

THE UNIVERSITY OF MANITOBA

Electrical Activity in the Phrenic Nerve and
Diaphragm of the Fetal Lamb in Utero

by

Andrej Bahoric, D.V.M.

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE IN PHYSIOLOGY

DEPARTMENT OF PHYSIOLOGY

WINNIPEG, MANITOBA

JANUARY, 1974

Acknowledgement

I would like to express my deep gratitude to my supervisor Dr. V. Chernick for his guidance, understanding and precious advice given during the course of experimentation as well as during the writing of this thesis. My colleague Mr. A. Jansen deserves special appreciation for fruitful consultations that helped me to solve many problems. I also wish to thank Mr. B. Russell for abundant technical help. Technical advice and help from Mr. H. Spencer was always welcomed. Secretarial work done by Miss Anna Gleeson is gratefully acknowledged.



ABSTRACT

ELECTRICAL ACTIVITY IN THE PHRENIC NERVE AND DIAPHRAGM OF THE FETAL LAMB IN UTERO.

Dawes et al (10) monitored tracheal pressure and volume change of fetal lambs in-utero and described respiratory movements with a frequency of 1 to 4 Hz associated with periods of REM sleep. The present study was undertaken to more directly monitor fetal respiratory center output in-utero by chronic recordings of phrenic and diaphragmatic electrical activity. Pregnant ewes (105 to 125 days gestation) were anesthetized and following hysterotomy a fetal thoracotomy performed on the marsupialized fetus. Bipolar recording electrodes were implanted on either the right or left phrenic nerve and diaphragm and shielded leads tunneled to the dorsum of the ewe. Intrapleural or intratracheal pressures were monitored through liquid filled catheters. One week was allowed for recovery from surgery and recordings were obtained from awake preparations for as long as three weeks. In contrast to phrenic nerve activity in newborn and adult animals, fetal phrenic nerve activity was not phasic but consisted of episodic bursts interspersed by quiescent periods lasting up to 30 minutes. Activity was present as early as 112 days gestation. These bursts were present in all fetuses and were coupled with diaphragmatic activity and changes in intrapleural or intratracheal pressure. Fetal asphyxia was associated with continuous phrenic nerve activity and intrapleural pressure changes. This study indicates the presence of phrenic nerve discharges during the latter third of fetal life in the sheep. It is suggested that the fetal respiratory center is active in utero but this activity is minimal when compared to that present during extra-uterine life.

TABLE OF CONTENTS

GENERAL INTRODUCTION	PAGE
PART I. LITERATURE REVIEW	
A. Development of Distinctive Respiration In-Utero	1
B. Intrauterine Respiration in Chronic Preparation	3
C. Intrauterine Respiration and Fetal Asphyxia	5
D. Excitability of the Fetal Respiratory Center	7
E. Inhibition of Intrauterine Respiratory Movements	9
F. Properties of Phrenic Motoneurons	11
G. Relationship Between Phrenic Activity and the Tidal Volume	15
PART II. EXPERIMENTAL STUDY	
A. METHODS	17
a) Surgical Procedure	17
b) Muscles	18
c) Exploration of the Abdominal Cavity	18
d) Exteriorisation of the Gravid Uterus	19
e) Fetal Surgery	19
f) Cervical Approach	23
g) Abdominal Wound Closure	24
h) Post Surgical Treatment	24
i) Recording	25
B. RESULTS	28
C. DISCUSSION	36
Influences of the Experimental Condition	36
Comparing of Present Results with Those Obtained by Others	38
BIBLIOGRAPHY	41

LIST OF FIGURES

1. The Experimental Preparation	27
2. Single Burst of Phrenic Activity	30
3. Episodic Irregular Burst of Phrenic Activity	30
4. Phrenic Nerve Activity Similar to those in Rhythmical Breathing	31
5. Continuous Rhythmical Phrenic Nerve Activity	31

LIST OF TABLES

Exp. 1 Fetus No. 234	32
Exp. 2 Fetus No. 219	33
Exp. 3 Fetus No. 47 (BB)	34
Exp. 4 Fetus No. 226	35

GENERAL INTRODUCTION

The presence or absence of normal intrauterine respiratory movements has been the subject of considerable interest and debate. The central question is whether or not breathing after birth is a continuation of normal intrauterine respiration or an entirely new phenomenon.

Experiments in the past have been inconclusive since the techniques employed to observe fetal respiration were very often associated with either anesthesia, sensory stimulation or fetal asphyxia. It has generally been accepted that respiration is absent in the normal fetus near term,

After reviewing the pertinent literature Avery (2) concluded that there were no sustained respiratory movements of the fetus in-utero: Adams and his co-workers (1) also doubted the existence of regular respiratory efforts in-utero stating: "A recent review of the literature has concluded that the fetus does not normally make respiratory-like movements in-utero, but does so only under condition of distress".

Recently Dawes and co-workers (10) measured tracheal pressure and volume changes of the non-anesthetized fetal sheep in-utero from 100 days gestation to term. In addition to the respiratory pattern of 1-3 gasps per minute, the authors reported the presence of a rhythmic irregular breathing that had a frequency of 1-4 Hz. associated with the REM stage of sleep. Since the liquid filled fetal lung is in a sense enclosed in a liquid filled plethysmograph, it is possible that recorded changes in tracheal pressure or liquid flow do not accurately represent the output of the fetal respiratory center. We have therefore undertaken a study of the electrical activity of the phrenic nerve and diaphragm of the fetal sheep in-utero.

LITERATURE REVIEW

Since at the present time the knowledge concerning the presence or absence of the regular respiratory movements of the fetus in-utero remains somewhat controversial the following review of the pertinent literature on that subject is submitted.

Development of Distinctive Respiration In Utero:

In 1936 Barcroft and Barron (5) described the respiratory behavior of the early sheep fetus. Ewes were operated under light chloralose or spinal anesthesia, and the uterus delivered into a saline bath at 40°C. Sheep fetuses between the 36th and 49th day of gestation exhibited spontaneous rhythmic trunk movements associated with respiratory efforts. In this period rhythmic movements could not be elicited by asphyxia but this became possible after 50 days of gestation at which time spontaneous rhythmic movements of the fetus were absent. The early spontaneous movements at 36th--37th day were described as massive total movement; primarily rapid extensions of the neck, thorax and tail. With advancing gestation age the movements became more rhythmic and typical of respiratory movements with a frequency of 30-45 per minute. At about 40 days of gestation the movements became biphasic so that extension was followed by the flexion. Later, the extensor phase disappeared leaving only the flexor phase. By the 46th day truncal contractions were followed by active diaphragmatic contraction, as judged by inspection of the rib cage. Toward the 49th day the frequency of contraction became more rapid and powerful and easier to elicit. According to the authors, the fetus presented the appearance of an animal which was breathing continuously, but the depth and frequency varied with the amount of external stimuli.

After 50 days gestation spontaneous respiratory movements ceased.

In subsequent investigations Barcroft and Barron (3) established the chronic fetal maternal preparation. They sectioned the central nervous system at different sites in fetuses of various gestational ages. They came to the conclusion that the absence of rhythmic movements of the fetus after 50 days of gestation was due to gradual inhibition of activity by the CNS as gestation proceeded. This inhibition was brought about by the fiber systems that descended into the spinal cord. A further inhibition was provided by the development of the forebrain centres. They suggested that asphyxiation of the fetus due to impaired umbilical circulation increased the rhythmic activity of a dormant fetus by removing the inhibiting influences of higher centres, and not by direct stimulation of the lower centers. When the fetus was little over 50 days of gestation and was exposed to asphyxial stimulation sustained movements typical for the 50 days normal fetus appeared, followed by movements typical for the 40 day fetus (jerky movements). They therefore hypothesized that endogenous stimulation initially removed CNS inhibition and then directly stimulated the motor cortex. The authors also suggested that if the fetus after 55 days of gestation was stimulated, the respiratory movements dissociated from the muscular movements due to increased independence of the corresponding centres.

Barcroft and Barron (4) in another work pointed out that changes in fetal respiratory movements were also brought about by the onset of function in the "centers" located in the caudal end of the medulla, pons, midbrain and the forebrain. Until 45 days the respiratory movements of the diaphragm were part of a general muscular contraction involving the righting and postural reflexes. Impulses arising in the proprioceptor

system of the muscles stimulated the contraction of the diaphragm. Diaphragmatic activity ceased when general muscular activity stopped. From about 45 days onward the contractions of the diaphragm were still part of the general muscular movements but with a higher frequency (60-66/min). The sustained muscular activity in this stage produced longer sensory impulse volleys from muscle proprioceptors and excited the respiratory movements. The phase of inhibition of respiratory movements after 50 days as a part of general inhibition was caused by developing of the higher centers. After 65 days of gestation the respiratory movements dissociated from the general movements as a result of increased independence of the midbrain centers.

Intrauterine Respiration in Chronic Preparations :

Dawes et al (10) repeated Barcroft's experiment with fetal lambs exteriorised into a saline bath, and also made chronic implantations of tracheal and intrapleural catheters and tracheal flow meters in the fetus. Fetuses between 95-140 days of gestation were examined. They noticed that fetal lambs under both circumstances exhibited spontaneous breathing movement with a frequency of 1-4 Hz and the transpulmonary pressure often reached 30mm Hg during inspiration. The tidal volume, frequency and presence or absence of respiratory movements were not related to the fetal blood gas values, but asphyxiated fetuses developed deep pleural pressure changes and sustained respiratory movements in-utero. There was however a relationship between the fetal EEG and respiration. Rapid eye movements of the fetus during sleep were associated with rhythmic breathing movements.

In another paper Dawes and co-authors (10) described two types of

respiratory activity; first the "gasp" consisting of a single brief relatively deep inspiratory effort occurring irregularly 1-3 per minute, and the second type described as rapid irregular breathing with a frequency of 1-4 Hz and lasting from 10 seconds to one hour. A fall in esophageal pressure with no change in the amniotic fluid pressure was considered as proof of fetal respiratory movements. Fetal respiratory movements were related to changes in blood gases only when the fetal carotid PO_2 was less than 10mm Hg. It was thus concluded that fetal respiratory movements were not normally determined or regulated through the activity of the central or peripheral chemoreceptors. The rapid irregular fetal breathing was unaffected by section or blockade of the cervical vagi but was abolished by general anesthesia. During inspiration tracheal flow reached 6mm/sec but the inspiratory movements were so brief (0.2 sec) that tidal volume was very small (0.5ml). There was also some outward flow of fluid associated with a brief rise in the tracheal pressure once or twice per hour.

Dawes and others(10) measured the tracheal fluid flow, number of fluid pulses per minute, the respiratory minute volume and the duration of the respiratory movements. The experiment was carried out on sheep fetuses between 100-147 days of gestation. The authors stated that there was clear evidence of a circadian rhythm with the most active periods occurring in the late evening and the early hours of the morning. Other observations also suggested that the peak periods of the fetal breathings was associated with an increase in both rate and depth of the respiration. The total volume of the tracheal fluid flow elicited by respiratory movements in 24 hours was about 12 liters in the lambs at term. The fluid flow increased with the gestational age and showed differences

between the lambs of the same gestational age.

Interpretation of much of the published data depends on the ability to demonstrate that a normal fetal environment has been re-established after chronic fetal-maternal preparation has been made. This subject has been discussed by Heather T. Shelly (38). The fetus can survive a severe degree of hypoxia and hypoglycemia. Thus fetal survival cannot be a reliable index of fetal normalcy in the chronic preparation. After successful surgery at least 5-7 days were needed by the fetus and the ewe for complete recovery. The fetal and maternal blood gas values and pH had been established at relatively good levels within 24 hours. However 3-4 days was required before other parameters like plasma glucose and fructose levels in the ewe and fetus as well as the fetal lactate level reached their normal values. The author thus tried to establish criteria for assessing the state of the fetal preparation at the time of experiments. The plasma levels of glucose, fructose and lactate were recommended as good criteria for assessing the state of the chronic fetal-maternal preparation.

Intrauterine Movements Under Various Conditions

Snyder and Rosenfeld (32) made direct observations of intrauterine movements of fetal rabbits under various conditions. They noticed rhythmic excursions of the chest wall and diaphragm in term fetuses (32 days) in-utero or when exteriorized in a warm saline bath. Induced maternal hypercapnia failed to stimulate the fetal respiration beyond the maximum rate characteristic of the age of the fetus. Hypocapnia sharply reduced the fetal respiratory rate to the point of apnea. Hypoxemia depressed respiration in the fetus but stimulated respiration in the does. These authors therefore, concluded that the fetus bears a

striking resemblance to an adult lacking functional carotid bodies. Rosenfeld and Snyder (29) also made an attempt to trace the transition between the fetal and adult type of respiratory responses to various respiratory stimuli. Working on rabbits as experimental subjects the authors found that fetal respiratory activity at 29 days gestation in-utero was not sensitive to hypercapnia and hypoxia while anoxemia was purely depressant when acting on the "gaspings" fetuses at 29 days of gestation. The transition from the intrauterine to the adult type of respiratory response occurred abruptly at birth. Since respiration in the newborn was stimulated by an excess of CO_2 , hypoxia and cyanide, they postulated that carotid body function was inhibited in the fetus in-utero and birth activated a depressed carotid body. The same authors (29) believed that the respiratory movements of the fetus which were present early in the gestation, played an important role in the development of the lung alveoli. In bronchial obstruction the normal inflow of the amniotic fluid was impaired, leading to the incomplete dilatation of the alveoli. However, Edith Potter and co-workers (28) described several clinical cases in which normal alveolar development occurred despite the lack of a connection between the lungs and the amniotic cavity.

Windle and Monnier (37), working on pregnant cats concluded that the fetus was apneic under normal conditions. They suggested that adverse experimental conditions interfered with oxygen supply through the placental circulation and that the consequent increased PCO_2 and lowered PO_2 elicited the rhythmic respiratory movements in-utero.

Another approach to the study of intra-uterine respiration done by the same authors was the injection of contrast material into the amniotic

cavity (36). The absence of contrast material in the fetal lungs of guinea pigs was observed in fetuses kept under normal physiological condition. Experimental anoxemia caused respiratory-like movements in the fetus in-utero which was not always associated with the appearance of contrast material in the lungs. King and Becker (21) repeated Windle's experiment using pregnant rats with a calcodur blue dye injected into the amniotic sac. The dye under normal condition did not appear in the respiratory tract but it was abundant in the digestive tract. Experimentally induced intrauterine hypoxia was associated with immediate appearance of the dye in the lung. The same authors (21) thus postulated that there were many factors in such an experiment which may cause various degrees of hypoxia. They concluded that the decision of whether or not the intrauterine respiratory movements are spontaneous events connected to certain stages of the gestation, had to be solved on a statistical basis.

Excitability of the Fetal Respiratory Center:

There have been no previous direct studies of fetal respiratory center activity or phrenic motor neurones activity in-utero. So on the basis of a short literature review concerning the activity of main elements of the respiratory complex we speculate that there is no cardinal difference in development between an exteriorized fetus or an adult, and the fetus in-utero, especially one in the last third of gestation. Chernick et al (9) examined the respiratory center of the exteriorized fetal lamb by direct electrical stimulation. Focal stimulation of a diffuse area in the fetal medulla at low stimulating current induced an inspiratory gasp in the apnoeic fetus. Such a stimulation in the spontaneously breathing newborn lamb caused an apnaeic

response. Pentobarbitone anesthesia increased the threshold required to elicit the gasp in the fetus, but it reduced the threshold for apnea in the newborn. On the basis of these findings the authors concluded that the medullary respiratory structure must be well developed in the fetal lamb near term. The significant difference in ventilatory responses between the apneic and breathing animals then was due to different functional state of the respiratory neurones.

Pitts(26) studied cats with the brain stem transected at either of two levels: a rostral intercollicular section and a caudal section that passed through the acoustic tubercles dorsally and either caudal pons or trapezoid body ventrally. He divided the central respiratory mechanism into two major systems: a) bulbar respiratory center--that regulates the depth of inspiration by controlling the frequency of impulses in the phrenic motor units as well as the number of active units. It is situated in the ventral part of the inferior olivary nucleus and is sensitive to the CO₂ changes in its fluid environment, b) vagal inhibition system that causes periodic inhibition of the activity of the respiratory center motoneurone system by conducting the impulses from the stretch receptors in the lung. Impulses carried in the vagus were relayed through the nucleus solitarius and stimulated the expiratory center in the brain stem. The inhibitory system located in the ventral lateral tegmentum of the medulla and pons depressed inspiration. Pitts (25) in another experiment on anesthetised cats recorded the impulses in the phrenic motor neurones after stimulation of the respiratory center. During the inspiratory phase of eupneic respiration, phrenic neurones responded repetitively with a slowly augmenting frequency of discharge with sudden decrement as expiration started. Any increase in the activity of the neurones of the respiratory

center increased the frequency of discharge of already active phrenic neurones and activated quiescent neurones. Each phrenic nerve motor neurone excited by the respiratory center is influenced by a number of separate pathways and the level of excitation of the phrenic neurones depends upon the number of these pathways. The rates at which neurones fired were a function of their level of excitation, and the time necessary for recovery of the excitability once the impulse had been discharged.

Because of extensive synaptic interconnections each phrenic neurone is functionally related at least potentially with each one of the constituent neurones of the inspiratory center. Neurones of the expiratory center exert their inhibitory influence on the inspiratory center and act by "withdrawing" excitation from phrenic neurones.

Inhibition of Intrauterine Respiratory Movements :

Ponte and Purves (27) in a recently published paper stated that spontaneous respiratory movements with frequency of 10-20 per minute were recorded in the fetal lambs from ewes given a spinal anesthetic. Respiratory movements were elicited by lowering intratracheal pressure and abolished by elevating the pressure in the airways. They concluded that stretch receptors in the fetal lung or lower airways were stimulated by lowering the intrathoracic pressure since they were able to record regular afferent vagal discharge of 15-45 impulses per second. There was an indication that the vagal discharge had both an excitatory and an inhibitory influence on respiration. The authors pointed out that changes in the intratracheal pressure due to spontaneous respiratory movement had to be clearly distinguished from the artifacts in the intratracheal or intraesophageal pressure records. These could be

caused by heart beat or fasciculation of the intercostal or scalene muscles, giving rise to a noisy signal with a frequency of up to 5HZ and with an amplitude equivalent to ± 4 mm Hg. The effect of immersion in liquid on fetal respiration has been studied under various conditions. Tchobroutsky et al (34) have found that animals under general anesthesia with heads immersed in the warm saline developed a reduction in respiratory frequency or apnea, bradycardia and an elevation in arterial blood pressure. These changes appeared in spite of the free access of air through a tracheal cannula. The same reactions were elicited when water was applied to the glottis and larynx and were absent when the glottis was anesthetised, or the superior and external laryngeal nerve transected. Bradycardia always followed apnea. The onset of bradycardia and apnea was independent of arterial oxygen tension before immersion and the fall in arterial PO_2 during apnea was related to the duration of the apnea. When respiratory movements occurred during immersion their occurrence was not influenced by the level of PO_2 . In the lamb the bradycardia response was dependent on the vagus nerve. Section of both vagi did not affect respiration but abolished bradycardia. The authors therefore suggested that the fetus in-utero immersed in amniotic fluid may be apneic because of the so-called diving reflex. Respiration inhibition is elicited by contact of the fluid with the face and glottis.

Harned and his coworkers (17) studied the same problem and experimented with newborn breathing lambs as well as fetuses. The authors immersed the subject in a water bath or introduced fluid into the trachea. The temperature of the fluid was kept between 39° and 40° (approximate temperature of amniotic fluid). In newborn lambs they demonstrated a depression in ventilation following immersion of the

head or snout in the water while the rest of the body was outside. When the fetal lamb was totally immersed and the cord occluded significant respiratory depression was evident in comparison with non-immersed fetuses. On the basis of these results these authors concluded that the presence of fluid in the upper respiratory passage depressed respiration and that the state of complete immersion of the fetus in its amniotic fluid environment must strongly inhibit fetal respiration. They suggested that these inhibitory effects have to be removed at birth otherwise they might oppose the effects of other stimuli and represent a threat for the neonatal survival.

Properties of Phrenic Motoneurons:

Gill and Kuno (15) attempted to classify the phrenic motor neurones of the cat according to their properties, and by comparing them to the tonic and phasic motor neurones of the lumbosacral part of the spinal cord. The resting membrane potential of the phrenic motor neurones were in range of 40-70mV, and the impulses created in the medullary respiratory center normally produced a rhythmic inspiratory discharge in phrenic motor neurones with a frequency of 5-30/sec. Phrenic motor axons had axonal conduction velocities that were slower than that of phasic motor neurones of the cord; however phrenic motoneurons also had the ability to discharge at high frequency up to 400/sec following tetanic stimulation of the respiratory center. The period of time between spikes (refractory period) was in the range of that of lumbosacral phasic motoneurons. The average membrane resistance of phrenic motoneurons obtained was $3-3M \Omega$ with a range of $2.0-4.2M \Omega$. This was greater than for lumbosacral motor neurones. These authors concluded that phrenic motor neurones cannot be classified either with tonic or phasic motoneurons

of the lumbosacral spinal cord.

In another paper the same authors (16) concluded that there were a few factors that controlled the respiratory activity of the phrenic motor neurones. Their activity could be depressed by:

1. Hyperpolarisation following each discharge.
2. excess CO_2
3. inhibitory control from the expiratory center and
4. by segmental inhibition (at a cervical segment) due to apparent impulses in the contralateral phrenic nerve. Excitation comes from the medullary inspiratory center. However, the major factor in determining the discharge pattern of phrenic motor neurones during the quiet breathing seemed to be the slow depolarisation during the inspiratory phase.

Duron and Caillof (13) recorded the electrical activity of intact branches of the phrenic nerve during spontaneous breathing in cats. In 72% of cases afferent activity in the phrenic nerves during expiration was observed, the frequency of such activity increased toward the end of expiration. Discharges come from the stretched diaphragmatic spindles. During inspiration action potentials in the phrenic nerve opposite to that of motor discharges were recorded 25% of the time. The frequency of discharges decreased toward the end of inspiration. Also recorded was some activity independent of inspiratory activity (3%) and composed of high frequency potentials.

Reflexes That Influence the Phrenic Nerve Activity:

Kinderman and Pleschka (20) working with anaesthetised, paralysed and artificially ventilated dogs, showed that there was increased activity, of the phrenic nerves, expressed as a frequency change when the calf muscles were stimulated by stretching M. triceps surae. It was suggested that excited muscle receptors stimulated the reticular

formation generally and indirectly facilitated the activity of the inspiratory neurones. By applying different CO_2 concentrations during the stretch receptor stimulation the authors concluded that the afferents from the stretch receptors act independently of the actual CO_2 concentration and the phrenic nerve response to the stretch receptors was the same under both hypercapnia and normocapnia.

Duron (13) also studied the postural and ventilatory functions of intercostal muscles in unrestrained cats. The author found that the external intercostal muscles and most of the internal intercostal muscles showed electrical discharges not related to respiratory rhythm but rather were result of the posture. The independence between the diaphragm and the intercostal muscles were visualized by bilateral multisegmental sectioning of spinal dorsal roots. Such lesions did not affect the respiratory activity of the diaphragm and intercostal muscles but abolished spontaneous or reflex activity of intercostal muscles. In contrast, stimulation of the points in the bulbar pyramids in decerebrated cats caused activation of intercostal muscles but did not interfere with diaphragmatic activity.

Tomori et al (35) elaborated the reflex effects that may be activated by applying irritants on the nose and pharynx of the experimental rabbits and cats. These are the sneezing, apneic and the aspiration reflex. Experimenting with rabbits and cats these investigators measured EMG of the diaphragm and other respiratory muscles as well as cardiovascular, respiratory and glottal changes. The authors concluded that because of the evident complexity, the reflex mechanisms are not clear. The components of the apneic reflex apnea, tachycardia, hypertension, closure of the glottis, somatomotor inhibition and tonic expiratory

muscle activity that were caused by nasal stimulation were depressed by adrenergic or stimulated by cholinergic blockade.

The aspiration reflex is very resistant and cannot be suppressed by physical and pharmacological influences. Irani et al (18) showed that stimulation of the caudal intercostal muscle nerves elicited "intercostal to phrenic reflex" with a discharge in the phrenic motoneurons. Stimulation of all intercostal nerves caused "intercostal to recurrent laryngeal reflex" with discharges in the recurrent nerve. If the stimulus was applied to the superior laryngeal nerve a discharge from the recurrent nerve was stimulated while the phrenic motoneurone discharge was abolished. The so-called self excitation of the phrenic motoneurone was elicited by the brief stimulus applied on the peripheral end of the phrenic nerve at the end of inspiration. After a latency of 50-60 sec the discharge in the phrenic nerve was recorded, together with a concomitant excitation of the laryngeal motoneurons. It was speculated that such a reflex can arise under normal circumstances from the impulses elicited by these contractions and by the diaphragm stimulated caudal intercostal motoneurone activity. The authors concluded that "self excitation" is a generalized reflex mechanism for certain motoneurons allied to respiration.

Respiratory Movements of the Human Fetus In-Utero:

Respiratory movements of the human fetus during intrauterine life and their influence on the development of the fetal lung have also been studied. Reifferscheid and Schmiemann (30) injected fluorescent material into the amniotic fluid of women pending abortion. By using fluoroscopy the appearance of the fluorescent substance in the fetal lung was demonstrated. This finding was confirmed by microscopical examination of the lung after the abortion.

Davis and Potter (12) infused contrast material into the maternal amniotic cavity and roentgenograms of delivered human fetuses were taken. The authors suggested that respiratory activity of the human fetus was present and similar to that after delivery. The movements were intermittent, irregular and shallow leading to introduction of the amniotic fluid and the contrast material into the lungs. Materials were concentrated in the lungs as a result of the fluid absorption by the prealveolar and alveolar circulation while some of the aspirated fluid escaped back to the amniotic cavity. Since these studies were done on women with impending abortion or requiring cesarean section, the precise physiological state of the fetus was unknown.

Boddy and Mantell (7) detected respiratory movements of the human fetus with the use of diagnostic ultrasound. Observations were made on women during the second half of pregnancy and maternal abdominal movements were attributed to the fetal breathing. Fetal breathing movements of 40-70 per minute were detected in 34 out of 100 patients.

Relationship Between Phrenic Activity and the Tidal Volume:

Fitzgerald (14) elaborated the relationship between tidal volume and phrenic nerve and diaphragmatic activity in response to hypercapnia and hypoxia in cats. This investigator concluded that the rate of phrenic nerve activity was correlated with tidal volume increase. However, the duration of the phrenic discharge was not correlated with tidal volume increase.

The phrenic nerve response to tidal volume increased during induced hypercapnia was less active than in normocapnia. In case of hypoxia the diaphragm needed more neural energy per unit of time to generate the same tidal volume than in normal condition.

The relationship between the diaphragmatic and phrenic nerve activity and tidal volume during obstructed breathing was studied by Lourenco et al (23). Under normal circumstances the electrical activity in the phrenic nerve and the diaphragm increases linearly with tidal volume. When an inspiratory load was applied, increased electrical activity was coupled with a decrease in tidal volume. During complete obstruction, the electrical activity of the phrenic nerve increased even though there was no air flow. The authors concluded that during obstructed breathing minute volume is no longer a reliable measure of electrical output from the respiratory center.

METHODS

Experiments were attempted on fetuses from 30 cross-bred pregnant ewes (Dorsett-Suffolk) ranging in gestational age from 105-125 days. Younger fetuses proved to be too small for the extensive surgery, and were not used. Fetuses over 135 days of gestation were avoided since we noted early delivery and a very high rate of post-surgical fetal mortality.

Estimation of gestational age was based on a dye mark during mating. The ewe was given 100 mg of an aqueous suspension of medoxyprogesterone acetate* intramuscularly 24 hours before surgery. In addition 30 mg was given 24 hours postoperatively to prevent abortion. Since the potential effect of Medoxyprogesterone upon the respiratory center of the fetal animal had not been studied we tested the drug 14 days after fetal surgery during the period of phrenic activity. There was no evidence of changes in fetal phrenic activity 2-6 and 12 hours after maternal injection of the drug.

Surgical Procedure

Ewes were fasted for 12 hours prior to surgery. The ewe was anesthetized with sodium pentobarbital (20 mg/Kg BW) through the jugular vein. Supplemental doses (2-3 mg/Kg) were given periodically during the course of the surgery.

In three ewes, epidural analgesia was induced. After the initial dose of Pentobarbital 4 ml of 2% xylocaine hydrochloride with epinephrine 1:100,000 warmed to body temperature was injected slowly in the subarachnoidal space. With the use of spinal analgesia subsequent doses of Pentobarbital were minimal and the eructating reflex remained

*DEPOMEDROL: UPJOHN Company of Canada

intact thus preventing bloating.

The skin over the left paralumbal fossa and lateral abdomen was closely clipped and shaved. The region was also scrubbed with Bridine* surgical scrub and disinfected with 70% alcohol. The animal was then placed on the surgical table and covered with sterile operating drapes. Surgery was done under sterile conditions.

A skin incision about 20-25 cm long was made diagonally from the middle portion of the cranial border of the iliac muscle and extended cranioventrally toward the junction between the 13th rib and its cartilage. This allowed an easy access to both uterine horns with minimal intestinal or omental prolapse.

Muscles: M. cutaneous, m. obliquus abdominis externus and m. obliquus abdominis internus were transected separately. The edges of transected muscles were covered with moist sterile drapes. The peritoneum was then incised.

Exploration of the abdominal cavity :

After the abdominal cavity had been opened, usually small amounts of serous pinkish fluid poured from the wound, and it proved to be the best lubricant for the exteriorised uterus. The portion of the uterus which was visible depended upon the site of pregnancy, gestational age and on the degree of ruminal distention. The gravid horn appeared as a large, moist, bluish-gray sack with well ramified blood vessels and cotyledons which were barely visible through the semi-transparent uterine wall. Bicornual in type, the pregnant and non-pregnant uterus is completely situated in the abdominal cavity. Due to the extent of the broad

*Bridine Surg. Scrub. BPS Pharmaceuticals Toronto.

ligaments, the horns have limited freedom of movement, however there was enough movement to allow complete exteriorization of the gravid horn without exerting pressure or tension on the remaining tissues. The uterine wall was palpated to detect the position, and the number of fetuses while the uterus was still in the natural position.

Exteriorisation of the gravid uterus:

The gravid horn was entirely withdrawn from the abdomen by gentle but firm traction on its cranial portion containing fetal parts. The delivered horn was placed upon a special stand covered with surgical drapes and sterile towels moistened with fluid from the abdominal cavity or sterile physiological saline. Delivery of the entire uterine horn from the abdominal cavity permitted easier manipulation of the horn and hastened the operation. One side of each horn has an attached broad ligament, vascularly abundant and with numerous cotyledons, while the other side has a limited number of blood vessels and sparse cotyledons. Exteriorization of the horn allowed us to choose the side of the horn to be employed in surgery. The uterine horn was always incised on the least vascularised side.

Fetal Surgery

The intrathoracic surgery on the fetus for chronic preparation has not been previously described. Therefore the surgical procedure employed in the present study is described in full detail.

The cervical approach used in adult animals to reach one of the roots of the phrenic nerve was found unsuitable in the fetal sheep because it required a large uterine incision and extensive fetal muscular dissection. An intrathoracic approach to the phrenic nerve allowed the application of electrodes to both phrenic nerve and diaphragm through

the same incision.

The main concern was the prevention of loss of amniotic fluid during surgery. To achieve this the method of marsupialisation described by D. Jackson, Egdahl and Richmond (19) was used.

With the uterine wall still intact the fetal body was straightened out from its flexed position, so that full exposure of the proposed surgical site on the fetal chest was obtained. A 6-0 plastic suture with an atraumatic needle was used to make a continuous suture through the uterine wall, placenta and fetal skin, forming a square between the long head of musculus triceps, processes transversi of thoracic vertebrae, the last rib (13th) and the sternum. Cotyledones were carefully avoided and if any were situated in the sutured zone they were gently pushed out of the area. Once suturing was complete, an incision was made within this area through the uterine wall and placenta. The edges of the wound were clamped with forceps to prevent bleeding.

Following the uterine and placental incision the fetal skin and cutaneous muscle were incised in the 7, 8th or 9th intercostal space. The incision was made from the level of processes transversi of the thoracic vertebrae to the level of costochondral junction. The subcutaneous fat was incised and separated from the underlying fascia by blunt dissection. The cut edges of the fetal skin, placenta and uterus were then sewn together with 6-0 suture and loss of amniotic fluid and tearing of the fragile membranes was thus prevented. Next the latissimus dorsi muscle was transected in the upper part of the incision with the incision running vertical to the muscular fibres. Some fibres of serratus dorsalis muscle and obliquus abdominus externus muscle were then transected, elevated and retracted exposing the ribs and intercostal muscles.

At this point the precise intercostal space for intrathoracic penetration was determined. Because it was very easy to cut the diaphragm and penetrate the abdominal cavity instead of the thorax if the wrong intercostal space was chosen, the proper site was carefully chosen by counting and palpation. The intercostal muscles of the area were incised close to the cranial edge of the rib thus avoiding the intercostal vessels. The pleura was perforated with a pair of hooks and adjacent ribs lifted up with gentle traction; scissors were used to complete the pleural incision. The ribs were firmly retracted to provide access to the thoracic cavity, and the diaphragmatic lobe of the lung was packed off with a special retractor to allow more working room at the operation site. In the fetal sheep the diaphragm is attached to the sternum at the level of the 8th chondrosternal junction and then to the 7th and 8th cartilage in an upward direction. The attachment then crosses the 9th cartilage near its costal end reaching the 10th and 11th rib near their ventral ends. After crossing the 12th and 13th rib the diaphragm terminates as far back as the cranial border of the 1st lumbar transverse processes (31). The high intra-abdominal pressure and the position of the fetal lamb made the centrum tendineum of the diaphragm protrude deeply into the thoracic cavity. By elevating the ribs at the intercostal incision the diaphragm was stretched and the sites to be used for electrode implantation were exposed.

The phrenic nerves (31) are formed by the union of two or three roots arising from the ventral branches of the fifth (inconsistent), sixth, and seventh cervical nerves. In the thoracic cavity the course of the phrenic nerve is not the same on both sides. The right nerve after crossing the pericardium courses backward along the inferior vena cava toward the diaphragm. In this region the phrenic nerve is situated in the special

fold given off from the plica venae cava inclining slightly to the ventral side of the vein. Thus when the right side of the fetal thorax was used the anatomical situation required careful separation of the right phrenic nerve from the inferior vena cava. The nerve was first separated and then stripped of the remaining serous envelopes. The left phrenic nerve accompanied by the vagus nerve reaches the diaphragm at the tendinous center considerably left of the median plane. The phrenic nerves terminate by dividing into several branches. When the implantation of the electrodes on the phrenic nerve and diaphragm had been completed and the retractor removed a mixture of blood and saline was aspirated from the thoracic cavity. Small bruises of the lung margins were sometimes noticed after the displaced diaphragmatic lobe assumed its normal anatomical position.

To close the thoracic wall of the fetus number three chromic gut was used. Because the periosteum and the pleura are not capable of holding the suture satisfactorily, the first layer of sutures was placed into the intercostal spaces of adjacent ribs in form of figure 8 tension suture. The edges of the wound were drawn together by uniform pulling of the free ends of the gut. The electrode leads arising from the wound were fixed to the ribs with a #3 chromic gut preventing traction on the implanted device. In the fetuses a silastic catheter was left in the pleural space for subsequent measurement of intrapleural pressure.

At this point the protective sutures between the edges of the skin and uterine incision were removed and each successive layer of muscles and their fascia closed with a continuous suture. The skin was sutured with vertical mattress suture fixing the silastic tubing and the electrical leads between the edges of the wound. No attempt was made to remove air from the pleural cavity; firstly because it was difficult

to make the areas around the leads and the tubing airtight and secondly, there was concern about the possibility of creating an artificial negative pressure in the pleural cavity which would have altered normal physiological conditions. Thus, pleural air was allowed to absorb slowly after returning the fetus to the uterus. Intrathoracic drains were also not used. In all fetuses examined at post-mortem the lung adhered to the incision on the costal pleura and to the implanted electrodes due to the pleuritis caused by the surgery.

The cervical approach:

In two fetuses instead of an intrapleural cannula, an intratracheal cannula was used as well as a catheter in the common carotid artery. The incision on the uterus and the skin beneath was followed by a blunt separation of the tissue between the brachiocephalic and sternomandibular muscle. The common carotid artery is situated in the fascia beneath the jugular vein and omohyoid muscle intimately connected with the vagosympathetic nerve trunk and the recurrent laryngeal nerve. The nerves were carefully separated before the artery was ligated and catheterized. The catheter was then sutured to the arterial wall and the adjacent muscles, preventing flexion and obstruction at any point.

Situated somewhat deeper than the carotid artery and in close proximity to the esophagus, the trachea was imbedded in the deep fascia of the neck. A suture was placed around the trachea, and a small cut was made between two tracheal rings. A silastic cannula was inserted through the hole into the tracheal lumen and filled with tracheal fluid. The tracheostomy was then closed and the cannula sutured to the adjacent interannular spaces. It was therefore possible to measure tracheal pressure changes without obstructing the tracheal passage and liquid flows

After suturing the fetal skin, the uterine wall was sutured with very dense continuous suture (4-0 chromic gut) in order to prevent loss of the amniotic fluid. Following this first layer, the sutures of the protective square were removed and Lambert's single intestinal sutures used for final closure of the uterine incision. After closure of the uterine incision a single injection of 100 mg of Gentamycin into the amniotic fluid was made. Electrical leads and cannulas were partially pushed back into the amniotic fluid and secured to the uterus with a short layer of a single Lambert's suture. The uterine horn was cleared of the remaining blood and lubricated by flushing it with normal saline. The horn was then gently pushed back into the abdominal cavity through the dilated incision. A few coils of leads and cannulae were left in the abdominal cavity to avoid traction if the heavy uterine horn moved, when the ewe assumed the standing position.

Abdominal Wound Closure:

Chromic gut and a U-suture were used for closing the peritoneum and each successive layer of muscles. The electrical leads and cannulae were tunnelled under the skin to the dorsum of the sheep, and the skin was finally closed with N^o 1 Vetafil sutures in the form of a vertical U-stitch. A large belt made from thick sterile cloth was then applied to protect the wound and the devices from damage and contamination.

Post Surgical Treatment

Antibiotics were routinely administered. 800,000 units of Penicillin G and 100 mg of Gentamycin were injected i.m. while the animal was still on the table together with 30 mg of DepoMedrol. The same dose of antibiotics was repeated daily for the next few days.

Recording :

Following fetal thoracotomy bipolar platinum electrodes were applied to one phrenic nerve and the diaphragm. The recording electrodes for the phrenic nerve consist of two open platinum wire rings attached to a connecting cable and embedded in a silastic medical adhesive silicone type A.* (24)

The silicone rubber does not coat the wire rings on the inner surface, so that a nerve trunk treated into this space comes in intimate contact with the electrodes. The distance between the wire rings was 3-5 mm. The diaphragm electrodes were made by placing two stainless steel hooks connected with cables in silicone rubber.

After implantation electrodes were secured to the surrounding tissue with silk sutures. The connecting cables for conducting electrical signals consisted of a shielded multiconductor braided cable covered in transparent silastic tubing. In two fetuses a saline filled catheter was placed in the pleural cavity. In other two fetuses, small fluid filled catheters were introduced into the trachea. Simultaneous recordings of phrenic nerve and diaphragm muscle activity and either tracheal or pleural pressure of the fetus in-utero were not started before the seventh post operative day.

Recordings were done at random times during the day and evening for as long as three weeks. During recording the ewe was awake, and standing comfortably in a pen. The electrical impulses from the phrenic nerve and diaphragm electrodes, were amplified by Grass P15 pre-amplifiers, displayed on a Telequipment D.M. 64 oscilloscope, and stored on tape (4 channel Hewlett-Packard, 3960 instrumentation recorder).

Pleural or tracheal pressure were recorded by Statham pressure transducers

* Dow Corning Corporation, Midland Michigan, U.S.A.

connected to the fluid filled catheters (Figure 1). Impulses from the pressure transducer were amplified by the electronic amplifier (Model EEP.8 Electronic for Medicine, Inc.) and stored on tape.

- A1 PREAMPLIFIER (DIAPHRAGM)
- A2 PREAMPLIFIER (PHRENIC NERVE)
- B PRESSURE TRANSDUCER (TRACHEA OR PLEURAL SPACE)
- C OSCILLOSCOPE
- D CAMERA
- E TAPEREORDER

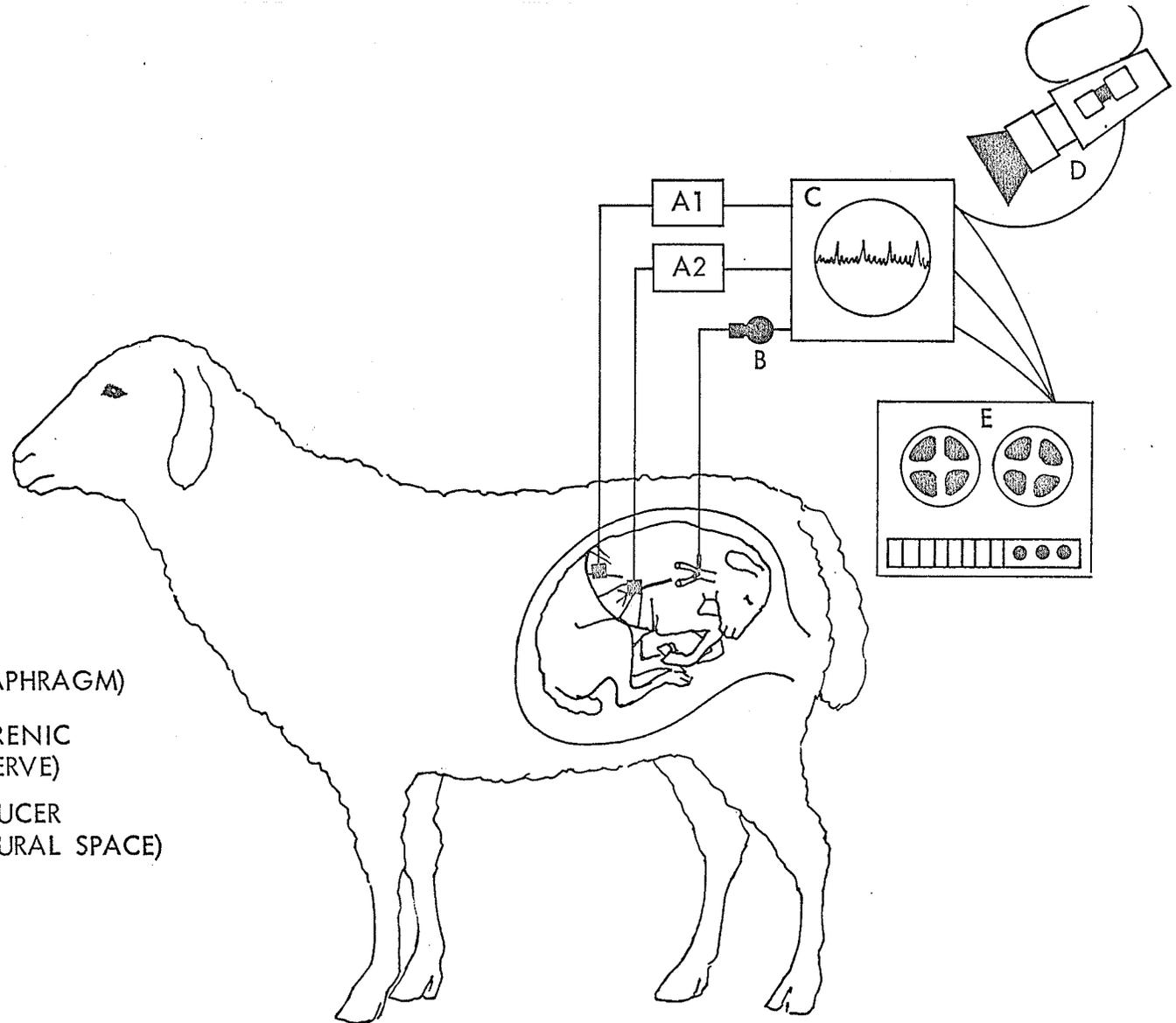


Figure 1

RESULTS

The record of phrenic motoneuronal discharge during the experiment demonstrated two types of activity: the first a relative short activity, lasting for an average of 267.0 m sec and the second a relatively long lasting activity, with an average of 1.9 sec, with a highest amplitude of 250 μ V. These action potentials generally appeared in three forms: as a single burst of activity, as irregular episodic bursts of activity or as a continuous rhythmical activity. Each single burst of activity (Fig. 2) appeared without a stable pattern interspersed among the activities of two other forms or alone, during the quiescent periods. The repetitive non-rhythmical bursts of activity (Fig. 3) were the most prevalent type of activity seen during the experiments. They demonstrated a totally irregular pattern both in frequency and duration and independent of the time of day, or gestational age. Such bursts of activity were interspersed with quiescent periods of various duration. Occasionally a single brief burst of activity appeared during quiescent periods. Continuous rhythmic phrenic nerve activity (Fig. 5) was recorded in two out of four fetuses, at the end of the experimental period of 16 and 20 days respectively. This type of activity was in addition to the other two types of activity recorded previously. These two fetuses died about 12 hours after the appearance of this type of activity. The frequency of the activity recorded was 18 and 21 bursts per minute and each burst lasted up to 480 m sec. They were interspersed by very brief quiescent periods and by single bursts. The total reported recording time was 29.4 hours. During this period there was a total of 1313 bursts of phrenic activity of less than 500 m sec duration (an average of 267.8 m sec), and 462 periods of activity of more than 500 m sec duration. This gives an average of 1.01 phrenic bursts per

minute. Since the tracheal or pleural pressure changes, as well as EMG of the diaphragm were used only as a confirmation of the activity recorded from the phrenic electrodes previous calibration on these parameters were deemed necessary.

Fig. 2

An example of a single burst of phrenic nerve activity. The upper trace is pleural pressure, middle trace phrenic nerve activity, and bottom trace diaphragmatic activity. One hundred m sec time marks are indicated. Note also the EKG on the phrenic nerve trace.

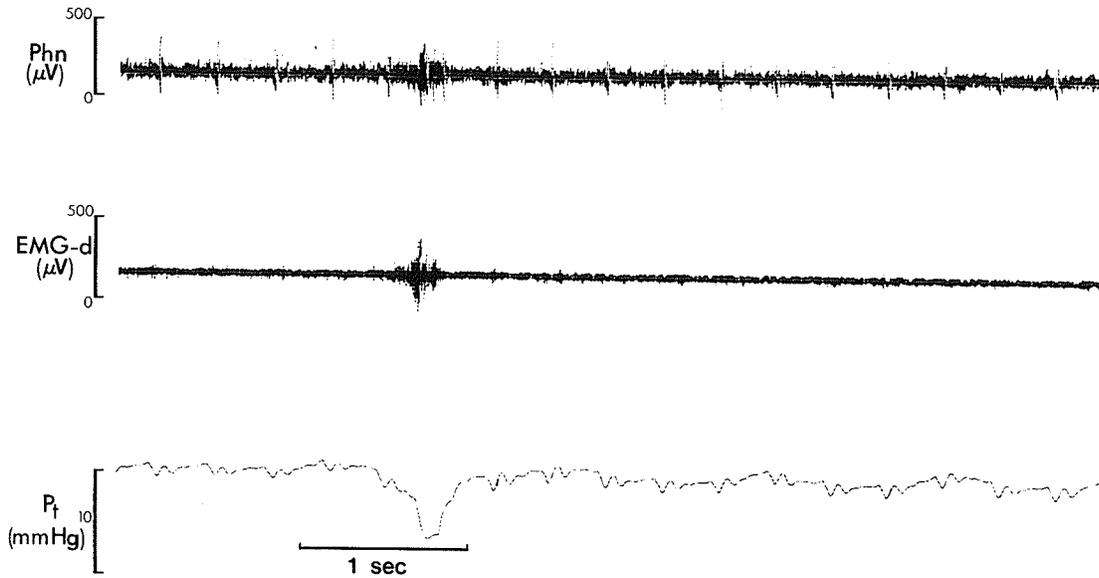


Fig. 3

The following Figure illustrates the pattern of episodic, irregular bursts of activity. This was the most prevalently seen pattern during the experiments.

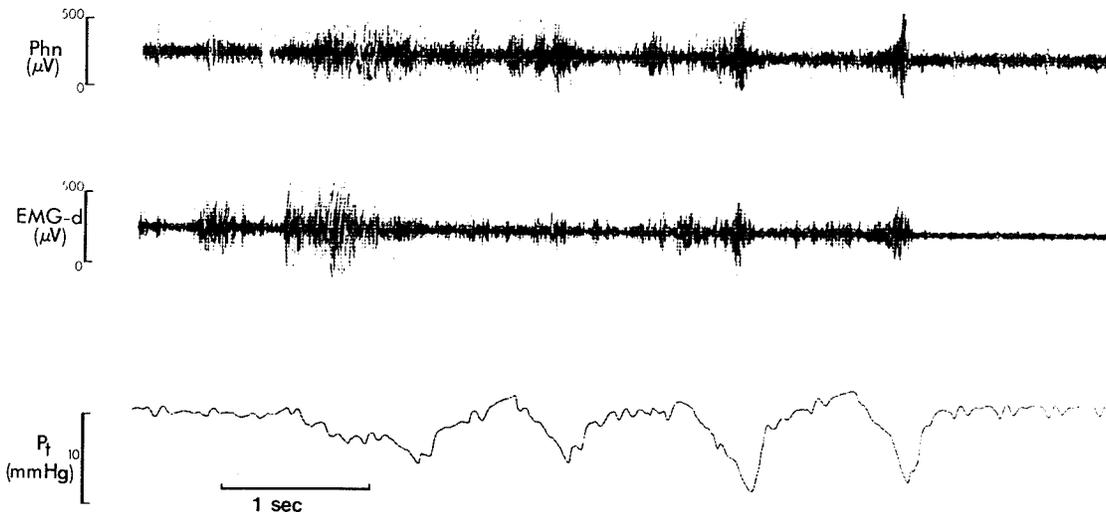
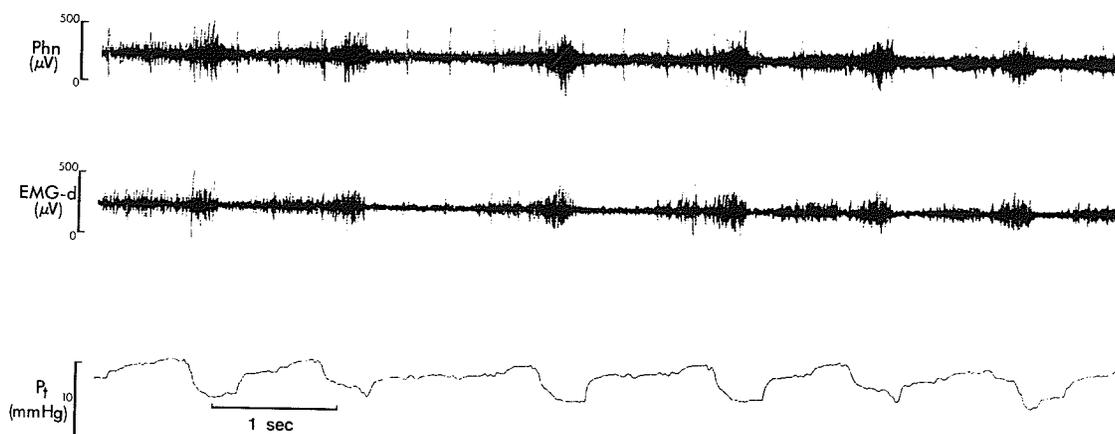


Fig. 4

Continuous rhythmical activity was recorded from fetuses of two experimental ewes that showed early signs of labor. The fetuses in both cases died about 12 hours after the appearance of such respiratory activity. The record was taken 16 and 20 days after the recording devices had been implanted.



Exp. 1. Fetus No. 234

Record Number	Gestation (Days)	Duration of the record (min)	Number and average duration of the phrenic nerve activity		Blood gases	Comments
			<500m sec	≥500m sec		
1	132	75	31 (225.8)	22 (1321.0)		Operated May 18/73 Gestational age: 125 d. First record May 25/73
2	132	75	13 (180.7)	4 (875.3)		
3	134	75	55 (219.5)	10 (1900.1)		
4	137	75	51 (236.7)	18 (1533.4)		
5	137	75	199 (343.0)	13 (851.2)		
6	143	75	70 (235.7)	18 (1763.5)		
7	143	75	76 (280.2)	5 (975.0)	pH 7.38	
8	145	75	35 (195.0)	12 (2093.1)	PO ₂ 25 PCO ₂ 39	
9	145	75	61 (268.4)	35 (1143.6)	pH 7.32 PO ₂ 25 PCO ₂ 41	
10	145	75	54 (184.3)	21 (2159.2)	pH 7.09 PO ₂ 26	
11	145	25	4 (143.7)	0 (0)	PCO ₂ 55	Fetus died.
Total		775	649 (228.9)	158 (1461.5)		

Exp. 2 Fetus No. 219

Record Number	Gestation (days)	Duration of the record (min)	Number and average duration of the phrenic nerve activity		Blood gases	Comments
			< 500m sec	> 500m sec		
1	135	60	19 (199.0)	3 (1100.0)		Operated May 4/73 Gestational age 126 days First record May 13/73
2	136	60	59 (365.3)	0 (0)		
3	137	60	59 (371.0)	25 (578.7)		
4	138	60	60 (347.8)	19 (800.6)	pH 7.25 PO ₂ 26 PCO ₂ 40	
5	138	60	87 (239.0)	12 (1229.0)		
6	139	90	Rhythmic phrenic nerve activity- Average duration 480m sec			Fetus died
Total		350	294 (304.4)	56 (927.0)		

Exp 3. Fetus No. 47 (BB)

Record Number	Gestation (days)	Duration of the record (min)	Number and average duration of the phrenic nerve activity		Blood gases	Comments
			<500msec	>500msec		
1	131	70	14 (221.4)	13 (4516.9)		Operated April 4/73 Gestational age 121 days First record April 14/73
2	136	70	17 (282.0)	38 (3678.4)		
3	136	70	37 (276.7)	17 (3900)		
4	136	70	17 (222.3)	22 (5840.9)		
5	139	70	122 (275.6)	51 (1070.5)		Fetus died.
Total		350	207 (255.6)	141 (3773.3)		

Exp. 4 Fetus No. 226

Record Number	Gestation (days)	Duration of the record (min)	Number and average duration of the phrenic nerve activity		Blood gases	Comments
			< 500m sec	>500m sec		
1	135	70	44 (292.6)	36 (1755.5)		Operated May 7/73 Gestational age 127 days First record May 15/73
2	137	70	39 (232.3)	10 (1687.5)		
3	137	70	50 (283.5)	46 (1898.1)		
4	139	70	30 (323.3)	15 (1588.3)		
Total		280	163 (282.9)	107 (1732.3)		

Discussion

The phrenic nerve activity is a fraction of the total nervous output from the respiratory center (23). Every change in the respiratory center discharge brings about a corresponding change in the phrenic nerve activity.

The data obtained in the experiments reported in this thesis demonstrated the existence of infrequent and irregular phrenic nerve activity of the fetal lamb in-utero. This data suggests that the main elements of the respiratory complex are fully developed in the last third of fetal life; however, the output from the respiratory center is quite different from the regular rhythmic discharge of breathing animals.

It is pertinent to consider how the results of the experiments might have been influenced by the experimental conditions, and further to consider the results of the present study in the light of general knowledge about fetal respiratory movements.

Influences of the Experimental Condition:

It is well known that the behavior of the fetus is largely dependent upon the integrity of its environment. Thus the main object of the experimental design in the present study was to make observations and measurements of the unanesthetized fetus in-utero in as close to normal physiological conditions as possible. In most previously reported studies on fetal sheep exteriorisation of the uterus has been a practical necessity(19). It is very difficult to make any observations on the fetus, and almost impossible to make any measurement or distinguish fetal respiratory movements through the intact uterine wall. Incision of the uterine wall represents a disturbance of the fetal environment which

may be sufficient to trigger the activity of the fetal respiratory center. However we allowed 7 days for recovery following fetal surgery, several longer than the recovery period recommended by Shelley (37). It was reported that subsequent to fetal exteriorization there is a decrease in fetal oxygenation alteration in placental transfer of gases and fall in fetal blood pressure (19). Such deterioration of the exteriorized fetus can be associated with sporadic or even rhythmical respiratory center discharge. In any work concerning the activity of the fetal respiratory complex the effect of anesthesia deserves special emphasis. The depressive effect of almost all known anesthetics upon respiration has been elaborated by many investigators (9). Normal activity of the respiratory center cannot be expected if anesthesia of any type is employed. Thus the negative finding concerning fetal respiration cannot be considered as conclusive unless sufficient time is allowed for recovery from anesthesia. This lead some authors (3) to use a method of total removal of the fetus from the uterus with subsequent replacement. Such an effort of obtaining data from the non-anesthetised fetus has rarely been successful because of total loss of amniotic fluid and marked contraction of uterus following loss of its content. There is at least some suggestive evidence that the loss of amniotic fluid per se may be significant in the alteration of growth and development of the fetus (19). Knowing the dependence of data obtained from the fetus upon all previously discussed factors, the importance of carrying out the fetal physiologic studies under chronic condition and without loss of amniotic fluid becomes obvious. If the operation was carried out in the proper way the fetus could be maintained in good general condition, and normal fetal-maternal relationships remain undisturbed. The development of the

chronic preparation made possible the collection of data concerning the general condition of the fetus over a long period of time and the validity of any physiologic study.

Dawes and his co-workers (10) frequently used the chronic preparation in their research of prenatal life, and also specifically studied fetal respiratory movements. By measuring the tracheal pressure and flow with devices chronically implanted into the fetus, they considered that they were measuring the respiratory activity of the fetus. However by measuring the tracheal pressure and flow changes one cannot accurately say that such change represent only the output of the fetal respiratory center. The fetal lung is filled with liquid and the fetus is surrounded by the amniotic liquor. Thus, pressure and even flow changes in the trachea may reflect transmitted pressure changes from influences other than respiratory movements. Thus in a sense the fetal lung is acting like a liquid plethysmograph. Therefore the experiment presented in this thesis was undertaken to study the electrical activity of the fetal phrenic nerve whose discharge is a direct reflection of the fetal respiratory center activity. The experiment was carried out under a chronic condition to minimize the effects of anesthesia, surgery and other mechanical and thermal influences upon the fetus and its environment.

Despite extensive fetal surgery a one week recovery period should have allowed sufficient time for the fetus to recover from the operative procedure.

Comparing of Present Results with Those Obtained by Others :

It is still an open question whether or not the fetus normally makes respiratory movements in utero. If it does what is the

frequency and length of such activity.

Results and arguments presented in this thesis extend the observations on the behavior of normal fetuses and enable us to distinguish fetal respiratory movements from potential artifacts.

It would be interesting to compare data presented in this thesis with those of other authors. Some investigators believe that triggering of the fetal respiratory center activity can be elicited only under condition of fetal distress (1). Fetal blood gases have been generally accepted as criteria for fetal normality. Thus deterioration of fetal blood gases is the most reliable expression of the deterioration of the fetal physiological state. Accordingly it might be expected that fetal respiratory movements would only be initiated when blood gases were not within normal limits. However Dawes and his collaborators observed respiratory movements in all fetal lambs in good physiological condition as judged by their blood gases. That is identical with our findings. In the present experiment fetuses were situated in an environment with well preserved fetal maternal relationship and abundantly surrounded by amniotic fluid. Even under such optimal experimental conditions fetuses experienced infrequent but well developed respiratory movement as judged by the phrenic nerve activity and changes in the pleural or tracheal pressure.

These data evidently do not confirm the inhibition hypothesis described by Barcroft (1946) (4) as well as Tchobroutsky's (34) suggestion that the inhibition of the respiratory movements in utero may be regarded as an expression of the diving reflex provoked by the contact of the glottis with liquid.

The main purpose of the current experiment was to accurately define the frequency and the duration of fetal respiratory movements in utero. Dawes and his co-workers reported (10,11) a type of respiratory movement described as a gasp. The gasp appeared irregularly with a slow rate of 1-3 per minute as a short, deep inspiratory effort. The second type of respiratory activity that Dawes observed was a shallow, irregular rhythmic respiratory activity with a frequency of 3-4 Hz and lasting from a few seconds to an hour. Findings presented in this thesis suggest the existence of infrequent and irregular respiratory movements which are synchronous with fetal phrenic nerve discharge.

In many respects concerning fetal respiratory movements the findings previously described in this thesis fit well with those of Dawes and his collaborators. We can agree with Dawes about the existence of irregular relatively deep inspiratory efforts because similar activities were seen during our experiment coupled with phrenic nerve activity.

We can also say that short bursts of phrenic nerve activity was sometimes accompanied with short but very fast changes in tracheal pressure, that in a sense, may fit in a range of frequency between 1-4 Hz. However this activity has to be clearly distinguished from so-called "irregular rhythmic" respiratory movements. We did see tracheal pressure changes similar to that described by Dawes. However, there was no phrenic nerve activity associated with such pressure changes. Even in the presence of "irregular rhythmic" pressure changes a burst of phrenic nerve activity caused a typical inspiratory effort. Thus, we agree with the suggestion made by Ponte and Purves (27) that the artifacts in the fetal intra-tracheal or intra-esophageal pressure records may be due to heart beat or fasciculation of intercostal or scalene muscle.

BIBLIOGRAPHY

1. ADAMS, F.H., DESILET, T.D., TOWERS, B. Control of flow of fetal fluid at the laryngeal outlet. *Resp. Physiol.* 2: 302-309, 1967.
2. AVERY, M.E. The lung and its disorders in the newborn infant. W.B. Saunders Comp. Philadelphia and London, 1964.
3. BARCROFT, J. and BARRON, D.H. Movements in midfetal life in the sheep embryo. *J. Physiol.* 91: 329-351, 1937.
4. BARCROFT, J. and BARRON, D.H. Observations on the functional development of the fetal brain. *J. Comp. Neurol.* 77: 431-452, 1942.
5. BARCROFT, J. and BARRON, D.H. The genesis of respiratory movements in the fetus of the sheep. *J. Physiol. (London)*. 88: 56-61, 1936.
6. BARCROFT, J. and BARRON, D.H., COWIS, A.T. and FORSHAM, P.H. The oxygen supply of the fetal brain and the effect of asphyxia in the fetal respiratory movements. *J. Physiol. (London)*. 97: 338-346, 1940.
7. BODDY, K. and MANTELL, C.D. Observations of fetal breathing movements transmitted through maternal abdominal wall. *The Lancet*. 1219-1220, 1972.
8. CARTER, W., BECKER, R.F., KING, J.E. and BARRY, W.F.: Intrauterine respiration in the rat fetus. II Analysis of roentgenological techniques. *Am. J. Obst. Gynec.* 90: 247-256, 1964.
9. CHERNICK, V., HAVLICEK, V. and PAGTAKHAN, R. Respiratory response to electrical stimulation of the brain stem of the fetal and neonatal sheep. *Ped. Res.* 7: 20-27, 1973.
10. DAWES, G.S., FOX, H.E., LEDUC, B.M., LIGGINS, G.C. and RICHARDS, R.T. Respiratory movements and rapid eye movements sleep in the fetal sheep. *J. Physiol.* 220: 119-143, 1972.
11. DAWES, G.S. Fetal and neonatal respiration. In *Recent Advances in Pediatrics*. 4th Edition. Eds. D. Gairdner and D. Hull. E.A. Churchill Ltd. London. 1-22, 1971.
12. DAVIS, M.E. and POTTER, E.L. Intrauterine respiration of the human fetus. *J.A.M.A.* 131: 1194-1201, 1946.
13. DURON, B. and CAILLLOL, M.C. Investigation of afferent activity in the intact phrenic nerve with bipolar electrodes. *Acta. Neurol. Exp.* 33: 427-432, 1973. 355-380, 1973.
14. FITZGERALD, R.S. Relationship between tidal volume and phrenic nerve activity during hypercapnia and hypoxia. *Acta. Neurol. Exp.* 33: 419-425, 1973.
15. GILL, P.K. and KUNO, M. Properties of phrenic motoneurons. *J. Physiol.* 168: 258-273, 1963.

16. GILL, P.K. and KUNO, M. Excitatory and inhibitory actions on phrenic motoneurons. *J. Physiol.* 168: 274-289, 1963.
17. HARNED, H. Jr., HARRINGTON, R. and FERREIRO, J. The effects of immersion and temperature on respiration in newborn lambs. *Pediat.* 45: 598-605, 1970.
18. IRANI, B., MEGIRIAN, D. and SHERREY, J. An analysis of reflex changes in excitability of phrenic, laryngeal and intercostal motoneurons. *Exp. Neurol.* 36: 1-13, 1972.
19. JACKSON, B.T. and EQDAHL, R. The performance of complex fetal operations in utero without amniotic fluid loss or other disturbances of fetal-maternal relationship. *Surgery* 48: 564-569, 1960.
20. KINDERMANN, W. and PLESCHKA, K. Phrenic nerve responses to passive muscle stretch at different arterial CO₂ tensions. *Resp. Physiol.* 17: 227-237, 1973.
21. KING, E. and BECKER, R. Intrauterine respiration of the rat fetus. III Aspiration and swallowing of calcodur blue dye. *Am. J. Obstet. and Gynec.* 90: 257-263, 1964.
22. LARRABEE, M.G., and KNOLTON, G.C. Excitation and inhibition of phrenic motor neurones. *J. Neurophysiol.* 5: 75-88, 1941.
23. LOURENCO, R., CHERNIACK, H.S., MALM, J.R. and FISHMAN, A. Nervous output from the respiratory center during obstructed breathing. *J. Appl. Physiol.* 21: 517-533, 1966.
24. McCARTY, L.P. Stimulating electrodes for nerves. *J. Appl. Physiol.* 20(3): 542-543, 1965.
25. PITTS, R. Excitation and inhibition of phrenic motor neurones. *J. Neurophys.* 5: 75-88, 1942.
26. PITTS, R. The function of components of the respiratory complex. *J. Neurophysiol.* 5: 404-413, 1942.
27. PONTE, J. and PURVES, M.J. Types of afferent nervous activity which may be measured in the vagus nerve of the sheep fetus. *J. Physiol.* 229: 51-76, 1973.
28. POTTER, E. and BOHLENDER, G. Intrauterine respiration in relation to development of the fetal lung. *Am. J. Obstet. and Gynec.* 14-22, 1942.
29. ROSENFELD, H. and SNYDER, F. Stages of development of respiration regulation and changes occurring at birth. *Am. J. Physiol.* 119: 406-415, 1937.

30. REIFFERSCHIED, K. and SCHMIEMANN, R. Surg. Gynec and Obstet. (Internal Abstract) 71: 278, 1940.
31. SISSON, S. and GROSSMAN, J.D. Anatomy of the domestic animals. W.B. Saunders Com. Philadelphia and London 4th Ed.
32. SNYDER, F. and ROSENFELD, H. Direct observations of intrauterine respiratory movements of the fetus and the role of carbon dioxide and oxygen in their regulation. Proc. Soc. Exper. Biol. and Med. 33: 516-598, 1936.
33. SOMA, L.R., WHITE, R.J. and KANE, P.B. Surgical preparation of a chronic maternal-fetal model in pregnant sheep; a technique for measurement of middle uterine blood flow, umbilical blood flow, and fetal sampling in the awake sheep. J. Sneg. Res. 11: 85-94, 1971.
34. TCHOBROUTSKY, C., MERLET, C. and REY, P. The diving reflex in rabbit, sheep and newborn lamb and its afferent pathways. Res. Phys. 8: 108-117, 1969.
35. TOMORI, Z., JAVORKA, K. and STRANSKY, A. Reflex stimulation of the upper respiratory tract. Acta. Neurolbiol. Exp. 33: 57-69, 1973.
36. WINDLE, W., BECKER, R.F., BARTH, E.E. and SHULTZ, M. Aspiration of amniotic fluid by the fetus. An experimental roentgenological study in the guinea pig. Surg. Gynec. Obst. 69: 705-712, 1939.
37. WINDLE, W.F. and MONNIER, M. Fetal respiratory movements in cat. Physiol. Vol 11: 425-433, 1938.

CONCLUSION

The present experiments clearly indicate that regular rhythmic respiration is not normally present in the fetal sheep in-utero but may be present during fetal distress. However, respiratory center activity is present during the latter third of the fetal life in the form of irregular repetitive bursts of electrical activity in the phrenic nerve or in the form of a single brief electrical activity interrupted with quiescent periods of various duration. These two patterns are quite different from the regular rhythmic respiratory discharge of breathing animals. These data suggest that although the capability for regular rhythmic respiration is present during later fetal life, the respiratory apparatus requires some triggering stimulation before regular respiration is initiated. Irregular respiratory center discharge might occur de novo or as a result of input from higher centers but further experiments are required to clarify this point.