THE INFLUENCE OF VASOPRESSIN AND PROLACTIN ON THE MOVEMENT
OF WATER AND SODIUM THROUGH THE ISOLATED AMNION
OF THE FETAL GUINEA-PIG

by

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ABSTRACT

Unidirectional flux of tritiated water across isolated guinea-pig amnion was studied in a perfusion cell supplied with amniotic saline at 37°C by means of a continuous double-circulation system. The diffusional permeability in the absence of an osmotic gradient was $3.09 \pm 0.15 \times 10^{-4}$ cm sec$^{-1}$ for maternal-fetal flow (10 experiments), and $2.62 \pm 0.14 \times 10^{-4}$ cm sec$^{-1}$ for fetal-maternal flow (13 experiments). The addition of vasopressin (50-500 mU/ml) to the fetal side of sixteen membranes set up between amniotic and maternal salines increased isotopic water flux in the maternal-fetal direction against an osmotic gradient of 28 m0sm/l. Treatment of eight other membranes with 10 μg/ml prolactin reduced fetal-maternal water flow by up to 17.0% at the end of 3 hours, in the absence of an osmotic gradient. This contrasted with five control experiments, in which fetal-maternal flow increased by 5.1% at the end of 3 hours. Therefore, the prolactin appeared to reduce diffusional fetal-maternal water flow by up to 22.1% within 3 hours.

In twelve further experiments, net flow (fetal-maternal) was measured gravimetrically. The membranes were set up between amniotic and maternal salines, in conditions which paralleled the natural ionic environment, and the natural
hydrostatic and osmotic gradients. In five experiments, the addition of prolactin (20 μg/ml) to the fetal surface of the amnion, caused a decrease in fetal-maternal water flow of almost 60% at 3 hours. In contrast, seven control studies showed no such effects.

Permeability of isolated amnion to sodium was studied over the course of gestation by the use of radioactive sodium (22NaCl). Maternal-fetal sodium movement was found to increase by a factor of about 35 fold from day 57 to day 70. The treatment of membranes with vasopressin (500 mU/ml) produced an average decrease in maternal-fetal sodium movement of 12.2 ± 7.8% in the third hour (2 experiments). Thus, water movement in this direction did not seem to be coupled to sodium flux. When prolactin (10 μg/ml) was added to seven membranes, an increase in maternal-fetal sodium flux of 21.3 ± 8.3% was recorded in the third hour. Control membranes, however, showed a similar increase of 21.3 ± 14.9% in the third hour (7 experiments). Therefore, comparison of experimental and control preparations suggests that prolactin probably does not affect maternal-fetal sodium movement. In contrast to this, treatment of five other membranes with prolactin (10 μg/ml) produced an average increase in fetal-maternal sodium flux of 53.6 ± 10.1% in the third hour. Since five control membranes showed an increase of only 15.1 ± 15.9% in this same
time period, prolactin seemed to be responsible for producing an overall increase of 38.5% in unidirectional flux of sodium.

Preliminary experiments indicate that neurohypophyseal hormone can stimulate an increase in unidirectional water flux across other fetal membranes and tissues. Vasopressin (100 mU/ml) increased water flow across the isolated fetal urinary bladder in the mucosal-serosal direction by \( 49.4 \pm 17.7\% \) at the end of an hour (3 experiments); a control membrane showed an increase of only 9.7%. The addition of vasopressin (500-1000 mU/ml) increased serosal-mucosal water movement across isolated fetal skin by up to 30% in 60 minutes (4 experiments).

At the present time the effects of vasopressin and prolactin on water and/or sodium movement across isolated amnion, fetal urinary bladder, and fetal skin must be regarded as pharmacological. It seems probable, however, that some of the responses may be physiological since high levels of hormone are found in fetal blood (i.e. vasopressin) and amniotic fluid (i.e. prolactin). This study suggests that hormonal regulation of fetal hydro-mineral metabolism may explain the enigma of how hydro-osmotic homeostasis is achieved in the intrauterine compartments.
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INTRODUCTION

1. The intrauterine fluid compartments

In the primate, intrauterine water is divided into three compartments, viz., the fetal, the placental, and the amniotic fluid compartments (Kerpel-Fronius, 1970). Out of a total water volume in the human intrauterine cavity of about 3,533 ml, 2,400 ml are found in the fetal compartment, 700 ml in the amniotic fluid compartment and 433 ml in the placental compartment at 37 weeks of gestation. In the last trimester of pregnancy in the human being there are approximately 756 mEq of exchangeable Na\(^+\) and 171 mEq of exchangeable K\(^+\) (MacGillivray and Buchan, 1958). Both water and ions are in a state of dynamic flux between the three compartments.

Vosburgh et al. (1948) showed that human amniotic fluid does not form a static and stagnant pool, but one which is constantly being renewed or replaced. They demonstrated that there is a rapid exchange of heavy water and various isotopic electrolytes between the amniotic fluid and the other intrauterine compartments. Vosburgh et al. (1948) found that 35% of the water in human amniotic fluid is renewed each hour, and thus, water would be replaced about every 2.9 hours. According to Plentl and Hutchinson (1953) the period of renewal of Na\(^+\) and K\(^+\) in human amniotic fluid is about 8 hours. Hutchinson et al.
(1955) calculated that water transfer across human amnion occurs at a rate of about 420 ml/hr, while Na\(^+\) transfer was 12 mEq/hr and K\(^+\) transfer was 0.6 mEq/hr.

A number of fetal tissues and organs have been implicated as playing a role in hydro-mineral exchange between the fetal and amniotic compartments. These include:

a) the fetal lung and tracheobronchial tract,
b) the fetal salivary glands,
c) the fetal skin surface,
d) the umbilical cord,
e) the fetal gastrointestinal tract,
f) the fetal kidney and

... probably only a few of these structures, however, participate in any large scale exchange of fluid between the fetal and the other compartments.

2. Fetal tissues and organs involved in hydro-mineral exchange:

A. The fetal lung and tracheobronchial tract

During fetal life the lung can act as an exocrine gland that secretes liquid of fairly large volume in some species of mammals. This liquid is of relatively constant and unique composition. The flow rate is quite variable, and the high flow rate seems to be correlated with respiratory movements in the fetus (Adamson et al., 1973). The importance of the lung as a secretory organ varies considerably amongst species. Fetal lambs are able to secrete tracheal fluid at a rate of 50-80 ml per day
(Abramovich, 1973), while fetal monkeys produce only about 0.23 ml per day (Reynolds et al., 1971). Fetal tracheal fluid has an osmolality and sodium concentration approximately equal to fetal plasma while its pH and pCO2 are lower and its chloride concentration is 40-50 mEq higher in fetal lambs near term (Adamson et al., 1969). Olver et al. (1973) found evidence that the liquid of the fetal lung is secreted by a process involving an active transport of Cl⁻ and HCO₃⁻ by the alveolar epithelium of the lamb. Cl⁻ seems to be transported from fetal plasma in exchange for HCO₃⁻. The similarity between alveolar epithelium and gastric mucosa with regards to active transport of Cl⁻ and HCO₃⁻ may be a reflection of the common origin of the two epithelia from the embryonic foregut.

Gluck et al. (1971) demonstrated that tracheobronchial fluid is secreted into the amniotic fluid compartment. They found that an active lecithin present in amniotic fluid was similar in fatty acid composition to lecithin found in tracheal effluents, but different from lecithins found in fetal membranes, fetal urine, maternal blood, and the placenta of the human being. Alveolar or tracheal secretion probably does not contribute significantly to amniotic fluid volume in the human being (Bourne, 1962; Jeffcoate and Scott, 1959). In other species of mammals such as the guinea-pig and goat, the fetal lung may make a
fairly large contribution to amniotic fluid volume in the last 1/3 of pregnancy (Adams et al., 1967). The fact that the lungs of many fetal mammals secrete relatively small volumes of fluid means that respiratory secretions are probably unimportant in osmoregulation or in maintaining acid-base balance. Goodlin and Perry (1966) feel that in the fetal rabbit, however, the lung can contribute to osmoregulation.

B. The fetal salivary glands

Arthur (1969) found that ungulates actively secrete saliva into the amniotic sac. In the earlier stages of gestation in fetal calves, urine passes out of the urinary bladder by two outlets, one going to the allantois and the other going to the amniotic cavity. At about midterm a sphincter in the fetal bladder closes so that urine can no longer pass to the amniotic compartment. Instead it goes along the urachus into the allantoic sac. As gestation proceeds towards term allantoic fluid becomes more urineline, while amniotic fluid becomes a colorless, glairy, liquid strongly resembling saliva. Lind and Hytten (1972) discovered that the human fetus may also contribute salivary gland secretion to the amniotic compartment since amylase was found in amniotic fluid.

The significance of salivary gland secretion into the
amniotic sac is not known, but it may be involved in some form of fluid or ion exchange. In adult mammals acetylcholine induces water and ion transport in the salivary glands by activating a NaCl pump at the luminal membrane or by increasing the calcium influx. There also seems to be a pump that extrudes Na⁺ and accumulates K⁺ (Peterson, 1971). Although in some mammals, such as the ungulates, saliva can make a large contribution to amniotic fluid volume, it probably does not play a major role in hydro-mineral transfer in other fetal mammals.

C. The fetal skin surface

The skin serves as an outer covering, a storage organ, a regulator of body temperature, a sense organ, and a secretory organ in the adult mammal. Fetal skin differs from adult skin in that transitional and cornified layers are thinner (Gleiss, 1970). Prior to keratinization fetal skin is very permeable to water. The fact that permeability coefficients for fetal skin (0.72×10⁻⁴ cm/sec), amnion (2.88×10⁻⁴ cm/sec), and chorion leave (1.3×10⁻⁴ cm/sec) in the human being are similar suggest that the skin may serve as a significant exchange site between fetal fluids and amniotic fluid (Lloyd et al., 1969; Seeds, 1972b). At 12 to 16 weeks of gestation in the human fetus (term
approx. 44 weeks) the periderm cells contain an abundance of microvilli, Golgi apparatus, mitochondria, endoplasmic reticulum, globular protrusions, and membrane-bound vesicles containing mucopolysaccharides and glycogen (Breathnach and Wyllie, 1965; Hoyes, 1967; 1968 a). The ultrastructural evidence would tend to suggest that fetal skin is capable of either secretion or absorption. In fact, Lind and Hytten (1970) describe the periderm cells of the human fetus as having a strong resemblance to renal tubular cells under the influence of vasopressin (see Ganote et al. 1968). Lind et al. (1972) showed that during the first half of pregnancy, Na$^+$ as well as water can readily pass through fetal skin. Although no potential difference could be measured in vivo or in vitro there could be a water solute coupling which causes movement of Na$^+$ and water across a membrane without generation of a potential difference (see Diamond, 1971).

Prior to midterm in the human fetus, the fetal skin seems to be capable of altering amniotic fluid composition and volume (Lind and Hytten, 1970; 1972; Lind, 1973). At this stage of gestation amniotic fluid appears to be a dialysate of fetal plasma, and might be considered merely an extension of the fetal extracellular fluid space (Seeds, 1972 a; Lind, 1973). The only barrier between amniotic fluid and the fetal extracellular fluid space is the skin,
and prior to midterm it may function as a semipermeable membrane. Since the volume of amniotic fluid varies directly with the surface area of the fetus, and since the fetal ectoderm and the amnion are derived from the same germ layer there is good indication that fetal skin may alter the volume, and perhaps composition, of amniotic fluid (Harrison and Malpas, 1953; Saunders and Rhodes, 1973).

Changes in the composition of liquor amnii have been correlated with changes in fetal skin (Lind et al., 1969). At 17-20 weeks of gestation cornification begins, and there is a reduction in the number of microvilli and an increase in the number of skin layers in the human fetus. Skin becomes more closely bound at the desmosomes restricting the passage of water and solutes. At 25 weeks human fetal skin is relatively impermeable to water and solutes due to the presence of a stratum corneum (Parmley and Seeds, 1970). The fact that liquor amnii becomes hypotonic to fetal and maternal plasma suggests that amniotic fluid is no longer an ultrafiltrate of plasma (Lind et al., 1969). When skin becomes keratinized in the second half of pregnancy the amniotic fluid becomes exteriorized because water and solutes can no longer pass between body fluids of the fetus and the amniotic fluid (Lind, 1973).

Prior to keratinization, the skin of the mammalian
fetus may serve as an important osmoregulatory structure. In large measure it controls the exchange of water and ions between the extracellular fluid space of the fetus and the amniotic fluid surrounding it.

D. The umbilical cord

Since the lining of the umbilical cord is derived from the same germ layer as the fetal ectoderm and amnion it might be expected to show similar characteristics. The cord epithelium consists of a single layer of cells with little differentiation between 8 and 10 weeks of gestation in the human being (Hoyes, 1969). At the end of the third month the cells undergo differentiation, and the epithelium becomes bilaminar. The cells at this stage contain endoplasmic reticulum, multivesicular bodies, cytoplasmic vesicles, glycogen deposits, Golgi apparatus, villous folds and microvilli. These cells are similar in appearance to periderm cells of fetal skin, and likewise, are thought to play a role in water exchange. The functional activity of fetal periderm declines steadily in the process of keratinization and the formation of the stratum corneum (Hoyes, 1968a). Keratinization does not occur in cord epithelium, however, except in a region in close proximity to the fetus; the remainder consists of simple squamous epithelium (Hoyes, 1969).
Although fetal skin readily absorbs isotopic water in early gestation, there is a decrease in water transfer as the skin begins to keratinize. Prior to 18 weeks of gestation the human umbilical cord reabsorbs little or no water \textit{in vivo} or \textit{in vitro}, but as the skin begins to keratinize, the umbilical cord begins to play an increasingly important role in reabsorption of water. Between 3 and 59 ml per hour may be exchanged across the human umbilical cord at term (Plentl, 1961; Abramovich, 1973). Between 22 and 26 weeks of gestation zonulae occludentes begin to disappear, villous folds develop in the intercellular spaces, and cytoplasmic vesicles appear in the basal cells of cord epithelium (Hoyes, 1969). These morphological changes occurring in cord epithelial cells between 22 and 26 weeks of gestation in the human fetus may explain the observation that the umbilical cord begins to play a significant role in water transfer when the skin is becoming keratinized (i.e. in weeks 17-25 of human gestation).

Hutchinson \textit{et al.} (1959) injected isotopic water into the human amniotic sac, and found that large amounts of the tritium isotope were concentrated in Wharton's jelly of the umbilical cord. Gadd (1966) provided further evidence that the umbilical cord plays a role in water exchange between the fetal and amniotic compartments. He injected
sulfonamides into the amniotic sac. He then measured the concentration in the fetal membranes and umbilical cord, and found that the sulfonamides were concentrated to a greater extent in the umbilical cord. Although the umbilical cord has been postulated as a site of exchange between fetal circulation and amniotic fluid it probably does not represent a major site. The surface area of the umbilical vessels is small compared to a capillary bed (Seeds, 1972 b). Since the surface area of the cord epithelium is small compared to that of fetal skin, the cord is not able to completely compensate for the loss of skin as an exchange site when keratinization occurs. This is reflected by the fact that after midterm amniotic fluid is no longer an ultrafiltrate of fetal plasma.

E. The fetal gastrointestinal tract

Evidence for fetal swallowing near term had been provided in the 19th century by the observation that epithelial cells, lanugo hairs, and vernix caesoa had been found in the gut of fetuses (Minot, 1892). In the early twentieth century, Wislocki (1920) discovered that when trypan blue was injected into the amniotic cavity of fetal guinea-pigs and cats that the colloidal dye was found in the gastrointestinal and respiratory tracts. Jeffcoate
and Scott (1959) and McLain (1963) provided evidence that not only does the human fetus swallow amniotic fluid, but that it can also absorb fluid across the wall of the stomach. They introduced a radio-opaque dye into the amniotic compartment, and X-rayed the fetus in utero a number of hours later; dye was found to be concentrated in the stomach. McLain (1963) also observed that although it took the dye 8 hours to reach the colon at 34 weeks of gestation, by 37 to 40 weeks only $4^{1/2}$ to 7 hours were required for passage of the dye to the colon.

The degree of water reabsorption by the gastrointestinal tract seems to vary greatly amongst species. Wright and Nixon (1961) calculated that the gastrointestinal tract of the fetal lamb may absorb as much as 32 liters of amniotic fluid between 80 days of gestation and term (approx. 145 days). Pritchard (1965) injected chromium-tagged maternal erythrocytes into the amniotic cavity and estimated that the gastrointestinal tract of the human fetus may absorb as much as 2-10 ml of fluid per hour. According to Abramovich (1970), however, the human fetus swallows only about 2 ml of amniotic fluid per day at 17 weeks of gestation and 13 ml per day at 20 weeks.

Wright and Nixon (1961) demonstrated that the fetal lamb can absorb the Na$^+$ from swallowed amniotic fluid via the intestinal tract. Mellor (1969) found that the potential difference across the stomach of the fetal lamb
seems to affect the amniotic fluid potential difference. This would suggest that an active ion pump may exist in the fetal stomach. In late gestation the stomach of the human fetus may contribute hydrogen ions in the form of \( \text{HCl} \) to amniotic fluid (Lind, 1973). The concentration of hydrogen ions in human amniotic fluid increases from 59.34 ± 1.25 nEq/l at 10-23 weeks of gestation to a concentration of 78.45 ± 2.68 nEq/l at term (Seeds and Hellegers, 1968).

The importance of deglutition in altering amniotic fluid composition and volume has not been well established. Abramovich (1970) has provided information gained in a study of 8 anencephalic fetuses and 1 microcephalic fetus that swallowing plays a very minor role in the polyhydramnios of anencephaly. Fetal disorders such as diabetes mellitus may be present in the anencephalic and exert a greater influence on amniotic fluid volume than swallowing (Abramovich, 1973). When the amniotic fluid volume lies within the normal range of values, however, swallowing of fluid and reabsorption by the gastrointestinal tract can play a major role in regulating fluid volume. Pritchard (1966) calculated that when the amniotic fluid volume was 849 ml that the human fetus swallowed about 453 ml of the fluid per day.
F. The fetal kidney

Hippocrates was responsible for the oldest and the most simple explanation for the source of amniotic fluid; he suggested that it was a product of fetal micturition. Abramovich (1968) provided evidence that the kidneys of the human fetus may be functional at 11 weeks of gestation since urine was found in the bladder of fetuses of this age. MacDonald and Emery (1959) suggested that at 18 to 22 weeks of gestation, 80% of the glomeruli in the human fetal kidney appear to be functional based on histological features. Abramovich (1970) estimated that fetuses of this gestational age void between 7-11 ml of urine per day, based on a bladder capacity of 0.3-0.7 ml. The kidney glomeruli and tubules appear to be functional at 60 days of gestation in the fetal lamb (Alexander and Nixon, 1961).

Fetal urine is hypotonic to plasma, and the hypotonicity increases as gestation advances in animals like the lamb. The osmolarity of fetal lamb urine at 81 to 93 days is 239 mOsm/l, and falls to an osmolarity of 166 mOsm/l at 130 to 142 days (Alexander and Nixon, 1961). Na\(^+\) and Cl\(^-\) reabsorption from the glomular filtrate presented to the renal tubules of fetal lambs increases from 60% at 61 days to 90% at term. K\(^+\) decreased in a similar fashion from 61 days to term. Carpek et al. (1968) showed by utilization of
micropuncture techniques that the proximal renal tubule of the 30 day old rat reabsorbs more Na\(^+\) than the 20 day old rat post partum. Although water reabsorption by the kidneys of the fetal lamb amounts to 92% at 142 days, only 28% of the water in the glomerular filtrate was reabsorbed by the tubules at 61 days (Alexander and Nixon, 1961). The hypotonicity of fetal urine seems to be the result of reabsorption of electrolytes in excess of water at certain stages of gestation in some mammals (Kleinman, 1970). The hypotonicity of fetal urine may also be attributed to a low solute load rather than an increased volume of water present in the tubules since some of the solute may be excreted by the human fetus via the placenta (Seeds, 1965). Edelman and Barnett (1960) theorized that the poor concentrating ability of the fetal or neonatal kidney is due to an unsatisfactory gradient of urea between the cortex and the papilla of the counter-current multiplier system. Since the human fetus or infant has a high anabolic rate it excretes less nitrogen, and thus, the medullary urea concentration is lower than in the adult (Kleinman, 1970). Edelman (1967) demonstrated that if the protein intake of the human infant's diet is increased so that there is more urea in the medulla, that the urine osmolarity can be greatly increased.

The fact that the fetal kidney is functional in utero
suggests that it could alter amniotic fluid volume. Seeds (1972a) provides the following evidence that the kidney of the human fetus makes a significant contribution to the amniotic compartment in the last half of gestation: 1. From the 20th week until term in the human being the total solute concentration in amniotic fluid decreases. 2. Fetal urine in utero decreases in osmolality as gestation advances to a value 80-140 mOsm/l, lower than maternal and fetal plasma at term. 3. Creatinine increases in both fetal urine and amniotic fluid to values higher than in maternal or fetal blood. 4. Creatinine and urea can pass through the placental membranes. 5. Kerr et al. (1969) found that concentration of taurine in amniotic fluid of the rhesus monkey, increases in the later stages of gestation, and is high in fetal urine. 6. Amniotic fluid becomes increasingly acidic from the middle of gestation to term, and fetal urine has been found to have a low pH at term. Klopper (1972) provided further evidence that urine is passed into the amniotic cavity. He showed that unconjugated glucosiduronate, and sulfate fractions of estriol in fetal urine and amniotic fluid were of very similar levels.

Indication that fetal micturition can influence amniotic fluid volume was provided by Alexander et al. (1958)
who measured urine flow in fetal lambs. They found that urine flow was about 0.14 ml/min at 61 days, 0.64 ml/min at 117 days, and 0.14 ml/min at term. These flow rates approximate the changes in amniotic fluid volume at these stages of gestation. A number of workers have sited cases of bilateral renal agenesis, cystic dysplasia, and congenital urethral atresia being associated with oligohydramnios as evidence that fetal micturition can play a major role in controlling amniotic fluid volume (Blain and Scott, 1960; Lind et al., 1971). Abramovich (1973) cautions against drawing a parallel between fetal disorders such as renal agenesis and oligohydramnios because the two are not always linked, and sometimes the presence of other fetal disorders with or without renal agenesis can lead to a decrease in amniotic fluid volume. When amniotic fluid volume strays from the expected value for a certain gestational age, as in oligohydramnios, the role of the fetal kidney in controlling amniotic fluid volume becomes ambiguous.

G. The fetal urinary bladder

Although kidney urine of the fetal guinea-pig is hypotonic to fetal plasma, bladder urine is slightly hypertonic (Kleinman, 1970). Osmolarity of bladder urine
of the fetal guinea-pig is 368 mOsm/l at one stage of gestation while fetal guinea-pig plasma has an osmolarity of 292 mOsm/l. The hypertonicity of bladder urine in the fetal guinea-pig is suggestive of water reabsorption by the bladder.

Stanier (1971) found that bladder urine of fetal pigs is hypotonic to the urine in the renal pelvis. Pelvic urine had an osmolarity of 120-250 mOsm/l. Bladder urine had 20% to 50% the osmolarity of pelvic urine early in gestation and increased to over 50% at 100 days of gestation. Bladder Na\(^+\) concentration was found to be 15 mEq/l while Na\(^+\) concentration in the renal pelvis was 53 mEq/l. These observations seemed indicative of ion reabsorption by the fetal bladder. France et al. (1972) carried out radioisotope experiments using \(^{22}\)Na\(^+\) and \(^{24}\)Na\(^+\) in order to measure ion fluxes across the urinary bladder of the fetal pig in vitro. There was a net efflux of Na\(^+\) (i.e. reabsorption), and since the ratio of efflux for tritiated water was different from that of sodium, water movement did not seemed to be coupled to sodium movement.

3. **Contribution of fetal tissues and organs in regulating amniotic fluid volume**

Abramovich (1973) estimated that at 17 to 19 weeks of human gestation that amniotic fluid volume is increasing
at a rate of 11-13 ml per day. Since the fetus swallows about 4-11 ml per day, there must be 11-24 ml of fluid added to the amniotic compartment per day in order to account for the volume of amniotic fluid present. The kidneys have been estimated to add only 7-14 ml of urine per day to the amniotic sac at this stage of gestation. Thus, 4-10 ml of fluid must come from other sources, such as the lungs, the salivary glands, the skin, the umbilical cord, the fetal membranes, or the fetal side of the placenta.

4. **Comparison of osmoregulatory structures of the fetal mammal with those of lower vertebrates**

The mammalian fetus is comparable to teleost fishes in its practice of swallowing fluid from the external medium, and reabsorbing water and ions across the gastrointestinal tract (see Utida et al., 1972). It also seems to resemble anuran amphibians with regards to natriferic and hydro-osmotic interactions of the skin surface and the urinary bladder with the fluid environment (see Bentley, 1966; Maetz, 1968; and Turner and Bagnara, 1971). The similarity in function of various fetal structures to osmoregulatory organs of lower vertebrates may represent an example of ontogeny recapitulating phylogeny. More probably, however, this may be a case of parallel evolution in which organisms develop similar adaptations in response to similar problems.
5. **The fluid exchange between intrauterine compartments**

We have determined the relative contribution various fetal structures make to hydro-mineral exchange between the fetal and other fluid compartments in the intrauterine cavity. We have also considered the influence of various fetal tissues and organs in controlling amniotic fluid volume and composition. We can now examine the interplay and exchange between the three compartments, in order to determine how intrauterine fluids accumulate and are disposed of.

The amniotic sac becomes the major fluid space surrounding the human fetus by the end of the ninth week of gestation. The chorionic and yolk sac are almost gone, while an allantoic sac never grows to a significant degree in the human being (Seeds, 1972 a). According to the data of Elliot and Inman (1961), amniotic fluid volume is about 5-10 ml at 8 weeks of gestation, rising to 1000 ml at 38 weeks, and dropping to 244 ml at 43 weeks in the human being. The significance of the changes in fluid volume is still obscure.

Since water increases at a rate of 30-40 ml per day in the human intrauterine cavity near term, water must be transferred from the maternal compartment. Intrauterine or fetal metabolic sources would be too small to contribute
significantly to intrauterine water volume (Seeds, 1973). Exchange rates between mother and fetus increase between mid pregnancy and term, while exchange rates between mother and amniotic fluid, and between fetus and amniotic fluid increase at a slower rate as gestation proceeds. Na\(^+\) and K\(^+\) were found to exchange more slowly than water (Kerpel-Fronius, 1970).

Schuefer et al. (1972) provided evidence that there is a dynamic exchange occurring between the amniotic fluid compartment and other compartments. When they replaced amniotic fluid with solute-free water in the rhesus monkey, there was a reduction in fluid volume of the amniotic cavity and the appearance of a large quantity of solutes. By the end of 20 hours the solute concentration in the amniotic sac had returned to pre-experimental levels. How solute and water exchange occurs between the amniotic and other compartments is not well understood.

6. **Factors affecting inter-compartmental fluid exchange**

Battaglia et al. (1960) showed that one could experimentally stimulate water or sodium transfer between the mother and the fetus, or between the fetus and mother across the placenta. They accomplished such a transfer by infusing the mother with mannitol, hypertonic saline, isotonic
glucose or 5% glucose. Infusion of the mother with a hypertonic saline solution was found to cause a 2% to 4% loss in total body water of the fetal rabbit, and an 18% decrease in extracellular fluid. Infusion of pregnant rhesus monkeys with hypertonic saline or disaccharide solutions caused a 4% decrease in fetal body water, as did infusion of the amniotic cavity with a disaccharide solution (Seeds et al. 1964). Bruns et al. (1963) showed that experimental dehydration of the fetal rabbit caused a decrease in amniotic fluid volume. Water has also been found to move in bulk flow across the perfused guinea-pig placenta in vivo as a linear function of hydrostatic pressure (Dancis et al. 1962). The fact that water transfer can occur in bulk flow in response to experimental osmotic and hydrostatic gradients between the three compartments suggests that water could be supplied for fetal development by in vivo chemical and physical gradients.

The osmolarity of fetal plasma (292.7 mOsm/l) and maternal plasma (289.1 mOsm/l) are approximately equal in the human being (Kerpel-Fronius, 1970). In early stages of gestation amniotic fluid is essentially isotonic to fetal plasma. Thus, there would appear to be no osmotic gradients present in utero capable of driving water in bulk form from one compartment to another in early stages of gestation.
Net flux of water can be driven by hydrostatic as well as osmotic pressure gradients. Reynolds (1960) determined that umbilical hydrostatic pressures did not indicate that water could be driven from mother to fetus in the sheep. Ramsay et al. (1959) measured hydrostatic pressures in the human being by intervillous space sampling, and could find no suggestion that gradients existed that were large enough to drive fluid from mother to fetus. Existing hydrostatic gradients would, if anything, cause water movement from fetus to mother. Thus osmotic and hydrostatic gradients do not explain the accumulation of intrauterine water.

Although there is no gradient in total solute concentration a difference in colloid osmotic gradients could cause a net flux of water from the mother to the fetus (Barnes, 1968). Meschia (1955) found a higher colloid osmotic pressure in the maternal plasma of sheep and goats than in the fetal plasma during the last half of pregnancy. Colloid osmotic pressure or plasma protein concentration of fetal blood increases throughout gestation from 2.5% to 6% at term in the human fetus. These values are still lower than maternal values, however, so water movement would be favored in the fetal to maternal direction (Westin, 1959). Therefore existing osmotic, hydrostatic, or colloid osmotic gradients between mother and fetus do not explain how water could accumulate in the intrauterine cavity.

There is also very little information available on how
hydro-mineral balance is achieved in the intrauterine cavity, and what controls the rate of water and ion exchange between the three compartments.

7. The role of hormones in controlling fetal hydro-mineral metabolism

In the adult mammal many forms of metabolism, including those involved with hydro-mineral balance, are controlled by hormones. The role of hormones in controlling fetal metabolism, however, is not well understood. It is only recently that any detailed investigation has been initiated to determine hormonal function in the fetus. Comline et al. (1970) have suggested that the fetus may control the level of ions in allantoic fluid by fetal adrenal gland secretion of corticosteroids. Basset and Hinks (1969) found that reduction of glucose in the plasma of the 6 week-old neonatal lamb stimulated the adrenal gland to secrete corticosteroids. An increase in glucose concentration in maternal plasma leads to an increase in sodium concentration in allantoic fluid of the fetal lamb (Mellor and Slater, 1971). A decrease in plasma glucose levels leads to a decrease in sodium concentration and an increase in potassium concentration in allantoic fluid. Since Mellor and Slater (1971) suggest that solute movement in and out of the allantoic sac is associated with fluid volume,
corticosteroids may influence water exchange between the allantoic sac and other fluid compartments in ungulates. Alexander and Williams (1968), however, found evidence in favor of control of allantoic fluid volume by maternal secretion of sex steroids. They observed that allantoic fluid accumulated in ovariectomized sheep maintained on low doses of intramuscularly administered progesterone. Increasing the amount of progesterone injected or injection of oestradiol benzoate reduced allantoic fluid volume to normal. Amniotic fluid volume was not altered by maternal ovariectomy. Thus, there is some indication that fetal or maternal hormones may directly or indirectly influence hydro-mineral exchange in some species of fetal mammals.

STATEMENT OF THE PROBLEM

The present investigation was undertaken in order to determine whether or not pituitary hormones could be implicated in influencing hydro-mineral exchange in the fetal guinea-pig. Emphasis was placed on determining whether or not a hormone could affect water and/or sodium movement across fetal membranes and tissues. Mechanisms of hormone action were not investigated. In order to avoid complications from possible interactions of hormones with other metabolites, an in vitro preparation was used. Membranes
and tissues were taken from fetal guinea-pigs at various stages of gestation. Thus, membrane sensitivity to hormone could be studied as a function of gestional age. This also allowed one to determine whether or not membrane permeability changed over the course of gestation.
GENERAL METHODS

1. **Unidirectional water flux experiments**

A. **The isolated amnion preparation**

(a) Dissection:

Pregnant guinea-pigs, between midterm and term, were anaesthetised with ether, and the uterine horns, containing fetuses, were removed and placed in maternal saline at 37°C (see page 38). The uterine wall and yolk sac were removed, and an incision was made in the amnion; a viton o-ring (0.3 cm., internal diameter) was slipped under the membrane, and the section of the amnion adhering to the ring was freed from the rest of the membrane. The ring, with its membrane attached, was inserted into a perfusion cell. Care was taken to prevent any drying of the membrane, and exposure to air was minimal. Although approximate ages were known, the accepted gestational age of the membrane was determined according to the data of Draper (1920) and Ibsen (1928). Whenever possible, two preparations from the same amnion were set up side by side, in identical cells, one to act as the experimental preparation, the other as control.

(b) The perfusion cells:

Fig. 1 shows the perfusion cells used in some of the later studies with vasopressin and all of the studies with
Figure 1. The Perfusion Cell used for studying Radioisotope Flux through the Isolated Guinea-Pig Amnion

Stippling represents the concentration of radioisotope. The "hot" reservoir receives the initial injection of radioisotope; the "cold" reservoir contains saline with no radioisotope. The arrows show the direction of flow of saline, with both circuits driven at the same flow rate by a common pump. Radioisotope which passes through the amniotic membrane is collected in the fraction collector.
prolactin. The perfusion cell employed in most of the early experiments with vasopressin differed from the setup shown in Fig. 1 in that the inflow and the out-flow tubings were directed perpendicular to one another. The setup was modified to improve the circulation of fluid, and to eliminate dead space and unstirred layers in front of the isolated membrane. The membranes were supported between two viton O-rings (0.3 cm inner diameter); these were clamped between the two halves of the plexiglass cells (2.5 cm outside diameter, 5.2 cm overall length), which were held together lightly but firmly, in a vise. The membrane formed the central division of a spherical chamber, 0.3 cm in diameter, of approximately 20 µl capacity for the perfusion cell in Fig. 1. The entire assembly was immersed in a constant temperature bath at 37 °C ± 0.01 °C (Model CTC circulator, Bronwill, Rochester, N.Y.). When the isolated membranes were in place, they were perfused on one side with maternal saline (see page 38) and on the other side with amniotic saline at 37 °C. In experiments with 22Na+, and in all experiments with prolactin, the membranes were perfused on both sides with amniotic saline at 37 °C. An identical rate of flow (12 ml/hr) was maintained on both sides of the membrane by a Buchler Dekastaltic pump (Buchler Instruments, Fort Lee,
N.J.), which was common to both flows. The circulation on one side of the isolated amniotic membrane of the fetal guinea-pig was a closed, recirculating system, suitable for the initial radioisotope containing saline; it included a 6 ml or 12 ml reservoir (37°C) to which hormone preparations could be added, and this was aerated to ensure both oxygenation and mixing. The circulation on the opposite side of the isolated amniotic membrane was open; saline, free of radioisotope was contained in an aerated 25 ml reservoir at 37°C (for the vasopressin studies) and aerated 100 ml reservoir at 37°C (for the prolactin studies); it was pumped past the amnion, where it received any radioisotope which permeated the membrane, and the out-flow was collected in either 1.0 ml or 2.0 ml samples in a fraction collector (L.K.B. UltroRac, Type 7000; Stockholm, Sweden). The samples were checked for any variations in perfusion rate, and then measured for radioisotope content.

B. Experimental procedure for studying unidirectional flux of water

100 μCi of tritiated water (³H₂O; New England Nuclear, Boston, Mass.) was diluted into 12.0 ml of maternal saline, and added to the reservoir of the closed-circuit circulation. Amniotic saline, free of radioisotope, was placed in the
open-circuit circulation. The membranes were inserted, and the salines allowed to flow for a 60 minute equilibration, during which time effluent samples were collected in the fraction collector. At 60 minutes, vasopressin was added to the reservoir of the open circulation, to give a final concentration of 50-500 mU/ml; normally it circulated over the fetal side of the amnion. The experiment continued for up to 35 minutes after the addition of vasopressin; during this time samples were collected in 1 ml amounts from the fetal side of the amnion and estimated for tritium content.

Experiments dealing with the effects of prolactin on unidirectional flux of water differed in several respects from the procedure outlined above for vasopressin. Firstly, tritiated water (100 μCi) was diluted in amniotic rather than maternal saline since both sides of the membrane were bathed with amniotic saline in the prolactin studies. Secondly, the salines were allowed to flow for a 90 minute, rather than a 60 minute, equilibration period. At 90 minutes, prolactin was added to the reservoir to give a final concentration of either 10 or 20 μg/ml; normally it circulated over the fetal side of the amnion. Thirdly, unlike vasopressin studies which lasted 35 minutes, the prolactin experiments continued for up to 3 hours after
addition of hormone. During this time samples were collected in 2 ml aliquots from either the maternal or the fetal side of the amnion, and estimated for concentration of tritiated water.

C. Estimation of unidirectional flux of water

Unidirectional flux was obtained by measuring tritiated water concentrations on the open circuit (normally fetal) side of the membrane; the measurements were made on the samples collected in the fraction collector. 200 μl aliquots were removed from each centrifuge tube and placed in scintillation vials containing 10.0 ml of "Aquasol" (New England Nuclear Corp.). The vials were kept in the dark overnight, and counted to over 10,000 counts in a liquid scintillation counter (Isocap 300; Nuclear Chicago Corp.). The channels ratio method was used for quench correction, in order to express data in d.p.m.. Data was analyzed by a PDP-11/45 computer (Digital Equipment Corp., Maynard, Mass.).

The permeability coefficient ($K_{trans}$) for $^{3}$H$_{2}$O passing across the amnion was calculated by computer from the Fick equation (see Hays and Leaf, 1962):

$$K_{trans} = \frac{Qw}{(C_1 - C_2)A}$$

Where:

$Qw =$ the net increase in isotopic water per unit time on the side opposite to which radioisotope was initially added.
\( C_1 \) and \( C_2 = \) the mean concentrations of radioisotope on the two sides of the membrane during the period of measurement.

\( A = \) the cross-sectional area of the chamber (0.07 cm\(^2\)).

The unidirectional flux was calculated by multiplying \( K_{\text{trans}} \) by the molar concentration of water in the saline (55.2 mM/cm\(^3\)) and the partial molal volume of water (18 \( \mu L/mM \)). Values for unidirectional flux were calculated as \( \mu L/cm^2/hr \).

2. **Net water flux experiments**

Net flux of water through amnion was determined by the weight-change method of Vizsolyi and Perks (1974). The original method was modified in two ways: firstly, weight changes were determined over longer, one hour time intervals since prolactin is often slow acting; secondly, the long-term weight changes were determined by direct weighing on a Mettler balance.

The amnion, attached across a glass supporting-tube (0.9 cm diameter; 9.5 cm long), was maintained at 37\(^\circ\)C, with amniotic saline on the inner, fetal side, and with aerated maternal saline on the outer maternal surface, as described by Vizsolyi and Perks (1974). (For salines, see page 38). After one hour of equilibration, the preparation was weighed; care was taken to drain excess fluid, and to
weigh rapidly. The preparation was replaced, in fresh maternal saline, and the inner amniotic saline was substituted by a new sample which contained prolactin at 20 µg/ml. Weight changes were determined at hourly intervals for three hours. All values for net flux were expressed as µl/cm²/hr, for comparison with the unidirectional measurements. A control preparation, whenever possible from the same fetus, was set up alongside the experimental membrane; it received equivalent treatment, but the prolactin was replaced by saline of the same volume subjected to the pH treatment, or serum albumen, at the same concentration as the prolactin, and adjusted in the same manner.

3. Unidirectional sodium flux experiments

A. Experimental procedure for studying unidirectional flux of sodium

50 µCi of radioactive sodium (²²NaCl; Amersham/Searle Corp., Arlington Heights, Illinois) was diluted into 6.0 ml of amniotic saline, and was added to the reservoir of the closed-circuit circulation. Amniotic saline, free of radioisotope, was placed in the open circulation. The membranes were inserted, and the salines allowed to flow for a 90 minute equilibration period, during which time
samples were collected in the fraction collector. At 90 minutes, hormone was added to the saline reservoir bathing the fetal side of the amnion to give a final concentration of either 500 mU/ml vasopressin or 10 μg/ml prolactin. The experiments continued for up to 3 hours after addition of hormone. During this time samples were collected in 2 ml amounts from the fetal side of the amnion and estimated for $^{22}\text{Na}^+$ content.

B. Estimation of unidirectional sodium flux

Unidirectional flux of sodium was determined by measuring the concentration of $^{22}\text{Na}^+$ in samples collected in the fraction collector. 1.8 ml aliquots were removed from each centrifuge tube and dispensed into planchets. Saline containing radioisotope was evaporated to a solid counting source in planchets by means of an infrared lamp. Samples were counted to 10,000 counts for at least two cycles in a gas flow Geiger counter (Model 1042; Nuclear Chicago Corp.). Sample counts did not exceed 5000 cpm since coincidence loss in the Geiger-Mueller detector becomes significant over this value (see Wang and Willis, 1965).

The rate of $^{22}\text{Na}^+$ movement across the amnion was calculated by means of the following equation (see Snell, Shulman, Spencer and Moos, 1965):
\[ J_{i12} = \frac{V_2 \frac{dC_i^*}{dt}}{A} \times \frac{C_i^*}{C_i^1} \]

Where:

\( J_{i12} \) = the rate of isotopic flux across the membrane from compartment 1 to compartment 2 of the perfusion cell.

\( V_2 \) = the volume of solution 2 in ml (i.e. that collected in the fraction collector).

\( \frac{dC_i^*}{dt} \) = the time of change in radioisotope concentration (i.e. the rate of appearance of \( \text{Na}^+ \) in cpm into solution 2).

\( A \) = the cross sectional area of the chamber (0.07 cm\(^2\)).

\( \frac{C_i^*}{C_i^1} \) = the specific activity of \( \text{Na}^+ \) in solution 1 (i.e. the radioisotope reservoir).

\( C_i^* \) = the concentration of radioisotope in solution 1 (in cpm/ml).

\( C_i^1 \) = the concentration of unlabeled Na\(^+\) in solution 1 (i.e. 125 \( \mu \)Eq/ml).

Values for unidirectional flux of sodium were calculated in terms of \( \mu \)Eq/cm\(^2\)/hr.

4. **Hormone preparations**

A. **Vasopressin**

The vasopressin used in the present studies consisted of Pitressin (lots Ck 217 and Ej 112; Parke-Davis & Comp. Ltd. Brockville, Ontario). This is an aqueous solution of arginine and lysine vasopressin at a concentration of 20 pressor units/cc. Solubility of the hormone in solution is
maintained by adjustment of the pH with acetic acid. Before the vasopressin solution was added to the membrane preparation, its pH was brought to neutrality by addition of NaOH. 62.5-625.0 μl of Pitressin was diluted into the 25.0 ml of amniotic saline in the open reservoir (dilution: $\frac{1}{40}$-$\frac{1}{400}$). A volume of 0.25 acetic acid equal to that of the hormone solution was brought to neutral pH by addition of 0.2 N sodium hydroxide, and was added to the control membranes. The Pitressin solution described above will be referred to, henceforth, as "vasopressin".

B. **Prolactin**

A purified prolactin preparation (NIH P-S-11, 26.4 IU/mg) was kindly supplied by Dr. R. Bates, National Institute of Health, Bethesda, Md. It was dissolved in amniotic saline at 1 mg/ml by careful adjustment to pH 9. In the radioisotope experiments, the saline contained $^3$H$_2$O or $^{22}$NaCl at the same specific activity as that in the closed-circuit reservoir when studying fetal-maternal flux. In those experiments investigating maternal-fetal water or sodium movement, prolactin solution was added to the open-circuit reservoir, free of radioisotope. In the unidirectional water and sodium flux experiments hormone dilution into the saline reservoir was $\frac{1}{100}$, while in net water flow experiments
the dilution was $^{1/50}$. Controls consisted of amniotic saline adjusted to the same pH in the same way as for hormone, or serum albumen at the same concentrations as the prolactin, and adjusted in the same manner.

5. **Salines**

Salines were designed to parallel the appropriate natural fluids, and consisted of:

A. **Amniotic saline**

\[ \begin{align*}
\text{Na}^+ & \quad 125.0; \quad \text{K}^+ \quad 6.2; \quad \text{Ca}^{2+} \quad 4.5; \quad \text{Mg}^{2+} \quad 2.3 \text{ mEq/l}; \\
\end{align*} \]

all were added as chlorides. Glucose was added at 1.0 g/l. A phosphate buffer, pH 7.8 (0.2M NaH$_2$PO$_4$/Na$_2$HPO$_4$) was added at 10 ml buffer/l of saline. The final pH was 7.4 and the osmolarity was 286 mOsm/l.

B. **Maternal saline**

\[ \begin{align*}
\text{Na}^+ & \quad 150.0; \quad \text{K}^+ \quad 5.5; \quad \text{Ca}^{2+} \quad 4.4; \quad \text{Mg}^{2+} \quad 2.6 \text{ mEq/l}; \\
\end{align*} \]

all were added as chlorides. Glucose and phosphate were added as for the amniotic saline. The final pH was 7.4, and the osmolarity was 314 mOsm/l.
SECTION I

THE EFFECT OF VASOPRESSIN ON WATER AND SODIUM MOVEMENT THROUGH THE ISOLATED AMNIOTIC MEMBRANE OF THE GUINEA-PIG

INTRODUCTION:

Vizsolyi and Perks (1974) demonstrated that the isolated membrane of the guinea-pig is sensitive to both arginine vasotocin and arginine vasopressin. When arginine vasotocin (8-100 mU/ml) was added to the media bathing the fetal side of the membrane it slowed, and could reverse fetal-maternal water movement through the amnion. Fetal pituitary extracts and synthetic arginine vasopressin also stimulated water movement across the amniotic membrane of the guinea-pig. Since the threshold dose for arginine vasopressin was lower than for arginine vasotocin (1.0 mU/ml v.s. 6.4 mU/ml), it seemed to be more effective in stimulating water movement across the membrane. Oxytocin (100 mU/ml) was without effect. Reversals of water flow against an osmotic gradient of 28 mOsm/l and a hydrostatic gradient of 2 cm of water were noted for a number of membrane preparations in response to neurohypophyseal hormone. This suggested that water was not moving by strictly passive means (e.g. bulk flow), but rather by an active process (e.g. perhaps being driven in a water-solute coupling by a pump).
An investigation was undertaken to determine whether or not vasopressin could stimulate an increase in the unidirectional flux of tritiated water from the maternal to the fetal side of the isolated amniotic membrane of the fetal guinea-pig. If the unidirectional flux of water showed a response to treatment with vasopressin, the next proposed step was to determine whether water movement could be linked to a possible sodium transport.
RESULTS:

1. **Permeability coefficients for the guinea-pig amnion**

   The permeability of the isolated amnion to water was determined by measuring transmembrane movement of tritiated water in response to a tracer gradient; the perfusion cell used in determining permeability coefficients is shown in Fig. 1 (see methods). Fluxes were measured in both directions across the membrane by inserting the amnion with either its fetal or its maternal surface in contact with the radioisotope-containing closed circulation. Although the membrane ranged from 0.62 of term until apparently overdue, there was little evidence for any obvious relationship between permeability to water and gestational age over the period studied. In ten experiments, the permeability coefficient, $K_{\text{trans}}$, for water movement from the maternal to the fetal side of the membrane was calculated to be $3.09 \pm 0.15 \times 10^{-4}$ cm sec$^{-1}$. In thirteen experiments $K_{\text{trans}}$ in the reverse direction was $2.62 \pm 0.14 \times 10^{-4}$ cm sec$^{-1}$. The difference in the permeability coefficient in the two directions was statistically significant (Student's t test, $P<0.05$). This implied that water movement would be easier from mother to fetus than from fetus to mother.
2. **The action of vasopressin; effects on unidirectional flux of water through the amnion**

The addition of vasopressin (50-500 mU/ml) to the fetal side of the perfusion chamber caused an increase in water movement through the amnion in the maternal-fetal direction as judged by use of tritiated water. A total of 18 different membranes were tested. Fig. 2 shows the means (i.e. $\bar{x}$) ± the standard error of the means (i.e. S.E.M.) for all the responses obtained to the various doses of vasopressin, as listed in Table I. The responses were calculated as percentage change in water movement over the 35 minute period after addition of hormone or neutralized acetic acid (i.e. sodium acetate); the equilibration period from 30 to 60 minutes after setting up the membrane was averaged for each preparation, and was used as the "base line" (i.e. 0% change). Just as in earlier studies concerned with the effect of neurohypophyseal hormones on the net flux of water across the amnion (see Vizsolyi and Perks, 1974), the response to hormone occurred within 5-10 minutes of addition of vasopressin. The fact that water movement across the amnion from the maternal saline (314 mOsm/litre) to the amniotic saline (286 mOsm/litre) increased in response to hormone
Figure 2. The Effect of Different Doses of Vasopressin on Unidirectional Water Flux through the Guinea-Pig Amnion

The amniotic membranes (N = 18) are arranged in order of increasing doses of vasopressin. At the origin, the membranes received either acetic acid adjusted to neutral pH with sodium hydroxide (i.e. forming sodium acetate), or vasopressin (50-500 mU/ml) on their fetal side. The period just prior to hormone addition (i.e. 60 min) was taken as the "base line" value (0 % change) from which the percentage change in water flux was calculated after the addition of sodium acetate or vasopressin. The values are expressed as the mean ± the standard error of the mean. Ordinates: percentage change in maternal-fetal water movement from the "base line" value. Absissae: time from addition of sodium acetate or vasopressin, in minutes.
TIME: MINUTES
treatment indicated that water could move against electrochemical gradients. This supported the observation in the net flux experiments of Vizsolyi and Perks (1974) that water movement could be reversed from the fetal-maternal to the maternal-fetal direction in response to vasopressin.

The magnitude of the change in water movement seemed to be a function of the dose of vasopressin used (see Table I). When one plots the log dose of vasopressin against the percentage increase in maternal-fetal water movement one obtains a lineal relationship such as shown in Fig. 3. The threshold dose appears to be about 46 mU/ml. The fact that one obtains a definite log dose/response relationship for both net and unidirectional flux of water across amnion seems indicative of hormone sensitivity of the membrane being genuine and not an artifact.

It should be pointed out that these experiments studying the effect of vasopressin were carried out in an early version of the perfusion cell shown in Fig. 1. Dead space and unstirred layers resulted in lower transfer rates than were recorded with the apparatus used for determining permeability values for the amniotic membrane to water. However, the responses were clear, and thus, were calculated as percentage change so that they could be compared directly with later data.
Figure 3. The Relationship between the Logarithm of the Dose of Vasopressin added to Amniotic Membranes and the Percentage Increase in Unidirectional Flux of Water

The curve was calculated from data shown in Table I. Ordinate: percentage increase in unidirectional water flux from the "base line" value for the 35 minute period subsequent to hormone addition. Absissae: logarithm of the dose of vasopressin (in mU/ml) added to the membranes.
LOG DOSE OF VASOPRESSIN.

% INCREASE IN WATER FLOW.
TABLE I
THE EFFECT OF VASOPRESSIN ON MATERNAL-FETAL WATER MOVEMENT THROUGH THE ISOLATED AMNION OF THE GUINEA-PIG

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of preparations</th>
<th>% increase in water movement in 35 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>sodium acetate</td>
<td>2</td>
<td>0.4 ± 0.4 a</td>
</tr>
<tr>
<td>50 mU/ml vasopressin</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>100 mU/ml vasopressin</td>
<td>2</td>
<td>3.8 ± 1.9</td>
</tr>
<tr>
<td>125 mU/ml vasopressin</td>
<td>2</td>
<td>4.7 ± 2.9</td>
</tr>
<tr>
<td>250 mU/ml vasopressin</td>
<td>3</td>
<td>9.1 ± 0.6</td>
</tr>
<tr>
<td>500 mU/ml vasopressin</td>
<td>8</td>
<td>12.3 ± 1.5</td>
</tr>
</tbody>
</table>

a \( \bar{x} \pm S.E.M. \)

3. The action of vasopressin; effects on unidirectional flux of sodium through the amnion

Since water was moving against an osmotic gradient of 28 mOsm/l in response to hormone it seemed to suggest a water-solute interaction in which water could be "dragged" across the membrane by an ion such as sodium. Studies were carried out with the perfusion chamber shown in Fig. 1 in order to determine whether or not vasopressin could increase maternal-fetal movement of sodium across the amnion. In contrast to the previous studies which were concerned with unidirectional flux of water, hormone (500 mU/ml vasopressin) was not added until after a 90 minute, rather than a 60 minute, equilibration period.
In addition to this, both sides of the amnion were bathed with amniotic saline. At 35 minutes after the addition of vasopressin to the fetal side of two membranes an increase of only \(0.7 \pm 0.9\%\) was recorded for sodium movement in the maternal-fetal direction. Since this value is not significantly different from control values over this same time interval it did not appear that vasopressin could stimulate an increase of sodium flux in this direction under the ionic conditions employed in the experiment. It seemed possible, however, that the membranes may have been slow in responding to the hormone, and thus it seemed auspicious to study the preparations over a longer period of time than the usual 35 minutes after hormone addition. Contrary to what was expected, a decrease in maternal-fetal sodium movement across the amnion was observed over the longer time period. In the two membrane preparations studied, a decrease in water movement of \(1.4 \pm 0.6\%\) was recorded in the first hour, followed by a \(12.4 \pm 8.2\%\) and a \(12.2 \pm 7.8\%\) decrease in the second and third hours respectively, after hormone treatment. Both of the membranes showed a reduction of maternal-fetal sodium movement after the addition of vasopressin, but the response of one was more marked than the response of the other.
DISCUSSION:

The results given here confirm the findings of Vizsolyi and Perks (1974) that vasopressin can increase the maternal-fetal flow of water through the amnion. The water movement does not seem to be coupled to sodium transport. In addition, some of the permeability characteristics of the freshly dissected membranes were determined.

1. The diffusional permeability of the isolated guinea-pig amnion

Previous studies on the permeability of the isolated amnion concentrated on human preparations taken after delivery. The permeability coefficients obtained for water movement were $2.88 \pm 0.19 \times 10^{-4}$ cm sec$^{-1}$ (Lloyd et al., 1969) and $0.5$ to $4.0 \times 10^{-4}$ cm sec$^{-1}$ (Page et al., 1974 a). The values obtained with the perfusion cell shown in Fig. 1, for freshly dissected guinea-pig amnion, taken prior to birth, agree well with the human results. These measurements made on guinea-pig amnion with the improved perfusion cell shown in Fig. 1 were considered to be relatively dependable since they were carried out in a small chamber, perfused continuously by a double circulation system designed to reduce dead space, unstirred layers, and back fluxes, all of which were known to have effects on the
results (see Page et al., 1974 a). In addition, and unlike previous studies, the permeabilities were calculated for both directions across the membrane. It was found that diffusional permeability in the maternal-fetal direction $(3.09 \pm 0.15 \times 10^{-4} \text{cm sec}^{-1})$ was higher than that from fetus to mother $(2.62 \pm 0.14 \times 10^{-4} \text{cm sec}^{-1})$. Differences of this type have been noted in other membranes, such as the toad bladder, where the discrepancies were of a closely similar order of magnitude (mucosal to serosal $= 2.00 \times 10^{-4} \text{cm sec}^{-1}$, serosal to mucosal $= 1.48 \times 10^{-4} \text{cm sec}^{-1}$; Hays, 1972). At present, it is not clear whether this asymmetry is of any importance in the functioning of the amnion; however, the statistically significant greater ease with which water could pass in, towards the fetus, might help to ensure the fluid environment of the fetus, whilst the greater difficulty with which water could leave would tend to retain any water which accumulated.

The permeability of the amniotic membrane to water did not seem to change significantly over the course of gestation, although there was some indication of a very slight increase in late stages of pregnancy. Gillibrand (1969 a) found a large and linear increase in water transfer between 14 and 26 weeks, and later a plateauing or a leveling off of the transfer rate as term approached in the human being.
The apparent discrepancy between the guinea-pig and human amnion could represent a species difference or a result of differences in methods of measuring water transfer. Rather than using an in vitro membrane preparation, Gillibrand (1969a) introduced deuterium oxide into the amniotic sac and determined water transfer by measuring concentration of deuterium oxide in amniotic fluid and maternal blood several hours later. A criticism of this method is that Gillibrand (1969a) was not actually measuring membrane permeability of amnion, in a strict sense. In the in vivo study the fetus could have altered the amount of deuterium oxide passing from the amniotic sac to maternal circulation by either swallowing or absorption across the skin surface. Complications in interpretations of results were eliminated in the in vitro studies described here, in the sense that water movement could occur only across amniotic membrane. Another factor which might explain why Gillibrand (1969a) observed a large increase in water transfer over gestation, while only a very small increase was observed in the present study, may be a result of the range in gestational ages studied. In the present investigation membranes were taken only between 0.62 and term, while in the study with human amnion transfer, rates were calculated between 0.32 and
term. Thus, there may be a definite increase in water movement across guinea-pig amnion prior to 0.62 of term.

2. The action of vasopressin on water flow through the amnion

Vizsolyi and Perks (1974) found that not only could treatment of amniotic membranes with vasopressin slow net fetal-maternal water movement, but it could also, on occasion, cause a reversal of flow. In the present investigation vasopressin was found to be capable of stimulating an increase in unidirectional water flux across amnion in the maternal-fetal direction. Indication that the responses observed represent a true sensitivity of the amnion to hormone can be seen by the fact that membranes showed no significant increase in water movement (approx. 0.4%) in response to control saline containing sodium acetate. Further indication that the response of amnion to vasopressin is real comes from the fact that there is a definite log dose/response relationship showing a linear regression lacking an overlap of the standard errors of the means. This was also noted in the net flux studies of Vizsolyi and Perks (1974). One difference between the two studies is the fact that in the unidirectional flux studies the threshold dose was calculated to be approxi-
mately 46 mU/ml, while in the net flux studies it was calculated to be about 1 mU/ml. Part of the differences in these values may be explained on the basis of differences in the mechanics of diffusional and bulk flow. Page et al. (1974 b) have shown that net flow through the amnion takes place as the organised movement of water known as bulk flow. In contrast, the radioisotope method measures flow resulting from the random movement of water molecules, or diffusional flow. A number of workers have shown that hormones can have different effects on the two types of flow; for example, neurohypophyseal principles increased net flux of water across amphibian skin by 160 %, whilst the unidirectional flux increased little if at all (see Maetz, 1968). Therefore the relatively small changes seen in the radioisotope studies (maximum = approx. 12.3%) compared to the net flux experiments (maximum = approx. 200 %), and the discrepancy between threshold values for vasopressin in the two experiments, are less important than the fact that amnion shows a log dose/response to vasopressin in both types of studies. After having verified the fact that water could move against prevailing osmotic gradients in response to hormone the next step was to determine how such a process was accomplished.
3. **Water-solute coupling**

One means by which water can move against an osmotic gradient is by coupling of water transfer to active solute transport. Such a coupling depends on "dead-end channels" in the form of lateral spaces, basal infoldings, or intracellular canaliculi such as seen in gall bladder, vertebrate intestine, renal tubules, ciliary body, salivary gland striated duct, liver, pancreas, stomach, avian salt gland, and gills of aquatic animals (Hochachka and Somero, 1973). When ions, such as sodium, are pumped into these closed channels, the fluid inside becomes hypertonic. Diffusion of ions down the concentration gradient within the channel towards the open end causes water to move across the channel walls to the inside in response to the osmotic gradient. Depending on such factors as the radius and the length of the channels, permeability of channel walls to water, and orientation of the pumps, the fluid emerging from the open end of the channel will be either isotonic or hypertonic (Diamond, 1971).

Histological and electron microscope studies of Danforth and Hull (1958), Bourne and Lacy (1960), Bourne (1962), and Hoyes (1968 b) indicate that the amnion may be involved in active secretion or absorption of water and ions. Bourne and Lacy (1960) and Bourne (1962) observed that the amniotic epithelium changes as gestation advances from...
simple epithelial cells to complex cells in later stages of pregnancy. The epithelial cells of the human amnion in later stages of gestation possess complicated intercellular canals, villous folds in the intercellular spaces, secretory granules, membrane-bound vesicles, endoplasmic reticulum, Golgi apparatus, and surface microvilli (Hoyes, 1968b). Although in early pregnancy the epithelial cells could allow an easy passage of fluid through them, in later stages of pregnancy the cells would probably be more selective due to their more complex make-up. Since the cells of the human amnion possess closed end channels there is a possibility for water-solute coupling. Unfortunately, Scoggin et al. (1964) could find no indication that water could cross the human amnion except in response to osmotic or hydrostatic gradients. If the cells of the guinea-pig amnion were similar to those of the human amnion in possessing closed end channels then perhaps vasopressin could stimulate water movement against the prevailing osmotic gradient by means of a water-solute coupling. In order to test this hypothesis the effect of vasopressin on maternal-fetal sodium movement was studied.

Vasopressin was found to produce a decrease rather than an increase in sodium movement from the maternal to the fetal side of the amnion in the absence of an osmotic
gradient. Although only two membranes were used in the study of vasopressin's effect on sodium movement, further experiments with sodium or other ions were not carried out. The reason for not elaborating on the vasopressin studies was the fact that vasopressin could not be detected in the amniotic fluid of the guinea-pig at levels high enough to elicit a response in the in vitro water flux experiments (Plath, personal communication). Inability to detect vasopressin in amniotic fluid of the guinea-pig does not seem to be the result of vasopressinase activity since this enzyme has not been found in amniotic fluid of mammals other than the primates. Vasopressin has not been found in human amniotic fluid although very low levels of vasopressinase activity have been detected (Rosenbloom et al., 1975).

4. **Hormones present in amniotic fluid**

Although it seems unlikely that vasopressin occurs in amniotic fluid of the guinea-pig in levels high enough to affect in vitro water movement, the present studies demonstrate hormone sensitivity of the membrane. This indicated that perhaps some other hormone(s) present in amniotic fluid could mediate water and/or ion movement across the membrane. Some of the hormones that have been detected in amniotic fluid include: estriol (Michie and Robertson, 1971), cortisone (Nicholas et al., 1963), cortisol
(Murphy et al., 1975), pregnanediol and pregnedione (Patti and Stein, 1964), testosterone (Giles et al., 1975), human chorionic gonadotrophin (Crosignani et al., 1970, 1971), human placental lactogen (Josimovich, 1971), and prolactin (Tyson et al., 1972). At least a few of the hormones found in amniotic fluid have been implicated in hydro-mineral balance in mammals and other vertebrates. Thus it seemed reasonable that one or more of the hormones might regulate passage of water and/or ions across the membrane. In order to test this hypothesis the effect of prolactin on hydro-mineral movement across the amnion was investigated.
SECTION II

THE EFFECT OF PROLACTIN ON WATER AND SODIUM MOVEMENT THROUGH
THE ISOLATED AMNIOTIC MEMBRANE OF THE GUINEA-PIG

INTRODUCTION:

Although prolactin has long been known to have reproductive functions in mammals, there is much evidence for an effect on osmoregulation in sub-mammalian vertebrates, such as fish. Prolactin promotes survival of certain hypophysectomized teleosts in fresh water, probably by regulating plasma electrolyte levels (Ball, 1969; Lam, 1972); it influences water and ion movement across gills (Lam, 1969), renal tubules (Stanley and Fleming, 1967; Lahlou and Giordan, 1970), intestine (Utida et al., 1972), and the urinary bladder of some fresh-water teleosts (Johnson et al., 1972, 1974; Johnson, 1973). Despite this, the effects in mammals are concerned mainly with the mammary glands and corpus luteum, and any connections with osmoregulation are indirect or uncertain (Jørgensen, 1968).

Recently, two factors emerged that were suggestive of a salt-water effect of prolactin in mammals. Firstly, it has been shown that prolactin occurs in high amounts in human amniotic fluid (Friesen et al., 1972; Josimovich, 1973; Josimovich et al., 1974; Parke, 1973). Secondly,
the amniotic membrane has been found to be sensitive to hormonal control, since neurohypophyseal peptides are capable of causing an active uptake of water into the amniotic cavity by the amniotic epithelium (Vizsolyi and Perks, 1974). Therefore, it seemed possible that prolactin might combine its importance in reproduction with its phylogenetic role in osmoregulation, by a direct action on the amnion during pre-natal life. It raised the interesting possibility that prolactin might parallel its role in hydro-mineral metabolism in fish by influencing water and/or ion movement in the mammal during its early period of "aquatic" existence.

The present investigation was initiated to determine whether or not mammalian prolactin could influence water and/or sodium movement through the isolated amniotic membrane of the guinea-pig.
RESULTS:

1. The action of prolactin: effects on unidirectional fetal-maternal water movement through the amnion

The addition of prolactin (10 µg/ml) to the fetal side of the amniotic membrane was found to cause a clear decrease in fetal-maternal water movement (for example see membrane 8 in Fig. 4). In two other preparations studied subsequently (membranes 1 and 3 in Fig. 4) the decrease in water movement was not as great as for membrane 8. Since the hormone might be slow in exerting an effect, it seemed advantageous to extend the time interval of study after hormone addition from 90 minutes to 3 hours. Fig. 4 shows the responses obtained, arranged in order of increasing gestational ages. There was a time lag of 30-60 minutes before the onset of a response, so that prolactin was slower acting than vasopressin. The exact form of the responses varied in different preparations, with some showing a clear effect in the second hour, whilst others gave a smaller response, which became marked in the third hour. In contrast, all five control experiments showed the opposite effect, with a slow, progressive rise in flow throughout the three hour period.

The five three-hour experiments with prolactin,
Figure 4. The Effect of Prolactin on Unidirectional Water Flux through the Guinea-Pig Amnion in the Fetal-Maternal Direction

The amniotic membranes are arranged in order of increasing proportion of term, as indicated on each graph (bottom left). At the arrows, membranes 1 to 8 received prolactin at 10 μg/ml, on the fetal side; treatment continued until the end of the experiment. In the same way, membranes 9 to 13 received control saline or albumen (10 μg/ml), adjusted for pH as for prolactin. Water fluxes were measured by the use of tritiated water. Dotted lines indicate the initial period of equilibration of radioisotope. Ordinates: fetal-maternal water flow, μl of water per cm² of amnion, per hour. Absissae: time from addition of radioisotope, in minutes.
HORMONE

Prolactin
(10 μg/ml)

CONTROLS

A. amniotic saline

B. albumin

TIME: MINUTES.
included in Fig. 4, could be analyzed statistically. If the resting flow was taken as the average flow in the 30 minute period prior to prolactin administration, the average fall in flow in the second and third hours was 3.5% and 9.7% respectively. Statistical analysis of all individual readings showed that the fall was significant in both the second and third hours (Student's t test of paired comparisons, Steel and Torrie, 1960; P < 0.05).

In the third period of one hour, the controls showed a rise of 5.1%; therefore, the comparison between experimental and control preparations, during the final hour of the experiments, suggested that prolactin accounted for an overall reduction in flow of 14.8% - and there was no indication that this fall would not have increased further with time.

However, this estimate of the magnitude of the changes in Fig. 4 is probably too low, since it left out the three particularly clear responses seen in the shorter 90 minute experiments (membranes 1,3,8). If a conservative assumption is made that the flow through these three membranes would have remained unchanged, at its final level, during a third hour - and this is clearly conservative, since all five three hour experiments showed a continual fall during this additional period - then the average response
for all eight experiments would be estimated at a 13.5% fall by the second hour, and a 17% fall by the third hour. This final value constituted a 22.1% factor between experimental and control values. This value is the best estimate possible for the effect of prolactin in all eight responses shown in Fig. 4.

In three further experiments, prolactin was added to the maternal side of the amnion; no responses were seen. Fig. 4 suggests that the response to prolactin tended to increase with gestational age, but the relationship was only rough, and further studies are needed. Nevertheless, attention is drawn to the exceptionally strong response given to membrane number 8, which was judged to be past its expected birth date, and about three days overdue, if one accepts the data of Draper (1920) that gestation in the guinea-pig is 65 days in length.

2. The action of prolactin; effect on net flux of water through the amnion

Since the essential importance of changes in water flux lie in the overall movement and distribution of water within the body, the effect of prolactin on net water flow through the amnion was investigated. This was carried out on an isolated amniotic membrane, attached across a
supporting tube, and immersed in fluids which reproduced natural conditions as closely as possible (see Methods). At the onset of the experiments measurements of weight changes showed that there was a movement of water from the amniotic saline within the tube to the aerated saline which surrounded the preparation (average value = 37.0 \mu l/cm^2/hr, 10 experiments). This movement followed the small hydrostatic and osmotic gradients which were present (2 cm water; 28 mOsm/l, respectively). In five experiments the resting flow was recorded for one hour, after which time both bathing salines were replaced, and prolactin at 20 \mu g/ml was included in the inner amniotic saline, which contacted the fetal surface of the amnion. In every case, the prolactin appeared to cause a marked and constant reduction in fetal-maternal water flow (Fig. 5). In four cases, there was an initial latency of about one hour, followed by a profound fall in fetal-maternal flow, which remained depressed at the termination of the experiments (3 hours). This was similar to the findings for unidirectional fluxes. In one case (Fig. 5 membrane 4), the final flow fell to zero. In one additional preparation, where the resting flow was unusually low (Fig. 5 membrane 1), there was an immediate fall to zero during the first hour after prolactin treatment.
Figure 5. The Effect of Prolactin on Net Water Flux through the Guinea-Pig Amnion in the Fetal-Maternal Direction

The amniotic membranes are arranged in order of increasing proportion of term, as indicated on each graph (bottom left). Hormone-treated and control membranes 1 and 6, 2 and 7, 3 and 8 are pairs taken from the same fetuses. At the arrows, membranes 1 to 5 (black columns) received prolactin at 20 μg/ml on their fetal side; treatment continued until the end of the experiment. In the same way, membranes 6 to 10 (light hatching) received control saline, adjusted for pH as for prolactin. Fetal-maternal flow was measured gravimetrically. Ordinates: fetal-maternal water movement, μl of water per cm² of amnion per hour. Absissae: time from the onset of the experiment, in one hour intervals.
Although most membranes were close to term, one amnion, which was judged to be overdue by two days, showed a remarkably strong response (Fig. 5; membrane 5); this is in agreement with findings obtained during the unidirectional flux experiments. In the five experiments, the average fall over the three hour period of prolactin was 59.4 %. Statistical analysis showed that the reductions in flow during the second and third hours were significant (Student's t test of paired comparisons; 95 % level of probability).

Five control experiments showed no changes parallel to prolactin effects; there was often an increase in fetal-maternal flow. For the controls to which pH adjusted saline had been added there was a 3.6 ± 0.5 % decrease in water movement in the second hour, and a 1.3 ± 0.2 % increase in the third hour. When 20 µg/ml albumen solution was added to two other control preparations there was a 6.7 ± 2.3 % increase in the second hour and a 4.8 ± 3.9 % increase in the third hour. The comparison of experimental and control data was particularly notable in three cases, where it was possible to study control membranes from the same fetuses as the experimental preparations (Fig. 5, membranes 1 and 6; 2 and 7; 3 and 8). Statistical analysis confirmed that there were no significant effects in the
control preparations (Student's t test of paired comparisons 95% confidence level).

3. The action of prolactin; the comparison of the effects on unidirectional and net fetal-maternal water flux through the amnion

Fig. 6 shows a comparison of the changes induced by prolactin in both types of experiment, when the results from unidirectional flux were converted into the same form used to express net flow (one hour periods). Five three hour experiments are averaged for each graph. It is clear that prolactin acts on the fetal surface of the amnion to cause a consistent fall in fetal-maternal water flow as judged by either unidirectional or net flow, and the latency and form of the flow is similar in both cases. Statistical analysis (Student's t test of paired comparisons, 95% level of probability) showed that in both types of experiment the changes in the first hour were not significant, whilst in the second and third hours were significant at the 95% confidence levels. Control experiments showed no significant changes at any time.

However, comparison of the results of the two methods showed that the decrease after prolactin was approximately five to six times greater when judged by net flow. This
Figure 6. The Comparison of the Effect of Prolactin on Net Flux and Unidirectional Flux of Water through the Guinea-Pig Amnion in the Fetal-Maternal Direction

**Top:** Average Values for Net Water Flux (Gravimetric)

**Bottom:** Average Values for Unidirectional Flux (Tritiated Water)

All measurements are for the fetal-maternal direction of flow, and are expressed by the same parameters. Each graph represents an average of five complete three-hour experiments. All membranes were over 0.62 of term. Black columns show experiments with prolactin treatment (20 μg/ml for net flux; 10 μg/ml for unidirectional flux). The period of treatment lasted from the arrows to the end of the experiment. Columns with light hatching show corresponding control experiments carried out with saline, pH adjusted as for prolactin. The values above the columns show the significance of the change from the resting flow, as determined by Student's t test of paired comparisons; NS = not significant. Ordinates: fetal-maternal water movement, μl of water per cm² of amnion, per hour. Absissae: time from the onset of the experiment, in one hour intervals.
difference is probably due, firstly, to the higher dose of prolactin used in the net flow experiments (20 μg/ml, net flow; 10 μg/ml, unidirectional flow), and, secondly, to differences between bulk flow and diffusional flow (see page 54 and the discussion). However, it should be remembered that the two sets of experiments were carried out in different ionic conditions, and direct comparisons are difficult.

Evidence that the decrease in fetal-maternal water movement is due to a response of the membrane to prolactin, and not due to a change in the osmotic pressure, comes from two types of observation. Firstly, the membranes treated with albumen showed an increase rather than a decrease in fetal-maternal water movement. Secondly, the osmotic pressures of amniotic saline, pH adjusted amniotic saline, pH adjusted amniotic saline containing 10 μg/ml prolactin, and pH adjusted amniotic saline containing 20 μg/ml prolactin were not significantly different when measured with an osmometer (Model 31LAS, Advanced Instruments, Newton, Mass.). The differences were within the precision of error of the machine used.
4. **The action of prolactin: effect on unidirectional maternal-fetal water flux through the amnion**

In eighteen early experiments in which prolactin (20 μg/ml) was added to the fetal side of the perfusion chamber there was an indication that prolactin might be able to increase maternal-fetal water movement. At the end of 30 minutes after hormone addition there was a $0.9 \pm 1.1\%$ increase in water movement, followed by a $5.2 \pm 4.8\%$ and $4.4 \pm 3.6\%$ increase at 60 and 90 minutes respectively. Interpretation of the results was difficult because no control membranes had been set up for these experiments. 

The study was repeated at a later date using controls, and a longer time interval (3 hours v.s. 90 minutes), since fetal-maternal investigations had indicated that the hormone was often slow acting. Upon addition of 10 μg/ml prolactin to the fetal side of five membranes, an increase in maternal-fetal water flow of $10.2 \pm 20.3\%$ was recorded in the second hour and $8.5 \pm 20.2\%$ in the third hour. Six control membranes that had been treated with 10 μg/ml albumen showed an average decrease in water flow of $2.2 \pm 7.6\%$ in the second hour and an increase of $1.0 \pm 10.0\%$ in the third hour (see Fig. 7). Statistical analysis (Student's t test of paired comparisons, 95% confidence
Figure 7. The Effect of Prolactin on Unidirectional Water Flux through Guinea-Pig Amnion in the Maternal-Fetal Direction

At the arrows, membranes received either 10 μg/ml prolactin (N = 5) or 10 μg/ml albumen (N = 6) on their fetal side; treatment continued until the end of the experiment. Data were averaged over one hour periods, and expressed as the mean ± the standard error of the mean, so that comparison could be made with Fig. 6. Statistical analysis (Student's t test of paired comparisons, 95% confidence interval) shows that all flux values subsequent to treatment with prolactin or albumen are not significant (N S). Ordinates: maternal-fetal water movement, μl of water per cm² of amnion, per hour. Absissae: time from the onset of the experiment in one hour intervals.
MATERNAL - FETAL DIRECTION

**Prolactin (10 μg/mL)**

<table>
<thead>
<tr>
<th>TIME (HOURS)</th>
<th>WATER MOVEMENT (μL/cm²/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1000</td>
</tr>
<tr>
<td>1</td>
<td>900</td>
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<tr>
<td>2</td>
<td>1000</td>
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<tr>
<td>3</td>
<td>1100</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
</tr>
</tbody>
</table>

**Albumen (10 μg/mL)**

<table>
<thead>
<tr>
<th>TIME (HOURS)</th>
<th>WATER MOVEMENT (μL/cm²/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1000</td>
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<tr>
<td>1</td>
<td>900</td>
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<tr>
<td>2</td>
<td>1000</td>
</tr>
<tr>
<td>3</td>
<td>1100</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
</tr>
</tbody>
</table>

**Legend:**
- **NS** indicates no significant difference.
level), however, revealed that none of the changes in water movement recorded for either experimental or control preparations were significant at any time. There was also no indication of a dose/response relationship between the early preparations to which 20 μg/ml prolactin had been added and later ones, which were treated with 10 μg/ml prolactin when equivalent time intervals were compared.

5. Permeability of the amnion to sodium

Permeability of the isolated amniotic membrane to sodium was determined by measuring transmembrane flux of radioactive sodium. In every case the permeability value was taken as the flux rate of sodium across the amnion 90 minutes after the preparation was set up (i.e. just prior to the addition of hormone or albumen). Membrane permeability to sodium was found to change markedly over the course of gestation (see Fig. 8). From day 30 to day 55 permeability of amnion to sodium in the fetal-maternal direction seemed to decrease by a factor of about 5. The permeability in this direction seemed to increase by a factor of about 13 between days 55 to 70, but from day 57 to day 70 the increase was only about 4 fold. Membranes had not been taken from fetuses younger than 40 days when studying maternal-fetal movement of sodium, and thus, it
Figure 8. The Permeability of Guinea-Pig Amnion to Sodium at Different Gestational Ages

Unidirectional flux of radioactive sodium ($\text{Na}^{22}$) was studied in both directions across the isolated amniotic membrane of the guinea-pig at different gestational ages. The flux rate at 90 minutes, just prior to hormone addition, was used as an indication of permeability for each membrane. Ordinates: unidirectional sodium movement, $\mu$Eq of sodium per cm$^2$ of amnion, per hour. Absissae: gestational age in days for the fetal guinea-pigs from which amniotic membranes were taken.
UNIDIRECTIONAL SODIUM FLUX: \( \mu \text{Eq/cm}^2/\text{hr} \)

GESTATIONAL AGE: DAYS

MATERNAL - FETAL DIRECTION
was not possible to determine whether permeability in this direction decreases between days 30 to 55. Between days 57 to 68 maternal-fetal sodium movement increased by a factor of approximately 8; and from day 57 to day 70 permeability increased 35 fold. More experiments are needed to determine if there is a significant difference in membrane permeability to sodium in the two directions.

6. The action of prolactin; effect on unidirectional maternal-fetal sodium flux through the amnion

In order to make a comparison of prolactin's effect on sodium movement with its effect on water movement possible, data were expressed as percentage change in ion flux following hormone treatment. The value for sodium movement at 90 minutes, just prior to the addition of prolactin or albumen was taken as the "base line" (i.e. 0 % change). Upon addition of prolactin (10 \( \mu g/ml \)) to seven membrane preparations increases of sodium flux in the maternal-fetal direction of 6.2 \( \pm \) 3.5 %, 11.3 \( \pm \) 6.7 %, and 21.3 \( \pm \) 8.3 % were recorded in the first, second, and third hours respectively. Changes of 5.9 \( \pm \) 4.1 %, 10.8 \( \pm \) 8.1%, and 21.3 \( \pm \) 14.9 % were observed in the first, second, and third hours for membranes treated with albumen (see Fig. 9). Statistical analysis (Student's \( t \) test of paired comparisons, 95 % confidence interval) revealed that there was a
At the origin, the membranes received either albumen (10 µg/ml) or prolactin (10 µg/ml), on their fetal side. Sodium fluxes were measured by the use of Na\(^{2+}\). The period just prior to hormone addition (i.e. 90 min) was taken as the "base line" value (0 % change) from which percentage change in sodium movement was calculated after the addition of albumen or prolactin. Values were measured in the maternal-fetal direction (N = 14) and in the fetal-maternal direction (N = 10), and expressed as the mean ± the standard error of the mean. Ordinates: percentage change in sodium movement from the "base line" value. Absissae: time from addition of albumen or prolactin, in minutes.
MATERNAL - FETAL DIRECTION

- prolactin (10μg/mL)
- albumen (10μg/mL)

FETAL - MATERNAL DIRECTION

- prolactin (10μg/mL)
- albumen (10μg/mL)

TIME: MINUTES.
significant increase in maternal-fetal sodium movement in the first, second, and third hours after addition of prolactin. The controls, however, showed a similar trend of increase after addition of albumen, and statistical analysis (Student's t test of unpaired comparisons, 95% confidence level) showed that there was no significant difference between experimentals and controls at any time. Thus comparison of prolactin-treated membranes with albumen-treated membranes suggested that prolactin probably does not influence maternal-fetal sodium movement.

7. The action of prolactin; effect on unidirectional fetal-maternal sodium flux through the amnion

When prolactin (10 μg/ml) was added to five isolated amniotic membranes from fetal guinea-pigs, between 30 and 57 days old, a much greater change in fetal-maternal sodium movement was observed than was noted in the maternal-fetal direction. The rate of sodium movement increased to $22.7 \pm 12.4\%$ in the first hour, $22.4 \pm 14.4\%$ in the second hour, and $53.6 \pm 10.1\%$ in the third hour. Control membranes treated with albumen showed smaller changes that were similar to those recorded in the maternal to the fetal direction. Values of $8.0 \pm 6.4\%$, $8.3 \pm 7.3\%$, and $15.1 \pm 15.9\%$ were recorded in the first, second, and third
hours, respectively for controls. Although there was no significant increase in fetal-maternal sodium movement in either the first or second hours after addition of prolactin there was a very significant increase in the third hour (Student's t test of paired comparisons; $p < 0.01$). This value was significantly different from that of control membranes in the third hour (Student's t test of paired comparisons, $p < 0.05$). At no time did control membranes treated with albumen show a significant increase over the "base line" value of sodium flow. Comparison of hormone and control preparations suggests that prolactin can cause a 38.5% increase in fetal-maternal sodium movement. A membrane that was judged to be from a 70 day-old guinea-pig showed no response to treatment with prolactin. Further experiments are necessary, however, in order to determine whether "overdue" membranes are not able to increase sodium flux in response to hormone; permeability to sodium at this stage may be maximal, and cannot be increased by hormone addition.
DISCUSSION:

The results given here suggest that prolactin is capable of modifying water and sodium movement through the amnion. It is able to slow water flow in the fetal-maternal direction by decreasing osmotic and diffusional permeabilities to water. The hormone also stimulates an increase in fetal-maternal sodium movement. Changes in membrane permeability to sodium over the course of gestation were also studied.

1. The action of prolactin on water flux through the amnion

The present experiments were initiated in order to determine whether or not the amniotic membrane of the guinea-pig could alter its permeability to water and ions in response to treatment with prolactin. It had been previously noted that the membrane was sensitive to neurohypophyseal hormones, but it did not appear that vasopressin occurred in amniotic fluid in sufficient quantities to elicit an *in vitro* response. Although it is often dangerous to apply data from one species to another, the fact that prolactin occurs in human amniotic fluid in amounts similar to those used here (i.e. 10 μg/ml) suggests that the responses of the guinea-pig amnion may prove to be of physiological significance.

The results obtained are in general agreement with
the hydro-mineral effects observed in some teleost fishes. Prolactin has been found to reduce net influx of water in the stickleback by reducing osmotic permeability of the gill to water (Lam, 1969). It is able to increase urine flow in some fish by either decreasing reabsorption of water by the renal tubules, or by increasing glomerular filtration rate (Stanley and Fleming, 1967). Utida et al. (1972) have shown that injection of prolactin into the sea-water adapted eel, *Anguilla japonica* decreases water reabsorption across the isolated intestine. In particular the results with the amnion agree with the results of Johnson et al. (1972) and Johnson (1973). These workers found that prolactin produced a decrease in water efflux from the isolated urinary bladder of the starry flounder (*Platichthys stellatus*); the 60% decrease in net flux of water (Johnson, 1973) was close to the average decrease of 59.4% found during the final hour of the amnion experiments reported here. The main difference in the two sets of experiments is that prolactin did not act *in vitro* in the fish studies, but had to be injected into the whole animal prior to the experiment. Maximal effects in decreasing the permeability of the flounder bladder did not occur until two days after injection of prolactin, although it lasted for about four days (Johnson *et al.*).
1974). At least twelve hours were required in order to observe any significant change in permeability. However, prolactin is known to act *in vitro* in other preparations, such as the toad bladder (Snart and Dalton, 1973) so that the sensitivity of the isolated amnion is not unreasonable. Perhaps the strongest evidence that the effects of prolactin noted here are genuine was provided by the very recent findings of Josimovich and Merisko (1975) which were announced at the 22nd Annual Meeting of the Society for Gynecological Investigation in March of this year. They found that water shifts could be induced between the amniotic fluid and the fetal rhesus monkey within 2 hours after injection of prolactin into the amniotic sac.

The most striking effect of prolactin in the guinea-pig study reported here was shown on the net flux of water, where, on the average, flows were reduced by almost 60% by the third hour after treatment. Occasionally values of zero were recorded. These effects were five to six times greater than in the radioisotope experiments. However, this discrepancy was not entirely unexpected, for a number of reasons. The dose of prolactin (20 μg/ml) given in the net flow experiments was twice that in the radioisotope studies, so that a larger response was reasonable. However, the apparent log dose/response curve for the action of prolactin on water movement in other tissues is nowhere
near as steep as this discrepancy would require (see data of Johnson et al., 1972). It is possible that part of the problem reflects differences in the mechanics of diffusional and bulk flow, as were noted in the studies with vasopressin (see page 54). Therefore, the relatively small changes seen in the radioisotope studies, and the discrepancy between the unidirectional and net flow experiments, are less important than the fact that consistent responses were produced by prolactin, even in terms of diffusional flow.

In any case, it is worth pointing out, that it is the net flow, which was strongly influenced by prolactin, which has the greater relevance to the functioning of the fetus itself.

2. The permeability of the amniotic membrane to sodium

The permeability of the amniotic membrane of the guinea-pig to sodium was found to change markedly over the course of gestation. From day 57 to day 70 maternal-fetal sodium movement increases by a factor of about 35. This agrees with the findings of Flexner and Gellhorn (1942). These workers found that the transfer rate of radioactive sodium into amniotic fluid of the guinea-pig increases by a factor of between 20 and 30 fold from the first third of pregnancy to term. Although increases of up to 70 fold have been recorded for sodium movement across human placenta
from week 9 to 36 (Flexner et al., 1948), permeability changes of isolated human amnion to sodium seem less dramatic (see Lind et al., 1972). This may be a result of the fact that only two stages of gestation were studied in the human investigation (viz. at 0.36 term and term). The values given here for sodium movement across the guinea-pig amnion are very similar at 30 (0.44 term) and 68 days (term), and therefore, the selection of these two periods would not indicate that any clear permeability changes took place. If one assumes that the length of gestation in the guinea-pig is between 65 days (Draper, 1920) and 69 days (Illingworth et al., 1974) one can compare human values with guinea-pig values for sodium movement across amnion at term. Transfer rate for sodium across the isolated amniotic membrane of the guinea-pig was 20 μEq/cm²/hr at 64 days and 22 μEq/cm²/hr at 68 days. These values are comparable to the mean of 16.3 μEq/cm²/hr reported for sodium movement across isolated human amnion at term (Lind et al., 1972).

It is quite difficult to hypothesize what might be responsible for the observed changes in permeability of guinea-pig amnion to sodium without knowledge of possible ultrastructural changes occurring. Only the human amnion has been well studied at the ultrastructural level so that
considerations of the guinea-pig amnion are very speculative. If one makes the assumption that guinea-pig amnion may be similar to human amnion then there may be a morphological basis for the physiological changes noted in the present investigation. Hoyes (1968) demonstrated that there were sites of open communication between the intercellular spaces and the amniotic cavity, and that the time of formation of the villous folds and their change in width seemed to be correlated with total volume of amniotic fluid. The villous folds appear at the time of maximum accumulation of amniotic fluid. If one associates the appearance of villous folds with an increase in permeability then this might explain why the guinea-pig amnion showed high fetal-maternal permeability to sodium in early gestation (i.e. at 30 days). Closure of the intercellular spaces in human amnion occurred at a time when the high volume of amniotic fluid is being maintained. If closure of the spaces would decrease permeability to sodium, then the slow movement of sodium across the guinea-pig amnion between days 45-60 might also have a morphological basis. In the last weeks of human pregnancy the intercellular spaces become wider, and this seems to be related to the reduction in the total volume of fluid at this time. This observation might also explain the increased
permeability of guinea-pig amnion between day 60 and term. The large increase noted for membranes that were judged to be 70 days old might represent merely a degeneration of the membrane and concomitant loss of selectivity.

The changes in rates of sodium movement have been referred to as due to permeability changes rather than being due to changes in an active pump. Although no attempt was made to determine whether sodium movement was active, it was assumed to be passive for a number of reasons. Firstly, Scoggin et al. (1964) and Lind et al. (1972) could find no evidence of active sodium transport across human amnion in vitro. Secondly, Mellor (1969) could measure no potential difference across the amnion, the uterine wall, or the yolk sac splanchnopleur of the guinea-pig in vitro. The negative potential difference of guinea-pig amniotic fluid appears to arise from electromotive forces contributed by the fetal gastric mucosa and the placenta. The secretion of HCl by the fetal stomach into the amniotic sac probably accounts for the high chloride concentration of amniotic fluid. The potential difference of guinea-pig amniotic fluid decreases by over 50% from day 60 to term. Mellor (1969) attributed this decrease to either a degeneration of the electrogenic ion pump or to an increase in ionic permeability that would short-circuit the pump. The second explanation seems to
be in agreement with the findings here, where the permeability of the amnion to sodium increases on about the 60th day of gestation (see page 79).

3. **The action of prolactin on sodium flux through the amnion**

The effect of prolactin in stimulating sodium movement across the amniotic membrane is in agreement with results obtained in lower vertebrates. Prolactin promotes survival of some hypophysectomized fish in fresh water by maintenance of normal plasma osmolality and concentration of NaCl in blood (Ball and Ensor, 1965; Pickford et al., 1970; Johnson et al., 1970). Prolactin stimulates an active uptake of sodium by the gills of hypophysectomized *Fundulus kansae* (Ball, 1969). In the stickleback, injection of prolactin causes an increase in urine flow and a decrease in urine sodium (Lam, 1969). Utida et al., (1971) found that prolactin decreases water reabsorption across the isolated intestine of *Anguilla japonica*, while at the same time, promoting reabsorption of NaCl. Johnson et al. (1974) determined that a single injection of prolactin into the starry flounder (*Platichthys stellatus*) caused an increase in sodium absorption by the isolated urinary bladder, and a decrease in urine osmolality and sodium concentration. Effects for sodium movement across the
bladder were not observed until 24 hours after injection. Maximal effects were noted at about 96 hours after injection when sodium movement increased by 101%. As in the case of prolactin's effect on water movement, there was a much shorter lag period for hormone action on sodium movement across the amnion than is noted for tissues of fish. But again it might be mentioned in defense of the present results that Snart and Dalton (1973) found that sodium movement across the in vitro toad bladder preparation increases about 30 minutes after addition of prolactin to saline bathing the membrane.

There is some indication that prolactin might play a role in regulating hydro-mineral balance in the adult mammal. Mainoya et al. (1974) demonstrated that injection of the rat, the hamster, and the guinea-pig with 1 mg of ovine prolactin stimulated enhancement of fluid and NaCl absorption across the isolated jejunum. Prolactin was found to increase the mucosal Na⁺ transfer by about 110% across the jejunum of the rat, if experimental and control values are compared. The 41.6% increase in sodium movement across the jejunum of the adult guinea-pig is in agreement with the 38.5% increase in sodium movement across the amnion after prolactin treatment. The results given on prolactin's effect on water and ion movement across the intestine of
the adult mammal are suggestive of hormone sensitivity. Whether or not this would represent a physiological action of prolactin in the adult remains to be proven, however, since rather massive doses of the hormone were required to elicit a response. Since Mainoya et al. (1974) injected each rat with at least 1 mg of prolactin, levels of the hormone in the blood of a 250 g rat could approximate 60 µg/ml, if one assumes complete entry of the hormone into the circulatory system and a total blood volume of 16.7 ml. According to Bast and Melampy (1972) the total concentration of prolactin in the blood of the rat amounts to about 48 ng/ml at diestrous. Making the assumptions mentioned above for prolactin injection, the dose administered by Mainoya et al. (1974) could approach 1000 times the physiological level in the blood. In contrast to this, the dose of prolactin (10 µg/ml) used in the present study has been detected in the amniotic fluid of some species of mammals (Friesen et al., 1972). It may be that the tissues of the fetal mammal are sensitive to prolactin's hydro-mineral control, but that the sensitivity decreases with age. The sensitivity of the adult intestine to prolactin, in a gross sense, may represent a carry over from fetal development that has lost its relevance to the physiological well being of the organism as an adult mammal.
SECTION III

PRELIMINARY EXPERIMENTS STUDYING VASOPRESSIN'S EFFECT ON
OTHER FETAL TISSUES

INTRODUCTION:

The studies of Vizsolyi and Perks (1974) were undertaken in an attempt to determine the significance of high levels of neurohypophyseal hormones in the blood of the fetal mammal. Although no detectable levels of vasopressin have been found in amniotic fluid to date, very high levels have been found in fetal circulation. During the last trimester of pregnancy in the rhesus monkey, the neurohypophysis of the fetus maintains a high rate of vasopressin secretion (Skowsky et al., 1973). Not only does the fetal secretory rate of vasopressin exceed the maternal rate, but also the osmolar and volume receptors controlling vasopressin release are functional at birth (Fisher et al., 1963). This suggests that although vasopressin may not be able, by itself, to exert a direct effect on the amniotic membrane of the guinea-pig, that it could influence water and ion movement across fetal tissues in contact with the circulatory system.
1. **The effect of vasopressin on water flux through the isolated fetal bladder**

**Introduction:**

It was mentioned that bladder urine of the guinea-pig is hypertonic to kidney urine (Kleinman, 1970). The bladder of the adult mammal acts as a storage organ for urine but seems to have no reabsorptive capacity. This is in contrast to the urinary bladder of some anuran amphibians and fish. These animals are able to reabsorb water and/or ions across the bladder wall, and regulate the reabsorptive process hormonally. Since the fetal mammal has been found to have certain characteristics in common with lower vertebrates it seemed reasonable that water reabsorption by the urinary bladder of the fetal guinea-pig might be under endocrine control.

**Results:**

In order to test this hypophysis urinary bladders were removed from fetal guinea-pigs ranging in age from 0.75 to 0.91 term, and set up in a perfusion chamber. The bladders were bathed on their serosal side by maternal saline and on their mucosal side by amniotic saline. At the end of a 60 minute equilibration period vasopressin
(100 mU/ml) was added to the serosal side of three bladders, and was found to increase water flux by almost 50% at the end of an hour. The addition of sodium acetate to a control preparation, however, was followed by less than a 10% rise in water flow (see Table II and Fig. 10).

**TABLE II**

**THE EFFECT OF VASOPRESSIN ON UNIDIRECTIONAL MUCOSAL-SEROSAL WATER MOVEMENT THROUGH THE ISOLATED URINARY BLADDER OF THE FETAL GUINEA-PIG**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of preparations</th>
<th>% increase in water movement in 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>sodium acetate</td>
<td>1</td>
<td>9.7</td>
</tr>
<tr>
<td>100 mU/ml vasopressin</td>
<td>3</td>
<td>49.4 ± 17.8 a</td>
</tr>
</tbody>
</table>

*\bar{x} ± S.E.M.*

Discussion:

Although the results are only preliminary, and should be verified by further experiments, there is some indication that vasopressin can influence water movement across the isolated urinary bladder of the fetal guinea-pig. If such a role for vasopressin should prove to be physiological then the hormone could aid water conservation in the fetal guinea-pig by promoting reabsorption of water across the bladder wall into the blood. Effects of vasopressin on the fetal bladder of the lamb had been noted previously by France
Figure 10. An Example of the Effect of Vasopressin on Percentage Increase in Unidirectional Water Flux through the Urinary Bladder of the Fetal Guinea-Pig

At the origin, the isolated urinary bladders received either 100 mU/ml of vasopressin (N = 1) or sodium acetate (N = 1) on their serosal side. Water fluxes were measured by the use of tritiated water. The period just prior to hormone addition (i.e. 60 min) was taken as the "base line" value (0 % change) from which percentage change in water flux was calculated after the addition of sodium acetate or vasopressin. One membrane from the three described in Table II was chosen as representative of the effect of vasopressin on water movement across the fetal bladder. Ordinates: percentage change in mucosal-serosal water movement from the "base line" value. Absissae: time from addition of sodium acetate or vasopressin, in minutes.
et al. (1972). These workers found that addition of the hormone to the serosal side of the isolated urinary bladder influenced the flux of isotopic ions across this organ. Vasopressin caused a net influx of sodium across the bladder of early fetuses and a net efflux in late fetuses. The natriuretic response noted by France et al. (1972), and the water resorptive response described here for fetal urinary bladder to neurohypophyseal hormone is typical of some anuran amphibians (see Bentley, 1966; and Turner and Bagnara, 1971). The fact the frog bladder is more sensitive to arginine vasotocin than to arginine vasopressin suggests the possibility that the fetal bladder may also be more sensitive to vasotocin. This may provide a role for the high levels of arginine vasotocin found in the pituitary of fetal mammals by Vizsolyi and Perks (1969). During its early "aquatic" existence the fetal mammal may resemble frogs and toads in its ability to regulate water and ion movement across the urinary bladder by secretion of arginine vasotocin. The fact that the fetal urinary bladder demonstrates hormone sensitivity suggests a possible role for prolactin, since this hormone regulates hydro-mineral movement across the bladder wall of certain teleost fishes. The possible roles of arginine vasotocin and prolactin in an endocrine control of the fetal urinary bladder should be investigated in
future experiments.

2. The effect of vasopressin on water flux through isolated skin of the fetal guinea-pig

Introduction:

Ultrastructural studies of human fetal skin suggests that it is capable of either secretion or absorption (Breathnach and Wyllie, 1965; Hoyes, 1967, 1968 a). Unlike adult skin, fetal skin is very permeable to water prior to midterm (Lloyd et al., 1969, and Seeds, 1972 a). Lind et al. (1972) demonstrated that Na⁺, as well as water, can readily diffuse through human fetal skin prior to keratinization. The fact that Lind and Hytten (1970) describe the periderm cells of the human fetus as having a strong resemblance to renal tubule cells under the influence of vasopressin suggested a possible hydro-mineral effect of neurohypophyseal hormones.

Results:

Skin was removed from the back region of fetal guinea-pigs ranging in age from 0.46 term to 0.51 term by sharp dissection, and was placed in a perfusion chamber, where it was bathed by amniotic and maternal salines. At the end of a 90 minute equilibration period vasopressin (500 mU/ml and 1000 mU/ml was added to the amniotic saline bathing
the serosal side of four preparations, and was found to increase water flux across fetal skin (see Table III and Fig. 11).

TABLE III
THE EFFECT OF VASOPRESSIN ON UNIDIRECTIONAL SEROSAL-MUCOSAL WATER MOVEMENT THROUGH THE ISOLATED SKIN OF THE FETAL GUINEA-PIG

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of preparations</th>
<th>% increase in water movement in 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mU/ml vasopressin</td>
<td>2</td>
<td>14.3 ± 8.9 a</td>
</tr>
<tr>
<td>1000 mU/ml vasopressin</td>
<td>2</td>
<td>30.4 ± 13.7</td>
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</tbody>
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a $\bar{x}$ ± S.E.M.

Histological sections were prepared from portions of skin removed from the back region of each animal used in this study. Sections for histological study were taken in close proximity to those used in the transport studies, and were stained with Chevremont-Frederick's stain for SH groups and Mallorie's stain. In this way one could determine whether or not skin used in the flux experiments was keratinized. There was no indication of keratinization.

Discussion:

Although more experiments are needed, including controls, there seems to be some indication of hormonal influence on serosal-mucosal water movement across isolated skin of the fetal guinea-pig. The fact that doubling the dose of vasopressin seemed to produce a doubling of the
Figure 11. Examples of the Effect of Vasopressin on Percentage Increase in Unidirectional Water Flux through Skin of the Fetal Guinea-Pig

At the origin, fetal skin received either 500 mU/ml (N = 1) or 1000 mU/ml (N = 1) of vasopressin on their serosal side. Water fluxes were measured by the use of tritiated water. The period just prior to hormone addition (i.e. 90 min) was taken as the "base line" value (0 % change) from which percentage change in water flux was calculated after the addition of vasopressin. Two membranes from the four described in Table III were chosen as representative of the effect of vasopressin on water movement through isolated fetal skin. Ordinates: percentage increase in serosalmucosal water movement from the "base line" value. Absissae: time from addition of vasopressin, in minutes.
response tends to support the hypothesis that fetal skin is sensitive to hormone at the gestational ages studied. If these findings prove to be of physiological significance to the fetal guinea-pig, then the fetus may be able to increase water reabsorption from the amniotic sac across its skin by secretion of vasopressin in response to fetal dehydration. A short note appearing after the experiments mentioned above indicated that vasopressin could stimulate an increase of the influx of sodium across the skin of fetal sheep (France, 1974). Thus, the present findings with regards to vasopressin control of water movement across fetal skin would seem reasonable. Since the fetal skin is bathed with amniotic fluid containing high concentrations of prolactin in some species, it would be interesting to investigate a possible control of hydro-mineral movement across fetal skin by prolactin, in view of hormone-sensitivity of fetal guinea-pig skin.
GENERAL DISCUSSION

The experiments described in the preceding sections of this thesis suggest the possibility that vasopressin and prolactin can influence water and sodium movement across isolated membranes and tissues of the fetal guinea-pig. Although a number of hormonal effects have been noted that parallel the actions seen in other vertebrates, it is probably very dangerous at this time to assume that the findings in the guinea-pig study will apply to other species of fetal mammals.

The results described in section I indicate that vasopressin can influence water movement through the amniotic membrane. In the subsequent discussion of hormonal influence on the amniotic membrane, however, only prolactin will be dealt with in detail. It is still too early to determine whether or not vasopressin can be released into the amniotic sac of the fetal mammal in amounts capable of exerting an in vivo response. It may be that if vasopressin is found in the amniotic fluid of the fetal guinea-pig in amounts too small to cause an effect on its own, that it may be acting in synergism with another hormone (e.g. prolactin). This is reasonable in view of the findings of Horrobin et al. (1973) that prolactin can act in synergism with vasopressin in the production of antidiuresis in Merino ewes.
1. **The physiological significance of the effects of prolactin on the amnion**

At the present time, the *in vitro* effects of prolactin on the amnion must be regarded as pharmacological. However, there is a strong possibility that they may prove to be physiological. Studies on human amniotic fluid, from early stages of pregnancy, have shown unusually high levels of prolactin, sometimes 227 times higher than those of maternal serum (Friesen *et al.*, 1972; Josimovich, 1973; Josimovich *et al.*, 1974; Parke, 1973). The values range from 1.2 to 10 μg/ml at 20 weeks, and this last dose was shown to be capable of having a positive effect on the isolated amnion, in the present experiments.

Nevertheless, the possible importance of prolactin in normal amniotic control must be a matter of speculation. Certainly the action of prolactin in slowing fetal-maternal water flow could partly aid in allowing amniotic fluid to build up. The actual significance of maintaining a certain volume of liquor amnii is not well understood. Evidence that a certain volume must be maintained is demonstrated by the fact that removal of the fluid at midterm will cause death of the fetus (Adolph, 1967). Some of the proposed roles for amniotic fluid include the following: 1. It provides thermal insulation and serves as a cushion to
protect the fetus from shock and abrasion by the uterine wall (Reynolds, 1972). 2. It may act as a moist spacious environment in which the fetus can grow in a relatively weightless condition. 3. Amniotic fluid may play a role in causing distention of the uterus, which may be necessary for not only fetal development, but also uterine and placental development (Kerpel-Fronius, 1970). 4. Liquor amnii may act as a safeguard against fetal water imbalance, so that if large quantities of water accumulate in the fetus excess water may be diverted to the amniotic sac. Water may also be removed from the amniotic sac in the case of fetal dehydration (Seeds, 1965). Bruns et al. (1963) showed that experimental dehydration of the fetal rabbit caused a decrease in the volume of amniotic fluid, suggesting that water had been transferred from the amniotic compartment to the fetal compartment.

It was mentioned in the general introduction that retention of water in the amniotic sac is difficult to explain; the existing osmotic, colloid osmotic, and hydrostatic gradients, as well as Staverman (reflection) coefficients for solutes, all tend to favor the passage of water from the amniotic compartment to the mother (Seeds, 1973). The action of vasopressin in promoting water movement into the sac, and the ability of prolactin to slow
water loss from it, could help to explain the enigma of how amniotic fluid volume is maintained. This is not meant to imply that the fetus is able to directly control amniotic fluid volume by secretion of prolactin into the amniotic sac. On the contrary, it appears that in the rhesus monkey the maternal circulation may be the major source of amniotic fluid prolactin (Josimovich et al., 1974). No attempt has been made yet to determine whether or not the mother is able to regulate the release of prolactin into the amniotic compartment in response to stimuli such as change in osmotic pressure of amniotic fluid. Prolactin deposition into amniotic fluid may occur for reasons other than the physiological control of hydro-mineral balance. The net result, however, would still be a slowing of water flow out of the amniotic sac. Although the amount of prolactin present in human amniotic fluid decreases over the course of gestation (Friesen et al., 1972), sensitivity of the amniotic membrane to hormonal stimulation seems to increase in apparently "overdue" membranes (see results, page 65) so that low levels don't rule out the possibility of a response to hormone at this stage of gestation.

In addition, it is possible that prolactin affects the levels of certain solutes in amniotic fluid. Isotopically labeled nitrogenous solutes such as creatinine have a
rapid and extensive bidirectional exchange between the three intrauterine compartments of the pregnant rhesus monkey (Pitkin and Reynolds, 1975). In the first half of human pregnancy, creatinine is present in amniotic fluid at approximately the same level as that of maternal plasma. At about midterm there is a gradual increase in creatinine concentration, and between $34$ to $37$ weeks there is a rapid increase so that levels are $2$ to $4$ times higher than those of early stages of gestation (Reynolds et al., 1954). The relationship between amniotic fluid creatinine and gestational age has lead to use of amniotic fluid creatinine level as an index of fetal maturity (Gauthier et al., 1972). Although urea can be rapidly exchanged between amniotic fluid and maternal circulation, it accumulates in amniotic fluid near term to levels $3$ to $4$ times higher than found in the fetus or mother (Reynolds, 1972). How solutes like creatinine and urea accumulate in the amniotic compartment, when the amnion is relatively permeable to their egress, has been an enigma.

Leaf and Hays (1962) found a close parallel in the passage of water and urea through the toad bladder, and both were influenced by vasopressin. Seeds (1973) describes the placental membranes as having significant porous channels through which there is a bulk flow of
solvent water in a fashion similar to that for toad bladder. Thus, the action of prolactin (and perhaps, vasopressin) might lead to a slowing of the movement of urea out of the amniotic sac of the guinea-pig. This hypothesis needs to be tested, however, since in tissues other than the toad bladder, urea and water follow different routes. Rocha and Kokko (1974) found that the pathways of urea movement are not the same as the principal pathways of water movement across the papillary collecting duct epithelium of the rabbit. Vasopressin was found to have no effect on increasing permeability of the nephron to urea. According to Hays and Levine (1974), the solvent drag effect noted in the toad bladder for water and urea movement may be the result of inadequate stirring in conventional diffusion chambers. There is no apparent solvent drag effect noted for acetamide, an amide closely related to urea, when toad bladder is studied in mechanically stirred chambers. The apparent solvent drag effect may have been due to the accumulation of isotopically labeled solute in unstirred layers near the luminal membrane, when water moves from the mucosal to the serosal side of the toad bladder. Studies should, thus, be carried out in order to determine whether or not prolactin can slow the fetal-maternal movement of isotopically labeled
nitrogenous solutes across the amniotic membrane in a chamber with adequate stirring.

Although no significant changes in maternal serum sodium have been observed during the period of pregnancy, alterations have been reported for concentration of sodium in amniotic fluid. Gillibrand (1969 b) found that there was a very significant decrease in amniotic fluid sodium concentration as gestation advanced in the human being. Between 38 and 44 weeks there was a mean deficit of 8.8 mEq/l in amniotic fluid compared to maternal serum sodium. In other species, such as the rat, the rabbit, and the guinea-pig, Na\(^+\) distribution between amniotic fluid and maternal plasma is not according to electrochemical equilibrium (Mellor, 1969). In the rat the observed amniotic fluid sodium concentration is higher than the calculated value (0.88 v.s. 0.57), and in the rabbit and guinea-pig, the observed concentration is lower than the calculated value (0.89 v.s. 1.41 and 0.92 v.s. 6.25 respectively). Sensitivity of the amniotic membrane's permeability to sodium under action of prolactin might explain the decrease in sodium concentration in guinea-pig amniotic fluid with advancing gestation as reported by Bates (1963) and Mellor (1969). The results of the present study tend to suggest that prolactin can increase
fetal-maternal sodium movement while not affecting maternal-fetal movement. Sensitivity of the membrane to hormonal stimulation seemed to increase up to at least 57 days of gestation. This would tend to cause a net movement of sodium out of the amniotic sac and thus decrease the concentration of sodium in amniotic fluid.

It is concluded that prolactin can cause slowing of unidirectional and net flux of water in the fetal-maternal direction across the isolated guinea-pig amnion, since the doses required have been shown to exist in some amniotic fluids. Prolactin may be partly responsible for the retention of water, and perhaps other substances, within the amniotic sac of the intact mammal. There is a possibility that prolactin may also interact with other hormones present in amniotic fluid. According to Lam (1972) prolactin may require some pituitary synergist(s) for its full action on the renal tubules of the eel, *Anguilla anguilla*. Although vasopressin may not be found in amniotic fluid of the guinea-pig in high enough concentration to evoke responses observed in the in vitro studies with amnion, it may act in synergism with a hormone such as prolactin. The trend from isotonicity to hypotonicity of amniotic fluid and decrease in amniotic fluid sodium concentration, with respect to maternal serum, occurs in spite of the fact that there is a free and rapid
movement of various ions and nitrogenous waste products in and out of the amniotic sac (Reynolds, 1972). Prolactin may be responsible for increasing fetal-maternal sodium movement as gestation advances in the guinea-pig. Therefore permeability studies on the isolated amnion, in normal salines, free of hormone may give a false impression of the in vivo situation.

Finally, there is some indication that responsiveness of guinea-pig amnion to prolactin and/or other hormones present in amniotic fluid may help to initiate birth. According to some workers such as Schwarz et al. (1975a), the fetal membranes may be the key to the initiation of labour. Indication for such a role comes from the finding that not only does the amniotic fluid contain quite large quantities of cortisol (Murphy et al., 1975), but also the human fetal membranes contain steroid receptors (Schwarz et al., 1975b). Steroids such as cortisol have been implicated in the initiation of human labour. Arachidonic acid, the precursor of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been isolated from the human chorioamnion (Schultz et al., 1975a). Partuition in the guinea-pig does not seem to be under the control of progesterone, estrogen, corticosteroids or oxytocin (Illingworth et al., 1974). Partuition can be induced, however, by infusion of guinea-pigs with
PGF$_{2\alpha}$, PGE$_2$, or I.C.I. 80,996 (a potent analogue of PGF$_{2\alpha}$). Since arachidonic acid present in the amniotic membrane can form PGF$_{2\alpha}$, which is elevated during human labour (Schultz et al., 1975 b), such a conversion may act as a trigger for partuion in the guinea-pig. Prolactin, which is known to enhance lipid and protein biosynthesis (Winter et al., 1975) might be speculated to stimulate an increase in the fatty acid concentration (e.g. arachidonic acid) in fetal membranes, and thereby initiate a series of reactions leading to the induction of labour in the guinea-pig. Strauss et al. (1975) found that treatment of pregnant rats with PGF$_{2\alpha}$ on days 19 and 20 of pregnancy resulted in premature delivery on day 21. Bast and Melampy (1972) discovered that serum prolactin levels in pregnant rats doubled on day 20 and tripled on day 21 of pregnancy, indicating that prolactin might play a role in initiating labour. Such a speculated role for prolactin in the guinea-pig deserves attention. It may be that the radical change in membrane permeability to sodium for overdue amnions may be related to a birth response.

The present experiments have suggested that prolactin may combine its reproductive functions in mammals with its hydro-mineral activities in lower vertebrates by acting
on the amniotic membrane of the fetal mammal. Preliminary experiments outlined in section III indicate that fetal guinea-pigs show similarities to certain anuran amphibians in their ability to influence water movement across the urinary bladder and the skin surface. Further studies are required to elaborate the role of hormones in controlling fetal hydro-mineral balance. High levels of vasopressin in fetal blood may also exert an influence on water and/or ion movement through the yolk sac of the guinea-pig, since unlike the amnion, this membrane is in contact with the fetal circulatory system. Renfree et al. (1975) suggest that the yolk sac membrane may be more important than the amniotic membrane in regulating the volume and the biochemical composition of amniotic fluid in the mouse. An investigation of possible endocrine influence on the yolk sac membrane is left for future study. In conclusion, it appears that hormonal control of fetal hydro-mineral metabolism may help to explain the enigma of how fluids accumulate in the intrauterine compartments, and how hydro-osmotic homeostasis in the internal milieu is achieved.
REFERENCES


