ELECTROCHEMICAL EQUILIBRIUM POTENTIALS OF EXOGENOUS ELECTROLYTES USED TO DISTINGUISH THE MATERNOFETAL ELECTRICAL POTENTIAL DIFFERENCE FROM THE TRANSPLACENTAL POTENTIAL DIFFERENCE IN SHEEP AND GUINEA PIGS

by

Nancy D. Binder

A THESIS

Presented to the Department of Physiology and

the Graduate Division of the University of Oregon Health Sciences Center

in partial fulfillment of the requirements for the degree of

Master of Science

June 1979

Approved:

. . . . . [redacted] . . . . . .

(Professor in Charge of Thesis)

. . . . . [redacted] . . . . . .

(Chairman, Graduate Council)

This thesis is dedicated to

I. Lloyd Hererra

who kept the spark alive

ACKNOWLEDEMENTS

I would like to thank Dr. J. J. Faber and Dr. K. L. Thornburg for their invaluable inspiration and assistance. I would also like to thank Thomas J. Green for the technical assistance in these experiments and Mary E. Harris for typing this thesis.

TABLE OF CONTENTS

INTRODUCTION

During its prenatal existence the mammal is completely dependent on its mother for obtaining the materials necessary for its growth and metabolism and for providing a route for its excretion. After very early embryonic development this exchange of materials takes place across a placenta that contains and separates the maternal and fetal circulations. In order to understand the process of transplacental exchange it will be necessary to know something about the properties of the placental exchange barrier and about the physical forces affecting the movement of materials across the barrier. The research described in this thesis was conducted in order to gain a better understanding of the extent to which electrical forces influence the transfer of charged molecules across the placental exchange barrier.

A difference in electrical potential has been measured between the mother and the fetus of several species. The reported values show considerable interspecies variation. The guinea pig fetus is approximately 20 mV negative with respect to the mother (18, 28, 30), the sheep fetus is about 50 mV negative (19, 34, 35) and the goat fetus is approximately 70 mV negative (19, 22). The rat fetus is about 15 mV positive with respect to its mother (18). No potential difference has been found between the mother and fetus in humans (20) or in rabbits (18, 37).

A potential difference across a living membrane is usually generated by the active transport of one ionic species or by the active exchange of two, while other ionic species are distributed across the membrane in conformance with the Nernst equation. It has been assumed that the potential difference recorded between mother and fetus is generated at

the placental exchange barrier, although the technically difficult direct measurement of the potential difference between maternal and fetal blood in the microvasculature of the placenta has never been made. If a potential difference of the magnitude commonly measured between mother and fetus was generated at the placental exchange barrier, it would be a major force affecting the passive movement of ions and charged molecules across the placenta. The electrolyte composition of fetal plasma should show evidence of such a force and knowledge of the concentrations of ions in maternal and fetal plasmas should allow one to predict the electrical potential that is governing the distribution of ions at the barrier.

Previous studies in guinea pigs (36) and sheep (1, 2, 6, 16) have shown electrolyte compositions that are virtually identical in maternal and fetal plasma (Tables 1 and 2). If electrolytes are passively distributed then these results indicate that any potential difference across the placental exchange barrier itself is much smaller than the potential difference that has been measured between maternal and fetal extracellular fluids. If this were not so then one would have to postulate an energy requiring mechanism to maintain similar concentrations of electrolytes in maternal and fetal plasmas in the face of a large transplacental difference in potential. Because the transplacental potential difference (transmembrane potential) need not necessarily be the same as the maternofetal potential difference, a distinction will be made between these two terms.

In the present experiments the transmembrane potential in the placenta was evaluated by the calculation of equilibrium potentials for electrolytes not normally present in plasma. Salts of bromide,

sulfate, rubidium and lithium were injected into pregnant guinea pigs and sheep and the concentrations of these ions in maternal and fetal plasma were measured at various times after injection. The assumption was made that these ions would be passively distributed across the placental exchange barrier and would be in electrochemical equilibrium after sufficient time had elapsed for transient effects to vanish. The transplacental difference in electrical potential was calculated from the Nernst equation (24) and the measured steady state concentrations of each of these ions in maternal and fetal plasmas. The calculated transplacental differences were then compared to the differences in potential measured between electrodes placed in maternal and fetal extracellular fluids.

MATERIALS AND METHODS

Animals

Pregnant guinea pigs in the second half of gestation were obtained from a commercial breeder. The sizes of the guinea pig fetuses were estimated by palpation of the abdomens of the sows. Pregnant ewes of mixed western breed were purchased from a rancher. The gestational ages of the sheep fetuses were estimated from a roentgenogram (12). Surgery was performed on the ewes when gestation was estimated to be greater than 100 days.

Ionic Tracers

[^{86}Rb] rubidium chloride, [^{35}S] sodium sulfate, and [^{82}Br] ammonium bromide in solution were obtained from New England Nuclear, Boston, Massachusetts. Analytical grade lithium chloride was obtained from the J. T. Baker Chemical Company, Phillipsburg, New Jersey.

Processing of Samples

The blood samples were centrifuged immediately and the supernatant plasma were removed. The activity of $^{82}Br^-$ in a 0.5 ml aliquot of plasma was determined by gamma spectrometry in a Packard Model 3002 scintillation spectrometer. The plasma proteins in samples containing $^{86}Rb^+$ and $^{35}SO_4^=$ were precipitated by addition of 0.5 ml of 10% trichloroacetic acid to 0.5 ml of plasma. The solution was thoroughly mixed and centrifuged. An aliquot of 0.5 ml of the supernatant was dissolved in fluor (Aquasol®, New England Nuclear) and was counted in a Packard Tricarb Model 3200 liquid scintillation spectrometer. The remainders of the plasma samples were coded and sent to the Department of Clinical

Pathology for the determination of lithium concentration by atomic absorption spectrometry. Because the half life of ^{82}Br is only thirty-six hours, the counts per minute of samples containing bromine were corrected for the radioactive decay that occurred during the period of counting the samples. In samples containing both ^{86}Rb and ^{35}S the activity of each was determined by the channels ratio method. The activities (counts per minute per milliliter plasma) of all samples containing radioisotopes were corrected for background counts. All concentrations and activities were corrected for dilution by the heparin solution in the dead space of the sampling syringes.

Surgical Preparation of Guinea Pigs

Each sow was anesthetized with 2% halothane in a two to one (v/v) mixture of nitrous oxide and oxygen, and a 1 mm outside diameter polyvinyl catheter was placed in one of her carotid arteries. The skin was closed with metal clips and the catheter was secured with an adhesive tape collar.

Surgical Preparation of Sheep

Techniques for implanting chronic catheters in sheep fetuses were first described in 1965 (23). Modifications of these basic techniques were used for these experiments.

The ewes were kept in a small pen in the laboratory and were given food and water ad libitum until 18 hours prior to surgery. Anesthesia was induced with 4% halothane delivered by face mask. The ewe was tied to the surgery table in the supine position and her trachea was intubated. Anesthesia was maintained during surgery with 1.5% halothane in a 2/1 (v/v) mixture of nitrous oxide and oxygen delivered

through the endotracheal tube. The ewe's belly was shaved, scrubbed with an iodophor and draped. Strict sterile precautions were observed at all times.

The uterus was exposed through a midline abdominal incision. A purse string suture was placed through the myometrium and an incision was made through the myometrium and the allantoic and amniotic membranes.

A bilateral nephrectomy was performed on most single fetuses and on one fetus of each twin pair. This was done by delivering the hindquarters of the fetus through the uterine incision and tightening the purse string suture around the fetus to prevent leakage of amniotic fluid. Care was taken to insure that circulation in the umbilical cord was not compromised. The nephrectomies were performed through flank incisions placed just lateral to the vertebral column and were done without entering the peritoneal cavity. The adrenal glands were not removed. The hindquarters were then returned to the amniotic sac.

Vascular catheters were placed in all fetuses. One hind limb or the head was brought through the uterine incision and the purse string suture was tightened around the fetal part. Indwelling polyvinyl catheters filled with heparinized saline were placed in a femoral artery and vein or in a carotid artery and jugular vein by standard techniques (21). The catheters were sutured to the skin after the wound had been closed.

A catheter with several side openings was anchored to the fetal skin to permit later access to the amniotic fluid compartment. A catheter was also placed in the allantoic fluid compartment through a separate uterine incision and purse string suture.

Before closing the uterus the extrafetal membranes were gathered

around the exiting catheters and tied with a silk suture. The seal thus formed was checked for leaks which were repaired when necessary. A single dose of 1,000,000 Units penicillin G was then injected into the amniotic fluid via the catheter. No additional antibiotics were given at any time during these experiments.

In two cases silver/silver chloride electrodes were implanted. One electrode was placed in the peritoneal cavity of the fetus and brought out through the membranes and uterus with the catheters. The other electrode was sutured in place inside the ewe's peritoneal cavity.

The catheters and electrode wires were led through a hole in the peritoneum and abdominal musculature lateral to the midline incision and then through a subcutaneous tunnel to exit on the flank of the ewe. The catheters were coiled into a bag fastened to the ewe's side. The peritoneum was sutured and the skin was closed with metal clips. After closure of the ewe's abdomen catheters were placed in her carotid artery and jugular vein.

Experimental Protocol for Guinea Pigs

After the sow had regained consciousness a saline solution containing 100 μCi [^{86}Rb] rubidium chloride and 50 μCi [^{35}S] sodium sulfate or 100 μCi [^{82}Br] ammonium bromide was injected via the catheter in the carotid artery. The catheter was thoroughly flushed with heparinized saline. Some of the sows received an intraperitoneal injection of lithium chloride (1 meg/kg) alone or in addition to the radiotracer. After the injection the sow was given food and water ad libitum.

Blood samples were obtained from the conscious sow thirty minutes after the injection, immediately before she was re-anesthetized for

the purpose of fetal sampling, and at appropriate intermediate times. A final maternal blood sample was also obtained after fetal blood sampling had been completed. Before each maternal sample was taken, 2 ml of blood was withdrawn from the catheter to rinse the catheter deadspace. Then a 2 to 3 ml sample was withdrawn into a heparinized syringe and saved for analysis. The blood withdrawn prior to sampling was returned to the sow and the catheter was flushed with heparinized saline. Blood samples were centrifuged and the supernatant plasma removed for processing within three hours.

When the last blood sample had been taken from the conscious sow she was re-anesthetized and her uterus was exposed through a midline abdominal incision. A small incision was made in the uterus overlying the abdomen of a fetus. The fetal membranes and skin were grasped with a hemostat so that amniotic fluid could not leak into the fetal peritoneal cavity and a small stab wound was made through the fetal skin.* Amniotic fluid which spilled onto the uterine surface was absorbed with gauze sponges in order to prevent the establishment of a short circuit across the uterine wall.

Difference in electrical potential between the mother and the in situ fetuses were measured with silver/silver chloride electrodes and a Keithley Model 602 electrometer which has an input impedance of 10^{14} ohms, specified by the manufacturer. (It has been determined that the concentrations of chloride in maternal and fetal plasmas are nearly the same [36]). The reference electrode was placed in the maternal

---

*Anesthetics pass through the placenta with such ease that a general anesthetic given to the mother anesthetizes the fetuses also.

peritoneal cavity and the other electrode was inserted into the fetal peritoneal cavity. The asymmetry potential of the electrode pair was measured by placing both electrodes into the same saline solution before and after all electrical measurements had been made.

The uterus and fetal membranes were then opened, blood samples were withdrawn from the umbilical vein of each fetus into heparinized syringes and the times of sampling were noted. The final blood sample was then withdrawn from the mother. The fetuses and placentas were weighed.

Measurements of plasma concentrations of injected tracers were determined at several times during the experiment for each guinea pig sow and once for each group of fetuses from a single sow at the end of the experiments. Before analyses of the samples were made, a fetal sample was rejected if there was any question whether the uterus or placenta was perfused, as determined by inspection of the blood vessels at the time of laparotomy.

Experimental Protocol for Sheep

After the recovery from anesthesia, the ewes were kept in stanchions in bathtubs on casters for the duration of the experiments. This method of housing sheep allowed the radioactive excretement to be safely contained.

One or more tracers were injected as a single bolus or slowly infused with a Gilson Minipuls-II pump into the maternal jugular vein catheter. The rates of infusion of lithium chloride varied between 0.42 and 3.7 g/day, but most ewes received 0.7 and 0.9 g/day. These rates of infusion did not result in any observed toxic effects in

the ewes.

At intervals, 4 ml blood samples were withdrawn through the maternal and fetal arterial catheters under strict sterile conditions. Before withdrawing a blood sample a volume of fluid equal to at least three times the catheter volume was withdrawn and discarded in order to rinse the catheter dead space. On some occasions fluid samples were also withdrawn from the amniotic and allantoic catheters. The blood samples were centrifuged for 10 minutes and the supernatant plasmas were removed. The plasma and fluid samples were stored at -20°C until needed for processing. Several paired measurements on maternal and fetal plasmas were thus obtained for each ewe in the course of the experiments.

Electrical potential differences between the mothers and the fetuses with implanted silver/silver chloride electrodes were measured with the Keithley Model 602 electrometer described in the experimental protocol for guinea pigs. The small difference in chloride concentrations in maternal and fetal sheep plasmas (Table 1) would be expected to result in approximately 1.5 mV difference in electrical potential between the electrodes if chloride activities are assumed to be proportional to the concentrations in the plasmas (4). The asymmetry potential difference between the two electrodes placed in the same saline solution was measured before implantation of the electrodes and was found to be less than ± 2 mV on both occasions.

RESULTS

Guinea Pigs

Twenty-four sows with eighty-two fetuses were studied. Thirteen of the fetuses were excluded from the study for the following reasons: seven fetuses, including two with meconium staining, were rejected because of inadequate placental perfusion, four were so small that it was not possible to obtain an adequate volume of blood from them, one was dead and one was discarded because of the possibility that the blood sample had been contaminated with amniotic fluid. The sixty-nine remaining fetuses appeared to be in good condition. The weight of the fetuses ranged from 22 to 134 grams.

One sow was injected with [^{35}S] sodium sulfate alone, three received only [^{86}Rb] rubidium chloride, six were given $^{86}Rb^{+}$ and $^{35}SO_4^{=}$, four were injected with [^{82}Br] ammonium bromide, two were given lithium chloride only, three received $^{82}Br^{-}$ and Li^{+} and five received $^{86}Rb^{+}$, $^{35}SO_4^{=}$, and Li^{+}. Lithium was used in addition to radiotracers only for sows with large fetuses to ensure that a sufficient volume of fetal blood would be obtained to determine the concentration of both tracers. In spite of attempts to select sows with fetuses of adequate size by palpation of the mother's abdomen, there were three cases in which there was not enough plasma left after the removal of an aliquot for radiotracer counting to determine the plasma Li^{+} concentration for each fetus. In these cases the remaining plasmas from the entire litter were pooled and treated as one sample.

Figures 1 through 4 show the concentrations of these exogenous electrolytes in maternal and fetal plasmas as functions of time. In

order to normalize the data, we arbitrarily set the concentration of each substance found in the maternal plasma thirty minutes after injection equal to one; all other maternal and fetal plasma concentrations for that sow were expressed as ratios of the maternal plasma concentration at thirty minutes.

Figure 1 shows the results for guinea pigs that received $^{82}Br^{-}$. Immediately after the intravenous injection of the tracer, the maternal concentrations were very high. They rapidly declined during the period of distribution to the various fluid compartments of the body. The fetal concentrations were initially low, but in six hours they rose to the same level as the maternal concentrations. After equilibration of the bromide in maternal and fetal plasmas there was no detectable difference in tracer concentration between the mother and the fetus.

The results for $^{35}SO_4^{=}$ are shown in Figure 2. In this case the concentrations continued to decline after the period of distribution because of rapid excretion of sulfate ion by the mother. The radiosulfate concentration in fetal plasma reached the level of the maternal concentration in about two hours, and then declined at the same rate as the maternal concentration.

Similar results are shown for $^{86}Rb^{+}$ in Figure 3. However, there was a large variability in the concentrations measured at any particular time. During the course of these experiments we observed that the concentration of rubidium in maternal plasma always rose after the sow had been anesthetized. We surmised that the intracellular concentration of rubidium was very much higher than the extracellular concentration and that the observed rise in plasma concentration was due to a small shift of rubidium ions from their intracellular location under

the influence of a slight decrease in cell membrane potential. Fetal samples were taken only when the animals were anesthetized. Thus it can be expected that the tracer concentration in fetal plasma would be higher than the concentration in the plasma of a different, unanesthetized, sow at the same time. This systematic difference in the plasma concentrations of $^{86}Rb^{+}$ in addition to the normal variability in different animals, made it more difficult to determine the exact time at which fetal concentrations had reached the same level as maternal concentrations. However, it appeared that the concentrations of $^{86}Rb^{+}$ were the same in maternal and fetal plasmas eight or more hours after injection.

Figure 4 shows the results for guinea pigs who were injected with lithium chloride. The concentration of lithium in maternal plasma rose as it was absorbed from the peritoneal cavity and then declined as it was distributed to the various fluid compartments and excreted. No fetal blood samples were obtained prior to six hours. By that time it appeared that maternal and fetal plasmas had equilibrated.

The results revealed a similar pattern for all four electrolytes. The concentrations in fetal plasma rose until they were essentially the same as the concentrations in maternal plasma. After equilibration, concentration changes in maternal plasma were followed by equal changes in fetal plasma concentrations. Differences in equilibration time for the different ions reflected factors such as placental permeability to the ion and the volume of distribution of the ion. Changes in plasma concentration after equilibration probably reflected the rate of excretion of the electrolyte by the mother.

The mean difference in electrical potential between mother and

fetus as measured with electrodes in sixty-two pairs was -33 ± 2 (S.E.M.) mV, where the minus sign indicates that the fetus was negative with respect to the mother. There was a small positive correlation between potential difference and fetal weight ($r = 0.315$, $t = 2.47$, $p < 0.01$; the absolute value of the potential difference was less for larger fetuses. The order in which potential differences were measured within a litter did not correlate with the magnitude of the potential difference ($r = 0.106$, $t = 0.778$, $p > 0.1$). There was therefore no evidence of deterioration of the preparations during the short period of anesthesia necessary for the measurements.

Sheep

Experiments were performed on twelve fetuses carried by nine ewes. The mean fetal weight was 2.8 kg ± 0.8 kg (S.D.). Arterial blood gases were checked in 10 fetuses. These showed a mean ± S.E.M., pH = 7.35 ± 0.03, PO_2 = 20 ± 4 torr, and PCO_2 = 51 ± 4 torr. These results are similar to values previously reported for chronically catheterized sheep fetuses considered to be in good condition (8, 11, 23). The difference in electrical potential was measured between mother and fetus in the two pairs with implanted electrodes. One fetus was -49 mV with respect to its mother and the other was -68 mV. The asymmetry potential of the two electrodes placed in the same saline solution was less than ± 2 mV. These results are in good agreement with previously reported maternofetal potential differences in the sheep (19, 34, 35).

[^{35}S] Sodium sulfate was injected into one ewe who carried a non-nephrectomized fetus and into four ewes carrying four nephrectomized fetuses. [^{82}Br] Ammonium bromide was injected into three ewes with

three nephrectomized fetuses and into one ewe carrying one nephrectomized and one intact fetus. Lithium chloride was infused into seven ewes carrying nine fetuses. All of these fetuses, except for two that were the seconds of twin pairs, were nephrectomized. Rubidium chloride was not used in any of the sheep experiments.

Figure 5 shows the results of the first experiment in which radiosulfate was injected into the ewe; the logarithm of the concentration of $^{35}SO_4^{=}$ in maternal fetal plasma samples is plotted as a function of time. The dashed line indicates the concentration of radiosulfate in fetal plasma that would be in electrochemical equilibrium with radio sulfate in maternal plasma at a given time if there had been a potential difference of -50 mV across the placental exchange barrier. For a divalent anion such as sulfate, this can be predicted from the Nernst equation:

$$\Delta E = \frac{RT}{zF} \cdot \ln \frac{[SO_4^{=}]_M}{[SO_4^{=}]_F} \qquad (1)$$

Which yields, after substitution of the appropriate values for R, T, z, F, and E, at 39°C:

$$\log [SO_4^{=}]_M - \log [SO_4^{=}]_F = 1.61 \qquad (2)$$

The subscripts M and F refer to the concentrations in maternal and fetal plasma.

Figure 5 shows that when the first sample was taken, eighteen hours after injection, the concentration in fetal plasma had already risen above the electrochemical equilibrium at -50 mV. However, the concentration in fetal plasma then began to fall instead of continuing to rise towards the maternal concentration, but the decline in the fetal concentration did not appear to parallel the decline in maternal

concentration. Two possible explanations for this observation were considered. There might have been a transplacental potential difference of a lesser magnitude than the approximately -50 mV that has been recorded between the mother and fetus (19, 34, 35) or the fetal plasma might have been losing radiosulfate to other fetal fluid compartments at a rate that exceeded the rate of transplacental acquisition. The concentrations of radiosulfate found in samples of amniotic and allantoic fluids taken at the time the third set of samples were obtained lent support to the second alternative. The concentrations in these extrafetal fluids were many times higher than those in the fetal plasma, probably due to sulfate excretion by the fetal kidney. To reduce the complexities of a multicompartment system of intrafetal and extrafetal fluids, we performed fetal nephrectomies on all singletons and one fetus in each set of twins in subsequent experiments.

Figure 6 shows the results obtained in the next four experiments which were all performed on ewes with nephrectomized fetuses. The mean duration of the four experiments, measured from the time of injection of radiosulfate to the time the last samples were taken was 168 hours (7 days). In order to make the results from different animals mutually comparable on the same graph, we normalized the concentrations by setting the maternal concentration at 84 hours equal to 100 units and by adjusting all other concentrations proportionally. The dashed line indicates the fetal concentration which would be in electrochemical equilibrium with the maternal concentration if there was a transplacental potential difference of -50 mV.

Figure 6 shows that the sulfate concentrations in fetal plasma continued to rise after having reached the electrochemical equilibrium

concentrations predicted by the Nernst equation for a potential difference of -50 mV until they reached the level of the maternal concentration on the third day after injection of the tracer into the ewe. After this time the concentration in fetal plasma began to decline, although it did not decline as rapidly as the maternal concentration did. This was apparently due to tracer being lost from the maternal plasma at a rate greater than the rate at which sulfate could be transferred across the placenta. In these experiments the concentrations of radiosulfate in amniotic and allantoic fluids were less than the concentrations in fetal plasma until after fetal plasma concentrations achieved equality with maternal plasma concentrations and did not exceed fetal plasma concentrations to any significant degree at any time. These results support the idea that an elevated concentration of radiosulfate in the extrafetal fluids of the first fetus occurred as a result of renal excretion.

These experiments did not support the existence of a large potential difference across the exchange barrier of the chorioallantoic placenta of the sheep. However, it was impossible to ascertain from these experiments what the transplacental potential difference is. The rapidly changing maternal sulfate concentrations precluded the establishment of a steady state; therefore, no equilibrium potentials could be calculated from the results of these experiments. Three ewes were given continuous infusions of radiosulfate in an attempt to establish constant maternal plasma concentrations. These experiments were terminated before this was achieved when it was discovered that very large amounts of radiosulfate were excreted by the ewes and that the desired information could be obtained more easily and more safely by injecting radiobromine

into the sheep.

The results of the first experiments in which a bolus of [^{82}Br] NH_4Br was given to a ewe carrying a nephrectomized fetus are shown in Figure 7. The concentration of $^{82}Br^-$ in the first sample of fetal plasma was already greater than the concentration predicted by the Nernst equation for electrochemical equilibrium with maternal plasma at a hypothetical transplacental potential of -50 mV. Unlike sulfate, bromide did not have a short biological half life in maternal plasma. Thus it appears that radiobromide was virtually equilibrated between maternal and fetal plasmas for the period of time after the second fetal sample had been taken.

The results of all five experiments with radiobromide are compiled in Figure 8. The mean duration of the experiments was 170 hours (7 days). The results were normalized by setting the maternal concentrations at 85 hours equal to 100 and by making proportional adjustments in all of the concentrations. The normalized concentrations are plotted on a linear scale in this figure to emphasize the time course of equilibration of fetal and maternal plasma concentrations. The fetal plasma concentrations in the non-nephrectomized fetuses were indistinguishable from those in the nephrectomized twin, so they have been included in these results. Amniotic and allantoic fluid concentrations of $^{82}Br^-$ were measured on some occasions and did not exceed fetal plasma concentrations to any significant degree at any time during the experiments.

Lithium chloride was given as a constant intravenous infusion to seven ewes carrying seven nephrectomized and two non-nephrectomized fetuses. A constant infusion was given in order to maintain a steady

concentration of lithium in maternal plasma at a level that could be easily measured and below the level at which the ewe would show signs of toxicity. The mean duration of the experiments, from the start of the infusion to the last fetal sample, was 257 hours (10.7 days). Rates of infusion varied from 0.42 to 3.7 grams per day, but most ewes received 0.7 and 0.9 grams of LiCl per day.

Figure 9 shows the results of a typical experiment where LiCl was infused into a ewe carrying a single nephrectomized fetus. The concentration of Li^+ in fetal plasma rose to a level close to that in maternal plasma, but no further. In none of these experiments did we observe fetal concentrations that exceeded maternal concentrations, except temporarily during an acute fall in maternal concentration when the rate of infusion was reduced or the infusion was terminated, such as is shown in Figure 9. The concentration of Li^+ was occasionally measured in amniotic and allantoic fluids and found to parallel fetal plasma concentration in nephrectomized fetuses but to exceed fetal plasma concentration in the fetuses with kidneys. In those intact fetuses, fetal plasma levels rose towards maternal plasma levels more slowly than in their nephrectomized twins, otherwise, there was no difference. Spontaneous fluctuations in maternal plasma concentrations occurred from time to time in spite of constant rates of infusion, probably because of variations in renal excretion. Parallel changes were then observed in fetal plasma concentrations (Figure 9).

The results obtained for Li^+ and $^{82}Br^-$ in the sheep were very similar to the results obtained in guinea pigs. Initially, the fetal plasma concentration of the exogenous ion increased until it approximately equaled the maternal plasma concentration and then changes in

the fetal concentration paralleled changes in the maternal concentration. More time was required for equilibration of maternal and fetal plasmas in the sheep than in the guinea pig and there appeared to be a significant lag time before a change in maternal plasma concentration was reflected by a change in fetal plasma concentration. This probably resulted from differences in ion permeability between the epitheliliochorial placenta of the sheep and the hemochorial placenta of the guinea pig (2, 7, 30, 32).

Electrochemical Equilibrium Potentials

If it is assumed that ions are in passive equilibrium across the placental exchange barrier, then the Nernst equation can be used to calculate the electrical potential that governs the distribution of ions across the barrier:

$$\Delta E = [RT/(zF)] \cdot \ln([ion]_M/[ion]_F) \qquad (3)$$

E is the calculated electrical potential, R is the gas constant, T is the absolute temperature, z is the valence of the ion, F is the Faraday constant, and $[ion]_M$ and $[ion]_F$ refer to the concentrations of the ion in maternal and fetal plasmas.

For each mother and fetus a potential difference across the placental exchange barrier was calculated from the concentrations of the tracer ion in maternal and fetal plasmas at the time of fetal blood sampling. A correction was made in the maternal concentrations of guinea pigs by calculating the maternal concentration at the time of fetal blood sampling by linear interpolation between the concentration found immediately before anesthesia and the concentration in the sample taken after all fetal blood samples were taken. Since the time between these two samples was

less than fifteen minutes, this calculation also helped to reduce random errors in the measurements of plasma concentrations. In the sheep, which were not anesthetized at the time of sampling, maternal and fetal samples were drawn almost simultaneously and thus no interpolation was necessary.

Figure 10 shows the transplacental potentials calculated from the Nernst equation as a function of time after injection of rubidium, lithium, bromide, and sulfate ions in the guinea pig. Figure 11 shows the similarly calculated transplacental potential as a function of time after injection of ammonium bromide or after the beginning of the lithium chloride infusion in the sheep. Initially, the calculated potential is positive for the positive ions and negative for the negative ions. This is the result expected from the effect of the polarity of the ion on the calculated potential differences when there is still diffusional transfer from the mother to the fetus. For every ion studied, the magnitude of the calculated potential decreases with time and reaches a limiting value of approximately zero at the time that equilibration between fetal and maternal plasmas occurs. The dashed line in Figure 10 indicates the mean maternofetal potential difference between guinea pig mother and fetus measured during these experiments.

Table 3 gives the mean calculated transplacental potential difference and assumed time for equilibration for each of the exogenous electrolytes used in experiments on guinea pigs. The means were calculated from all paired maternal-fetal post equilibration samples. The time until equilibration was determined from Figure 1 through 4 by noting the time from which changes in fetal plasma concentration paralleled changes in maternal concentration. The exact time of

equilibration was not determined, but an estimate of the time at which it was reasonably certain that equilibration had occurred was made. The actual time of equilibration was probably earlier than this estimate. The mean calculated transplacental potential was not significantly different from zero for rubidium, bromide, or lithium, but was slightly different from zero for sulfate. In animals that received injections of both a positive and a negative ion there was no reliable correlation between the potentials calculated from the concentrations of the positive ion and the potentials calculated from the concentrations of the negative ion ($r = 0.268$, $t = 1.275$, $p > 0.1$). Thus it appears that slight differences in calculated equilibrium potentials can be attributed to random variation. There was no reliable correlation between the electrically measured maternofetal potential difference and the calculated electrochemical equilibrium potential difference across for sulfate ($r = 0.229$, $t = 1.00$, $p > 0.1$), lithium ($r = -0.224$, $t = 0.91$, $p > 0.1$), bromide ($r = 0.062$, $t = 0.20$, $p > 0.5$), or rubidium ($r = -0.017$, $t = 0.15$, $p > 0.5$).

Table 4 gives the mean transplacental potential difference calculated from paired maternal and fetal plasma samples taken after steady state conditions had been reached after the injection of bromide or after the beginning of the infusion of lithium in the sheep. Equilibration times given in Table 4 were estimated from Figures 8 and 9. These calculated transplacental potential differences were slightly larger than those calculated for the guinea pig placenta, but were significantly different from the approximately -50 mV potential difference that has been measured between the ewe and her fetus in this and other experiments (19, 34, 35).

DISCUSSION

Applicability of Methods

The Nernst equation applies only to situations in which there is thermodynamic equilibrium. However, it is frequently used to describe physiological systems which can be considered to be in a steady state (24). Under steady state conditions, concentrations of substances in the plasmas on both sides of the exchange barrier and the voltage across the barrier would be invariant with respect to time and the net flux of a tracer substance across the exchange barrier would be equal to zero.

It is unlikely that there is ever a true steady state established for transplacental exchange. A healthy fetus is constantly growing. A continually expanding fetal volume of distribution requires that there be a net influx of materials to the fetus if constant plasma concentrations are to be maintained and growth is to be sustained. A major constraint on the rate of growth of sheep fetuses appears to be the rate at which it can acquire the electrolytes Na^+ and Cl^- (9). Thus, the diffusional gradients that are established in this situation would lead to apparent equilibrium potential differences that are negative (fetus with respect to mother) for negative ions and positive for positive ions when the Nernst equation is applied in these non-steady state conditions (Tables 1, 2, 3, & 4).

For this reason, one would predict that the actual transplacental difference in electrical potential lies somewhere between the equilibrium potentials for the positive ions and the negative ions. Although it is impossible to determine what the exact value of the electrical potential difference across the placental exchange barrier is, the best

estimates would be ± 1 mV for the guinea pig and within a few mV of zero for the sheep.

The results of these experiments are consistent in their failure to demonstrate an approximately -50 mV potential difference (19, 34, 35) across the placenta of the sheep or an approximately -20 mV potential difference across the placenta of the guinea pig (18, 28, 30). Thus it appears that the electrically measured potential difference between mother and fetus in these and other experiments (18, 19, 28, 30, 34, 35) is not the same as the transmembrane potential difference across the placental exchange barrier.

Evidence from Other Studies

Ussing was the first to derive an equation explicitly from simple kinetic considerations which provided a general method of characterizing the transport of a substance across a membrane as either "passive" or "active" (33). The flux-ratio equation makes it possible to consider the forces influencing the transport of a substance through the membrane without the requirement that all parameters of the membrane be known.

The assumptions made in the derivation of this equation are that the diffusing substances all pass through the same pores in a continuous membrane, that they are in the same chemical state within the membrane as they are on both sides of the membrane, and that they are in thermodynamic equilibrium at the interfaces of the solutions with the membrane (15). After a steady state has been established, the flux of an ion across an area perpendicular to the direction of diffusion is:

$$J_i = \frac{AUa_i}{G}\left(-\frac{d\mu}{dx} - zF\frac{d\Psi}{dx} - \frac{dP}{dx}\right) \qquad (4)$$

where J is the flux of the ion (moles·cm^{-2}·sec^{-1}), A is the area of the membrane (cm^2), U is the mobility of the ion in a unit electrochemical potential gradient, a is the activity of the ion, G is a coefficient incorporating the friction of the ion with water and with the membrane pores, x is the distance from the membrane surface within the membrane, μ is the chemical potential, z is the valence of the ion, F is the Faraday constant, Ψ is the electrical potential, and P is the hydrostatic pressure. The total electrochemical potential, $\tilde{\mu}$, of an ion is defined by:

$$\tilde{\mu} = RT \ln a + zF\Psi \qquad (5)$$

If one assumes that the transport of the ion across the membrane as a result of a bulk flow of solution through the pores is negligible in comparison to its transport by diffusion, and makes the appropriate substitution for the electrochemical potential gradient, the flux equation becomes:

$$J_i = - \frac{RTAUa_i}{G} \left(\frac{d \ln a}{dx} + \frac{zF}{RT} \frac{d\Psi}{dx} \right) \qquad (6)$$

This equation cannot be solved directly because it contains too many unknowns. However, for chemically identical ions it is possible to derive an expression for the ratio of the flux from solution 1 to solution 2 and the flux from solution 2 to solution 1.

It is useful to consider the situation that arises when two different isotopes of the same ion are diffusing from opposite sides of the membrane. Initially, each isotope is present only on one side of the membrane. If one makes the assumptions that the frictional force, G, the ionic mobility, U, and the change of electrochemical potential within the membrane, du/dx, are the same for both ions, and integrates over the thickness of the membrane, and as was shown by

Ussing (33) the ratio of the fluxes is:

$$\frac{J_1}{J_2} = \frac{a_1}{a_2} \; e^{(zF/RT)(\Psi_1 - \Psi_2)}$$

If one makes the assumption that the activity coefficient, , is the same for the ions in the solutions on both sides of the membrane, the equation reduces to a form that contains expressions for measurable variables only:

$$\frac{J_1}{J_2} = \frac{c_1}{c_2} \; e^{(zF/RT)(\Psi_1 - \Psi_2)}$$

where c is the concentration of the ion. Thus, measurement of the fluxes of ions across a membrane will provide useful information about the electrical potential difference across that membrane.

Recently, the bidirectional fluxes of sodium across the placenta of the sheep have been measured (34). The mean ($\pm$ S.E.M.) flux from the ewe to the fetus was 0.137 $\pm$ 0.015 mM/min and from the fetus to the ewe was 0.142 $\pm$ 0.029 mM/min. The simplest explanation of these observed equal fluxes is that there is a negligible difference in electrical potential across the membrane where placental exchange takes place.

Other investigators have measured the bidirectional fluxes of cations across the perfused placentas of guinea pigs after the fetuses had been removed. Within the limits of experimental errors the fluxes have been found to be equal for Na^+, (3, 7, 26), K^+ (3, 7), and Rb^+ (3, 7). The physiological significance of these findings is not certain because it is possible that some of the forces governing fluxes across the perfused placentas were different than those in the intact guinea pig.

A Model

The results of these experiments raise several intriguing questions. Where is the potential difference between mother and fetus generated if not at the placental barrier that separates them? Does the existence of a potential difference between them necessarily affect the fluxes of electrolytes across the exchange barrier of the placenta? Does it affect the placental permeabilities measured with isotopically labeled electrolytes such as $^{22}Na^{+}$ and $^{36}Cl^{-}$?

It will be easier to answer these questions with a concrete conceptual model such as the one shown in Figure 12. This model is based on data obtained from experiments on sheep and it would in no way be applicable to the guinea pig. Because there is insufficient evidence at the present time to give a complete explanation of the observed facts, the model has been proposed solely for the purpose of examining the above questions.

In this model it is assumed that there is an ion pump, located in the tissue separating the fetal allantoic fluid compartment from the maternal extracellular fluid. This site was chosen for the location of an ion pump because Crawford and McCance have demonstrated the existence of a sodium pump of the correct polarity in the chorio-allantoic membrane of the pig (10), and Mellor has demonstrated in the sheep that the source of the potential difference must be close to the placental cotyledons (19). Mellor did not find a sodium pump in the chorioallantoic membranes of sheep and goats, but it is not clear which areas of the membranes were tested in his studies. Finally, Stacey and his colleagues have shown that the potential difference between the fetus and ewe is maintained when the fetus is killed and

when the mother is killed that it disappears (27). This strongly suggests that the "pump" depends on maternal blood for its supply of oxygen. Although the chorioallantoic membrane may be considered to be the most likely site for the pump that generates the large potential difference between the fetus and ewe, the reasoning in the discussion that follows is still valid as long as the pump is in a location other than the placental exchange barrier (Figure 12).

Once the model has been defined, it is instructive to calculate the electrical resistance of the exchange barrier. This is possible because there are well defined relationships between the equivalent ionic conductivity of an ion and its coefficient of free diffusion in water (25) and therefore between its ionic conductance and its diffusional permeability in the placental barrier. Table 5 shows a compilation of the variables used in the calculation of the resistance of the exchange barrier. It is assumed that the frictional forces acting on an ion moving under the influence of an electrical gradient are the same as those acting on an ion moving under the influence of a diffusional gradient. The contribution of sodium ions to the total electrical conductivity of the barrier, G, is given by the product of the equivalent ionic conductivity of sodium, Λ_{Na^+}, the sodium concentration in plasma, $[Na^+]$, and the ratio of the placental permeability for Na^+, P, and the coefficient of free diffusion of Na^+, D, i.e., $(P/D)_{Na^+}$:

$$G_{Na^+} = \Lambda_{Na^+} \cdot [Na^+] \cdot (P/D)_{Na^+} \qquad (9)$$

The ratio P/D incorporates not only the geometry of the transmembrane passages for Na^+ but also a possible partition coefficient between membrane water and plasma. By substituting the values from Table 5

for the variables, one finds that G_{Na^+} = 2.73 mho/kg. The conductance is expressed per kilogram because placental permeabilities are expressed per kilogram fetal weight. Similarly, G_{Cl^-} = 3.29 mho/kg. The combined contributions of the sodium and chloride conductances to the total membrane conductance is therefore 6.02 mho/kg or about 18 mho for a typical 3 kg fetus. The corresponding inverse is the placental resistance of 0.06 ohm. Taking into account the conductances of the other electrolytes present in plasma would only slightly lower this value.

Except for the fact that the electrical potentials of the allantoic fluid and the fetal extracellular fluid are about -90 and -50 mV with respect to the ewe (19), it is not known how the voltages in the circuit depicted in Figure 12 are distributed. However, they must be distributed in proportion to the distribution of electrical resistances in the circuit. Therefore, the very low electrical resistance is in complete accord with the conclusion that there is almost no difference in electrical potential across the barrier where exchange of materials between mother and fetus takes place.

Suppose that the potential difference across the placental exchange barrier is -5 mV; this is the largest possible value permitted by the 95% confidence limits for the transplacental potential difference calculated from the electrochemical equilibrium potential for $^{82}Br^-$. It follows that there must be a potential difference of 45 mV in the remainder of the circuit between the ewe and the lamb. A placental resistance of 0.06 ohm and a potential difference of 5 mV represent a current of 83.3 mA; the total resistance of the remainder of the circuit must therefore be 0.48 ohm. In Figure 12, one-half of this value has

been arbitrarily assigned to the resistance between the maternal placental capillaries and the maternal extracellular fluid at the site where the potential difference is generated. A current of 83.3 mA requires that there be a resistance of 0.54 ohm to account for the difference in electrical potential between the allantoic fluid and the fetal extracellular fluid. The actual current is almost certainly less than 83.3 mA because it is unlikely that the potential difference across the exchange barrier is as high as -5 mV. The larger the transplacental potential difference the lower the other calculated resistances in the circuit will be. In this model the allocation of the total resistance between fetus and mother is arbitrary, except for the value of the resistance of the placental membrane itself, but the actual values of particular resistances in other parts of the circuit will not affect the conclusions below.

The only way in which a remote ion pump can affect the ionic fluxes across the exchange barrier is by changing the transbarrier potential difference. The results show that the absolute value of the transmembrane potential is unlikely to exceed 2 mV (Tables 1 and 4). A potential difference of 2 mV corresponds to a concentration ratio of 1.08 for a monovalent ion in maternal and fetal plasmas. One may conclude, therefore, that if the concentration ratios of monovalent ions are greater than 1.08, that the effect of the electrical potential difference on the resulting fluxes is negligible. Because the concentration ratios of common plasma electrolytes are of magnitudes comparable to the value of 1.08 (Table 1) it is a matter of some physiological consequence whether the transmembrane potential is -2 mV or only 0.2 mV.

Unfortunately, there are no certain methods at the present time for determining the exact value of the transmembrane potential at the exchange barrier.

It is possible to make a definite statement about the effect of the transplacental potential difference on the measured values of permeability of the placenta to radiolabeled monovalent electrolytes. If the concentration ratios are much greater than 1.08 (as is the usual case when making such measurements), the electrical potential difference across the exchange barrier can be ignored.

Thus, the results of these experiments refute the commonly held notions that the large difference in electrical potential that has been measured between mother and fetus is generated at the placental exchange barrier itself and that this potential difference requires active transport of one or more ions between the mother and her fetus.

SUMMARY AND CONCLUSIONS

If sufficient time is allowed to reach a steady state the transplacental electrical potential calculated from maternal and fetal plasma concentrations of bromide, sulfate, rubidium and lithium ions in the guinea pig and of bromide and lithium ions in the sheep is essentially zero. This agrees with the transplacental potential calculated from the concentrations of sodium, potassium, magnesium and chloride ions normally found in maternal and fetal plasmas. If the large electrical potential difference between mother and fetus is generated at the placental exchange barrier the concentrations of the electrolytes in the fetal plasma would have to be maintained by the expenditure of energy. It is highly unlikely that specific ionic pumps would exist for each of the ions not normally found in plasma or that existing pumps would have the appropriate affinity for these exogenous ions to maintain equal concentrations of them in maternal and fetal plasmas. These results are best explained by the presence of no more than a very small transplacental difference in electrical potential and the generation of a potential difference between mother and fetus at a site other than the exchange barrier.

TABLE 1

Distribution of electrolytes across the placenta of the sheep under steady state conditions (2).

Electrolyte	Concentration in maternal plasma (mM/l)	Concentration in fetal plasma (mM/l)	Equilibrium potential* (mV)
Na^+	145.9	143.5	+ 0.4
K^+	4.5	3.5	+ 3.6
Mg^{++}	0.83	0.80	+ 0.5
Cl^-	107.6	102.2	- 1.4

* A positive sign indicates that fetal plasma is positive and a negative sign indicates that it is negative with respect to maternal plasma in the placental capillaries.

TABLE 2

Distribution of electrolytes across the placenta of the guinea pig under steady state conditions (36).

Electrolyte	Concentration in maternal plasma (mM/kg water)	Concentration in fetal plasma mM/kg water)	Equilibrium potential* (mV)
Na^+	142.2	140.8	+ 0.5
K^+	4.5	4.4	+ 0.6
Mg^{++}	1.1	1.0	+ 1.3
Cl^-	102.7	103.3	+ 0.2

* A positive sign indicates that fetal plasma is positive and a negative sign that it is negative with respect to maternal plasma in the placental capillaries.

TABLE 3

Mean (± S.E.M.) electrically measured potential differences between guinea pig mother and fetus and transplacental potential differences calculated from steady state concentrations of exogenous electrolytes (6).

Tracer	Time after injection (hr)	Calculated transplacental potential difference (mV)	Measured maternofetal potential difference (mV)	Fetal weight (g)
$^{35}SO_4^{=}$ (n)	≥ 2	-0.87 ± 0.3 * (21)	-33 ± 2 * (19)	71.7 ± 6.3 (21)
$^{86}Rb^{+}$ (n)	≥ 8	+0.85 ± 0.7 + (10)	-34 ± 3 * (10)	87.8 ± 5.5 (10)
$^{82}Br^{-}$ (n)	≥ 6	-0.34 ± 0.9 + (12)	-44 ± 3 * (12)	51.5 ± 10.3 (12)
Li^{+} (n)	≥ 7	0 ± 5 + (17)	-37 ± 3 * (17)	57.4 ± 6.0 (17)

*Significantly different from zero, $p < 0.01$

+ Not significantly different from zero, $p > 0.1$

TABLE 4

Mean transplacental potential differences calculated from the steady state concentrations of exogenous electrolytes in maternal and fetal plasmas of sheep (31).

Tracer	Time after injection (hr)	Calculated transplacental potential (± S.E.M.) (mV)
$^{82}Br^-$	≥ 85	-2.2 ± 0.8
Li^+	≥ 60	+5.2 ± 2.2

TABLE 5

Quantities and estimated values used in calculation of the placental resistance of the sheep. These values are at 39° C and at 0.3 M in water where applicable (31).

Quantity and units	Value for Na^+	Value for Cl^-	Reference
Equivalent ionic conductivity, $cm^2\ ^{-1}\ eq^{-1}$	51	77	(25)
Coefficient of free diffusion, $cm^2\ s^{-1}$	1.9×10^{-5}	2.9×10^{-5}	(17)
Permeability in sheep placenta*, $cm^3\ s^{-1}\ kg^{-1}$	6.8×10^{-3}	1.2×10^{-2}	(14, 32)
Plasma concentration, $eq\ cm^{-3}$	1.45×10^{-4}	1.05×10^{-4}	(2)

*Expressed per kg fetal weight

Figure 1. Maternal and fetal plasma concentrations of $^{82}Br^-$ expressed as ratios of the concentration in maternal plasma thirty minutes after i.v. injection into the sow (6).

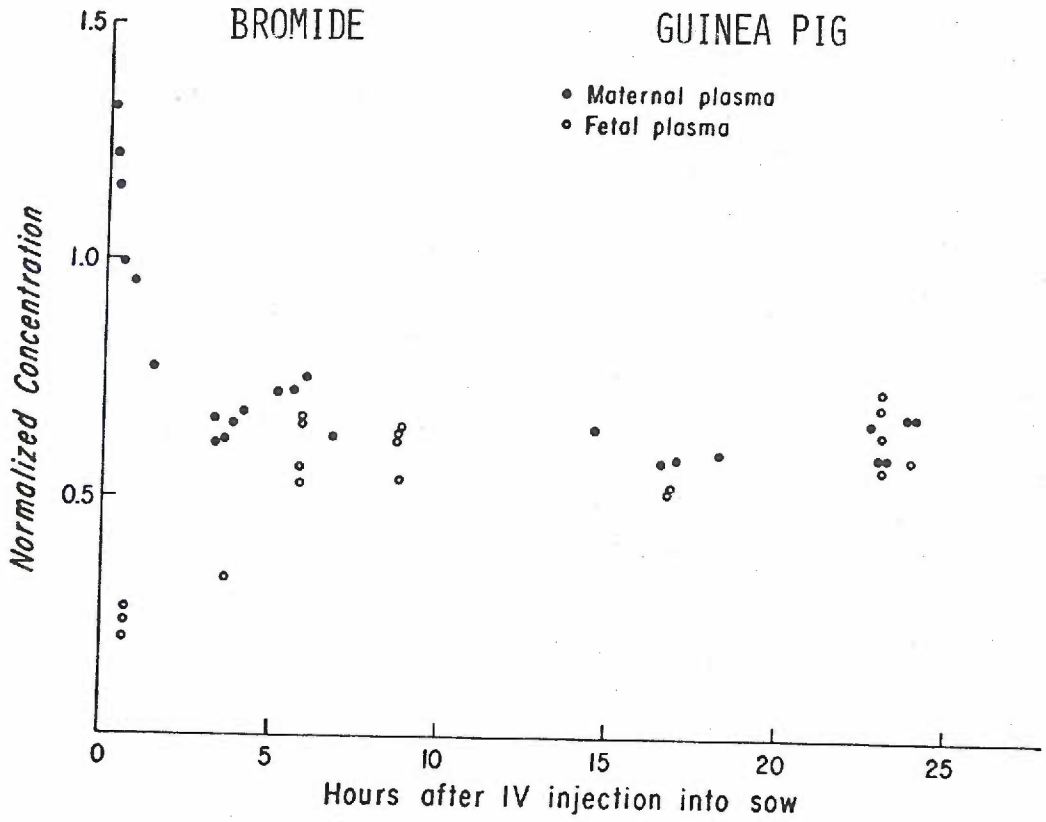

BROMIDE
GUINEA PIG
• Maternal plasma
○ Fetal plasma
Normalized Concentration
1.5
1.0
0.5
0
5
10
15
20
25
Hours after IV injection into sow

Figure 2. Maternal and fetal plasma concentrations of $^{35}SO_4^=$ expressed as ratios of the concentration in maternal plasma thirty minutes after i.v. injection into the sow (6).

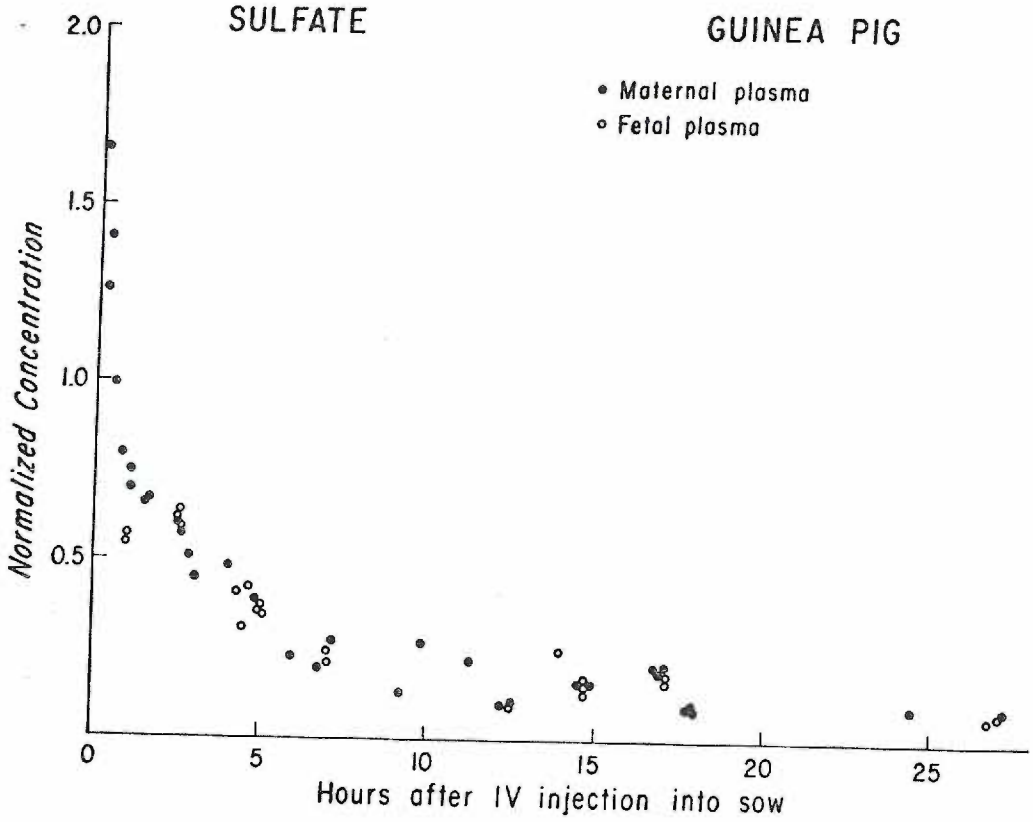

SULFATE
GUINEA PIG
• Maternal plasma
○ Fetal plasma
Normalized Concentration
2.0
1.5
1.0
0.5
0
5
10
15
20
25
Hours after IV injection into sow

Figure 3. Maternal and fetal plasma concentrations of $^{86}Rb^{+}$ expressed as ratios of the concentration in maternal plasma thirty minutes after i.v. injection into the sow (6).

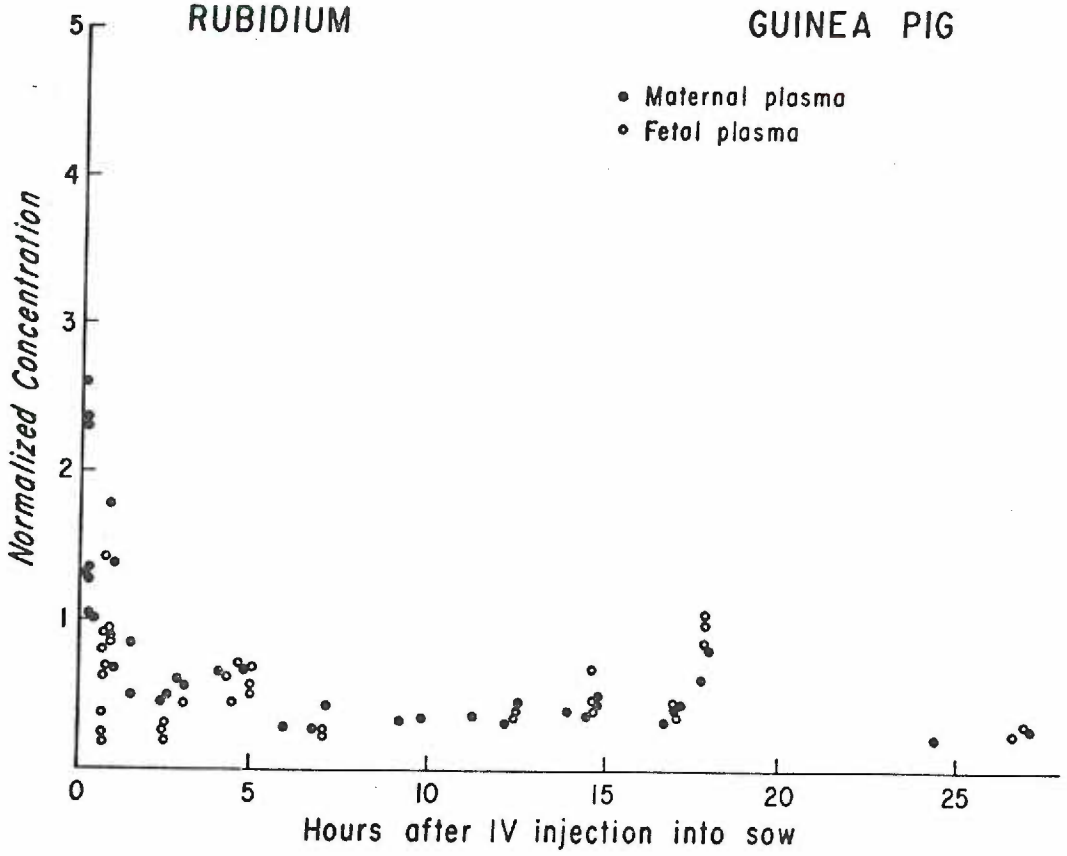

RUBIDIUM
GUINEA PIG
• Maternal plasma
○ Fetal plasma
Normalized Concentration
5
4
3
2
1
0
5
10
15
20
25
Hours after IV injection into sow

Figure 4. Maternal and fetal plasma concentrations of Li^+ expressed as ratios of the concentration in maternal plasma thirty minutes after i.v. injection into the sow (6).

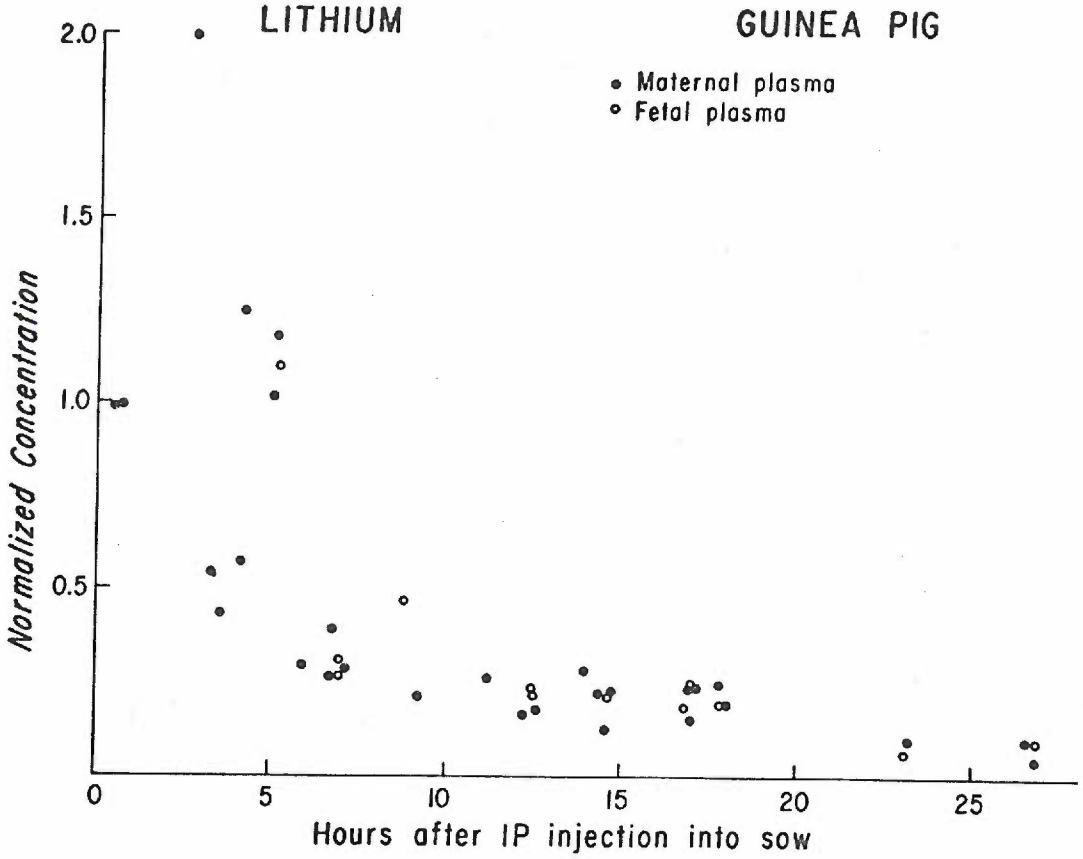

LITHIUM
GUINEA PIG
Maternal plasma
Fetal plasma
Normalized Concentration
2.0
1.5
1.0
0.5
0
5
10
15
20
25
Hours after IP injection into sow

Figure 5. Concentrations of $^{35}SO_4^{=}$ in maternal and fetal plasmas and extrafetal fluids after i.v. injection of the tracer into the ewe at time zero. The concentration scale is logarithmic. The dashed line indicates the concentration at which radiosulfate in fetal plasma would have been in electrochemical equilibrium with radiosulfate in maternal plasma, if there had been an electrical potential difference of -50 mV across the exchange barrier. The fetus in this experiment was not nephrectomized (31).

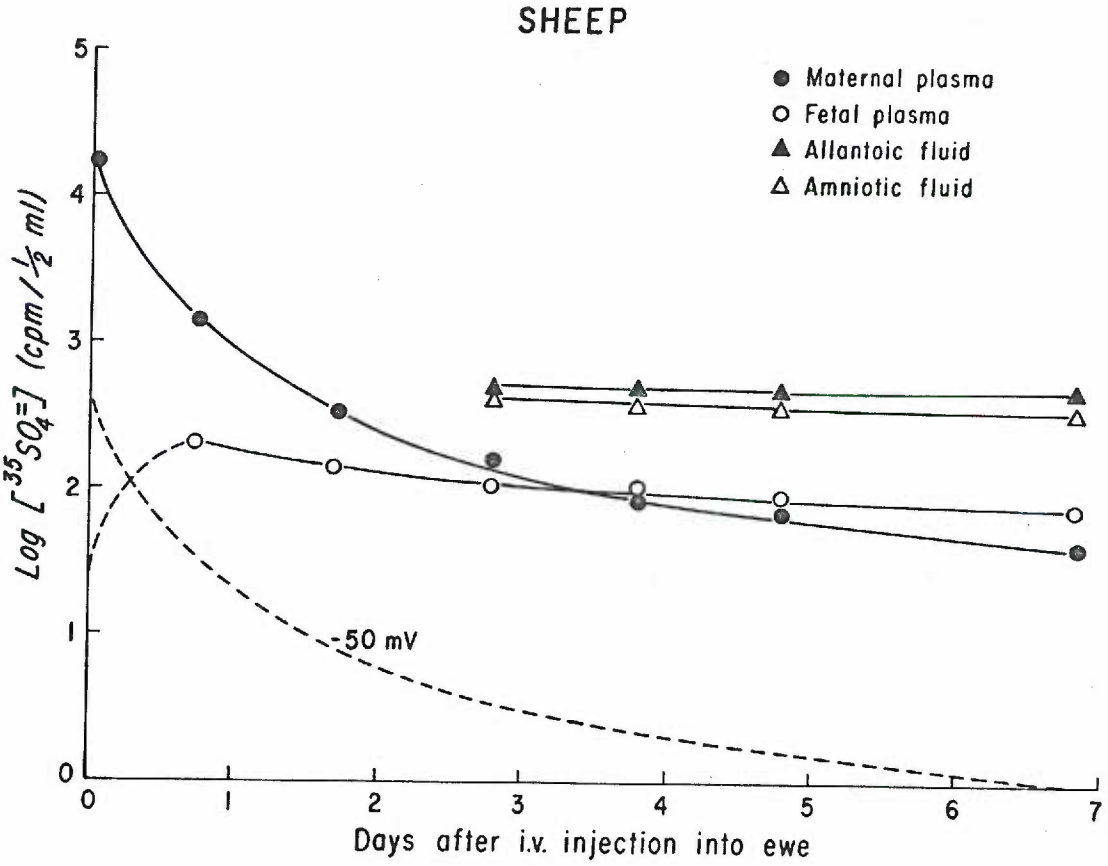

SHEEP
Maternal plasma
Fetal plasma
Allantoic fluid
Amniotic fluid
Log [35SO4=] (cpm/½ ml)
-50 mV
Days after i.v. injection into ewe
0
1
2
3
4
5
6
7

Figure 6. Concentrations of $^{35}SO_4^{=}$ in maternal and fetal plasmas after i.v. injection of the tracer into four ewes carrying four nephrectomized fetuses at time zero. The concentrations in maternal plasmas sampled at 3½ days were set equal to 100 units and other concentrations were normalized by making proportionate adjustments. The concentration scale is logarithmic. The dashed line indicates the concentration at which radiosulfate in fetal plasma would have been in electrochemical equilibrium with radiosulfate in maternal plasma, if there had been a difference in electrical potential of -50 mV across the exchange barrier (31).

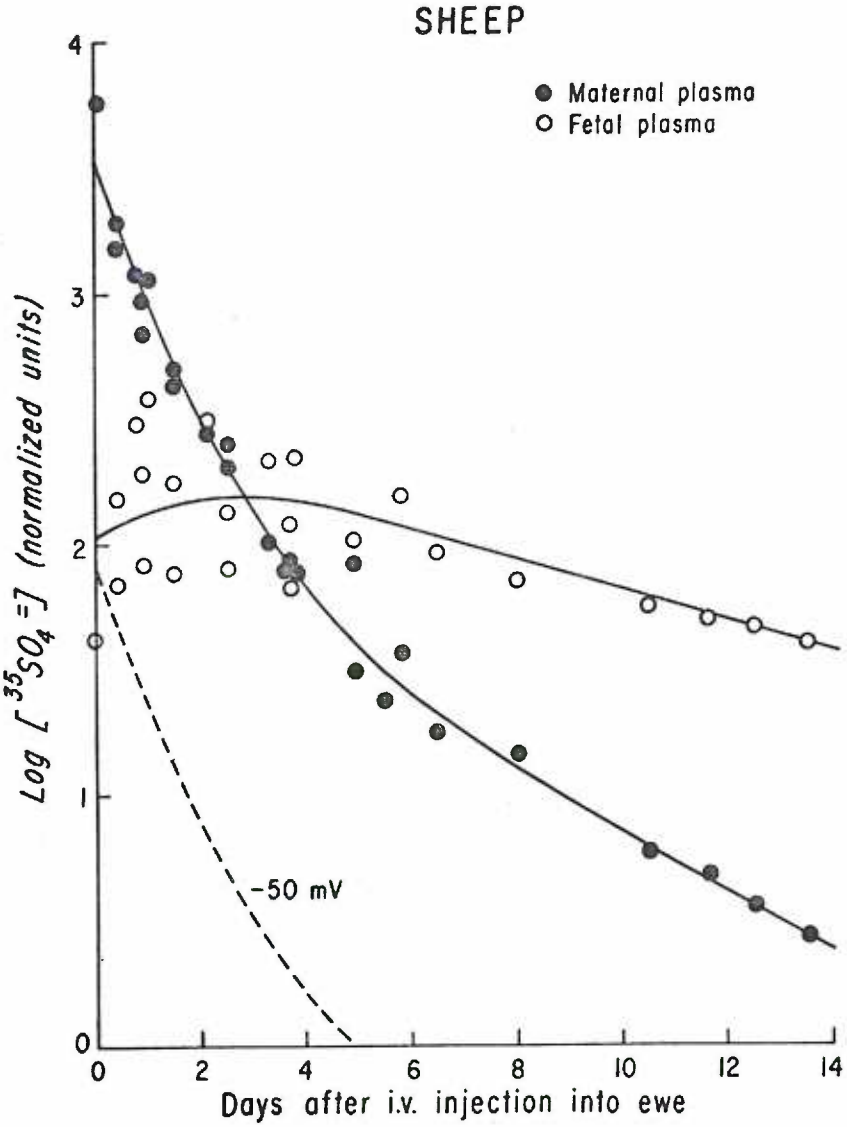

SHEEP
Maternal plasma
Fetal plasma
Log [$^{35}SO_4^{=}$] (normalized units)
4
3
2
1
0
-50 mV
0
2
4
6
8
10
12
14
Days after i.v. injection into ewe

Figure 7. Concentrations of $^{82}Br^-$ in maternal and fetal plasmas and amniotic fluid after i.v. injection of the tracer into the ewe at time zero. The concentration scale is logarithmic. The dashed line indicates the concentration at which radiobromide in fetal plasma would have been in electrochemical equilibrium with radiobromide in maternal plasma if there had been a difference in electrical potential of -50 mV across the exchange barrier. Concentrations of $^{82}Br^-$ in the amniotic fluid of this nephrectomized fetus paralleled those in fetal plasma (31).

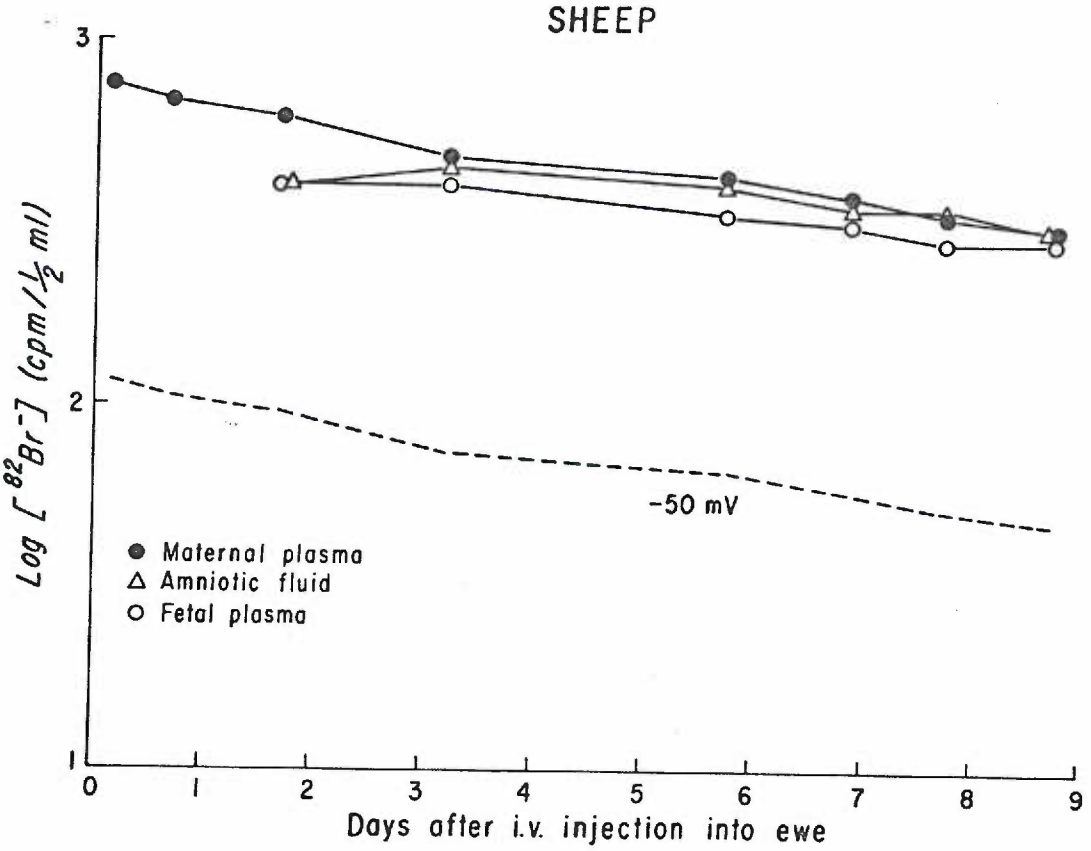

SHEEP
3
2
1
Log [$^{82}Br^-$] (cpm/½ ml)
-50 mV
Maternal plasma
Amniotic fluid
Fetal plasma
0
1
2
3
4
5
6
7
8
9
Days after i.v. injection into ewe

Figure 8. Concentrations of $^{82}Br^-$ in maternal and fetal plasmas after i.v. injection of the tracer into four ewes carrying one intact and four nephrectomized fetuses at time zero. The concentrations in maternal plasmas sampled at 3½ days were set equal to 100 units and other concentrations were normalized by making proportionate adjustments. In this figure, the concentration scale is linear (31).

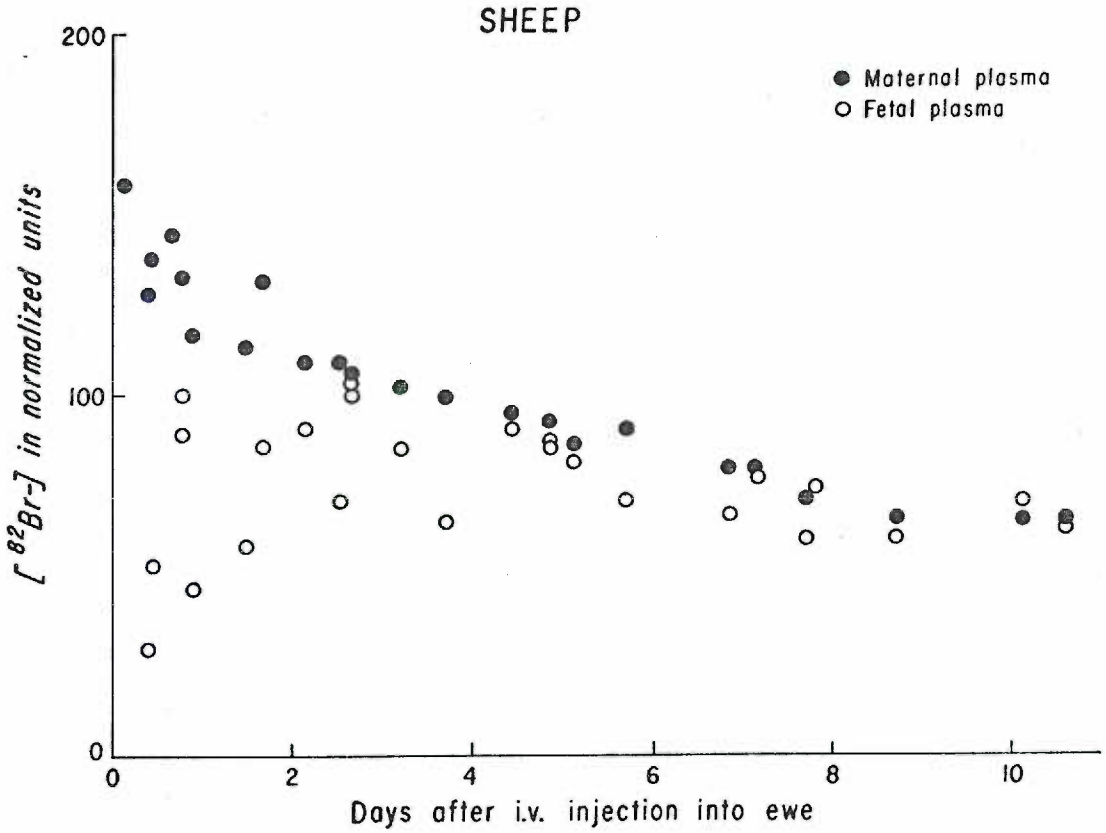
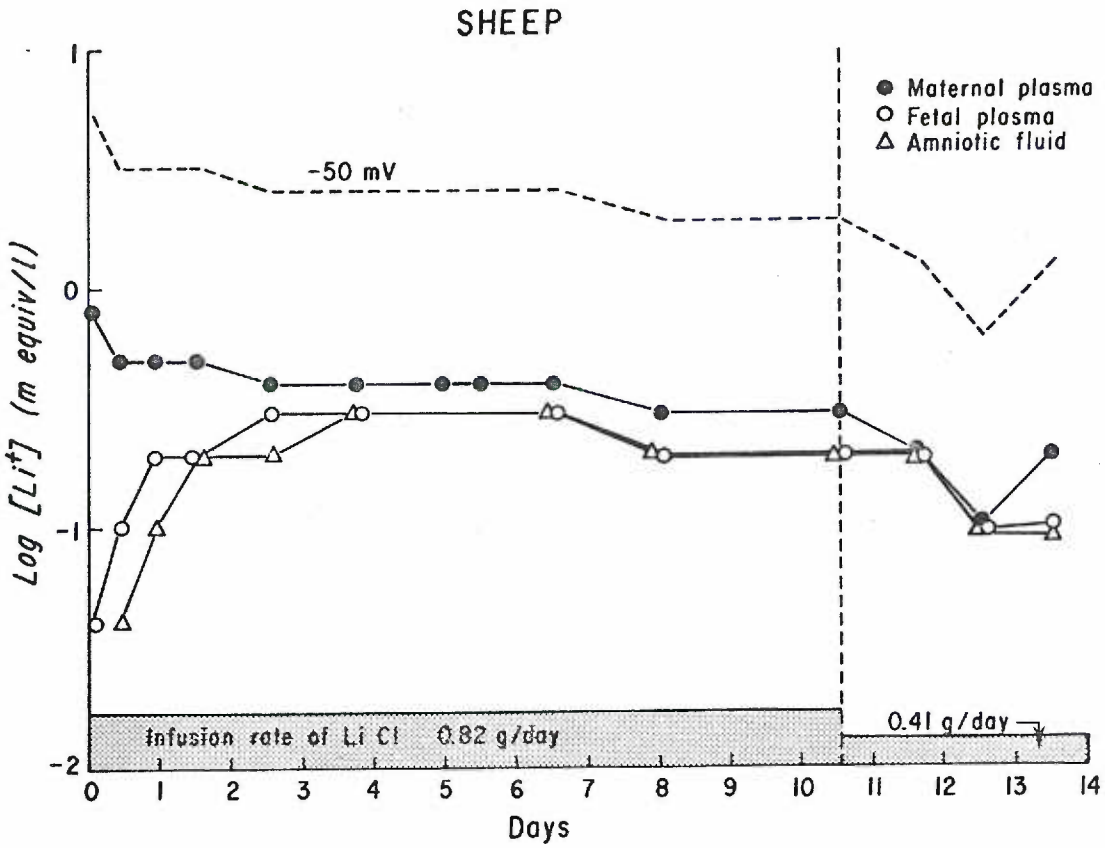
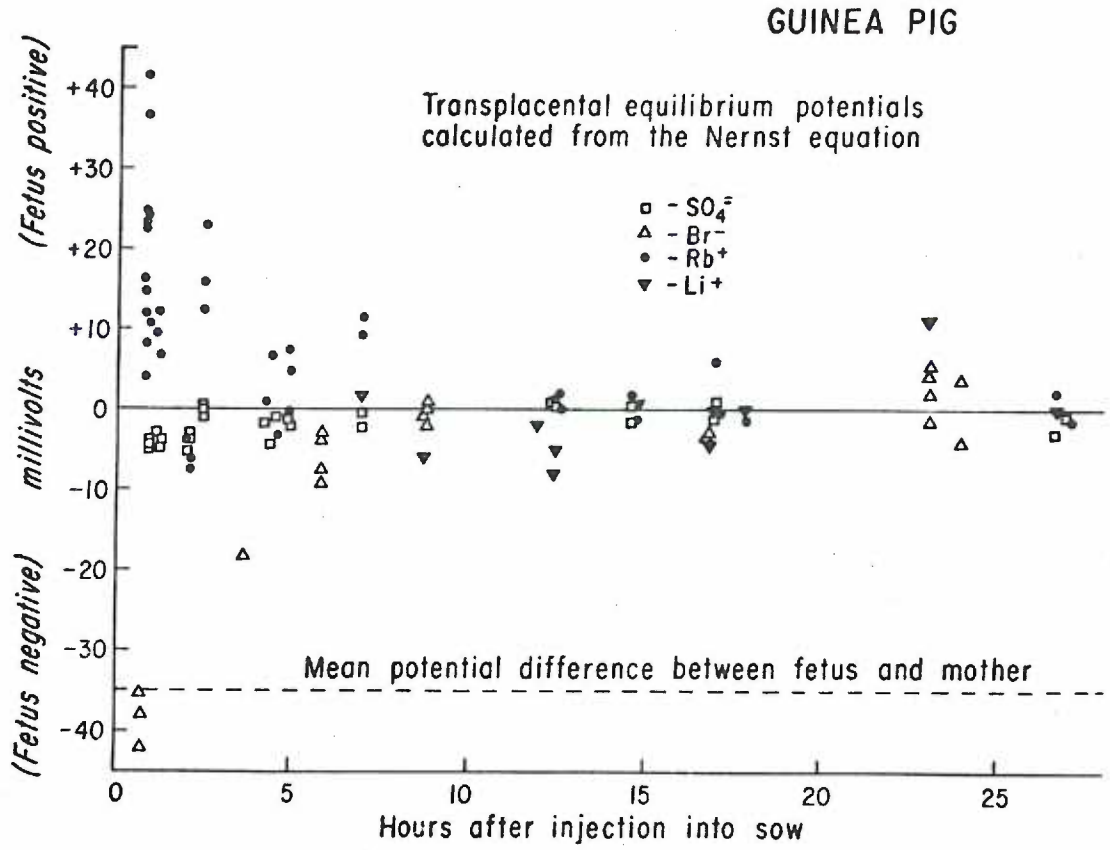

SHEEP
Maternal plasma
Fetal plasma
200
100
0
[82Br-] in normalized units
0
2
4
6
8
10
Days after i.v. injection into ewe

Figure 9. Concentrations of Li^+ in maternal and fetal plasmas after beginning a continuous i.v. infusion of LiCl into the ewe at time zero. The concentration scale is logarithmic. The dashed line indicates the concentration at which Li^+ in fetal plasma would have been in electrochemical equilibrium with Li^+ in maternal plasma, if there had been a difference in electrical potential of -50 mV across the exchange barrier. In this nephrectomized fetus the concentrations of Li^+ in amniotic fluid paralleled those in fetal plasma. Decreases in fetal plasma concentration, following decreases in maternal plasma concentration, show that the failure of fetal plasma concentration to rise to the level of the dashed line is not due to a diffusional limitation of the exchange barrier (31).


SHEEP
Maternal plasma
Fetal plasma
Amniotic fluid
-50 mV
Log [Li+] (m equiv/l)
1
0
-1
-2
infusion rate of Li Cl 0.82 g/day
0.41 g/day
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days


GUINEA PIG
Transplacental equilibrium potentials
calculated from the Nernst equation
□ - $SO_4^=$
△ - Br^-
● - Rb^+
▼ - Li^+
(Fetus positive)
millivolts
(Fetus negative)
+40
+30
+20
+10
0
-10
-20
-30
-40
Mean potential difference between fetus and mother
0
5
10
15
20
25
Hours after injection into sow

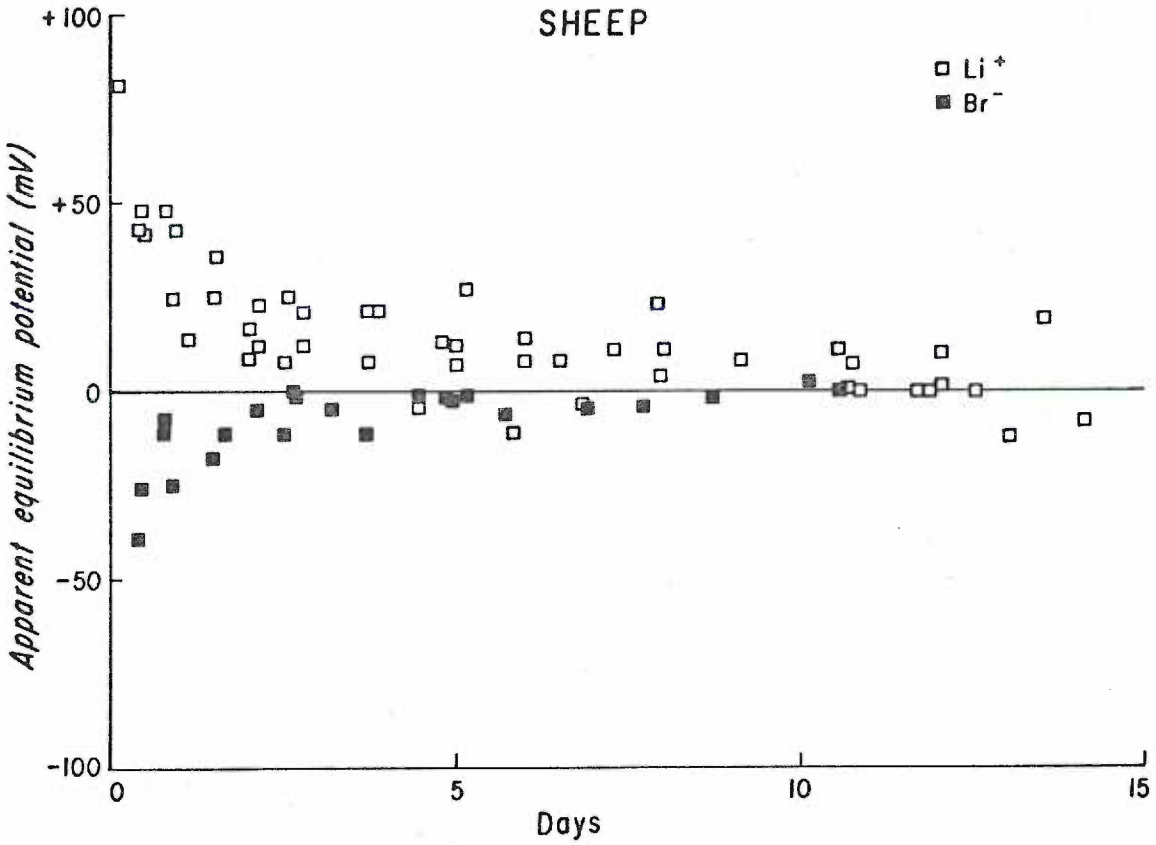
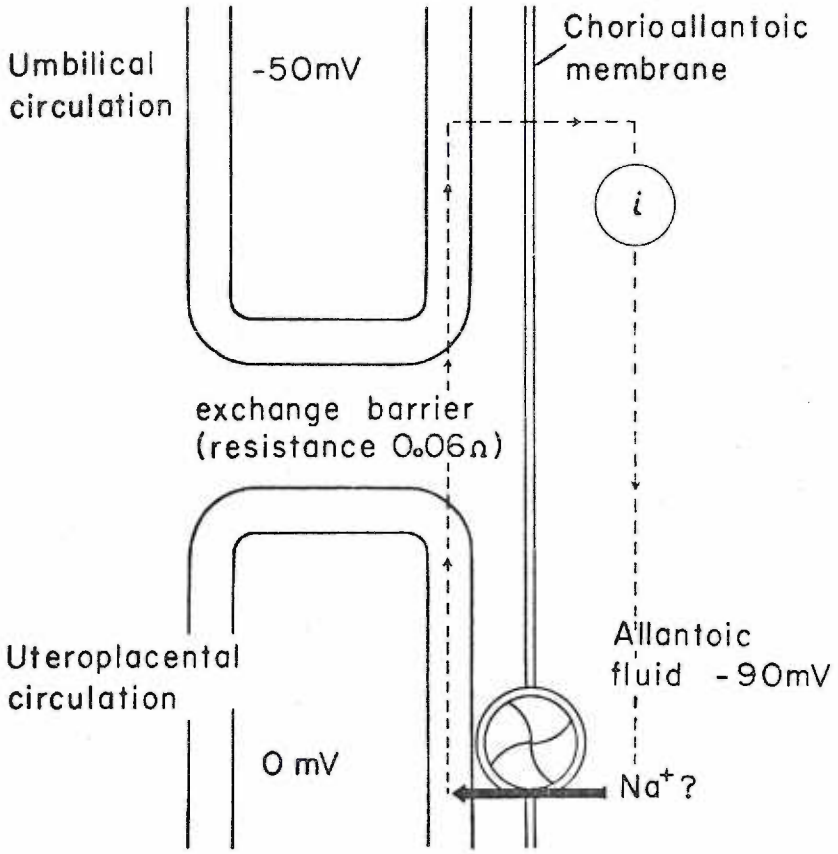

SHEEP
Li+
Br−
+100
+50
0
-50
-100
Apparent equilibrium potential (mV)
0
5
10
15
Days

Figure 12. One of several models that would fit presently available data. In this model, the electrogenic "pump" is located in the chorioallantoic membrane that separates the allantoic fluid and the maternal extracellular fluid compartments of the sheep. In this location a single ion pump could account for the ca. -90 mV potential difference between allantoic fluid and maternal extracellular fluid and the ca. -50 mV potential difference between fetal extracellular fluid and maternal extracellular fluid. The current, i, in the circuit must be the same everywhere in the circuit since the capacitances are negligible. Voltage distribution therefore reflects the distribution of electrical resistances in various parts of the circuit in accordance with Kirkhoff's second law. The fact that Mellor (19) demonstrated that the difference in electrical potential between fetus and ewe disappears when the umbilical cord is occluded makes it probable that the circuit depicted here is located at or near the cotyledonary level. The resistance between the fetal extracellular fluid compartment and the allantoic fluid compartment would have to be equal to one half of the total resistance of the circuit to account for the distribution of voltages, unless as it is probable, there are external shunts on the circuit that are not incorporated in this diagram. Unless the resistance of the entire circuit is less than 1½ ohm, the potential drop across the exchange barrier will be less than 2 mV (31).


Umbilical
circulation
-50mV
Chorioallantoic
membrane
i
exchange barrier
(resistance 0.06Ω)
Uteroplacental
circulation
0 mV
Allantoic
fluid -90mV
Na+?

BIBLIOGRAPHY

1. Alexander, D. P., Nixon, D. A., Widdas, W. F., & Wohlzogen, F. X. Gestational variations in the composition of the foetal fluids and foetal urine in the sheep. J. Physiol. London, 1958. 140, 3-13.

2. Armentrout, T. A., Katz, S., Thornburg, K. L., and Faber, J. J. Osmotic flow through the placental barrier of the chronically prepared sheep. Am. J. Physiol., 1977. 233, H466-H474.

3. Bailey, D. J., Bradbury, M. W. B., France, V. M., Hedley, R., Saroj, N., & Parry, H. Cation transport across the guinea-pig placenta perfused in situ. J. Physiol. London, 1979. 287, 45-56.

4. Barry, P. H., & Diamond, J. M. Junction potentials, electrode standard potentials, and other problems in interpreting electrical properties of membranes. J. Membrane Biol, 1970. 3, 93-122.

5. Binder, N. D., Faber, J. J., & Thornburg, K. L. The transplacental potential difference as distinguished from the maternal-fetal potential of the guinea-pig. J. Physiol. London, 1978. 282, 561-570.

6. Bradbury, M. W. B., Crowder, J., Desai, S., Reynolds, J. M., Reynolds, M., & Saunders, N. R. Electrolytes and water in the brain and cerebrospinal fluid of the foetal sheep and guinea-pig. J. Physiol. London, 1972. 227, 591-610.

7. Bradbury, M. W. B., France, V. M., Hedley, R., & Parry, H. Transport of cations by the guinea-pig placenta perfused in situ. J. Physiol. London, 1977. 272, 22P-23P.

8. Comline, R. S., & Silver, M. Daily changes in foetal and maternal blood of conscious pregnant ewes, with catheters in umbilical and uterine vessels. J. Physiol. London, 1970. 209, 567-586.

9. Conrad, E. E., and Faber, J. J. Water and electrolyte acuisition across the placenta of the sheep. Am. J. Physiol., 1977. 233, H475-H487.

10. Crawford, J. D., & McCance, R. A. Sodium transport by the chorio-allantoic membrane of the pig. J. Physiol. London, 1960. 151, 458-471.

11. Dawes, G. S. Foetal blood gas tensions and pH. In Foetal and neonatal physiology. Oxford: Year Book Medical Publishers, Inc., 1969. pp. 106-116.

12. Evans, H. E., & Sack, W. O. Prenatal development of domestic and laboratory animals. Growth curves, external features, and selected references. Anat., Histol., Embryol., 1973. 2, 11-45.

13. Faber, J. J., & Green, T. J. Foetal placental blood flow in the lamb. J. Physiol. London, 1972. 223, 375-393.

14. Faber, J. J., & Thornburg, K. L. Fetal homeostasis in relation to placental water exchange. Ann. Rech. Vet., 1977. 8, 353-360.

15. Hoshiko, T., & Lindley, B. D. The relationship of Ussing's flux-ratio equation to the thermodynamic description of membrane permeability. Biochim. Biophys. Acta, 1964. 79, 301-317.

16. Kaiser, I. H., & Cummings, J. N. Plasma electrolytes of the pregnant ewe and fetal lamb. Am. J. Physiol., 1958. 193, 627-633.

17. Longsworth, L. G. Diffusion in liquids. In D. E. Gray (Ed.) American Institute of Physics Handbook. New York, N. Y.: McGraw, 1957. pp. II 189 - II 195.

18. Mellor, D. J. Potential differences between mother and foetus at different gestational ages in the rat, rabbit, and guinea-pig. J. Physiol. London, 1969. 204, 395-405.

19. Mellor, D. J. Distribution of ions and electrical potential differences between mother and foetus at different gestational ages in goats and sheep. J. Physiol. London, 1970. 207, 133-150.

20. Mellor, D. J., Cockburn, F., Lees, M. M., & Blagden, A. Distribution of ions and electrical potential differences between mother and foetus in the human at term. J. Obstet. Gynaec., 1979. 76, 993-998.

21. Mellor,. J., & Slater, J. S. The use of chronic catheterization techniques in foetal sheep. Brit. Vet. J., 1973.

22. Meschia, G., Wolkoff, A. S., & Barron, D. H. Difference in electrical potential across the placenta of goats. Proc. Natn. Acad. Sci. U.S.A., 158. 44, 483-485.

23. Meschia, G., Cotter, J. R., Breathnach, C. S., & Barron, D. H. The hemoglobin, oxygen, carbon dioxide, and hydrogen ion concentrations in the umbilical bloods of sheep and goats as sampled via indwelling plastic catheters. Quart. J. Exp. Physiol., 1965. 50, 185-195.

24. Noble, D. The initiation of the heartbeat. Oxford: Clarendon, 1975.

25. Robinson, R. A., and Stokes, R. H. Electrolyte solutions (2nd Ed.). London: Butterworths, 1959.

26. Schröder, H., Stolp, W., & Leichtweiss, H. P. Measurements of Na^+ transport in the isolated artifically perfused guinea-pig placenta. Am. J. Obstet. Gynec., 1972. 114, 51-57.

27. Stacey, T. E., Boyd, R. D. H., Ward, R. H. T., & Weedon, A. P. Placental permeability in the sheep. Ann. Rech. Vet., 1977. 8, 345-352.

28. Štulc, J., Rietveld, W. J., Soeteman, D. W., & Versprille, A. The transplacental potential difference in guinea pigs. Biol. Neonat., 1972. 21, 130-147.

29. Štulc, J., & Švihovec, J. Effects of potassium cyanide, strophanthin or sodium-free perfusion fluid on the electrical potential difference across the guinea-pig placenta perfused in situ. J. Physiol. London, 1973. 231, 403-415.

30. Štulc, J., & Švihovec, J. Placental transport of sodium in the guinea-pig. J. Physiol. London, 1977. 265, 691-703.

31. Thornburg, K. L., Binder, N.D., & Faber, J. J. Distribution of ionic sulfate, lithium, and bromide across the sheep placenta. Am. J. Physiol., 1979. 236, C58-C65.

32. Thornburg, K. L., Binder, N. D., & Faber, J. J. Diffusion permeability and ultrafiltration reflection coefficient of Na^+ and Cl^- in the near term placenta of the sheep. J. Devel. Physiol., 1979. In press.

33. Ussing, H. H. Distinction by means of tracers between active transport and diffusion. The transfer of iodide across the isolated frog skin. Acta Physiol. Scand., 1949. 19, 43-56.

34. Weedon, A. P., Stacey, T. E., Ward, R. H.T., & Boyd, R. D. H. Bidirectional sodium fluxes across the placenta of conscious sheep. Am. J. Physiol., 1978. 235, F536-F541.

35. Widdas, W. F. Transport mechanisms in the foetus. Br. Med. Bull., 1961. 17, 107-111.

36. Woods, L. L., Thornburg, K. L., & Faber, J. J. Transplacental gradients in the guinea pig. Am. J. Physiol., 1978. 235, H200-H207.

37. Wright, G. H. The potential difference between amniotic fluid and maternal extracellular fluid in the rabbit. Biol. Neonat., 1966. 10, 193-199.