INITIATION OF RESPIRATION

PART I. Review of the Literature

Part II. Experimental Study

A. Appraisal of the Role of Arterial Blood Pressure

B. Relative Contribution of Changes in Arterial Gas Tensions

A Thesis

Presented to

The University of Manitoba

In Partial Fullfillment

of the Requirements for the Degree

Master of Science in Physiology

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by

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October 1969

TO MY WIFE AND PARENTS

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to his Adviser, Dr. Victor Chernick, for his keen interest in this study from its onset until its completion and for his criticism during the preparation of the manuscript. The assistance of Dr. Edmund Faridy has been invaluable. The author is also grateful to Professor Arnold Naimark, Chairman, Department of Physiology, University of Manitoba. The technical assistance of Mr. Brian Russell and the patience of Miss Marilyn Davison in typing the manuscript are gratefully acknowledged.

The work was performed under the Medical Research Council of Canada grant held by Dr. V. Chernick and during the author's tenure as Research Fellow in the Department of Pediatrics, University of Manitoba.

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PART I

A review of the literature on the initiation of respiration reveals that the peripheral and central chemoreceptor and baroreceptor reflex mechanisms are indeed functional during the perinatal period and, hence, their potential role in the onset of breathing at birth. The weight of evidence favors the view that the fetus *in utero* does not perform rhythmic respiratory movements until such time as it is stimulated to breathe at the time of birth. Asphyxia at parturition triggers the first breath but the individual contribution of each chemical change has yet to be differentiated. Furthermore, both physical and chemical stimuli appear to play a role in the initiation of this crucial event. The precise importance of each factor requires investigation. For example, the evidence for the part played by such sensory stimuli as changes in blood pressure has not been conclusive. It was thus of interest to investigate not only the relative importances of each chemical factor but also the influence of fluctuations in blood pressure, alone or jointly, in the onset of the first breath.

PART II

Phase One of the investigation delineated the influence of changes in mean systemic pressure in 14 exteriorized fetal lambs. Respiratory efforts were monitored using a liquid plethysmograph. Changes in mean systemic pressure in the absence of changes in blood gas tensions were produced on 15 occasions by rapid infusion or withdrawal of blood, infusion

of norepinephrine or occlusion of one umbilical artery. Despite change of mean blood pressure of as much as ±50%, breathing was not initiated. On 18 occasions alterations in mean blood pressure with concomitant hypercapnia and hypoxemia were produced by either occlusion of the umbilical vein, both umbilical arteries, entire umbilical cord or the maternal abdominal aorta. In all instances fetal gasping was evoked indicating that the initiation of breathing is independent of the site of interruption of fetal-placental gas exchange. By several methods of analysis there was no relationship between the mean blood pressure and the arterial PCO_2 and PO_2 at the time of gasp. Also, mean blood pressure did not influence the latent period. It is concluded that changes in arterial systemic mean pressure per se cannot initiate respiration. Furthermore, there is no interaction between hypercapnia-hypoxemia and mean blood pressure in the initiation of breathing of fetal lambs.

Phase Two of the experimental study evaluated the relative importances of arterial gas tensions in 14 exteriorized fetuses. The fetus was cross-circulated with artificially ventilated newborn lamb at a flow rate of 250-300 ml/min. During cross-circulation with the the newborn donor breathing room air, the fetus maintained its arterial gas tensions and pH at normal levels and remained in the normal apneic state for as long as 25 minutes even if the umbilical cord was occluded throughout this period. This observation conclusively showed for the first time that cord occlusion per se cannot initiate breathing. Fetal liquid ventilation was initiated on 29 occasions by adjusting the alveolar ventilation and composition of the inspired gas of the newborn donor. At the time of the first breath, there was a positive relationship between the levels of fetal P_aO_2 and P_aCO_2. This relationship appeared to be linear: $P_aO_2 = 0.13\ (P_aCO_2) + 0.24$; ($r = + 0.80$; $p < 0.001$). At a fetal P_aCO_2 below 40 mm Hg, the first breath did not occur until the P_aO_2 was 5 mm Hg or less; at level of P_aCO_2 between 40 and 100 mm Hg, the P_aO_2 at the time of the first breath was always above 5 mm Hg and ranged from 6 to 16 mm Hg; and at fetal P_aCO_2 above 100 mm Hg, the first breath was initiated at P_aO_2 above 16 mm Hg and as high as 20 mm Hg. It is concluded that there is an interaction between arterial PO_2 and PCO_2 in the initiation of respiration in sheep fetus.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

At birth, after the placenta can no longer provide oxygen and remove carbon dioxide for the fetus, the onset of breathing is the most crucial act essential for survival. Sir Joseph Barcroft (1) in 1947 classically pictured this momentous event as "the earnest of a new vital principle" and "the necessary initiation of life in a new environment". The precise mechanisms that trigger this event have long been of vital interest to the physiologist.

Information on the nature of the initial respiratory stimulus or stimuli is also apropos to the clinician's choice of resuscitative measures for the newborn born with depressed respiratory function. Indeed, such knowledge should have a highly practical clinical significance since human neonatal death, highest in the first 48 hours after delivery, is more commonly associated with failure of respiratory adaptation than with any other adaptational failure once the fetus has attained a gestational age compatible with extrauterine existence (2,3). It is thus curious to note that relatively little attention has been directed to the study of this problem that has both physiological and clinical importance, compared to the enormous advances recently made in the understanding of the regulation of respiration in the adult (4).

Heretofore, the influence of changes in systemic arterial blood pressure on the onset of breathing has not been delineated. Neither has the role of umbilical cord occlusion per se nor the relative contribution of either hypoxemia, hypercapnia or acidemia been clearly differentiated

(5,6,7). Yet occlusion of the umbilical cord, which invariably initiates respiration (1,7,8,9,10), is accompanied by hypoxemia, hypercapnia acidemia and fluctuations in blood pressure.

The present experiments on sheep fetus have been designed to obtain conclusive answers to the specific issues raised above. The first phase of the investigation deals with the influence of changes in blood pressure and the second phase concerns itself with the relative importances of alterations in arterial gas tensions and the role of cord occlusion per se.

A survey of the literature, divided into two sections, will be given to clarify the background of the present investigation. The first considers the morphologic and the functional bases of perinatal respiration. The second section considers the various hypotheses that have been proposed in the genesis of the first breath.

The critical review of the literature and the analysis of the experimental results constitute the mainstay of this thesis.

PART I. REVIEW OF THE LITERATURE

I. Morphologic and Physiologic Bases of Perinatal Respiration

II. Consideration of the Hypotheses for the Initiation of the First Breath

PART I

A REVIEW OF THE LITERATURE

I. Morphologic and physiologic bases of perinatal respiration

In this section are reviewed the functional morphology of the lung as an organ of gaseous exchange and certain aspects of development of the neural respiratory apparatus. Some aspects of respiratory control in the neonate are discussed in relation to known experimental results in the adult.

A. Development of the lung as the organ of gas exchange

Fundamental to the establishment of air breathing are the anatomical and the functional preparedness of the lungs. Lung originates from the gut endoderm and from the mesoderm (11,12). In the human fetus, an outgrowth of cells from the endoderm appears at 24 days of gestation. This collection of cells later develops into the epithelial portion of the lung. The rest of the lung develops from the mesoderm. By the twenty-eighth week of gestation, a sufficient number of capillaries appear in close contact with the future air spaces. This marks the beginning of the alveolar period in lung development which was heralded by the earlier glandular (series of bronchial tubes) and canalicular (canalicular spaces destined to be the respiratory portion of the lung) periods. Both the combined alveolar surface area and the number of capillaries in contact with the alveolar surfaces are structurally not adapted for pulmonary gaseous exchange prior to six months of gestation, but they continue to grow from this time onwards throughout intrauterine life. Therefore, the human lung at term can efficiently assume its main function as the organ of gas exchange.

The degree of pulmonary development varies among different species (7). Thus, the lungs of sheep are relatively more developed than that of man at birth. Inasmuch as the human lung is morphologically ready for gaseous exchange at birth, it can be inferred that the sheep lung must be similarly prepared at term.

However, the lungs need to develop not only air spaces but also surface active material (surfactant) before they can be effectively ventilated. In the sheep fetus, Born and co-workers (13) and Howatt et al (14) showed that this material, which is a complex of proteins and lipids particularly dipalmitoyl lecithin and is believed to be produced by the granular alveolar epithelial cells, first appeared at 125 days gestation (0.85 of term). The importance of this surface active material has been demonstrated by Reynolds and Strang (15). They observed that in immature lambs of 110-120 days of gestation (0.75 of term), the lungs collapsed completely on deflation whereas near term (140 days) the alveoli remained distended following the first inflation. Further, they showed on a Wilhelmy surface-tension balance that the extract from immature lung had a higher surface tension on compression of the film than that of the mature lung. The absence of surfactant in immature lambs explains the higher surface tension which in turn provides an explanation for the complete collapse of the lungs on deflation. In contrast, the presence or absence of surfactant does not affect the pressure required to inflate the lungs initially. Therefore in immature lungs, the opening pressure for subsequent breaths will be as great as that for the first breath. In contrast, a large transpulmonary pressure is not required for the second breath in term lungs because the term lungs retain residual air. Consequently, there is greater work of breathing with each breath in the immature group.

B. Development of the neural respiratory apparatus

In the sheep fetus, Barcroft (1) in collaboration with Barron were able to elicit by mechanical stimulation of the face, respiratory neuromuscular movement of the diaphragm as early as the 38th day (0.27 of term) of gestation (term = 145-147 days) and showed that by the 60th day onwards the lamb was set for the first breath as indicated by its response to oxygen-want. Thus, the stage is set for the first gasp although only less than half of the gestation period has elapsed.

Pronin (16) observed spontaneous respiration in rabbit fetuses on the 21st day of intrauterine development (term = 31 days) and concluded that the respiratory center became functional at this time. Moreover, he demonstrated that respiratory movements could be induced by tactile and painful stimulation of the skin as early as the 19th day (0.61 of term) of gestation.

The conclusions of these investigators regarding the functional state of the fetal respiratory center have been arrived at using indirect methods. To date, there is no data defining the activity of respiratory neurons in the developing fetus.

C. Chemical receptors influencing ventilation

It is well established in the adult that both the peripheral and central chemoreceptors regulate respiration through blood oxygen tension and acid-base shifts (17). Thus, 10% oxygen breathing gives rise to a sustained hyperventilation when the peripheral chemoreceptors are intact. If these receptors are denervated ventilation is no longer affected by the same degree of hypoxia, but extreme hypoxia depresses respiration. Moreover, "physiological denervation" of these receptors can be achieved by increasing the oxygen concentration of the inspired gas above 21%; 100% O_2 to the inspired gas can effectively abolish the normal peripheral chemoreceptor drive and decreases minute ventilation (18). The carotid bodies also react to infusion of hypercapnic blood with transient increase in ventilation in adult dogs (19,20,21,22) and with initiation of respiratory movements in the exteriorized fetal guinea pig and lamb (23). Recently, Fitzgerald and co-workers (24) showed in adult dogs that transient vertebral infusion of hypercapnic blood also increased ventilation. Thus, whereas hypoxemia stimulates respiration normally only through the peripheral chemoreceptors, hypercapnia acts on both the peripheral and central receptors. The significance of chemoreceptors and their physiologic role have more recently gained the increasing attention of researchers in perinatal biology.

1. Peripheral chemoreceptors

There is abundant experimental evidence for the presence of functional carotid and aortic bodies in various fetal and newborn animals. In 1948, Barcroft and Karvonen (25) examined the response of anesthetized fetal sheep at 60 and 70 days gestation and at full term to cyanide injected into the umbilical vein and to CO_2 administered via the mother. Early in fetal life, cyanide induced vigorous respiratory movements of the same type and rhythm as that induced by 7 to 8% CO_2 in air administered via a tracheal cannula to the mother. Their observations following cyanide were attributed to a functioning carotid body chemoreflex at this early age in the fetus. They postulated that carbon dioxide was acting at the central and peripheral levels. However, they did not provide direct evidence for this hypothesis such as repeating their experiments following peripheral chemoreceptor denervation. To support their conclusion, they made an analogy with the experiments of Comroe (26) who had earlier shown in adult animals that the momentary stimulation of respiratory movements by cyanide, a known depressant of the respiratory center, was entirely due to the peripheral chemoreceptors mainly those of the carotid body.

In mature fetal lambs, cyanide failed to elicit a respiratory response presumably due to inhibition from some higher centers at term (1). Reynolds and Mackie (27) made similar observations, but showed that cyanide was an effective respiratory stimulant even in the term animal in the presence of other cutaneous sensory stimuli. Later, Dawes and co-workers (28) showed that the level of anesthesia must be taken into consideration. In lightly anesthetized fetal lambs (gestational age 120-141 days), they showed that cyanide produced respiratory movements which persisted after section of the vagi and cervical sympathetics but were abolished by carotid sinus nerve transection. The dose had to be increased by threefold in order to obtain a response in the youngest group (89-91 days gestation). This work suggests that either the peripheral chemoreceptors become more responsive with increasing gestational age or there is less inhibition by central mechanisms.

Although it may be ineffective when used alone in the term fetus, cyanide elicits a brisk respiratory response in anesthetized lambs shortly after birth (27). Dawes and Mott (29) and Biscoe and Purves (30) have furnished further experimental evidence for the presence of a functional peripheral chemoreflex at birth. The former group of investigators showed that intravenous injection of cyanide, lobeline or nicotine in newborn rabbits caused an increase in ventilation. The latter investigators showed that the unanesthetized newborn lamb had decreased ventilation when breathing 100% oxygen, increased when breathing low concentrations of oxygen and had an increased sensitivity to carbon dioxide when hypoxic. In both experiments, the responses were abolished or markedly diminished following transection of the carotid sinus and vagus nerves.

The first direct recordings from the carotid sinus nerves were done by Cross and Malcolm (31) in 1952. They found an increase in impulse activity in the sinus nerves in newborn animals on exposure to low partial pressure of oxygen. The same finding was confirmed by Biscoe and Purves (32) in 1967 also in newborn lambs. Cross and Malcolm (31) also demonstrated an increase in sinus nerve activity in full term sheep fetuses following exposure of their mother to a hypoxic gas mixture. In 1965, Biscoe and Purves (33) measured and compared the carotid chemoreceptor activity in 6 term fetal lambs before and after the first breath. The activity was found to be low prior to the first breath and increased with the onset of respiration. Harned et al (9) similarly showed in term fetal lamb an increase in chemoreceptor discharge in whole carotid sinus nerve immediately after cord occlusion.

The foregoing direct recordings in both the fetus and the newborn were, however, made from multifiber preparations of sinus nerve. The chemoreceptor origin of impulse activity recorded from such large strands of nerve is open to question. Indeed, Biscoe, Purves and Sampson (34) showed in their study of 22 mature fetal sheep using single unit receptor nerve recording that one type (they discussed 3 types) of activity was irregular in nature and had no clear relation to the pulse pressure wave during control conditions and was initially considered as chemoreceptor in origin. However, further tests such as inducing large changes in PO_2 in blood or saline injected into the carotid sinus at control pressure and injecting KCN, NaCN, KCl or nicotine all failed to affect the nerve activity. They then discovered that rapid elevation of the carotid sinus pressure elicited an increase in this type of impulse activity later followed by a period of quiescence. Such a sequence is typical of a baroreceptor response. They did, however, delineate one type of activity which was purely chemoreceptor in nature as established by the foregoing chemical tests demonstrating that the fetal carotid body indeed responds to cyanide. Single-fiber peripheral chemoreceptor discharges have also been shown recently by Schwieler (35) in newborn kittens exposed to varying gas mixtures.

Recently, Dawes and co-workers (36) likewise demonstrated that the aortic chemoreceptors are also active during the latter half of fetal development. These receptors in the sheep fetus receive their blood supply from the left heart only. Thus, following injection of minimal effective doses of cyanide into the left atrium of the fetal lamb, arterial blood pressure rose along with the femoral vasoconstriction and complex changes in heart rate, but rarely did any respiratory movement occur. These responses were greatly reduced or abolished by cervical vagotomy. On the other hand, injection of the same doses of cyanide into a jugular vein, the right ventricle, pulmonary or common carotid arteries caused negligible cardiovascular and respiratory effects, whereas injection into the carotid of newborn lambs elicited a profound hyperpnea. It has been shown in the adult that the aortic chemoreceptors are mainly concerned with cardiovascular regulation, and apparently not directly concerned with respiration (37). In the fetus the influence of aortic chemoreceptors (through their effects on hemodynamics) upon the respiratory response to carotid body stimulation should be taken into consideration.

Indeed, Dawes et al (36) hypothesized that the aortic chemoreceptors probably serve as the first line of defence in fetal blood gas homeostasis. This hypothesis assumes greater meaning in view of the features of the fetal circulation which is unique in that the left ventricular blood is a mixture of highly oxygenated venous blood with blood of lower oxygen content from the lungs and the inferior part of the fetus. For example, a fall in umbilical blood flow secondary to fetal hypotension should result in a decrease of left ventricular blood PO_2. The fall in arterial PO_2 would then excite the aortic chemoreceptors causing reflex femoral vasoconstriction, increase in arterial pressure, preferential redistribution of cardiac output to the placenta and eventually a rise in umbilical flow. This sequence of changes would tend to keep the arterial PO_2 at normal fetal levels. This maintenance of blood gas homeostasis by the aortic chemoreceptor mechanism tends to prevent stimulation of the carotid body before birth. This mechanism would appear to be important only in near term fetuses who have functional carotid chemoreceptors. The latter is suggested since Biscoe, Purves and Sampson (34) failed to record tonic carotid chemoreceptor activity nor could they elicit such activity with clamping of the cord in fetal lambs less than 125 days gestation in contrast to the study of Barcroft and Karvonen (25) who used cyanide stimulation. It could be that asphyxic changes in gas tension are relatively less intense stimuli than cyanide particularly early in fetal life.

To test their hypothesis, Dawes et al (38) further studied the effect of hypoxemia in term fetal lambs before and after denervation of the peripheral receptor afferents. Relative hypoxemia in the fetus was induced by giving the ewe lower oxygen mixtures or room air to breathe for 6 to 10 minutes after initially breathing 100% O_2 or an O_2-enriched air mixture. A fall in fetal carotid arterial PO_2 from 37.8 ± 0.5 to 28.4 ± 0.9 mm Hg was associated with tachycardia, femoral vasoconstriction, and an increase in arterial pressure which persisted after bilateral carotid sinus nerve transection but were abolished by cutting the vago-sympathetic trunks and/or aortic nerves. Indeed, after section of the latter nerves, hypoxemia caused a fall in blood pressure and femoral vasodilatation.

Thus during the last third of gestation the aortic chemoreceptors reflexly control the circulation to maintain normal gas tensions. It is likely that the carotid body in the sheep fetus becomes responsive to changes in arterial gas tensions by 125 days gestation.

2. Central Chemoreceptors

In adult, Nicholson and Sobin (39) and Nicholson (40) demonstrated that nicotine or lobeline applied to the posterior floor of the fourth ventricle instantly depressed ventilation; cocaine also depressed ventilation after a period of hyperpnea. Thus,they proposed the presence of chemoreceptors on the posterior floor of the fourth ventricle which includes the area postrema. Later, Mitchell et al (41) demonstrated brisk respiratory responses to increased hydrogen ion concentration or PCO_2 and to rapid injection of nicotine, lobeline or cyanide into mock cerebrospinal fluid perfusing the ventrolateral surface of the medulla. Thus, they considered the site of chemoreceptor action to be in the subarachnoid space and not in the fourth ventricle nor in the region of the area postrema.

Notwithstanding the uncertainty regarding the site and morphology of central chemoreceptors, their importance in the control of respiration in the adult is well accepted (17). Further, studies have shown that CO_2, known to have both peripheral and central effects in the adult, increases ventilation in the newborn and can also stimulate the fetus (23). Thus, central receptors are probably also functional during the perinatal period and conceivably play a significant part in the initiation of breathing at birth.

In an effort to elucidate the role of central stimulation in the fetus and neonate, Hodson and co-workers (42) postulated, on the basis of information derived from studies in adult (41), that an alkaline cerebrospinal fluid (CSF) pH would inhibit breathing in utero and a rapid decrease in CSF pH could initiate respiration at birth.

However, their data showed that the acid-base relationships between blood and cerebrospinal fluid in 20 fetal lambs studied were similar to that in the adult. The CSF pH remained acidic during the last third of gestation and yet no respiratory movements were seen. Neither did alterations in CSF pH between 7.10 and 7.30 induce a respiratory response.

The findings of Hodson and co-workers (42) should not nullify or diminish the suggestion of Biscoe and Purves (30) that the initiation of breathing in sheep fetus by cord occlusion could be due not only to peripheral chemoreceptor stimulation but also to direct central activation of the mid-brain and medulla or both. To support their supposition the latter investigators showed that newborn lambs increased their ventilation in response to CO_2 in spite of carotid sinus nerve section.

Obviously, simply cutting the carotid sinus nerves but leaving the vagi intact cannot rule out a possible influence from aortic and/or pulmonary chemoreceptors. Schwieler (35) investigated this particular problem. He confirmed earlier observations that 3% CO_2 in the inspired air of newly born kittens increased their ventilation. Shortly after transecting both the carotid sinus nerves and vagi, the same hypercapnic stimulus again increased respiratory frequency and amplitude, indicating indeed a central effect. On the other hand, administering 10% O_2 usually caused a diminution of ventilation. Not infrequently, however, hypoxia resulted in a gasp-like breathing pattern of considerable duration.

Just as a gasp-like breathing pattern can occur in response to hypoxia even in the absence of all known peripheral chemoreceptors, in intact animals an increasing carotid chemoreceptor discharge due to persistent hypoxia does not guarantee increasing or continuing ventilation.

Indeed, Schwieler (35) has demonstrated in newborn kittens exposed to hypoxia an increasing chemoreceptor discharge even after respiration had ceased. In an indirect sense, this observation supports the importance of the concept of central chemosensors in the neonate, but much more direct study is required.

D. Baro- or presso-receptors

As recently as 1968 (43), the role of sino-aortic baroreceptors in postnatal respiratory excitation has been considered but has remained unsettled. In the adult, they are known to have a direct influence on the ventilatory response since an increase in baroreceptor activity reflexly inhibits ventilation and a decrease in discharge augments respiration (37). However, these receptors are mainly concerned with cardiovascular regulation. Typically they increase their rate of firing in response to increased mean arterial pressure and increase rate of change of arterial blood pressure (44). Impulses from these receptors travel up the sinus nerves to the medulla where they inhibit the activity of cardioaccelerator and vasoconstrictor centers, allowing unopposed action of the cardioinhibitory centers resulting in bradycardia, peripheral vasodilation and hypotension. Conversely, the fibers cease firing when blood pressure falls below 40 mm Hg (45), permitting uninhibited activity of vasoconstrictor and cardioaccelerator centers.

Does baroreceptor function appear early in life? Mott (46) seemed to have answered this question when she stated "the circulation of the newborn mammal is not less efficient than that of the adult". In the newborn period, a persuasive piece of evidence bearing on baroreceptor function was the demonstration of a positive linear correlation between the level of induced increase in carotid sinus pressure and the fall in systemic mean arterial pressure. Such information has been furnished by Downing (47) who studied nine newborn rabbits from 12 hours to 9 days old. When the static pressure was raised by 30 - 40 mm Hg in one carotid sinus isolated from the rest of the circulation but with its afferent nerve intact, he observed a significant fall in systemic arterial pressure. Denervation of the sinus in five animals completely abolished the response. Furtheremore, he showed that the discharge frequency of recorded action potentials from a single fiber preparation increased with an increase in arterial pressure and decreased with a decrease in pressure. Moreover, he showed that the response occurred even at the low mean arterial pressure of the newborn. That considerable tonic afferent baroreceptor activity existed in both the aortic depressor and carotid sinus nerves was indicated by the observation of significant increase in blood pressure (about 13%) on consecutive section of these nerves. Bloor (48), aside from confirming Downing's findings of a positive linear relationship between baroreceptor discharge frequency and systemic blood pressure and of a higher threshold in older rabbits, showed no difference in baroreceptor sensitivity (baroreceptor sensitivity was defined as change in discharge frequency per unit change in pressure) at various age groups with either absolute or relative blood pressure changes.

The baroreceptor reflexes may be quite responsive even during intrauterine life. This was first suggested in 1945 by Barcroft and Barron (49) who observed in the near term sheep fetus that a rise of arterial pressure caused by injection of epinephrine or norepinephrine was often associated with acute bradycardia. The bradycardia was abolished by bilateral vagotomy. About a decade later, Dawes, Mott and Rennick (50) similarly showed in the sheep that the same phenomenon could be demonstrated as early as 90 days of gestation. Moreover, sustained tachycardia has been observed in this same age fetus when subjected to hemorrhage (51). The foregoing observations only indirectly reflect the presence of functional baroreceptors in fetal life.

A few years earlier in 1952, Cross and Malcolm (31) had approached the issue more directly by measuring the action potentials recorded from proximally severed carotid sinus nerves. They observed in two near term lambs the presence of baroreceptor activity following carotid occlusion with rise in blood pressure. In 1961, Cross (52) illustrated in premature sheep fetus (130 days gestational age) tonic baroreceptor activity that was rendered more conspicuous when the mother received 100% O_2 which abolished the chemoreceptor action potentials. Harned et al (9) published similar baroreceptor recordings in fetal lambs before and after clamping the umbilical cord. All the nerve potentials were, however, obtained from multiple-fiber preparations.

Very recently, Biscoe, Purves and Sampson (34) reported their investigations into the various types of carotid sinus nerve activity from single unit. In all 22 fetal lambs studied (120-147 days gestational age) baroreceptor afferent activity was identified. Two types of such activity were described: 1) regular baroreceptor afferent in which the activity is synchronous with the pulse pressure wave, closely follows local (carotid artery) changes in arterial pressure and is not affected by large changes in arterial PO_2 or intracarotid injection of cyanide or nicotine, and 2) irregular presso-receptor afferent in which there is irregular discharge without clear relation to the pulse pressure wave but otherwise exhibits the same properties as the first. Moreover, they showed that following cord clamping the responses of both the regular baroreceptor and irregular presso-receptor afferents were immediate in contrast to carotid chemoreceptor response which was delayed for about 5 seconds and coincided with the fall in arterial PO_2. Also, they found that stimulation of the sympathetic nerves increased baroreceptor activity. This latter observation is at variance with the response in adult cat (53).

E. Summary

The lung must reach the alveolar stage of development before there can be efficient gas exchange to support the newborn animal. This stage is fully reached at term gestation at which time surfactant essential for lung stability is also available. Review of the literature reveals that the peripheral and central chemoreceptor and baroreceptor reflex mechanisms are indeed functional during the perinatal period and, hence, their potential role in the onset of breathing at birth must be considered. It should be emphasized, however, that their functional presence does not confer to them a major role in the initiation of respiration. This realization leads to a critical consideration of the various hypotheses which have been invoked to explain the first breath.

II. Consideration of the hypotheses for the initiation of breath

In general four postulates have been proposed to explain the mechanism of the initial postnatal breath—henceforth to be referred to simply as the first breath. These are: 1) the first breath is a continuation of intrauterine respiratory movements; 2) it is a reaction to sudden flow of sensory impulses acting from outside the body or from within; 3) it is a response to asphyxia which accompanies delivery; and 4) it is a joint result of both asphyxia and sudden sensory bombardment at the time of birth.

A. The first breath is a continuation of intrauterine respiratory movement

This hypothesis implies that neonatal respiration is not initiated at the time of delivery, that is, the first (postnatal) breath is not the "first breath". Although seemingly a paradox, this postulate is based upon the belief that the fetus *in utero* normally performs rhythmic respiration which is continued after birth. A question is immediately raised: Does intrauterine respiratory activity normally occur? The answer to this problem has been sought from two main categories of investigations (54). Firstly, observations were made directly through the abdominal and/or uterine walls. Under these circumstances precise distinction of generalized body movements from those due to the respiratory act must be difficult. Furthermore, one would have to be assured that the open abdominal and/or uterine walls do not interfere with transplacental gas exchange. Secondly, the study of fetal respiratory passages for aspirated radiopaque dye following its injection into the amniotic sac was undertaken.

The controversy over the presence or absence of intrauterine respiratory movements has resolved itself into two fundamental views. One view holds that intrauterine respiration is a normal spontaneous event. This concept was initially suggested by Ahlfeld (55) in 1888. His viewpoint was based upon inferences drawn from tambour tracings of a rhythmic movement pattern picked up from the unopened abdominal walls of unanesthetized pregnant women. These rhythmical movements proved to be of the same frequency and amplitude as the respiratory movements of the newborn infant. Ahlfeld's observations were extended by Bonar, Blumenfeld and Fenning (56). They observed through the exposed intact uterus distinct fetal respiratory movements in 10 rabbits, 5 dogs and 13 rats. A further proponent of the same school of thought is Pronin (16) who recorded spontaneous fetal respiration by means of a thermistor which recorded the entry of air into the lung in 22-day old rabbit fetus with membranes and placental circulation intact. Their views were also shared in 1937 by Snyder and Rosenfeld (57) who studied the problem in rabbit and guinea pig fetuses. These investigators likewise observed rhythmical respiratory movements within the uterus. It should be noted, however, that their technique required the uterus to be delivered into a bath of Ringer's solution at 37°C. It is indeed likely that this manipulation interfered with placental gas exchange.

Becker and co-workers (58) noted that in most studies which favor the conclusion that the fetus normally performs respiration the investigators had interrupted the pregnancies for a variety of pathological causes. This leads to a consideration of the opposite view which holds that the fetus is lying inert insofar as respiratory movements are concerned until such time as it is stimulated to breathe by stress or other changes in its control mechanisms. The observation of Barcroft and Karvonen (25) that not all fetuses show rhythmic respiration at all times supports this view. More critical studies recently done in rats and guinea pigs (58,59) have firmly strengthened this second viewpoint. Becker and co-workers (58) compared the behavior of 75 guinea pig litters in which the uterine vessels to one horn were clamped near term. Prior to uterine vascular occlusion of one horn, the oxygenated fetuses in both horns never engaged in intrauterine respiratory-like behavior. In contrast, the group whose placental blood supply was later compromised showed intrauterine respiration. The observations were consistent for each paired guinea pig specimen, although somewhat uncertain in rat fetuses. Even with the rats, however, respiratory movements could be counted with far greater degree of certainty in fetuses from clamped horns than in fetuses from unclamped horns.

In addition to these direct observations made through the semi-transparent uterine walls and necessarily opened abdominal walls, Carter and his collaborators (59) employed roentgenological techniques. They injected thorium dioxide into the amniotic sacs of guinea pig and rat fetuses and looked for evidence of aspirated contrast medium in the lung. As before, one group was from the horn with uterine vessels clamped; the fetuses in the opposite unclamped horns served as oxygenated controls. Evidence of aspiration of the dye in the hypoxic fetus and absence of aspiration in the oxygenated group was most convincing among guinea pigs but equivocal with the rat fetuses. In order to obviate the drawback of flake formation of thorium dioxide in the rats' amniotic fluid that could prevent its aspiration even by the hypoxic fetuses, they performed further experimental study in pregnant rats using a different contrast material (60). The amniotic sacs were injected with Calcodur blue dye which does not cause flaking nor crosses the circulation. It can only find its way to the gut or lungs if directly swallowed or inhaled. The dye almost invariably appeared in the fetal lungs when hypoxia supervened; otherwise, the dye normally found its way into the fetal digestive tract. In human fetuses in utero, localization of the fetal abdomen may be accomplished by injecting a radiopaque dye into the amniotic fluid (61). The dye is swallowed by the fetus and concentrated in the digestive tract. It is common knowledge although not formally reported that the contrast material never gets into the fetal lung under normal circumstances. These data favor the view that intrauterine respiratory movement is not a spontaneous, normally occurring phenomenon. Consequently, it appears more logical to consider the first neonatal breath as an event that interrupts the normal fetal apnea.

B. The first breath is a reaction to sudden sensory bombardment

Undoubtedly, the fetal respiratory neuroregulatory apparatus is organized early in fetal life and set for coordinated function at birth. Moreover, it is known that reflex pathways can exist before they are brought into active use (47). If in fact the fetus is wholly inactive in utero, at least two possibilities exist to explain the full picture. Either 1) the fetal medullary respiratory centers are normally directly inhibited from some higher centers, or 2) the sensory impulses from all sources into the respiratory center are insufficient. The possibility of inhibition near term from some higher areas was tested by Barcroft (1). Using twin-bearing ewes near term with the unoperated fetus serving as a control, he showed that following section of the brain stem just caudal to the pons respiratory movements were produced only on mechanical stimulation of the facial area. In contrast, spontaneous almost continuous respiratory movement ensued following section at a level just rostral to the upper border of the pons. This observation led him to conclude that the locus of inhibition of the medullary mechanisms resides rostral to the pons. His observations also support the view that intrauterine respiration does not normally occur in the near term fetus.

The alternative explanation of insufficient peripheral sensory stimuli resulting in ineffective traffic of general activity within the brain stem reticular formation has received a number of supporters since first proposed by Burns and Salmoiraghi (62). These investigators demonstrated in decerebrate, decerebellate adult cats that the periodic discharge of respiratory neurons was maintained in the absence of periodic activity in vagal and proprioceptive afferent pathways. Furthermore, they showed that progressive neurological isolation of the brain stem from the rest of the nervous system brought about a progressive decline in the number of periodically active respiratory neurons within the medullary respiratory area. Their findings suggested to them that in the unborn animal the respiratory system remains stable because of the extremely low level of general activity with the brain stem. Moreover, they proposed that the sudden increase in sensory input to the nervous system at the moment of birth could explain the onset of postnatal breathing. This proposal has found current adherents in Avery (6), Dawes (7) and Purves (63).

1. Tactile and proprioceptive stimuli

From studies in adults, it is known that active or passive movements of the limbs with receptors probably in the joints (64) and certain passive total body motions such as vibration in high speed aircraft (65) can increase minute ventilation. That such movements could, indeed, contribute to the initiation of respiration in the fetus has been raised as a possibility by Polgar (61). These movements would probably be playing only a minor role in view of the observations of Burns and Salmoiraghi (62,67) that the periodic discharge of respiratory neurons persisted in the absence of proprioceptive afferent pathways.

Additional evidence has been provided by Lymans'kyi (68) who obtained, using glass microelectrode, intracellular recordings from the neurons of reticularis formation in adult cats. He showed that streams of proprioceptive impulses from joints and muscles had only slight influence on activation of the neurons, whereas the flow of cutaneous afferent impulses were essential in the neuronal activation. His findings together with that of Burns and Salmoiraghi (62,67) strongly support the early observations of Barcroft (1) who was able to elicit gasp from painful pinching of the rabbit fetus ear and of Pronin (16) who showed the same response from tactile and painful stimulations of the skin of the rabbit fetus. Studies similar to that of Lymans'kyi should be done in the fetus to define the precise afferent systems that activate the fetal respiratory neurons.

2. Thermal stimuli

That cold can be an important respiratory stimulant was known to

Barcroft (1). However, it was Dawes (7) who elegantly demonstrated that, indeed, cold alone can initiate breathing in sheep fetus. He delivered the fetus by Caesarean section under light chloralose or local anesthesia. By application of ice cubes or use of an elctric fan, he could reduce the rectal temperature of the mature fetal lamb from 39.5° - 40°C to 35.5° - 36.5°C. At this latter temperature shivering and respiratory movements occurred. When cooled to shivering but prior to the onset of the respiratory response, application of ice cubes or ice-cold water to the mouth or face could immediately elicit rhythmic respiration, but none occurred when warm metal or warm water was used.

The mechanism by which cooling initiated respiration has not been defined. Changes in arterial gas tensions and pH could not be incriminated inasmuch as they remained normal during the procedure. Neither could it be due to the tactile effect of air movement produced by the electric fan since switching a fan on or off would start or stop breathing movements only when the lamb's coat was wet, indicating that it was the cooling per se that was critically important. He suggested that cooling by itself resulted in intense cutaneous stimulation that activated the medullary respiratory neurons. In fact, it is readily understandable that the cold air of the extra-uterine atmosphere represents a drastic change from the warm intrauterine environment.

A possible alternate explanation for the effect of cold may be derived from the recent work of Melmon and co-workers (69). They discussed the role of bradykinin as a possible mediator of neonatal circulatory changes in the human and proposed as one mechanism for the production of the kinin the temperature drop in umbilical vessel blood at the time of birth. Recently, Capek (70) studied the pharmacologic effects of bradykinin on the central nervous system of adult cats and described the consistent occurrence of tachypnea in addition to the other obvious signs of central nervous system excitation. This dual information might be considered as an explanation of the mechanism involved in the initiation of breathing by cooling alone. Moreover, the exact role of bradykinin in the onset of postnatal respiration merits further investigation.

The influence of high temperature has not been directly studied in the fetus and neonate. In adult animals and man, moderate hyperthermia is known to produce an increase in ventilation (18). The mechanism in animals is thought to be an early inhibition of inspiratory activity which results in tachypnea. Thus, it seems unlikely that hyperthermia would initiate an inspiratory gasp in the fetal lamb. Indeed, Dawes (7) was unable to initiate breathing by the application of heat to the face of the term fetal lambs. It is of interest that Kramer and Millikan (71), investigating the effect of high temperature on the oxygen saturation of the blood in the newborn lamb, observed that the heat from an electric radiator reduced respiratory activity. Thus, the ventilatory response to hyperthermia may change with age.

3. Sensory stimulus arising in the umbilical cord

Another stimulus that has been suggested in the past is cord occlusion per se. Could the ventilatory response be due in part to a neural mechanism arising in the cord? There is no known central nervous system supply to the cord. However, Fox and Jacobson (72) have recently demonstrated, by means of a modified methylene blue technique, neural fibers in the human umbilical cord, but the role of such fibers remains purely speculative at this time. On the other hand, there has been no convincing evidence which excludes a role of cord occlusion per se, whatever the mechanism involved, in the initiation of breathing.

4. Neural discharge from mechanoreceptors in the thorax

In addition to the sensory stimuli from outside the body, there are also certain stimuli from within which the fetus experiences for the first time at birth. Compression of the thorax during the passage of the fetus through the vagina might trigger the inflation reflex of Hering and Breuer mediated by the vagus. This would not explain why fetuses delivered by Caesarean section begin to breathe. Mechanical expansion of the chest could also initiate internal sensory afferent impulses which are carried by the vagus to the respiratory center (1). What initially triggers the chest expansion remains to be shown.

A gasp reflex similar to the "paradoxical reflex", first described by Head (73) in 1889 to distinquish it from the usual respiratory inhibition produced by inflation, has very recently been described in the sheep fetus by Hughes et al(74). They demonstrated that tracheal inflation to 40 mm Hg invariably elicited an inspiratory gasp. The response was reversibly inhibited by vagal cooling or local lidocaine anesthesia to the air passages. Bilateral vagotomy permanently abolished the response, indicating that this gasp response indeed involved a vagal reflex pathway. It has been postulated that the "frog swallowing" movements of air into the fluid-filled trachea at birth -- described by

Bosma, Lind and Gentz (75) in the newborn infant -- could initiate the gasp reflex at the time of parturition. The exact role of this reflex in the initiation of breathing at birth remains to be established.

5. Sudden changes in systemic blood pressure

Another internal stimulus that has been considered is the rise in systemic arterial blood pressure following severance of the low-resistance placental circulation. Krafka (76) in 1933 originally proposed that the rise in systemic pressure with consequent increase in cerebral blood flow and greater delivery of carbon dioxide to the brain was essential for the first breath. Later, Barclay and co-workers (77) subscribed to Krafka's hypothesis and to Barcroft's (78) original study which apparently showed that following occlusion of the umbilical cord the rise in pressure preceded the onset of respiration. Since chemical changes also simultaneously occurred, the exact role of the rise in blood pressure has to be delineated. Barcroft (1) assigned to this factor at most only a minor role. He made observations of instances of initiation of breath in the absence of hypertension. Purves (63) showed increased afferent impulses from baroreceptors following cord compression and suggested that changes in blood pressure might in this manner contribute to the flood of sensory impulses that surround and activate the medullary respiratory neurons at birth.

Recently, however, Harned and co-workers (79) perfused the carotid sinus region of term fetal lambs at high pressures and did not observe any respiratory response. Moreover, in adults an increase in pressure in the carotid sinus regions decreases ventilation; conversely, a decrease in pressure causes hyperpnea (37). Furthermore, the rise in pressure following cord occlusion is only transient and soon followed by a return to normal.

Thus, it is remarkable to note that in the absence of conclusive data, the prompt rise in blood pressure following cord occlusion has continued to be mentioned in recent writings on the subject of the first breath as one of cardinal importance (5). The influence on the first breath of the changes in pressure has not been systematically studied. Studies designed to produce circulatory alterations similar to those taking place following occlusion of the cord but under normal fetal oxygenation and acid-base state are required.

C. <u>The first breath is a response to asphyxia</u>

The evidence for functional chemoreflex and baroreflex mechanisms during fetal life has already been discussed. It has been pointed out that reaction to sensory stimulation such as cooling alone, which does not disturb the normal fetal arterial gas tensions and pH, can initiate breathing. However, it is not implied in any way that the chemical changes in the blood at the time of birth are not important. Indeed, the most favored hypothesis states that the first breath is a reaction to asphyxia at the time of birth. The term asphyxia is meant to encompass the triad of hypoxemia, hypercapnia and acidemia which occur following cessation of placental gas exchange.

In the experimental animals, the inauguration of respiration is definitely ushered in by cord occlusion which is invariably associated with asphyxia in addition to changes in blood pressure. This phenomenon has long been known to Windle (8), Barcroft (1), Dawes (7), Harned (9) and Howatt (10) and their collaborators. It is curious to note their observations that undisturbed fetuses at term (with intact umbilical circulation) very seldom make any respiratory effort and usually do not respond even to vigorous tactile stimulation. Reasons have already been given for considering the possible role of fluctuations in systemic blood pressure and of cord occlusion per se. Whether the striking and consistent effect of cord clamping in chemically initiating breathing results from hypoxic, acidic or hypercapnic stimulation of the chemoreceptors, hypercapnic stimulation of the respiratory center, or from some combination of hypoxemia, hypercapnia and acidemia is not yet settled.

Previous works in fetal animals involved the induction of fetal hypoxemia and hypercapnia through the administration of gas mixtures to the mothers but the results are conflicting. Barcroft and colleagues (25) administered high CO_2 mixtures to pregnant sheep but obtained no fetal respiratory movements. Their sheep, however, were anesthetized with urethane and the possibility of respiratory center depression by this anesthetic must be considered. In fact, Barcroft and Kramer (80) later showed that urethane could, indeed, depress the center. In contrast to Barcroft's observations, Snyder and Rosenfeld (57) were able to demonstrate that high CO_2 administered to pregnant rabbits could elicit fetal respiratory movements in one-third (8/24) of their animals. Windle (8) studied the problem in cats. Some fetal cats were delivered keeping the placental circulation intact and others were observed through the unopened uterus. Gas mixtures containing 8-10% CO_2 in O_2 given to the mothers invariably initiated respiration. Moreover, 5-10% O_2 in N_2

gas mixture also induced breathing. The latter observation was in agreement with those of Barcroft and co-workers (25) who showed a similar response in guinea pigs exposed to O_2-poor atmosphere but in conflict with those of Snyder and Rosenfeld (57) who observed that anoxemia failed to elicit fetal respiration.

The foregoing observations were without direct fetal blood gas measurements. Although the occurrence of fetal hypercapnia or hypoxemia when mothers respectively breathed CO_2-enriched or O_2-poor gas mixture was correctly assumed, neither the degree of change or the respective concurrent alteration in fetal arterial PO_2 or PCO_2 was given due consideration. Recently, Harned and his colleague (81) studied the effect of varying the maternal gas environment on the gaseous composition of the fetal blood and related the changes in the latter to the onset of breathing in fetal lambs. They showed that 6.5% CO_2 in air administered to the mothers caused hyperventilation and an increase in arterial PO_2 so that the fetal arterial PO_2 rose passively as the PCO_2 increased. Thus, when the fetal arterial PCO_2 increased to a mean value of 52.9 mm Hg (maximum was 63 mm Hg), the PO_2 also increased to an average of 25.4 mm Hg. There was no fetal respiratory response. Administration of 10% O_2 in N_2 also caused maternal hyperventilation and resulted in a concurrent fall in maternal and fetal arterial PO_2 and PCO_2. The fetal arterial PO_2 decreased to a mean value of 11.6 mm Hg (lowest was 6.6 mm Hg) and the PCO_2 also decreased to a mean of 37.0 mm Hg. Again, no gasp was observed in the fetus. Could this mean that the stimulating effect of hypercapnia was masked by the accompanying increase in PO_2 and the stimulating effect of hypoxemia was masked by the concomitant drop in in PCO_2? In newborn lambs, Biscoe and Purves (30) have shown that hypoxia potentiated the effect of hypercapnia in augmenting ventilation and that higher concentration of oxygen than normal decreased respiration. Thus, the conditions under which Harned and his co-workers (81) studied the separate effect of hypercapnia and hypoxemia upon the initiation of breathing in the fetal lamb were the least favorable to observe a positive effect. Moreover, these observations underscore the importance of making direct and simultaneous measurements of arterial PO_2 and PCO_2 when studying their influence on the onset of the first breath.

D. The first breath is a result of a combination of physical and chemical stimuli

Reasons have already been given for considering the individual part played by physical and chemical stimuli upon the onset of the first breath. Since these stimuli might interact to produce the response, one obvious situation to investigate is the combined influence upon the initiation of breathing of changes in blood pressure and gas tensions which accompany cord occlusion.

It is known in the adult that not only the chemical composition of the blood perfusing the peripheral chemoreceptors but also the blood pressure in these regions affect their discharge (82,83). Gernandt and co-workers (82) reported that the discharge fell when the blood pressure was raised by large doses of adrenaline. Conversely, Landgren and Neil (83) showed a massive increase in discharge after hemorrhage. Recently, Lee, Mayou and Torrance (84) demonstrated that in adult cats the rate of discharge of aortic body chemoreceptors in response to hypoxia was inversely related to the prevailing blood pressure when this pressure was altered mechanically over a wide range. Moreover, it is well established in the adult that changes in blood pressure reflexly affect ventilation. Thus, strong arguments exist for considering an interaction between chemical changes and alterations in blood pressure in the initiation of breathing.

E. Summary

The weight of evidence favors the view that the fetus *in utero* does not perform rhythmic respiratory movements until such time as it is stimulated to breathe at the time of birth. Whether the normal fetal apnea is due to inhibition of the respiratory center from some higher centers or due to insufficient traffic of peripheral sensory impulses into the medullary respiratory neurons is not clearly known. Asphyxia at parturition triggers the first breath but the individual contribution of each chemical change has yet to be differentiated. It is further known that certain sensory stimuli such as cold alone can initiate breathing in the presence of normal fetal arterial gas tensions and pH. Indeed, both physical and chemical stimuli appear to play a role in the initiation of this crucial event. Teleologically, it seems wise to assume that Nature would not rely upon a single mechanism for this vital event. The precise importance of each factor requires investigation. For example, the evidence for the part played by such sensory stimuli as changes in blood pressure has not been conclusive. It thus becomes of interest to investigate not only the relative importance of each chemical factor but also the influence of fluctuations in blood pressure, alone or jointly, in the onset of the first breath. These problems provide the basis of the present investigation the results of which and their interpretations form Part II (Experimental Study) of this thesis.

PART II. EXPERIMENTAL STUDY: INITIATION OF RESPIRATION

I. Appraisal of the Role of Systemic Arterial Mean Pressure

II. Relative Contribution of Changes in Arterial Gas Tensions

PART II

INITIATION OF RESPIRATION: APPRAISAL OF THE ROLE OF ARTERIAL BLOOD PRESSURE AND GAS TENSIONS

The precise mechanism that triggers the first breath has remained unsettled. The preceding review of the literature has illustrated that several factors are involved in the genesis of this crucial event at the time of birth. The current experiments, in two phases, were undertaken in order to assess the role of arterial blood pressure and the relationship between arterial PO_2 and PCO_2 at the time of the first breath. Phase One of the investigation delineates the influence of changes in fetal blood pressure. The manuscript is now in press in Respiration Physiology and is essentially reproduced below. Phase Two of the study evaluates the relative contribution of changes in fetal arterial gas tensions and will be presented in a separate section.

I. PHASE ONE: APPRAISAL OF THE ROLE OF SYSTEMIC ARTERIAL BLOOD PRESSURE

A. INTRODUCTION

Initiation of respiration invariably occurs following occlusion of the umbilical cord (1,7,8,9,10). The accompanying hypoxemia, hypercapnia and acidemia have been favoured as the key stimuli (1,5,7,8,9). On the other hand, Dawes (7) has demonstrated the initiation of regular liquid breathing in mature fetal lambs by cooling with no significant change in arterial pH and gas tensions. Both manoeuvres -- occlusion of the cord and application of cold -- caused a transient rise in systemic blood pressure. As yet, the relative contribution of changes in systemic arterial blood pressure has not been satisfactorily elucidated. Krafka (76) postulated that the immediate rise in pressure which accompanies cord occlusion at birth was of crucial importance. In contrast, observations by Barcroft (1) suggested that the rise in blood pressure had only a minor role. Studies in adult animals, however, have shown that an increase in systemic arterial pressure inhibits breathing and a decrease in pressure stimulates respiration (37,85). In order to delineate the influence of changes in systemic arterial mean pressure on the initiation of breathing in fetal sheep, this phase of the study was designed to produce circulatory alterations similar to those taking place following occlusion of the cord but under normal fetal oxygenation and acid-base state.

B. METHODS

Fourteen near-term lambs (gestational age 135 to 145 days), weighing 2.3 to 4.6 kg and measuring 46 to 54 cm, were exteriorized by Caesarean section from 11 pregnant ewes anesthetized with intravenous pentobarbital, 20 to 25 mg/kg body weight. Supplemental doses of pentobarbital, 2 to 3 mg/kg, were given to the ewes as required during the experiment. The ewe was placed on her right side and was spontaneously breathing a mixture of oxygen-enriched air.

Immediately after delivery, the fetal nose was capped with a rubber condom filled with warm saline. Care was taken to avoid undue traction of the umbilical cord and it was covered with a few layers of saline-wet gauze. The fetus was covered with a heating blanket to maintain the rectal temperature between 38.5° and 40°C. A tracheostomy was performed and a clamped cannula filled with warm saline was inserted into the trachea. The cannula was connected to a volume plethysmograph consisting of a 30-liter capacity bottle containing 2 to 3 liters of saline and the clamp was then removed from the cannula (10). This procedure prevented any loss of pulmonary fluid. The height of the fluid in the plethysmograph was adjusted to prevent flow of liquid to or from the lungs unless breathing occurred. Liquid breathing by the fetus caused a decrease in pressure in the plethysmograph sensed by a differential pressure transducer (Statham Model PM 5) attached to the air space above the liquid and recorded on an Electronics for Medicine recorder. Volume calibration was done at a frequency of 5 to 10 cycles per minute using a syringe attached to the plethysmograph. Removal of 10 ml of saline caused less than 0.5 cm of water fall in pressure so that the fetus experienced no significant elastic pressure change during inspiration.

Heparin, 10 mg/kg body weight, was administered intravenously to all lambs. Both femoral arteries were cannulated, one for continuous recording of the mean intra-arterial pressure (using a Statham transducer, Model P23 Db) and the other for withdrawal of blood samples. Blood samples were obtained immediately prior to any procedure and thereafter at intervals of 30 seconds and at the onset of gasp. In the absence of a gasp response, blood sampling was continued for 3 to 5 minutes. All samples were immediately analysed for pH, PO_2, PCO_2 using a Radiometer micro-electrode system. The temperature of the system was kept within 0.5°C of the fetal rectal temperature. Calibration of the system was done daily using buffer solutions of known pH and gases of known composition.

The influence of changes in systemic arterial mean pressure on the initiation of respiration was studied under two conditions: 1) Alterations in systemic arterial mean pressure with simultaneous changes in arterial gas tensions and pH. This condition was induced by one of the following procedures: occlusion of the intra-abdominal umbilical vein, occlusion of both intra-abdominal umbilical arteries, occlusion of the whole cord or occlusion of the maternal abdominal aorta. 2) Alterations in systemic arterial mean pressure without accompanying significant change in arterial gas tensions and pH. This condition was induced by the following procedures: rapid withdrawal of blood (50-60 ml) from the femoral artery, rapid infusion of maternal venous blood (50-60 ml), intra-arterial infusion of norepinephrine (0.2 ÷ 0.3 mg in 10 ml of saline), or occlusion of only one intra-abdominal umbilical artery.

When more than one procedure was tested in the same fetus, a minimum of 15 minutes were allowed between the procedures for the arterial gas tensions and pH and systemic arterial pressure to return to near-baseline values. There was no significant variation between the initial and the subsequent baseline values for arterial gas tensions, pH and systemic mean pressure during the course of the experiment. A particular procedure was terminated following the onset of breathing. When no ventilatory response was observed following a procedure, the observation of pressure change was continued for 3 to 5 minutes.

Statistical analyses (t-test and test for correlation between two variables) were performed and the relationship between variables determined at the 5% level of significance.

C. RESULTS

1. Alterations in Systemic Arterial Mean Pressure with Simultaneous Changes in Arterial Gas Tensions and pH

On 18 occasions in 11 fetal lambs the placental gas exchange was severely compromised by one of the following procedures: occlusion of the intra-abdominal vein in 6 instances, of both intra-abdominal umbilical arteries in 5, of the whole cord in 4 and of the maternal abdominal aorta in 3. In all instances the fetus gasped within 1 to 3 minutes from the onset of the procedure with a mean time of 1 minute and 45 seconds. The respiratory movements consisted of 3 to 6 respiratory efforts per minute with an average tidal volume of 5 ml. The blood gas tensions and pH at the time of the first gasp were similar in the four methods used to induce respiration (Table I, Fig. 1). The mean (± S.E.) arterial PO_2, PCO_2 and pH at the time of the first breath from all procedures (9.3 ± 0.63 mm Hg, 68.1 ± 2.34 mm Hg, and 7.13 ± 0.02 units, respectively) and the average control values (26.8 ± 1.57 mm Hg, 51.5 ± 2.28 mm Hg and 7.22 ± 0.01 units,respectively) obtained immediately prior to the procedures are shown in Table II.

The pattern of change in systemic arterial mean pressure prior to the onset of gasp was variable with the greatest change following occlusion of the umbilical arteries (Fig. 2). The change in blood pressure was analysed in three ways:

a. The pressure at the time of gasp with the control value. The mean pressure decreased in two instances, increased in 10 instances and was essentially equal to the control on 6 occasions, (less than 5% change

TABLE I

FEMORAL ARTERIAL GAS TENSIONS AND pH AT THE TIME OF INITIATION OF BREATHING FOLLOWING INTERRUPTION OF PLACENTAL GAS EXCHANGE AT FOUR DIFFERENT SITES

Site of Vascular Occlusion	Number of Observations	PO_2 (mm Hg)	PCO_2 (mm Hg)	pH
Whole umbilical cord	4	10.2* (6-13)**	68.2 (52-83)	7.08 (7.02-7.15)
Umbilical vein	6	9.3 (5-14)	69.2 (59-81)	7.16 (7.08-7.25)
Umbilical arteries	5	8.8 (7-13)	64.4 (55-74)	7.13 (7.01-7.23)
Maternal abdominal aorta	3	8.7 (6-11)	71.7 (56-87)	7.11 (7.04-7.23)

* Mean
** Range

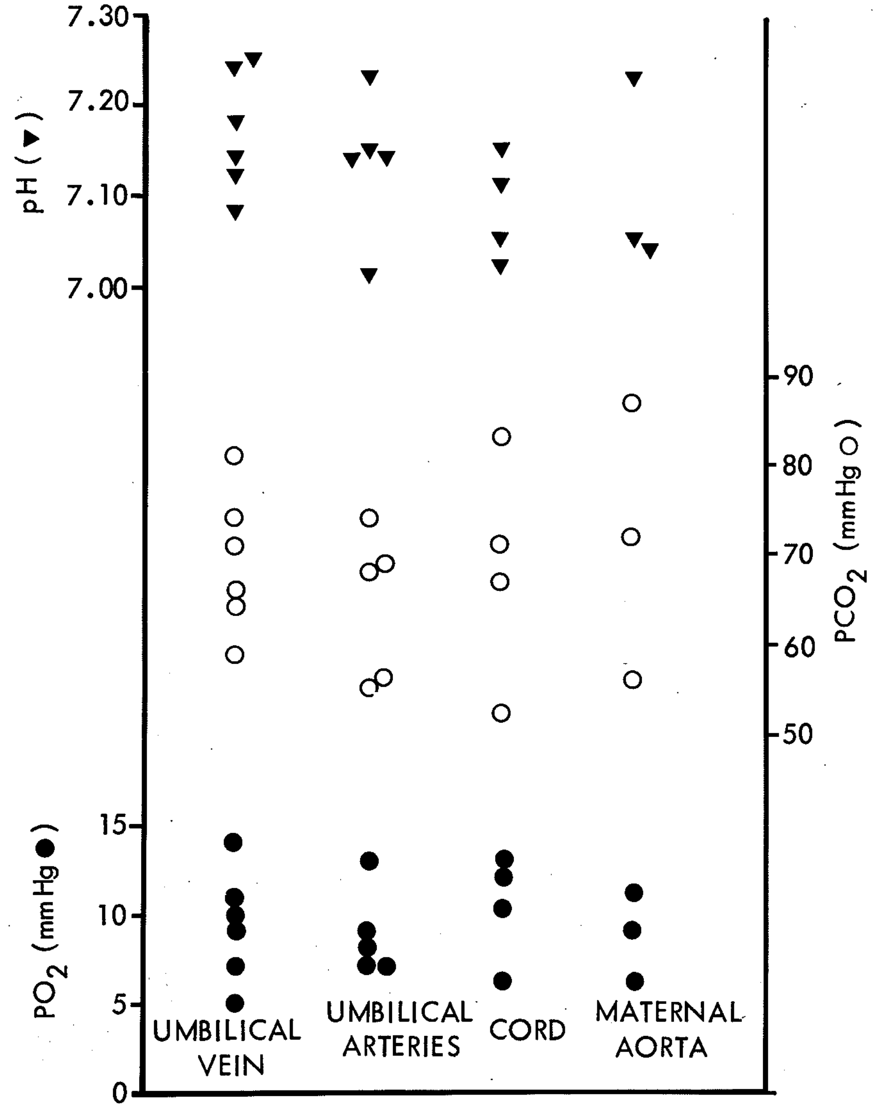

Figure 1. Comparison of the arterial gas tensions and pH at the onset of gasp induced by either occlusion of the umbilical vein, both umbilical arteries, whole umbilical cord or the maternal abdominal aorta.

TABLE II

FEMORAL ARTERIAL GAS TENSIONS AND pH DURING VARIOUS CONDITIONS: A. CONTROL, B. AT THE TIME OF GASP AND C. FOLLOWING PROCEDURES NOT ASSOCIATED WITH GASP

Conditions	Number of Observations	PO_2 (mm Hg)	PCO_2 (mm Hg)	pH
A	18	26.8±1.6*	51.5±2.3	7.22±0.01
B	18	9.3±0.6	68.1±2.3	7.13±0.02
C	15	25.3±1.0	44.4±2.6	7.25±0.01

* Mean ± Standard Error

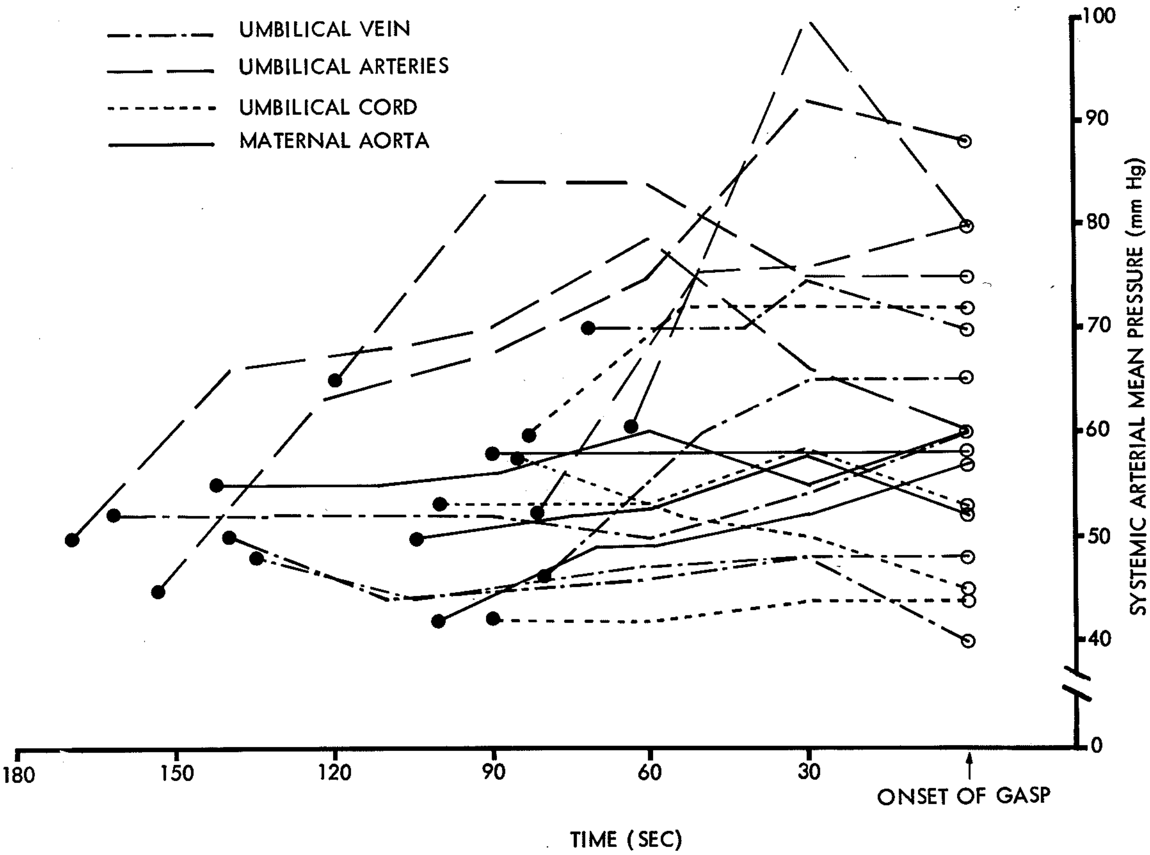

Figure 2. Pattern of change in systemic arterial mean pressure prior to the onset of gasp induced by the four procedures plotted against the time from the onset of the procedure (closed circles) to the point of gasp (open circles).

from control). The range of blood pressure change was from -20% to +95%. Whether the mean blood pressure was higher, lower or equal to control values, the arterial gas tensions and pH at the time of gasp were similar (Fig. 3). Furthermore, there was no correlation between the absolute mean blood pressure at the time of gasp and the arterial gas tensions and pH (Fig. 4).

b. The direction of change in systemic mean pressure during the first 30 seconds following the procedure. This was classified as either decreasing, stationary or increasing (Fig. 2). The pressure changes ranged from -10.5% to +61% of the control.

c. The pattern of pressure change during the final 30 seconds immediately preceding the gasp. Similarly, this was classified as either decreasing, stationary, or increasing (Fig. 2). The changes in pressure from the preceding 30 seconds to the point of gasp ranged from -20% to +13%. Whether the pattern of pressure change considered was during the first or final 30 seconds, blood gas tensions and pH at the time of the gasp were similar in instances of decreasing, stationary or increasing pressure (Fig. 5). Furthermore, using the 3 methods of analysing the change in blood pressure, there was no relationship between the systemic mean pressure and the time delay from the onset of the procedure to the time of gasp. (Fig. 2 & 6).

2. Alterations in Systemic Arterial Mean Pressure without Significant Change in Arterial Gas Tensions and pH

This condition was studied on 15 occasions on 6 fetal lambs (Fig. 7). Blood pressure was decreased by rapid withdrawal of blood on 5 occasions

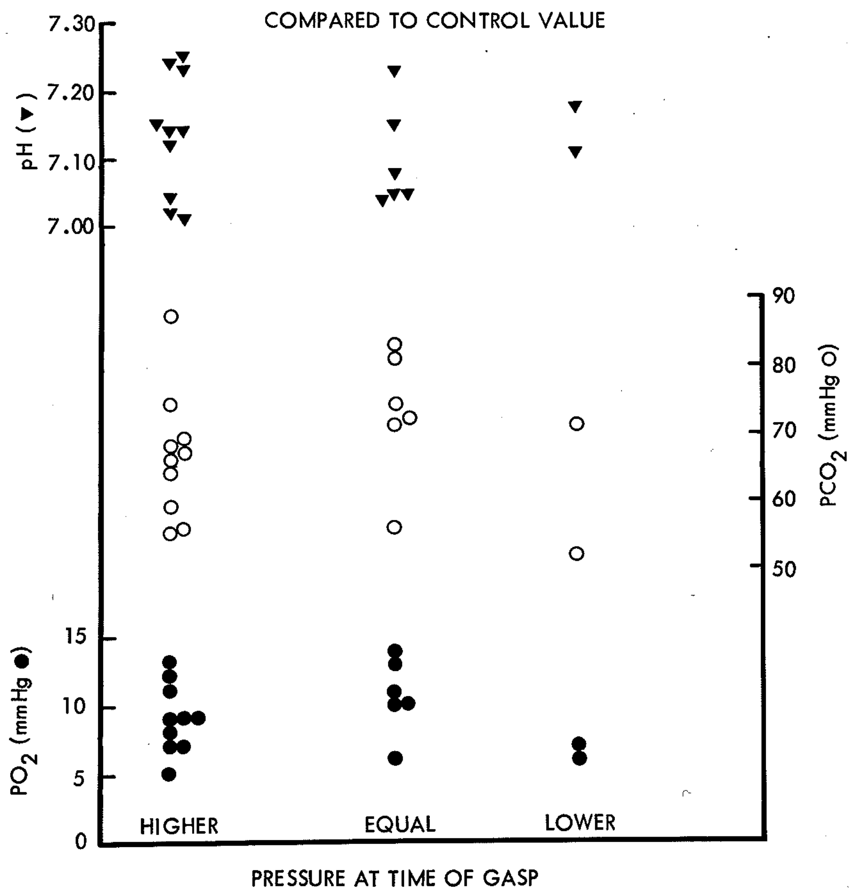

Figure 3. Comparison of the arterial gas tensions and pH at the time of gasp when the mean pressure at the onset of gasp was either higher, equal or lower than the control pressure.

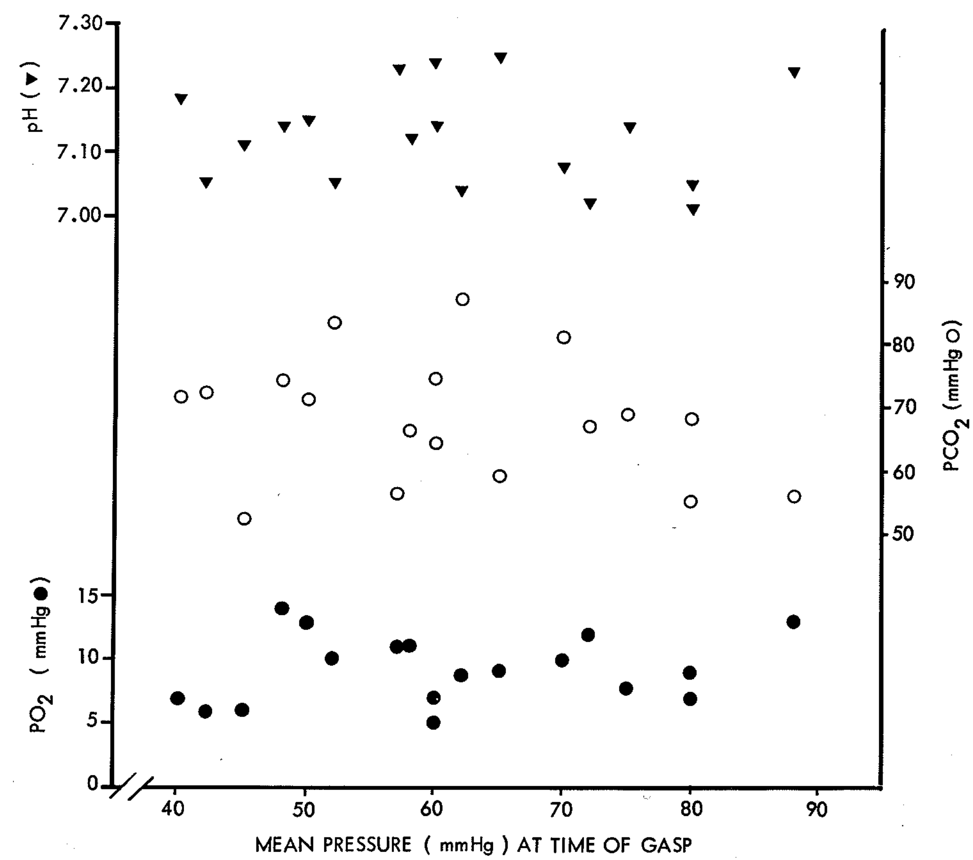

Figure 4. Relationship between the absolute mean blood pressure and the arterial gas tensions and pH at the time of gasp.

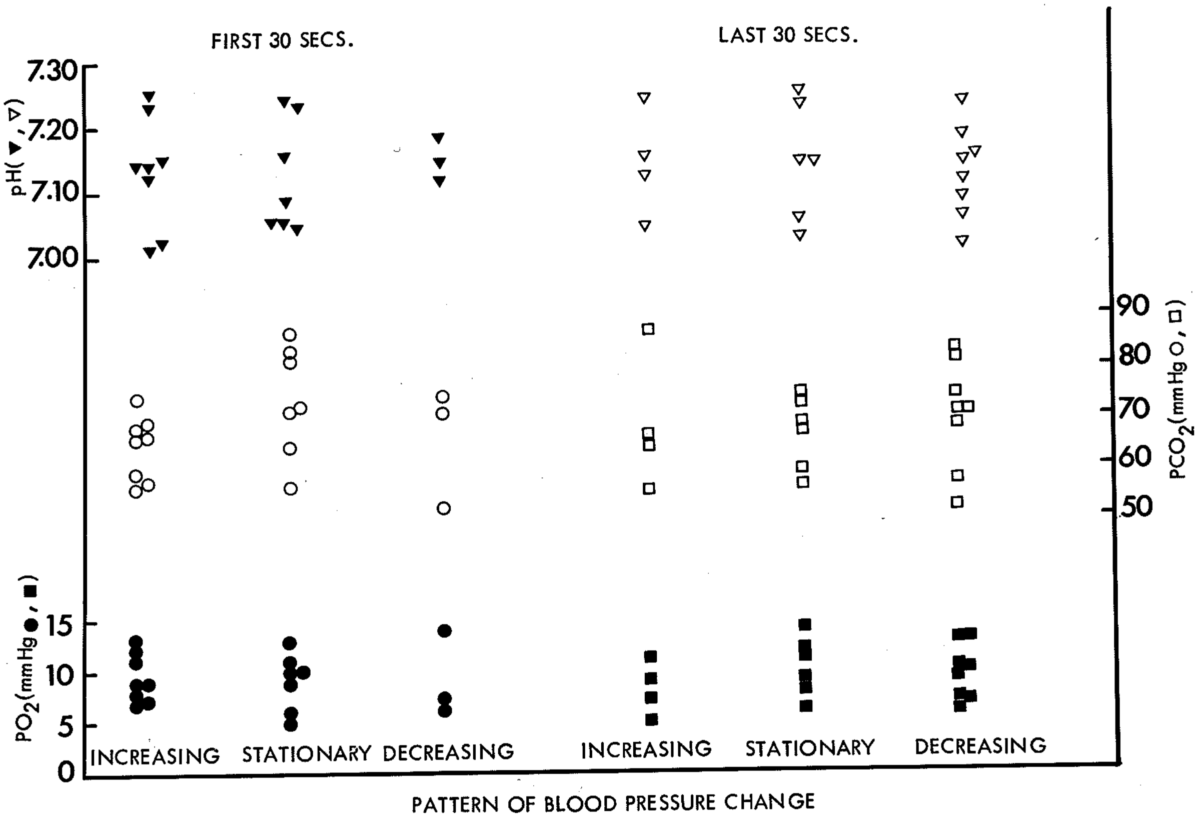

Figure 5. Arterial gas tensions and pH at the time of gasp for different patterns of pressure change a) during the first 30 seconds following onset of a procedure or b) during the 30 seconds immediately preceding the initiation of respiration.

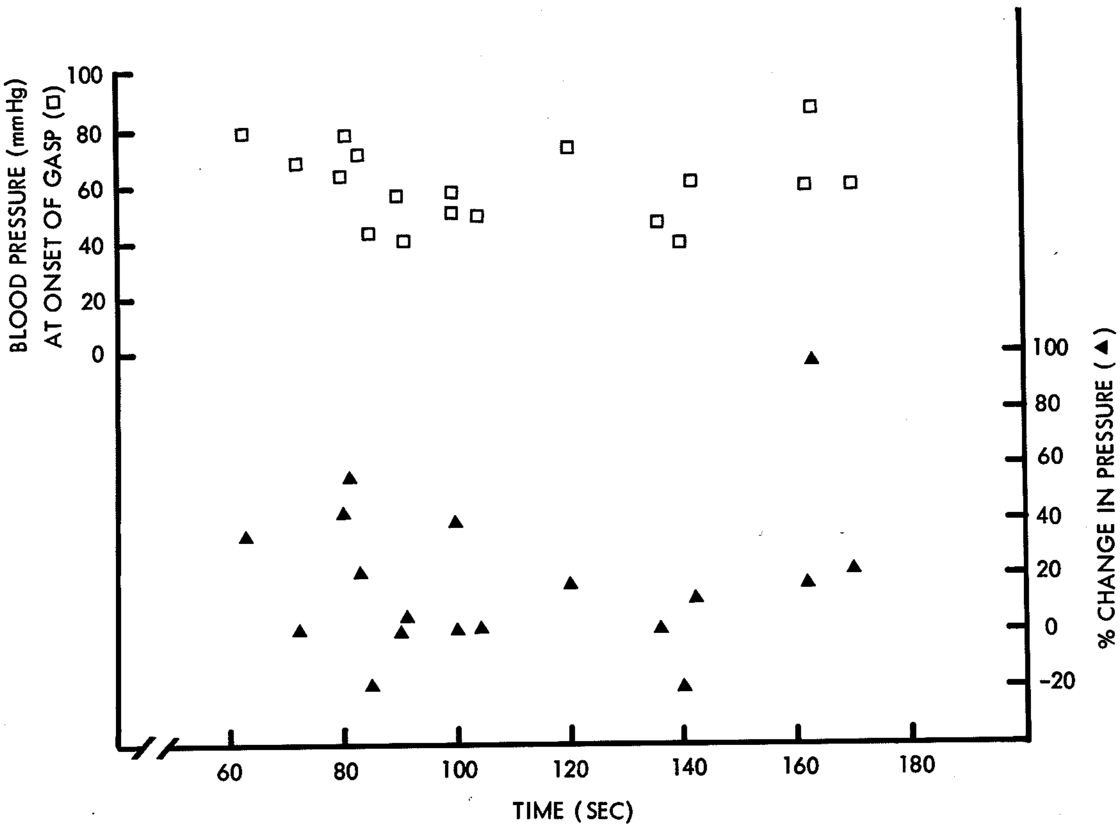

Figure 6. Upper portion: Relationship between the systemic arterial mean pressure and the time delay from the onset of a procedure to the time of gasp (latent period). Lower portion: Relationship between percent change in pressure and latent period.

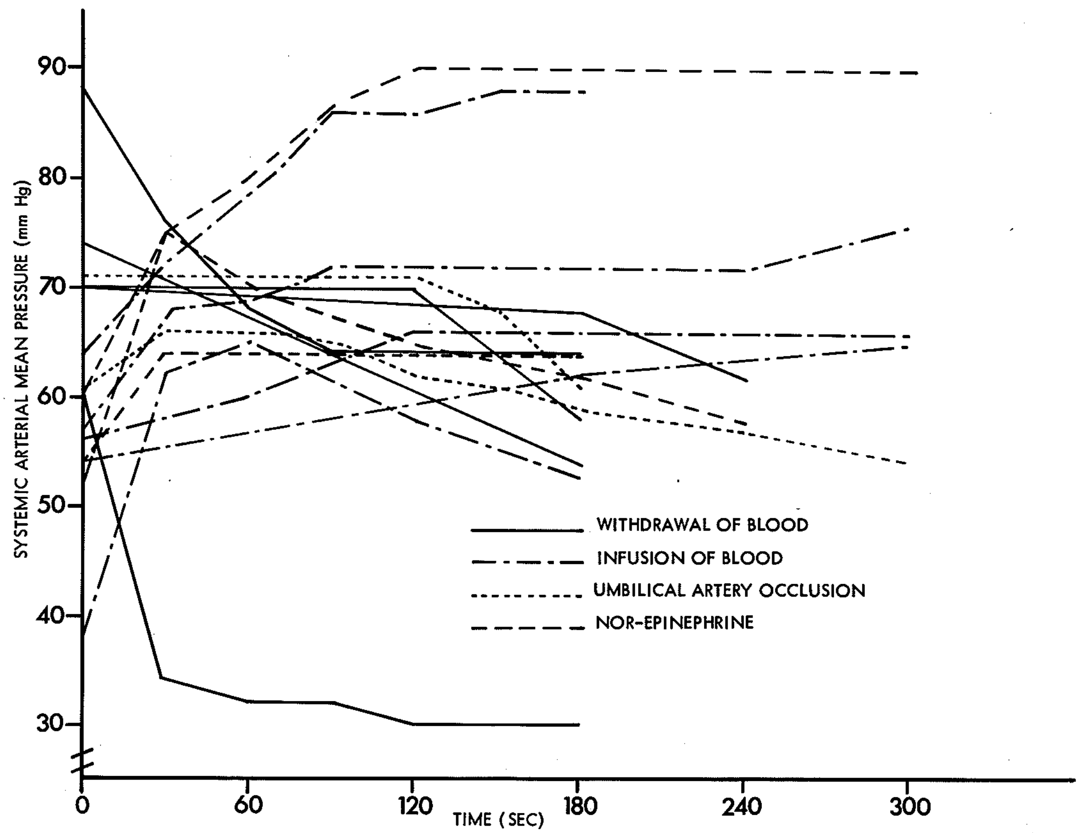

Figure 7. Pattern of change in systemic arterial mean pressure following the four procedures which did not induce respiration, plotted agent time.

and by occlusion of the intra-abdominal portion of one umbilical artery in 2 instances. The maximum decrease in pressure ranged from 11 to 50%. On 8 occasions the systemic mean pressure was raised above the control value by infusion of norepinephrine (3 instances) and by infusion of maternal venous blood (5 instances). The maximum increase in pressure ranged between 12 and 52%. Although the observation for pressure change was continued for 3 to 5 minutes no ventilatory response was seen in any of the fetal lambs. Manipulation of the systemic mean pressure by these methods did not significantly alter the blood gas tensions and pH. The mean (± S.E.) PO_2, PCO_2 and pH were 25.3 ± 1.02 mm Hg, 44.4 ± 2.64 mm Hg, and 7.25 ± 0.01 units, respectively (Table II, Fig. 8).

D. DISCUSSION

These experiments demonstrate that complete interruption of the placental circulation uniformly initiated gasp in the fetal lambs, and are consistent with the observation of Barcroft (1), Dawes (7), Windle (8), Harned (9) and Howatt (10) and their co-workers. To interrupt the placental gas exchange, either the whole umbilical cord, both umbilical arteries, the umbilical vein or the maternal abdominal aorta was occluded. The initiation of gasp with each of these procedure indicates that the site of interruption of placental flow, whether fetal or maternal, is not a determinant factor. Invariably, hypoxemia, hypercapnia and acidemia accompanied the onset of breathing. The level of arterial gas tensions and pH were similar to previously reported values, with the PO_2 less than 15 mm of Hg in all instances (9,10).

Simultaneously, variable changes in systemic arterial mean pressure

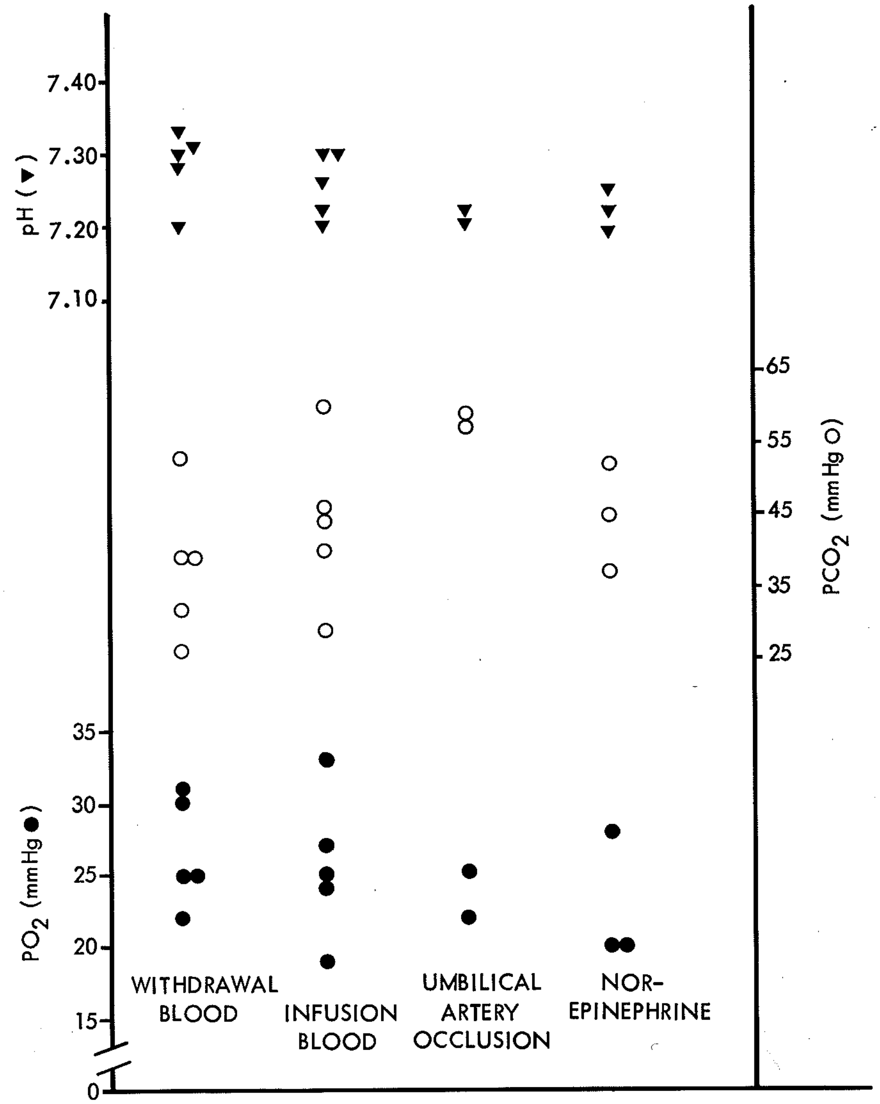

Figure 8. Arterial gas tensions and pH just prior to the conclusion of the four procedures not associated with respiration.

occurred. Removal of the low-resistance vascular bed of the placenta causes an increase in systemic vascular resistance (86). The immediate rise in systemic arterial mean pressure which we observed particularly following occlusion of both umbilical arteries might be explained on this basis. Accumulation of fetal blood in the placenta with reduction in fetal blood volume, as would be expected following occlusion of the umbilical vein, could explain the immediate fall in systemic arterial pressure seen in two instances with the latter procedure. However, the pattern of blood pressure change particularly following cord occlusion was more variable and a more complex explanation is required. The change in direction of ductus blood flow to left-to-right could explain an initial fall in pressure. Hypoxemia produces a transient rise in systemic blood pressure followed by hypotension in newborn puppies and monkeys (87,88). Recently, Stahlman, et al (89) showed that hypoxemia and hypercapnia lowered systemic vascular resistance in newborn lambs. A complex interaction between these factors could provide an explanation for the variable blood pressure patterns seen with all the procedures from their onset to the induction of respiration.

Could similar changes in blood pressure alone initiate respiration? In the presence of normal fetal blood gas tensions a decrease or increase in systemic arterial mean pressure did not evoke a gasp response in any of the fetal lambs, although the pressure changes were similar in magnitude to those observed during the initiation of breathing. The animals were viable since they gasped on subsequent cord occlusion. These observations indicate that marked changes in systemic mean pressure per se cannot initiate breathing in fetal sheep.

Indeed, it appears that chemical changes are the prime stimuli for the initiation of the first breath. As yet, the relative importance of each chemical stimulus and their interaction and locus of action remain unsettled. The carotid chemoreceptors have been shown to play an important role in the onset of respiration (79). In view of this, the demonstrations in adult animals by Gernandt, Liljestrand and Zotterman (82), Landgren and Neil (83) and more recently by Lee, Mayou and Torrance (84) of an inverse relationship between the prevailing systemic blood pressure and the peripheral chemoreceptor discharge in response to hypoxia suggested a possible secondary influence of alterations in blood pressure. To test this possibility, the changes in blood pressure were analyzed in three ways and were related to the level of blood gas tensions and pH at the onset of gasp and also to the time delay from the onset of a procedure to the initiation of response. Whether the pressure change considered was the initial compared to the final, the trend during the first 30 seconds or the 30 seconds immediately preceding the gasp, no relationship between the chemical changes and the systemic arterial mean pressure was seen. Similarly, the time course from a procedure to response was independent of the blood pressure change by the three methods of analysis. These results indicate that changes in systemic arterial mean pressure cannot, per se, initiate respiration and do not influence the chemical stimuli to the onset of breathing in fetal sheep.

The influence of pentobarbital in the present experiments must be considered. General anesthesia of the mother is known to delay the onset of rhythmic breathing. It is possible that pentobarbital anesthesia may mask an influence of the systemic blood pressure on the initiation of of respiration. However, during the present experiments baroreceptor activity was definitely present in the fetal lambs since an increase in blood pressure was associated with a bradycardia and a decrease in blood pressure with a tachycardia. This preservation of baroreceptor activity suggests that the present finding of a lack of influence of the systemic mean pressure on the initiation of respiration is valid.

II. PHASE TWO: RELATIVE CONTRIBUTION OF CHANGES IN ARTERIAL GAS TENSIONS

A. INTRODUCTION

Phase One of the study confirmed and extended earlier observations that interruption of placental gas exchange invariably initiated respiration (1,7,8,9,10). The levels of fetal hypercapnia, hypoxemia and acidemia at the time of the first gasp were independent of the site of interruption. In the presence of normal fetal blood gas tensions and pH, respiration was not initiated despite the large changes in blood pressure and the extensive operative manipulations during the preparation of the specimen. These observations support the view that asphyxia is the primary stimulus for the initiation of breathing (1,7,9).

The relative contribution of changes in arterial gas tension to the onset of breathing have not been defined. Harned et al (81) have shown that neither fetal hypercapnia nor hypoxemia alone of moderate degree elicited gasp in the sheep fetus. Although induced separately, the conditions of their experiments were such that arterial PO_2 and PCO_2 changed in the same direction within a narrow range and were not precisely controlled. The present phase of the study was undertaken in order to more closely define the relationship between P_aO_2 and P_aCO_2 at the time of the first breath.

B. METHODS

Fourteen near-term pregnant ewes were used. The ewes were anesthetized and the fetal lambs exteriorized as described in detail in Phase One. Care of the fetus after exteriorization, sampling of blood for determination of arterial gas tensions and pH, and recordings of fetal blood pressure and respiratory response were as described in Phase One.

Newborn lambs, one to two weeks of age, were anesthetized with pentobarbital, 10 mg/kg body weight intraperitoneally and a tracheostomy performed. The tracheostomy cannula was connected to a Harvard respirator (Model 607) and the inspired gas adjusted as desired with the use of specially prepared gas cylinders which supplied a reservoir bag attached to the inflow value of the respirator. Composition of the gas mixtures was analyzed by the Scholander method. In contrast to the ewes, the newborn did not as a rule require supplemental doses of pentobarbital during the duration of the experiment.

It was essential that changes in fetal arterial gas tensions could be induced at widely varying and precise levels. To achieve this, the newborn lamb -- henceforth referred to as donor -- was substituted for the placenta as the organ of gas exchange (Fig. 9). Polyvinyl catheters filled with heparinized saline were used to connect a carotid artery of the donor to a femoral vein of the fetus and a fetal femoral artery to a jugular vein of the donor. A dual-head roller pump interposed

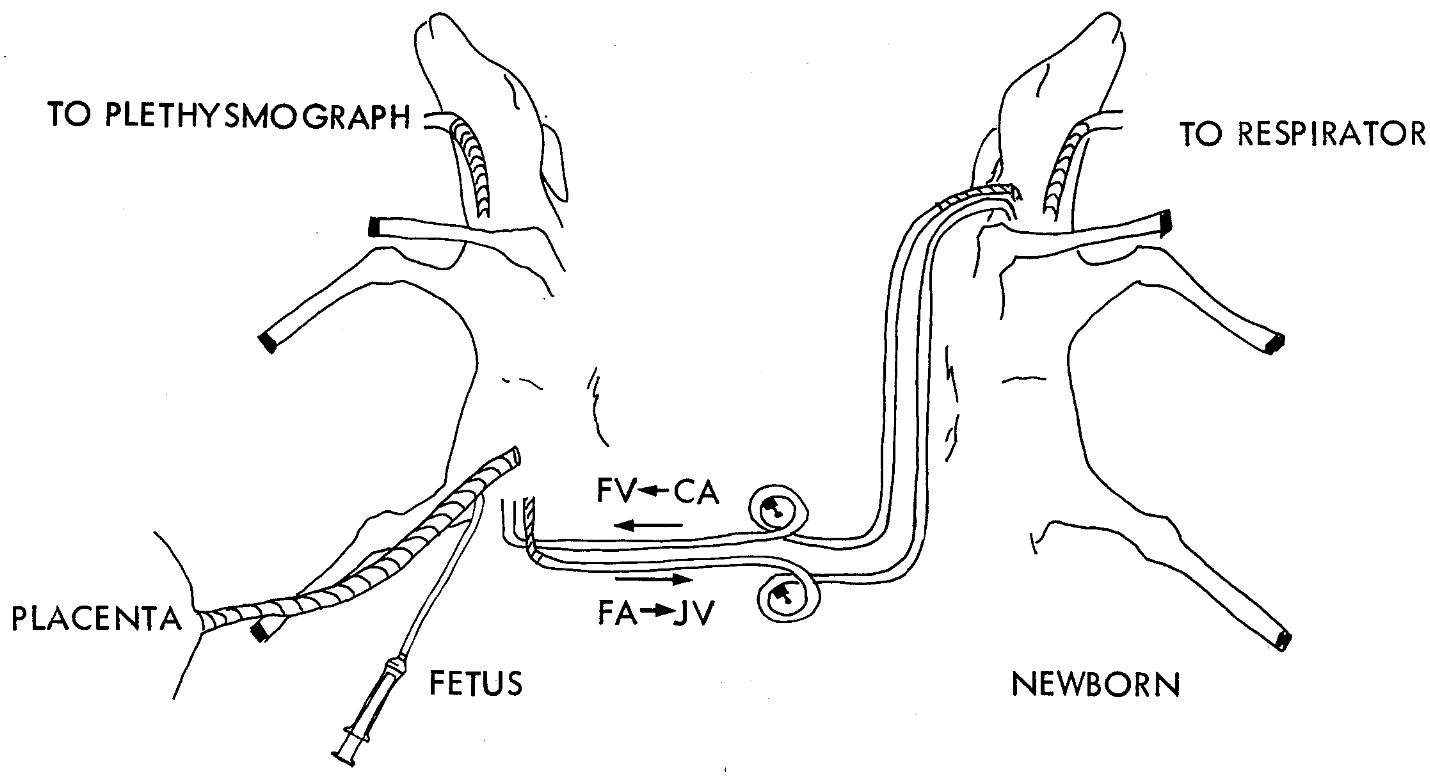

Figure 9. Diagram of the cross-circulation method utilizing a newborn lamb as the donor for the exteriorized fetus.

between the donor and the fetal circulations controlled the direction of flow from the donor artery to the fetal vein and from the fetal artery to the donor veing at a rate of 250-300 ml/min. Thus, oxygenated blood continued to be supplied to the fetus via the inferior vena caval return to the heart as in the normal fetal state. Less oxygenated blood in the fetal femoral artery was cross-circulated to the donor for gas exchange. During cross-circulation with the donor on room air, the fetal blood gas tensions and pH could be maintained at normal fetal levels, and the fetus remained apneic for at least 25 minutes even when the umbilical cord remained occluded (Fig. 10).

The newborn donor was artificially ventilated with room air and end-tidal PCO_2 was continuously monitored in an infra-red CO_2 analyzer (Godart capnograph). Exact arterial PCO_2 was determined by direct blood sampling. By altering alveolar ventilation and composition of the inspired gas of the donor animal during cross-circulation, the end-tidal PCO_2 could be adjusted to the desired level in the donor animal and hence in the fetus. For example, in order to initiate respiration when arterial PCO_2 was elevated, the donor was ventilated with 10% CO_2 in air and the end-tidal PCO_2 monitored. Serial blood samples were also taken. When P_aCO_2 was over 100 mm Hg it was usually necessary to add nitrogen to the reservoir bag in order to lower fetal P_aO_2 to a level which initiated respiration. In order to initiate respiration when P_aCO_2 was low, the donor was hyperventilated with air until P_aCO_2 was at the desired level. When P_aCO_2 was less than 40 mm Hg it was necessary to hyperventilate the donor using 100% nitrogen in order to start respiratory efforts in the fetus. Blood pressure of both the donor and the fetus were

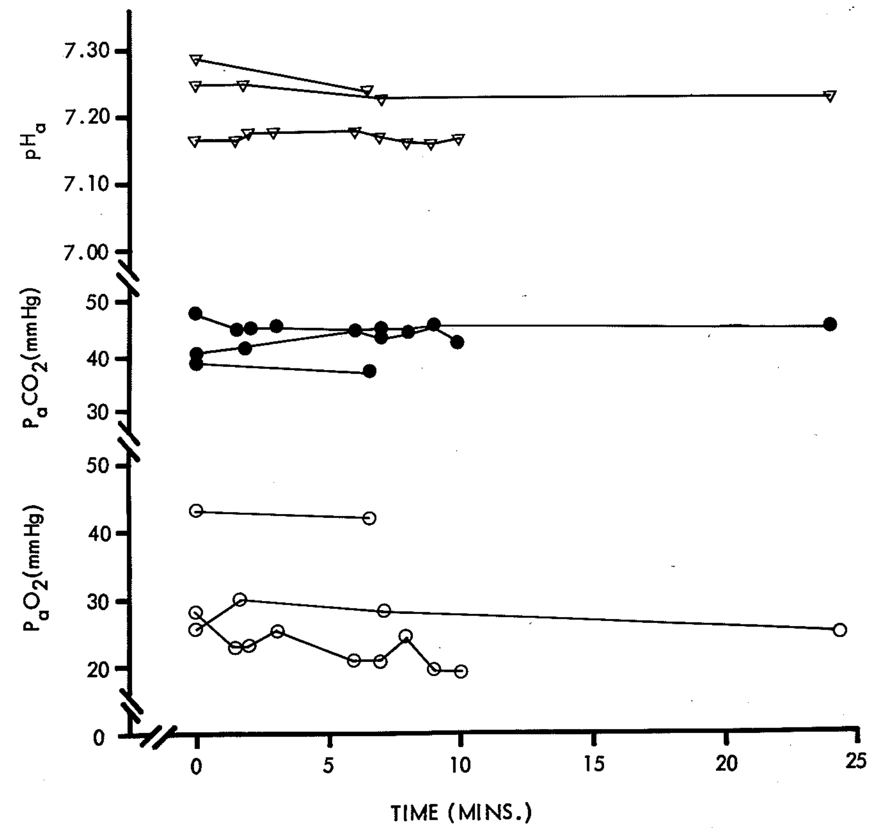

Figure 10. Arterial PO_2, PCO_2 and pH during cross-circulation of three fetuses for varying lengths of time. The donor was breathing room air and the umbilical cord occluded throughout.

continuously monitored on an oscilloscope and maintained as closely as possible to baseline levels in order to prevent a large change in blood volume in each animal.

At the time of the first breath, blood was immediately withdrawn from a separate fetal femoral artery and subsequently analyzed for PO_2, PCO_2 and pH. Following the onset of gasp, it was possible to return to the apneic fetal state by adjusting blood gases back to normal fetal levels. This was achieved by continuing the cross-circulation with the donor breathing room air and adjusting minute ventilation so that the end-tidal PCO_2 returned to normal levels. Subsequently, the umbilical cord clamp was released and the cross-circulation stopped. It was therefore possible to perform more than one experiment on one fetus. Subsequent analysis revealed no differences in the pattern of ventilatory response between experiments in the same fetus. Similarly the response was not influenced by the sequence of blood gas levels used to initiate respiration.

All respiratory responses were analyzed by computing the breath-by-breath minute ventilation, that is, by multiplying the inspired volume by the frequency. The frequency was calculated as the product of 60 and the reciprocal of the duration between 2 successive gasps. All the first, second and third breaths were analyzed in this manner and the effects of arterial gas tensions and pH were tested on each breath.

C. RESULTS

During cross-circulation with the donor breathing room air, in the absence of umbilical blood flow for as long as 25 minutes, the fetal arterial gas tensions and pH remained near baseline values (Fig. 10). No gasp was observed during this period.

During cross-circulation with the donor breathing various gases at varying alveolar ventilation, gasp was induced on 29 occasions in 14 fetal lambs. At the time of gasp, the P_aCO_2 ranged from 20 to 100 mm Hg and the P_aO_2 from 2 to 20 mm Hg. There was a direct linear relationship between P_aO_2 and P_aCO_2 at the time of the first breath ($r = +0.80$, $p < 0.001$) (Fig. 11). At P_aCO_2 less than 40 mm Hg, the P_aO_2 was always 5 mm Hg or less; at P_aCO_2 level between 40 mm Hg and 100 mm Hg, the P_aO_2 ranged from 6 to 16 mm Hg; and at P_aCO_2 levels greater than 100 mm Hg, the P_aO_2 was always above 16 mm Hg.

The positive relationship between P_aCO_2 and P_aO_2 at the time of the first breath was also demonstrated within individual animals in whom it was possible to do more than one experiment (Fig. 12). Furthermore, the sequence of alterations in P_aCO_2 did not influence this gas tension relationship.

During periods when the fetus was not being cross-circulated with the donor animal, occlusion of the umbilical cord invariably initiated breathing on 10 occasions in 9 fetal lambs at levels of gas tensions and pH similar to that previously observed during the first phase of the study. The P_aO_2 ranged from 7 to 14 mm Hg, the P_aCO_2 from 44 to 87 mm Hg and the pHa from 6.70 to 7.17 with mean values (± standard error) of 9.2 ± 0.8 mm Hg, 66.9 ± 4.3 mm Hg and 7.04 ± 0.04, respectively. A plot of P_aO_2 against P_aCO_2 suggested a relationship between the degrees of hypoxemia and hypercapnia at the time of gasp but this apparent relationship was statistcally not highly significant ($p < 0.05 > 0.01$) (Fig. 13). However, these values

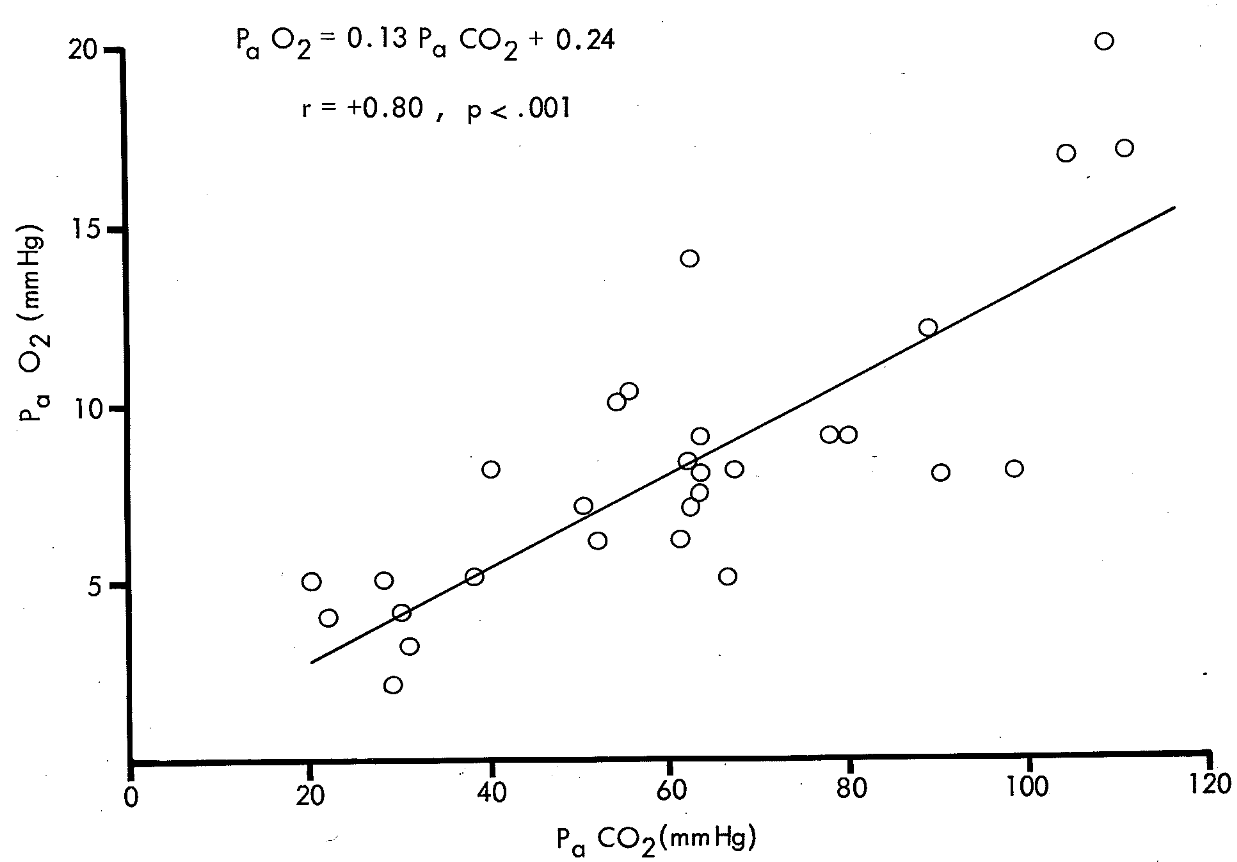

Figure 11. Relationship between the arterial gas tensions at the time of gasp initiated during cross-circulation. The slope of the regression line is highly significant.

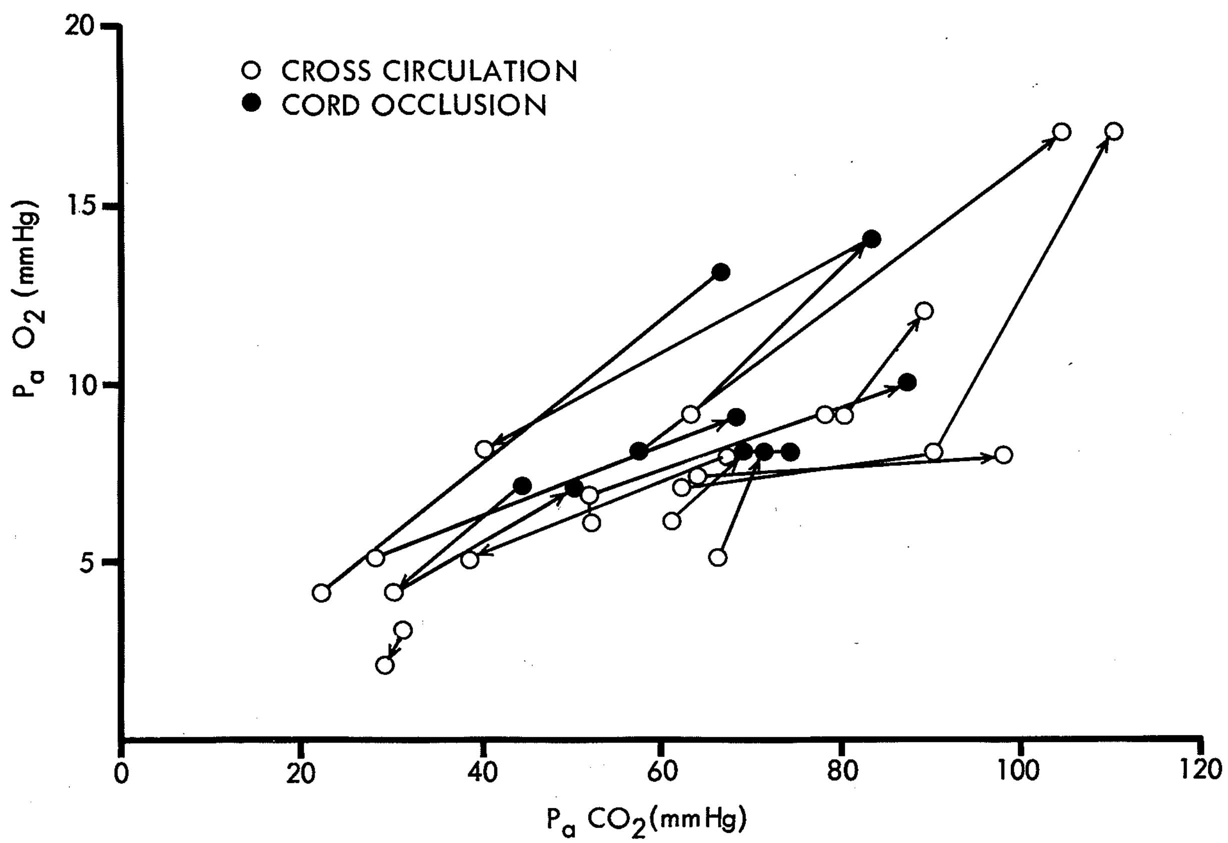

Figure 12. Relationship in 13 individual fetal lambs between the arterial gas tensions at the time of gasp obtained following cord occlusion (●) and during cross-circulation (O). The direction of arrows indicates the sequence with which different paired levels of P_aO_2 and P_aCO_2 initiated respiration. Each fetus demonstrated a positive slope for the relation between the levels of gas tensions.

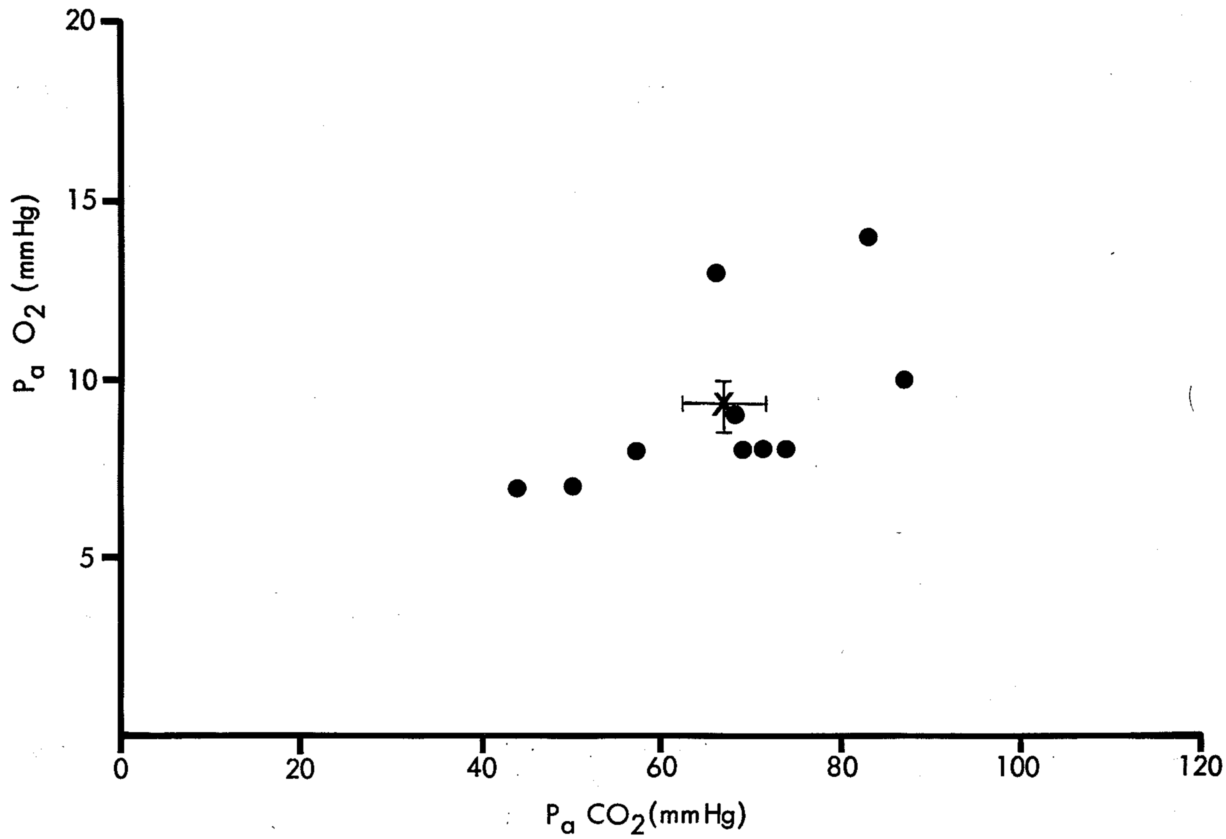

Figure 13. A scatter diagram of the levels of arterial gas tensions obtained at the time of gasp following cord occlusion in the absence of cross-circulation. The average P_aO_2 and P_aCO_2 (± S.E.) are shown. The correlation coefficient +0.62 is not highly significant ($p < 0.05 > 0.01$).

of arterial gas tensions fell along the regression line derived from data obtained during cross-circulation (Fig. 14).

Figure 15 shows that the relationship between P_aO_2 and $[H^+]$ at the time of gasp was similar to that between P_aO_2 and P_aCO_2 whether obtained during cross-circulation or cord occlusion. Since no attempt was made to keep $[H^+]$ constant, it is impossible to separate out a direct effect of P_aCO_2 from that of pH.

The sequence of arterial gas tensions and pH obtained from a single fetus during cross-circulation and simple cord occlusion is shown in Figure 16 and illustrates that a threshold value of blood gases must be reached in order to initiate respiration. Furthermore, it shows that the interrelation between the P_aO_2 and the P_aCO_2 at the time of gasp was maintained within this individual fetus.

Table III shows the frequency, tidal volume and breath-by-breath minute ventilation of the first, second and third liquid breaths during the initial and the subsequent experiments in the same fetus. In the initial experiments, the frequency, tidal volume and minute ventilation of the first breath did not differ from that of the second and third breaths. In the subsequent experiments in the same fetus, the tidal volume and minute ventilation of the second and third breaths were greater than that of the first breath ($p < 0.01$); there was no difference in the frequency. The first breath of the initial and that of the subsequent experiments differed only in regard to the tidal volume, that is, the first breath of the initial experiment had greater tidal volume than that of the subsequent experiment in the same fetus ($p < 0.05$).

There was no relationship between the levels of arterial PO_2, PCO_2

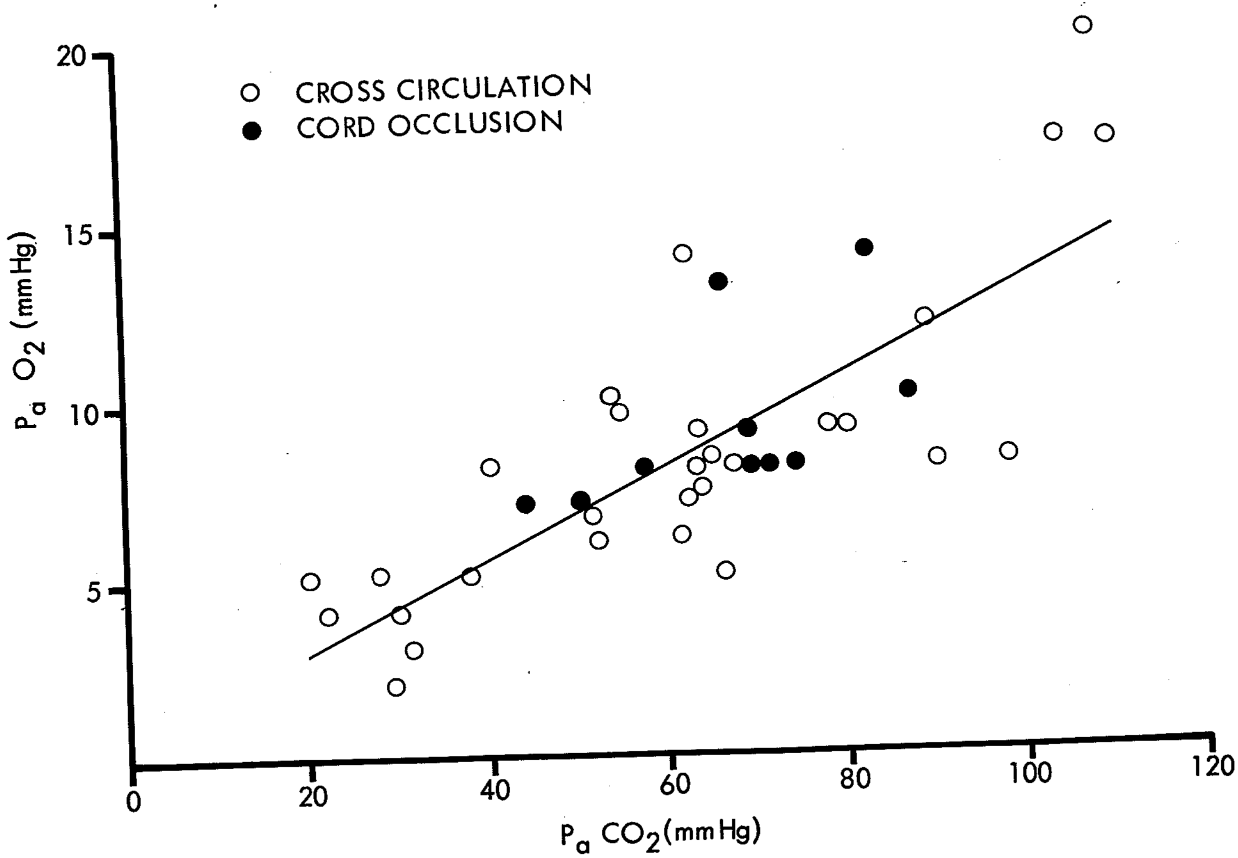

Figure 14. A scatter diagram which shows that the levels of arterial gas tensions obtained at the time of gasp following cord occlusion (●) fall along the regression line of data obtained at the time of gasp during cross-circulation (O) ($P_aO_2 = 0.13\ P_aCO_2 + 0.24$; $r = +0.80$, $p < 0.001$).

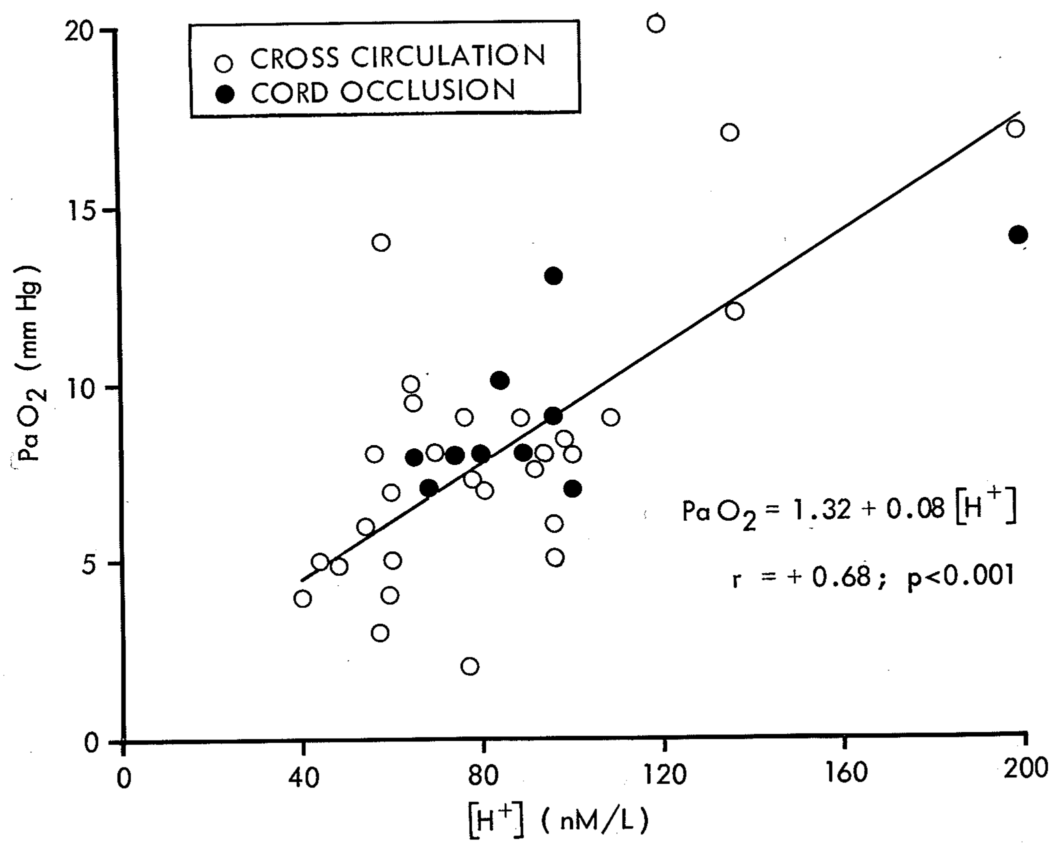

Figure 15. Relationship between the arterial hydrogen ion concentration and partial pressure of oxygen at the onset of gasp initiated following cord occlusion (●) and during cross-circulation (O).

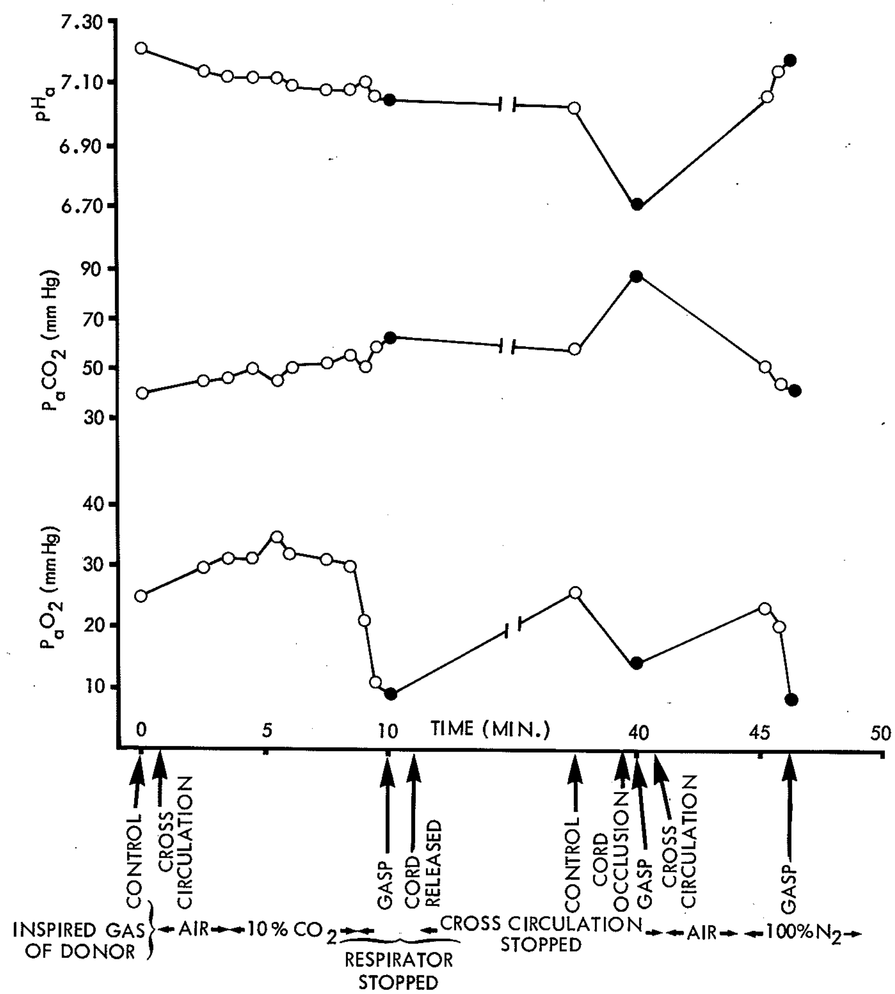

Figure 16. Diagram showing the sequence of changes in arterial gas tensions and pH prior to (O) and at the onset of gasp (●) in an individual fetus following cord occlusion alone and during cross-circulation with donor on various gases. Following the onset of gasp the arterial gas tensions and pH could be returned to near control values and a subsequent experiment was then possible. In this example 3 experiments were done in the same fetus over a period of about 50 minutes.

TABLE III

FREQUENCY (f), TIDAL VOLUME (V_T) AND BREATH-BY-BREATH MINUTE VENTILATION (V_I) OF THE FIRST, SECOND AND THIRD LIQUID BREATHS OF THE INITIAL AND SUBSEQUENT EXPERIMENTS IN THE SAME FETUS

Sequence of Breath	Initial Experiments				Subsequent Experiments			
	N*	f (per min)	V_T (ml)	V_I (ml/min)	N	f (per min)	V_T (ml)	V_I (ml/min)
First	11	2.7** (1.9-4.2)***	2.4 (0.6-5.3)	6.2 (1.2-12.5)	16	3.8 (1.3-10.7)	1.4 (0.6-2.8)	4.6 (1.9-12.1)
Second	10	3.3 (1.4-6.7)	3.1 (0.8-5.3)	9.8 (3.8-22.8)	16	4.1 (1.6-9.2)	2.1 (0.8-4.4)	7.5 (2.6-17.5)
Third	5	2.8 (1.3-4.2)	2.9 (0.8-4.5)	7.1 (2.1-14.0)	14	4.2 (1.8-13.3)	2.4 (0.8-5.2)	8.3 (3.5-16.7)

* Number of Observations
** Mean
*** Range

and pH at the time of the first breath and the frequency, tidal volume and minute ventilation of the first three breaths during the initial or subsequent experiments.

D. DISCUSSION

An obvious limitation of this study concerns the physiologic conditions of the experiment. Even during the best of experimental conditions, it is exceedingly difficult if not actually impossible to expose and maintain the fetus in a state comparable with that *in utero*. Interference with natural conditions took three forms in this study: 1) exteriorization of the fetus from the uterus, 2) surgical procedures during the preparation of the specimen and 3) use of pentobarbital anesthesia.

The sheep fetus when removed from the uterus and prevented from breathing maintains blood gas tensions and pH within their usual range of normal values for 4 to 5 hours provided the umbilical cord is left intact. Thus, it has been generally assumed that the exteriorized fetus remains in a normal physiological state. Heymann and Rudolph (90) have recently demonstrated that hemodynamic changes such as a decrease in umbilical flow may occur with minimal or no alterations in arterial pH, PO_2 and PCO_2. Since concern in the present study was not with measurements of flow but mainly with the levels of gas tensions which were determined by cross-circulation during the experimental procedures, exteriorization does not appear to be a major limitation.

The various surgical procedures during the preparation of the specimen conceivably contribute to the flow of sensory input to the respiratory center. Burns and Salmoiraghi (62,67) have demonstrated that peripheral sensory stimuli are important in determining the level of neuronal activity within the respiratory area in the brain stem. Their proposal regarding the importance of sensory input to the nervous system at birth has found adherents in Avery (6), Dawes (7) and Purves (63). It is interesting, however, that despite these surgical manipulations no ventilatory response was seen provided the arterial gas tensions and pH remained within normal baseline values.

The first phase of the study has shown that the levels of hypercapnia, hypoxemia and acidemia at the time of the first gasp were independent of the site of interruption of placental gas exchange. The foregoing observations indicate, indeed, that the chemical changes of cord occlusion are the prime stimuli for the initiation of the first breath. Measurement of gas tensions and pH within the cells of the central and peripheral chemoreceptors is beyond the scope of the present study but measurements of arterial gas tensions and pH ascertain the degree to which these receptors are influenced by the chemical changes in the blood which reaches its.

At the time of gasp induced by cord occlusion alone, the levels of arterial gas tensions only suggested but did not reveal a clear correlation between P_aO_2 and P_aCO_2. The levels of P_aCO_2 ranged from 44 mm Hg to 87 mm Hg. With the aid of a cross-circulation technique it was possible to extend the range of P_aCO_2 values at the time of the first breath over a much wider range than heretofore possible. With the use of this technique in this phase of the study the experiments clearly indicate a correlation between P_aO_2 and P_aCO_2 at the time of the first breath. This positive linear relationship between the levels of arterial gas tensions in initiating breathing is not surprising since hypoxemia and hypercapnia are known to augment each other's effect on ventilation in both the adult (4,91,92) and newborn animals (30,35). Furthermore, this P_aO_2-P_aCO_2 interrelation at the time of gasp provides an explanation for the observations of Harned and co-workers (81) who failed to elicit gasp in the sheep fetus by inducing fetal hypercapnia or hypoxemia through the ewe. Although induced separately, the conditions of their experiments were such that when the fetal P_aCO_2 was raised to study the effect of hypercapnia alone, fetal P_aO_2 was also raised and vice versa. Thus, their experimental conditions were the least favorable to observe a positive response.

The regression line relating arterial PO2 and PCO_2 at the time of the first breath had a slope of 0.13. This suggests that under the present experimental conditions, a 1mm Hg change in P_aO_2 was seven-or eightfold more potent a stimulus for the onset of breathing than a 1mm Hg change in P_aCO_2, but this slope might have been influenced by pentobarbital. Recently, Florez and Borison (93), utilizing tidal volume as the index of CO_2 responsivity, demonstrated in cats that pentobarbital reduced the slope of the CO_2 response curve. Indeed, it is possible that the use of pentobarbital in the ewe and the newborn donor altered the responsiveness of the fetal respiratory center to both chemical and mechanical stimuli. Windle (8) and Barcroft (1) have shown that doses of pentobarbital which anesthetize the mother depress the respiratory response of the fetus to mechanical stimulation. This effect may become magnified under acidemia which causes accumulation of barbiturates in lipids and deepens anesthesia. Thus, although an interaction was shown between P_aO_2 and P_aCO_2 at the time of the first breath, it is likely that this interaction was obtunded by the presence of pentobarbital.

The cross-circulation technique itself does not appear to have played a role since fetal respiration was not initiated during the procedure for as long as 25 minutes provided the arterial gas tensions and pH remained at normal fetal levels. Since the umbilical cord remained occluded throughout this time, the absence of breathing also provides conclusive proof for the first time that cord occlusion per se cannot initiate respiration. Further, this observation indicates that adequate fetal gas exchange was possible using the newborn donor as the placenta. Moreover, the observation that the arterial gas tensions at the time of gasp initiated by cord occlusion also fell along the same regression line derived from data obtained during cross-circulation indicates that the procedure itself did not influence the P_aO_2-P_aCO_2 relationship.

The direct linear relationship between P_aO_2 and P_aCO_2 was also demonstrated individually in 13 of the 14 fetal lambs studied and was independent of the sequence used in varying the arterial gas tensions. This suggests that the gasp response remained dependent on the interaction between P_aO_2 and P_aCO_2 despite several "first breaths" in the same fetus.

The tidal volume of the first breath in the initial experiment on a single fetus was greater than the tidal volume of the first breath in subsequent experiments. The reason for this is obscure since the level of blood gas tensions and pH at the time of gasp did not influence the frequency, inspired tidal volume or breath-by-breath minute ventilation of either the first, second or third liquid breath whether the initial or subsequent experiments within the same fetus were considered. These observations appear to indicate that the first few breaths constitute an all-or-none phenomenon. The influence of blood gas tensions on the ventilation after the first three breathes was not studied since blood gas levels were quickly readjusted to normal fetal levels following a response.

It is likely that the optimal experimental design requires investigation of the fetus in utero without the influence of anesthesia. However, for technical reasons this was not possible in the present series of experiments but does not detract from the value of new observations on a fundamental physiological problem. Thus, although precise quantitation is difficult there undoubtedly exists an interaction between hypoxemia and hypercapnia at the time of the first breath.

GENERAL SUMMARY AND CONCLUSION

GENERAL SUMMARY AND CONCLUSION

The present work is an investigation of the role of arterial systemic mean blood pressure and gas tensions in the initiation of respiration in the sheep fetus. Near-term pregnant ewes and newborn lambs were used.

Part I gives a review of the morphologic and physiologic bases of perinatal respiration and of the various hypotheses which have been invoked to explain the first breath.

Part II describes in two phases the initiation of breathing by various experimental procedures. Respiratory efforts were monitored using a liquid plethysmograph. Phase One delineated the influence of changes in mean systemic pressure in 14 exteriorized fetal lambs. Changes in mean systemic pressure in the absence of changes in blood gas tensions were produced on 15 occasions by rapid infusion or withdrawal of blood, infusion of norepinephrine or occlusion of one umbilical artery. Despite change of mean blood pressure of as much as ±50%, breathing was not initiated. On 18 occasions alterations in mean blood pressure with comcomitant hypercapnia and hypoxemia were produced by either occlusion of the umbilical vein, both umbilical arteries, entire umbilical cord or the maternal abdominal aorta. In all instances fetal gasping was evoked indicating that the initiation of breathing is independent of the site of interruption of fetal-placental gas exchange. By several methods of analysis there was no relationship between the mean blood pressure and the arterial PCO_2 and PO2 at the time of gasp. Also, mean blood pressure did not influence the latent period. It is concluded that changes in arterial systemic mean pressure per se cannot initiate respiration. Furthermore, there is no interaction between hypercapnia-hypoxemia and mean blood pressure in the initiation of breathing of fetal lambs.

Phase Two of the experimental study evaluated the relative importances of arterial gas tensions in 14 exteriorized fetuses. The fetus was cross-circulated with artificially ventilated newborn lamb at a flow rate of 250-300 ml/min. During cross-circulation with the newborn donor breathing room air, the fetus maintained its arterial gas tensions and pH at normal levels and remained in the normal apneic state for as long as 25 minutes even if the umbilical cord was occluded throughout this period. This observation conclusively showed for the first time that cord occlusion per se cannot initiate breathing.

Fetal liquid ventilation was initiated on 29 occasions by adjusting the alveolar ventilation and composition of the inspired gas of the newborn donor. At the time of the first breath, there was a positive relationship between the levels of fetal arterial PO_2 and PCO_2. This relationship appeared to be linear: $P_aO_2 = 0.13\ (P_aCO_2) + 0.24$; $(r=+0.80,\ p<0.001)$. At a fetal P_aCO_2 below 40 mm Hg, the first breath did not occur until the P_aO_2 was 5 mm Hg or less; at level of P_aCO_2 between 40 and 100 mm Hg, the P_aO_2 at the time of the first breath was always above 5 mm Hg and ranged from 6 to 16 mm Hg; and at fetal P_aCO_2 above 100 mm Hg, the first breath was initiated at P_aO_2 above 16 mm Hg and as high as 20 mm Hg. The interaction between P_aO_2 and P_aCO_2 was also demonstrated in 13 of the 14 individual animals studied. It is concluded that there is an interaction between arterial PO_2 and PCO_2 in the initiation of respiration in sheep fetus.

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