THE EFFECTS OF EPINEPHRINE, AVP, NOREPINEPHRINE
AND ACETYLCHOLINE ON LUNG LIQUID PRODUCTION IN *IN VITRO*
PREPARATIONS OF LUNGS FROM
FETAL GUINEA PIGS (*Cavia porcellus*)

by

Birgitta A. Woods
BSc. Queen's University, 1987

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES
Department of Zoology

We accept this thesis as conforming to
the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
April, 1991
©Birgitta Woods, 1991

THE EFFECTS OF EPINEPHRINE, AVP, NOREPINEPHRINE
AND ACETYLCHOLINE ON LUNG LIQUID PRODUCTION IN *IN VITRO*
PREPARATIONS OF LUNGS FROM
FETAL GUINEA PIGS (*Cavia porcellus*)

by

Birgitta A. Woods
BSc. Queen's University, 1987

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES
Department of Zoology

We accept this thesis as conforming to
the required standard

....

....

....

....

THE UNIVERSITY OF BRITISH COLUMBIA
April, 1991
©Birgitta Woods, 1991

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of _Zoology_

The University of British Columbia
Vancouver, Canada

Date _April 19 91_

1. ABSTRACT

This study examined the effects of epinephrine, norepinephrine, AVP and ACh on fluid movement by the lungs of the late-term guinea pig fetus. Catecholamines and AVP are secreted in high amounts by the fetus during delivery, and could be important with respect to fetal lung fluid removal; this event is vital at the time of birth.

The lungs were supported *in vitro* for a duration of three hours, and production rates were measured using a dye-dilution technique. The average resting production rate in terms of ml/kg·h declined with gestational age (54-67 days gestation; n=171). There was a lesser decline in the average resting production rate in terms of ml/h. The average production rate of untreated preparations in the first hour was 1.60 ± 0.26 ml/kg body weight per hour, and rates did not change significantly during the remaining two hours of experimentation (n=30). This rate is comparable to those reported from chronically catheterized fetal sheep.

Treatment was administered during the second hour of experimentation, following an ABA design. Lungs (n=36) were transferred to fresh Krebs-Henseleit saline containing one of the following concentrations of epinephrine: (a) 10^{-5} M; (b) 10^{-6} M; (c) 10^{-7} M; (d) 5×10^{-8} M; (e) 10^{-8} M; and (f) 10^{-9} M. With the exception of the top dose, epinephrine treatment caused an immediate reduction in fluid secretion, or fluid reabsorption. Sodium followed the movement of water in all cases. The effect of epinephrine at 10^{-7} M was maximal, and the threshold dose for epinephrine was calculated at 1.78×10^{-11} M. Phentolamine and propranolol had no effect in control preparations. However, phentolamine completely blocked the effect of epinephrine, whereas propranolol was ineffective. Isoproterenol had no effect on pulmonary fluid production. Alpha-adrenergic receptors apparently mediate the effect of epinephrine on pulmonary fluid movement in the fetal guinea pig lung. This conclusion is different from that obtained in fetal sheep, in which beta-adrenergic receptors are utilized.

A possible synergism between epinephrine and AVP was examined. Lungs (n=12) were transferred to fresh Krebs-Henseleit saline containing either (a) 0.6 mU/ml AVP, or (b) 0.6 mU/ml AVP combined with epinephrine at 10^{-7} M. Treatment with AVP caused a slow, prolonged reduction in fluid production. Treatment with AVP together with epinephrine did not demonstrate synergism.

The effect of norepinephrine (NE) was examined. Lungs (n=36) were transferred to fresh Krebs-Henseleit saline containing one of the following concentrations of NE: (a) 1.24×10^{-5} M; (b) 1.24×10^{-6} M; (c) 1.24×10^{-7} M; (d) 5.24×10^{-8} M; (e) 1.24×10^{-8} M; and (f) 1.24×10^{-9} M. In all preparations, treatment with NE resulted in an immediate reduction in fluid production, and reabsorptions were observed at the higher doses. Sodium followed the movement of water in every case. The threshold dose was calculated at 3.16×10^{-10} M. Phentolamine blocked the effect of NE, reinforcing the importance of pulmonary alpha-adrenergic receptors in the fetal guinea pig. There was no relationship between age and degree of response with treatment of either epinephrine or NE, but fetuses under 78.0 g did not respond to NE.

The effect of ACh was examined. Lungs (n=24) were transferred to fresh Krebs-Henseleit saline containing one of the following concentrations of ACh: (a) 10^{-4} M; (b) 10^{-5} M; (c) 10^{-6} M; and (d) 10^{-8} M. At the three top doses, immediate and powerful reabsorptions of pulmonary fluid were observed in older fetuses (60 days gestation and above); significant falls were observed in the younger fetuses. This result was unexpected, as it was hypothesized that ACh would stimulate fluid production. The threshold dose for ACh was between 10^{-6} M and 10^{-8} M. Phentolamine blocked the effect of ACh. This result suggested that reabsorption is a result of an indirect effect of ACh acting through pulmonary alpha receptors.

The results in this study show that epinephrine, NE, AVP and ACh are all important promoters of fetal pulmonary fluid removal in the fetal guinea pig. Pulmonary alpha-adrenergic receptors

mediate the effects of epinephrine, NE and ACh (indirectly). The conclusions drawn from this study emphasize the importance of species' comparison in fetal research.

LIST OF ABBREVIATIONS

AVP   Arginine Vasopressin
NE   Norepinephrine
DOPA   dihydroxyphenylalanine
PNMT   Phenylethanolamine n-methyltransferase
ACh   Acetylcholine

2.   TABLE OF CONTENTS

3. LIST OF FIGURES

4. ACKNOWLEDGEMENTS

5. INTRODUCTION

5.1   Origin of Fetal Lung Fluid

Since the late 1700's it has been known that the fetal lung is filled with fluid (Wislow, 1787,

quoted by Preyer, 1885). For many years, the origin of this fluid was thought to be amniotic in

nature, brought into the respiratory tract and lungs as a result of fetal breathing movements

(Preyer, 1885). In the early 1900's, doubts were cast on this conclusion. Addison and How

(1913) observed that respiratory movements would not be strong enough to draw fluid into

potential air spaces, especially those in the terminal regions in which this fluid had been

identified. Respiratory movements were ruled out altogether as a mechanism of fluid entry into

the lung by Windle and co-workers in 1939. These researchers concluded that the fetus did not

exhibit breathing movements, except under abnormal conditions. Finally, Potter and Bohlender

(1941) observed fetuses whose lungs were fluid-filled and properly developed, despite the fact

that entry of amniotic fluid had been prevented due to an obstruction of the trachea. These

observations eliminated the possibility that pulmonary fluid was amniotic in origin.

Whitehead and co-workers (1942) were among the first to suggest that the fluid was produced

within the lung itself. It was reasoned that there would be flow of fluid from the lung outward

into the amniotic space, and not the reverse as was originally postulated. Jost and Policard

(1948) substantiated this hypothesis in studies using fetal rabbits. With decapitation of the

fetuses and ligation of the trachea *in utero* , a progressive distention of the alveoli was observed,

presumably as a result of fluid production within the lung. Despite the severity of their

technique, these observations were supported by other researchers. Reynolds (1953) placed a

rubber nose-bag over the head of the fetus (fetal lambs delivered by Cesarean section; placental

circulation left intact, i.e. "exteriorized") and observed a clear, viscous fluid in the bag. He

concluded that the naso-pharyngeal and buccal cavities produced this liquid, which in turn

served as a substantial source of amniotic fluid. Although Reynolds underestimated the role of

the lung, he demonstrated nonetheless that there was a net flow of liquid out of the fetus through the respiratory tract. Studies on goats and guinea pigs by a direct-flow technique confirmed the role of the lung as the source of the fluid therein (Setnikar et al. 1959).

Production of lung fluid was found to be essential to the fetus for several reasons. First, lung liquid provides a substantial source of amniotic fluid. Fluid production can reach rates as high as 200 to 400 ml/day (fetal sheep), a rate comparable to that of fetal urine (Barnes, 1976). Lung fluid passes along the trachea and into the pharynx where it may be swallowed or enter the amniotic sac (Harding et al. 1984). Second, it has been suggested that lung fluid is important for salt and water regulation in the fetus (Cassin and Perks, 1982). These observations are of particular importance to those animals who do not have a functioning kidney until late in fetal life. Third, lung fluid production is essential for proper lung development and growth (Potter and Bohlender, 1941; Alcorn et al. 1977).

5.2   The Production of Lung Fluid

It has been suggested that fetal lung fluid may be an ultrafiltrate of the blood in a system not unlike the mammalian kidney (Adams et al. 1963; Avery, 1968). Adams and co-workers (1963) compared the composition of lung fluid of fetal lambs to that of the amniotic fluid and plasma. They demonstrated that lung fluid differed markedly from amniotic fluid in that the former was distinctly more acid (lung fluid: pH of 6.43; amniotic fluid: pH of 7.06), and had a lower CO_2 level (4.4 mEq/litre compared to 18.0 mEq/litre for amniotic fluid). In addition, lung fluid was higher in sodium and chloride concentration and lower in potassium, protein, sugar and urea than amniotic fluid. They did not demonstrate a marked difference between lung fluid and plasma, with the exception that lung fluid had a lower protein content. It was also shown that NaI and NaCl were rapidly excreted by the fetal lung. These observations provided evidence that fetal lung fluid could be an ultrafiltrate of blood, with selective reabsorption or secretion by the lung.

More extensive lung fluid composition studies showed it to be much more distinct from plasma than was originally believed (Adamson et al. 1969). Not only was lung fluid more acidic than plasma (lung fluid: pH of 6.27; plasma: pH of 7.34), but lung fluid also had a higher potassium and chloride concentration (lung fluid: K^+=6.3 mEq/kg H_2O, Cl^-=157 mEq/kg H_2O; plasma: K^+=4.8 mEq/kg H_2O, Cl^-=107 mEq/kg H_2O), and was lower in protein and bicarbonate ions (lung fluid: protein=0.03 g/100 ml, HCO_3^-=2.8 mEq/kg H_2O; plasma: protein=6.27 g/100 ml, HCO_3^-=24 mEq/kg H_2O). The differences in ion concentrations across the blood/pulmonary epithelial border suggested the presence of an ion pump. These studies did not elucidate the ions involved in fluid secretion, but Cl^- ions appeared to be important.

The low protein content of lung fluid was explained by lung permeability studies. Normand et al. (1971) injected radioactively labelled test substances into the plasma, lung lymph, or alveolar liquid of fetal lambs, and determined that pores, 5.5 A and 150.0 A in radius, existed in the alveolar epithelium and lung capillaries respectively. The small size of the alveolar pores is thought to prevent proteins and other plasma solutes from penetrating alveoli. In addition, studies using exteriorized fetal lambs showed that tight intracellular junctions appeared as early as 39 days gestation (term=147 days), and continued throughout gestation (Schneeberger et al. 1978). The fetal lamb lung had a remarkably low permeability to small non-electrolytes; it was selective in the solutes it allowed to penetrate the lung, and this selectivity did not change with fetal development (Olver et al. 1981). Egan et al. (1976) confirmed these findings, although they also noted a transient large increase in pore size at birth. They recorded no further postnatal changes in lung permeability. The low permeability of the lung to plasma solutes, together with the findings of Adamson et al. (1969) indicated that lung fluid could not be produced by filtration of blood as proposed by Adams et al. (1963), but must be actively manufactured by the lung itself, possibly via a chloride pump (Adamson et al. 1969; Strang, 1977; Olver and Strang, 1974).

5.2.1   The Mechanism of Production of Lung Fluid

To test the possible involvement of a chloride pump, ion movements across the pulmonary epithelium were studied by Olver and co-workers (1974, 1975, 1977 and 1981). In the earlier study, labelled isotopes of several cations including Na^+, K^+ and Ca^+, and several halides such as Cl^-, Br^- and I^- were injected into either the lung liquid or into the plasma of exteriorized fetal lambs (Olver et al. 1974). By following changes in ion concentrations in these fluids, bidirectional fluxes and permeability constants were calculated. They concluded that the halides, together with K^+ and Rb^+ were actively transported from plasma to lung liquid, and that Cl^- was quantitatively the most important ion. In contrast, Na^+ and Ca^{2+} ions moved passively through the epithelium. In their model, the active transport of Cl^- ions from plasma to lung liquid was coupled with HCO_3^- movement in the reverse direction. This establishes an electrochemical gradient (-1.0 to -10.0 mV, with the lung side more negative), which allows Na^+ and Ca^{2+} ions to move passively into the lung. Water then moves across in response to the osmotic gradient established by NaCl. In addition, it was observed that the luminal side of tracheal epithelium in dogs was electrically negative relative to the submucosal side, due to the net flux of Cl^- ions towards the tracheal lumen (Olver et al. 1975). Two years later, they showed this movement occurred in the alveolar epithelium, when evidence suggested a chloride pump operating from the blood to the alveolar space (Olver, 1977). Finally, Olver and co-workers (1981) showed that the fetal sheep lung can actively transport Cl^- into the lung lumen by 84-87 days gestation (term=147 days). These researchers concluded that a chloride pump was the major driving force for fetal lung fluid production.

Gatzy (1983) has elaborated on chloride transport activity in the lung using the shark rectal gland as a model for Cl^- transport (Silva et al. 1977). The shark rectal gland appears to transport Cl^- in the same way as the adult canine trachea, and probably fetal alveolar cells (Widdicombe et al. 1979). In the model described by Gatzy (1983), Na^+ is actively transported out of the epithelial cell along the basolateral membrane by a Na^+/K^+ ATP-dependent pump, rendering the cell

interior electronegative relative to both the blood or the lumen. The Na^+ reenters the cell through the basolateral membrane due to a favorable gradient. Chloride ions enter the cell against their electrochemical gradient due to the fact that they are neutrally coupled to Na^+. Chloride ions accumulate inside the cell, and then enter the lung lumen down a concentration gradient. Sodium is removed from the cell in exchange for K^+ via the Na^+/K^+ pump, after which K^+ leaves the cell passively through channels in the basolateral membrane. The apical membrane is not permeable to Na^+ in the cell-to-lumen direction, however Na^+ does enter the lumen, possibly through a paracellular route (refer to figure 1).

It is not known how accurately the above model describes chloride secretion by the fetal lung. However, this system appears to be operative in the tracheal epithelium of fetal and adult species (Olver and Robinson, 1986). There have been some insightful studies using loop diuretics on the fetal lung (Cassin et al. 1986). Bumetanide and furosemide specifically inhibit chloride secretion on the apical side of the loop of Henle in the kidney (Imai, 1977). Cassin and co-workers applied either bumetanide or furosemide directly into the lung fluid of the chronically catheterized near-term fetal lamb. They found that, in the presence of bumetanide, fluid secretion significantly reduced or fluid was reabsorbed, particularly in older fetuses. Fluid reabsorption was observed in the presence of a high dose of furosemide. Similar results were found in isolated lungs from fetal guinea pigs (Thom and Perks, 1990). These findings further support that fetal lung fluid secretion involves the transport of Cl^- out into the lumen of the lung.

The ability of an organ to generate an electrochemical gradient depends on the ability of the epithelium to prevent a passive back-flux of the actively secreted ions. The permeability studies done by Normand et al. (1971), Egan et al. (1976), Schneeberger et al. (1978) and Olver et al. (1981) revealed that an appropriate barrier exists in the fetal lung. Fromter and Diamond (1972) reported a leaky epithelium in the fetal lung. Olver and Strang (1974) believed that the pores of 5.5 A in the alveolar epithelium (Normand et al. 1971) represent these leaks, and described these

Figure 1 **A cellular model of ion transport in Cl⁻ secreting epithelium.**

In this model, Na^+ is actively transported out of the epithelial cell across the basolateral membranes via a Na^+/K^+ ATP-dependent pump, rendering the cell negative relative to the blood. The Na^+ reenters the cell due to a favorable gradient. Chloride accumulates beyond its electrochemical equilibrium inside the cell as a result of secondary active transport across the basolateral membrane via a $Na^+,K^+/2Cl^-$ co-transport system. Regulation of ion transport is achieved by second-messanger gating of apical Cl⁻ channels, which when open allow Cl⁻ ions to exit down its concentration gradient into the lumen. The apical membranes of epithelial cells are impermeable to Na^+ in the cell-to-lumen direction, but Na^+ can enter the lung lumen via a paracellular route. Ouabain and loop diuretics inhibit the Na^+/K^+ ATP-dependent pump and $Na^+,K^+/2Cl^-$ co-transport system respectively. Amiloride blocks Na^+ channels in the apical membranes.

Lumen (-)

Blood (+)

Cell

Na$^+$

Secretory

ouabain

ATP

Na$^+$

K$^+$

Na$^+$

ADP

K$^+$

Cl$^-$

Cl$^-$

K$^+$

Na$^+$ } Loop diuretics

Cl$^-$

ca^{++}

Absortive

ouabain

Na$^+$

ATP

K$^+$

Na$^+$

ADP

Amiloride

K$^+$

Cl$^-$
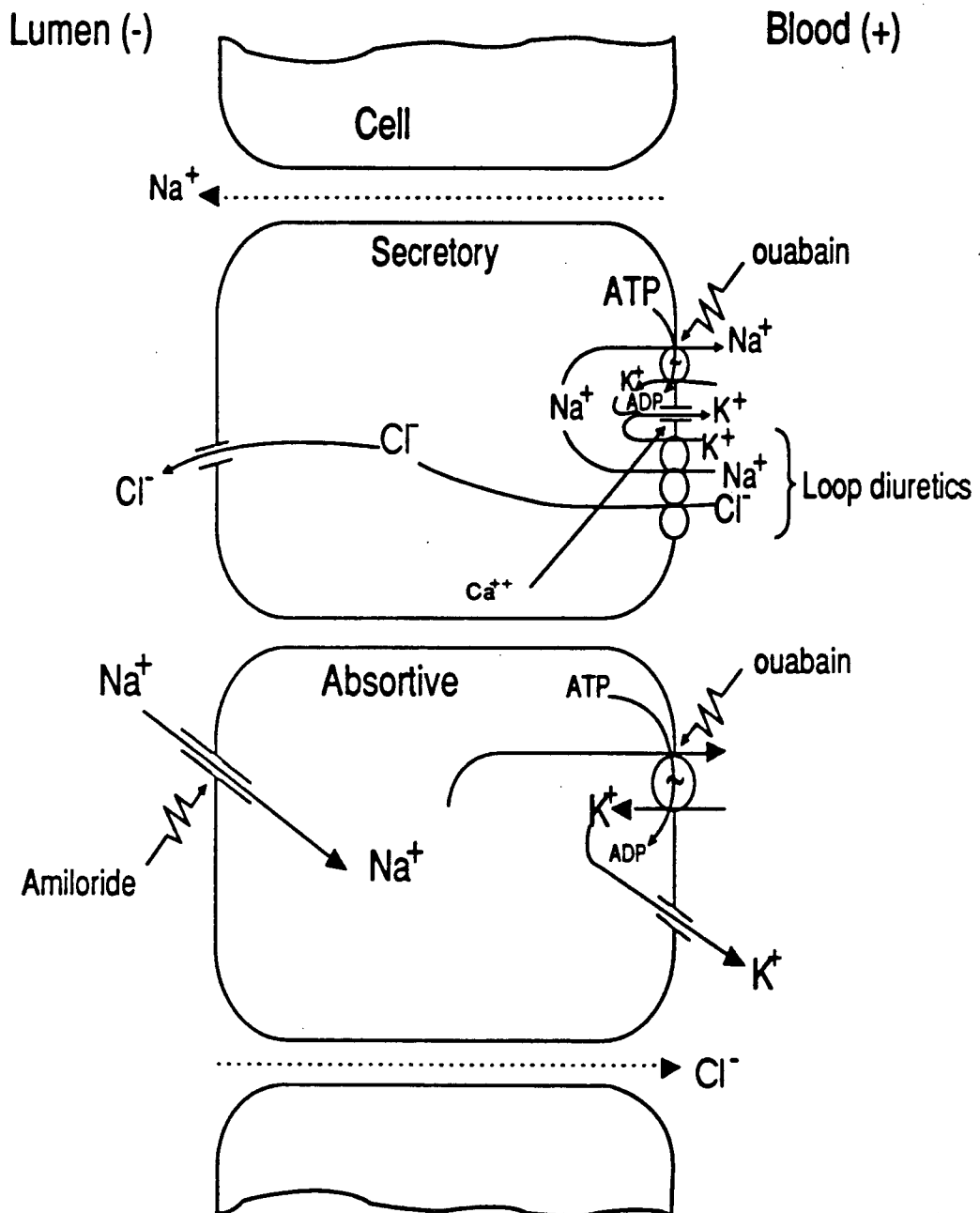
as water-filled cylindrical channels carrying fixed negative charges. These pores could participate in ion transport and lung lung secretion.

The cells responsible for fluid secretion in the lung epithelium have not been conclusively identified. Adamson et al. (1969) were among the first to postulate a Cl⁻ pump, but they could not verify its specific location. They noted no variations in fluid composition anywhere in the lung, so it seemed reasonable to conclude that the entire lining of the lung was responsible for maintaining the observed electrolyte balance between lung fluid and plasma. Olver (1983) suspected the Cl⁻ pumps were on the alveolar type II cells, which line parts of the alveolar walls and are capable of considerable metabolic activity (Mason et al. 1982). Dome formation, which is characteristic of transporting epithelium *in vitro*, was observed in cultured alveolar type II cells (Mason et al. 1982). In addition, constant removal of fetal lung fluids in the lamb produced a significant increase in the number of these cells (Alcorn et al. 1977).

5.3   The Absorption of Lung Fluid

Lung fluid is secreted at a rate of 3.1 ± 2.2 (SD) ml/kg·h in the fetal lamb (Perks and Cassin, 1985a); at 2.5 to 4.0 ml/kg·h in the fetal goat (Normand et al. 1971); and at 2.14 ± 0.08 (SE) ml/kg·h in the fetal guinea pig (Perks et al. 1990). At birth, the neonate must convert from placental to pulmonary gas-exchange, an event which requires the complete removal of fetal lung fluid. There is evidence that this process begins before birth. Kitterman et al. (1979) have reported that secretion of tracheal fluid slows seven days before birth in fetal sheep. Brown et al. (1983) supported these findings, and Perks et al. (1990) reported a decline in lung fluid production prior to term in fetal guinea pigs.

A dramatic reabsorption of lung fluid is observed at the time of birth (Adams et al. 1971; Olver et al. 1974; Brown et al. 1981). Humphreys et al. (1967) reported total lung clearance ensued within four to six hours after delivery in the lamb. However, it seems likely that most of the

fluid must be cleared from the alveolar spaces much more rapidly, otherwise efficient gas exchange would not be possible (Olver et al. 1974). Adams et al. (1971) observed that the gross appearance of the lungs appeared completely aerated within ten minutes after delivery in rabbits, and Brown et al. (1981) reported that lung fluid clearance rate accelerated to 20 ml/h as the fetal parts became visible during delivery in lambs. Humans showed a functional residual capacity and efficient gas exchange within a few minutes of birth (Olver, 1977).

5.3.1   The Mechanism of Absorption of Lung Fluid

The route and mechanisms by which fluid leaves the lung have been studied extensively. Humphreys et al. (1967) followed the path of lung liquid during reabsorption with a dye, and observed large amounts of dye in the thoracic duct and to a lesser extent in the right lymph duct. Since an increase in pulmonary lymph flow was noted upon ventilation, these authors concluded that lung liquid was cleared from interstitial spaces by the lymphatics. However, Olver and Strang (1974) demonstrated that changes in pulmonary vascular pressure sufficient to double lung lymph flow had no effect on lung liquid movement. Bland et al. (1982) observed that fluid does not drain from the trachea, nor does it enter the pleural space and speculated that liquid must flow from potential air spaces into the interstitium and then into the microcirculation of the lungs. Hydrostatic pressure, as well as protein osmotic pressure gradients across the alveolar epithelium undoubtedly play some role in lung fluid reabsorption (Gee and Williams, 1979). However, Egan et al. (1975) pointed out that whatever their magnitude, the removal rate of liquid would be limited by the movement of Cl^- and Na^+ into the interstitium. They suggested that spontaneous ventilation was associated with the opening of water-filled cylindrical pores. If associated with a fall in lung fluid secretion, the colloid osmotic gradient across the epithelium would facilitate withdrawal of lung liquid through these open pores. These authors also noted that this increase in lung permeability appeared to be related to the expansion of the lungs following birth. Perks and Cassin (1985b) have shown that a minimum expansion of 18.6% of total volume is required to cause any significant decrease in fluid production. Expansions

greater than 18.6% result in an increase in sodium transport from the lumen towards the interstitium. The movement of water then follows that of sodium.

Sodium movement from the lung lumen towards the interstitium seems to be an important factor in fluid reabsorption. It has been suggested that a stimulation of intracellular cAMP production activates Na^+-selective channels in the apical membrane of alveolar type II cells (Olver et al. 1986). This would allow Na^+ to diffuse more rapidly to the Na^+ pump in the basolateral membranes, and so be extruded into the circulation with Cl^- and water following passively. The exact trigger(s) causing this rise in intracellular cAMP at birth are still being investigated. Other mechanisms for Na^+ absorption by the fetal lung epithelium include a Na^+/H^+ exchange (Butcher et al. 1988), and Na^+-glucose co-transport (Barker et al. 1989).

5.3.2   Factors that Affect Lung Fluid Reabsorption

Fetal lung fluid reabsorption has been a focus of much research due to the importance of this event at birth. It is vital that fluid clearance is rapid at birth so that proper pulmonary gas exchange ensues in post-natal life. The mode of delivery seems to play a substantial role in lung fluid clearance. Delivery by Cesarean section significantly retards the rate at which even the mature newborn attains normal blood gases (Oliver et al. 1961). Adams et al. (1971) observed that the gross appearance of the lungs in vaginally delivered rabbits seemed completely aerated after ten minutes of breathing, whereas lungs from the Cesarean-delivered group took several hours to appear completely aerated. In addition, Brice and Walker (1977) concluded that a portion of lung volume occupied by liquid is greater in babies born by elective Cesarean section, which may contribute to the increased incidence of transient tachypnea associated with this form of delivery. Milner et al. (1978) reported a higher initial thoracic gas volume of 33 ml/kg in vaginally delivered neonates compared to 20 ml/kg in newborns delivered by Cesarean section. Infants born by Cesarean section showed a significant increase in the incidence of Hyaline Membrane Disease (Jones et al. 1975). However, Adams et al. (1971) found insignificant

differences between the two types of delivery in terms of long-term fluid removal; by the sixth hour post-delivery, pulmonary water content was similar in neonates born either vaginally or surgically. Despite this observation, it seems that labor is extremely important in initiating the removal of fluid from the lungs at birth.

Since labor seems important in initiating lung fluid reabsorption, it is reasonable to question the role of agents that are highly active during this time as possible triggers for fetal fluid reabsorption. One such agent is epinephrine, which is secreted in high amounts by both mother and fetus at birth (Lagercrantz and Bistoletti, 1973). Mean plasma catecholamine levels of 62.1 nmol/l in the umbilical artery and 29.3 nmol/l in the umbilical vein have been measured in full-term infants during uneventful vaginal delivery; these values are considerably higher than those in adults during heavy exercise (Lagercrantz and Bistoletti, 1973). Plasma catecholamines are elevated by the stress of delivery which includes the squeezing of the fetus in the birth canal (Lagercrantz and Bistoletti, 1973) as well as intermittent fetal hypoxemia and acidemia due to changes in fetal arterial blood pressure induced by uterine contractions (Irestedt et al. 1982). Some of the known responses of the fetus to catecholamines include a rise in plasma glucose, free fatty acids and plasma lactate levels, as well as transient bradycardia followed by tachycardia, all occurring within minutes of catecholamine release (Comline and Silver, 1972).

Walters and Olver (1978) showed that epinephrine can significantly slow lung fluid production or cause reabsorption in the fetal lamb, especially in older fetuses. Isoprenaline, a beta-adrenergic receptor agonist (Weiner, 1985b), also caused inhibition of secretion or reabsorption of fluid. These responses could be blocked by propranolol, a beta-adrenergic receptor antagonist (Weiner, 1985a), so these researchers reasoned that the effect of epinephrine was mediated via beta-adrenergic receptors. These receptors have been identified in late-term fetal lamb lung tissue (Warburton et al. 1987c). These results were collaborated by Brown et al. (1981; 1983), Olver et al. (1981) and Walters et al. (1982). Olver and co-workers (1981) provided evidence that epinephrine-induced reabsorption involved a Na^+/K^+ pump. Amiloride at 10^{-4} M

consistently and reversibly blocked the reabsorptive effect of epinephrine. Since amiloride blocks Na^+ channels in the apical membrane (Soltoff and Mandell, 1983), these authors suggested that Na^+ was denied access to its active transport sites at the basolateral membrane and thus reabsorption was inhibited. Perhaps this hormone is responsible for a rise in intracellular cAMP (a known mediator of epinephrine), which in turn activates Na^+ channels necessary for fluid reabsorption (Olver et al. 1986). Interestingly, control experiments for amiloride increased secretion by fifty percent, suggesting that fluid movement might be the result of a balance between a secretory process and a reabsorptive process. A relationship between gestational age and degree of response was noted in this study. Before 130 days gestation, epinephrine slowed secretion, while after 130 days gestation epinephrine caused reabsorption. These results were limited to work on fetal sheep, however the overall effect of epinephrine on lung fluid secretion has been confirmed in near-term fetal goats (Perks and Cassin, 1982; 1989).

Although the ability of epinephrine to inhibit secretion and cause reabsorption is widely acknowledged, the relative importance of this hormone in regulating fluid balance near parturition is not certain. Bland et al. (1982) sacrificed lambs before and during labor and found that there was no difference in plasma catecholamine levels between the two groups. McDonald et al. (1986) observed that blockage of beta-adrenergic receptors in fetal rabbits did not prevent the normal decrease in lung fluid during labor. In addition, the slowing of secretion rate before birth in lambs is not associated with an increase in fetal plasma catecholamine concentrations (Brown et al. 1981). Roberts et al. (1983) claim that this discrepancy reflects an increased sensitivity of the lung epithelium to epinephrine due to a coincident increase in the number of beta-receptors (in rabbits). However, this argument should be used with caution since the functional maturity of sympathetic innervation varies widely among species. Sympathetic innervation occurs after birth in rats, remains incomplete at birth in humans, and lambs develop a mature sympathetic nervous system by term (Lagercrantz and Slotkin, 1986). Thus, information gained from animal models should be applied with caution.

Some researchers claim that epinephrine has an indirect effect through the release of surfactant. Surfactant is a lipid-containing substance secreted by the mature fetal lung. This substance reduces surface tension in the alveoli, and is necessary for pulmonary gas exchange in post-natal life (Rooney, 1985). Epinephrine infused into chronically catheterized fetal lambs inhibited lung liquid secretion by actually increasing surfactant release (Lawson et al. 1978). McDonald et al. (1986) concluded that endogenous catecholamines participate in the release of surfactant but not water flux in the rabbit fetus, and this is supported by studies using beta-receptor agonists. Intramuscular injection of isoxsuprine, a synthetic beta-receptor agonist, three hours before delivery in rabbits not only significantly decreased the amount of lung liquid at birth, but it also doubled the lecithen/sphingomyelin ratio of the liquid while decreasing surface tension by thirty percent (Enhorning et al. 1977). The administration of the $beta_2$-agonists salbutamol or terbutaline during preterm labor decreased the incidence of Hyaline Membrane Disease (DeLemos et al. 1969; Bergman and Hedner, 1978). This disease is associated primarily with surfactant deficiency, as well as inadequate lung fluid clearance (Gandy et al. 1970). Surfactant stores are significantly depleted with the administration of terbutaline, suggesting a stimulation of surfactant release (Ekelund et al. 1983) In their studies on fetal lambs, Warburton et al. (1987a;b;c) reported that $beta_2$-agonists increase surfactant in the lung by activating protein kinases through cAMP, which, in turn, depletes glycogen stores. They suggested that after $beta_2$-stimulation, pulmonary glycogen deposits become a source of energy to drive the active processes of surfactant production (1987b). They also observed a down-regulation of pulmonary beta-receptors, which might play a role in the homeostasis of intracellular cAMP concentration (1987c).

Glucocorticoids may prepare the fetal lung for the actions of epinephrine. There is a progressive rise in fetal plasma cortisol prior to birth (Liggins, 1976). Glucocorticoids increase the number of beta-adrenergic receptors in alveolar cells, thereby augmenting surfactant release in response to catecholamines (Cheng et al. 1980). In addition, phenylethanolamine n-methyltransferase (PNMT), an enzyme responsible for the final step in epinephrine synthesis, has been identified in

the fetal lung (Padbury et al. 1983a). Studies using SKF29661, an inhibitor of this enzyme, show that a decrease in the production of epinephrine is associated with a decrease in alveolar phophatidylcholine (Padbury et al. 1983b). These results suggest that endogenous epinephrine release plays an important role in the regulation of surfactant. Endogenous cortisol production might augment the concentration of PNMT in the fetal lung (Padbury et al. 1983a).

Arginine vasopressin (AVP) is another hormone secreted in high amounts at birth. Levels as high as 533 uU/ml and 570 uU/ml were measured in cord blood of human infants during vaginal delivery (Parboosingh et al. 1982; Pohjavuori and Fyrquist, 1980 respectively). Increased fetal plasma AVP concentrations during delivery have also been recorded in sheep (Stark et al. 1977). As with epinephrine, plasma AVP levels are higher in vaginally delivered neonates, and hypersecretion ensues as a result of fetal stress such as fetal hypoxia (Parboosingh et al. 1982).

The effects of AVP on fetal lung fluid production have been studied. Infusions of AVP at physiological levels into fetal goats showed slow but extended periods of reabsorption of lung fluid (Perks and Cassin, 1982). The effects were age-dependent, as only fetuses over 131 days gestation responded. These authors subsequently confirmed these results on fetal goats and fetal sheep (Perks and Cassin, 1989; 1985a respectively). The age-dependent relationship between plasma AVP concentration and responsiveness was evaluated more extensively by Wallace et al. (1990). AVP infusions into fetal lambs before 135 days gestation had no effect, whereas there was a 40.8% inhibition of fluid secretion between 136 and 140 days gestation, and a 78.4% inhibition at gestational ages above 140 days. These authors did not report a reabsorptive effect of AVP. The above studies all report a slow, prolonged effect of AVP, so it is possible that AVP is responsible for the mopping-up of residual lung fluid in the hours after birth.

The mechanism of action of AVP is not certain. In one study, sodium moved with water, and potassium moved into the lungs with AVP-induced reabsorption suggesting a Na^+/K^+ pump (Perks and Cassin, 1982). Wallace and co-workers (1990) suggested a relationship between V_2

receptor stimulation, adenylate cyclase activation, and cAMP production. An increase in intracellular cAMP production, caused by V_2 receptor stimulation, may activate Na^+ channels on the luminal surface of the pulmonary epithelium. This, in turn, would increase the movement of Na^+ from lung lumen to plasma. The increased ion flux would be associated with an alteration in the osmotic gradient and thus produce a slowing of secretion or the reabsorption of liquid. Alternatively, AVP could be acting via a mechanism similar to that seen in the kidney (Guillon et al. 1982). In this case, AVP stimulation of the V_2 receptor results in an increase in intracellular cAMP concentrations which, following the activation of phosphokinase and appropriate protein phosphorylation, increases water permeability by inserting specific water channels into the luminal cell membranes. Wallace et al. (1990) also suggested that the gestational age-related effect of AVP on fetal lung liquid secretion results from increased plasma cortisol concentrations. Cortisol increased beta-adrenergic receptors (Cheng et al. 1980), so it is possible that cortisol could also induce the formation of AVP receptors on the pulmonary epithelium (Wallace et al. 1990).

Epinephrine and AVP have been given substantial attention in studying fetal lung fluid balance. It is possible that the mechanical events at delivery also play a role in lung fluid clearance at this time. These events include expansion of neonatal lungs upon initial inspiration, and a dramatic temperature drop experienced by the newborn.

Perks and Cassin (1985b) reported a fall in lung fluid secretion in fetal goats with a minimum lung expansion (with saline) of 18.6%. This fall changed to reabsorption at about 47% expansion. Based on the assumption that fetal lung volume approximates the functional residual capacity of the air lung (Strang, 1977), these authors concluded that expansions of 68% are normal and that these effects are within physiological limits. It was observed that Na^+ moved with water out of the lung during reabsorption and K^+ moved into the lung against its concentration gradient, which supports reabsorption brought about by the activation of a Na^+/K^+ pump (Olver et al. 1981). More recent studies on isolated fetal guinea pig lungs supported these

findings (Garrad, unpublished thesis). A minimum expansion of 18% caused a significant reduction in fluid secretion, and expansions of 72% caused fluid reabsorption (Garrad, unpublished thesis). Amiloride placed directly into the lung blocked the effect of expansion-induced reabsorption, thus confirming the importance of Na^+ movement out of the lung with fluid removal (Garrad, unpublished thesis).

The neonate experiences a temperature change from those *in utero* (body temperature, 37°C) to those in ambient conditions (room temperature, 20°C) immediately following delivery. In doing so, the core temperature of the newborn drops by 2 to 3°C (Adamsons, 1966). A 2°C drop in temperature caused a reabsorption of lung fluid in the guinea pig, and this effect could be blocked by amiloride (Garrad, unpublished thesis). Again, this study emphasized the importance of the passage of Na^+ out of the lung during reabsorption.

In summary, epinephrine, AVP, lung expansion and temperature drop all seem to play a role in converting the lung from fetal fluid production to post-natal gas-exchange. Chloride secretion is involved in the production of lung fluid, whereas a Na^+/K^+ pump is essential for fluid removal. Most studies have been performed on intact animals, with ruminants as the most popular model for the human condition, even though ruminant physiology differs in many ways from most mammalian groups. The guinea pig, on the other hand, is a superior animal to use not only as a reproductive model (Martensson, 1984), but especially in terms of fetal lung physiology (Stith and Das, 1982; Sosenko and Frank, 1987).

Until recently, the guinea pig had been used in only a single study of fetal lung liquid production (Setnikar et al. 1959). In this study, intact preparations were used in combination with a direct flow technique, a method which gives erratic results due to problems with drainage and siphoning. Perks et al. (1990) developed an *in vitro* preparation of fetal guinea pig lungs, which has many advantages. While fetal guinea pig lungs are large enough to perform experiments by the same methods used on intact animals, they are small enough to use *in vitro*. Large litter sizes

also prevent the undo sacrifice of adult animals. There are several limitations to using intact preparations: first, there is a possibility that maternal hormonal and other factors may affect the fetus; second, the test agent infused into the fetal circulation may be destroyed or lost into the maternal circulation; and third, agents that are harmful, if not fatal, to other organs cannot be tested on intact animals. Chemicals such as ATP-ase inhibitors are good examples because their effects on the heart are deleterious. These limitations are eliminated using an *in vitro* technique.

It was decided to extend the studies of Perks and co-workers (1990) using isolated lungs from the fetal guinea pig. The effects of epinephrine on lung fluid production are well known in the intact fetal sheep and goat (Walters and Olver, 1978; Brown et al. 1981, 1983; Olver et al. 1981; Walters et al. 1982; Perks and Cassin, 1982; 1989). In addition, the effects of AVP have been examined in these species (Perks and Cassin, 1982; 1985a; Wallace et al. 1990). In this study, the effects of these hormones on fetal lung fluid production are examined in the fetal guinea pig.

Less attention has been paid to the effects of norepinephrine (NE) on lung fluid production. Like epinephrine, this catecholamine is also secreted in high amounts at birth (Lagercrantz and Slotkin, 1986). In the chronically catheterized late-term fetal lamb, infusions of NE into the fetus had no detectable effect on lung fluid secretion (Walters and Olver, 1978). However, in one recent study on the fetal lamb, NE infusions caused a reduction in lung fluid flow by 45% (Higuchi et al. 1987). In the study presented here, NE is applied to guinea pig fetal lungs *in vitro*.

Acetylcholine (ACh) enhanced lung conditioning and surfactant production in late-term fetal lungs (Kolobow et al. 1987; Oyarzun and Clements, 1977). However, little is known about any relationship between fetal pulmonary fluid balance and cholinergic pathways. Kitterman et al. (1979) reported that neither atropine treatment of the fetus nor bilateral section of the cervical vagosympathetic trunk affected tracheal fluid production in fetal sheep. Further insight into cholinergic control of pulmonary fluid balance in the guinea pig is explored in this study.

6. STATEMENT OF THE PROBLEM

The purpose of this study was to examine the effects of epinephrine, AVP, NE and ACh on fetal pulmonary fluid production. An *in vitro* approach is relatively new in fetal research, and the use of the late-term guinea pig is relatively unexplored. Epinephrine, NE and AVP are secreted in high amounts by the fetus at birth. Epinephrine and AVP have been shown to reduce fluid production, or cause reabsorption in some species. It was hypothesized that epinephrine, NE and AVP would reduce lung fluid production or cause reabsorption in the late-term fetal guinea pig. ACh acts as an antagonist to epinephrine and NE under many circumstances. It was hypothesized that this neurotransmitter would stimulate lung fluid production in the late-term fetal guinea pig.

7.   METHODS AND MATERIALS

7.1   Animals

Pregnant albino guinea pigs (*Cavia porcellus*) of an inbred departmental stock were given food and water *ad libitum* (guinea pig chow, Ralston-Purina; supplemented by fresh vegetables and vitamin C). Experiments were carried out on 171 fetuses between the ages of 54 and 67 days (term) gestation, and an average body weight of 102.85 ± 19.74 (SD) g. The majority of gestational ages were calculated from previous delivery dates, as guinea pigs enter estrus immediately following delivery. In cases where this information was not known, gestational ages were estimated from the average fetal weight and size of the litter, according to the methods of Ibsen (1928).

7.2   Basis of the Method

Measurements of lung fluid production were based on an impermeant tracer technique used by Normand and co-workers (1971), Martins and co-workers (1975) and Liu and Chiou (1981). The impermeant tracer used in this study was Blue Dextran 2000 (Pharmacia, Dorval, Que.: Stokes' radius, 270 angstroms (1 angstrom=0.1 nm); radius of gyration, 380 angstroms; molecular mass, 2 000 000 Daltons). This method has been used in previous studies of lung fluid secretion in fetal sheep and goats *in vivo*, and was checked with the simultaneous use of ^{125}I-labelled albumin (Cassin and Perks 1982; Perks and Cassin 1985a, 1985b, 1989).

7.3   Surgical Procedures

Pregnant guinea pigs of late gestation were anesthetized with halothane until the corneal reflex was extinguished. A Caesarean section was performed and the fetuses were extracted with the amnion intact around the head of each fetus. A ligature around the neck of each fetus was tied as

a second precaution against fetal breathing. A mid-line incision along the thorax exposed the fetal lungs and trachea, and the latter was ligated rostrally. Just below the ligature a small incision was made on the ventral surface of the trachea, through which the trachea was cannulated with 1.5-2.0 cm polyethylene tubing (PE 50; Intramedic, Clay Adams, Parsippany, N.J.). The cannula was attached to a 1.0 ml tuberculin syringe via an 18G hypodermic needle and a 3-way stop-cock (K75, Pharmaseal, Puerto Rico). The cannula was positioned just above the bifurcation of the bronchi and secured with two ligatures. The heart and cannulated lungs were then carefully excised, and following the separation of the heart the isolated lungs were suspended in a 50 ml bath containing Krebs-Henseleit saline (pH 7.4). The saline was well oxygenated with 95% O_2/5% CO_2 and maintained at a constant temperature of 37° C to further mimic optimum *in vivo* conditions (refer to figure 2). The time required for these surgical procedures took 3 to 4 minutes, during which time the lungs were kept moist and warm by frequent washes with saline at 37° C.

7.4 Experimental Procedures

Lung fluid (0.2-0.6 ml) was drawn into the syringe of the cannula assembly and redistributed to the upper cup of the 3-way stop-cock. A 10 ul sample of lung fluid was withdrawn from this reservoir using a fixed volume syringe (1701 NCH gas-tight fixed volume syringe; Hamilton Co. Reno, Nevada) as a blank for spectrophotometry. Blue Dextran 2000 (0.1 ml; prepared as 50 mg/ml in 0.9% NaCl) was then added to the cannula assembly via the upper cup and thoroughly mixed with the lung fluid in the syringe before being passed into the lungs. A 30 minute interval was allowed for equilibration, during which time the lung fluid was "mixed" (by withdrawing lung fluid into the syringe and then returning it to the lungs - 3 times) at 5 minute intervals to ensure proper distribution of Blue Dextran throughout the lungs. In addition, the saline bath was changed after 15 minutes and again after 30 minutes.

After dye equilibration, the lungs were maintained for 3 hours with the supporting saline being renewed at the start of each hour. Samples of lung fluid (10 ul) were taken thereafter at 10 minute intervals in the same fashion as that of the blank, and lung fluid was "mixed" at 5 minute intervals to ensure even distribution of dye. The samples were placed in polyethylene micro test-tubes (250 ul Eppendorf C3515-7, Brinkman Instruments (Canada) Ltd., Rexdale, Ont.), and diluted by a factor of 20 with distilled water. The samples were sealed, well mixed with a vortex (Vortex-Genie, Fisher Scientific, N.Y.) and centrifuged at 250 G for 10 minutes (clinical centrifuge, Model CL, International Equipment Co., Needham Heights, Mass.). The supernatant was removed by syringe, and the concentration of Blue Dextran in each sample was estimated using spectrophotometry (Guilford 250, Oberlin, Ohio; 250 ul quartz microcells, Type 10972, NSG Precision Cells Inc., Farmington, N.Y.; wavelength=620 nm). In this way, a trend in lung fluid movement could be ascertained. The 3 hour experiment followed an A/B/A design: (1) saline alone, (2) treatment in saline and (3) saline alone. The samples from the first, second and third hours gave a resting rate, a treatment rate and a recovery rate respectively.

The first part of this study tested the effect of epinephrine on lung fluid production. At the start of the treatment hour, lungs were transferred to a saline bath containing one of the following concentrations of epinephrine hydrochloride (Adrenalin, Parke-Davis Inc., Scarborough, Ont.): 10^{-5} M, 10^{-6} M, 10^{-7} M, 5 x 10^{-8} M, 10^{-8} M and 10^{-9} M. Lungs were also tested using: epinephrine 10^{-8} M combined with propranolol 10^{-7} M (Inderal, Ayerst Laboratories, Montreal, Que.), epinephrine 10^{-7} M combined with phentolamine 1.78 x 10^{-6} M (Rogitine, CIBA-Geigy Canada Ltd., Mississauga, Ont.) or isoproterenol 10^{-7} M (Isuprel, Winthrop Laboratories (Sterling Drug Ltd.), Aurora, Ont.) to examine the receptor mediation of epinephrine's effect. Controls (A/B/A) were done for both inhibitors. Finally, lungs were treated with AVP 0.6 mU/ml (Pitressin, Parke-Davis, Scarborough, Ont.) or a combination of epinephrine 10^{-7} M and AVP 0.6 mU/ml.

The second part of this study tested the effect of NE on lung fluid production. At the beginning of the treatment hour, lungs were transferred to a bath containing one of the following concentrations of NE bitartrate: 1.24×10^{-5} M, 1.24×10^{-6} M, 1.24×10^{-7} M, 5.24×10^{-8} M, 1.24×10^{-8} M and 1.24×10^{-9} M. Lungs were also exposed to a mixture of NE 1.24×10^{-6} M and phentolamine 1.78×10^{-5} M during the treatment hour.

The last part of this study tested the effect of ACh on lung fluid production. At the beginning of the treatment hour, lungs were transferred to a bath containing one of the following concentrations of ACh chloride (BDH Chemicals Ltd., Poole, England): 10^{-4} M, 10^{-5} M, 10^{-6} M and 10^{-8} M. A mixture of ACh 10^{-6} M and phentolamine 1.78×10^{-5} M was also tested.

7.5 Quantification of Results and Statistical Methods

Fluid production rates were determined from the change in concentrations of Blue Dextran (Cassin and Perks, 1982; Perks and Cassin, 1989). Total volumes (in ml) of fluid present in the lungs were calculated, taking into account the addition of Blue Dextran at the onset of experimentation and making appropriate sequential adjustments every 10 minutes for the removal of fluid and Dextran during sampling throughout incubation. Total volumes (expressed as a percentage of the total volume present at sample #6) were plotted against time, and slopes over 1-h intervals were calculated by linear regression, fitted by the method of least squares (Steel and Torrie, 1970; Hewlett-Packard program SD-03A, or by Apple II computer). Fluid production rates in ml/kg·h for each hour were determined as the change in total volume (in ml) over time relative to the weight of the fetus. The significance of changes in rate were estimated from changes in slope, analyzed by a t-test for differences between two regression (Steel and Torrie, 1970; for details, see Cassin and Perks, 1982). In addition, significances were determined by analysis of variance (ANOVA), followed by Newman-Keul's test (Zar 1984). Statistical significance was accepted at $p \leq 0.05$. Combined data were obtained by averaging the total volumes (in %) at each 10 minute interval for a given set of experiments, as well as

averaging the rates for each hour. Averages are reported with standard errors for all combined results.

7.6 Chemical Methods

After estimating Blue Dextran concentration, samples were analyzed for sodium and potassium concentrations, by atomic absorption spectrophotometry (Model AA 120, Varian Technicon Pty Ltd., Melbourne, Australia). The rates of secretion of these ions were calculated as described above, but using total milliequivalents rather than total volume. Significance of changes in rate were estimated by a t-test for differences between two regressions (Steel and Torrie, 1970), and significance was accepted at $p \leq 0.05$.

figure 2 **Apparatus for the maintenance of the *in vitro* guinea pig lung preparation.**

The isolated lung was cannulated by way of the trachea, and the cannula was connected to a 1 cc tuberculin syringe via a 3-way stop-cock. Lung fluid could be withdrawn into the syringe, and redistributed to the upper cup of the stop-cock for sampling. The assembly was clamped such that the lung was supported freely in a bath containing Krebs-Henseleit saline. The saline was maintained at a constant temperature of $37^{\circ}C$, and a gas mixture of 95% O_2/5 % CO_2 bubbling in the bath kept the lungs well oxygenated.

3-WAY
STOP-COCK

CLAMP

OXYGEN

1cc TUBERCULIN
SYRINGE

CANNULA

TRACHEA (with double
ligatures)
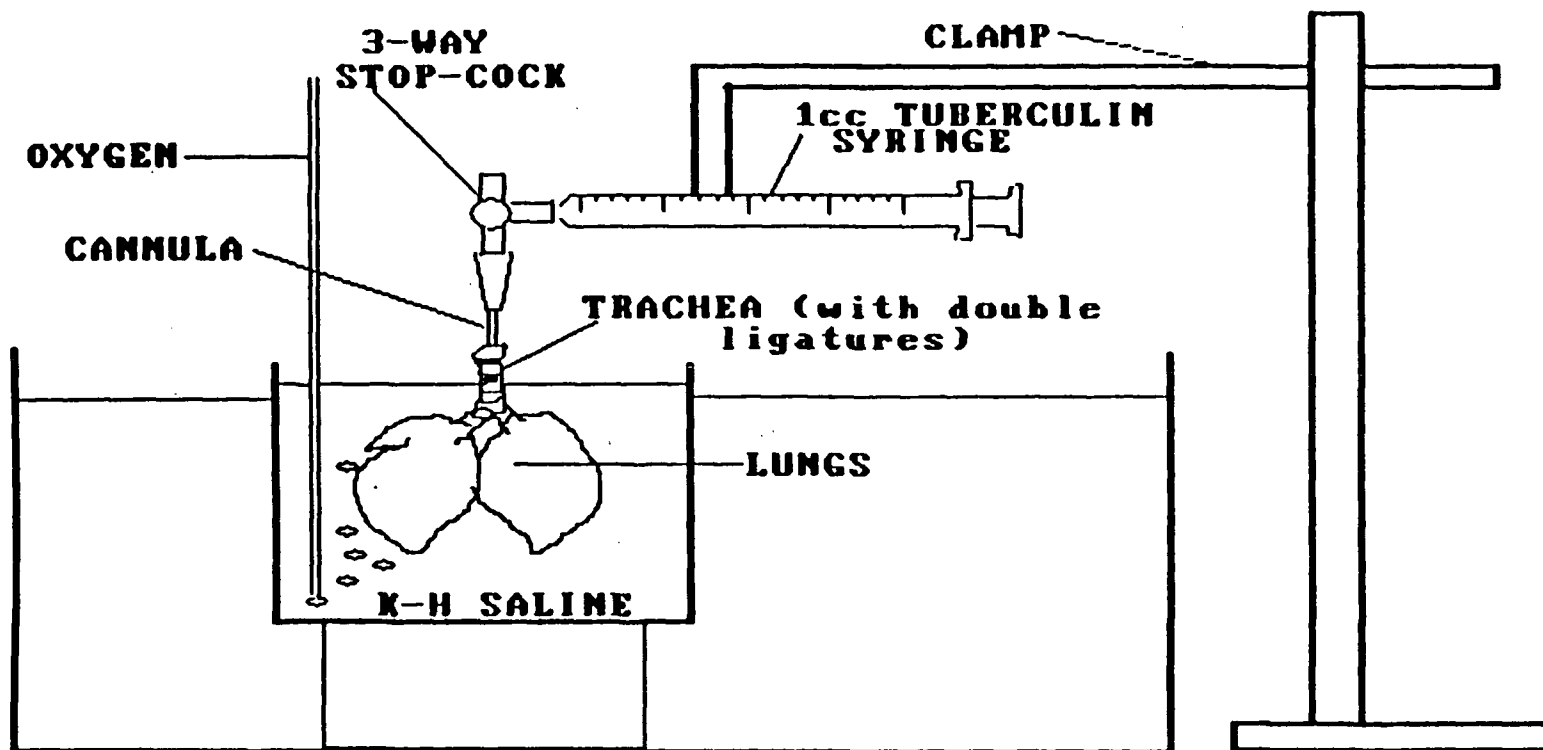
LUNGS

K-H SALINE

8. RESULTS

8.1 Fluid Production by *in vitro* Lungs from Fetal Guinea Pigs

Fetal lungs were supported in Krebs-Henseleit saline for the three hours of experimentation. The first hour gave the resting rate of fluid production in each preparation. The average resting secretion rate for all lungs used in this study was 1.68 ± 1.44 (SD) ml/kg·h (n=171; 54-67 days gestation; average body weight of 102.85 ± 19.75 (SD) g). This value is comparable to those obtained in intact animals (Setnikar et al. 1959; Cassin and Perks, 1982; Perks and Cassin, 1989; Scarpelli et al. 1975; Normand et al. 1971; Platzker et al. 1975; for details see discussion), thus demonstrating healthy lung function *in vitro*. The data showed a clear decline in resting rate with age in terms of ml/kg·h (Figure 3A), and a lesser decline in terms of ml/h (Figure 3B). This suggests that there is a reduction in the secretory activity of the lung tissue with age, but this reduction is partly compensated for by the increase in lung size. This trend has been observed in earlier experiments (Perks and Cassin, 1985a; Perks et al. 1990).

Experimental controls consisted of supporting fetal lungs in Krebs-Henseleit saline for the entire three hour duration of incubation (n=30; 58-66 days gestation; term=67 days; average body weight of 99.02 ± 19.68 (SD) g). At the start of each hour, the lungs were transferred into a fresh bath of saline. During the first hour of experimentation, a combined secretion rate of 1.60 ± 0.26 ml/kg body weight per hour was calculated (see Figure 4). The preparations continued to secrete without significant change during the second and third hours at combined rates of 1.67 ± 0.24 ml/kg·h and 1.66 ± 0.24 ml/kg·h respectively. These significances were verified using both regression analysis and ANOVA. Fluid production rates are maintained by the fetal guinea pig lung throughout the three hours of experimentation; this observation substantiates the validity of this technique.

Figure 3 **Secretion rates of *in vitro* lungs from fetal guinea pigs at different gestational ages.**

Average values from the first hour of incubation ($\pm$SE) from 171 fetuses, 54-67 days gestation, and an average body weight of 102.85 ± 19.74 (SD) g (outer limits, 62.6-166.8 g). Upper graph: rates in ml/kg body weight per h. Lower graph: rates in ml/h. Overall average rates, 1.68 ± 0.11 ml/kg·h, or 0.17 ± 0.01 ml/h.
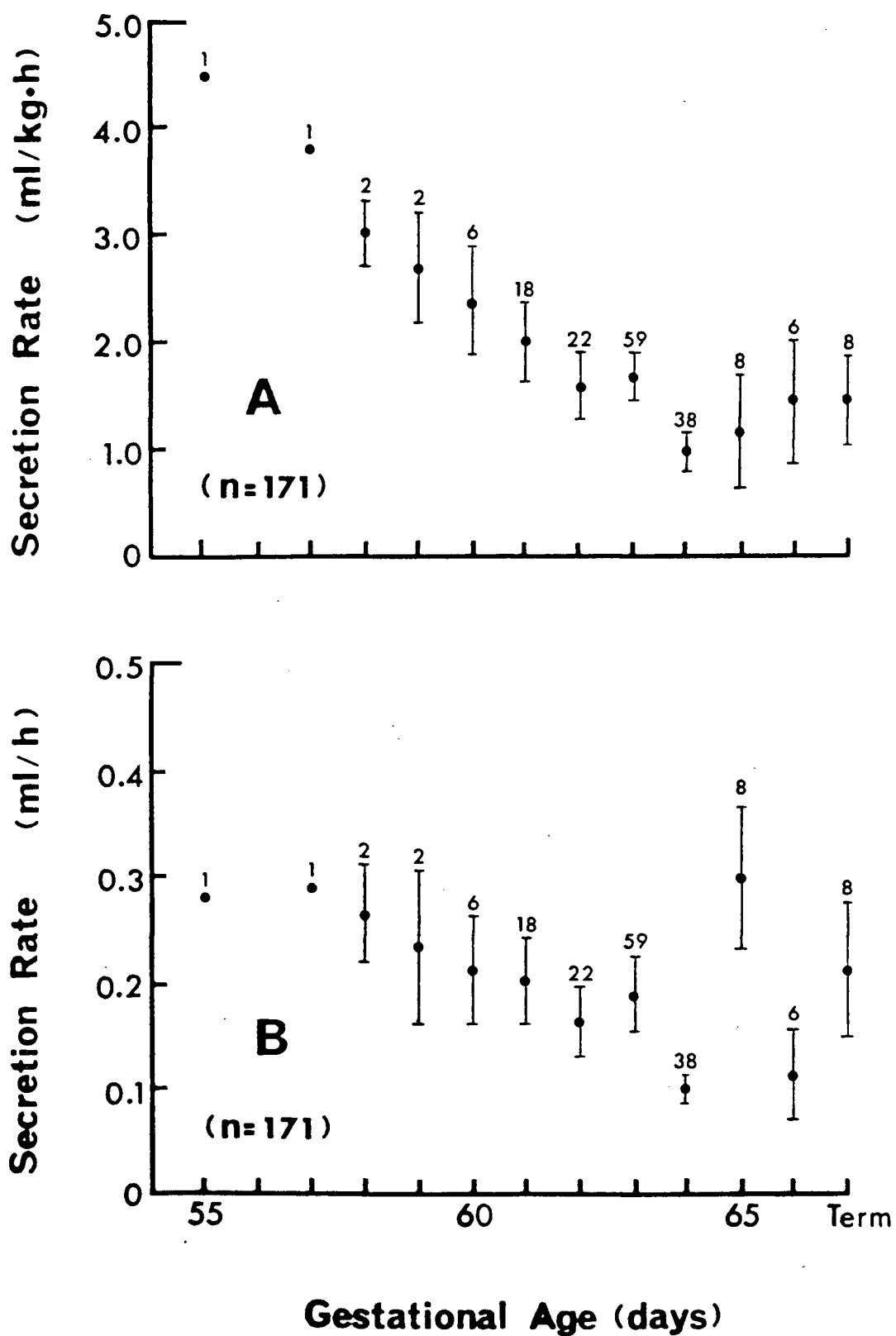
Figure 4 **Lung liquid production over a 3-h period by** *in vitro* **lungs from fetal guinea pigs**

Based on 30 fetuses, 60-64 days gestation, average body weight of 99.0 ± 19.7 (SD) g. Ordinate: total volume of lung fluid (mean ± SE) expressed as a percentage of that present at the end of the first hour, where 100% was 0.84 ± 0.05 ml. Abscissa: time in hours. All regressions are lines of best fit (method of least squares; Steel and Torrie 1970). Slopes respresent the secretion rates; the values below the lines give the average rates in ml/kg·h. The dotted line shows the original slope of the first hour; there were no significant changes in slope in the last 2 h (t-test for differences in two regressions; significance accepted at $p \leq 0.05$; Steel and Torrie 1970).
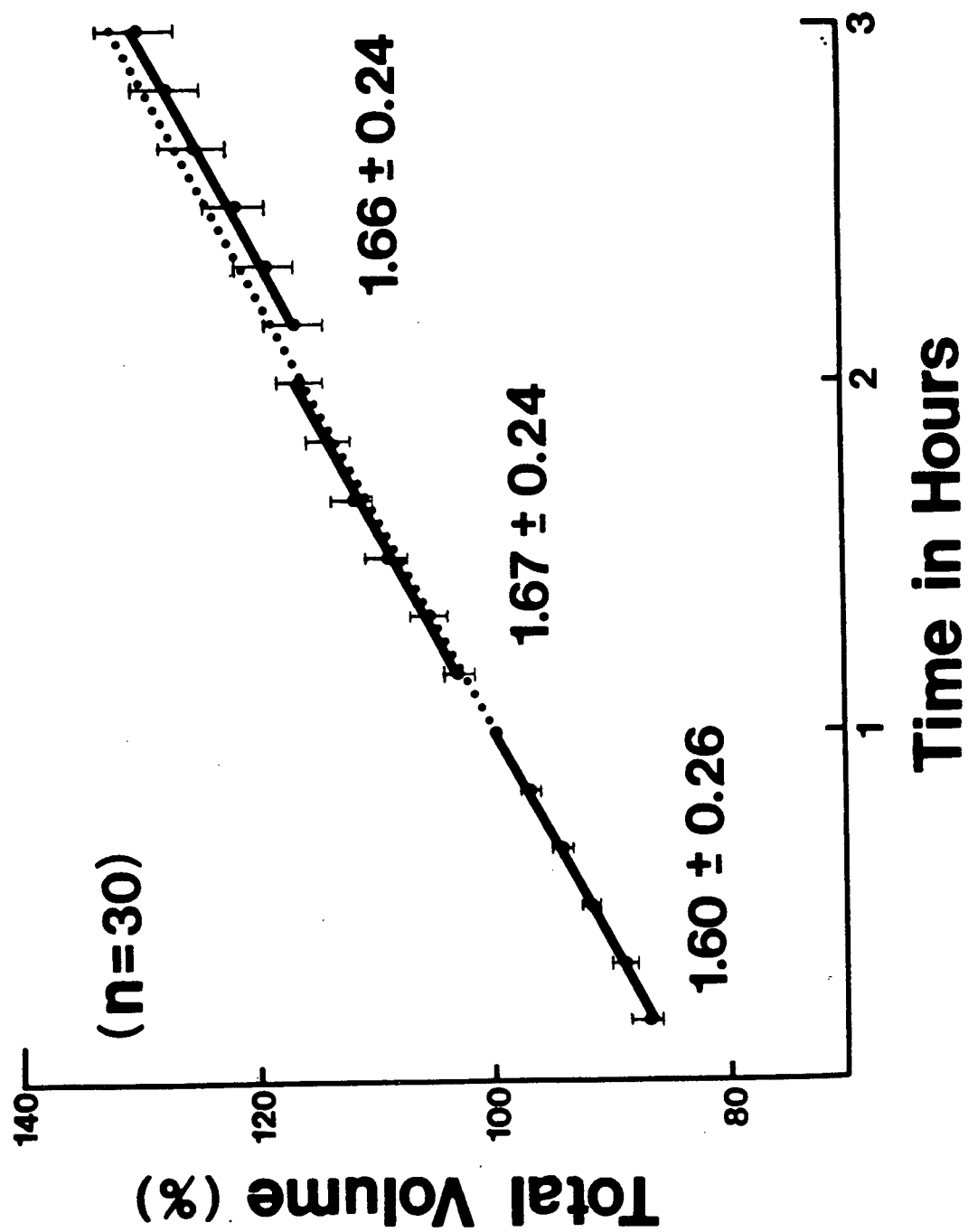
8.2 The Effect of Epinephrine on Lung Fluid Production.

To test whether epinephrine plays a role in controlling fetal lung fluid secretion in the fetal guinea pig, the pleural surfaces of fetal lungs were treated with different concentrations of epinephrine during the second ("treatment") hour of experimentation. Six concentrations of epinephrine were used: 10^{-5} M, 10^{-6} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, and 10^{-9} M (see Figure 5). The responses increased with concentrations up to 10^{-7} M, above which the responses (surprisingly) reduced in size. A dose/response curve using only the concentrations up to 10^{-7} M gave a threshold value for epinephrine at 1.78×10^{-11} M. A relationship between gestational age or body weight and degree of response was not observed.

Epinephrine at 10^{-5} M (Figure 5A)

Fetal lungs were exposed to epinephrine at 10^{-5} M during the treatment hour (n=6; 62-67 days gestation; average body weight of 105.32 ± 27.68 (SD) g). The combined rates for each hour were as follows: 1.06 ± 0.09 ml/kg·h (h1), 0.98 ± 0.12 ml/kg·h (h2; treatment hour) and 0.99 ± 0.23 ml/kg·h (h3). The average fall in secretion rate during the treatment hour was 7.55 ± 6.66 %. The effect of epinephrine at this concentration was minimal and the slight drop in secretion rate during the treatment hour was not significant by either regression analysis or ANOVA. The secretion rate in the third hour was not significantly different from those in the second or first hours. Clearly epinephrine at this concentration had little or no effect. For individual data, refer to Table C.1 (appendix C).

Epinephrine at 10^{-6} M (Figure 5B)

Fetal lungs were exposed to epinephrine at 10^{-6} M during the treatment hour (n=6; 60-67 days gestation; average body weight of 94.86 ± 12.08 (SD) g). The combined secretion rates for each hour were as follows: 3.38 ± 0.72 ml/kg·h (h1), 1.27 ± 0.31 ml/kg·h (h2; treatment hour) and

1.01 $\pm$ 0.24 ml/kg·h (h3). The average drop in secretion rate during the treatment hour was 62.43 $\pm$ 8.25 % which was significant by both regression analysis (p<0.0005) and ANOVA (p<0.05). The effect of epinephrine at this concentration was more marked, and in individual experiments there was a consistent fall in secretion with treatment in all cases (refer to Table C.2 in appendix C). There was a significant drop in the secretion rate in the third hour compared with that of the second hour (p<0.0005; regression analysis), thus recovery from hormone treatment at this concentration was not observed.

Epinephrine at 10^{-7} M (Figure 5C)

Fetal lungs were exposed to epinephrine at 10^{-7} M during the treatment hour (n=6; 62-63 days gestation; average body weight of 102.48 $\pm$ 9.04 (SD) g). The combined secretion rates for each hour were as follows: 3.67 $\pm$ 0.51 ml/kg·h (h1), 0.42 $\pm$ 0.57 ml/kg·h (h2; treatment hour) and 1.76 $\pm$ 0.48 ml/kg·h (h3). The average drop in secretion rate during the treatment hour was 88.56 $\pm$ 13.33 %. The degree of response was maximal at this concentration, and the drop in secretion rate during the treatment hour was highly significant by both regression analysis (p<0.0005) and ANOVA (p<0.005). In all individual experiments there was a marked reduction in secretion rate, with reabsorption in two preparations (-1.31 and -0.84 ml/kg·h; refer to Table C.3 in appendix C). When the hormone was removed in the third hour there was a significant rise in secretion rate (p<0.0005; regression analysis), thus recovery from hormone treatment was observed.

Epinephrine at 5 x 10^{-8} M (Figure 5D)

Fetal lungs were exposed to epinephrine at 5 x 10^{-8} M during the treatment hour (n=6; 60-66 days gestation; average body weight of 101.58 $\pm$ 14.61 (SD) g). The combined rates for each hour were as follows: 1.26 $\pm$ 0.30 ml/kg·h (h1), 0.35 $\pm$ 0.31 ml/kg·h (h2; treatment hour) and 0.49 $\pm$ 0.15 ml/kg·h (h3). The average drop in secretion rate during the treatment hour was 72.22

± 28.44 % which proved to be significant by both regression analysis (p<0.0005) and ANOVA (p<0.025). The effect was still marked at this concentration as reabsorption occurred in three preparations (-0.023, -0.55 and -0.11 ml/kg·h; refer to Table C.4 in appendix C) and the remaining three showed a fall in secretion. The secretion rate during the third hour did not significantly change from that in the second hour, thus recovery was not observed after hormone treatment. However, there was a slight rise in secretion rate during the third hour.

Epinephrine at 10^{-8} M (Figure 5E)

Fetal lungs were exposed to epinephrine at 10^{-8} M during the treatment hour (n=6; 61-65 days gestation; average body weight of 104.78 ± 16.06 (SD) g). The combined secretion rates for each hour were as follows: 2.71 ± 0.97 ml/kg·h (h1), 1.11 ± 0.44 ml/kg·h (h2; treatment hour) and 1.29 ± 0.50 ml/kg·h (h3). The average fall in secretion rate during the treatment hour was 59.04 ± 18.62 %, which was significant by regression analysis (p<0.0025) and ANOVA (p<0.025). In individual experiments, one preparation reabsorbed fluid upon treatment (-0.09 ml/kg·h) and secretion rate fell in the remaining five lungs (three of which were significant; refer to Table C.5 in appendix C). There was no significant change in secretion rate during the third hour, although a slight rise in fluid production was observed after the hormone was removed.

Epinephrine at 10^{-9} M (Figure 5F)

Fetal lungs were exposed to epinephrine at 10^{-9} M during the treatment hour (n=6; 62-66 days gestation; average body weight of 104.55 ± 14.55 (SD) g). The combined secretion rates for each hour were as follows: 1.08 ± 0.18 ml/kg·h (h1), 0.64 ± 0.07 ml/kg·h (h2; treatment hour) and 0.47 ± 0.07 ml/kg·h (h3). The average drop in secretion rate during the treatment hour was 40.74 ± 6.51 %, which was significant by both regression analysis (p<0.05) and ANOVA (p<0.005). All lungs showed a drop in secretion rate with treatment (in four lungs this fall was within the minimal limit of significance; refer to Table C.6 in appendix C) so there was a

Figure 5 **The effect of epinephrine on fluid production by *in vitro* lungs from fetal guinea pigs.**

Based on 36 fetuses, 60-67 days gestation, average body weight of 102.27 ± 15.90 (SD) g. During the middle hour the preparations were transferred to saline containing one of the following concentrations of epinephrine: (A) 10^{-5} M (n=6); (B) 10^{-6} M (n=6); (C) 10^{-7} M (n=6); (D) 5×10^{-8} M (n=6); (E) 10^{-8} M (n=6); (F) 10^{-9} M (n=6); (G) control saline. Ordinates: total volume of lung fluid expressed as a percentage of that present at the end of the first hour, just prior to treatment, where 100% was (A) 0.97 ± 0.07 ml; (B) 1.76 ± 0.16 ml; (C) 1.88 ± 0.16 ml; (D) 0.74 ± 0.10 ml; (E) 1.36 ± 0.31 ml; (F) 0.80 ± 0.05 ml; (G) 0.68 ± 0.02 ml. All regressions are lines of best fit (method of least squares, Steel and Torrie, 1970). The slopes represent the secretion rates; the values below the lines give the average rates in ml/kg·h. Asterisks above the lines in hour 2 show significant changes from the original slope (dotted lines)(significance accepted at $p \leq 0.05$). Asterisks above the lines in hour 3 show significant changes from the treatment hour slope. Standard errors of means are omitted for clarity; however, they averaged for (A) 1.61 ± 0.16; (B) 1.68 ± 0.18; (C) 2.27 ± 0.37; (D) 2.41 ± 0.24; (E) 1.71 ± 0.17; (F) 1.85 ± 0.17; (G) 3.24 ± 0.41 (units as above); corresponding coefficients of variation were (A) 3.60 ± 0.33 %; (B) 4.24 ± 0.53 %; (C) 5.54 ± 0.82 %; (D) 5.72 ± 0.62 %; (E) 4.16 ± 0.45 %; (F) 4.34 ± 0.39 %; (G) 7.09 ± 0.76 %.

EPINEPHRINE
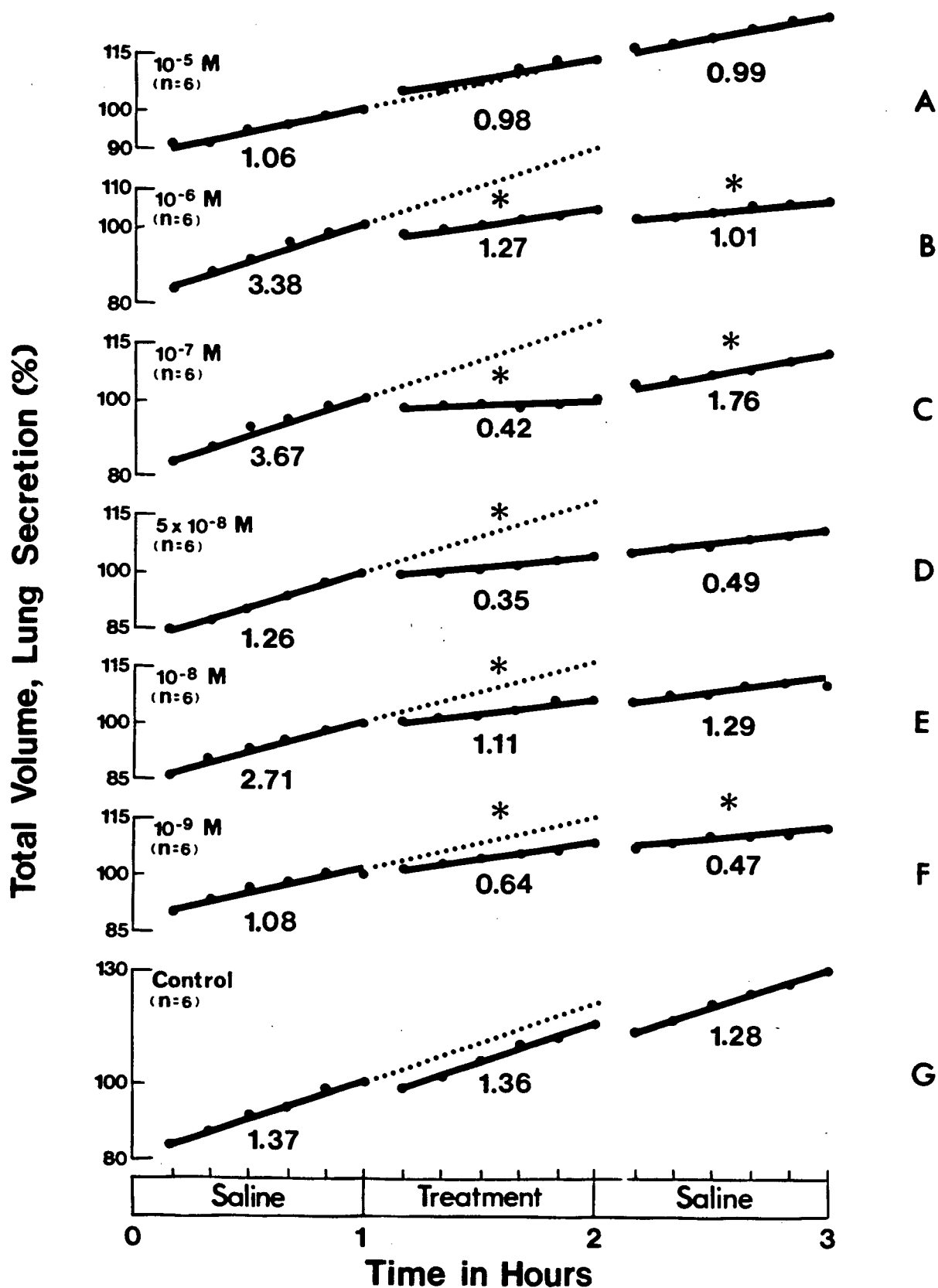
consistent response with treatment. As with epinephrine at 10^{-6} M, there was a fall in secretion during the third hour which was significant (p<0.05; regression analysis).

Controls (Figure 5G)

Fetal lungs were incubated in saline during each hour of experimentation. At the start of each hour, the lungs were transferred into a fresh bath of saline. The secretion rate for each hour were as follows: 1.37 ± 0.30 ml/kg·h (h1), 1.36 ± 0.30 ml/kg·h (h2) and 1.28 ± 0.27 ml/kg·h (h3). There was no significant change in fluid production in any hour (verified by both regression analysis and ANOVA), thus demonstrating constant fluid production by the preparations over the 3-hour experimental period. These results support those shown in Figure 4. For individual data, refer to Table C.26 (appendix C). Sodium movement followed that of water in all cases, and there was no significant change in the rate of secretion of this ion throughout the experiments. Potassium, which was secreted at a much lower rate, did not follow the movement of water as closely as sodium (see Table D.11 in appendix D).

8.2.1 The Effect of Epinephrine on Ion Movement

The movements of sodium and potassium ions closely followed that of water in all experiments involving treatment with epinephrine. The secretion rates of both ions fell significantly with each concentration of epinephrine, and sodium showed a slight but insignificant recovery in the third hour (see Table D.1 in appendix D).

8.2.2 The Effect of Epinephrine and Adrenergic Receptor Blockers on Lung Fluid Production

It is clear that epinephrine at 10^{-9} M to 10^{-6} M caused a slowing of fluid production (and in some cases fluid reabsorption) by the fetal guinea pig lung. Whether this effect is mediated by alpha- or beta-adrenergic receptors was tested using propranolol, a non-selective beta-adrenergic

antagonist (Weiner, 1985a), and phentolamine, a non-selective alpha-adrenergic antagonist (Weiner, 1985a).

Propranolol Alone (Figure 6C)

The pleural surfaces of fetal lungs were exposed to propranolol at 10^{-7} M (n=6; 62-64 days gestation; average body weight of 104.95 $\pm$ 20.77 (SD) g). This level of propranolol has been used in other studies (Sheppard and Burghardt, 1970). The combined secretion rates for each hour were as follows: 1.21 $\pm$ 0.58 ml/kg·h (h1), 1.24 $\pm$ 0.24 ml/kg·h (h2; treatment hour) and 0.63 $\pm$ 0.22 ml/kg·h (h3). There was an overall slight rise in secretion rate with treatment, but this was not significant by regression analysis or ANOVA. There was a significant drop in secretion rate during the final hour (p<0.025; regression analysis) which was curious, but since fluid secretion did not change significantly during the treatment hour, any immediate effect of propranolol appeared to be negligible. For individual data, refer to Table C.8 (appendix C).

Epinephrine Combined With Propranolol (Figure 6B)

Fetal lungs were exposed to a mixture of epinephrine at 10^{-8} M and propranolol at 10^{-7} M (n=6; 62-64 days gestation; average body weight of 101.78 $\pm$ 15.03 (SD) g). To ensure that beta receptors were blocked to epinephrine, propranolol at 10 times the concentration of epinephrine was used. This concentration of propranolol is sufficient to block beta-stimulated cAMP production (Sheppard and Burghardt, 1970). The secretion rates for each hour were as follows: 1.97 $\pm$ 0.48 ml/kg·h (h1), 0.75 $\pm$ 0.12 ml/kg·h (h2; treatment hour) and 0.46 $\pm$ 0.04 ml/kg·h (h3). There was an average fall in secretion rate during the treatment hour of 61.93 $\pm$ 10.53 %, which was significant by both regression analysis (p<0.0005) and ANOVA (p<0.005). This fall is comparable to that observed with epinephrine at 10^{-8} M treatment alone (59.04 $\pm$ 18.62 %; Figure 6A), which suggests that epinephrine is not working through beta-adrenergic receptors. Upon removal of treatment during the third hour, a continued and significant fall in secretion rate

Figure 6 **The effect of epinephrine and propranolol on fluid production by *in vitro* lungs from fetal guinea pigs.**

Based on 18 fetuses, 61-65 days gestation, average body weight of 103.84 ± 16.47 (SD) g. During the middle hour the preparations were transferred to saline containing one of the following: (A) epinephrine 10^{-8} M (n=6); (B) epinephrine 10^{-8} M/propranolol 10^{-7} M (n=6); (C) propranolol 10^{-7} M (n=6); (D) control saline. Ordinates: total volume of lung fluid expressed as a percentage of that present at the end of the first hour, just prior to treatment, where 100% was (A) 1.36 ± 0.31 ml; (B) 0.86 ± 0.05 ml; (C) 0.96 ± 0.12 ml; (D) 0.68 ± 0.02 ml. All regressions are lines of best fit (method of least squares, Steel and Torrie, 1970). The slopes represent the secretion rates; the values below the lines give the average rates in ml/kg·h. Asterisks above the lines in hour 2 show significant changes from the original slope (dotted lines)(significance accepted at $p \leq 0.05$). Asterisks above the lines in hour 3 show significant changes from the treatment hour slope. Standard errors of means are omitted for clarity; however, they averaged for (A) 1.71 ± 0.17; (B) 1.28 ± 0.23; (C) 4.74 ± 0.60; (D) 3.24 ± 0.41 (units as above); corresponding coefficients of variation were (A) 4.16 ± 0.45 %; (B) 3.20 ± 0.70 %; (C) 11.05 ± 1.31 %; (D) 7.09 ± 0.76 %.
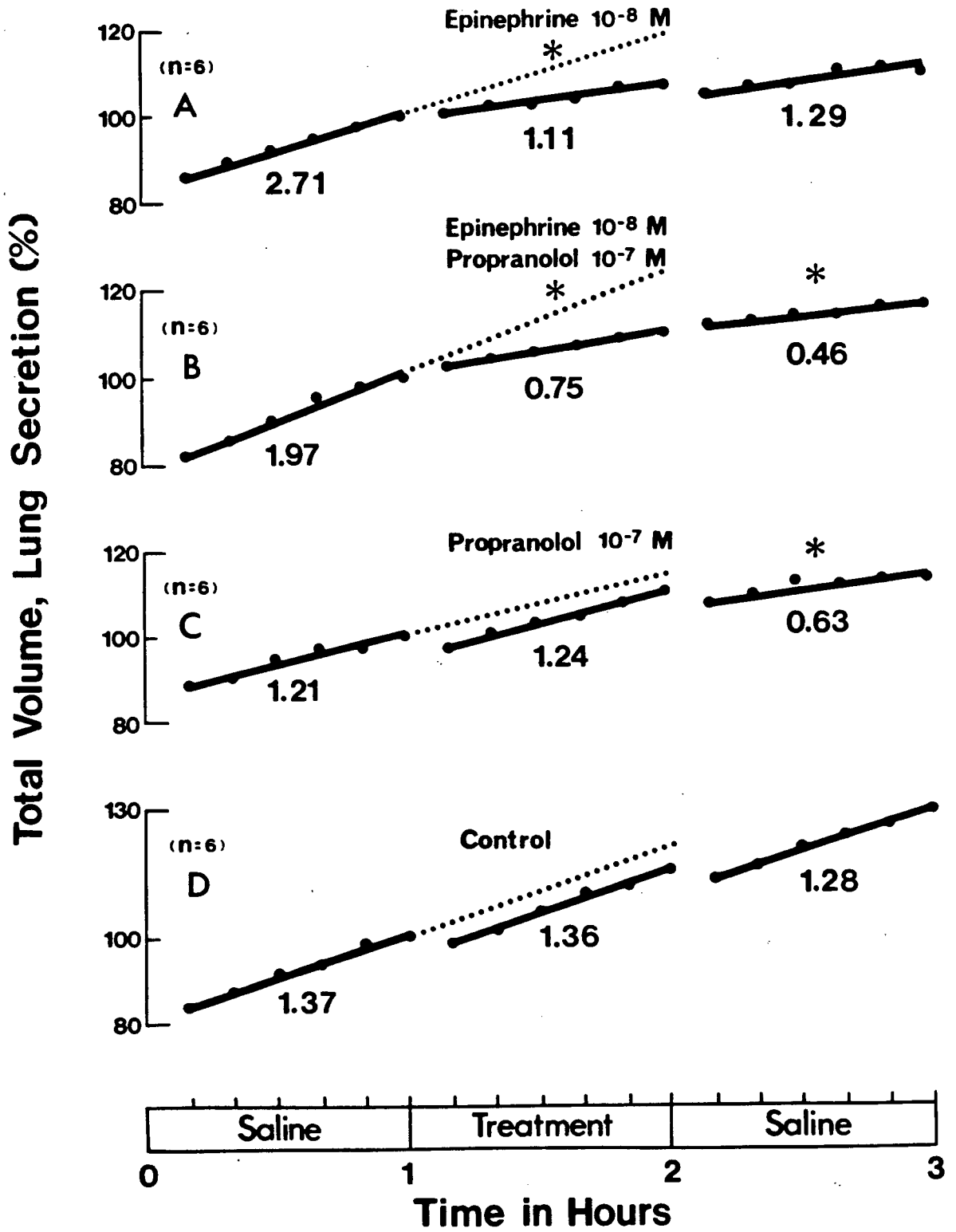
was observed (p<0.0025; regression analysis). This fall was also seen in the propranolol controls, so this could be due to a delayed effect of propranolol. Despite this fact, it appears that epinephrine can cause a fall in fluid production by the fetal lung, even after beta-receptor blockade. For individual data, refer to Table C.7 (appendix C).

Phentolamine Alone (Figure 7C)

Fetal lungs were exposed to several trial doses of phentolamine from 10^{-5} M to 10^{-6} M, all of which produced no effect on fluid production. These levels have been used in other *in vitro* studies (Alwmark and Ahrein, 1987; Siegl and Orzechowski, 1981). The phentolamine controls finally used were 1.78 x 10^{-5} M (n=7; 61-64 days gestation; average body weight of 117.24 ± 16.63 (SD) g). The secretion rates for each hour were as follows: 2.20 ± 1.02 ml/kg·h (h1), 2.20 ± 0.59 ml/kg·h (h2; treatment hour) and 1.19 ± 0.44 ml/kg·h (h3). There was no significant change in fluid production during any of the three hours of experimentation (verified by regression analysis and ANOVA), which demonstrates that phentolamine by itself does not affect lung fluid secretion. For individual data, refer to Table C.20 (appendix C).

Epinephrine Combined With Phentolamine (Figure 7B)

Fetal lungs were exposed to a mixture of epinephrine at 10^{-7} M and phentolamine at 1.78 x 10^{-6} M (n=6; 62-64 days gestation; average body weight of 100.07 ± 18.84 (SD) g). The effect of epinephrine 10^{-7} M alone was maximal (Figure 5C;7A), and a concentration of phentolamine 10 times higher than epinephrine was used in order to ensure that sufficient alpha receptors were blocked to epinephrine. This level of phentolamine is reported to antagonize alpha-mediated responses (Siegl and Orzechowski, 1981). The secretion rates for each hour were as follows: 0.56 ± 0.21 ml/kg·h (h1), 1.17 ± 0.37 ml/kg·h (h2; treatment hour) and 0.39 ± 0.09 ml/kg·h (h3). There was a surprising overall rise in secretion rate with treatment which was significant by regression analysis (p<0.0025), but not by ANOVA. Out of six lungs, only two lungs showed a

Figure 7 **The effect of epinephrine and phentolamine on fluid production by *in vitro* lungs from fetal guinea pigs.**

Based on 19 fetuses, 61-64 days gestation, average body weight of 107.16 ± 16.65 (SD) g. During the middle hour the preparations were transferred to saline containing one of the following: (A) epinephrine 10^{-7} M (n=6); (B) epinephrine 10^{-7} M/phentolamine 10^{-6} M (n=6); (C) phentolamine 10^{-5} M (n=7); (D) control saline. Ordinates: total volume of lung fluid expressed as a percentage of that present at the end of the first hour, just prior to treatment, where 100% was (A) 1.88 ± 0.16 ml; (B) 0.64 ± 0.09 ml; (C) 0.96 ± 0.22 ml; (D) 0.68 ± 0.02 ml. All regressions are lines of best fit (method of least squares, Steel and Torrie, 1970). The slopes represent the secretion rates; the values below the lines give the average rates in ml/kg·h. Asterisks above the lines in hour 2 show significant changes from the original slope (dotted lines)(significance accepted at $p \leq 0.05$). Asterisks above the lines in hour 3 show significant changes from the treatment hour slope. Standard errors of means are ommitted for clarity; however, they averaged for (A) 2.27 ± 0.37; (B) 2.89 ± 0.44; (C) 4.17 ± 0.51; (D) 3.24 ± 0.41 (units as above); corresponding coefficients of variation were; (A) 5.54 ± 0.82 %; (B) 6.44 ± 3.82 %; (C) 9.34 ± 0.99 %; (D) 7.09 ± 0.76 %.
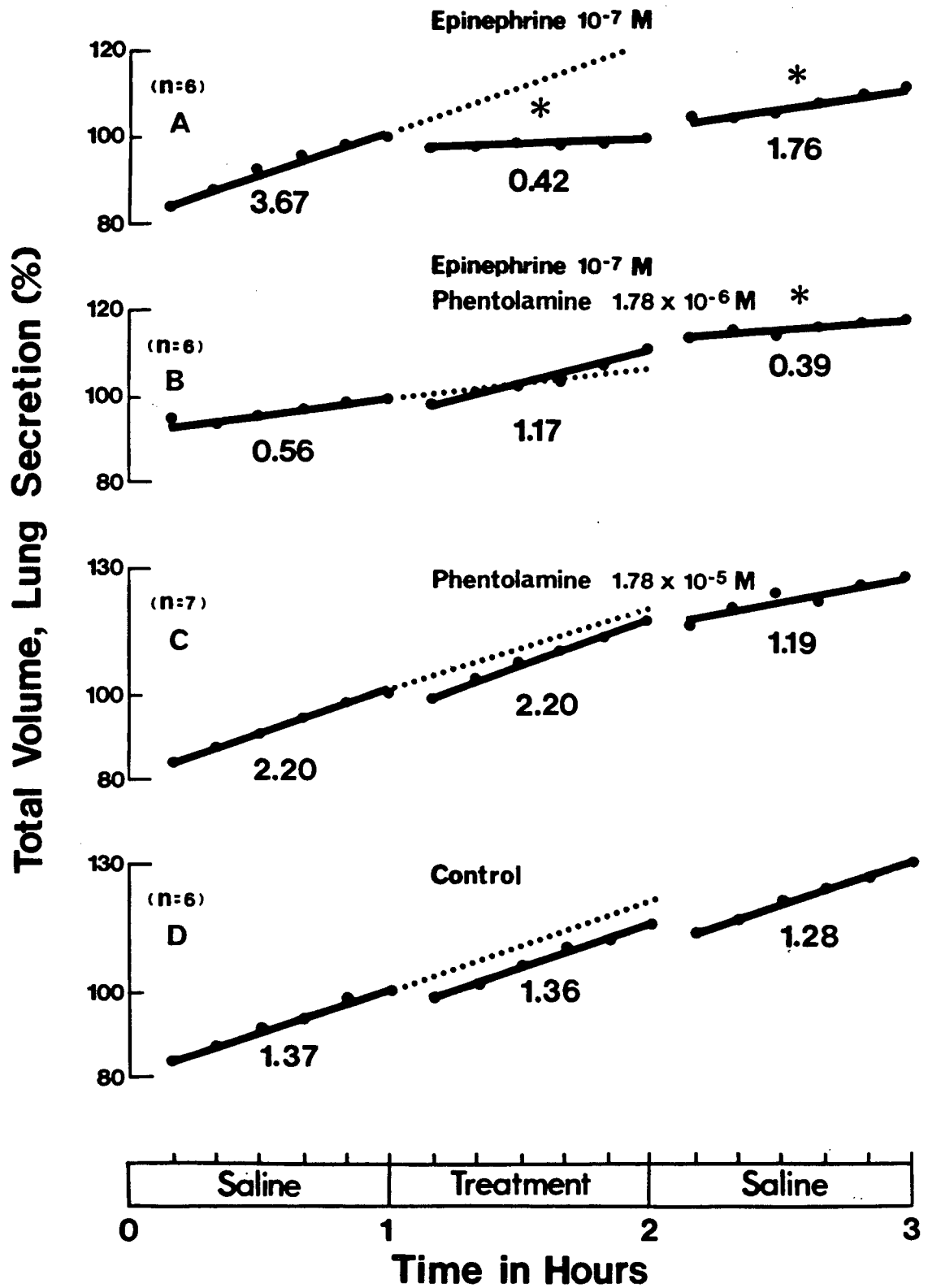
fall in fluid production in hour 2 (one fall was significant; one was not), and the remaining four lungs showed a significant rise in fluid secretion (refer to Table C.9 in appendix C). This clearly demonstrates that an epinephrine-induced fall (or reabsorption) in secretion can be blocked by phentolamine; this indicates that epinephrine is working through alpha receptors in the fetal guinea pig. The secretion rate fell to resting rate levels upon the removal of treatment (p<0.0005; regression analysis).

8.2.3 The Effect of Epinephrine and Adrenergic Receptor Blockers on Ion Movement

Secretion rates of both ions fell significantly after treatment with joint epinephrine and propranolol (see Table D.3 in appendix D); the changes were in parallel with water. There was a significant recovery of sodium secretion during the third hour not seen in the water results. Potassium followed water movement during the recovery period in these experiments. The movements of these ions in preparations treated with propranolol alone were identical to those of untreated lungs. Sodium and potassium secretion did not follow that of water with the epinephrine/phentolamine experiments. Where there was a rise in fluid secretion, there was a significant fall in sodium movement and no change in potassium movement (see Table D.4 in appendix D). Fluid secretion fell in the third hour, as did potassium secretion, but sodium secretion showed a rise during this time. Both ions were unaffected by phentolamine treatment alone.

8.2.4 The Effect of Isoproterenol on Lung Fluid Production

The experiments using adrenergic receptor blockers indicate that epinephrine is working via alpha receptors. Considering that other researchers working on fetal sheep have reported that the effect of epinephrine is mediated through beta adrenergic receptors (Walters and Olver, 1978), it seemed important to check these unusual results by an independent method. Therefore, tests were made with the selective beta-agonist, isoproterenol (Weiner, 1985b).

Isoproterenol at 10⁻⁷ M (Figure 8B)

Isoproterenol at a concentration equivalent to epinephrine at its maximal effect (10^{-7} M) was tested on the isolated lungs (n=6; 62-64 days gestation; average body weight of 105.47 $\pm$ 18.83 (SD) g). This concentration of isoproterenol has been used in other *in vitro* studies (Siegl and Orzechowski, 1981), and equivalent concentrations of isoproterenol versus epinephrine have been used in studies on the intact animal (Walters and Olver, 1978). The secretion rates for each hour were as follows: 1.39 $\pm$ 0.52 ml/kg·h (h1), 1.00 $\pm$ 0.35 ml/kg·h (h2; treatment hour) and 1.14 $\pm$ 0.41 ml/kg·h (h3). There was a small drop in secretion rate of 28.06 $\pm$ 9.76 % with treatment, but this was not significant by regression analysis or ANOVA. Clearly, lung fluid production remained relatively unaffected by isoproterenol treatment. These results further substantiates that a reduction in fluid secretion by the fetal guinea pig lung is not mediated by beta adrenergic receptors. For individual data, refer to Table C.10 (appendix C).

8.2.5   The Effect of Isoproterenol on Ion Movement

The secretion rates of both ions were unaffected by isoproterenol. As the fluid movement into the lungs decreased slightly with treatment, there was a corresponding decrease in sodium movement (see Table D.5 in appendix D). The movement of both ions did not change significantly during the third hour.

8.2.6   The Effect of Epinephrine and AVP on Lung Fluid Production

Fetal lungs were exposed to AVP alone, or to a mixture of epinephrine and AVP to test a possible synergism between these two hormones.

Figure 8 **The effect of isoproterenol on fluid production by *in vitro* lungs from fetal guinea pigs.**

Based on 12 fetuses, 62-67 days gestation, average body weight of 103.98 ± 14.17 (SD) g. During the middle hour the preparations were transferred to saline containing one of the following: (A) epinephrine 10^{-7} M (n=6); (B) isoproterenol 10^{-7} M (n=6); (C) control saline. Ordinates: total volume of lung fluid expressed as a percentage of that present at the end of the first hour, just prior to treatment, where 100% was (A) 1.88 ± 0.16 ml; (B) 0.98 ± 0.12 ml; (C) 0.68 ± 0.02 ml. All regressions are lines of best fit (method of least squares, Steel and Torrie, 1970). The slopes represent the secretion rates; the values below the lines give the average rates in ml/kg·h. Asterisks above the lines in hour 2 show significant changes from the original slope (dotted lines)(significance accepted at $p \leq 0.05$). Asterisks above the lines in hour 3 show significant changes from the treatment hour slope. Standard errors of means are omitted for clarity; however, they averaged for (A) 2.27 ± 0.37; (B) 2.31 ± 0.24; (C) 3.24 ± 0.41 (units as above); corresponding coefficients of variation were (A) 5.54 ± 0.82 %; (B) 5.47 ± 0.53 %; (C) 7.09 ± 0.76 %.
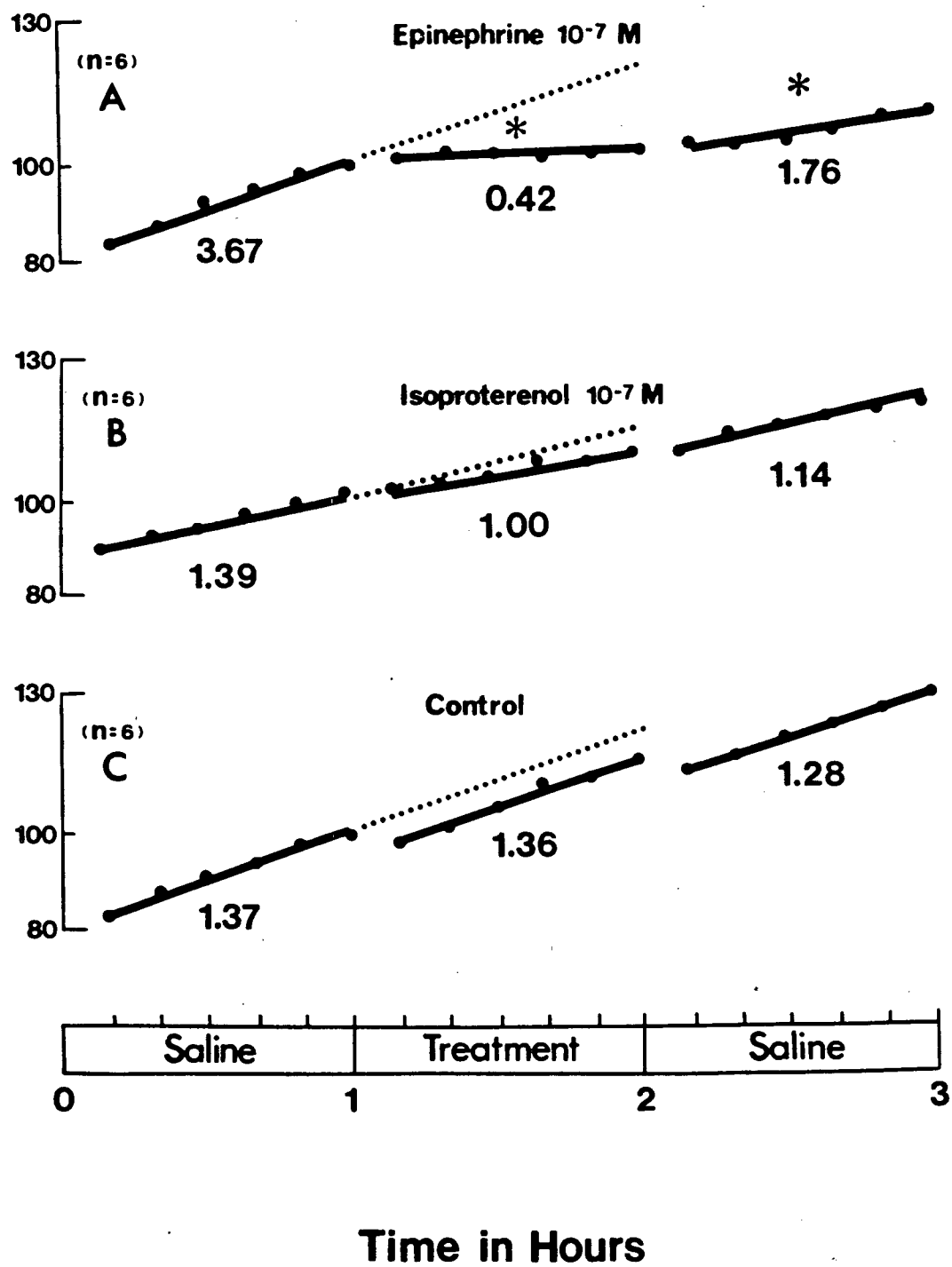
AVP Alone (Figure 9C)

Fetal lungs were exposed to AVP at 0.6 mU/ml (n=6; 63-67 days gestation; average body weight of 127.97 $\pm$ 24.05 (SD) g). This concentration of AVP is not beyond the physiological limits of fetal plasma AVP levels that have been reported at the time of birth (Pohjavuori and Fyhrquist, 1980; Pohjavuori 1983). The secretion rates for each hour were as follows: 1.58 $\pm$ 0.54 ml/kg·h (h1), 0.96 $\pm$ 0.41 ml/kg·h (h2; treatment hour) and 0.39 $\pm$ 0.36 ml/kg·h (h3). There was an average drop in secretion rate of 39.24 $\pm$ 20.66 % which was significant by regression analysis ($p<0.005$). The ANOVA was not significant due to some variation in individual experiments. One lung reabsorbed fluid with treatment, but there was no evidence for an age-dependent AVP response (for individual data, refer to Table C.12 in appendix C). There was a continued fall in fluid secretion in the third hour which was significant ($p<0.01$; regression analysis). This slow effect of AVP has been observed in other studies (Perks and Cassin, 1982, 1985a).

Epinephrine Combined With AVP (Figure 9B)

Fetal lungs were exposed to a mixture of epinephrine at 10^{-7} M and AVP at 0.6 mU/ml (n=6; 64 days gestation; average body weight of 102.28 $\pm$ 7.95 (SD) g). The combined secretion rates for each hour were as follows: 1.15 $\pm$ 0.48 ml/kg·h (h1), 0.23 $\pm$ 0.79 ml/kg·h (h2; treatment hour) and 0.67 $\pm$ 0.24 ml/kg·h (h3). The average drop in secretion rate with treatment was 80.00 $\pm$ 45.20 % which was significant by regression analysis ($p<0.005$) and by ANOVA ($p<0.005$). Out of six lungs, two were highly reabsorbing and four showed a fall in secretion (two were significant; refer to Table C.11 in appendix C). This treatment was not more effective in causing the lung to reabsorb or reduce fluid production than when compared to epinephrine treatment alone (Figure 9A), although it was more effective than AVP treatment alone (Figure 9C). There was a curious rise in fluid production during the recovery period, but this rise was not significant.

Figure 9 **The effect of epinephrine and AVP on fluid production by *in vitro* lungs from fetal guinea pigs.**

Based on 18 fetuses, 62-67 days gestation, average body weight of 110.91 $\pm$ 19.18 (SD) g. During the middle hour the preparations were transferred to saline containing one of the following: (A) epinephrine 10^{-7} M (n=6); (B) epinephrine 10^{-7} M/AVP 0.6 mU/ml (n=6); (C) AVP 0.6 mU/ml (n=6); (D) control saline. Ordinates: total volume of lung fluid expressed as a percentage of that present at the end of the first hour, just prior to treatment, where 100% was (A) 1.88 $\pm$ 0.16 ml; (B) 0.83 $\pm$ 0.06 ml; (C) 1.28 $\pm$ 0.22 ml; (D) 0.68 $\pm$ 0.02 ml. All regressions are lines of best fit (method of least squares, Steel and Torrie, 1970). The slopes represent the secretion rates; the values below the lines give the average rates in ml/kg·h. Asterisks above the lines in hour 2 show significant changes from the original slope (dotted lines)(significance accepted at $p \leq 0.05$). Asterisks above the lines in hour 3 show significant changes from the treatment hour slope. Standard errors of means are ommitted for clarity; however, they averaged for (A) 2.27 $\pm$ 0.37; (B) 3.64 $\pm$ 0.46; (C) 2.15 $\pm$ 0.23; (D) 3.24 $\pm$ 0.41 (units as above); corresponding coefficients of variation were; (A) 5.54 $\pm$ 0.82 %; (B) 8.58 $\pm$ 4.20 %; (C) 5.20 $\pm$ 0.60 %; (D) 7.09 $\pm$ 3.22 %.
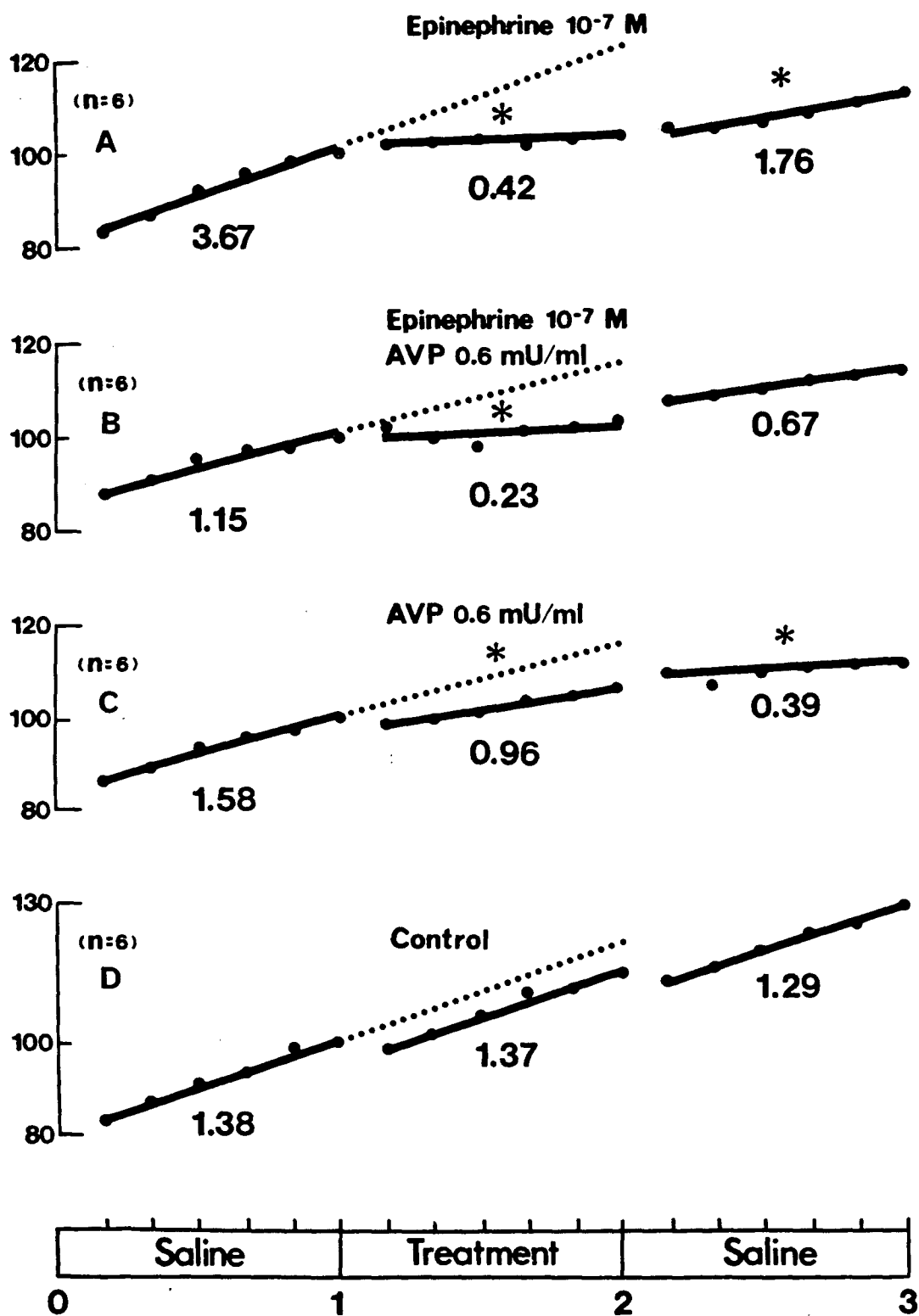
8.3   The Effect of Norepinephrine on Lung Fluid Production

To test whether NE is involved in controlling lung fluid production, the pleural surfaces of fetal lungs were treated with different concentrations of NE during the treatment hour of experimentation. Six concentrations of NE were used: 1.24×10^{-5} M, 1.24×10^{-6} M, 1.24×10^{-7} M, 5.24×10^{-8} M, 1.24×10^{-8} M and 1.24×10^{-9} M (see Figure 10). A dose/response curve estimated a threshold dose of NE at 3.16×10^{-10} M. There was no clear relationship between fetal age and degree of response, but it was found that there was little effect of NE with fetuses under 78.0 g. Only fetuses above this weight were used in this part of the study.

NE at 10^{-5} M (Figure 10A)

Fetal lungs were exposed to NE at 1.24×10^{-5} M during the treatment hour (n=6; 60-65 days gestation; average body weight of 102.21 ± 19.25 (SD) g). The combined secretion rates for each hour were as follows: 1.86 ± 0.30 ml/kg·h (h1), -0.31 ± 0.15 ml/kg·h (h2; treatment hour) and 0.08 ± 0.99 ml/kg·h (h3). The average fall in secretion rate during the treatment hour was 116.67 ± 6.40 %, where 100% represents a fall to zero production, and higher percentages, reabsorption. This reduction was significant by both regression analysis (p<0.0005) and ANOVA (p<0.005). The effect of NE was maximal at this concentration, and in five out of six lungs there was immediate reabsorption of fluid with treatment, and in the remaining lung there was a significant fall in secretion (refer to Table C.13 in appendix C). There was a recovery in the third hour which was significant (p<0.005; regression analysis).

NE at 10^{-6} M (Figure 10B)

Lungs were exposed to NE at 1.24×10^{-6} M during the treatment hour (n=6; 62-64 days gestation; average body weight of 97.18 ± 17.94 (SD) g). The combined secretion rates for each hour were as follows: 1.97 ± 0.60 ml/kg·h (h1), 0.22 ± 0.18 ml/kg·h (h2; treatment hour) and

0.73 ± 0.19 ml/kg·h (h3). The average fall in secretion rate with treatment was 88.83 ± 13.52 % which was significant by both regression analysis (p<0.0005) and ANOVA (p<0.01). The effect of NE at this concentration was strong, as one lung showed immediate reabsorption of lung fluid, and the remaining five showed a significant fall in secretion (refer to Table C.14 in appendix C). There was a recovery upon removal of NE which was significant (p<0.0005; regression analysis).

NE at 10^{-7} M (Figure 10C)

Lungs were exposed to NE at 1.24 x 10^{-7} M during the treatment hour (n=6; 61-64 days gestation; average body weight of 89.15 ± 7.87 (SD) g). The combined secretion rates for each hour were as follows: 1.70 ± 0.19 ml/kg·h (h1), 0.41 ± 0.11 ml/kg·h (h2; treatment hour) and 0.58 ± 0.17 ml/kg·h (h3). The average fall in secretion rate with treatment was 75.88 ± 12.57 % which was significant by both regression analysis (p<0.0005) and ANOVA (p<0.005). Individual experiments showed a fall in secretion rate in every case, five of which were significant (refer to Table C.15 in appendix C). Again, there was a recovery upon removal of treatment which was significant (p<0.05; regression analysis).

NE at 5 x 10^{-8} M (Figure 10D)

Lungs were exposed to NE at 5.24 x 10^{-8} M during the treatment hour (n=6; 61-63 days gestation; average body weight of 89.95 ± 11.02 (SD) g). The combined secretion rates for each hour were as follows: 1.84 ± 0.37 ml/kg·h (h1), 0.47 ± 0.16 ml/kg·h (h2; treatment hour) and 0.62 ± 0.12 ml/kg·h (h3). The average fall in secretion rate with treatment was 74.46 ± 9.96 % which was significant by both regression analysis (p<0.0005) and ANOVA (p<0.01). There was a slight reabsorption of fluid in one lung during treatment and the remaining five lungs showed a fall in secretion (three of which were significant; refer to Table C.16 in appendix C). There was a recovery of fluid secretion seen in the third hour, but this was not significant.

NE at 10^{-8} M (Figure 10E)

Lungs were exposed to NE at 1.24 x 10^{-8} M (n=6; 62-64 days gestation; average body weight of 94.80 ± 9.37 (SD) g). The combined secretion rates for each hour were as follows: 1.12 ± 0.28 ml/kg·h (h1), 0.77 ± 0.19 ml/kg·h (h2; treatment hour) and 0.62 ± 0.23 ml/kg·h (h3). The average fall in secretion rate with treatment was 31.25 ± 17.58 % which was significant only by regression analysis (p<0.01). Individual data were inconsistent at this concentration: three lungs showed a rise in secretion with treatment, two showed a fall and one slightly reabsorbed fluid. These responses had no relation to the age or weight of the fetus (refer to Table C.17 in appendix C). There was no significant change in fluid secretion in the third hour of experimentation.

NE at 10^{-9} M (Figure 10F)

Lungs were exposed to NE at 1.24 x 10^{-9} M during the treatment hour (n=6; 61-65 days gestation; average body weight of 112.13 ± 25.89 (SD) g). The combined secretion rates for each hour were as follows: 1.08 ± 0.28 ml/kg·h (h1), 0.96 ± 0.26 ml/kg·h (h2; treatment hour) and 0.75 ± 0.32 ml/kg·h (h3). The average drop in secretion rate with treatment was 11.11 ± 5.09 % which was not significant by regression analysis or ANOVA. The response from each lung with treatment varied: three lungs showed a fall in secretion (two of which were significant) and three showed a rise in secretion (one of which was significant; refer to Table C.18 in appendix C). There was a significant fall in fluid production during the third hour (p<0.025; regression analysis).

8.3.1   The Effect of NE on Ion Movement

The movement of sodium followed that of water with each concentration of NE (see Table D.6 in appendix D). Where there was movement of water out of the lungs with the top dose of NE, there was a corresponding withdrawal of sodium. This suggests that the reabsorption of lung

Figure 10 **The effect of norepinephrine on fluid production by *in vitro* lungs from fetal guinea pigs.**

Based on 36 fetuses, 60-65 days gestation, average body weight of 97.57 ± 17.22 (SD) g. During the middle hour the preparations were transferred to saline containing one of the following concentrations of NE: (A) 1.24×10^{-5} M (n=6); (B) 1.24×10^{-6} M (n=6); (C) 1.24×10^{-7} M (n=6); (D) 5.24×10^{-8} M (n=6); (E) 1.24×10^{-8} M (n=6); (F) 1.24×10^{-9} M (n=6); (G) control saline. Ordinates: total volume of lung fluid expressed as a percentage of that present at the end of the first hour, just prior to treatment, where 100% was (A) 0.78 ± 0.07 ml; (B) 0.70 ± 0.03 ml; (C) 0.68 ± 0.04 ml; (D) 0.80 ± 0.04 ml; (E) 0.71 ± 0.07 ml; (F) 0.86 ± 0.10 ml; (G) 0.68 ± 0.02 ml. All regressions are lines of best fit (method of least squares, Steel and Torrie, 1970). The slopes represent the secretion rates; the values below the lines give the average rates in ml/kg·h. Asterisks above the lines in hour 2 show significant changes from the original slope (dotted lines)(significance accepted at $p \leq 0.05$). Asterisks above the lines in hour 3 show significant changes from the treatment hour slope. Standard errors of means are omitted for clarity; however, they averaged for (A) 3.38 ± 0.47; (B) 2.69 ± 0.33; (C) 1.84 ± 0.17; (D) 2.34 ± 0.21; (E) 2.37 ± 0.37; (F) 2.43 ± 0.33; (G) 3.24 ± 0.41 (units as above); corresponding coefficients of variation were (A) 8.45 ± 1.14 %; (B) 6.66 ± 0.92 %; (C) 4.48 ± 0.48 %; (D) 5.65 ± 0.54 %; (E) 5.34 ± 0.75 %; (F) 5.58 ± 0.70 %; (G) 7.09 ± 0.76 %.

NOREPINEPHRINE
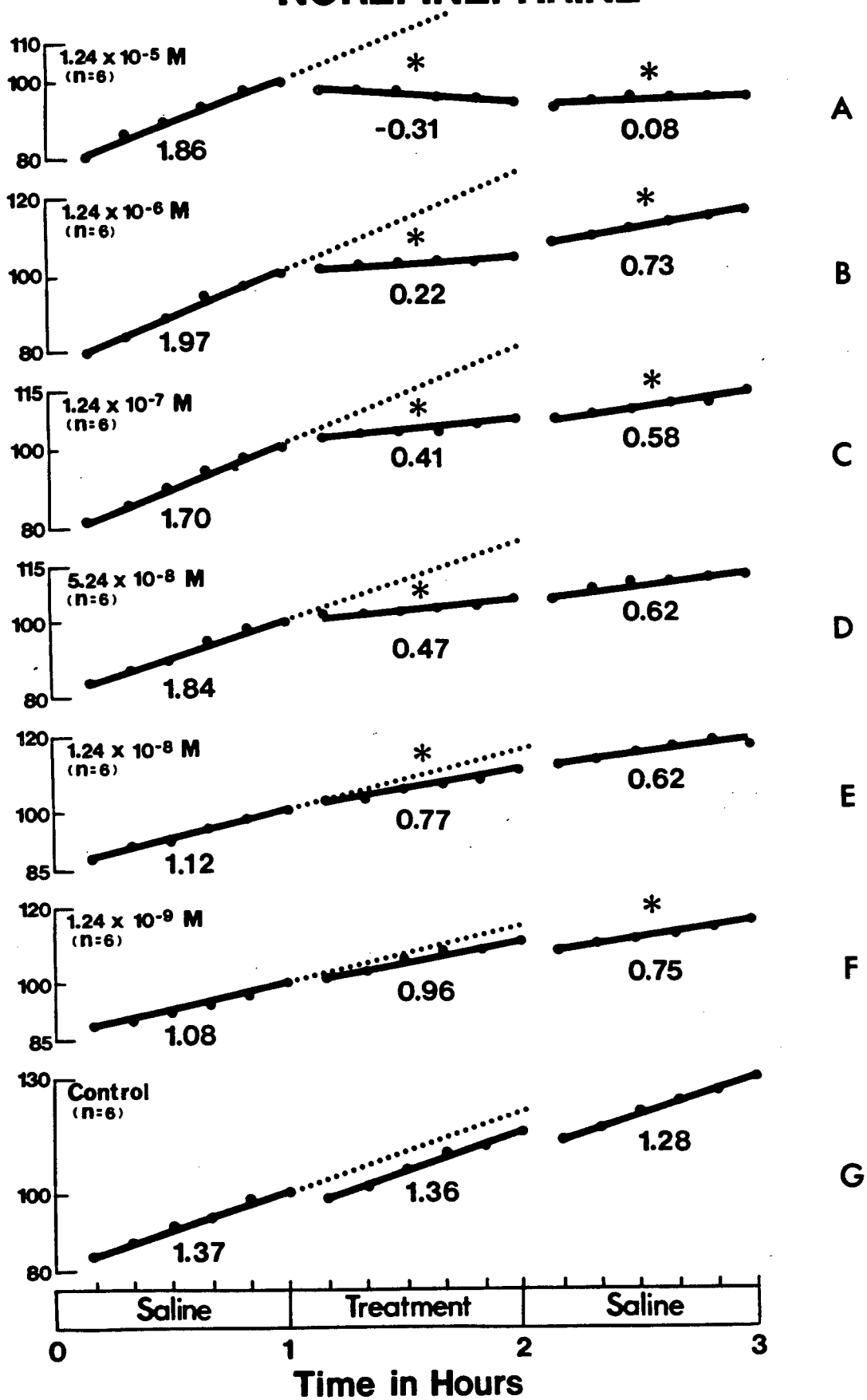
fluid requires the removal of sodium out of the lung. There was a significant fall in potassium secretion in every case (see Table D.7 in appendix D). Sodium secretion closely followed that of water during the third hour where there was a recovery, whereas potassium secretion remained low.

8.3.2 The Effect of NE Combined With Phentolamine on Lung Fluid Production (Figure 11B)

To test whether NE was working through alpha-adrenergic receptors, lungs were exposed to a mixture of NE at 1.24×10^{-6} M and phentolamine at 1.78×10^{-5} M during the treatment hour (n=6; 62-65 days gestation; average body weight of 96.18 ± 13.20 (SD) g). A concentration of phentolamine 10 times more concentrated than NE was used in order to ensure that alpha receptors were blocked to NE. This level of phentolamine is reported to antagonize alpha-mediated responses (Siegl and Orzechowski, 1981). The combined secretion rates for each hour were as follows: 0.77 ± 0.27 ml/kg·h (h1), 0.99 ± 0.18 ml/kg·h (h2; treatment hour) and 0.74 ± 0.15 ml/kg·h (h3). There was a rise in secretion rate with treatment, but this effect was not significant by regression analysis or ANOVA. In individual experiments, there were two lungs that showed significant rises in secretion rate with treatment, but the remaining four lungs showed no significant change in fluid production (see Table C.19 in appendix C). Clearly, phentolamine blocked the effect of NE; this demonstrates that NE is working through alpha-adrenergic receptors. Controls for phentolamine have been described above, and are illustrated in Figure 11C.

8.3.3 The Effect of Combined NE and Phentolamine on Ion Movement

The movements of sodium and potassium into the lung was unaffected by the joint treatment of NE and phentolamine. The movements of these ions with phentolamine treatment alone have been described above (see Table D.8 in appendix D)

Figure 11 **The effect of norepinephrine and phentolamine on fluid production by *in vitro* lungs from fetal guinea pigs.**

Based on 19 fetuses, 61-65 days gestation, average body weight of 104.26 ± 18.26 (SD) g. During the middle hour the preparations were transferred to saline containing one of the following: (A) NE 10^{-6} M (n=6); (B) NE 10^{-6} M/phentolamine 10^{-5} M (n=6); (C) phentolamine 10^{-5} M (n=7); (D) control saline. Ordinates: total volume of lung fluid expressed as a percentage of that present at the end of the first hour, just prior to treatment, where 100% was (A) 0.70 ± 0.03 ml; (B) 0.76 ± 0.09 ml; (C) 0.96 ± 0.22 ml; (D) 0.68 ± 0.02 ml. All regressions are lines of best fit (method of least squares, Steel and Torrie, 1970). The slopes represent the secretion rates; the values below the lines give the average rates in ml/kg·h. Asterisks above the lines in hour 2 show significant changes from the original slope (dotted lines)(significance accepted at $p \leq 0.05$). Asterisks above the lines in hour 3 show significant changes from the treatment hour slope. Standard errors of means are omitted for clarity; however, they averaged for (A) 2.69 ± 0.33; (B) 2.90 ± 0.31; (C) 4.17 ± 0.51; (D) 3.24 ± 0.41 (units as above); corresponding coefficients of variation were (A) 6.66 ± 0.92 %; (B) 6.58 ± 0.68 %; (C) 9.34 ± 0.99 %; (D) 7.09 ± 0.76 %.

Total Volume, Lung Secretion (%)

A (n=6)

Norepinephrine 1.24 × 10⁻⁶ M

120 100 70

1.97   * 0.22   * 0.73

B (n=6)

Norepinephrine 1.24 × 10⁻⁶ M
Phentolamine 1.78 × 10⁻⁵ M

120 100 80

0.77   0.99   0.74

C (n=7)

Phentolamine 1.78 × 10⁻⁵ M

130 100 80

2.20   2.20   1.19

D (n=6)

Control

130 100 80

1.37   1.36   1.28

Saline   Treatment   Saline

0   1   2   3

Time in Hours
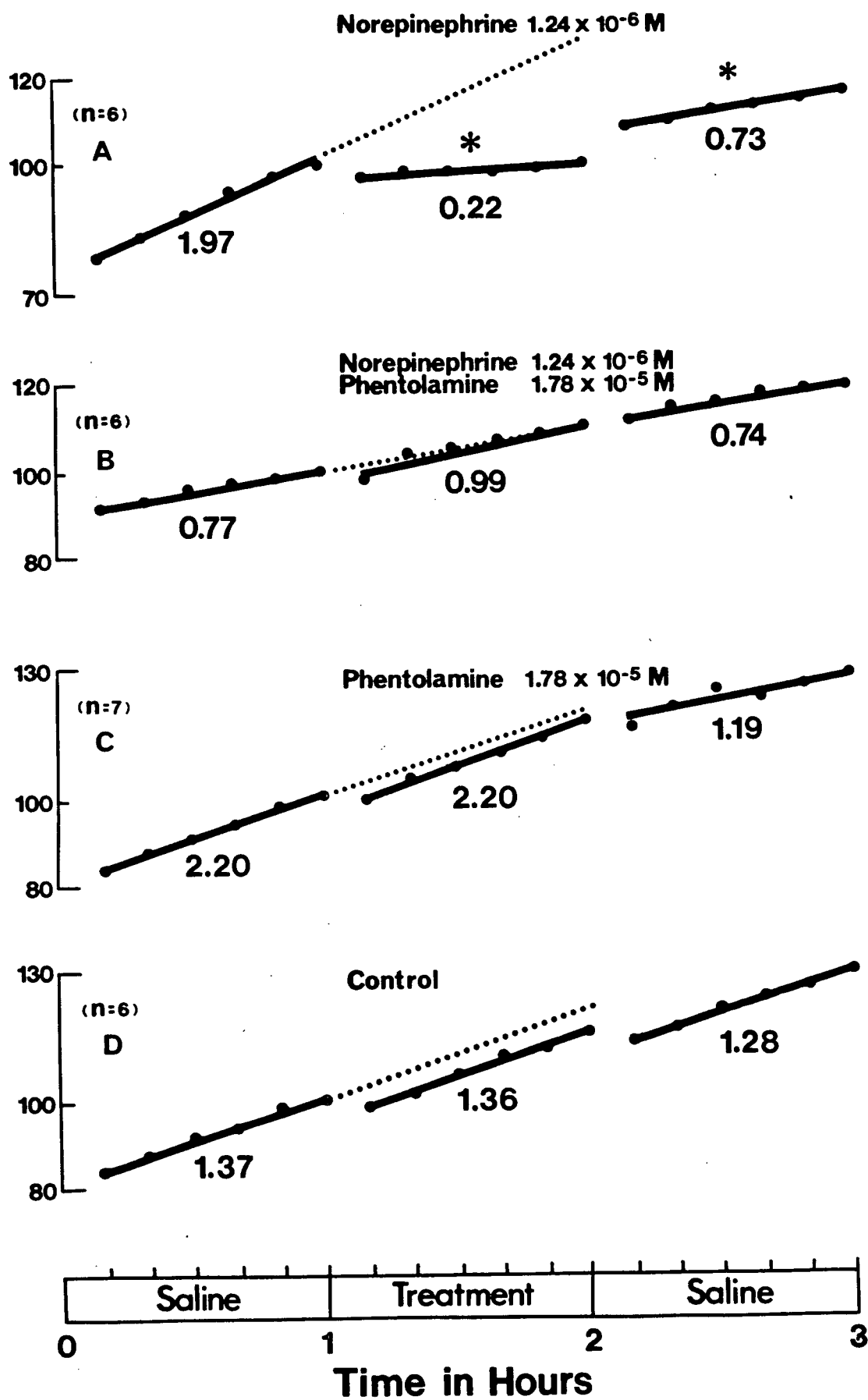
8.4 The Effect of Acetylcholine on Lung Fluid Production

The pleural surfaces of fetal lungs were treated with different concentrations of ACh during the treatment hour of experimentation. Four concentrations were used: 10^{-4} M, 10^{-5} M, 10^{-6} M and 10^{-8} M (see Figure 12). At the three top concentrations, maximum responses were observed in fetuses at or above 60 days gestation (0.88 of term), but a relationship between fetal weight and degree of response was not observed. A threshold dose for ACh was estimated between 10^{-6} M and 10^{-8} M.

ACh at 10^{-4} M (Figure 12A)

Lungs were exposed to ACh at 10^{-4} M during the treatment hour (n=6; 58-64 days gestation; average body weight of 96.03 ± 28.37 (SD) g). The combined secretion rates for each hour were as follows: 3.51 ± 0.85 ml/kg·h (h1), -0.83 ± 0.65 ml/kg·h (h2; treatment hour) and 2.11 ± 0.56 ml/kg·h (h3). The effect was maximal at this concentration and the average drop in secretion rate with treatment was 123.65 ± 11.16 % which was highly significant by both regression analysis (p<0.0005) and ANOVA (p<0.025). Individual experiments were consistent: four lungs showed immediate reabsorption with treatment (60-64 days gestation) and the two remaining lungs showed a significant fall (refer to Table C.21 in appendix C). There was a significant recovery of fluid secretion upon removal of treatment (p<0.005; regression analysis). Not only was the effect of ACh clear, but it was very unexpected as well.

ACh at 10^{-5} M (Figure 12B)

Lungs were exposed to ACh at 10^{-5} M during the treatment hour (n=6; 57-64 days gestation; average body weight of 92.53 ± 15.11 (SD) g). The combined secretion rates for each hour were as follows: 2.24 ± 0.52 ml/kg·h (h1), -0.11 ± 0.95 ml/kg·h (h2; treatment hour) and 1.50 ± 0.21 ml/kg·h (h3). Again, the effect was marked and the average fall in secretion rate with treatment

was 104.91 ± 21.16 % which was significant by both regression analysis (p<0.0005) and

ANOVA (p<0.005). Reabsorption occurred in three lungs with treatment (61-64 days gestation)

and there was a fall in secretion in the remaining three lungs (two of which were significant;

refer to Table C.22 in appendix C). There was a significant recovery of fluid secretion when the

lungs were returned to untreated conditions (p<0.0005; regression analysis).

ACh at 10^{-6} M (Figure 12C)

Lungs were exposed to ACh at 10^{-6} M (n=6; 63-67 days gestation; average body weight of 94.13

± 14.55 (SD) g). The combined secretion rates for each hour were as follows: 1.20 ± 0.48

ml/kg·h (h1), -0.43 ± 0.34 ml/kg·h (h2; treatment hour) and 0.64 ± 0.09 ml/kg·h (h3). The effect

of ACh at this concentration was still marked and the average drop in secretion rate with

treatment was 135.83 ± 37.71 % which was significant by regression analysis only (p<0.0005).

Analysis of variance proved to be not significant due to an inconsistency in one experiment.

Four lungs showed reabsorption of fluid (63-67 days gestation; refer to Table C.23 in appendix

C). There was a significant recovery of lung secretion upon removal of treatment (p<0.0005;

regression analysis).

ACh at 10^{-8} M (Figure 12D)

Lungs were exposed to ACh at 10^{-8} M during the treatment hour (n=6; 59-66 days gestation;

average body weight of 87.26 ± 8.09 (SD) g). The combined secretion rates for each hour were

as follows: 1.32 ± 0.42 ml/kg·h (h1), 1.52 ± 0.37 ml/kg·h (h2; treatment hour) and 1.01 ± 0.17

ml/kg·h (h3). There was a slight rise in secretion rate with treatment but this was not significant

by either regression analysis or ANOVA. This set of experiments were similar to control

experiments (Figure 12E), which demonstrates that the threshold dose for ACh is between 10^{-6}

M and 10^{-8} M. For individual data, refer to Table C.24 (appendix C).

Figure 12 **The effect of acetylcholine on fluid production by** *in vitro* **lungs from fetal guinea pigs.**

Based on 24 fetuses, 57-67 days gestation, average body weight of 92.49 ± 17.20 (SD) g. During the middle hour the preparations were transferred to saline containing one of the following concentrations of ACh: (A) ACh 10^{-4} M (n=6); (B) ACh 10^{-5} M (n=6); (C) ACh 10^{-6} M (n=6); (D) ACh 10^{-8} M (n=6); (E) control saline. Ordinates: total volume of lung fluid expressed as a percentage of that present at the end of the first hour, just prior to treatment, where 100% was (A) 0.98 ± 0.24 ml; (B) 0.77 ± 0.05 ml; (C) 0.70 ± 0.09 ml; (D) 0.82 ± 0.06 ml; (E) 0.68 ± 0.02 ml. All regressions are lines of best fit (method of least squares, Steel and Torrie, 1970). The slopes represent the secretion rates; the values below the lines give the average rates in ml/kg·h. Asterisks above the lines in hour 2 show significant changes from the original slope (dotted lines)(significance accepted at $p \leq 0.05$). Asterisks above the lines in hour 3 show significant changes from the treatment hour slope. Standard errors of means are ommitted for clarity; however, they averaged for (A) 2.61 ± 0.27; (B) 2.51 ± 0.25; (C) 2.23 ± 0.25; (D) 2.28 ± 0.28; (E) 3.24 ± 0.41 (units as above); corresponding coefficients of variation were; (A) 6.24 ± 0.68 %; (B) 6.22 ± 0.70 %; (C) 5.55 ± 0.65 %; (D) 5.36 ± 0.72 %; (E) 7.09 ± 0.76 %.
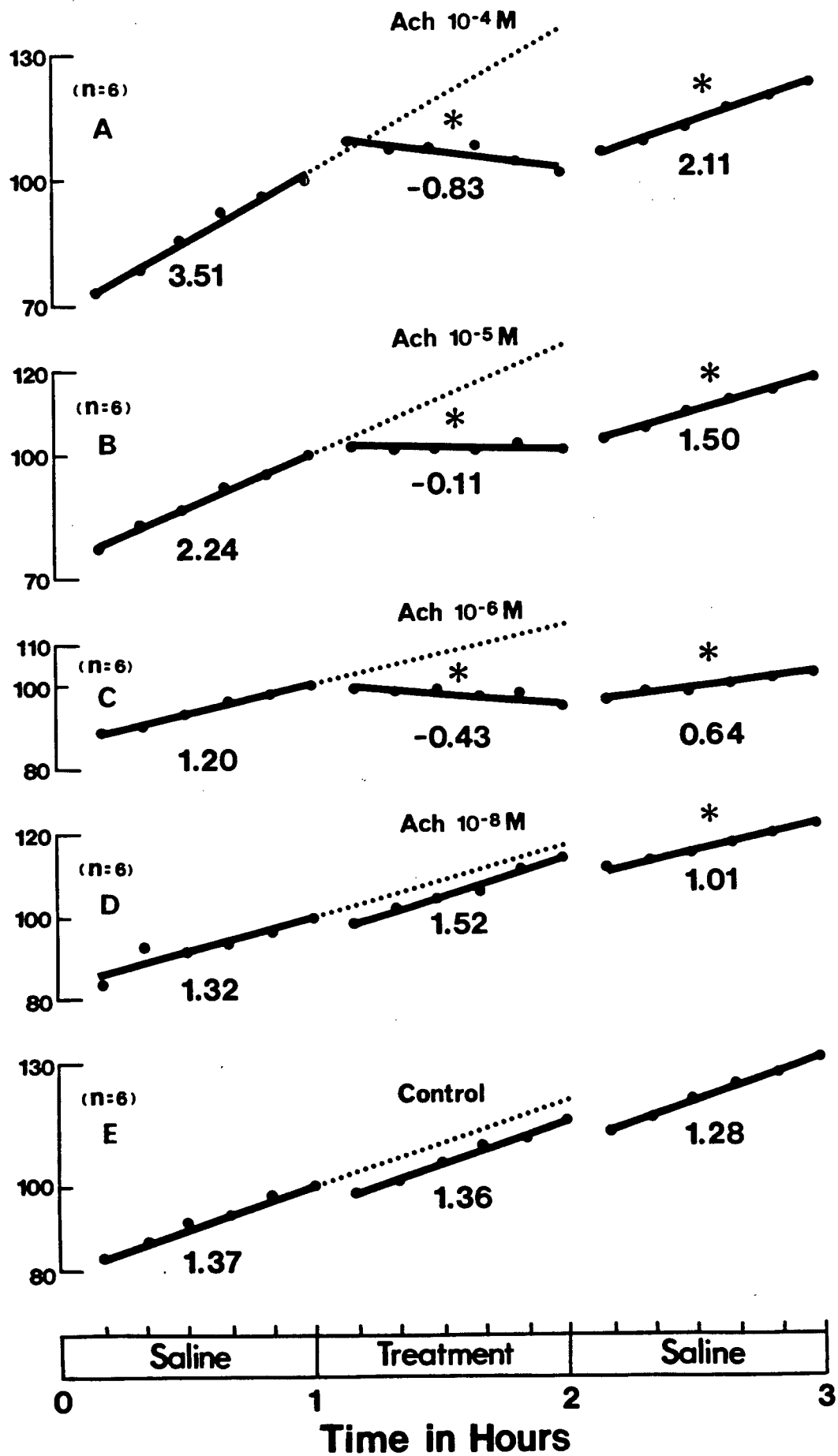
8.4.1   The Effect of Acetylcholine on Ion Movement

Sodium movement was not analyzed for the two top concentrations of ACh, however this ion closely followed that of water at ACh 10^{-6} M and at ACh 10^{-8} M. Potassium movement into the lung fell in every case, which resembled this ion's response to epinephrine and NE. In addition, sodium and potassium secretion into the lung at ACh 10^{-8} M was identical to that of control experiments, further substantiating that ACh at this concentration is below threshold. There was a recovery of sodium secretion upon removal of ACh 10^{-6} M, as there was with fluid secretion, and there was a recovery of potassium secretion observed only at ACh 10^{-4} M (see Table D.9 in appendix D).

8.4.2   The Effect of ACh Combined With Phentolamine on Lung Fluid Production (Figure 13B)

The effect of ACh on pulmonary secretion was unexpected and curious. Since the effect of ACh was similar to that of the catecholamines, it was postulated that ACh might be working indirectly through them. In order to test whether alpha-adrenergic receptors were mediating the effect of ACh, lungs were exposed to a mixture of ACh at 10^{-6} M and phentolamine at 1.78×10^{-5} M during the treatment hour (n=6; 63-64 days gestation; average body weight of 119.80 ± 28.94 (SD) g). As with the catecholamines, a concentration of phentolamine 10 times more concentrated than ACh was used to ensure that alpha-adrenergic receptors were blocked. The combined secretion rates for each hour were as follows: 0.85 ± 0.14 ml/kg·h (h1), 0.62 ± 0.27 yml/kg·h (h2; treatment hour) and 0.58 ± 0.20 ml/kg·h (h3). There was an average drop in secretion rate of 27.06 ± 17.89 % with treatment, but this was not significant by regression analysis or ANOVA. Clearly, phentolamine inhibited the effect of ACh when compared to the effect of ACh 10^{-6} M alone (Figure 13A), which raises the possibility that ACh could be working indirectly through catecholamines acting on alpha-adrenergic receptors. For individual data, refer to Table C.25 (appendix C). Controls for phentolamine have been described above and are illustrated in Figure 13C.

8.4.3   The Effect of Combined ACh and Phentolamine on Ion Movement

Both sodium and potassium secretion paralleled the movement of water, although the fall in secretion was significant with the ions. There was a significant recovery of sodium secretion during the third hour, but not of potassium. Phentolamine controls have been described above (see Table D.10 in appendix D).

Figure 13 **The effect of acetylcholine and phentolamine on fluid production by** *in vitro* **lungs from fetal guinea pigs.**

Based on 19 fetuses, 61-67 days gestation, average body weight of 105.69 ± 34.09 (SD) g. During the middle hour the preparations were transferred to saline containing one of the following: (A) ACh 10^{-6} M (n=6); (B) ACh 10^{-6} M/phentolamine 10^{-5} M (n=6); (C) phentolamine 10^{-5} M (n=7); (G) control saline. Ordinates: total volume of lung fluid expressed as a percentage of that present at the end of the first hour, just prior to treatment, where 100% was (A) 0.70 ± 0.09 ml; (B) 0.92 ± 0.16 ml; (C) 0.96 ± 0.22 ml; (D) 0.68 ± 0.02 ml. All regressions are lines of best fit (method of least squares, Steel and Torrie, 1970). The slopes represent the secretion rates; the values below the lines give the average rates in ml/kg·h. Asterisks above the lines in hour 2 show significant changes from the original slope (dotted lines)(significance accept at $p \leq 0.05$). Asterisks above the lines in hour 3 show significant changes from the treatment hour slope. Standard errors of means are omitted for clarity; however, they averaged for (A) 2.23 ± 0.25; (B) 3.28 ± 0.47; (C) 4.17 ± 0.51; (D) 3.24 ± 0.41 (units as above); corresponding coefficients of variation were (A) 5.55 ± 0.65 %; (B) 7.15 ± 0.93 %; (C) 9.34 ± 0.99 %; (D) 7.09 ± 0.76 %.
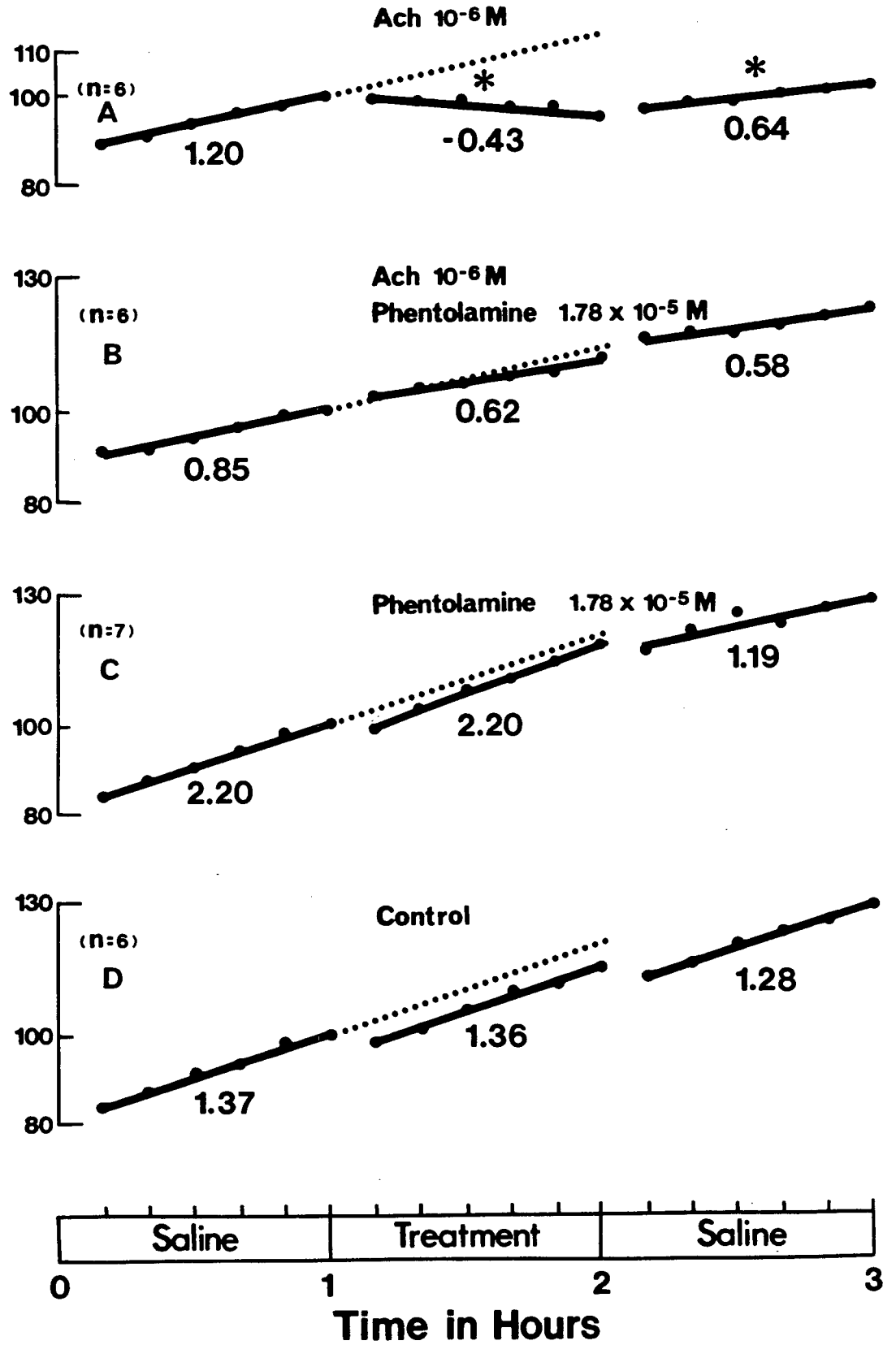
9. DISCUSSION

9.1 Fluid Production by Lungs from Fetal Guinea Pigs

The sheep has been a popular model used in fetal research. However, the intact ruminant may not be the optimum experimental model to use for the human condition, considering the differences in reproductive physiology (Martensson, 1984), and the presence of many important differences in glucose metabolism between the two species (Bergman, 1973). The guinea pig on the other hand is an excellent model in terms of human reproductive physiology (Martensson, 1984), and regulation of gluconeogenesis in both species is similar (Bergman, 1973). Surprisingly, the guinea pig is relatively unexplored in fetal research and, compared to the ruminant, it is possibly a superior model for human physiology and development.

Extensive studies on fetal lung fluid balance in the sheep and goat have provided much insight into the controls and mechanisms involved in fluid production or reabsorption. However, it is important to extend these findings to other species, especially in light of the species differences as noted above. In this study, late-term fetal guinea pig lungs were used to examine possible controls for lung fluid reabsorption observed at the time of birth. The guinea pig lung is similar to the human lung in several ways. The functional maturity of the guinea pig and human lungs at birth are comparable (Collins et al. 1986), and the developmental profile of the surfactant system in the guinea pig is similar to that in the human (Sosenko and Frank, 1987). The guinea pig may be a preferred model for human lung development (Stith and Das, 1982).

The average resting secretion rate for all lungs used in this study was 1.68 ± 1.44 (SD) ml/kg·h (n=171). Using a direct flow technique and capillary tube, Setnikar et al. (1959) reported lung fluid secretion rates from 1.3 to 6.7 ml/kg·h in intact fetal guinea pigs. Secretion rates of 1.81 ml/kg·h and 2·0 ml/kg·h were reported in fetal goats using acute preparations (Cassin and Perks, 1982; Perks and Cassin, 1989 respectively). This preparation involves removing the fetus by

Cesarean section and leaving the placental circulation intact, a procedure that could put the animal under considerable stress due to anesthesia and surgery. The chronically catheterized animal has been used extensively in fetal lung research; this technique has an advantage over acute preparations since the fetus is not under stress at the time of experimentation. Using this preparation, lung fluid secretion rates of 1.5 ± 0.27 (SD), 2.16 ± 0.02 (SD) and 3.2 ± 1.6 (SD) ml/kg.h have been reported in fetal sheep (Scarpelli et al. 1975; Normand et al. 1971; Platzker et al. 1975 respectively). The resting secretion rate of 1.68 ± 1.44 (SD) ml/kg·h obtained in this study compares favorably with those obtained in the above studies using intact preparations. This observation demonstrates the ability of isolated lungs to operate at levels similar to those found in intact lungs.

It may be argued that the lungs cannot be maintained for three hours in an *in vitro* situation. However, several observations demonstrate that the isolated lungs remain in good condition for the duration of the experiment. First, the isolated lungs maintain a constant fluid production over three hours in control experiments. Second, there is no evidence of an increase in the rate of potassium ion entry into the lung fluid with time, as would be expected if tissue damage were occurring. Finally, there is no unusual production of lactate in these preparations, since the rate of loss to the alveolar space and supporting saline shows no significant rise throughout incubation (Perks et al. 1990).

There is a clear decline in lung fluid production in ml/kg·h with advancing gestational age in fetal guinea pigs. This trend has been demonstrated in earlier experiments in this species (Perks et al. 1990). Fetal sheep show similar changes in fluid production rates through gestation. Kitterman et al. (1979) reported a decline in tracheal fluid production starting a week prior to delivery, and a decline in lung fluid production was observed starting earlier, from at least 128 days gestation (term is 147 days in this species)(Perks and Cassin, 1985a). There is a transient rise in lung fluid production observed at 65 days gestation (0.97 of gestation) in the guinea pig. This observation has been reported in fetal sheep; there is a transient rise in lung fluid production

observed from 137 to 139 days gestation (approximately 0.94 of gestation)(Perks and Cassin, 1985a). While the lung fluid production rates decline in ml/h as well as ml/kg·h in fetal sheep (Perks and Cassin, 1985a), this correlation was not observed in the fetal guinea pig. There was a lesser decline in lung fluid production in ml/h than there was in ml/kg·h, suggesting that, unlike the sheep, this reduction in lung fluid production with age in the guinea pig is partly compensated for by the increase in lung size. Saccular expansion may be associated with an increase in fetal lung fluid secretion (Collins et al. 1986). This hypothesis may explain the increase in lung fluid production at 65 days gestation, since lung growth in the guinea pig is characterized by a high rate of saccular expansion during this time (Collins et al. 1986; Lechner and Banchero, 1982).

9.2   The Effect of Epinephrine on Lung Fluid Production

The sympathoadrenal system is activated in the fetus at birth, even in uncomplicated deliveries (Lagercrantz and Bistoletti, 1973; Lagercrantz and Slotkin, 1986). This response is due to the stress of the birth process, including the squeezing and squashing of the fetus in the birth canal (Lagercrantz and Bistoletti, 1973); these changes produce intermittent hypoxemia and acidemia during delivery (Irestedt et al. 1982). The surge of catecholamines during delivery is thought to have functional importance in neonatal adaption, including an increase in cardiac performance, particularly during hypoxemia (Lewis et al. 1982), the mobilization of glucose and free fatty acids (Comline and Silver, 1972), pulmonary surfactant release (Lawson et al. 1978), and pulmonary fluid reabsorption (Walters and Olver, 1978).

Although plasma epinephrine levels have been measured before delivery in the fetal guinea pig, they have not been measured during delivery. In the human neonate, epinephrine can reach levels of 2.31×10^{-8} M (23.14 nmol/l) in the limb blood flow during labor (Faxelius et al. 1984). During the first stage of labor, levels of epinephrine at 1.92×10^{-8} M (19.2 nmol/l) have been recorded in fetal scalp blood; by the second stage, the plasma catecholamine levels in the

umbilical artery are approximately twofold higher (Bistoletti et al. 1983). In fetal sheep,

epinephrine reaches levels 100 times prelabor values during delivery (Brown et al. 1981).

Prelabor values have been reported at $2.02 \pm 0.87 \times 10^{-9}$ M (0.37 ± 0.16 ng/ml; Jones, 1980).

In this study, epinephrine at 10^{-7} M produced a maximum response (i.e. falls in fluid production

or fluid reabsorption) by the fetal guinea pig lung, and concentrations in the range of 1 to 5 x 10^{-8} M produced strong responses. This level of epinephrine is comparable to those measured

during delivery in both the human and the sheep. The threshold value for epinephrine was

calculated at 1.78×10^{-11} M. The plasma levels of epinephrine measured in the late-term fetal

guinea pig are $1.53 \pm 0.71 \times 10^{-8}$ M (2.8 ± 1.3 ng/ml; Jones, 1980). These values are high

compared with the threshold value obtained in this study. However, Jones (1980) points out that

the values measured in his study may be significantly overestimated due to the stress of

experimentation and anesthesia.

The effect of epinephrine on lung fluid production has been extensively studied in the fetal sheep

and goat. Epinephrine can significantly slow lung fluid production or cause reabsorption in the

fetal lamb, especially in the older fetuses (Walters and Olver, 1978). The lamb is capable of

responding to epinephrine at 122 days gestation, and the reabsorptive effect of epinephrine

begins at approximately 130 days gestation (Walters and Olver, 1978). The lung is also capable

of recovering to approximate pretreatment fluid production rates upon the cessation of

epinephrine infusion (Walters and Olver, 1978). These results have been supported by Brown et

al. (1981; 1983), Olver et al. (1981; 1986) and Walters et al. (1982). Epinephrine causes a

reduction in fluid production or reabsorption in fetal goats, and recovery is observed after

treatment (Perks and Cassin, 1982; 1989). In contrast to the fetal sheep, fetal goats from 118

days gestation to term show no age-related response to epinephrine (Perks and Cassin, 1982;

1989). Instead, falls in fluid production occur at lower doses, and reabsorption is observed at

higher doses (Perks and Cassin, 1989). Epinephrine at 10^{-7} M reduced fluid production or

initiated reabsorption in fetal guinea pig lungs. The responses to epinephrine declined with

lower concentrations, and there was no observed relationship between response and gestational age; these observations are similar to those reported in goats. However, recovery from treatment was variable, and concentrations of epinephrine higher than 10^{-7} M were not as effective, which was unexpected.

Based on the studies performed on fetal sheep, it has been accepted that epinephrine is responsible for the rapid reabsorption of lung fluid observed in the neonate at birth. However, there are several observations that question the fundamental role of epinephrine in causing lung fluid to reabsorb during this time. Bland et al. (1982) did not find a significant difference between fetal plasma catecholamines before birth and during labor in lambs. In addition, the slowing of secretion rate before birth in lambs is not associated with an increase in fetal plasma catecholamine concentrations (Brown et al. 1981). Catecholamine levels typically start to decrease about 30 minutes after delivery and return to resting levels within 2 hours (Bistoletti et al. 1983); however, the lung is not cleared of fluid for at least 4 to 6 hours in the lamb (Humphreys et al. 1967), and this process can take up to several days in the newborn rabbit (Adams et al. 1971). It is unlikely that epinephrine is the sole factor responsible for lung fluid reabsorption in the neonate.

It is possible that large reabsorptions are not observed in the isolated lungs due to the absence of colloid osmotic forces in this preparation. However, changes in concentration of colloids in the alveolar instillate hardly affected fluid absorption in the anesthetized, intact fetal sheep (Matthay et al. 1982). Fluid transport out of the airspaces involves forces other than hydrostatic pressure or protein concentration differences between fluid and plasma in the adult, isolated, fluid-filled rabbit lung (Effros et al. 1987).

The effect of epinephrine was not blocked by propranolol, and isoproterenol had no effect on fetal guinea pig lungs. This suggests that epinephrine is not working through beta-adrenergic receptors. The effect of epinephrine was blocked by phentolamine, suggesting that epinephrine

is working through alpha-adrenergic receptors in the fetal guinea pig lung. Alpha-adrenergic receptors can mediate the effects of both epinephrine and NE (Lefkowitz and Caron, 1987), and alpha$_1$ receptors have been identified in the adult guinea pig lung (Barnes et al. 1979). These findings are different from those obtained in fetal sheep studies in which propranolol blocked the effect of epinephrine, and isoproterenol produced falls in lung fluid secretion or initiated reabsorptions (Walters and Olver, 1978). It appears that in the lamb, the effect of epinephrine is mediated via beta-adrenergic receptors.

The conclusion that epinephrine is affecting lung fluid production via alpha-adrenergic receptors in the fetal guinea pig lung and beta-adrenergic receptors in the fetal sheep lung commands interpretation. There are two possible explanations. First, it is possible that there are developmental differences between these two species. Several lines of evidence indicate immature tissues may have different adrenergic responses than mature tissues. Adenylate cyclase of tadpole but not frog erythrocytes was stimulated by isoproterenol (Rosen and Rosen, 1968). Mennon and co-workers (1972) showed that adenylate cyclase activity could be stimulated by sodium flouride in all human adult and fetal tissues examined. In contrast to amphibian erythrocytes, isoproterenol stimulated adenylate cyclase activity in all the adult tissues, but was only active in some fetal tissues; membranes prepared from the fetal heart, liver, and adrenal were capable of responding to isoproterenol, whereas the fetal lung, kidney, testis and brain were not (Mennon et al. 1972). Isoproterenol does not stimulate the fetal sheep lung before 122 days gestation (Walters and Olver, 1978). The efficacy of propranolol in some fetal tissues has not been fully determined and could be potentially quite different from that in the adult (Hooper and Harding, 1989). Myocardial membranes in the sheep fetus demonstrate a supersensitivity to exogenous NE when compared to adult membranes (Friedman and Kirkpatrick, 1977); this is also true in dogs (Buchthal et al. 1987) and piglets (Buckley et al. 1979). There is evidence that there are two classes of alpha$_1$ receptors in the canine heart: one class has a high affinity and limited capacity, and the other has a low affinity and greater capacity (Buchthal et al. 1987). The activity of the low affinity site is greater in the canine fetus

and neonate than in the adult (Buchthal et al. 1987). Physiological events in the immature fetus can be mediated by receptor systems different from those in the mature fetus and adult. For example, glycogenolysis in the liver is mediated by alpha-adrenergic receptors in the mature fetus and the adult (El-Refai et al. 1979), but by beta-adrenergic mechanisms in the immature fetus (Plas and Nuez, 1975; Blair et al. 1979). The maturational process in the liver is accompanied by a loss of functional beta-receptors and the gradual appearance of the alpha-adrenergic receptor system (Blair et al. 1979). It is possible that the tissues examined in this study were immature with respect to those in the late-term lamb fetus, and possible developmental distinctions between the two could give rise to different interpretations. However, this explanation is highly unlikely, as discussed below.

The developmental profile of adrenergic receptors has not been studied in the fetal guinea pig, although several observations indicate that the guinea pig fetus is physically and functionally mature in many respects before birth. At this time, guinea pigs have full coats of hair, open eyes, and the ability to regulate body temperature and to feed independently (Wagner and Manning, 1976). The lungs of newborn guinea pigs also show physical, physiologic and morphologic maturity (Sosenko and Frank, 1987; Collins et al. 1986). Sympathetic innervation is complete at the time of birth in guinea pigs (Coupland et al. 1982). Both $beta_1$ and $beta_2$ sub-type receptors appear in the fetal rabbit lung by of 0.81 gestation (Whitsett et al. 1981b) and in the fetal rat lung by 0.82 of gestation (Whitsett et al. 1982). Beta-adrenergic receptors (both sub-types) are found in the adult guinea pig lung (Engel, 1981). Based on these observations, it is assumed that receptor morphology is mature in the late-term fetal guinea pig. Fetal sheep respond to both alpha- and beta-adrenergic stimulation by mid-gestation (Barrett et al. 1972), and the sympathetic nervous system is considered to be complete at birth (Born et al. 1956). In addition, competent beta-adrenergic receptors are found in the late-term fetal sheep (Warburton et al. 1987c). It is likely that the late-term guinea pig fetus and late-term lamb fetus have comparable sympathetic nervous system and adrenergic receptor maturity.

Species differences probably account for the alpha-adrenergic mediation of pulmonary fluid movement in the guinea pig. Species vary in this regard: beta receptor blockade did not significantly change the lung fluid content in the fetal rabbit, either at or after birth (McDonald et al. 1986). Terbutaline, a beta$_2$-agonist, did not effect pulmonary fluid reabsorption in the the isolated, fluid-filled, adult rabbit lung (Effros et al. 1987). Innervation of the lung is species specific (Richardson, 1979). Pulmonary smooth muscle innervation may be similar in the human, guinea pig and sheep, but innervation of the pulmonary epithelium is uncertain and could vary depending on the species (Richardson, 1979). Adrenergic receptor populations in the pulmonary epithelium are species specific; beta$_1$: beta$_2$ ratios are 30:70 in the human (Engel, 1981), 22:78 in the guinea pig (Engel, 1981), 25:75 in the rat (Whitsett et al. 1981a), and 60:40 in the rabbit (Whitsett et al. 1981b). Other tissues show species differences with respect to receptor populations and physiological responses. For example, alpha$_1$-adrenergic receptors in canine myocardium are fewer in number and respond differently to exogenous epinephrine and prazosin than do alpha$_1$ receptors in rabbit and rat myocardial membranes (Mukherjee et al. 1983). Furthermore, glycogenolysis in the liver is stimulated by isoproterenol in the dog but not in the rat, despite the fact that epinephrine induces this response in both species (Hornbrook, 1970). These differences should be considered in comparing species' responses to adrenergic agonists and antagonists.

The mechanism of epinephrine-induced reabsorption has been carefully worked out in the fetal sheep. The thin-walled acini, which comprise 99% of the internal lung surface, provide the sites of ion transport and water movement (Olver et al. 1986). A Na^+/K^+ ATP-dependent pump at the basolateral surface of the pulmonary epithelium removes Na^+ from the epithelial cell into the plasma (Olver et al. 1986). Apical membranes are impermeable to Na^+ in the cell-to lumen direction, but Na^+ can enter the lung lumen via a paracellular route (Olver et al. 1986). This system is based on the model described by Gatzy (1983)(refer to section 5.2.1 in the introduction). In the mature fetus, stimulation of beta receptors with epinephrine induces cAMP formation, which in turn opens Na^+ channels in the apical membrane (Walters et al. 1990).

Sodium exits the lung lumen through these channels, then water follows transcellularly, thus

fluid reabsorption. The precise location of the rate-limiting step between the generation of

cAMP and the activation of Na^+ channels remains uncertain. The activity of cAMP-dependent

protein kinase is a possibility (Walters et al. 1990). Activation of cAMP-dependent protein

kinase C plays an important role in mediating effect of $beta_2$-agonists in the regulation of fetal

lung liquid and of surfactant production in fetal sheep (Warburton et al. 1987b). In the post-natal

lung, concentrations of epinephrine at resting conditions are sufficient to produce continuous

stimulation of absorption of lung liquid (Walters and Strang, 1987). Maturation of the

reabsorptive response could be due to several factors including the maturation of adrenergic

receptors, Na^+ channels, NaCl co-transport, and the Na^+ pump itself (Olver et al. 1986).

In general, reabsorption of fluid with epinephrine treatment was accompanied by reabsorption of

sodium. This suggests that, like the sheep, absorption of fluid from the guinea pig lung relies on

Na^+ transport out of the lung lumen. Water reabsorption involving Na^+ movement out of the

fetal lung through Na^+ channels has been demonstrated in the guinea pig. In the late-term fetal

guinea pig, amiloride blocked the reabsorptive effect of lung expansion at levels similar to those

of the first breath (Garrad, unpublished thesis), and also blocked the reabsorptive effect of a $2^{\circ}C$

temperature drop (Garrad, unpublished thesis). These observations suggest that although

epinephrine acts on different adrenergic receptors in the guinea pig and sheep, the end result in

both systems is the same, i.e. movement of sodium out of the lung with fluid reabsorption.

Since phentolamine is a non-selective alpha antagonist, it is not conclusive as to whether

epinephrine works through $alpha_1$ or $alpha_2$ receptors in the guinea pig lung. The $alpha_1$

receptors are implicated because first, pulmonary fluid reabsorption involves cAMP as a second

messenger in the sheep fetus (Walters et al. 1990) and second, $alpha_2$ receptors have an

inhibitory action on adenylate cyclase (Lefkowitz and Caron, 1987). $Alpha_1$ receptors exist in

the guinea pig lung (Barnes et al. 1979). Although $alpha_1$ receptor activation operates through

the polyinositol system in many tissues, it is also known to activate the production of cAMP via

a Ca^{2+}-sensitive adenylate cyclase (Fain and Garcia-Sainz, 1980). This response exists in

several systems including alpha$_1$ stimulation of adenylate cyclase in the rat erythrocyte and

hepatocyte (Sheppard and Burghardt, 1970; Hornbrook, 1970 respectively). It is likely that this

system exists in the guinea pig; propranolol and phentolamine block the effect of epinephrine-

induced cAMP production in the adult lung of this species (Palmer, 1971), suggesting that

epinephrine can induce cAMP formation through alpha and beta receptors in the lung. The late-

term fetal guinea pig lung is a rich source of cAMP-dependent protein kinases (Kuo, 1975) and

cAMP stimulates fluid reabsorption in the fetal guinea pig lung (Kindler, Ziabakhsh and Perks,

unpublished observations).

The effects of epinephrine at 10^{-6} M and 10^{-5} M are confusing. Pulmonary alpha-adrenergic

receptor density increases with advancing gestational age in the rat (Whitsett et al. 1982). It is

speculated that epinephrine at such high doses interacts with alpha$_2$ receptors as well as alpha$_1$

receptors. If this is the case, then alpha$_2$ receptors could be responsible for the decreasing

responses observed with increasing concentrations of epinephrine, due to their inhibitory action

on cAMP production (Lefkowitz and Caron, 1987). The adult guinea pig trachea contains

mainly alpha$_2$ receptors (Takayanagi et al. 1990), but the existence of these receptors in the fetal

guinea pig lung has not been examined. Alpha$_2$ receptors have been identified in the neonatal

rat lung (Latifpour et al. 1982; Bylund and Ray-Prenger, 1989).

9.3   The Effect of Epinephrine and AVP on Lung Fluid Production

The plasma concentration of arginine vasopressin (AVP) is markedly elevated in human and

lamb fetuses in association with hypoxemia, asphyxia, and hemorrhage (Alexander et al. 1972;

DeVane et al. 1982; Rose et al. 1983). Thus, a role for AVP in the fetal response to intrauterine

stress has been suggested. This hormone is also secreted in high amounts by the fetus at birth,

especially during vaginal delivery (Parboosingh et al. 1982). Intermittent fetal hypoxia and/or

asphyxia during labor is associated with elevated AVP levels (Pohjavuori et al. 1980; Stark et al.

1977), as well as elevated catecholamine levels (Irestedt et al. 1982). The role of AVP in fetal homeostasis at birth remains unclear. A rise in fetal arterial pressure (Wiriyathian et al. 1983) and fetal lung fluid clearance (Perks and Cassin, 1985a; 1989) have been demonstrated with AVP treatment.

Plasma AVP levels have not been measured in the guinea pig neonate during delivery. In the human neonate, levels at 0.55 mU/ml and 0.57 mU/ml have been measured in the cord blood during normal deliveries (Chard et al. 1971; Pohjavuori and Fyrquist, 1980); the maximal concentration was 1.3 mU/ml (Pohjavuori, 1983). Levels of AVP at 0.25 $\pm$ 0.52 mU/ml have been found in sheep fetuses at birth (Stark et al. 1977). AVP at 0.6 mU/ml has been used in this study to test the effect of this hormone on lung fluid production in the fetal guinea pig. This dose is within the range of plasma AVP reported at the time of birth in the human and sheep.

AVP reduced fluid production in the chronically catheterized fetal lamb (Perks and Cassin, 1985a; Wallace et al. 1990). The fetal pulmonary response to AVP was present by 135 days gestation in this species, and the response increased with increasing gestational age (Perks and Cassin, 1985a; Wallace et al. 1990). There was a 40.8% inhibition of fetal lung fluid production between days 136 and 140 of gestation, and older fetuses showed a 78.4% inhibition (Wallace et al. 1990). In the goat, AVP decreased the rate of lung fluid secretion, or caused reabsorption (Perks and Cassin, 1982; 1989). This response was present by 131 days gestation (Perks and Cassin, 1982; 1989). In both the sheep and goat, the effect of AVP was slow and persisted even after infusions had stopped (Perks and Cassin, 1982; 1985a; 1989). The findings in this study collaborate these observations since AVP caused a significant fall in lung fluid production, which persisted after AVP treatment. However, there were no age-related responses observed.

There is evidence that the fetal responses to stress are the result of AVP and catecholamines working together. The hemodynamic response of the late-term fetal lamb to asphyxia is accompanied by an increase in the blood flow to the brain, heart and adrenals, no change in

blood flow to the placenta, and a decrease in blood flow to the lungs, kidney, spleen and gut (Reuss et al. 1982). Reuss and co-workers (1982) observed that these responses persisted with induced hypoxia, despite alpha-adrenergic blockade. The maximum hemodynamic responses occur within five minutes of induced moderate fetal hypoxemia (Wiriyathian et al. 1983), although maximum AVP levels were not achieved until after the hemodynamic responses had stabilized (Wiriyathian et al. 1983). These observations suggest that catecholamines initiate fetal responses to hypoxemic stress, and AVP sustains these responses. Fetal responses to hemorrhage are blocked by AVP-antagonists, suggesting that AVP is important for catecholamine responses to volume depletion in the fetus (Rose et al. 1983). Catecholamines and AVP may have combined effects on fetal lung fluid balance. Hooper and Harding (1989) observed that pulmonary fluid production can be inhibited by either induced asphyxia or epinephrine infusion in the late-term fetal lamb. Because beta-blockade had no effect on asphyxia-induced inhibition but antagonized the epinephrine-induced inhibition, these authors concluded that reductions of fetal lung fluid production associated with asphyxia does not solely result from catecholamine stimulation of pulmonary beta-receptors. It was suggested that AVP, as well as catecholamines, are involved in this response. The inhibitions of lung fluid by epinephrine and AVP were additive and synergistic in the acute, late-term fetal goat (Perks and Cassin, 1989). In contrast, joint AVP/epinephrine treatment of fetal guinea pig lungs were not synergistic. However, AVP alone caused reductions or reabsorptions in fetal lung fluid production, and these effects persisted after treatment. It appears that in the sheep, goat and guinea pig, AVP is involved in the continued reabsorption of fetal pulmonary fluid after birth.

The mechanism(s) by which AVP exerts its effects cannot be ascertained in this study. The mechanism of action of AVP on fetal pulmonary fluid movement is uncertain, but a Na^+/K^+ pump appears to be involved (Perks and Cassin, 1982). Wallace and co-workers (1990) suggest that cAMP acts as a second messenger in response to pulmonary V_2 receptor stimulation. Increased intracellular cAMP may activate Na^+ channels located on the apical membranes of the pulmonary epithelium, which in turn would allow Na^+ movement out of the lung lumen. This

would allow water reabsorption, due to the resulting change in the osmotic gradient. This system is identical to that proposed with pulmonary beta-receptor stimulation in the fetal lamb (Walters et al. 1990). Alternatively, these authors suggest that the stimulation of V_2 receptors could ultimately induce the insertion of specific water channels in the apical membranes of the pulmonary epithelium, in a system not unlike the mammalian kidney (Guillon et al. 1982). These channels would increase the permeability of the lung to water and, together with osmotic forces, the movement of water would favor reabsorption. In both hypotheses cAMP is the second messenger involved. It is clear that further investigations are needed to elucidate the mechanism of action of AVP.

9.4   The Effect of Norepinephrine on Lung Fluid Production

The effect of NE on fetal pulmonary fluid has not been studied in great detail, a surprising fact considering it is secreted in high amounts by the fetus in response to the stress of delivery (Bistoletti et al. 1983; Brown et al. 1981), and plays a relatively more important role in the fetus than in the adult (Friedman and Kirkpatrick, 1977; Jones and Robinson, 1975). The fetal heart is supersensitive to exogenous NE (Friedman and Kirkpatrick, 1977), and in this regard NE is thought to enhance cardiovascular tone in response to hypoxemia experienced during labor (Jones and Robinson, 1975). The adrenal medulla stores predominantly NE until innervation of this tissue is complete (Comline and Silver, 1961), and there is a substantial contribution of this catecholamine from paraganglia at all stages of fetal development (Jones, 1980). Extra-adrenal chromaffin tissue, especially paraganglia, is particularly evident in fetal life in most species (Coupland, 1952). Paraganglia are prominent in the human, not prominent but present in the rat, and absent in the sheep (Lagercrantz and Slotkin, 1986; Cohen et al. 1982).

There are species differences with respect to the development of innervation of the adrenal medulla. The adrenal medulla is not innervated in the newborn rat (Lagercrantz and Slotkin, 1986), incomplete in the human neonate (Lagercrantz and Slotkin, 1986), and complete in the

neonatal sheep and guinea pig (Comline and Silver, 1961; Coupland et al. 1982 respectively). Catecholamine release proceeds by a non-neurogenic mechanism in the immature adrenal medulla, a system which is not present in mature animals (Slotkin and Seidler, 1988). NE is the predominant catecholamine stored in the adrenal medulla of the neonatal lamb (Comline and Silver, 1961), guinea pig (Coupland et al. 1982) and rabbit (Coupland et al. 1982). Extra-adrenal chromaffin tissue synthesizes and stores mainly NE in all species, yet innervation of this tissue is species specific (Coupland et al. 1982). In the rabbit, mouse and dog, abdominal extra-adrenal chromaffin bodies are not innervated even though they may persist throughout the lifespan of the animal (Coupland and Weakley, 1968; Mascorro and Yates, 1971; 1977 respectively). Extra-adrenal chromaffin tissue is innervated in the guinea pig, although there are fewer cholinergic nerve endings per unit cell area than in the adrenal medulla (Coupland et al. 1982). The main extra-adrenal bodies, which are lying adjacent to and usually posterior to the left renal vein, can be readily identified in the guinea pig during the first two weeks of life (Coupland et al. 1982).

Although plasma NE concentrations have been measured in the late-term guinea pig fetus, they have not been measured during labor. In the human, levels of NE at 9.97×10^{-8} M (99.7 nmol/l) have been recorded in fetal scalp blood during the first stage of labor (Bistoletti et al. 1983). The plasma catecholamine levels were found to be twofold higher by the second stage of labor, and in cases of fetal acidemia (blood pH<7.26) were significantly elevated (Bistoletti et al. 1983). NE reached levels of 1.31×10^{-7} M (131.12 nmol/l) in the limb blood of the human neonate during vaginal delivery (Faxelius et al. 1984). In the sheep, NE reached approximately four times prelabor values (Brown et al. 1981). NE concentrations were $3.13 \pm 1.42 \times 10^{-9}$ M (0.53 $\pm$ 0.24 ng/ml) in the umbilical vein of the late-term fetal sheep (Jones, 1980), and rose to 2.97 $\pm$ 0.62×10^{-7} M (50.34 $\pm$ 10.52 ng/ml) during hypoxemia (Cohen et al. 1982).

In the late-term fetal guinea pig, reabsorptions occurred in all but one case, which showed an 86% fall in production, with NE at 1.24×10^{-5} M. The effect was immediate, consistent, and recovery was generally observed. Significant falls in lung fluid production were observed even

at the lowest concentration used in this study (1.24×10^{-9} M), and a reabsorptive response was observed with NE at 1.24×10^{-8} M. NE at the top dose level is high from a physiological standpoint, but this catecholamine significantly affected fetal pulmonary fluid at levels recorded during delivery in the human and sheep. The threshold value for NE, 3.16×10^{-10} M, was comparable to resting levels of NE in the late-term guinea pig fetus (Jones, 1980). It is also comparable to the threshold value of epinephrine obtained in this study, indicating that the two catecholamines have similar potencies. Finally, there was a relationship between fetal weight and response to NE; fetuses below the weight of 78.0 g did not respond to this catecholamine. A correlation between weight and NE sensitivity has been observed in another species. Fetal goats below the weights of 1.58 kg did not respond to AVP infusions, and thereafter the fetal weight and absolute reduction in fetal pulmonary fluid were linearly related (Perks and Cassin, 1989).

The effects of NE on the fetal sheep lung are conflicting. Brown et al. (1981) observed no correlation between lung liquid absorption and NE concentrations during labor. Infusion rates of 0.18 up to 3.0 ug/min NE into the fetal jugular vein had no detectable effect on lung fluid secretion in the chronically catheterized late-term fetal lamb (Walters and Olver, 1978). However, in another study on late-term fetal lambs (also chronic preparations), infusions of NE at 3 ug/min into the jugular vein reduced lung fluid flow by 45% (Higuchi et al. 1987). Pretreatment with propranolol did not affect the results, so these authors concluded that NE decreases lung fluid net flow through alpha-adrenergic receptors (Higuchi et al. 1987).

Phentolamine blocked the effect of NE in fetal guinea pig lungs. This suggests that the effect of NE on fluid production is mediated via alpha-adrenergic receptors. Guinea pig alpha-adrenergic receptors mediate the effects of epinephrine and NE on fetal lung fluid production, but in the sheep, alpha- and beta-adrenergic receptors mediate the effects of NE and epinephrine on lung fluid production respectively.

In the fetal guinea pig lung, reabsorption of fetal pulmonary fluid with both NE and epinephrine treatment was linked to reabsorption of sodium. Therefore, the stimulation of pulmonary alpha receptors in guinea pigs affects fetal lung fluid production via sodium transport. As indicated above, the intracellular messenger(s) involved with $alpha_1$ stimulation in the fetal guinea pig lung is unknown. It is speculated that cAMP is implicated, since its production can be induced with alpha and beta adrenergic stimulation in the adult guinea pig lung (Palmer, 1971). Furthermore, cAMP can stimulate fluid reabsorption (Kindler, Ziabakhsh and Perks, unpublished observations). If this is the case, then cAMP could ultimately induce the opening of pre-existing sodium channels in the apical membrane of pulmonary epithelial cells, thus allowing Na^+ movement out of the lung. This system is operative in the fetal sheep (Walters et al. 1990).

Whether alpha and beta receptors exist on the same cell is uncertain. In the fetal rat, pulmonary $alpha_1$ receptors increase during late gestation and postnatally, reaching maximum concentrations by 15 days of postnatal age (Whitsett et al. 1982). These ontogenic changes contrast sharply with those of the pulmonary beta receptors in this species, which continue to increase between days 15 and 28 of postnatal age (Whitsett et al. 1981a). These findings suggest that alpha- and beta-adrenergic receptors are independently regulated in the lung and that they may exist on different cell populations. However, it should be noted that only beta receptor stimulation induced cAMP production in the rat lung (Palmer, 1971), while cAMP production can be induced by both alpha and beta receptor stimulation in the guinea pig lung (Palmer, 1971).

9.5   The Effect of Acetylcholine on Lung Fluid Production

It is widely acknowledged that control of airway smooth muscle tension in most species is dominated by excitatory neural inputs transmitted via cholinergic pathways (Coburn, 1987). The vagal cholinergic motor neurons innervate the smooth muscle cells of the tracheo-bronchial tree (Gabella, 1987). This part of the parasympathetic outflow to the lung operates via muscarinic

receptors (Gabella, 1987). However, the involvement of parasympathetic pathways extending to the lung epithelium are less well understood. Vagal stimulation increased alveolar phospholipid content in the adult lung (Oyarzun and Clements, 1977), but parasympathetic control of fetal pulmonary fluid balance has not been studied.

With few exceptions, effects of parasympathetic stimulation oppose effects of sympathetic stimulation (Weiner and Taylor, 1985). Since epinephrine and NE slowed secretion or induced reabsorption of fetal pulmonary fluid, it was hypothesized that acetylcholine (ACh) would cause a rise in fluid secretion in the fetal lung. It should be noted that tracheal fluid production does not seem to be under parasympathetic control in fetal sheep. Neither atropine treatment of the fetus nor bilateral section of the cervical vagosympathetic trunk affected tracheal fluid production (Kitterman et al. 1979). Tracheal fluid production was not affected by denervation of peripheral chemoreceptors, or denervation of lung receptors with afferent fibres in the vagus nerve (Murai et al. 1985). However, there is some question concerning these conclusions, since ACh has been shown to affect ion movements in the tracheal epithelium and these are inevitably linked to water flux (Marin et al. 1976; Olver et al. 1975).

Acetylcholine from 10^{-6} M to 10^{-4} M caused reabsorption, or significant falls in pulmonary fluid production in fetal guinea pig lungs. This was unexpected, but these responses were undeniably marked and consistent. Furthermore, recovery was observed after treatment. Reabsorptions of lung fluid in fetuses below 60 days of gestation were not observed, although there were significant falls in production in these cases. There were no responses to ACh at 10^{-8} M, thus it appears that the threshold dose for ACh is above this concentration, but below 10^{-6} M. ACh at 5 x 10^{-7} M induced a significant rise in unidirectional flux of Cl⁻ ions toward the lumen under open-circuit conditions in the canine tracheal epithelium (Marin et al. 1976; Olver et al. 1975), a response consistent with fluid secretion (Olver et al. 1975). Opposing responses in the trachea and lung to similar stimulation has been observed in another study. Beta-stimulation

caused an increase in tracheal fluid secretion, but absorption of pulmonary fluid (Olver and Robinson, 1986).

In fetal sheep, parasympathetic pathways become functional earlier than those of the sympathetic system (Jones and Robinson, 1975). Both M_1 and M_2 sub-type muscarinic receptors are found in the guinea pig and human lungs (Mak and Barnes, 1988). It is assumed that the guinea pig parasympathetic network is mature in the late-term fetus, just as the sympathetic network is. However, phentolamine blocked the effect of ACh, which indicates that this neurotransmitter was not acting at muscarinic receptors in the guinea pig lung, but rather the effect was mediated by alpha receptors, suggesting an indirect effect of ACh.

It is possible that ACh stimulated an endogenous source of catecholamines. Neuroendocrine cells have been identified in the pulmonary epithelium of the human, rabbit, mouse and guinea pig (Hage, 1976; Pele et al. 1989). These cells are part of a diffuse neuroendocrine system present in mammalian lungs (Hage, 1976). They are localized mainly in the epithelial lining of the bronchial tree, although some sub-types are found in the alveolar epithelium as well (Hage, 1976). Neuroendocrine cells have the ability to store and secrete biogenic amines, and sub-type characterization is based on secretory granule morphology (Hage, 1976). Neuroepithelial bodies are also considered to be part of the pulmonary neuroendocrine system (Sorokin and Hoyt, 1990). In response to cholinergic stimulation, these bodies secrete several hormones including gastrin-releasing peptide (mammalian bombesin) and calcitonin among others (Sorokin and Hoyt, 1990). Both neuroendocrine cells and neuroepithelial bodies are particularly evident during fetal life (Hage, 1976; Plowman et al. 1988). Neuroendocrine cells have been reported to store dihydroxyphenylalanine (DOPA), a precursor involved in catecholamine synthesis (Hage, 1976). Furthermore, these cells decarboxylate precursors resulting, in the case of DOPA, in NE synthesis (Hage, 1976). In addition, phenylethanolamine n-methyltransferase (PNMT) has been identified in the fetal ovine lung; this enzyme methylates NE to produce epinephrine (Padbury et al. 1983b). It is likely that these neuroendocrine cells synthesize and secrete catecholamines,

and the cells involved could be a source of extra-adrenal chromaffin tissue. These cells have been referred to as enterochromaffin-like cells by others (Ericson et al. 1972, in Hage, 1976). Discharge of neuroendocrine cell content occurs at the basolateral surface (Hage, 1976). Cholinergic innervation of neuroepithelial bodies is evident, but innervation of neuroendocrine cells is uncertain. Enterochromaffin tissue is innervated in the guinea pig (Coupland et al. 1982). If these pulmonary, enterochromaffin-like cells are also innervated, ACh may stimulate the local production and release of epinephrine (or NE) by these cells. If this is the case, then these catecholamines could activate alpha receptors in the submucosa. In support of this hypothesis, it was observed that lung fluid reabsorption with ACh treatment occurred in fetuses at or above 60 days gestation (0.88 of term); the reabsorptive effect of epinephrine began at approximately 0.88 of term in the fetal lamb (Walters and Olver 1978). This hypothesis remains speculative, and further investigation is necessary to reach any conclusions.

These studies show that both nervous and hormonal systems aid in fluid clearance from potential air spaces of the lungs. Guinea pig fetal pulmonary alpha-adrenergic receptors are important mediators for this physiological event, although the subsequent cellular events remain to be clarified. These findings differ from published results on fetal sheep. In this species, beta-adrenergic receptors mediate epinephrine-induced fluid reabsorption, and results for NE were conflicting. Clearly, comparison of species in fetal research is important, and the use of particular species as models for the human condition should be questioned.

10. REFERENCES

Adams, F.H., Moss, A.J. and Fagan, L., 1963, The tracheal fluid in the foetal lamb, Biol. Neonate, 5: 151-158.

Adams, F.H., Yanagisawa, M., Kuzela, D. and Martinek, H., 1971, The disappearance of fetal lung fluid following birth, J. Pediatr., 78: 837-843.

Adamson, T.M., Boyd, R.D.H., Platt, H.S. and Strang, L.B., 1969, 204: 159-168.

Adamsons, K., 1966, The role of thermal factors in fetal and neonatal life, Pediatr. Clin. N. Am., 13: 599-619.

Addison, W.H.F. and How, H.W., 1913, On the prenatal and neonatal lung, Am. J. Anat., 15: 199-241.

Alcorn, D., Adamson, T.M., Lambert, T.F., Maloney, J.E., Richie, B.C. and Robinson, R., 1977, Morphological effects of chronic tracheal ligation and drainage in the fetal lamb lung, J. Anat., 123: 649-660.

Alexander, D.P., Forsling, M.L., Martin, M.J., Nixon, D.A., Ratcliffe, J.G., Redstone, D. and Tunbridge, D., 1972, The effect of maternal hypoxia on fetal pituitary hormone release in the sheep, Biol. Neonate, 21: 219-228.

Alwmark, A. and Ahrein, B., 1987, Phentolamine reverses NPY-induced inhibition of insulin secretion in isolated rat islets, Eur. J. Pharm., 135: 307-311.

Avery, M.E., 1968, The fetal lung, In: The Lung and its Disorders in the Newborn Infant, W.B. Saunders Co., Philadelphia, pp. 18-23.

Barker, P.M., Boyd, C.A.R., Ramsden, C.A., Strang, L.B. and Walters, D.V., 1989, Pulmonary glucose transport in the fetal sheep, J. Physiol., 409: 15-27.

Barnes, R.A., 1976, Water and mineral exchange between maternal and fetal fluids, In: Fetal Physiology and Medicine, W.B. Saunders Co., London, pp. 194-214.

Barnes, P., Karliner, J., Hamilton, C. and Dollery, C., 1979, Demonstration of alpha$_1$-adrenoceptors in guinea pig lung using ^{3}H-Prazosin, Life Sci., 25: 1207-1214.

Barrett, C.T., Heymann, M.A. and Rudolph, A.M., 1972, Alpha and beta adrenergic receptor activity in the fetal sheep, Am. J. Obstet. Gynecol., 112: 1114-1121.

Bergman, E.N., 1973, Glucose metabolism in ruminants as related to hypoglycemia and ketosis, Corn. Vet., 63: 341-382.

Bergman, B. and Hedner, T., 1978, Antepartum administration of terbutaline and the incidence of hyaline membrane disease in preterm infants, Acta Obstet. Gynaecol. Scand., 57: 217-221.

Bistoletti, P., Nylund, L., Lagercrantz, H., Hjemdahl, P. and Strom, H., 1983, Fetal scalp catecholamines during labor, Am. J. Obstet. Gynecol., 147: 785-788.

Blair, J.B., James, M.E. and Foster, J.L., 1979, Adrenergic control of glucose output and adenosine 3'-5' monophosphate levels in hepatocytes from juvenile and adult rats, J. Biol. Chem., 254: 7579-7584.

Bland, R.D., Hansen, T.N., Haberkern, C.M., Bressack, M.A., Hazinski, T.A., Raj, J.V. and Goldberg, R.G., 1982, Lung fluid balance in lambs before and after birth, J. Appl. Physiol., 53: 992-1004.

Born, G.V.R., Dawes, G.S. and Mott, J.C., 1956, Oxygen lack and autonomic nervous control of the foetal circulation in the lamb, J. Physiol., 134: 149-166.

Brice, J.E.H. and Walker, C.H.M., 1977, Changing patterns of respiratory distress in the newborn, Lancet, 11: 752-754.

Brown, M.J., Olver, R.E., Ramsden, C.A., Strang, L.B. and Walters, D.V., 1981, Effects of adrenaline infusion and of spontaneous labour on lung liquid secretion and absorption in the fetal lamb, J. Physiol., 313: 13P-14P.

Brown, M.J., Olver, R.E., Ramsden, C.A., Strang, L.B. and Walters, D.V., 1983, Effects of adrenaline and of spontaneous labour on the secretion and absorption of lung liquid in the fetal lamb, J. Physiol., 344: 137-152.

Buchthal, S.D., Dilezikian, J.P. and Danilo, P., 1987, $Alpha_1$-adrenergic receptors in the adult, neonatal, and fetal canine heart, Dev. Pharmacol. Ther., 10: 90-99.

Buckley, N.M., Goodman, P.M. and Yellin, E.L., 1979, Age-related cardiovascular effects of catecholamines in anesthetized piglets, Circ. Res., 45: 282-292.

Butcher, P.A., Olver, R.E., Shaw, A.M., Steele, L.W. and Ward, M.R., 1988, pH sensitive transport of Na^+ into apical membrane vesicles prepared from fetal sheep alveolar type II cells, J. Physiol., 409: 57P

Bylund, D.B. and Ray-Prenger, C., 1989, Alpha-2A and alpha-2B adrenergic receptor subtypes: attenuation of cyclic AMP production in cell lines containing only one receptor subtype, J. Pharm. Exp. Ther., 251: 640-644.

Cassin, S., Gause, G. and Perks, A.M., 1986, The effects of bumetanide and furosemide on lung liquid secretion in fetal sheep, Proc. Soc. Exp. Biol. Med., 181: 427-431.

Cassin, S. and Perks, A.M., 1982, Studies of factors which stimulate lung fluid secretion in fetal goats, J. Dev. Physiol., 4: 311-325.

Chard, T., Hudson, C.N., Edwards, C.R.W. and Boyd, N.R.H., 1971, Release of oxytocin and vasopressin by the human fetus during labor, Nature (Lond), 23: 148-154.

Cheng, J.B., Goldfien, A., Ballard, P.L. and Roberts, J.M., 1980, Glucocorticoids increase pulmonary beta-receptors in the fetal rabbit, Endocrin., 107: 1646-1648.

Coburn, R.F., 1987, Peripheral airway ganglia, Am. Rev. Physiol., 49: 573-582.

Cohen, W.R., Piasecki, G.J. and Jackson, B.T., 1982, Plasma catecholamines during hypoxemia in fetal lamb, Am. J. Physiol., 243: R520-R525.

Collins, M.H., Kleinerman, J., Moessinger, A.C., Collins, A.H., James, L.S. and Blanc, W.A., 1986, Morphometric analysis of the growth of the normal fetal guinea pig lung, Anat. Rec., 216: 381-391.

Comline, R.S. and Silver, M., 1961, The release of adrenaline and noradrenaline from the adrenal glands of the foetal sheep, J. Physiol., 156: 424-444.

Comline, R.S. and Silver, M., 1972, The composition of foetal and maternal blood during parturition in the ewe, J. Physiol., 222: 233-256.

Coupland, R.E., 1952, The prenatal development of the abdominal para-aortic bodies in man, J. Anat., 86: 357-372.

Coupland, R.E., Kent, C. and Kent, S.E., 1982, Normal function of extra-adrenal chromaffin tissues in the young rabbit and guinea pig, J. Endocrin., 92: 433-442.

Coupland, R.E. and Weakley, B.S., 1968, Developing chromaffin tissue in the rabbit, an electron microscopic study, J. Anat., 102: 425-455.

DeLemos, R.A., Shermeta, D.W., Knelson, J.H., Kotas, R.V. and Avery, M.E., 1969, The induction of the pulmonary surfactant in the fetal lamb by the administration of corticosteroids, Pediatr. Res., 3: 505-506 (abst).

DeVane, G.W., Naden, R.P., Porter, J.C. and Rosenfeld, C.R., 1982, Mechanism of arginine vasopressin release in the sheep fetus, Pediatr. Res., 16: 504-507.

Effros, R.M., Mason, G.R., Hukkanen, J. and Silverman, P., 1987, Reabsorption of solutes and water from fluid-filled rabbit lungs, Am. Rev. Resp. Dis., 136: 669-676.

Egan, E.A., Nelson, R.M. and Olver, R.E., 1976, Lung inflation and alveolar permeability to non-electrolytes in the adult sheep in vivo, J. Physiol., 260: 409-424.

Egan, E.A., Olver, R.E. and Strang, L.B., 1975, Changes in non-electrolyte permeability of alveoli and the absorption of lung liquid at the start of breathing in the lamb, J. Physiol., 244: 161-179.

Ekelund, L, Burgoyne, R. and Enhorning, G., 1983, Pulmonary surfactant release in fetal rabbits: immediate and delayed response to terbutaline, Am. J. Obstet. Gynecol., 147: 437-443.

El-Retai, M.F., Blackmore, P.F. and Exton, J.H., 1979, Evidence for two alpha-adrenergic binding sites in liver plasma membranes. Studies with [^{3}H]-epinephrine and [^{3}H]-dihydroergocryptine, J. Biol. Chem., 254: 4375-4386.

Engel, G., 1981, Subclasses of beta-adrenoceptors - a quantitative estimation of beta$_1$ and beta$_2$ adrenoceptors in guinea pig and human lung, Postgrad. Med. J., 57 (suppl): 77-83.

Enhorning, G., Chamberlain, D., Contreras, C., Burgoyne, R. and Robertson, B., 1977, Isoxsurpine-induced release of pulmonary surfactant in the rabbit fetus, Am. J. Obstet. Gynecol., 129: 197-202.

Ericson, L.E., Hakanson, R., Larson, B., Owman, C. and Sundler, F., 1972, Fluorescence and electron microscopy of amine storing enterochromaffin-like cells in tracheal epithelium of mouse, Z. Zelforsch. Mikrosk. Anat., 124: 532-545.

Fain, J.N. and Garcia-Sainz, A., 1980, Mini review: role of phosphatidylinositol turnover in alpha$_1$ and of adenylate cyclase inhibition in alpha$_2$ effects of catecholamines, Life Sci., 26: 1183-1194.

Faxelius, G.F., Lagercrantz, H. and Yao, A., 1984, Sympathoadrenal activity and peripheral blood flow after birth: comparison in infants delivered vaginally and by Cesarean section, J. Pediatr., 105: 144-148.

Friedman, W.F. and Kirkpatrick, S.E., 1977, Fetal cardiovascular adaptation to asphyxia, In: Intrauterine Asphyxia and the Developing Brain (ed. L. Gluck), Year Book Medical Publishers Inc., p. 149.

Fromter, E. and Diamond, J., 1972, Route of passive ion permeation in epithelia, Nature, 235: 9-13.

Gabella, G., 1987, Innervation of airway smooth muscle: fine structure, Ann. Rev. Physiol., 49: 583-594.

Gandy, G., Jacobson, W. and Gairdner, D., 1970, Hyaline membrane disease, I: cellular changes, Arch. Dis. Child., 45: 289-310.

Garrad, E.P., 1990, The effects of temperature change and lung expansion on lung liquid production in *in vitro* preparations of lungs from fetal guinea pigs (Cavia porcellus), unpublished thesis.

Gatzy, J.T., 1983, Mode of chloride secretion by lung epithelia, Am. Rev. Resp. Dis., 127: S14-S16.

Gee, M.H. and Williams, D.D., 1979, Effect of lung inflation on perivascular cuff fluid volume in isolated dog lung lobes, Microvasc. Res., 17: 192-201.

Guillon, G., Butler, D., Cantau, B., Barth, T. and Jard, S., 1982, Kinetic and pharmacological characterization of vasopressin membrane receptors from human kidney medulla: relation to adenylate cyclase activation, Eur. J. Pharmacol., 85: 291-304.

Hage, E., 1976, Endocrine-like cells of the pulmonary epithelium, In: Chromaffin, Enterochromaffin and related cells (eds. R.E. Coupland and T. Fujita), Elsevier Scientific Co., London, pp. 317-332.

Harding, R., Bocking, A.D., Sigger, J.N. and Wicknam, P.J.D., 1984, Composition and volume of fluid swallowed by fetal sheep, Quart. J. Exp. Physiol., 69: 487-495.

Higuchi, M., Murata, Y., Miyake, Y., Hesser, J., Tyner, J., Keegan, K.A. and Porto, M., 1987, Effects of norepinephrine on lung fluid flow rate in the chronically catheterized fetal lamb, Am. J. Obstet. Gynecol., 157: 986-990.

Hooper, S.B. and Harding, R., 1989, Effect of beta-adrenergic blockade on lung liquid secretion during fetal asphyxia, Am. J. Physiol., 257: R705-R710.

Hornbrook, K.R., 1970, Adrenergic receptors for metabolic responses in the liver, Fed. Proc., 29: 1381-1385.

Humphreys, P.W., Normand, I.C.S., Reynolds, E.O.R. and Strang, L.B., 1967, Pulmonary lymph flow and the uptake of liquid from the lungs of the lamb at the start of breathing, J. Physiol., 193: 1-29.

Ibsen, H.G., 1928, Prenatal growth in guinea pigs, J. Exp. Zool., 51: 51-94.

Imai, M., 1977, Effect of bumetanide and furosemide on the thick ascending limb of Henle's loop of rabbits and rats perfused in vitro, Eur. J. Pharmacol., 41: 409-416.

Irestedt, L., Lagercrantz, H., Hjern, P., Hagnevik, K. and Belfrage, P., 1982, Fetal and maternal plasma catecholamine levels at elective Cesarean section in general or epidural anesthesia versus vaginal delivery, Am. J. Obstet. Gynecol., 142: 1004-1010.

Jones, C.T., 1980, Circulating catecholamines in the fetus, their origin, actions and significance, In: Biogenic Amines in Development (eds. H. Parvez and S. Parvez), Elsevier/North-Holland Biomedical Press, pp. 63-86.

Jones, M.D. Jr., Burd, L.I., Bowes, W.A. Jr., Battaglia, F.C. and Lubchenco, L.O., 1975, Failure of association of premature rupture of membranes with respiratory distress syndrome, N. Eng. J. Med., 292: 1253-1257.

Jones, C.T. and Robinson, R.O., 1975, Plasma catecholamines in foetal and adult sheep, J. Physiol., 248: 15-33.

Jost, A. and Policard, A., 1948, Contribution experimental a l'etude du developpement prenatal du poumon chez le lapin, Arch. Anat. Microsc., 37: 177-281.

Kitterman, J.A.P., Ballard, P.L., Clements, J.A., Mescher, E.J. and Tooley, W.H., 1979, Tracheal fluid in fetal lambs: spontaneous decrease prior to term, J. Appl. Physiol., 47: 985-989.

Kolobow, T., Solca, M., Chen, V., Buckhold, D.K. and Peirce, J.E., 1987, Enhancement of lung conditioning by acetylcholine in the prevention of respiratory disress syndrome in the preterm fetal lamb, Biol. Neonate, 51: 224-233.

Kuo, J.F., 1975, Changes in relative levels of guanosine-3':5'-monophosphate-dependent and adenosine-3':5'-monophosphate-dependent protein kinases in lung, heart, and brain of developing guinea pigs, Proc. Nat. Acad. Sci., 72: 2256-2259.

Lagercrantz, H. and Bistoletti, P., 1973, Catecholamine release in the newborn infant at birth, Pediatr. Res., 11: 889-893.

Lagercrantz, H. and Slotkin, T.A., 1986, The "stress" of being born, Sci. Amer., 254: 100-107.

Latifpour, J., Jones, S.B. and Bylund, D.B., 1982, Characterization of [3H]-Yohimbine binding to putative alpha-2 adrenergic receptors in neonatal rat lung, J. Pharm. Exp. Ther., 223: 606-611.

Lawson, E.E., Brown, E.R., Torday, J.S., Madansky, D.L. and Tauesch, H.W. Jr., 1978, The effect of epinephrine on tracheal fluid flow and surfactant efflux in fetal sheep, Am. Rev. Respir. Dis., 118: 1023-1026.

Lechner, A.J. and Banchero, N., 1982, Advanced pulmonary development in newborn guinea pigs (Cavia porcellus), Am. J. Anat., 163: 235-246.

Lefkowitz, R.J. and Caron, M.G., 1987, Molecular and regulatory properties of adrenergic receptors, Rec. Prog. Horm. Res., 43: 469-497.

Lewis, A.B., Evans, W.N. and Sischo, W., 1982, Plasma catecholamine responses to hypoxemia in fetal lambs, Biol. Neonate, 41: 115-122.

Liggins, G.C., 1976, Adrenocortical-related maturational events in the fetus, Am. J. Obstet. Gynecol., 126: 931-941.

Liu, K.H. and Chiou, C.U., 1981, Continuous, simultaneous and instant display of aqueous humor dynamics with a micro-spectrophotometer and sensitive drop counter, Exp. Eye Res., 32: 583-592.

Mak, J.C.W. and Barnes, P.J., 1988, Muscarinic receptor subtypes in guinea pig and human lung, Br. J. Pharm., 95: 777P

Marin, M.G., Davis, B. and Nadel, J.A., 1976, Effect of acetylcholine on Cl⁻ and Na⁺ fluxes across dog tracheal epithelium *in vitro*, Am. J. Physiol., 231: 1546-1549.

Martensson, L., 1984, The pregnant rabbit, guinea pig, sheep and rhesus monkey as models of reproductive physiology, Eur. J. Obstet. Gynecol. Reprod. Biol., 18: 169-182.

Martins, A.N., Ramirez, A. and Doyle, T.F., 1975, Comparison of radio-iodinated, serum albumen and Blue Dextran as indicators to measure rate of formation of cerebrospinal fluid, Exp. Neurol., 47: 249-256.

Mascorro, J.A. and Yates, R.D., 1971, Ultrastructural studies of the effects of reserpine on mouse abdominal sympathetic paraganglia, Anat. Rec., 170: 269-280.

Mascorro, J.A. and Yates, R.D., 1977, The anatomical distribution and morphology of extra-adrenal chromaffin tissue (abdominal paraganglia) in the dog, Tissue and Cell, 9: 447-460.

Mason, R.J., Williams, M.C. and Widdicombe, J.H., 1982, Secretion and fluid transport by alveolar type II epithelial cells, Chest, 81: 61S-63S.

Matthay, M.A., Landolt, C.C. and Staub, N.C., 1982, Differential liquid and protein clearance from the alveoli of anesthetized sheep, J. Appl. Physiol., 53: 96-104.

McDonald, J.V., Gonzales, L.K., Ballard, P.L. and Roberts, J.M., 1986, Lung beta-adrenoreceptor blockade affects perinatal surfactant release but not lung water, J. Appl. Physiol., 60: 1727-1733.

Mennon, K.M.J., Giese, S. and Jaffe, R.J., 1972, Hormone and fluoride sensitive adenylate cyclases in human fetal tissues, Biochim. Biophys. Acta, 304: 203-209.

Milner, A.D., Saunders, R.A. and Hopkin, I.E., 1978, Effects of delivery by Caesarean section on lung mechanics and lung volume in the human neonate, Arch. Dis. Child., 53: 545-548.

Mukherjee, A., Haghani, Z., Brady, J., Bush, L., McBride, W., Buja, L.M. and Willerson, J.T., 1983, Differences in myocardial alpha- and beta-adrenergic receptor numbers in different species, Am. J. Physiol., 245: H957-H961.

Murai, D.T., Wallen, L.D., Lee, C.H. and Kitterman, J.A., 1985, Denervation of peripheral chemoreceptors and lung receptors does not affect production of tracheal fluid, Pediatr. Res., 19: 159A.

Normand, I.C.S., Olver, R.E., Reynolds, E.O.R., Strang, L.B. and Welch, K., 1971, Permeability of lung capillaries and alveoli to non-electrolytes in the foetal lamb, J. Physiol., 219: 303-330.

Oliver, T.K. Jr., Dennis, J.A. and Bates, G.D., 1961, Serial blood gas tensions and acid-base balance during the first hour of life in human infants, Acta Pediatr. Scand., 50: 346-360.

Olver, R.E., 1977, Fetal lung liquids, Fed. Proc., 36: 2669-2675.

Olver, R.E., 1983, Fluid balance across the fetal epithelium, Am. Rev. Resp. Dis., 127: 33S-36S.

Olver, R.E., Davis, R., Marin, M.G. and Nadel, J.A., 1975, Active transport of sodium and chloride across the canine tracheal epithelium in vitro, Am. Rev. Resp. Dis., 112: 811-814.

Olver, R.E., Ramsden, C.A. and Strang, L.B., 1981, Adrenaline-induced changes in net lung liquid volume flow across the pulmonary epithelium of the fetal lamb: evidence for active sodium transport, J. Physiol., 319: 38-39P.

Olver, R.E., Ramsden, C.A., Strang, L.B. and Walters, D.V., 1986, The role of amiloride-blockable sodium transport in adrenaline-induced lung liquid reabsorption in the fetal lamb, J. Physiol., 376: 321-340.

Olver, R.E. and Robinson, E.J., 1986, Sodium and chloride transport by the tracheal epithelium of fetal, new-born and adult sheep, J. Physiol., 375: 377-390.

Olver, R.E., Schneeberger, E.E. and Walters, D.V., 1981, Epithelial solute permeability, ion transport and tight junction morphology in the developing lung of the fetal lamb, J. Physiol., 315: 395-412.

Olver, R.E. and Strang, L.B., 1974, Ion fluxes across the pulmonary epithelium and the secretion of lung liquid in the foetal lamb, J. Physiol., 241: 327-357.

Oyarzun, M.J. and Clements, J.A., 1977, Ventilatory and cholinergic control of pulmonary surfactant in the rabbit, J. Appl. Physiol., 43: 39-45.

Padbury, J.F., Jacobs, H., Lam, R.W., Jobe, A.H. and Fisher, D.A., 1983a, Endogenous epinephrine secretion regulates surfactant release, Clin. Res., 31: 137 (abst).

Padbury, J.F., Lam, R.W., Hobel, C.J. and Fisher, D.A., 1983b, Identification and partial purification of phenylethanolamine n-methyltransferase in the developing ovine lung, Pediatr. Res., 17: 362-367.

Palmer, G.C., 1971, Characteristics of the hormonal induced cyclic adenosine 3',5'-monophosphate response in the rat and guinea pig lung in vitro, Biochim. Biophys. Acta, 252: 561-566.

Parboosingh, J., Lederis, K. and Singh, N., 1982, Vasopressin concentration in cord blood: correlation with method of delivery and cord pH, Obstet. Gynecol., 60: 179-183.

Pele, J., Robidoux, C. and Sirois, P., 1989, Guinea pig lung cells, method of isolation and partial purification, identification, ultrastructure, and cell count, Inflamm., 13: 103-123.

Perks, A.M. and Cassin, S., 1982, The effects of arginine vasopressin and other factors on the production of lung fluid in fetal goats, Chest, 81: 63S-65S.

Perks, A.M. and Cassin, S., 1985a, The effects of arginine vasopressin on lung liquid secretion in chronic fetal sheep, In: The Physiological Development of the Fetus and Newborn (eds. C.T. Jones and P.W. Nathanielz), Academic Press, London, pp. 253-257.

Perks, A.M. and Cassin, S., 1985b, The rate of production of lung liquid in fetal goats and the effect of expansion of the lungs, J. Dev. Physiol., 7: 149-160.

Perks, A.M. and Cassin, S., 1989, The effects of arginine vasopressin and epinephrine on lung liquid production on fetal goats, Can. J. Physiol. Pharm., 67: 491-498.

Perks, A.M., Dore, J.J., Dyer, R., Thom, J., Marshall, J.K., Ruiz, T., Woods, B.A., Vanderhorst, E. and Ziabakhsh, S., 1990, Fluid production by *in vitro* lungs from fetal guinea pigs, Can. J. Physiol. Pharm., 68: 505-513.

Plas, C. and Nuez, J., 1975, Glycogenolytic response of glucagon of cultured fetal hepatocytes, J. Biol. Chem., 250: 5304-5311.

Platzker, A.C.G., Kitterman, J.A., Mescher, E.J., Clements, J.A. and Tooley, W.H., 1975, Surfactant in the lung of the fetal lamb and acceleration of its appearance by dexamethazone, Pediatr., 56: 554-561.

Plowman, L., Scheibner, T. and Sullivan, C.E., 1988, Neurochemicals in the infant lung, Prog. Clin. and Biol. Res., 263: 131-148.

Pohjavuori, M., 1983, Obstetric determinants of plasma vasopressin concentrations and renin activity at birth, J. Pediatr., 103: 966-968.

Pohjavuori, M. and Fyhrquist, F., 1980, Hemodynamic significance of vasopressin in the newborn infant, J. Pediatr., 97: 462-465.

Potter, E.L. and Bohlender, G.P., 1941, Intra-uterine respiration in relation to development of the foetal lung, Am. J. Obstet. Gynecol., 42: 14-22.

Preyer, W., 1885, Specielle physiologie des embryos, In: Th. Grieben's Verlag, (ed. F.L. Leipzig), p. 147.

Reuss, M.L., Parer, J.T., Harris, J.L. and Krueger, T.R., 1982, Hemodynamic effects of alpha-adrenergic blockade during hypoxia in fetal sheep, Am. J. Obstet. Gynecol., 142: 410-415.

Reynolds, S.R.M., 1953, A source of amniotic fluid in the lamb: the naso-pharyngeal and buccal cavities, Nature, 172: 307-308.

Richardson, J.B., 1979, Nerve supply to the lungs, Am. Rev. Resp. Dis., 119: 785-802.

Roberts, J.M., Jacob, N.M., Cheng, J.B., Barnes, P.J., O'Brien, A.T. and Ballard, P.J., 1983, Fetal pulmonary beta-adrenoreceptors: characterization in human in vitro modulation by glucocorticoids in rabbits, Pediatr. Res., 19: 212-216.

Rooney, S.A., 1985, The surfactant system and phospholipid biochemistry, Am. Rev. Respir. Dis., 131: 439-460.

Rosen, O.M. and Rosen, S.M., 1968, The effect of catecholamines on the adenyl cyclase of frog and tadpole hemolysates, Biochem. and Biophys. Res. Comm., 31: 82-91.

Rose, J.C., Jones, C.M., Kelly, R.T., Hargrave, B.Y. and Meis, P.J., 1983, A vasopressin antagonist blocks the norepinephrine and epinephrine response to hemorrhage in the fetus, Endocrin., 113: 2314-2316.

Scarpelli, G.W., Condarelli, S. and Cosmi, E.V., 1975, Lamb fetal pulmonary fluid. Validation and significance of method for determination of volume and volume change, Pediatr. Res., 9: 190-195.

Schneeberger, E.E., Walters, D.V. and Olver, R.E., 1978, Development of intercellular junctions in the pulmonary epithelium of the foetal lamb, J. Cell Sci., 32: 307-324.

Setnikar, I., Agostoni, E. and Taglietti, A., 1959, The fetal lung, a source of amniotic fluid, Proc. Soc. Exp. Biol. Med., 101: 842-845.

Sheppard, H. and Burghardt, C.R., 1970, The effect of alpha, beta, and dopamine receptor-blocking agents on the stimulation of rat erythrocyte adenyl cyclase by dihydroxyphenethylamines and their beta-hydroxylated derivatives, Mol. Pharm., 7: 1-7.

Siegl, P.K.S. and Orzechowski, R.F., 1981, Alpha- and beta-adrenergically mediated responses in isolated lung strips of guinea pigs, Res. Comm. Chem. Path. Pharm., 32: 459-470.

Silva, P., Stoff, J., Field, M., Fine, L, Forrest, J.N. and Epstein, F.H., 1977, Mechanism of active chloride secretion by shark rectal gland: role of Na^+-K^+-ATPase, Am. J. Physiol., 233: F298-F306.

Slotkin, T.A. and Seidler, F.J., 1988, Adrenomedullary catecholamine release in the fetus and newborn: secretory mechanisms and their role in stress and survival, J. Dev. Physiol., 10: 1-16.

Soltoff, S.P. and Mandell, L.J., 1983, Amiloride directly inhibits the Na^+, K^+ ATP-ase activity of rabbit kidney proximal tubules, Sci., 220: 957-959.

Sorokin, S.P. and Hoyt, R.F., 1990, On the supposed function of neuroepithelial bodies in adult mammalian lungs, News in Physiol. Sci., 5: 89-95.

Sosenko, I.R. and Frank, L., 1987, Lung development in the fetal guinea pig: surfactant, morphology, and premature viability, Pediatr. Res., 21: 427-431.

Stark, R., Hussain, K., Daniel, S., Milliez, J., Morishima, L. and James, L.S., 1977, Characteristics of vasopressin (AVP) release during adrenocorticotrophin (ACTH) induced parturition in the lamb, Pediatr. Res., 11: 412 (abs).

Steel, R.G.D. and Torrie, J.H., 1970, Principles and Procedures of Statistics, McGraw Hill, New York, 481 pp.

Stith, I. and Das, S.K., 1982, Development of cholinephophotransferase in guinea pig lung mitochondria and microsomes, Biochim. Biophys. Acta, 714: 250-256.

Strang, L.B., 1977, Neonatal Respiration; Physiological and Clinical Studies, Blackwell Scientific Publications, Oxford, 316 pp.

Takayanagi, I., Kawano, K. and Koike, K., 1990, Alpha$_2$-adrenoceptor mechanisms in the guinea pig trachea, Eur. J. Pharmacol., 182: 577-580.

Thom, J. and Perks, A.M., 1990, The effects of furosemide and bumetanide on lung liquid production by in vitro lungs from fetal guinea pigs, Can. J. Physiol. Pharm., 68: 1131-1135.

Wagner, J.E. and Manning, P.J., 1976, The Biology of the Guinea Pig, Academic Press, pp. 1-4.

Wallace, M.J., Hooper, S.B. and Harding, R., 1990, Regulation of lung liquid secretion by arginine vasopressin in fetal sheep, Am. J. Physiol., 258: R104-R111.

Walters, D.V. and Olver, R.E., 1978, The role of catecholamines in lung liquid absorption at birth, Pediatr. Res., 12: 239-242.

Walters, D.V., Ramsden, C.A., Brown, M.J., Olver, R.E. and Strang, L.B., 1982, Fetal lung liquid absorption during epinephrine infusion and spontaneous labor in the lamb, Chest, 81: 65S-66S.

Walters, D.V., Ramsden, C.A. and Olver, R.E., 1990, Dibutyryl cAMP induces a gestation-dependent absorption of fetal lung liquid, J. Appl. Physiol., 68: 2054-2059.

Walters, D.V. and Strang, L.B., 1987, The secretion and absorption of fetal lung liquid, In: Physiology of the Fetal and Neonatal Lung (eds. D.V. Walters and L.B. Strang), MTP Press Ltd., Lancaster, pp. 61-73.

Warburton, D., Parton, L., Buckley, S., Cosico, L. and Saluna, T., 1987a, Effects of beta$_2$ agonist on tracheal fluid flow, surfactant and pulmonary mechanics in the fetal lamb, J. Pharm. Exp. Ther., 242: 394-398.

Warburton, D., Parton, L., Buckley, S., Cosico, L. and Saluna, T., 1987b, Effects of beta$_2$ agonists on metabolic regulation in the fetal lamb lung, J. Pharm. Exp. Ther., 242: 389-393.

Warburton, D., Parton, L., Buckley, S., Cosico, L. and Saluna, T., 1987c, Beta-receptors and surface active material flux in the fetal lamb lung: female advantage, J. Appl. Physiol., 63: 828-833.

Weiner, N., 1985a, Drugs that inhibit adrenergic nerves and block adrenergic receptors, In: The Pharmacological Basis of Therapeutics, 7th ed. (eds. A.G. Gilman, L.S. Goodman, T.W. Rall and F. Murad), MacMillan Publishing Co., New York, pp. 181-214.

Weiner, N., 1985b, Norepinephrine, epinephrine and the sympathomimetic amines, In: The Pharmacological Basis of Therapeutics, 7th ed. (eds. A.G. Gilman, L.S. Goodman, T.W. Rall and F. Murad), MacMillan Publishing Co., New York, pp. 145-180.

Weiner, N. and Taylor, P., 1985, Drugs acting at synaptic and neuroeffector junctional sites, In: The Pharmacological Basis of Therapeutics, 7th ed. (eds. A.G. Gilman, L.S. Goodman, T.W. Rall and F. Murad), MacMillan Publishing Co., New York, pp. 66-99.

Whitehead, W.H., Windle, W.F. and Becker, R.F., 1942, Changes in lung structure during aspiration of amniotic fluid and during air-breathing at birth, Anat. Rec., 83: 255-265.

Whitsett, J.A., Manton, M.A., Darovec-Beckerman, C. and Adams, K., 1981a, Beta-adrenergic receptors and catecholamine sensitive adenylate cyclase in the developing rat lung, Life Sci., 28: 339-345.

Whitsett, J.A., Manton, M.A., Darove-Beckerman, C., Adams, K.G. and Moore, J.J., 1981b, Beta-adrenergic receptors in the developing rabbit lung, Am. J. Physiol., 240: E351-E357.

Whitsett, J.A., Machulski, A., Noguchi, A. and Burdsall, J.A., 1982, Ontogeny of alpha$_1$- and beta-adrenergic receptors in the rat lung, Life Sci., 30: 139-145.

Widdicombe, J.H., Basbaum, C.B. and Yee, J.Y., 1979, Localization of Na$^+$ pumps in the tracheal epithelium of the dog, J. Cell Biol., 82: 380-390.

Windle, W.F., Becker, R.F., Barth, E.E. and Schultz, M.D., 1939, Aspiration of amniotic fluid by the fetus, J. Gynecol. Obstet., 69: 705-712.

Wiriyathian, S., Porter, J.C., Naden, R.P. and Rosenfeld, C.R., 1983, Cardiovascular effects and clearance of arginine vasopressin in the fetal lamb, Am. J. Physiol., 245: E24-E31.

Zar, H., 1984, Biostatistical Analysis, 2nd ed., Prentice-Hall Inc., New Jersey, 718 pp.

APPENDIX A

PREPARATION OF KREBS-HENSLELEIT SALINE

A.1 <u>Preparation of Saline (1 litre)</u>

1. 10 ml $MgSO_4$ stock solution
2. 100 ml NaCl/KCl stock solution
3. 2.1 g $NaHCO_3$
4. 2.0 g Glucose

After adding the above solutions/solids into a 1 litre volumetric flask, fill the flask at least half-full with distilled water. Mix and ensure the solids have completely dissolved. Then add:

5. 10 ml KH_2PO_4 stock solution
6. 10 ml $CaCl_2$ stock solution

Fill the flask to the 1.0 litre mark with distilled water and bubble with 95% O_2/5% CO_2 for at least 30 minutes.

A.2 <u>Preparation of Stock Solutions</u>

1. 69.2 g NaCl and 3.5 g KCl in 1 L distilled water
2. 36.8 g $CaCl_2$ in 1 L distilled water
3. 15.0 g $MgSO_4$ in 1 L distilled water
4. 16.0 g KH_2PO_4 in 1 L distilled water

APPENDIX B

PREPARATION OF BLUE DYE

B.1 <u>Preparation of Blue Dye</u>

    1. Add 9.0 g NaCl to 1 L of distilled water

    2. Weigh out 5.0 g Blue Dextran 2000 dye crystals

    3. Add dye to 100 ml 0.9% NaCl

    4. Stir using a magnetic stirrer for at least 1 hour

B.2 <u>Preparation of Standard for Spectrophotometry</u>

    1. Put 1 ml of prepared blue dye in 100 ml distilled H_2O

    2. Remove 25 ml from above and add 25 ml distilled H_2O

APPENDIX C

EFFECT OF TREATMENT ON LUNG FLUID PRODUCTION
(Individual Experiments)

C.1   TREATMENT: Epinephrine 1×10^{-5} M

Age in days	Weight in grams	Rate in h1 ml/Kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
63	114.7	1.14	1.12	0.63	1.75	*ns	p<0.05	p<0.05
63	86.8	1.13	1.09	1.50	3.54	ns	ns	ns
63	96.8	1.19	1.32	0.75	---	ns	ns	p<0.025
62	82.6	0.63	0.64	2.08	---	ns	p<0.001	p<.0025
67	157.1	1.20	0.63	0.36	47.50	p<0.005	ns	p<0.005
63	93.9	1.08	1.22	1.30	---	ns	ns	ns

C.2   TREATMENT: Epinephrine 1×10^{-6} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
60	97.4	3.73	1.83	0.25	50.94	p<0.01	p<0.005	p<.0005
67	111.9	2.18	0.94	1.02	56.88	p<.0005	ns	p<0.025
60	105.4	0.86	0.40	1.35	53.49	ns	ns	ns
66	81.2	3.73	1.10	1.72	70.51	p<.0005	ns	p<0.005
60	86.3	3.70	2.66	1.62	28.11	ns	ns	ns
65	87.0	8.21	1.45	0.55	82.34	p<.0005	p<0.05	p<.0005

* ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX C

C.3 TREATMENT: Epinephrine 1×10^{-7} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
63	104.8	1.73	0.71	0.26	58.96	p<0.025	*ns	p<0.005
63	116.0	4.01	1.90	1.43	52.62	p<0.025	ns	p<0.05
63	102.1	4.73	-1.31	----	127.7	p<.0005	--	--
63	87.7	2.88	0.79	1.60	72.57	p<.0005	p<0.05	p<0.005
63	101.1	3.48	0.88	3.06	74.71	p<0.005	p<0.01	ns
62	103.3	5.19	-0.84	2.33	116.2	p<.0005	p<0.01	p<0.025

C.4 TREATMENT: Epinephrine 5×10^{-8} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
62	119.3	1.49	1.25	0.43	16.11	ns	p<0.025	p<0.025
66	100.7	0.78	0.33	0.07	57.69	p<0.005	p<0.25	p<.0005
60	81.7	2.26	-0.02	0.49	101.0	p<.0005	p<0.05	p<0.001
66	104.1	0.28	-0.55	0.52	296.4	ns	p<0.05	ns
66	115.1	0.86	-0.11	0.24	112.8	p<.0005	p<0.01	p<0.005
63	88.6	1.88	1.08	1.17	42.55	ns	ns	ns

* ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX C

C.5   TREATMENT: Epinephrine 1×10^{-8} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
65	98.5	4.37	0.68	1.42	84.44	p<.0005	p<0.05	p<0.001
61	105.3	5.50	2.04	3.18	62.91	p<0.025	ns	ns
61	88.9	4.65	2.81	2.08	39.57	p<0.05	ns	p<0.05
64	108.6	0.92	0.69	0.32	25.00	ns	ns	p≤0.05
64	93.4	0.58	0.52	0.47	10.34	ns	ns	ns
62	134.0	0.24	-0.09	0.13	137.5	ns	p<0.01	ns

C.6   TREATMENT: Epinephrine 1×10^{-9} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
63	98.3	1.09	0.73	0.34	33.03	ns	ns	p<0.05
66	127.3	1.01	0.45	0.22	55.45	p<0.05	p<0.05	p<0.05
62	89.0	1.91	0.74	0.41	61.26	p<0.05	ns	p<0.05
63	99.7	0.82	0.66	0.66	19.51	ns	ns	ns
64	95.8	0.94	0.43	0.35	54.26	p<0.05	ns	p<0.05
64	117.2	0.69	0.47	0.52	31.88	p<0.05	ns	ns

* ns=not significant; significance is taken at p≤0.05

APPENDIX C

C.7   TREATMENT: Epinephrine 10^{-8} M/ Propranolol 10^{-7} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
63	101.8	1.74	0.83	0.36	52.30	p<.0005	p<0.025	p<.0005
62	81.3	3.54	0.98	0.47	72.32	p<.0005	*ns	p<.0005
63	99.0	2.37	0.95	0.43	59.92	p<.0025	p<0.025	p<0.01
64	91.3	2.87	0.74	0.54	74.22	p<.0005	ns	p<.0005
63	115.4	0.62	0.55	0.31	11.29	ns	ns	ns
62	121.9	0.67	0.25	0.52	62.69	p<0.025	p<0.025	ns

C.8   TREATMENT: Propranolol 1 x 10^{-7} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
62	88.4	1.07	0.68	0.63	36.45	p<0.05	ns	p<0.05
64	81.9	1.06	1.25	0.45	---	ns	ns	ns
64	133.1	0.51	1.18	0.23	---	p<0.005	p<.0025	ns
63	95.2	4.01	2.32	0.62	42.14	p<0.05	p<0.01	p<.0025
63	104.6	0.38	1.08	0.25	---	p<0.025	p<0.01	ns
63	126.5	0.23	0.80	1.69	---	p<0.05	p<.0025	p<.0005

* ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX C

C.9   TREATMENT: Epinephrine 10^{-7} M/ Phentolamine 10^{-6} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
64	129.7	0.73	1.63	0.10	---	p<0.005	p<0.01	*ns
63	100.4	0.14	0.65	0.13	---	p<.0025	p<.0025	ns
63	89.2	0.37	0.23	0.24	37.84	ns	ns	ns
63	79.3	0.47	2.11	0.38	---	p<.0005	p<.0005	ns
63	114.1	0.12	0.64	0.42	---	p<.0025	p<0.05	p<0.025
62	87.8	1.51	1.12	1.23	25.83	p<0.001	ns	p<.0025

C.10   TREATMENT: Isoproterenol 1 x 10^{-7} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
62	90.4	3.88	2.48	2.80	36.22	ns	ns	ns
63	98.7	0.86	1.23	1.07	---	ns	ns	ns
63	133.8	1.44	1.01	1.81	29.86	ns	p<0.05	ns
62	88.2	1.14	0.99	0.14	13.16	ns	p<.0025	p<.0025
63	97.8	0.70	0.12	0.59	82.86	p≤0.001	p<0.025	ns
64	123.9	0.35	0.22	0.34	37.14	p<0.05	ns	ns

* ns=not significant; significance is taken at p≤0.05

APPENDIX C

C.11 TREATMENT: Epinephrine 10^{-7} M/ AVP 0.6 mU/ml

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
64	108.0	0.22	-0.83	0.33	47.27	p<0.05	p<.0025	*ns
64	110.6	1.39	1.30	1.67	6.47	ns	ns	ns
64	91.8	3.42	1.11	0.26	67.54	p<0.005	p<0.05	p<.0005
64	104.6	0.32	-1.92	0.93	700.0	p<0.025	p<0.001	ns
64	105.8	0.73	0.61	0.16	16.44	ns	ns	ns
64	92.9	0.84	0.63	0.53	25.00	ns	ns	ns

C.12 TREATMENT: AVP 0.6 mU/ml

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
64	124.6	0.30	1.13	-0.49	---	p<0.005	p<0.005	p<0.025
65	136.6	0.95	-0.33	0.37	134.7	p<.0025	p<0.05	p<0.05
64	122.1	0.36	0.34	0.30	5.56	ns	ns	ns
67	166.8	3.61	1.12	----	68.98	p<.0005	--	--
63	125.3	2.63	2.79	0.12	---	ns	p<0.05	p<0.05
63	92.5	1.63	1.00	0.96	38.65	p<0.05	ns	ns

* ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX C

C.13   TREATMENT: Norepinephrine 1.24×10^{-5} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
60	78.8	2.64	-0.38	1.21	114.4	p<.0025	p<0.01	p<0.025
63	80.9	1.38	0.20	-1.46	85.5	p<0.025	p<0.005	p<.0005
63	105.5	1.99	-0.53	-0.41	126.6	p<.0005	*ns	p<.0005
61	107.3	1.83	-0.26	0.44	114.2	p<.0005	ns	ns
63	111.6	0.41	-0.04	0.11	109.8	p<0.025	ns	p<0.01
65	129.2	2.90	-0.78	0.27	126.9	p<.0005	p<0.005	p<.0005

C.14   TREATMENT: Norepinephrine 1.24×10^{-6} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
64	122.8	0.49	0.05	0.13	89.80	p<0.01	ns	p<0.05
62	78.5	0.59	-0.46	0.47	178.0	p<.0025	p<.0025	ns
62	89.6	4.48	0.75	1.50	83.26	p<.0005	p<0.001	p<.0005
64	83.6	2.54	0.44	1.17	82.68	p<.0005	p<0.025	p<0.01
63	115.7	1.46	0.22	0.61	84.93	p<.0005	p<0.05	p<.0025
63	92.9	2.31	0.27	0.78	88.31	p<.0005	ns	p<0.005

* ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX C

C.15   TREATMENT: Norepinephrine 1.24×10^{-7} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
61	93.3	1.61	0.45	0.31	72.05	p<.0025	*ns	p<0.001
61	92.0	1.94	0.63	0.99	67.53	p<.0005	p<0.001	p<0.001
63	81.6	1.55	0.40	0.04	74.19	p<0.005	p<0.05	p<.0025
64	88.9	1.06	0.88	1.27	16.98	ns	ns	ns
64	78.9	1.72	0.08	0.65	95.35	p<.0005	p<0.05	p<.0025
63	100.2	2.47	0.16	0.52	93.52	p<.0005	p<0.01	p<.0005

C.16   TREATMENT: Norepinephrine 5.24×10^{-8} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
63	108.0	0.78	0.32	0.62	58.97	ns	ns	ns
61	78.4	1.20	0.46	0.74	61.67	ns	ns	ns
61	94.4	2.83	0.52	0.05	81.63	p<.0005	p<0.01	p<.0005
62	79.0	2.45	-0.21	0.88	108.6	p<.0005	p<0.005	p<0.01
62	87.9	2.64	0.90	0.86	65.91	p<0.005	ns	p<0.005
62	92.0	1.12	0.65	0.66	41.96	p<0.025	ns	p<0.05

* ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX C

C.17   TREATMENT: Norepinephrine 1.24×10^{-8} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
63	94.6	0.57	-0.06	0.02	110.5	p<0.005	*ns	ns
62	82.6	1.50	0.89	0.83	40.67	p<0.025	ns	p<0.025
62	105.6	0.45	0.65	-0.15	---	ns	ns	ns
64	87.5	0.88	0.94	1.24	---	ns	ns	p<0.025
63	105.6	2.30	1.45	0.83	36.96	p<0.025	p<0.01	p<0.001
63	92.9	1.04	1.17	1.11	---	ns	ns	ns

C.18   TREATMENT: Norepinephrine 1.24×10^{-9} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
65	150.8	1.47	0.39	-0.68	73.47	p<.0025	p<0.025	p<.0025
65	131.3	2.07	0.96	1.20	53.62	p<0.025	ns	ns
63	114.5	0.35	0.07	0.63	80.00	ns	p<0.025	ns
61	88.8	0.82	1.65	0.59	---	ns	p<0.05	ns
61	82.3	1.44	1.58	1.15	---	ns	ns	p<0.01
61	105.1	0.34	1.15	1.54	---	p<0.01	ns	p≤0.005

* ns=not significant; significance is taken at p≤0.05

APPENDIX C

C.19   TREATMENT: Norepinephrine 10^{-6} M/ Phentolamine 10^{-5} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
62	104.2	0.19	1.17	0.46	---	p<0.005	p<0.05	*ns
62	77.4	1.65	1.34	0.62	18.79	ns	ns	ns
65	95.5	1.45	1.61	1.42	---	ns	ns	ns
63	100.8	0.21	1.14	0.70	---	p<.0025	ns	p<.0025
63	113.9	0.87	0.50	0.60	42.53	ns	ns	ns
63	85.3	0.27	0.43	0.53	---	ns	ns	ns

C.20   TREATMENT: Phentolamine 1.78×10^{-5} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
64	135.8	0.46	0.35	-0.04	23.91	ns	p<0.005	p<0.005
61	105.1	0.88	0.71	0.40	19.32	ns	p<0.01	p<.0025
64	137.5	2.14	2.82	0.66	---	ns	p<0.025	p<0.05
64	103.9	0.03	1.98	1.66	---	p<.0025	ns	p<.0005
63	128.6	7.96	3.41	2.22	57.16	p<.0005	ns	p<.0005
63	113.6	1.60	3.17	2.67	---	p<0.025	ns	ns
63	96.3	1.07	0.52	0.13	51.40	p=0.05	ns	p<0.001

* ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX C

C.21   TREATMENT: Acetylcholine 1×10^{-4} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
58	62.6	4.53	0.42	1.31	90.73	p<.0025	p<0.02	p<.0005
61	101.4	2.89	-0.70	2.03	124.2	p<.0005	p<.0025	p<0.025
64	118.8	1.68	-0.42	1.21	125.0	p<0.005	p<.0005	*ns
63·	136.8	7.27	-4.73	4.55	165.1	p<0.001	p<.0005	ns
60	84.2	2.43	-0.59	2.47	124.3	p<.0005	p<.0005	ns
59	72.4	2.27	0.11	0.89	95.15	p<.0025	p<0.05	p<.0005

C.22   TREATMENT: Acetylcholine 1×10^{-5} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
63	110.7	2.65	-0.39	1.98	114.7	p<.0005	p<.0005	ns
63	101.6	1.73	0.46	0.82	73.41	ns	ns	ns
64	104.0	1.37	-0.76	1.12	155.5	p<.0005	p<.0005	ns
61	76.5	0.46	-0.61	1.50	232.6	p<0.025	p<0.005	p<0.05
57	74.7	3.83	0.27	1.98	92.95	p<0.01	p<0.05	p<0.025
58	87.7	3.41	0.85	1.13	75.07	p<0.01	ns	p<0.005

* ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX C

C.23   TREATMENT: Acetylcholine 1×10^{-6} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
63	84.6	3.25	-2.64	0.82	181.2	p<.0005	p<.0025	p<0.01
67	93.5	1.33	-0.51	0.52	138.4	p<0.005	p<0.025	*ns
64	72.6	1.71	-0.79	0.60	146.2	p<.0005	p<0.005	p<0.05
63	114.8	0.27	0.18	0.39	33.33	ns	p<.0005	ns
64	97.2	0.47	-0.33	0.41	170.2	p<.0005	p<.0005	ns
63	102.2	0.18	0.56	0.40	---	p<0.025	ns	p<0.05

C.24   TREATMENT: Acetylcholine 1×10^{-8} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
65	90.4	1.27	1.74	0.83	---	ns	p<0.05	ns
61	77.2	0.81	1.58	1.47	---	p<.0005	ns	p<0.005
60	78.4	1.14	1.76	0.91	---	ns	ns	ns
67	88.2	1.13	0.15	0.83	86.73	p<0.05	ns	ns
59	91.2	3.27	2.32	1.27	29.05	p<0.025	p<0.01	p<0.005
66	98.3	0.27	0.59	0.36	---	p<.0025	p<0.01	ns

* ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX C

C.25   TREATMENT: Acetylcholine 10^{-6} M/ Phentolamine 10^{-5} M

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
64	114.6	0.34	0.71	0.11	---	p<0.025	p<0.01	*ns
64	136.3	1.21	1.18	0.40	2.48	ns	ns	ns
64	119.7	0.69	0.56	1.41	18.84	ns	p<0.005	p<0.001
64	106.9	0.68	1.28	0.49	---	p<0.05	ns	ns
63	77.5	1.16	-0.55	0.23	147.4	p<.0005	p<0.025	p<0.025
63	163.8	1.00	0.56	0.71	44.00	ns	ns	ns

C.26   TREATMENT: Control

Age in days	Weight in grams	Rate in h1 ml/kg·h	Rate in h2 ml/kg·h	Rate in h3 ml/kg·h	Fall in %	Significance		
						h1 v h2	h2 v h3	h1 v h3
61	85.3	0.89	1.49	0.66	---	ns	ns	ns
64	112.1	1.73	0.30	0.71	82.65	ns	ns	ns
63	109.9	1.29	0.97	1.89	24.81	p<0.01	p<0.05	p<0.05
58	82.6	2.65	2.54	1.97	4.15	ns	ns	ns
64	113.4	1.06	1.12	1.11	---	ns	ns	ns
67	159.4	0.65	0.61	0.44	6.15	ns	ns	p<0.01

* ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX D

EFFECT OF TREATMENT ON SODIUM AND POTASSIUM MOVEMENT

D.1 Effect of Epinephrine on Sodium Movement

Ion	Treatment	Rate in h1 mE/kg·h	Rate in h2 mE/kg·h	Rate in h3 mE/kg·h	Significance		
					h1 v h2	h2 v h3	h1 v h3
Na+	Epi 10^{-5}M	0.262±0.005	0.093±0.013	0.097±0.038	p<0.01	*ns	p<0.01
Na+	Epi 10^{-6}M	0.815±0.214	0.290±0.080	0.295±0.095	p<.0005	ns	p<.0005
Na+	Epi 10^{-7}M	0.547±0.098	0.160±0.076	0.238±0.095	p<0.005	ns	p<0.005
Na+	Epi 10^{-8}M	0.680±0.122	0.092±0.191	0.190±0.285	p<.0005	ns	p<.0005
Na+	Epi 10^{-9}M	0.269±0.084	0.161±0.049	0.049±0.026	p<0.005	p<0.01	p<.0005

D.2 Effect of Epinephrine on Potassium Movement

Ion	Treatment	Rate in h1 mE/kg·h	Rate in h2 mE/kg·h	Rate in h3 mE/kg·h	Significance		
					h1 v h2	h2 v h3	h1 v h3
K+	Epi 10^{-5}M	0.053±0.014	0.014±0.006	0.006±0.018	p<0.005	p<0.01	p<.0005
K+	Epi 10^{-6}M	0.190±0.036	0.063±0.021	0.026±0.008	p<0.005	p<0.025	p<.0005
K+	Epi 10^{-7}M	0.081±0.016	0.016±0.010	0.003±0.008	p<0.005	ns	p<0.005
K+	Epi 10^{-8}M	0.149±0.049	0.042±0.041	0.016±0.025	p<0.005	ns	p<0.005
K+	Epi 10^{-9}M	0.063±0.008	0.032±0.016	0.012±0.004	p<0.005	p<0.01	p<.0005

*ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX D

D.3   Effect of Epinephrine and Propranolol on Ion Movement

Ion	Treatment	Rate in h1 mE/kg·h	Rate in h2 mE/kg·h	Rate in h3 mE/kg·h	Significance		
					h1 v h2	h2 v h3	h1 v h3
Na+	Epi/Prop	0.351±0.061	0.120±0.041	0.240±0.164	p<.0005	p<0.005	p<0.005
K+	Epi/Prop	0.067±0.012	0.025±0.003	0.020±0.018	p<.0005	*ns	p<.0005
Na+	Propranol	0.242±0.101	0.165±0.082	0.142±0.046	ns	ns	ns
K+	Propranol	0.053±0.008	0.031±0.007	0.002±0.008	p<0.005	p<0.005	p<.0005

D.4   Effect of Epinephrine and Phentolamine on Ion Movement

Ion	Treatment	Rate in h1 mE/kg·h	Rate in h2 mE/kg·h	Rate in h3 mE/kg·h	Significance		
					h1 v h2	h2 v h3	h1 v h3
Na+	Epi/Phent	0.108±0.049	0.005±0.039	0.044±0.027	p<0.005	ns	p<0.005
K+	Epi/Phent	0.033±0.008	0.028±0.007	0.002±0.014	ns	p<.0005	p<.0005
Na+	Phentol	0.282±0.168	0.302±0.132	0.152±0.110	ns	p<0.005	0<0.005
K+	Phentol	0.083±0.078	0.124±0.078	0.034±0.026	ns	p<0.025	p<0.05

D.5   Effect of Isoproterenol on Ion Movement

Ion	Treatment	Rate in h1 mE/kg·h	Rate in h2 mE/kg·h	Rate in h3 mE/kg·h	Significance		
					h1 v h2	h2 v h3	h1 v h3
Na+	Isoproter	0.177±0.033	0.072±0.035	0.048±0.038	p<0.005	ns	p<.0005
K+	Isoproter	0.028±0.020	0.022±0.009	0.024±0.080	ns	ns	ns

*ns=not significant; significance is taken at $p \leq 0.05$

APPENDIX D

D.6   Effect of Norepinephrine on Sodium Movement

Ion	Treatment	Rate in h1 mE/kg·h	Rate in h2 mE/kg·h	Rate in h3 mE/kg·h	Significance		
					h1 v h2	h2 v h3	h1 v h3
Na+	NE 10^{-5} M	0.322 ± 0.082	$-.009\pm0.017$	0.038 ± 0.070	p<.0005	p<0.005	p<.0005
Na+	NE 10^{-6} M	0.280 ± 0.105	0.075 ± 0.043	0.149 ± 0.032	p<.0005	p<0.025	p<0.005
Na+	NE 10^{-7} M	0.274 ± 0.046	0.083 ± 0.020	0.093 ± 0.031	p<.0005	*ns	p<0.005
Na+	NE 10^{-8} M	0.155 ± 0.048	0.091 ± 0.036	0.192 ± 0.030	p<0.01	p<0.005	ns
Na+	NE 10^{-9} M	0.244 ± 0.051	0.068 ± 0.190	0.112 ± 0.070	p<.0005	ns	p<0.005

D..7   Effect of Norepinephrine on Potassium Movement

Ion	Treatment	Rate in h1 mE/kg·h	Rate in h2 mE/kg·h	Rate in h3 mE/kg·h	Significance		
					h1 v h2	h2 v h3	h1 v h3
K+	NE 10^{-5} M	0.102 ± 0.024	0.066 ± 0.013	0.065 ± 0.067	p<0.05	ns	p<0.05
K+	NE 10^{-6} M	0.056 ± 0.017	0.014 ± 0.015	0.029 ± 0.017	p<.0005	p<0.025	p<.0005
K+	NE 10^{-7} M	0.067 ± 0.007	0.035 ± 0.004	$-.008\pm0.003$	p<0.005	p<.0005	p<.0005
K+	NE 10^{-8} M	0.035 ± 0.011	0.010 ± 0.007	0.019 ± 0.009	p<0.005	ns	p<0.025
K+	NE 10^{-9} M	0.028 ± 0.013	0.006 ± 0.048	$-.006\pm0.044$	p<.0005	ns	p<0.025

D.8   Effect of Norepinephrine and Phentolamine on Ion Movement

Ion	Treatment	Rate in h1 mE/kg·h	Rate in h2 mE/kg·h	Rate in h3 mE/kg·h	Significance		
					h1 v h2	h2 v h3	h1 v h3
Na+	NE/Phent	0.102 ± 0.066	0.131 ± 0.030	0.098 ± 0.023	ns	ns	ns
K+	NE/Phent	0.038 ± 0.007	0.017 ± 0.005	0.009 ± 0.008	p<.0005	ns	p<.0005
Na+	Phentol	0.282 ± 0.168	0.302 ± 0.132	0.152 ± 0.110	ns	p<0.005	p<0.005
K+	Phentol	0.083 ± 0.078	0.124 ± 0.078	0.034 ± 0.026	ns	p<0.025	p<0.05

*ns=not significant; significance is taken at p≤0.05

APPENDIX D

D.9 Effect of Acetylcholine on Ion Movement

Ion	Treatment	Rate in h1 mE/kg·h	Rate in h2 mE/kg·h	Rate in h3 mE/kg·h	Significance		
					h1 v h2	h2 v h3	h1 v h3
K+	ACh 10^{-4}M	0.100 ± 0.022	0.019 ± 0.006	0.051 ± 0.019	p<.0005	*ns	p<0.025
K+	ACh 10^{-5}M	0.079 ± 0.034	0.039 ± 0.019	0.040 ± 0.017	ns	ns	ns
K+	ACh 10^{-6}M	0.087 ± 0.034	0.010 ± 0.100	$-.003 \pm 0.005$	p<.0005	ns	p<0.005
K+	ACh 10^{-8}M	0.088 ± 0.020	0.068 ± 0.014	0.037 ± 0.009	ns	p<0.025	p<0.005
Na+	ACh 10^{-6}M	0.199 ± 0.059	0.024 ± 0.032	0.096 ± 0.027	p<.0005	p<0.05	p<0.01
Na+	ACh 10^{-8}M	0.378 ± 0.096	0.367 ± 0.108	0.312 ± 0.064	ns	ns	ns

D.10 Effect of Acetylcholine and Phentolamine on Ion Movement

Ion	Treatment	Rate in h1 mE/kg·h	Rate in h2 mE/kg·h	Rate in h3 mE/kg·h	Significance		
					h1 v h2	h2 v h3	h1 v h3
Na+	ACh/Phent	0.133 ± 0.021	0.077 ± 0.058	0.124 ± 0.040	p<0.05	p<0.05	ns
K+	ACh/Phent	0.042 ± 0.001	0.024 ± 0.003	0.011 ± 0.006	p<0.01	p<0.05	p<0.005
Na+	Phentol	0.282 ± 0.168	0.302 ± 0.132	0.152 ± 0.110	ns	p<0.005	p<0.005
K+	Phentol	0.083 ± 0.078	0.124 ± 0.078	0.034 ± 0.026	ns	p<0.025	p<0.05

D.11 Effect of Control Experiments on Ion Movement

Ion	Treatment	Rate in h1 mE/kg·h	Rate in h2 mE/kg·h	Rate in h3 mE/kg·h	Significance		
					h1 v h2	h2 v h3	h1 v h3
Na+	Control	$0.172 + 0.048$	$0.158 + 0.050$	$0.104 + 0.056$	ns	ns	ns
K+	Control	$0.071 + 0.010$	$0.034 + 0.017$	$0.002 + 004$	p<0.005	p<0.005	p<.0005

*ns=not significant; significance is taken at p≤0.05