	-735	34	- 73
124a	-44	-559	19	- 44
b	-55	—	38	- 11
130	-21	-227	90	- 62
130	-17[x]	-166	81	- 28
136	-19	-122	56	- 16
140	-28	-280	89	- 23

a and b are twins.

* The polarity of the p.d. is relative to the amniotic fluid.

[x] Values obtained by subtraction.

Negative signs indicate observed values are less than calculated values, and vice versa.

of a pump maintaining the $[\mathrm{Na}^+]$ below the electrochemical equilibrium value.

From 76 to 113 days the observed allantoic fluid $[\mathrm{K}^+]$ approximated to the value expected at electrochemical equilibrium with foetal plasma only (Table XVI). Between 113 and 124 days the $[\mathrm{K}^+]$ doubled. If the $[\mathrm{K}^+]$ 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 more than twice the magnitude of the $[\mathrm{K}^+]$ observed between 76 and 124 days inclusive (Table XVI). It appears, therefore, that 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. was sufficient to account for this rise, but such a joint action does not seem to have been adequate enough to account for the allantoic fluid $[\mathrm{K}^+]$ observed from 130 days until term (Table XVI).

Although there was a significant difference ($P < 0.001$) between the observed allantoic fluid $[\mathrm{Cl}^-]$ and that calculated using the mean of the allantoic fluid p.d. and the foetal e.c.f. to allantoic fluid p.d. (the calculated values were less than the observed values; Table XVII), 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 allantoic fluid $[\mathrm{Cl}^-]$ and the mean of these 2 potential differences. This suggests that a joint action of these 2 electrochemical gradients determined the allantoic fluid $[\mathrm{Cl}^-]$.

Table XIX. The amniotic fluid $[K^+]$ compared with that of the foetal urine at different gestational ages.

	$[K^+]$ (m-equiv/l)	
Gestational age (days)	Amniotic fluid	Foetal urine
	GOATS	
81a	4.8	14.3
b	5.4	10.4
94	5.2	8.8
110	7.9	15.4
124	7.3	9.1
141a	6.8	11.3
b	5.4	9.3
	SHEEP	
80	10.6	1.0
93a	7.7	7.7
b	6.8	3.6
95	8.6	10.0
113	6.5	8.6
121	4.3	11.9
124a	10.1	19.2
b	8.6	21.6
130	16.5	44.3
140	11.6	24.4

a and b are twins

By comparing the observed allantoic fluid $[Na^+]$, $[K^+]$, and $[Cl^-]$, with those calculated using the amniotic fluid to allantoic p.d., it has been possible to determine whether these ions were in electrochemical equilibrium across the amnioallantois (Table XVIII). Large differences in the ion concentrations strongly suggest that none of these ions was in electrochemical equilibrium across the amnioallantois.

Goat and Sheep Foetal Urine

The $[K^+]$ of foetal urine was determined and the values were compared with the amniotic fluid $[K^+]$ (Table XIX). In the goat at all ages the foetal urine $[K^+]$ was between 1.5 and 2.5 times that of the amniotic fluid. In the sheep, the foetal urine $[K^+]$ was less than or approximately equal to that of the amniotic fluid before 113 days, and thereafter foetal urine values were between 2 and 3 times greater than those of amniotic fluid.

Osmolarity Determinations

The osmolarities of maternal and foetal plasma, and amniotic and allantoic fluid, are given in Table XX.

There was no significant difference between the maternal and foetal plasma osmolarities of the goat, but sheep foetal plasma was significantly hypotonic ($0.01 > P > 0.001$; about 11 m-osmole/kg water lower) relative to the maternal plasma. In the goat, the osmolarities of amniotic fluid

Table XX. The osmolarity of maternal and foetal plasma, and amniotic and allantoic fluid, at different gestational ages.

Gestational age (days)	Maternal plasma	Foetal plasma	Amniotic fluid	Allantoic fluid
		GOATS		
81a	318	314	300	—
b		244	226	—
94	315	315	295	283
110	321	317	287	293
124	307	312	260	298
141a	330	319	288	300
b		329	289	296
mean ± S.D.	318 ± 8	307 ± 28	278 ± 26	294 ± 7
		SHEEP		
80	337	322	317	297
93a	319	317	244	293
b		317	232	294
95	324	325	295	282
113	318	313	269	283
121	—	305	262	292
124a	332	315	293	293
b		315	293	298
130	333	315	295	308
140	328	320	246	300
mean ± S.D.	327 ± 7	316 ± 5	271 ± 27	294 ± 8

a and b are twins

and allantoic fluid were less ($0.01 > P > 0.001$ in both cases) than the maternal and foetal plasma values by 30 to 40 m-osmole/kg water, and 14 to 24 m-osmole/kg water, respectively. In the sheep, the osmolarities of amniotic fluid and allantoic fluid were lower ($P < 0.001$ in both cases) than the maternal and foetal plasma values by 45 to 55 m-osmole/kg water, and 22 to 32 m-osmole/kg water, respectively. Therefore, the amniotic and allantoic fluids of both goats and sheep were hypotonic relative to the maternal and foetal plasmas.

DISCUSSION

PLACENTAE HAEMOCHORIALIS

Sites of Origin of Potential Differences

Rats

In the rat the magnitude and sign of the transplacental p.d. were the same both before and after uterotomy. Since foetal trophoblasts of haemochorial placentae are bathed directly in maternal blood, the site of origin of the p.d. appears to be the foetal placenta. However, it is not possible to establish the location and nature of the (active) mechanisms producing the transplacental p.d. with any greater precision.

The absence of any effect of uterotomy on the transplacental p.d. and the fact that the transplacental p.d. and amniotic fluid p.d. are the same, together show that no specific (active) mechanism is required for the production of the amniotic fluid p.d. This p.d. may arise indirectly from the transplacental p.d. because of a relatively free exchange of ions between the amniotic fluid and the foetal blood at the surface of the yolk sac splanchnopleur, or in the foetal stomach, or both.

Rabbits

In the rabbit the site of origin of the amniotic fluid p.d. does not seem to be the uterine wall, the inverted yolk sac splanchnopleur, or the amnion, since they display no spontaneous electrical activity *in vitro*. This confirms previous *in vitro* observations on rabbit amnion (Wright, 1963).

The foetal gastric mucosa, however, appears to be a source of electromotive force *in vitro*, the mucosal side being negative (Wright, 1962), and it has been suggested that this is the site of origin of the amniotic fluid p.d. (Wright, 1963). It has also been suggested that the fluctuations in the amniotic fluid p.d. are due to sphincter activity associated with swallowing, since such activity would cause changes in the potential drop occurring between the foetal stomach and the amniotic fluid (Wright, 1963). The *in vivo* and *in vitro* results presented here are in complete agreement with these hypotheses.

In order to account for the difference between the steady foetal stomach p.d. and the maximum amniotic fluid p.d. Wright (1963) postulated a positive transplacental p.d. He was, however, unable to detect such a p.d., and this was also the case in the more extensive study presented here. A transplacental p.d. would necessarily have the same magnitude as the difference between the foetal stomach p.d. and the foetal e.c.f. to foetal stomach p.d. Since no difference between these potential differences was found it can be concluded that there is no transplacental p.d. in the rabbit.

Humans

Similarly, the transplacental p.d. of the human is approximately zero mV. However, the situation is not the same as in the rabbit. The negative foetal stomach p.d. does not seem to greatly influence the amniotic fluid, since the amniotic fluid p.d. is also close to zero mV. A probable explanation is that the cardiac sphincter of the foetal stomach of the human is tightly closed. The lack of spontaneous electrical activity across the human amnion *in vitro* (Garby, 1957; Wright, 1964) agrees with this. Further experiments are required to determine whether the pattern of these potential differences before the 36th week of gestation is the same as that present at term.

Guinea-pigs

In the guinea-pig, as in the rat, the site of origin of the transplacental p.d. appears to be the foetal placenta, although the precise location and nature of the (active) mechanisms remains unestablished. However, the negative polarity in this case indicates that the mechanism is different from that of the rat.

The amniotic fluid p.d. of the guinea-pig does not appear to originate from the uterine wall, the yolk sac splanchnopleur, or the amnion, since no electrical activity was associated with these tissues *in vitro*. The presence of the foetal stomach p.d. suggests, therefore, that the foetal gastric mucosa is a source of electromotive force contributing to the amniotic fluid p.d., in addition to the contribution from the transplacental p.d.

Placental anatomy

Enders (1967) demonstrated that haemochorial placentae could be subdivided into several groups on the basis of trophoblastic layering. It appears that the rat has a 3-layered trophoblast (haemotrichorial), the rabbit 2 layers (haemodichorial), the human 1 layer (villous haemomonochorial), and the guinea-pig 1 layer (labyrinthine haemomonochorial). The transplacental p.d. of the rat is positive, there is no such p.d. in the rabbit and human, and this p.d. in the guinea-pig is negative. It is not known whether the species difference in these transplacental potential differences are associated with the fine structure of the individual trophoblasts, but the zero mV potential differences in the rabbit and human tend to suggest that this is not the case. Observation of transplacental potential differences in other representatives of the 4 placental types may clarify this point.

Sodium Passage between Mother and Foetus

It would clearly be of interest to relate the potential differences observed in this study to movement of ions between mother and foetus. Unfortunately available published information is largely restricted to transfer of Na^+, so that the subsequent discussion is confined to this ion species.

Rats

Net flux of Na^+ towards the foetus, which of course equals the rate of foetal Na^+ retention, is far exceeded by the total flux of Na^+ towards the foetus (Flexner & Pohl, 1941a; 1941b; 1941c). It is known that the foetal and maternal plasma $[\mathrm{Na}^+]$ are not significantly different (Thalme, 1967). This fact and the positivity of the foetal blood together indicate that the foetal plasma $[\mathrm{Na}^+]$ (about 150 m-equiv/l) is greater than the concentration expected at electrochemical equilibrium (about 85 m-equiv/l). The maintenance of high $[\mathrm{Na}^+]$ might be due to active Na^+ transport across the placenta. If this were the case, and if the transport were electrogenic, it would account for the positive transplacental p.d. This idea is supported by the close correlation between the decline towards zero mV of the p.d. on the 22nd day, and an abrupt decrease on the 22nd day in the total flux of Na^+ towards the foetus (Flexner & Pohl, 1941a). Following this decline, Na^+ enters the foetus at about 45% of its entry rate on the 21st day. The fall in the p.d. could be due to either a failure of the cation pump mechanism or to a large increase in ionic permeabilities which result in the electrogenic ion pump being short-circuited. The above observations would favour the former explanation. It will be appreciated, however, that this discussion is based on the assumption that the placenta is the main site of active Na^+ transfer to the foetus.

Rabbits

The absence of a transplacental p.d. in the rabbit is consistent with the suggestion of Faber & Hart (1967) and Faber, Hart & Pontala (1968) that Na^+ and Cl^- cross the placenta by passive diffusion. However, on about the 28th day of gestation, passive Na^+ exchange between the maternal and foetal blood streams in the placenta, which occurs at a rate of approximately 0.014 m-equiv/min (Faber & Hart, 1967), only accounts for 55 to 65% of the total flux of Na^+ towards the foetus in the intact animal (Flexner & Pohl, 1941b). It may be, therefore, that the placenta is not the only path by which Na^+ may reach the foetus. Na^+ is actively transported from the mucosal to the serosal surface of the foetal stomach (Wright, 1962). The negative foetal stomach p.d. may arise from this active Na^+ absorption and, after the 23rd day of gestation, from active Cl^- secretion (accompanied by H^+) into the gastric lumen (Wright, 1962). It seems reasonable to suggest that this activity of the foetal gastric mucosa is responsible for the observed amniotic fluid $[Na^+]$ being lower (138 m-equiv/l observed; 218 m-equiv/l calculated), and the $[Cl^-]$ being higher (107 m-equiv/l observed; 73 m-equiv/l calculated), than the values expected under conditions of electrochemical equilibrium (Table VI). It appears, therefore, that if Na^+ can reach the amniotic fluid by diffusion down the concentration and the electrochemical gradient from the maternal circulation and, after being swallowed, is actively absorbed from the foetal gastric lumen, this would produce a net flux towards the foetus. If this were to exceed Na^+ requirements, net Na^+ flux along at least one other transfer path would be towards the mother.

Humans

The zero mV transplacental p.d. in the human after the 36th week of gestation is consistent with the suggestion of McGaughey, Jones, Talbert & Anslow (1958) that Na^+ crosses the placenta passively. Maternal plasma and amniotic fluid Na^+ is in electrochemical equilibrium (Table VI), and is exchangable (Vosburgh <u>et al</u>, 1948). Swallowing of amniotic fluid occurs from the 15th week of gestation (Smith, 1959), so that if the negative foetal stomach p.d. arises from active Na^+ absorption, as it appears to in the rabbit, net flux of Na^+ along this transfer path would be towards the foetus. The total flux of Na^+ towards the foetus as measured by Flexner, Cowie, Hellman, Wilde & Vosburgh (1948) may, therefore, have arisen from fluxes towards the foetus along several pathways.

<u>Guinea-pigs</u>

If the negative transplacental p.d. in the guinea-pig is due to electrogenic Na^+ transport, net flux of Na^+ across the placenta would need to be from foetal to maternal blood. Since foetal Na^+ requirements are met, such a loss would need to be made good or exceeded by net Na^+ flux towards the foetus along other transfer paths. One of these paths may involve transfer from maternal blood to the amniotic fluid, which has been demonstrated (Flexner & Gellhorn, 1942b), followed by active absorption of Na^+ from the foetal gastric lumen, as in the rabbit. The negative foetal stomach p.d., the lower amniotic fluid $[Na^+]$ (134 m-equiv/l observed; 906 m-equiv/l calculated), and the higher $[Cl^-]$

(140 m-equiv/l observed; 16 m-equiv/l calculated), than those expected at electrochemical equilibrium between maternal plasma and amniotic fluid (Table VI) suggest similar gastric mucosal activity in the guinea-pig foetus, and this appears to be worthy of further study. Whatever the nature of the mechanisms involved, the total flux of Na^+ towards the foetus, as measured by Flexner & Pohl (1941c), may not be due to passage across the placenta only.

In general, the differences in the potential differences shown in these 4 species call in question the assumption that transfer of Na^+ towards the foetus results almost entirely from transplacental passage from mother to foetus.

PLACENTAE SYNDESMOCHORIALIS

Sites of Origin of Potential Differences

In goats and sheep the overall pattern of the potential differences 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, and potential differences associated with single cotyledons increased towards zero mV when the foetal vessel between the salt bridge and the cotyledon was occluded. Therefore, the transplacental p.d. as measured 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). Although the stomach of the foetal sheep has been shown to generate a negative p.d. (Wright & Nixon, 1961), an observation confirmed in 2 goats and 1 sheep in the present study, it does not seem to be responsible for the amniotic fluid p.d. since this 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. 2). 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 zero 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 amniotic fluid and foetal blood in the amnion or foetal skin vessels, and not from activity across the full thickness of membranes. The <u>in vitro</u> 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 this membrane displays no spontaneous electrical activity <u>in vitro</u>. 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. 2), so that a large proportion of the allantoic fluid p.d. seems to arise 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 allantoic fluid and foetal blood in the chorioallantois, or perhaps the amnion. The in vitro results agree with this. However, results discussed later (page 66) suggest that the amnion severely hinders the passage of solute particles. 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. 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 zero mV as gestation advances, in the goat (Meschia, Wolkoff & Barron, 1958).

It appears, therefore, that the amniotic fluid p.d., and the allantoic fluid p.d., are each generated by activity at 2 different sites linked by foetal blood. Since no p.d. seems to be 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.

Regulation of Ion Concentrations

The negative polarity of the transplacental p.d. suggests the presence of a cation pump directed towards the maternal blood, or an anion pump towards the foetal blood, or both. The identification of the precise mechanism requires further work.

Amniotic fluid

The rise of the foetal e.c.f. to amniotic fluid p.d. towards zero mV (Tables VII and VIII), the concomitant decline of the amniotic fluid $[Cl^-]$ towards that expected at electrochemical equilibrium with foetal plasma (Table X; Fig. 4), and the negative polarity of the amniotic fluid, together suggest the presence of an electrogenic 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 Cl^- pump, or to a large increase in the ionic permeabilities which result in the electrogenic pump being short-circuited.

Before 113 days, the volume of amniotic fluid increases (Malan, Malan & Curson, 1937; Cloete, 1939). In the goat, the amniotic fluid $[K^+]$ was initially less than the value expected at electrochemical equilibrium with foetal plasma, but from 124 to 141 days it approximated to the electrochemical equilibrium concentration (Table X). In the sheep before 113 days the $[K^+]$ was lower, and thereafter it was higher than the value expected at electrochemical equilibrium with foetal plasma (Table XV). In both species, therefore, it seems that the entry of K^+ into the amniotic fluid is not sufficient to keep pace with the initial volume changes. After 113 days, in the goat equilibrium is reached, but in the sheep the entry of K^+ exceeds that required for the maintenance of electrochemical equilibrium values. This may be due to the secretion of foetal urine (Alexander, Nixon, Widdas & Wohlzogen, 1958b) which has a higher $[K^+]$ than amniotic fluid (Table XIX).

The tendency of the amniotic fluid K^+ and Cl^- to equilibrate with foetal plasma (Tables X and XV), and not with maternal plasma or allantoic fluid (Tables XIII and XVIII), suggests that the passage of solute particles across the amnion as a whole is severely hindered. In the sheep, the progressive rise in the urea concentration of the amniotic fluid relative to that of the maternal and foetal plasma and allantoic fluid (Alexander _et al_, 1958a; Hervey & Slater, 1968), and the similar rise observed in goat amniotic fluid (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 zero mV of the foetal e.c.f. to amniotic fluid p.d. (Tables VII and VIII) and the continued maintenance of a low amniotic fluid $[Na^+]$ (Tables IX and XIV), 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 XX).

Allantoic fluid

The negative polarity of the allantoic fluid, and its low $[Na^+]$

together suggest the presence of an electrogenic Na^+ pump directed towards the foetal blood. The high allantoic fluid $[K^+]$ cannot be completely accounted for by 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 (Table XVI). Thus, it appears that K^+ may be pumped into the allantoic fluid. If this is the case, the negative polarity of the allantoic fluid suggests that Na^+ is being pumped at a faster rate than K^+. Whether the Na^+ and K^+ pumps are independent or coupled remains to be determined. The highly significant correlation between the $[Na^+]$ and $[K^+]$ of allantoic fluid (in sheep) could result from coupling of the pumps, or from the necessity to balance positive and negative charges. A conclusive demonstration of the presence of these pumps requires further work, and appears to be worthy of attention.

The low $[Cl^-]$ of allantoic fluid seems to be maintained passively by a joint action of the 2 electrochemical gradients (Tables XII and XVII). This suggests that the chorioallantois 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.

There appears to be a large difference between the concentration of allantoic fluid cations and the concentration of anions known to be present. The contributions of protein (Hervey & Slater, 1968), ionized amino acids (Slater, unpublished data), sulphate ion (Alexander <u>et al</u>, 1958a), inorganic phosphorus (Mellor, Slater & Hervey, unpublished data), bicarbonate (Mellor, Percy-Robb & Cockburn, unpublished data), and chloride (Tables IX and XIV), are known to be small, so that the substance or substances that supply the remaining negative charge await identification.

Regulation of Foetal Fluid Osmolarity

Alexander et al (1958a), in a discussion of the origin of the foetal fluids, suggested that their hypotonicity results from a relative impermeability of the membranes to water, coupled with a more rapid entry of water than of solutes in foetal secretions; also, that the expanding volumes of the foetal fluids result from direct addition of foetal urine and secretions of the nasopharyngeal and buccal cavities. However, it has been shown that the placenta of the sheep is far more permeable to water than it is to urea, Na^+, or Cl^- (Meschia, Battaglia & Bruns, 1967), and a greater permeability of foetal membranes to water than to solutes has been demonstrated in the pig (Stanier, 1965), the guinea-pig (Flexner & Gellhorn, 1942b), and the human (Vosburgh et al, 1948; Garby, 1957). Meschia & Setnikar (1959) found that water would flow across a collodion membrane from a solution of higher osmolarity into a solution of lower osmolarity when the membrane was permeable to the solute in the hypertonic solution and impermeable to that in the hypotonic solution. It seems reasonable to suggest, therefore, that the tonicity of the foetal fluids may be determined by the relative distributions of the more non-diffusible solutes between maternal and foetal plasma and amniotic and allantoic fluid. In the presence of such a mechanism, expansion of the volume of the foetal fluids would result from a relative addition of solute to the fluids, so that the solute contributed in foetal urine and other foetal secretions, and from all other sources, would appear to be of greater importance than the actual volume additions from these sources.

Sodium Passage between Mother and Foetus

Although the placenta offers considerable hindrance to the passage of Na^+ (Meschia *et al*, 1967), the total flux towards the foetus (Pohl, Flexner & Gellhorn, 1941) far exceeds foetal Na^+ requirements (Field & Suttle, 1967). If the negative transplacental p.d. is due to active Na^+ transport, net Na^+ flux across the placenta would need to be from foetal to maternal blood. Since foetal Na^+ requirements are met, such a loss would need to be made good or exceeded by net Na^+ flux towards the foetus along other transfer paths. A path involving the amniotic fluid does not seem likely to make a major contribution, since the amnion as a whole appears to be relatively impermeable to solute particles. There seems to be a dynamic cycle involving Na^+ entry into the amniotic fluid in foetal urine (Alexander *et al*, 1958a; 1958b) and in secretions of the nasopharyngeal and buccal cavities (Reynolds, 1953), its exchange between amniotic fluid and foetal blood in the amnion, 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 Na^+. However, a net flux towards the foetus would seem to result from diffusion of Na^+ from maternal blood into the allantoic fluid down electrochemical and concentration gradients, followed by active uptake into the foetal blood in the chorioallantois.

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), which indicates that activity in the chorion of one foetus could influence the potential differences associated with the allantoic sac of its neighbour. Considered in relation to the apparent involvement of the chorioallantois in foetal Na^+ uptake, this possibly indicates intrauterine competition for Na^+ between litter mates, or a relative increase in the surface area available for Na^+ transfer to the individuals of multiple foetus pregnancies, or both.

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REFERENCES

Alexander, D.P., Nixon, D.A., Widdas, W.F. & Wohlzogen, F.X. (1958a). Gestational variations in the composition of the foetal fluids and foetal urine in sheep. J. Physiol., Lond., 140, 1.

Alexander, D.P., Nixon, D.A., Widdas, W.F. & Wohlzogen, F.X. (1958b). Renal function in the sheep foetus. J. Physiol., Lond., 140, 14.

Caldwell, P.C. (1968). Factors governing movement and distribution of inorganic ions in nerve and muscle. Physiol. Rev., 48, 1.

Cloete, J.H.L. (1939). Prenatal growth in merino sheep. Onderstepoort J. vet. Sci. Anim. Ind., 13, 417.

Crawford, J.D. & McCance, R.A. (1960). Sodium transport by the chorioallantoic membrane of the pig. J. Physiol., Lond., 151, 458.

Csaky, T.Z. (1965). Transport through biological membranes. A. Rev. Physiol., 27, 415.

Edelman, I.C. (1961). Transport through biological membranes. A. Rev. Physiol., 23, 37.

Enders, A.C. (1967). A comparative study of the fine structure of the trophoblast in several haemochorial placentas. Am. J. Anat., 116, 29.

Faber, J.J. & Hart, F.M. (1967). Transfer of charged and uncharged molecules in the placenta of the rabbit. Am. J. Physiol., 213, 890.

Faber, J.J., Hart, F.M. & Pontala, A.C. (1968). Diffusional resistance of the innermost layer of the placental barrier of the rabbit. J. Physiol., Lond., 197, 381.

Field, A.C. & Suttle, N.F. (1967). Retention of calcium, phosphorus, magnesium, sodium and potassium by the developing sheep foetus. J. agric. Sci., Camb., 69, 417.

Flexner, L.B., Cowie, D.B., Hellman, L.M., Wilde, W.S. & Vosburgh, G.J. (1948). The permeability of the human placenta to sodium in normal and abnormal pregnancies and the supply of sodium to the human foetus as determined with radioactive sodium. Am. J. Obstet. Gynec., 55, 469.

Flexner, L.B. & Gellhorn, A. (1942a). The comparative physiology of placental transfer. Am. J. Obstet. Gynec., 43, 965.

Flexner, L.B. & Gellhorn, A. (1942b). The transfer of water and sodium to the amniotic fluid of the guinea-pig. Am. J. Physiol., 136, 757.

Flexner, L.B. & Pohl, H.A. (1941a). The transfer of radioactive sodium across the placenta of the white rat. J. cell. comp. Physiol., 18, 49.

Flexner, L.B. & Pohl, H.A. (1941b). The transfer of radioactive sodium across the placenta of the rabbit. Am. J. Physiol., 134, 344.

Flexner, L.B. & Pohl, H.A. (1941c). Transfer of radioactive sodium across the placenta of the guinea-pig. Am. J. Physiol., 132, 594.

Fuhrman, F.A. (1959). Transport through biological membranes. A. Rev. Physiol., 21, 19.

Garby, L. (1957). Studies on transfer of matter across membranes with special reference to the isolated human amniotic membrane and the exchange of amniotic fluid. Acta physiol. scand., 40, Suppl. 137.

Ginzburg, B.Z. & Hogg, J. (1967). What does a short-circuit current measure in biological systems? J. theor. Biol., 14, 316.

Heinz, E. (1967). Transport through biological membranes. A. Rev. Physiol., 29, 21.

Hervey, E.J. & Slater, J.S. (1968). The sources of sheep foetal fluids in the later stages of gestation. J. Physiol., Lond., 194, 40P.

Huggett, A. St. G. & Widdas, W.F. (1951). The relationship between mammalian foetal weight and conception age. J. Physiol., Lond., 114, 306.

Johnsen, V.K., Levi, H. & Ussing, H.H. (1952). The mode of passage of chloride ions through the isolated frog skin. Acta physiol. scand., 25, 150.

Johnsen, V.K. & Ussing, H.H. (1958). The nature of the frog skin potential. Acta physiol. scand., 42, 298.

MacRobbie, E.A.C. & Ussing, H.H. (1961). Osmotic behaviour of the epithelial cells of frog skin. Acta physiol. scand., 53, 348.

Malan, A.I., Malan, A.P. & Curson, H.H. (1937). Studies in sex physiology No. 19 - The influence of age on amount and nature and composition of the allantoic and amniotic fluids of the merino ewe. Onderstepoort J. vet. Sci. Anim. Ind., 9, 205.

McGaughey, H.S., Jones, H.C., Talbert, L. & Anslow, W.P. (1958). Placental transfer in normal and toxic gestation. Am. J. Obstet. Gynec., 75, 482.

Mellor, D.J. (1969). Vescular anastomosis and fusion of foetal membranes in multiple pregnancy in the sheep. Res. vet. Sci., 10, .

Meschia, G., Battaglia, F.C. & Bruns, P.D. (1967). Theoretical and experimental study of transplacental diffusion. J. appl. Physiol., 22, 1171.

Meschia, G. & Setnikar, I. (1959). Experimental study of osmosis through a collodion membrane. J. gen. Physiol., 42, 429.

Meschia, G., Wolkoff, A.S. & Barron, D.H. (1958). Difference in electrical potential across the placenta of goats. Proc. natn. Acad. Sci., U.S.A., 44, 483.

Pohl, H.A., Flexner, L.B. & Gellhorn, N.A. (1941). Transfer of radioactive sodium across the placenta of the goat. Am. J. Physiol., 134, 338.

Quigley, H., Phillips, L.L. & McKay, D.G. (1965). Transport of radioactive sodium across the rat placenta in experimental toxemia of pregnancy. Am. J. Obstet. Gynec., 91, 377.

Reynolds, S.R.M. (1953). A source of amniotic fluid in the lamb: the naso-pharyngeal and buccal cavities. Nature, Lond., 172, 307.

Shanes, A.M. (1958). Electrochemical aspects of physiological and pharmacological action in excitable cells. Part 1. The resting cell and its alteration by extrinsic factors. Pharmac. Rev., 10, 59.

Smith, C.A. (1959). The physiology of the newborn infant. 3rd Ed., Ch. VIII, p. 228. Blackwell, Oxford.

Stanier, M.W. (1965). Transfer of radioactive water, urea and glycine between maternal and foetal body fluids in rabbits and pigs. J. Physiol., Lond., 178, 127.

Thalme, B. (1967). Electrolyte and acid-base balance in foetal and maternal blood. Acta Obstet. Gynec. Scand., XLV, Suppl. 8.

Ussing, H.H. (1965). Transport of electrolytes and water across epithelia. The Harvey Lectures, Series 59. Academic Press, New York.

Ussing, H.H. & Windhanger, E.E. (1964). Nature of shunt path and active sodium transport path through frog skin epithelium. Acta physiol. scand., 61, 484.

Ussing, H.H. & Zerahn, K. (1951). Active transport of sodium as a source of electric current in the short-circuited isolated frog skin. Acta physiol. scand., 23, 110.

Vosburgh, G.I., Flexner, L.B., Cowie, D.B., Hellman, L.M., Proctor, N.K. & Wilde, W.S. (1948). The rate of renewal in women of the water and sodium of the amniotic fluid as determined by tracer techniques. Am. J. Obstet. Gynec., 56, 1156.

Whittam, R. (1964). Transport and diffusion in red blood cells. Physiological Society Monograph. Arnold.

Widdas, W.F. (1961). Transport mechanisms in the foetus. Br. med. Bull., 17, 107.

Wright, G.H. (1962). Net transfer of water, sodium, chloride and hydrogen ions across the gastric mucosa of the rabbit foetus. J. Physiol., Lond., 163, 281.

Wright, G.H. (1963). Differences in electrical potential between maternal and foetal fluid compartments in the rabbit. J. Physiol., Lond., 166, 20P.

Wright, G.H. (1964). Absorption and secretion in the foetal stomach. Ph.D thesis. University of London.

Wright, G.H. & Nixon, D.A. (1961). Absorption of amniotic fluid in the gut of foetal sheep. Nature, Lond., 190, 816.

APPENDICES

APPENDIX I

VASCULAR ANASTOMOSIS AND FUSION OF FOETAL MEMBRANES IN MULTIPLE PREGNANCY IN THE SHEEP

D. J. Mellor

A paper accepted for publication in the May number of Research in Veterinary Science.

SUMMARY. Fusion of adjacent chorions in sheep multiple pregnancies appears to occur normally, and in cases where chorions fail to unite the lack of fusion may not be due to membrane necrosis.

In the majority of cases the chorion of one foetus overlies regions of one or both fluid sacs of its neighbouring litter-mate.

There appears to be a series of relationships with regard to apposition of adjacent fluid sacs, and this may possibly be dependent upon the initial positioning of the blastocysts in the uterus.

There seems to be a higher incidence of true anastomosis of minor vessels between adjacent chorionic vasculatures in British breeds of sheep than has previously been reported.

Intermixing of the bloods of neighbouring foetuses does not appear to occur in 'shared' cotyledons, nor does any detectable admixture seem to arise from the presence of minor trans-suture vascular anastomosis.

LITTLE IS KNOWN of the relationship between foetal membranes in naturally occurring multiple pregnancies in sheep, and Robinson's (1951) description of membrane associations in superfoetation is restricted to the period before the 40th day of gestation. The origin and development of sheep foetal membranes, and the formation and degree of association of the amniotic and allantoic sacs in single pregnancies have been described in detail (Assheton, 1906; Amoroso, 1952; Boyd & Hamilton, 1952). No accurate diagrams of the sheep conceptus during the last 100 days of gestation are available. Meschia <u>et al</u> (1965) depict the allantoic sac as surrounding the amniotic sac, which is not borne out by the present findings; Alexander and Nixon (1961) imply a greater area of contact between the 2 sacs than is normally found; and Keller (1928) does not clearly define the extent of their association. Therefore, the relationships of membranes in a single pregnancy require description before proceeding to membrane associations in multiple pregnancies.

MATERIALS AND METHODS

Anatomy

Pregnant sheep uteri from British breeds were obtained intact and at random from the local abattoir. In each case, the uterine wall was removed carefully leaving the enclosed conceptus intact. In all, 108 conceptuses were examined, and these were derived from 9 single, 45 twin, and 3 triplet conceptuses, ranging in age from about 45 to 145 days (full term). Gestational ages were estimated according to Huggett and Widdas (1951) modified for twin and triplet pregnancies (Huggett; unpublished data). All examinations were carried out 4 hours or more after the death of the mother. The following characteristics were noted particularly in each case: the relationships between allantoic and amniotic sacs of individual and adjacent conceptuses; associations between apposed sacs of neighbouring foetuses and the region of fusion of adjacent chorions; the number of major and minor vessel anastomoses between each chorionic vasculature; and the number of cotyledons with associated blood vessels arising from both foetuses ('shared' cotyledons).

Degree of Blood Admixture between Adjacent Foetuses

Two half-bred (Border Leicester X Cheviot) ewes carrying twin foetuses of 139 days gestational age were used. In both cases the operative procedure was as follows: anaesthesia was induced by an intravenous injection of about 45 mg./kg. bodyweight of pentobarbitone sodium (Nembutal*), and maintained subsequently with cyclopropane; the uterus was exposed by laparotomy; and, one placental artery from each foetus was catheterised by the method of Meschia et al (1965). Five ml. of a 1% (w/v) solution of Evan's Blue dye (T-1824) in isotonic saline were injected into one foetal circulation, and simultaneous 4 ml. blood samples were withdrawn from both

* Abbott Laboratories Ltd., Kent.

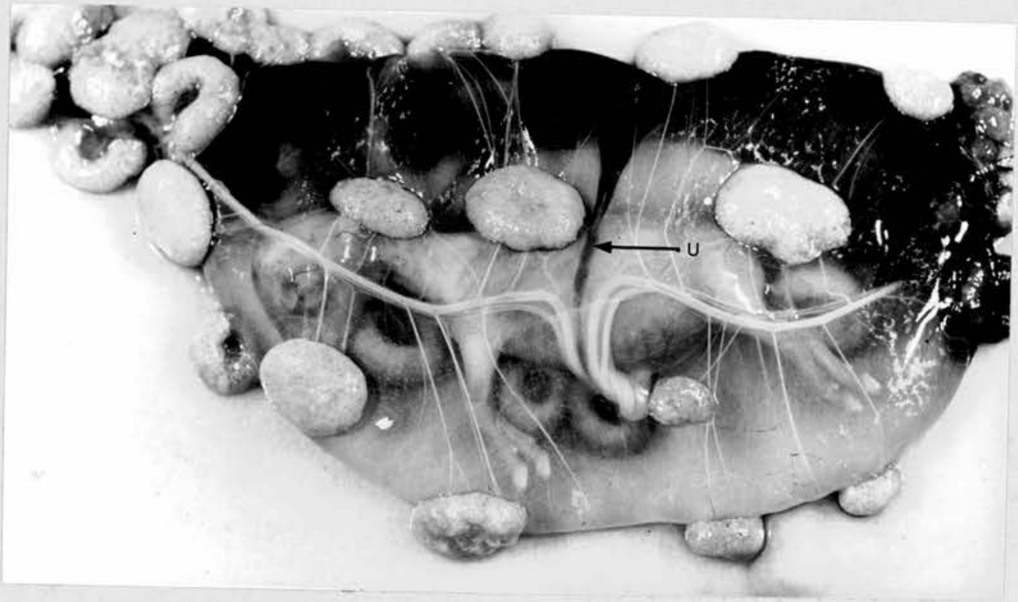

Fig. 1. Intact 70-day conceptus: note the allantoic isthmus (dark area; T-1824 dye), and the urachus (U) entering the umbilical cord.

foetal catheters, and from a catheter in a maternal jugular vein, at 30, 60, 120, 180 and 210 minutes after the dye injection. In each case, the condition of both foetuses, as judged by their heart rates, did not appear to deteriorate throughout the experiment. One ml. of plasma from each blood sample was diluted to a volume of 5 ml. with distilled water, and the optical density of the diluted plasma was read on an SP 500 Spectrophotometer[†] at a wavelength of 600 mμ.

Photographs

All the photographs have been reproduced from colour transparencies.

RESULTS

Single Conceptuses

In its most restricted form the regions of the allantoic sac occupying each uterine horn communicate with one another and with the urachus by a narrow tubular isthmus which is closely apposed to the surface of the amnion (Fig. 1). This situation is seen most frequently during the early stages of gestation. As pregnancy advances, the area of contact between the allantois and amnion becomes proportionately greater by a broadening of the allantoic isthmus along the whole or along part of its length (Fig. 2). In the conceptuses examined the allantois was never seen to be apposed to more than two-thirds of the amnion surface area, and in most cases the area of contact was considerably less than half, so that a large area of the chorion was apposed and adherent to the amnion in all cases.

[†] Unicam Ltd., Cambridge.

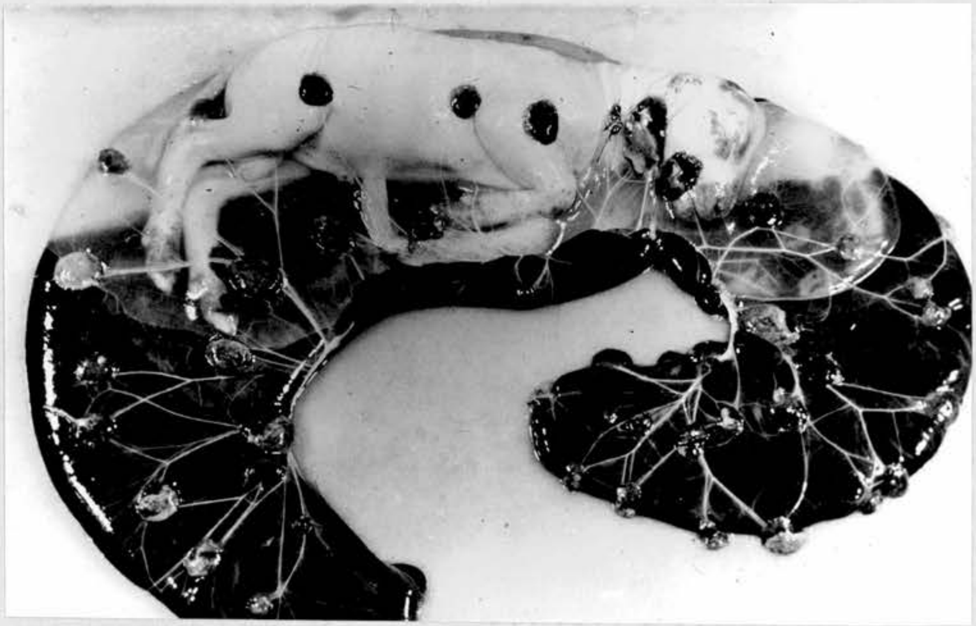

Fig. 2. Intact 140-day conceptus showing the relationship of the allantoic sac (dark area; T-1824 dye) to the amniotic sac (light area). This relationship has been emphasised by increasing fluid volumes.

Twin and Triplet Conceptuses

Fusion of Adjacent Chorions. It has been suggested that the terminal atrophy of the ends of the membranes (Jenkinson, 1906) would prevent their fusion in multiple pregnancies (Hammond, 1927). Subsequent reports (Keller, 1928; Williams, 1933; Kursonov, 1961) indicate, however, that fusion of adjacent chorions in sheep normally occurs, and this is supported by the finding in the present study that fusion of adjacent chorions failed to occur in only 2 of the 51 sets examined. This lack of fusion did not appear to be due to membrane necrosis, since the regions of apposition in both cases were highly vascular, and since there were no traces of necrotic tissue which might have prevented fusion of the membranes at the time of their initial contact.

Relationships of Adjacent Sacs to the 'Suture'. Neighbouring chorions meet at about 14 days post-conception (Assheton, 1906), and fusion appears to be complete on the 18th day (Bonnet, 1889; Robinson, 1951). At some time during or after the process of fusion, degeneration of the septum formed by the abutting chorions results in the formation of a patent tube of chorionic tissue. Throughout gestation the region of fusion normally remains as a clearly defined, continuous white line, or suture, around the chorion, and it is in the vicinity of this suture that the allantoic sacs of neighbouring foetuses meet on about the 23rd day of gestation (Kursonov, 1961). The present study indicates, however, that the relationship of the abutting fluid sacs to the chorionic suture is not as consistent as this might imply, and that in fact there appear to be 4 major variants.

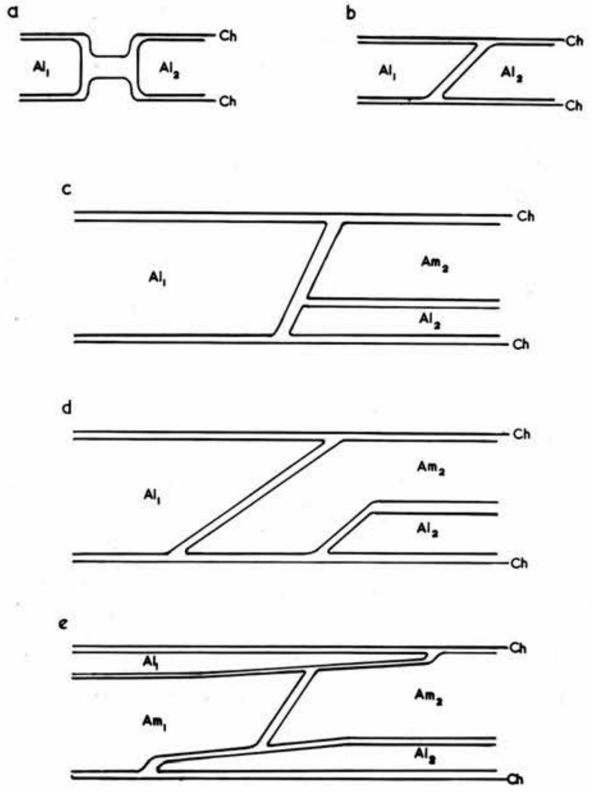

Fig. 3. Schematic representations of abutting sacs of adjacent foetuses: 3a - two allantoic sacs separated by a tubular segment of chorion; 3b - abutting allantoic sacs; 3c - allantoic sac in contact with the neighbouring allantoic and amniotic sacs; 3d - allantoic sac abutting on amniotic sac of neighbouring foetus; 3e - abutting amniotic sacs. Al - allantoic sac; Am - amniotic sac; Ch - chorion.

Firstly, in 31 cases where only the allantoic sacs of adjacent foetuses were involved, the following were observed: one case of a tubular segment of chorion 4 cm. long, containing the suture, separating neighbouring allantoic sacs (Fig. 3a); 5 cases in which the allantoic sacs abutted immediately under the suture; 3 cases of abutting membranes being within 3 to 5 cm. of the suture; and an additional 22 cases where the allantoic sac of one foetus encroached upon the chorionic territory of the other by at least 8 cm. (Fig. 3b). Of these 31 cases, 29 were in bicornuate twin pregnancies, and 2 in sets of triplets in the region of contact between the conceptus occupying the whole of one uterine horn and the closer of 2 conceptuses in the other horn.

Secondly, where the allantoic sac of one foetus abutted upon the allantoic sac and amniotic sac of its neighbouring litter-mate (Fig. 3c; Fig. 4), in only one case was the suture located within 3 to 5 cm. of the region of contact. The degree of overlap was greater than 5 cm. in another 7 cases, and of these, 2 were found with the suture girdling the allantoic sac of one foetus, while in 5 cases the suture encircled both the allantoic and the amniotic sac of one foetus. The 8 cases in this group were bicornuate twin pregnancies in which one foetus, usually located closer to the body of the uterus, had a less extensive allantoic sac than its litter-mate.

Thirdly, 5 cases were seen in which the allantoic sac of one foetus abutted only upon the amniotic sac of its neighbour, the 2 allantoic sacs being apposed to opposite sides of the abutting amniotic sac (Fig. 3d). In all cases the suture encircled the amniotic and the allantoic sac of one

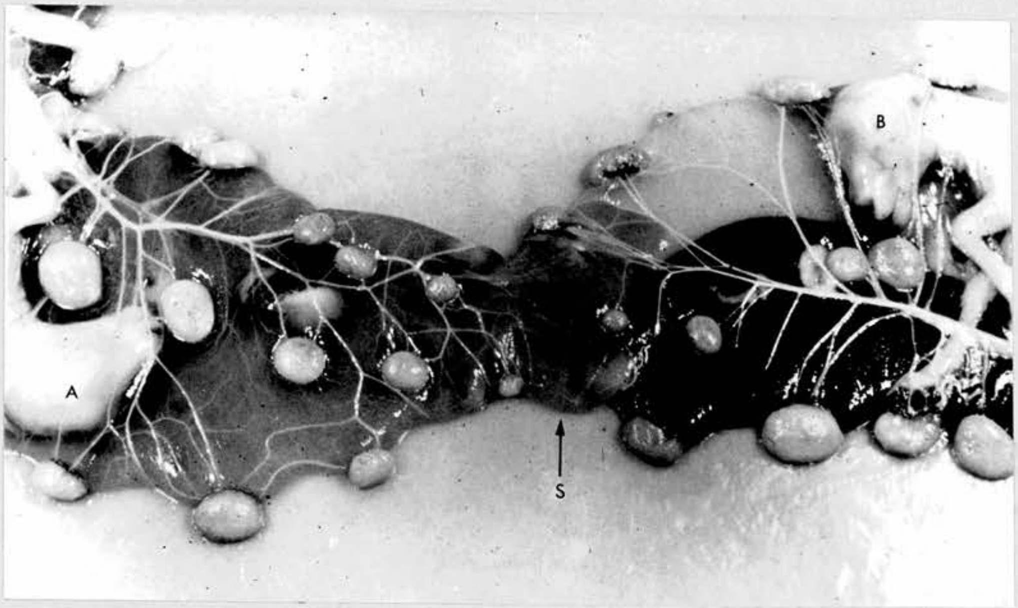

Fig. 4. Intact 110-day twin conceptus: allantoic sac (grey area) of foetus A abuts upon allantoic sac (dark area) and amniotic sac (light area) of foetus B. The suture (S) encircles allantoic sac of foetus A.

foetus more than 8 cm. from the centrally located extremity of its amnion, and in 3 of these the degree of overlap of the abutting allantoic sac was sufficient for it to be girded by the suture as well. The cases in this group arose from 3 bicornuate twin pregnancies in which one foetus was located in the body of the uterus, and from the areas of apposition between 2 conceptuses located in one uterine horn in 2 sets of triplets.

Finally, abutting amniotic sacs were observed in 5 cases, and in all of these a region of the allantoic sac of each foetus was apposed to the amniotic sac of its neighbour, but in such a way that the 2 allantoic sacs did not meet (Fig. 3e). In all cases, the suture was located 8 cm. or more from the abutting amnions, and girded both allantoic sacs and one amniotic sac. Four unicornuate twin pregnancies, and the regions of apposition of 2 conceptuses in one uterine horn in one set of triplets gave rise to this group.

In each of these 4 groups, the greatest degree of fluid sac encroachment upon the chorionic territory of neighbouring litter mates was observed most frequently in the cases where the foetuses were separated by the least distance, and a greater degree of overlap was found in unicornuate as apposed to bicornuate pregnancies. In all 49 cases the direction of overlap appeared to be quite random.

In 40 cases, therefore, there was a considerable degree of encroachment of one or several of the fluid sacs of adjacent foetuses upon the chorionic territory of their neighbours, so that the chorion of one foetus was located over regions of one or both fluid sacs of its neighbour. There was no apparent association between the degree of overlap and gestational age; indeed it appeared that the relationships of the various sacs were determined before the 45th day of gestation. The results indicate that the series of relationships is dependent upon the initial positioning of the blastocysts in the uterus. In bicornuate pregnancies, the allantoic sacs of adjacent foetuses are more likely to be apposed, but the closer the location of one foetus to the body of the uterus the more likely it is that its amniotic sac will become involved, to the extent that in unicornuate pregnancies adjacent amniotic sacs usually meet.

True and Pseudo-anastomosis between Adjacent Placental Vasculatures. The suture, arising as it does from the fusion of adjacent chorions, forms a relatively avascular border between neighbouring chorionic vasculatures, so that any blood vessels crossing between them can be seen easily with the naked eye. Trans-suture vascular elements appeared to be of 2 types: true anastomoses, which were seen to join vessels of both chorionic vasculatures, and which were shown to be patent by observation of the passage of their contained erythrocytes across the suture under the action of externally applied mechanical pressure; and, pseudo-anastomoses which, after crossing the suture, failed to unite with any vessels of the neighbouring vasculature and, either ended, or returned to the original side of the suture. Histological observations demonstrated that these vessels, both true and pseudo-anastomoses, were located in the vascular layer of the chorion, deep to the trophoblast.

No major vascular connections were seen crossing any of the 49 sutures

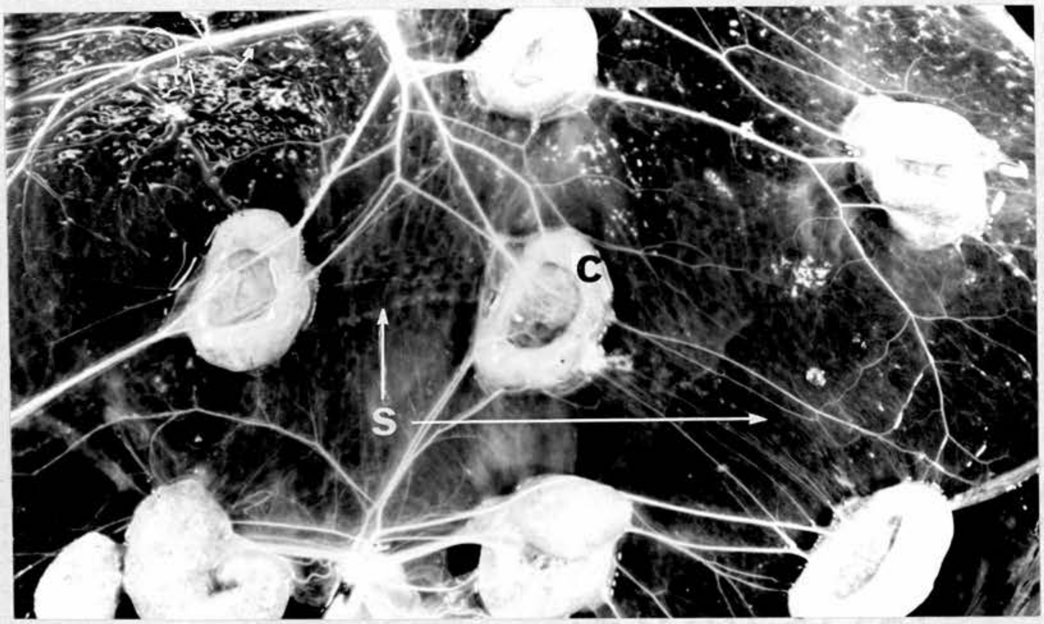

Fig. 5. Foetal side of the chorion from a twin conceptus: chorions have fused along the suture (S) on which a 'shared' cotyledon (c) is situated.

examined. In 32 cases, however, both true and pseudo- anastomoses less than 0.5 mm. in diameter were observed, and in 3 cases vascular elements having a diameter of approximately 0.5 to 0.8 mm. were seen, but in only one of these 3 cases were they found to be true anastomoses. Between one and 9 true anastomoses, and up to 8 pseudo-anastomoses were observed crossing different sutures at any one gestational age. There did not seem to be any particular association between relative numbers of the 2 types, except that in the majority of cases pseudo-anastomoses predominated. The number of true anastomoses crossing any one suture did not appear to bear any relationship to gestational age, nor did it appear to be associated with the circumference of the suture, which varied between about 4 and 40 cm.

'Shared' Cotyledons. Another feature of the relationship between adjacent chorionic vasculatures was the high incidence of cotyledons with associated placental blood vessels arising from both foetuses (Fig. 5). Between one and 6 of these 'shared' cotyledons were observed in 32 cases, and without exception they displayed the common characteristic of being located on the suture. There appeared to be no association between the presence of 'shared' cotyledons and the trans-suture vascular elements previously described. No anastomoses between the blood vessels of adjacent placental circulations were visible on the surface of 'shared' cotyledons, and vessels from one circulation did not appear to be associated with regions of 'shared' cotyledons on the opposite side of the suture. This indicated, therefore, that little or no intermixing of the bloods of adjacent foetuses occurs in 'shared' cotyledons, and this was verified experimentally in the 2 cases in which T-1824 was injected into the circulation of one foetus, and the bloods of both foetuses and the mother were sampled for a period of $3\frac{1}{2}$ hours following the injection.

Throughout both experiments the optical density readings of the samples obtained from the injected foetuses were greater than 0.6 units, indicating a very high plasma concentration of T-1824. On the other hand, no traces of T-1824 were detected in any of the other plasma samples. Post-mortem examination of both twin conceptuses revealed the presence of one large 'shared' cotyledon in each case, and a complete absence of any trans-suture anastomoses. The chorion and placental blood vessels of each injected foetus had a blue hue which ended abruptly at the suture. Under these experimental conditions in both of these cases, therefore, there appeared to be no intermixing of the bloods of adjacent foetuses within the 'shared' cotyledons. This tends to confirm the observation that the presence of anastomoses between the vessels of different foetuses within 'shared' cotyledons located on the suture is unlikely.

DISCUSSION

At about 23 days post-conception, in the majority of ovine multiple pregnancies, it appears that adjacent allantoic sacs meet (Kursonov, 1961) as a result of their rapid expansion (Assheton, 1906), while at the same stage amniotic sacs seem to have a relatively small volume (Boyd & Hamilton, 1952). This suggests, therefore, that even in the cases where apposed amnions were observed, the allantoic sacs had initially been in contact, but were later separated by the amniotic sacs as they increased their volume. The work of Robinson (1951) tends to confirm this observation.

The chorion of the sheep embryo apparently does not become vascular until the somatopleure fuses with the vascular allantoic splanchnopleure (Assheton, 1906). Accordingly, Kursonov (1961) suggested that vascular anastomosis between adjacent chorions in multiple pregnancies could not be established before the expanding allantoic sacs meet on about the 23rd day of gestation. He cited evidence indicating the early development of immunological incompatibilities between membranes and blood vessels of adjacent foetuses as a possible explanation of the low incidence (0.8 to 0.9%) of true anastomoses, and the relatively high occurrence (percentage unstated) of pseudo-anastomoses in his study. Indeed, the avascular nature of the suture, and the arc-like paths followed by most pseudo-anastomoses, are certainly suggestive of some form of repellent activity between neighbouring chorionic vasculatures. It seems, however, that the sequence of events leading to the formation of the suture is not as straight forward as this might imply.

If adjacent allantoic sacs abut in the immediate vicinity of the suture, the lack of fusion of adjacent chorionic vasculatures appears to have been adequately explained. In the majority of cases of abutting allantoic sacs, however, it seems that the suture is located some distance from where the 2 sacs meet. It may be suggested, therefore, that the allantois does not supply the chorion with vascular elements <u>en masse</u>, for if this were so, in every case the suture would be expected to be located in the immediate vicinity of the region of apposition of the 2 sacs. Since this does not occur, an alternative explanation appears to be necessary.

Embryological studies have revealed that the chorio-allantoic placenta arises from fusion of the mesodermal layer of the somatopleure with that of the allantoic splanchnopleure following expansion of the allantoic sac (Boyd & Hamilton, 1952). It seems possible, therefore, that the mesoderm of the allantoic splanchnopleure may simply have the role of providing vascular connections to join the embryo to the chorion, while the extent of the vasculature in fused adjacent chorions may be governed by the mesoderm of the somatopleure. Under these circumstances immunological reactions could prevent adjacent somatopleure mesoderms from uniting and may also force an expanding allantoic splanchnopleure to leave its mesodermal layer behind as it begins to encroach upon foreign chorionic territory. If this occurs, the allantois of one foetus could not vascularise the chorion of its neighbour.

Whatever the explanation of suture formation, the existence of 'shared' cotyledons clearly indicates that the 2 vasculatures are capable of quite intimate apposition without fusion. A simple coincidence of the suture lying across a maternal caruncle at the time of implantation appears to adequately account for the formation of 'shared' cotyledons, which Slee and Hancock (unpublished data) have also frequently observed.

Anastomosis of chorionic vessels not exceeding 0.8 mm. in diameter has been reported to occur at frequencies of 0.8% (Kursonov, 1961) and 15% (Slee, 1963). This variation may be due to breed differences, since the first percentage relates to Karakul and Romanov varieties, while the second largely refers to British breeds. British breeds were also examined in the present study, from which a 65% incidence of minor vessel fusion has arisen. There are 2 main factors which may account for this difference. Firstly, it was noted that trans-suture vessels not seen upon examination of conceptuses soon after death were visible upon re-examination several hours later. For this reason all the conceptuses in this study were examined no sooner than 4 hours after death. Secondly, with few exceptions, the conceptuses observed were obtained intact, so that blood contained in the placental vessels was available to fill any trans-suture vessels which might have dilated during the 4 hours before examination. In Slee's study, however, it appears that most of the placental membranes were examined under conditions which would not have facilitated observation of minor trans-suture vascular elements, since membranes ruptured by normal parturition were examined shortly after birth (Slee & Hancock, unpublished data). It appears, therefore, that trans-suture anastomosis between minor vessels occurs far more frequently in some British breeds of sheep than has previously been reported.

The presence of these minor vessel anastomoses is indicative of intermixing of the bloods of neighbouring foetuses, but the low incidence of erythrocyte mosaicism (Stormont _et al_, 1953) suggests that intermixing in these cases is minimal. This is supported by the low incidence of free-martinism in sheep (Kursonov, 1961). Free-martins are sterile females from twins of unlike sex between which placental vascular anastomosis has occurred. Free martinism has been demonstrated only in association with major vessel anastomosis, but the possible occurrence of covert free-martinism resulting from anastomosis of minor vessels was tested statistically by Slee (1963). He found no difference in fertility of ewes, whether born twin to males, or to females. It appears, therefore, that minor anastomoses between placentae of neighbouring foetuses do not permit mixing of the 2 blood streams in detectable amounts.

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REFERENCES

Alexander, D. P., & Nixon, D. A. (1961). Br. med. Bull., 17, 112.

Amoroso, E. C. (1952). In Marshall's Physiology of Reproduction, 3rd Ed., Vol. 2, Ch. 15. Longmans, Green & Co., London, N.Y., Toronto.

Assheton, R., (1906). Phil. Trans. R. Soc., 198, 143.

Bonnet, R. (1889). Cited by Lillie, F. R. (1917). J. exp. Zool., 23, 371.

Boyd, J. D., & Hamilton, W. J. (1952). In Marshall's Physiology of Reproduction, 3rd Ed., Vol. 2, Ch. 14. Longmans, Green & Co., London, N.Y., Toronto.

Hammond, J. (1927). The physiology of reproduction in the cow, pp. 159-160. Cambridge University Press, Lond.

Huggett, A. St. G., & Widdas, W. F. (1951). J. Physiol. Lond., 114, 306.

Jenkinson, J. W. (1906). Proc. zool. Soc. Lond., 1, 73.

Keller, K. (1928). Geburtshilfe bei den kleineren Haustieren Zweite Auflange, pp. 31-35. Urban & Schwarzenberg, Berlin, Wien.

Kursonov, K. M. (1961). Dokl. (Proc.) Acad. Sci. U.S.S.R. Biol. Sci. Section, 134, 711.

Meschia, G., Cotter, J. R., Breathnach, C. S., & Barron, D. H. (1965). Q. J. exp. Physiol., 50, 185.

Robinson, F. J. (1951). J. Agric. Sci. Camb., 41, 6.

Slee, J. (1963). Nature, Lond., 200, 654.

Stormont, C., Weir, W. C., & Lane, L. L. (1953). Science, N.Y., 118, 695.

Williams, W. L. (1933). Indian vet. J., 10, 90.

APPENDIX II

POTENTIAL DIFFERENCES BETWEEN MOTHER AND FOETUS AT DIFFERENT GESTATIONAL AGES IN THE RAT, RABBIT, AND GUINEA-PIG.

D. J. Mellor

A Paper accepted for publication in The Journal of Physiology, London.

SUMMARY

1. Potential differences associated with fluid compartments of rat, rabbit, and guinea-pig conceptuses have been measured. $[\mathrm{Na}^+]$ and $[\mathrm{Cl}^-]$ in maternal plasma and amniotic fluid from these 3 species were also determined.

2. Transplacental potential differences of about 15 mV (foetus positive) were found in the rat, of approximately zero mV in the rabbit, and of about 18 mV (foetus negative) in the guinea-pig.

3. Amniotic fluid potential differences appeared to arise indirectly from the transplacental p.d. in the rat, from the foetal gastric mucosa in the rabbit, and possibly from the foetal gastric mucosa and indirectly from the placenta in the guinea-pig.

4. The results are discussed in the context of Na^+ transfer to the foetus, and on this basis tend to question the general assumption that almost all Na^+ reaching the foetus passes across the placenta.

APPENDIX III

THE CHORIONIC SUTURE AND FREE-MARTINS - A BRIEF REVIEW

D. J. Mellor

Submitted for publication in The British Veterinary Journal

SUMMARY. Factors responsible for suture formation, which possibly express themselves before neighbouring chorions meet, may be of great importance in preventing the establishment of major placental vascular anastomosis.

IN A STUDY of fusion of foetal membranes in multiple pregnancy in sheep obtained from a Scottish abattoir (Mellor, 1969), fusion of adjacent chorions was the usual finding. The region of fusion was seen to remain throughout gestation as a clearly defined, continuous white line, or suture, around the chorion. This suture formed the border between neighbouring chorionic vasculatures, and in 65% of the cases true anastomoses less than 0.8 mm. in diameter were seen crossing the suture. Previously reported frequencies of minor vessel anastomosis have been at rates of 0.8% (Kursonov, 1961) and 15% (Slee, 1963).

The presence of minor vessel anastomosis is indicative of intermixing of the bloods of neighbouring foetuses, but the low incidence of erythrocyte mosaicism (Stormont, Weir & Lane, 1953) suggests that intermixing in these cases is minimal. This is supported by the low incidence of free-martins in sheep (Kursonov, 1961). Free-martins, sterile females from twins of unlike sex between which placental vascular anastomosis has occurred, have only been shown to be associated with major vessel anastomosis. However, the possible occurrence of covert free-martins resulting from anastomosis of minor vessels has been tested statistically, and no difference in fertility of ewes, whether born twin to males, or to females, was found (Slee, 1963). The significance of these minor vessels is unknown.

Major vessel fusion resulting in a considerable degree of blood intermixing is suggested by reports in sheep of erythrocyte mosaicism (Stormont, Weir & Lane, 1953), reciprocal skin homograft tolerance (Hraba, Hasek & Cumlivsky, 1956; Moore & Rowson, 1958; Slee, 1963), chromosomal variations (Gerneke, 1965; Bruere & Macnab, 1968), and by genital abnormalities (Fraser-Roberts & Greenwood, 1928; Ewen & Hummason, 1947; Bruere & Macnab, 1968), but proof of this in the majority of cases was not possible since the placentae were not examined. One case of limited placental vascular anastomosis between twins was observed by Rotermund (1933), but accounts of major vessel fusion appear to be limited to 4 cases of triplets; 3 recorded by Slee (1963), and 1 by Alexander & Williams (1964). Of these 4 sets of triplets, only 1 set arose from a normal mating, while the remaining 3 were produced artificially; one by superovulation, another by superovulation and quadruple mating, and a third by superovulation and quadruple mating following partial hysterectomy (Slee, 1963; Slee & Hancock, unpublished data; Alexander & Williams, 1964). In all 4 cases the suture was absent (Alexander & Williams, 1964) or not well defined (Slee & Hancock, unpublished data). Under normal circumstances, therefore, it seems that major vessel fusion occurs relatively infrequently. When it is observed, however, there appears to be a complete, or almost complete absence of a suture.

A suture is not normally visible even at very early gestational ages in bovine twins, and fusion of adjacent chorions giving rise to major placental vascular anastomosis regularly occurs (Lillie, 1917). Not unexpectedly, the incidence of bovine free-martins is correspondingly high, free-martins being observed in 80 - 90% of heterosexual twin pairs (Lillie, 1922). However, Lillie (1923) reports 6 cases of normal females co-twin to males with fused chorions, but in every case a well defined suture was present. A suture appears to be normally present in the fused chorions of adjacent foetal goats (Lillie, 1923; Keller, 1928; Williams, 1933), and there seems to be only one report of major placental vascular anastomosis in this species (Keller, 1928). Finally, it is of interest that William Harvey (1651) noted that adjacent chorionic vasculatures of deer twins do not unite even though the chorions fuse.

Therefore, in species in which fusion of adjacent chorions regularly occurs it appears that a suture, and major placental vascular anastomosis are mutually exclusive phenomena. Why a suture is normally present in fused chorions of the sheep, the goat, and possibly the deer, and not in the cow, is not clear, but it seems that the determinants of its formation express themselves during the very early stages of gestation, possibly even before neighbouring chorions meet.

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REFERENCES

Alexander, G., & Williams, D., (1964). Nature, Lond., 201, 1296.

Bruere, A.N., & Macnab, J. (1968). Res. Vet. Sci., 9, 170.

Ewen, A.H., & Hummason, F.A., (1947). J. Hered., 38, 149.

Fraser-Roberts, J.A., & Greenwood, A.W., (1928). J. Anat., 63, 87.

Gerneke, W.H. (1965). Jl S. Afr. Vet. Med. Ass., 36, 99.

Harvey, W. (1651). Anatomical exercises on the generation of animals (1651), Sydenham Society edition, translated by Willis, pp. 482-501. London: Adlard (1847).

Hraba, T., Hasek, M., & Cumlivsky, B., (1956). Folia. biol., Praha, 2, 276.

Keller, K. (1928). Geburtshilfe bei den kleineren Haustieren, 2nd Ed., pp. 31-35. Berlin, Wein: Urban & Schwarzenberg.

Kursonov, K.M. (1961). Dokl. (Proc.) Acad. Sci. U.S.S.R. Biol. Sci. Section, 134, 711.

Lillie, F.R. (1917). J. exp. Zool., 23, 371.

Lillie, F.R. (1922). Cornell Vet., 12, 332.

Lillie, F.R. (1923). Biol. Bull. mar. biol. Lab., Woods Hole, 44, 47.

Mellor, D.J. (1969). Res. Vet. Sci. (to be published).

Moore, N.W., & Rowson, L.E.A. (1958). Nature, Lond., 182, 1754.

Rotermund, H. (1929). Ueber Zwillingsfruchtsakentkleiner Wiederkauer, Inaug. Diss., Hannover. Cited by Williams, W.L. (1933). Indian Vet. J., 10, 90.

Slee, J. (1963). Nature, Lond., 200, 654.

Stormont, C., Weir, W.C., & Lane, L.L. (1953). Science, N.Y., 118, 695.

Williams, W.L. (1933). Indian Vet. J., 10, 90.